Genetics of Hereditary Ataxia in Scottish Terriers

G. Urkasemsin, D.M. Nielsen, A. Singleton, S. Arepalli, D. Hernandez, C. Agler, and N.J. Olby

Background: Scottish Terriers have a high incidence of juvenile onset hereditary ataxia primarily affecting the Purkinje neuron of the cerebellar cortex and causing slowly progressive cerebellar dysfunction.

Objective: To identify chromosomal regions associated with hereditary ataxia in Scottish Terriers.

Animals: One hundred and fifty-three Scottish Terriers were recruited through the Scottish Terrier Club of America.

Materials and Methods: Prospective study. Dogs were classified as affected if they had slowly progressive cerebellar signs. When possible, magnetic resonance imaging and histopathological evaluation of the brain were completed as diagnostic aids. To identify genomic regions connected with the disease, genome-wide mapping was performed using both linkage- and association-based approaches. Pedigree evaluation and homozygosity mapping were also performed to examine mode of inheritance and to investigate the region of interest, respectively.

Results: Linkage and genome-wide association studies in a cohort of Scottish Terriers both identified a region on CFA X strongly associated with the disease trait. Homozygosity mapping revealed a 4 Mb region of interest. Pedigree evaluation failed to identify the possible mode of inheritance due to the lack of complete litter information.

Conclusion and Clinical Importance: This finding suggests that further genetic investigation of the potential region of interest on CFA X should be considered in order to identify the causal mutation as well as develop a genetic test to eliminate the disease from this breed.

Key words: Canine; Cerebellar abiotrophy; Cerebellar ataxia; Neurodegeneration.

Hereditary ataxias are a heterogeneous group of neurodegenerative diseases that cause cerebellar ataxia with a wide range of clinical and pathological manifestations, constituting an important problem affecting purebred dogs. To date, mutations in 8 different genes have been associated with hereditary ataxias in more than 10 breeds of dog. These genes encode proteins that have a variety of different functions including protein degradation and autophagy (RAB24 in Old English Sheep dogs and Gordon Setters, SEL1L in Finnish hounds, and CAPN1 in Parson Russell Terriers), CNS cytoskeleton integrity (SPTBN2 in Beagles), cation trafficking (ITPR1 in Italian Spiones, GRM1 in Coton de Tulears, and KCNJ10 in Russell Terrier Group and smooth-haired Fox Terrier), and mitochondrial function and cholesterol trafficking (SERAC1 in Kerry Blue and Chinese Crested). Mutations include exonic nonsynonymous nucleotide substitution, retrotransposon insertion in the exon and intronic GAA repeat expansion. An autosomal recessive mode of inheritance has been identified in these breeds. An X-linked mode of inheritance has been reported in the English pointer dogs, but the mutation has not been identified.

Hereditary ataxia or hereditary cerebellar degeneration has been recognized in Scottish Terriers for more than a decade. The cerebellar cortex is the primary site of neurodegeneration, which is characterized by...
severe loss of Purkinje cells, depletion of granule cells and atrophy of the molecular layer as well as polyglucosan body accumulation. Clinical signs reflect cerebellar dysfunction including a wide-based stance, dysmetria producing a hypermetric gait, difficulty negotiating stairs and intention tremors. Onset of signs usually occurs during the first year of life and in many dogs, progression is slow with signs ultimately stabilizing, resulting in a lifelong relatively mild phenotype. The objective of this study was to investigate the genetic basis of hereditary ataxia in Scottish Terriers. We performed genome-wide microsatellite and single nucleotide polymorphism (SNP) genotyping in affected Scottish Terriers and their extended families to identify a chromosomal locus associated with the disorder using linkage and genome-wide association analyses.

Materials and Methods

This was a prospective study aimed at mapping the locus of cerebellar degeneration in Scottish Terriers following genotyping with microsatellites using linkage analysis in related families. To confirm findings of the linkage study, a broader population of dogs was then genotyped on single nucleotide polymorphisms and a genome-wide association study was performed.

