J Vet Intern Med 2014;28:871–877

A Homozygous *KCNJ10* Mutation in Jack Russell Terriers and Related Breeds with Spinocerebellar Ataxia with Myokymia, Seizures, or Both

D. Gilliam, D.P. O'Brien, J.R. Coates, G.S. Johnson, G.C. Johnson, T. Mhlanga-Mutangadura, L. Hansen, J.F. Taylor, and R.D. Schnabel

Background: Juvenile-onset spinocerebellar ataxia has been recognized in Jack Russell Terriers and related Russell group terriers (RGTs) for over 40 years. Ataxia occurs with varying combinations of myokymia, seizures, and other signs of neurologic disease. More than 1 form of the disease has been suspected.

Hypothesis/Objectives: The objective was to identify the mutation causing the spinocerebellar ataxia associated with myokymia, seizures, or both and distinguish the phenotype from other ataxias in the RGTs.

Animals: DNA samples from 16 RGTs with spinocerebellar ataxia beginning from 2 to 12 months of age, 640 control RGTs, and 383 dogs from 144 other breeds along with the medical records of affected dogs were studied.

Methods: This case-control study compared the frequencies of a *KCNJ10* allele in RGTs with spinocerebellar ataxia versus control RGTs. This allele was identified in a whole-genome sequence of a single RGT with spinocerebellar ataxia and myokymia by comparison to whole-genome sequences from 81 other canids that were normal or had other diseases.

Results: A missense mutation in the gene coding for the inwardly rectifying potassium channel Kir4.1 (*KCNJ10: c.627C>G*) was significantly ($P < .001$) associated with the disease. Dogs homozygous for the mutant allele all had spinocerebellar ataxia with varying combinations of myokymia and seizures.

Conclusions and Clinical Importance: Identification of the *KCNJ10* mutation in dogs with spinocerebellar ataxia with myokymia, seizures, or both clarifies the multiple forms of ataxia seen in these breeds and provides a DNA test to identify carriers.

Key words: Epilepsy; Kir4.1; Peripheral nerve hyperexcitability; Potassium channel.

Jack Russell Terriers, Parson Russell Terriers, and Russell Terriers are separately registered breeds believed to have descended from the stock of Parson Jack Russell, a 19th century British dog breeder. In this communication, we refer to members of these closely related breeds as the Russell group terriers (RGTs). Heritable ataxia has been recognized in RGTs for over 40 years.[1] The wide differences in ages at onset, clinical signs, and histopathologic changes reported for ataxic RGTs,[a,1–5] suggest that more than 1 heritable ataxia is segregating in these dogs. There is a cerebellar ataxia that becomes apparent at <2 weeks of age when the RGT puppies begin to walk.[a] It is an early-onset cerebellar ataxia with primary granule cell degeneration. There are also RGTs with a distinct spinocerebellar ataxia with an onset at 2–10 months of age and preservation of the granule cells. Many of these RGTs have exhibited myokymia, seizures, or both.[2,4–6] Because myokymia is an unusual clinical sign exhibited by human patients with voltage-gated potassium channel gene mutations,[7,8] part or all of 4 voltage-gated potassium channel genes (*KCNA1*, *KCNA2*, *KCNA6*, and *KCNQ2*) have been resequenced with DNA from RGTs that exhibited myokymia, but no causal mutation was identified.[9] A later onset spinocerebellar ataxia has been reported in 4 RGTs, with an age of onset between 9 and 36 months; no episodes of myokymia or seizures have been reported in this form of ataxia.[3] A missense mutation in *CAPN1* was strongly associated with a spinocerebellar ataxia phenotype beginning at 6–12 months of age that is sometimes referred to as "late-onset ataxia",[10] although the relationship with other reported cases of spinocerebellar ataxia in older RGTs is not clear.[3] No other clinical signs were reported in the *CAPN1*-associated spinocerebellar ataxia.[10] The *CAPN1* mutation was discovered with a genome-wide association study followed by the massively parallel sequencing of

Abbreviations:

EMG	electromyography
MRI	magnetic resonance imaging
RGT	Russell group terriers
SAMS	spinocerebellar ataxia with myokymia, seizures, or both

From the Department of Veterinary Pathobiology (Gilliam, GS Johnson, GC Johnson, Mhlanga-Mutangadura, Hansen) and the Department of Veterinary Medicine and Surgery, (O'Brien, Coates) College of Veterinary Medicine; and the Division of Animal Science, College of Agriculture, Food and Natural Resources, University of Missouri, Columbia, MO (Taylor, Schnabel). *Portions of the results were presented as abstracts at the 2013 ACVIM Forum, the 7th International Conference on Advances in Canine and Feline Genomics and Inherited Diseases, and the 63rd Annual Meeting of the American Society of Human Genetics.*

Corresponding author: D.P. O'Brien, Comparative Neurology Program, College of Veterinary Medicine, University of Missouri, 900 East Campus Drive, Columbia, MO 65211; e-mail: obriend@missouri.edu.