Study Population

Affected (case) and normal (control) Scottish Terriers were recruited through the Scottish Terrier Club of America. All participating dogs were privately owned pets. Dogs were classified as affected if they had slowly progressive cerebellar signs as described previously. The objective of this study was to investigate the genetic basis of hereditary ataxia in Scottish Terriers. We performed genome-wide microsatellite and single nucleotide polymorphism (SNP) genotyping in affected Scottish Terriers and their extended families to identify a chromosomal locus associated with the disorder using linkage and genome-wide association analyses.

Genome-Wide Microsatellite Genotyping and Linkage Analysis

Related dogs were genotyped with a genome-wide panel of 296 autosomal fluorescently labeled (FAM, VIC, NED, and PET) canine microsatellite markers from the minimal screening set (MSS-2) (representing approximately 10 cM resolution across the entire canine genome), organized into multiplex PCR groups. The PCR conditions used have been described elsewhere. PCR fragments were visualized by an ABI 3730xl DNA Analyzer, and genotypes were assigned by GeneMapper v3.7 software. Homozygous (uninformative) markers were excluded from further analysis.

Genome-Wide Association Study

Genome-wide SNP genotyping in the case and control dogs was performed with the Illumina® CanineSNP20 BeadChip (22,362 SNPs, n = 70 dogs) and Illumina® CanineHD genotyping BeadChip (173,662 SNPs, n = 59 dogs, 13 of which were also genotyped on the 22 K array). The assays were performed at the National Institutes of Health’s Laboratory of Neurogenetics (Bethesda, MD) following the manufacturer’s instructions. The amplified DNA products were imaged with a BeadArray Reader. The assay intensity data were then loaded into Illumina® BeadStudio software to score and generate the SNP genotypes.

The genome-wide association study (GWAS) and data pruning as well as the adjustment for multiple testing were carried out by PLINK v1.07 software package. A case-control GWAS was performed on the SNPs common to both the 22 K and 173 K platforms. These SNPs had to have identical alleles in the 13 duplicate dogs genotyped on both arrays. Data were pruned by removing individuals with a call rate below 96%, and SNPs with minor allele frequency (MAF) less than 1%, or with more than 10% missing genotypes. Individuals were clustered on the basis of genetic identity by Identical-by-state (IBS) clustering, and the Cochran-Mantel-Haenszel (CMH) association analysis was implemented to test SNP-disease association conditional on the IBS cluster provided. Bonferroni adjustment was used to correct multiple comparisons with adjusted P-values less than .05 considered significant. JMP® Genomics software (SAS; Cary, NC) was used to create QQ-plots from the genomic control (GC) data to assess the adequacy of correction of population structure.

Disease-segregating homozygous regions were identified based on the SNP genotypes by visual inspection of the data. The UCSC Genome Browser was used to identify regions of shared homozygosity.
Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway) was used to search for candidate genes in regions of interest (CanFam2.0).

**Results**

A total of 153 dogs (69 males, 84 females) were genotyped in this study. There are 46 cases (27 males, 19 females), 97 controls (36 males and 61 females), and 10 undetermined (6 males and 4 females). Hereditary ataxia was confirmed by histopathology in 11 cases, 3 of which had an MRI of the brain. An additional 3 cases underwent an MRI of the brain. The rest of the cases were diagnosed based on clinical history, and signs of cerebellar disease on videotapes of gait.

**Genome-Wide Microsatellite Genotyping and Linkage Analysis**

A total of 96 dogs from one pedigree were selected to be genotyped (Fig S1). There were 71 controls (25 males and 46 females), 22 cases (14 males and 8 females), and 3 undetermined status dogs (one 3-year-old male, and two 4- and 9-year-old females). The diagnosis was confirmed in 5 of the cases by histopathology, one of which was also examined by MRI. The rest of the cases were diagnosed based on clinical history, and signs of cerebellar disease on videotapes of gait.