Submitted December 26, 2013; Revised February 14, 2014; Accepted March 3, 2014.

DOI: 10.1111/jvim.12355

affected and control RGT DNA preparations enriched for the disease-associated chromosomal interval by hybridization capture.

We also made use of massively parallel sequencing, but without prior association mapping, to investigate RGTs with spinocerebellar ataxia with myokymia, seizures or both. In this study, we resequenced the genome of a single affected RGT and compared the detected variants with variants detected in 81 normal canids or dogs with other diseases that had also been resequenced. Among the sequence variants that were unique to the affected RGT was a homozygous transversion that predicts a missense mutation in *KCNJ10*, a gene that codes for a voltage-gated potassium channel Kir4.1. The *KCNJ10* mutation was found to be strongly associated with RGT spinocerebellar ataxia, which enabled us to describe the spectrum of disease signs associated with the genetically defined RGT ataxia.

Methods

The University of Missouri Animal DNA Repository contains samples from >100,000 individual dogs, including 2,868 from RGTs. At least 38 of the RGTs represented in the collection had a clinical history of cerebellar ataxia. The onset of ataxia was <2 weeks of age for 21 of these RGTs, strongly suggesting that these dogs had primary granule cell degeneration. The samples from these dogs were excluded from this study as was a sample from an ataxic RGT diagnosed with canine distemper encephalitis at necropsy. Included in this study were 16 samples from ataxic RGTs with an onset of ataxia between 2 and 12 months of age, 640 samples from control RGTs randomly selected from among the RGTs without a known clinical history of ataxia, and 383 samples from control dogs from 144 non-RGT breeds. All animal studies were conducted with approval of the University of Missouri, Animal Care and Use Committee and the informed consent of the owner.

The 16 cases were collected by JRC between 1996 and 2013 based on owner or clinician reports of spinocerebellar ataxia. When available, clinical examination and videotapes of the affected dogs were reviewed, and necropsies were performed when permitted. Other signs of neurologic disease, such as myokymia, neuromyotonia, excessive facial rubbing, hearing loss, or seizures, were also recorded. When available, the final outcome for the dogs was assessed by phone calls with the owners and referring veterinarians.

A DNA sample from an RGT with spinocerebellar ataxia and myokymia and an extensive clinical evaluation was selected for whole-genome resequencing. Two DNA libraries (fragment sizes of approximately 300 and 400 bp) were prepared for paired-end sequencing with a commercial kit (TruSeq DNA sample preparation kit[b]) and each library sequenced on a single flow-cell lane with a massively parallel DNA sequencer (HiSeq 2000[b]) at the University of Missouri, DNA Core Facility. Initial quality control on the sequence data involved removing exact duplicate sequence read pairs using a commercial software[c] and adapter trimming using custom Perl scripts. Unique adapter-trimmed reads were error corrected using MaSuRCA v1.9.5 software. [11] Error corrected reads were aligned to the reference CanFam3.1 genome assembly and variants called using a commercial software.[c] Variant calls were further processed using custom Perl scripts to filter likely false positives and then the variant calls were uploaded to a custom PostgreSQL database, which contained the variant calls for an additional 81 canid samples. Using custom SQL scripts, we identified variants that fit an autosomal recessive mode of inheritance such that the case is homozygous for an allele not observed (in either heterozygous or homozygous form) in any of the remaining 81 canids used as controls. This candidate variant list was further filtered to include only variants predicted to alter the amino acid sequence of protein coding genes. The sequence data from the 81 canid genomes used as controls were generated in a similar manner. The controls represented dogs for which the breed and disease phenotype status were known and included: 37 genomes from our group; 3 wild canid genomes provided by University of California, Los Angeles; 28 genomes provided by the Institute for Translational Genomic Research; 10 genomes provided by North Carolina State University; 3 genomes provided by the University of Pennsylvania. A detailed description of the data processing pipeline is in preparation and will be published elsewhere.

An apparent *KCNJ10:c.627C>G* sequence variant (GenBank accession XM_545752.3) was verified by PCR amplification with primers *5′-GCCAACATGCGGAAGAGCCT-3′* and *5′-TCGAAGT CACCCTCGCCACT-3′* and automated Sanger sequencing. The effect of the mutation on protein function was estimated using a tool for the prediction of the impact of an amino acid substitution on the structure and function of the orthologous human protein (PolyPhen-2) available at http://genetics.bwh.harvard.edu/pph2/.[12] A TaqMan allelic discrimination assay[13] was used to genotype DNA samples from individual dogs at *KCNJ10:c.627C>G*. For this assay, the PCR primer sequences were *5′-C GCCAACATGCGGAAGAG-3′* and *5′-GGTGGGTCTGAAGCAG CTT-3′* and the competing probes were *5′-VIC-CCTCCTCAT CGGCTGC-MGB-3′* (reference allele) and *5′-FAM-CTCCTCA TGGGCTGC-MGB-3′* (mutant allele). A second TaqMan allelic discrimination assay was used to genotype DNA samples at *CAPN1:c.344C>T* (GenBank accession XM_540866.3). For this assay, the PCR primer sequences were *5′-GAGGGTGAGGGAG GCAAT-3′* and *5′-GGGTCCCTCCCATCCCA-3′* and the competing probes were *5′-VIC-AGAAGCCAACAGTCCC-MGB-3′* (reference allele) and *5′-FAM-AGAAGCCAATAGTCCC-MBG 3′* (mutant allele). PCR amplification with primers 5′-AGGTTCTC TCTTTAGGAGCA-3′ and 5′-CACCACATTCAAAGGGCTA-3′ and automated Sanger sequencing, was used to genotype another apparent sequence variant, *KCNH7:c.C1180A,* p.394R>S (GenBank accession XM_005640254.1).

Electromyograms (EMG), motor nerve conduction measurements, and brainstem auditory evoked potentials were evaluated in 2 RGTs with myokymia according to previously described methods.[14,15] One-sided EMG recordings were made from the axial and appendicular muscles of the thoracic and pelvic limbs, as well as cranial and axial muscles. A commercially available electrophysiologic unit[e] was used for all electrodiagnostic recordings. All electrophysicologic recordings were performed under general anesthesia.

The brains of 3 RGTs were examined by magnetic resonance imaging (MRI) under general anesthesia. MRI was performed with a 1.5-Tesla unit[f] or a 3-Tesla unit.[g] Pulse sequences were selected to obtain 3D T1-weighted sequences or standard T1- and T2-weighted sequences in 3 planes. Complete necropsies were performed on 9 ataxic RGTs. The thoracic spinal cords were dissected, fixed, and embedded in paraffin. Sections of these samples were stained with hematoxylin and eosin plus either luxol fast blue-periodic acid Schiff or luxol fast blue-cresyl violet to detect neuronal fiber and myelin loss. In addition, spinal cord sections were immune-stained for neurofilament medium and for fibrillary acid protein to detect astrogliosis.

Results

Resequencing of the individual ataxic RGT case yielded an average of 33.9× coverage depth of the

CanFam3.1 reference genome with 99.4% of the mappable genome covered by at least one sequence read. A total of 7,440,532 putative variants between the case and the reference genome were detected. Of these, 20,652 were predicted to alter the amino acid sequence of proteins with only 23 of these homozygous in the case and the alternate allele not being present in any of the 81 control dog genomes. The homozygous unique coding variants are listed in Table S1. Among these 23, missense mutations in *KCNJ10* and *KCNH7* were considered most likely to be causal because they encode potassium channel genes and mutations in other potassium channel genes are known to cause ataxia, seizures, and myokymia in human patients.[7,8] The *KCNJ10:c.627C > G* transversion (Fig 1A) predicts a KCNJ10:p.I209M amino acid substitution. The presence of this missense mutation in the affected RGT was confirmed by PCR amplification and automated Sanger sequencing (Fig 1B). PolyPhen-2 predicted that this was "probably damaging" with a score of 0.979 on a 0–1 scale with 1 being most damaging.[12] The isoleucine at position 209 and the surrounding amino acids are conserved in all available orthologous mammalian sequences and in most vertebrate sequences; however, the isoleucine is replaced by leucine in some fish species (Table S2). Fourteen of the 16 samples from ataxic RGTs were homozygous for the *KCNJ10:c.627G* allele. The other 2 ataxic RGTs were *c.627C* homozygous siblings. The 640 randomly selected RGT samples were either heterozygous at *KCNJ10:c.627C > G* (n = 63) or homozygous for the reference *c.627C* allele (n = 577). Thus, among RGTs, there is a significant association between the ataxia phenotype and homozygosity for the *KCNJ10:c.627G* allele ($P < .001$; Fisher's exact test). All genotyped DNA samples from 383 individual dogs from 144 non-RGT breeds were *KCNJ10:c.627C* homozygotes.