The maximum LOD score from performing linkage analysis of the first 48 Scottish Terriers was 1.16 located on CFA 11 between markers C11.873 (66 Mb) and DGN13 (73 Mb). When the genotypes of an additional 47 dogs from the same pedigree were added, the LOD score in the same region decreased to 0.99. There were no LOD scores greater than 0.50 on any other autosomes. In contrast, LOD scores greater than 3 were found on CFA X between markers FH3027 (41.0 Mb) and REN75A05 (117.2 Mb). There was only 1 more informative marker in this region (FH2584 at 103.9 Mb), and the highest LOD score of 5.6 was located at this marker (Fig 1). Evaluation of the haplotypes in the region failed to identify clear segregation with disease using either a dominant or recessive model of inheritance. There were 9 genes within this 76.2 Mb disease-linked region (Table 1) that have been associated with X-linked syndromes in humans in which cerebellar degeneration or dysfunction was a component. However, none of these syndromes cause a pure cerebellar ataxia as seen in the Scottish Terrier.19–21

To determine whether the disease could be inherited as an X-linked dominant or recessive trait, dogs’ relationships in the pedigrees genotyped were re-examined. Full litter information was not available for most of the families; therefore, it was not possible to make a statistical comparison of actual and expected findings given different modes of inheritance. Overall, there were approximately twice as many females (46) as males (25) in the control dogs, while there were nearly twice as many males (14) as females (8) in the case dogs. If the control population was taken to be the baseline ratio of females to males, the ratio of male to female cases becomes more substantial. If the mode of inheritance is X-linked recessive trait, affected females would have affected male offspring. There was only 1 litter for which we had data on offspring from an affected female (Fig 2), and in this case, the male offspring was affected. Notably, in the litter with several male and female dogs (Fig 2), all the males were affected and all the females had a normal phenotype. These litters came from normal parents implying a recessive mode of inheritance, or incomplete penetration of a dominant mode. In one instance, the family structure is not consistent with an X-linked recessive mode of inheritance (Fig 2). In this family, normal parents (8-year-old dam and sire reported to be normal by the owners) gave rise to an affected female. There was only 1 subfamily in which an affected male and normal female produced normal male offspring. To investigate the results of the linkage study further a genome-wide association study was performed using SNP genotypes.

**Genome-Wide Association Study**

A total of 116 dogs (46 cases, 60 controls, and 10 undetermined) were genotyped on SNP chips, 59 (22 cases, 34 controls, and 3 undetermined) of which were included in the linkage analysis study. Thirty-four cases (15 females and 19 males), 34 controls (19 females and 15 males), and 2 undetermined dogs (1 female and 1 male) were genotyped on the 22 K array, whereas 24 cases (10 females and 14 males), 27 controls (16 females and 11 males), and 8 undetermined dogs (3 females, 5 males) were genotyped on the 173 K array. Thirteen dogs (6 female cases, 6 male cases, and 1 male control) were genotyped on both arrays for quality control. Before pruning, there were 19,215 SNPs common to both 22 K and 173 K platforms, all of which had identical alleles in the 13 duplicate dogs genotyped on both arrays. SNPs with MAF <1% and missing genotype calls >10% were removed from the analysis, resulting in a final data set of 14,365 SNPs that underwent case-control association analysis. The genotyping call rate in all individuals was more than 99%. As most of the dogs
were related and some dogs were closely related, popu-
lation stratification was expected. GWAS with CMH
testing for SNP-disease association conditional on IBS
was therefore performed. A significantly associated
region on CFA X was identified. It extended from 103
to 109 Mb (CanFam2.0) with the strongest $P_{raw}$ equal
to $5.249 \times 10^{-9}$ ($P_{genome} = 3.076 \times 10^{-5}$ and Bonferroni-
adjusted multiple testing significance value $= 7.497 \times 10^{-5}$). The Q-Q plot of the observed $p$-value (genomic control) against the expected $p$-value also demonstrated an appropriate adjustment for popu-
lation stratification (Fig 3A and B). This finding con-
firmed the results of the linkage analysis study. In
addition, there were weaker, but still significant signals
on CFA 2, 4, and 17 (Fig 3A).