The other candidate sequence variant that remained after data processing was a *KCNH7:c.1180C > A* transversion that predicts a p.394R>S substitution (GenBank accession XM_005640254.1). The *KCNH7:c.1180A* allele was only detected in the RGT DNA used for whole-genome sequencing. The other 15 ataxic RGTs were homozygous for the *KCNH7:c.1180C allele*. The *CAPN1:c.344T* allele, previously associated with a late-onset ataxia of RGTs,[10] was not detected in any of the 16 ataxic RGT samples or in any of the 44 genotyped control RGT samples.

Because 14 of the ataxic RGTs in this study were homozygous for the *KCNJ10:c.627G* allele, it is now possible to describe the associated disease phenotype in this genetically defined subpopulation of RGTs. Three of the dogs were female, 1 dog was a neutered male, and 10 dogs were intact males. All 14 affected dogs showed marked spinocerebellar ataxia (Video S1). The median age at onset of cerebellar ataxia in 12 dogs was 3 months (range, 2–6 months). The age of onset could not be ascertained for 2 dogs.

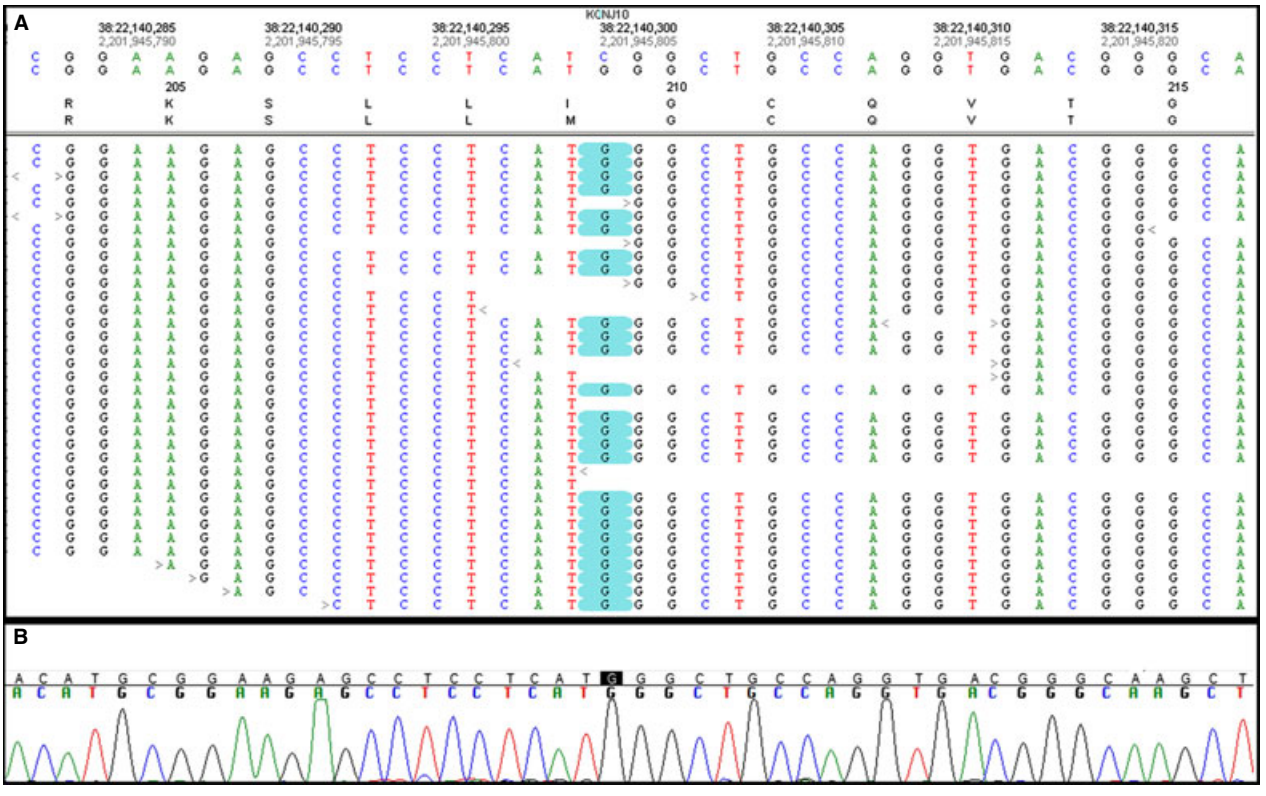

Fig 1. **(A)** Sequence reads from the Russell group terriers (RGT) whole-genome sequence aligned to a segment of canine chromosome 38 showing the homozygous *C > G* transversion in *KCNJ10*. **(B)** Automated Sanger sequence confirms the *C > G* transversion in DNA from the ataxic RGT.

Two dogs showed only signs of cerebellar ataxia without myokymia or seizure; one was euthanized at 4 months of age. Ten dogs showed muscle fasciculation characteristic of myokymia. The median age at onset for myokymia was 6 months (range, 3–8 months). Age of onset for myokymia could not be determined in 1 dog. Episodes of neuromyotonia were documented in 5 dogs. One of the dogs with myokymia also had a history of seizures, while 2 dogs had seizures, but no reported myokymia. The median age at onset for seizure was 2 months (range, 2–4 months). Excessive facial rubbing was not well documented in the histories, but it was noted in 2 dogs. Hearing loss was not evident in any of the dogs.

Brain MRIs in 3 *KCNJ10:c.627G* homozygous dogs were interpreted as normal. In 2 dogs that underwent electrodiagnostic testing, EMG showed myokymia in the form of semirhythmic bursts of doublet, triplet, or multiplet discharges of a single motor unit (Fig 2). A third dog with no clinical evidence of myokymia showed no abnormalities on EMG. Nerve conduction velocities and brainstem auditory evoked potentials were within normal limits. The diagnosis of spinocerebellar ataxia was confirmed by histopathologic examination of spinal cord sections in 9 RGTs, which revealed a bilateral myelopathy with loss of axons and myelin and astrogliosis in the dorsal and lateral portions of the lateral funiculus and in the ventral funiculus (Fig 3).

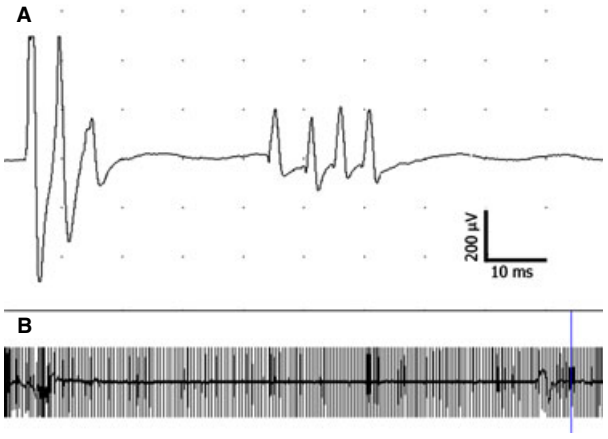

Fig 2. Electromyography from a Russell group terriers with myokymia. **(A)** Two waveforms consisting of trains of 3–4 motor unit action potentials. **(B)** Thirty-second sweep of the same recording showing the regularity of the firing pattern.