The regions of interest on CFA X, 2, 4, and 17 were
subjected to further investigation using homozygosity
mapping (Fig 4). The X-chromosome SNP genotypes of
all male dogs were displayed as homozygous, although
they are hemizygous because they only possess 1 X chro-
mosome. Genotype data were visually inspected for
regions of homozygosity shared only in the affected dogs.
There are 2 candidate regions from 103,456,993 bp to
103,530,501 bp and 104,813,634 bp to 106,189,685 bp.
While 93.47% of the cases in these 2 blocks are identical,
35% (103 Mb) and 31.67% (104–106 Mb) of the controls
also shared the same haplotype. Three female cases did
not share the haplotype of most cases (Fig 4 and
Table 2). When control dogs had the case genotype, this
was true across both regions of interest on the X chromo-
some. The SNP genotypes on CFA 2, 4, and 17 associ-
ated with a weaker GWAS signal were also evaluated,
but there was no segregation of genotype with
phenotype.

**Discussion**

Whole genome mapping using both linkage analysis
and GWAS identified a region on CFA X that is
strongly associated with hereditary ataxia in Scottish
Terriers. Linkage analysis revealed a region extending
from 41.0 to 117.2 Mb and GWAS confirmed the asso-
ciation on CFA X and narrowed the region down to
6 Mb (from 103 to 109 Mb). This finding appears to be
robust as it was duplicated with 2 separate mapping
techniques, but was unexpected given the apparent auto-
somal recessive mode of inheritance described previ-
ously. While the pedigrees were evaluated, the lack of
complete litter information prevented a segregation anal-
ysis from being performed. Several explanations could
be made to support an X-linked mode of inheritance for
this disease in Scottish Terriers. In X-linked recessive
traits, males have a higher risk of being affected than
females because males carry only 1 copy of the X chro-
mosome. In our population of cases, the number of
female cases was lower than males with an overall ratio
of 0.7 females to 1 male, but still higher than expected
for a recessive X-linked trait. The pedigree contains 3 lit-
ters with complete information (Figs 2 and S1, blue
ovals). In these litters, both parents were reported to be
normal, and only male offspring were affected, and the

---

### Table 1. X-linked disorders with cerebellar ataxia as a component

| Human X-linked syndrome with cerebellar ataxia | Human chromosomal locus | Dog chromosomal locus | Gene | Mutation |
|-----------------------------------------------|-------------------------|----------------------|------|----------|
| Oligophrenin-1 syndrome                        | chrX:67262186-67653299 | chrX:55372712-55980821 | OPHN1 | Point mutation |
| X-linked sideroblastic anemia with ataxia       | chrX:74376132-74627315 | chrX:61359945-61523487 | ABCB7 | Point mutation |
| X-linked Opitz/GBBB syndrome                   | chrX:10413350-10851809 | chrX:7041201-7198729 | MID1 | Unknown |
| X-linked lissencephaly type 1                  | chrX:11065540-11065546 | chrX:87469155-87586087 | DCX | Point mutation |
| Oral-facial-digital type I/X-linked Joubert syndrome | chrX:13752382-13787480 | chrX:10098404-10151431 | OFD1 | Unknown |
| X-linked Angelman-like syndrome                | chrX:135067586-135129428 | chrX:109487347-10953447 | SLC9A6 | Unknown |
| X-linked visceral heterotaxy                   | chrX:136648346-136648359 | chrX:10890225-11089603 | ZIC3 | Unknown |