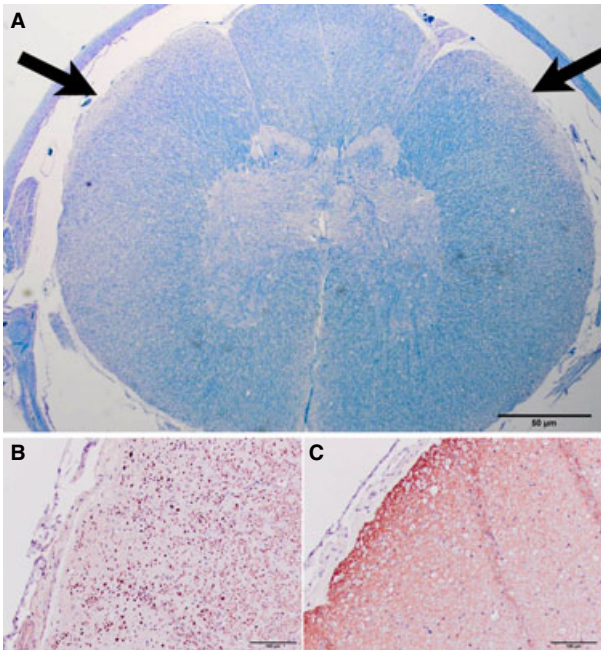

Fig 3. Histopathology of the spinal cord of an affected Russell group terriers. **(A)** Pallor of the dorsal portion of the lateral white matter. Luxol fast blue stain. **(B)** Immunohistochemical staining for neurofilament medium in an area of axonal loss. **(C)** Immunohistochemical staining for glial fibrillary acidic protein showing regional gliosis.

Discussion

We identified a missense mutation in *KCNJ10* in the whole-genome sequence of a single affected RGT and showed that homozygosity for this mutation was significantly associated with spinocerebellar ataxia with myokymia, seizures, or both in RGTs. *KCNJ10* encodes the inwardly rectifying potassium channel Kir4.1. Kir4.1 dysfunction would account for the clinical signs observed, and the similarities among the phenotype in RGTs, humans with *KCNJ10* mutations, and *Kcnj10* knockout murine models strongly support the *KCNJ10:c.627C>G* as the causal mutation. These findings confirm that more than one form ataxia is segregating in RGTs and permitted better delineation of the spectrum of clinical signs associated with *KCNJ10:c.627>G* mutation.

The *KCNJ10:c.627C>G* mutation predicts a p.I209M amino acid substitution in Kir4.1, an inwardly rectifying potassium channel that is expressed in glia, stria vascularis of the inner ear, renal distal convoluted tubules, and gastric parietal cells.[16] In glia, Kir4.1 is responsible for the highly negative resting membrane potential and the large K^+ conductance at rest.[17] In contrast to most potassium channels, inwardly rectifying channels primarily conduct K^+ into cells.[18] Because Kir4.1 is one of the "weak" inwardly rectifying channels, which does not inactivate at depolarized potentials, it can also conduct K^+ out of astrocytes. These properties allow astrocytes to spatially buffer the increased extracellular K^+ concentrations produced by neuronal activity (Fig 4). Interference with this buffering capacity can lead to increased extracellular K^+ and neuronal membrane depolarization. Glutamate transporters located in astrocytes also regulate neuronal excitability by removing glutamate at the synapse. Glutamate transport is strongly facilitated by the negative membrane potential, and impaired by decreased Kir4.1 function.[17] Kir4.1 is also important in the development of oligodendroglia and the maintenance of myelin, although the mechanisms are unclear.[17,19]

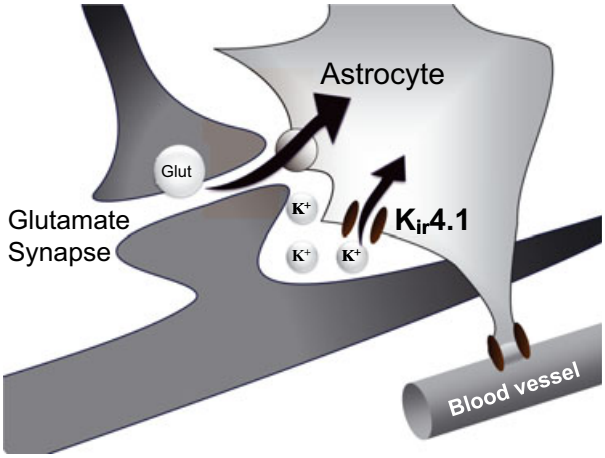

Fig 4. The Kir4.1 channel permits buffering of extracellular potassium by astrocytes. It also contributes to their highly negative resting membrane potential. This negative resting potential is essential for the uptake of glutamate from the synapse through glutamate transporters in astrocytes.

Mutations in human *KCNJ10* have been identified in patients with 2 autosomal recessive hereditary disease syndromes with overlapping signs: SeSAME (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalances)[20] and EAST (epilepsy, ataxia, sensorineural deafness, and tubulopathy).[21] *Kcnj10* knockout mice have severe ataxia, hind limb weakness, and die prematurely, whereas mice with a conditional knockout of *Kcnj10* in glia have stress-induced seizures and severe ataxia.[17,19] Several of the signs exhibited by patients with SeSAME and EAST and by the knockout mouse models are shared with the *KCNJ10:c.627G* homozygous RGTs (Table 1). The similarities between the clinical signs exhibited by the affected RGTs, the human patients with the *KCNJ10*-deficiency syndromes, and the *Kcnj10* knockout mouse models together with the highly significant association between the RGT spinocerebellar ataxia phenotype and homozygosity for the *KCNJ10:c.627G* allele strongly suggest that the *KCNJ10:c.627C>G* transversion is the mutation responsible for the autosomal recessive spinocerebellar ataxia in the 14 ataxic RGTs.

Thus, the hereditary ataxias in RGTs can be divided into at least 3 groups: (1) neonatal, granule cell degeneration,[a] (2) "late-onset" (6–12 months) spinocerebellar ataxia without other clinical signs associated with *CAPN1:c.344T*,[10] and (3) spinocerebellar ataxia with myokymia, seizures or both associated with *KCNJ10:c.627G*. Further studies will be necessary to determine what histologic changes are associated with the *CAPN1:c.344T*-associated ataxia and whether cases with an onset of ataxia over 12 months of age[3] are also associated with that mutation. Because our inclusion criteria focused on RGTs between 2 and 12 months of age, we cannot rule out the possibility that earlier or later onset ataxia, other breeds, or other clinical signs could also be associated with the *KCNJ10:cC>G.* mutation. Neither the *CAPN1:c.344T* allele nor the *KCNJ10:c.627G* allele was detected in 2 RGT siblings with a history of only spinocerebellar ataxia, indicating that there could be additional genetic factors responsible for ataxia in RGTs. No further information was available on these dogs, however, so acquired disease could not be excluded.

The identification of homozygosity at *KCNJ10:c.627G* as a probable cause of ataxia in RGTs allowed us to describe the disease phenotype in a genetically defined cohort. Episodes of myokymia were observed in 64% (9/14) of the *KCNJ10:c.627G* homozygous RGTs. Myokymia and neuromyotonia are common clinical signs in previous descriptions of spinocerebellar ataxia in RGTs.[2,4,22] The EMG recordings we generated from 2 *KCNJ10:c.627G* homozygous RGTs are similar to those from other studies of RGTs[2,4,6,22] and confirm that the episodic subcutaneous muscle rippling is indeed myokymia. Myokymia and neuromyotonia have not been reported in RGTs with primary granule cell degeneration[a] or in association with the *CAPN1* missense mutation.[10] In addition, we have not found reports of myokymia or neuromyotonia among the clinical signs of SeSAME/EAST patients or in descriptions of the *Kcnj10* knockout mouse phenotypes.[17,19–21] Mutations in human *KCNA1* and *KCNQ2* have been associated with myokymia along with episodic ataxia (*KCNA1*) or benign neonatal seizures (*KCNQ2*).[7,8] No unique homozygous coding variants were identified in these genes in our RGT whole-genome sequence. It is likely that the *KCNJ10* mutation causes both the ataxia and excessive membrane excitability resulting in repetitive firing of motor neurons and myokymia.

Table 1. Signs associated with *KCNJ10* mutations in different species.

	Cerebellar Ataxia	Seizures	Myokymia	Deafness	Mental Retardation	Electrolyte Imbalances	Weakness/ Paralysis	Premature Lethality
SeSAME human patients[20]	+	+	–	+	+	+	–	–
EAST human patients[21]	+	+	–	+	–	+	–	–
Knockout mice[19,24]	+	–	–	+	NE	+	+	+
Conditional knockout mice[17]	+	+	–	NE	NE	NE	+	+
Spinocerebellar ataxia with myokymia, seizures, or both in RGTs	+	+	+	–	–	NE	–	[a]

NE, not evaluated.

[a]Most dogs were euthanized because of poor quality of life.

Three of the 14 *KCNJ10:c.627G* homozygous RGTs (21%) had seizures, which are also characteristic of SeSAME/EAST in people. This is lower than the frequency of seizures (37%) reported in a European cohort of ataxic RGTs.[5] During severe episodes of myokymia (neuromyotonia), the dogs hold their limbs in rigid extension and are unable to rise from lateral recumbency. These episodes could be confused with a generalized seizure, but they last for up to several hours and the dog remains conscious during the episode. In contrast, the seizures observed by the author (DPO) were brief, generalized, tonic-clonic seizures with loss of consciousness and autonomic signs. As in the mice with the conditional *Kcnj10* knockout in glia,[17] the seizures in that dog appeared to be elicited by stress.