---

**Human X-linked syndrome**

**Gene product**

**Mutation**

---

**Human X-linked syndrome with cerebellar ataxia**

**Dog chromosomal locus**

---

**Gene**

---

**Gene product**

---

**Mutation**

---
females were normal. In another family, the female offspring from an affected dam was normal, whereas the male offspring was affected (Fig 2, black oval). These families suggest the disease could potentially be transmitted in an X-linked recessive manner. However, there is 1 affected female (Fig 2, pink oval) that has normal parents (as reported by the owners of the parents), which is inconsistent with an X-linked recessive mode of transmission.
inheritance, so an X-linked dominant trait with incomplete penetrance was considered. In female mammals, 1 of the X chromosomes is silenced in order to avoid overexpression of the genes compared to males.22 The process of X-chromosome inactivation (XCI) begins randomly in each cell of the embryo resulting in a genetic mosaic of cells from expression of either paternal or maternal X-chromosome origin. XCI is regulated initially by the long noncoding RNA which is transcribed from the X-inactivation center (Xic) locus on the X chromosome.23,24 However, about 15% of human X-linked genes can escape silencing by XCI and are expressed in both the active (Xa) and inactive (Xi) X chromosome in females.25 These escaped XCI genes are located mostly in the pseudoautosomal regions (PARs) on the X chromosome. PARs contain homologous nucleotide sequences between mammalian X and Y chromosomes, and they are inherited like autosomes. Nevertheless, in humans and mice, some of the escaped genes can be found distributed throughout the X chromosome.25–27 It is possible that the causal mutation of hereditary ataxia in Scottish Terriers is in an escaped gene on the X chromosome reflecting the apparent segregation of the disease in the population as an autosomal recessive trait. However, the identified region cannot be located in the PAR as the canine PAR spans approximately 6.6 Mb from the telomere of the Xp arm.28 Nonrandom XCI (skewed X-chromosome inactivation) in the female may occur by paternally or maternally biased inactivation of the X chromosome and can also result in tissue-specific skewing.29,30 This could also explain the affected female with normal parents. Unfortunately, the pattern of inheritance of the disease cannot be determined by examining the available pedigree. In order to accurately determine the mode of inheritance, a breeding colony of Scottish Terriers with hereditary ataxia would need to be established to perform selective breedings.

Table 2. Homozygosity mapping by examining dogs’ genotypes on the common 14,365 SNPs (116 dogs: 46 cases (19 females and 27 males); 60 controls (35 females and 25 males); and 10 undetermined dogs (4 females and 6 males)). F is female and M is male.

| Affected status | First haplotype | Second haplotype | Heterozygous |
|----------------|----------------|-----------------|--------------|
| 103.4–103.6 Mb (116 dogs: 58 F, 58 M) | 43 (18 F, 25 M) | 1 (F) | 2 (F) |
| Cases          | 43 (18 F, 25 M) | 1 (F) | 2 (F) |
| Controls       | 21 (6 F, 15 M) | 15 (6 F, 9 M) | 24 (F) |
| Undetermined   | 3 (1 F, 2 M)   | 4 (M) | 3 (F) |
| 104.8–106.2 Mb (116 dogs: 58 F, 58 M) | 43 (18 F, 25 M) | 1 (F) | 2 (F) |
| Cases          | 43 (18 F, 25 M) | 1 (F) | 2 (F) |
| Controls       | 19 (4 F, 15 M) | 15 (6 F, 9 M) | 26 (F) |
| Undetermined   | 3 (1 F, 2 M)   | 4 (M) | 3 (F) |

In female mammals, 1 of the X chromosomes is silenced in order to avoid overexpression of the genes compared to males.22 The process of X-chromosome inactivation (XCI) begins randomly in each cell of the embryo resulting in a genetic mosaic of cells from expression of either paternal or maternal X-chromosome origin. XCI is regulated initially by the long noncoding RNA which is transcribed from the X-inactivation-specific transcript (Xist) gene located in the
The possibility that more than one form of hereditary ataxia is segregating within the Scottish Terrier breed has also been considered. In addition, the lack of a definitive diagnosis made by MRI or histopathology in every dog means that some could have suffered from an acquired cause of cerebellar ataxia. However, the presence of phenocopies typically reduces the study power, making associated chromosomal loci impossible to detect.

Homozygosity mapping by visual inspection of the genotypes identified 2 nonoverlapping regions, within the 103 Mb and 104 to 106 Mb, in which the overwhelming majority of cases were homozygous. However, in both regions, approximately 30% of the controls were also homozygous for the same allele. This could be explained in 2 ways: Firstly, it was possible that the dogs had such a mild phenotype that it was unrecognized by their owners. Owners of control dogs were reluctant to send videotapes so it was difficult to confirm the reported phenotype of the control group. Secondly, as most of the controls that had the same haplotypes as cases were from the same family, the haplotype segregation could be explained by the presence of a common haplotype in this region with the mutation located within the haplotype.

In conclusion, linkage analysis, GWAS, and homozygosity mapping have identified a candidate region on CFA X associated with the hereditary ataxia in Scottish Terriers. Although, there was no perfect disease-segregating haplotype between cases and controls and no strong candidate gene within the region, it is worth investigating this region further using the new build of canine genome (CanFam3) and performing deep sequencing for this region to identify the potential mutations.

Footnotes

1. O'Brien DP, Zeng R, Schnabel RD, Taylor JF, Johnson GS. Identification of Two Breed-Specific Mutations Associated with Canine Multiple System Degeneration Using Whole Genome Resequencing. Proc ACVIM Forum 2013
2. QIAamp® DNA Blood Midi Kit: Qiagen, Valencia, CA
3. Oragene Animal kit: DNA Genotek, Kanata, Ontario
4. ND-1000 NanoDrop spectrophotometer: Thermo Scientific, Wilmington, DE
5. ABI 3730xl DNA Analyzer and GeneMapper v3.7 software: Applied Biosystems, Carlsbad, CA
6. Illumina® CanineSNP20 BeadChip, Illumina® CanineHD genotyping BeadChip, BeadArray Reader, and Illumina® BeadStudio software: Illumina Inc., San Diego, CA

Acknowledgments

This work could not have been completed without the help of veterinarians and Scottish Terrier breeders and owners. The work was supported by the Health Trust Fund of the Scottish Terrier Club of America and the American Kennel Club Canine Health Foundation.

Conﬂict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Urkasemsin G, Olby NJ. Canine hereditary ataxia. Vet Clin North Am Small Anim Pract 2014;44:1075–1089.
2. Agler C, Nielsen DM, Urkasemsin G, et al. Canine hereditary ataxia in old English sheepdogs and Gordon setters is associated with a defect in the Autophagy gene encoding RAB24. PLoS Genet 2014;10:e1003991.
3. Kyöstilä K, Cizinauskas S, Seppälä EH, et al. A SEL1L mutation links a canine progressive early-onset cerebellar ataxia to the Endoplasmic Reticulum-Associated Protein Degradation (ERAD) machinery. PLoS Genet 2012;8:e1002759.
4. Forman OP, De Risio L, Mellersh CS. Missense mutation in CAPN1 is associated with spinocerebellar ataxia in the parson Russell terrier dog breed. PLoS ONE 2013;8:1–8.
5. Forman OP, De Risio L, Stewart J, et al. Genome-Wide mRNA sequencing of a single canine cerebellar cortical degeneration case leads to the identiﬁcation of a disease associated SPTBN2 mutation. BMC Genet 2012;13:55.
6. Forman OP, De Risio L, Matiasek K, et al. Spinocerebellar ataxia in the Italian spinone dog is associated with an intronic GAA repeat expansion in ITPR1. Mamm Genome 2015;26:108–117.
7. Zeng R, Farias FH, Johnson GS, et al. A truncated retrotransposon disrupts the GRM1 coding sequence in Coton de Tulear dogs with Bandera’s neonatal Ataxia. J Vet Intern Med 2011;25:267–272.
8. Gilliam D, O’Brien DP, Coates JR, et al. A homozygous KCNJ10 mutation in jack Russell terriers and related breeds with spinocerebellar ataxia with Myokymia, seizures, Or both. J Vet Intern Med 2014;28:871–877.
9. Rohdin C, Gilliam D, O’Leary CA, et al. A KCNJ10 mutation previously identiﬁed in the Russell group of terriers also occurs in smooth-haired fox terriers with hereditary ataxia and in related breeds. Acta Vet Scand 2015;57:26.
10. O’Brien DP, Shibuya H, Zhou T, et al. X-linked cerebellar ataxia in English pointer dogs: Phenotype and linkage data. Canine Pract 1998;23:46.
11. van der Merwe LL, Lane E. Diagnosis of cerebellar cortical degeneration in a Scottish terrier using magnetic resonance imaging. J Small Anim Pract 2001;42:409–412.
12. Urkasemsin G, Linder KE, Bell JS, et al. Mapping of Purkinje neuron loss and Polyglucosan body accumulation in hereditary cerebellar degeneration in Scottish Terriers. Vet Pathol 2012;49:852–859.
13. Urkasemsin G, Linder KE, Bell JS, et al. Hereditary cerebellar degeneration in Scottish Terriers. J Vet Intern Med 2010;24:565–570.
14. Clark LA, Tsai KL, Steiner JM, et al. Chromosome-specific microsatellite multiplex sets for linkage studies in the domestic Dog. Genomics 2004;84:550–554.
15. Thompson EA. MCMC estimation of multi-locus genome sharing and multipoint gene location scores. Int Stat Rev 2000;68:53–73.
16. Olby N, Blot S, Thibaud JL, et al. Cerebellar cortical degeneration in adult American Staffordshire Terriers. J Vet Intern Med 2004;18:201–208.
17. Strauch K, Fimmers R, Kurz T, et al. Parametric and non-parametric multipoint linkage analysis with imprinting and two-locus-trait models: Application to mite sensitization. Am J Hum Genet 2000;66:1945–1957.
18. Purcell S, Neale B, Todd-Brown K, et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575.

19. Finsterer J. Ataxias with autosomal, X-chromosomal or maternal inheritance. Can J Neurol Sci 2009;36:409–428.

20. Zanni G, Bertini ES. X-linked disorders with cerebellar dysgenesis. Orphanet J Rare Dis 2011;6:24.

21. Del Bigio MR, Halliday WC. Multifocal atrophy of cerebellar internal granular neurons in Lesch-Nyhan disease: Case reports and review. J Neuropathol Exp Neurol 2007;66:346–353.

22. Lyon MF. Sex chromatin and gene action in the mammalian X-chromosome. Am J Hum Genet 1962;14:135–148.

23. Arthold S, Kurowski A, Wutz A. Mechanistic insights into chromosome-wide silencing in X inactivation. Hum Genet 2011;130:295–305.

24. Pontier DB, Gribnau J. Xist regulation and function explored. Hum Genet 2011;130:223–236.

25. Carrel L, Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 2005;434:400–404.

26. Berletch JB, Yang F, Distech CM. Escape from X inactivation in mice and humans. Genome Biol 2010;11:213.

27. Yang F, Babak T, Shendure J, Distech CM. Global survey of escape from X inactivation by RNA-sequencing in mouse. Genome Res 2010;20:614–622.

28. Young AC, Kirkness EF, Breen M. Tackling the characterization of canine chromosomal breakpoints with an integrated in-situ/in-silico approach: The canine PAR and PAB. Chromosome Res 2008;16:1193–1202.

29. Desai V, Donsante A, Swoboda KJ, et al. Favorably skewed X-inactivation accounts for neurological sparing in female carriers of Menkes disease. Clin Genet 2011;79:176–182.

30. Orstavik KH. X chromosome inactivation in clinical practice. Hum Genet 2009;126:363–373.

31. Lazzaro MA, Todd MAM, Lavigne P, et al. Characterization of novel isoforms and evaluation of SNF2L/SMARCA1 as a candidate gene for X-linked mental retardation in 12 families linked to Xq25–26. BMC Med Genet 2008;9:11.

**Supporting Information**

Additional Supporting Information may be found online in the supporting information tab for this article:

**Fig S1.** A Scottish Terrier pedigree. Ninety-six dogs were genotyped (green border). Females are ovals, and males are rectangles. Affected (case) dogs are solid red, normal (control) dogs are white, and undetermined status dogs are solid yellow. The vertical black oval highlights the sub-family in which the affected dam has an affected male and a normal female offspring. The horizontal blue ovals highlight litters containing affected males and normal females. The pink circle highlights a female affected dog from normal parents.