Varying degrees of hearing impairment have been reported in SeSAME/EAST patients, but the exact cause has not been determined.[20,21] *Kcnj10* is expressed in the stria vascularis of the cochlea where it is thought to play a role in the maintenance of the endonuclear potential.[23] *Kcnj10* knockout mice have cochlear degeneration and sensorineural deafness.[24] Deafness has not been reported in RGTs with ataxia and hearing impairment was not included in the clinical histories of any of the 14 *KCNJ10:c.627G* homozygotes in this study. Others have recorded abnormal brainstem auditory evoked potentials from ataxic RGTs with ataxia and myokymia.[4,5] Nonetheless, the presence of waveforms indicates that these dogs have at least some cochlear function and the abnormal wave forms more likely correlate with histologic changes in central auditory pathways.[1,5]

The excessive face rubbing reported in some RGTs with ataxia could represent a sensory paresthesia.[2,6] Paresthesias were not reported in SeSAME/EAST.[20,21] An itching sensation has been reported in humans with myokymia[25] and scratching reported in dogs with myokymia from other causes,[6] but excessive facial rubbing occurred in 1 *KCNJ10:c.627G* homozygote that did not show myokymia. In mice, inactivation of Kir4.1 in the trigeminal ganglia leads to pain behaviors, suggesting that the Kir4.1 deficiency could also affect sensory functions.[26]

KCNJ10 is also expressed in the distal convoluted tubules of the human kidney where it is essential for the maintenance of the resting electrical potential.[27] Loss of Kir4.1 function in SeSAME/EAST syndromes leads to hypokalemic metabolic alkalosis and hypomagnesemia.[20,21] Altered electroretinograms from EAST patients and nullizygous *Kcnj10* mice are attributed to impaired Kir4.1 function in retinal Muller cells.[28,29] Future examination of the renal and retinal functions of RGTs that are homozygous for *KCNJ10:c.627G* could reveal similar disease signs.

Kcnj10 knockout mice show hypomyelination, vacuolation, and axonal swellings in the spinal cord and cerebellum, predominantly in the white matter adjacent to the gray matter.[17,19] We and others have found similar, but less severe, changes in RGT with ataxia.[1,5,6] No postmortem findings have been reported in people with SeSAME/EAST. Some affected children show T2 hyperintensity of cerebellar roof nuclei and mild atrophy of the cerebellum, corpus callosum, brainstem, and spinal cord on MRI.[30] Such changes were not apparent in the RGTs imaged in this study nor in many of the human cases.[21]

To distinguish the autosomal recessive spinocerebellar ataxia investigated in this report from the other ataxias that occur in RGTs, we propose the acronym "SAMS" for Spinocerebellar Ataxia with Myokymia, Seizures, or both. The identification of the *KCNJ10:c.627C>G* mutation as the probable cause of SAMS confirms the suspicion that multiple forms of hereditary ataxia segregate in these breeds,[5,10] and it will provide the basis for DNA tests that can facilitate the diagnosis of this disease in RGTs. In addition, RGT breeders can utilize DNA tests for the *KCNJ10:c.627G* and the *CAPN1:c.344T* alleles to identify carriers of either alleles and avoid producing dogs with spinocerebellar ataxia associated with either of these mutations. As discussed above, however, 2 RGT siblings in which the client reported signs of spinocerebellar ataxia did not have either of the identified mutant alleles. Further research is needed to clarify whether additional genetic causes of spinocerebellar ataxia segregate in these breeds and to identify the mutation responsible for the neonatal, primary granule cell degeneration. Understanding how mutations in *KCNJ10* affect the function of the Kir4.1 inwardly rectifying potassium channel and the buffering of extracellular potassium by astrocytes could shed light on excessive neuronal membrane excitability in other forms of ataxia, epilepsy, or myokymia.

Footnotes

[a] Coates JR, Carmichael KP, Shelton GD, O'Brien DP, Johnson GS. Preliminary characterization of a cerebellar ataxia in Jack Russell Terriers. *J Vet Intern Med* 1996;10:176 (abstract)
[b] Illumina, San Diego, CA
[c] NextGENe® v2.3.2, SOFTGENETICS®, State College, PA
[e] Sierra Wave version 8.0; Cadwell Laboratories, Kennewick, WA
[f] Signa, General Electric Healthcare, Milwaukee, WI
[g] Trio, Siemens Medical Solutions, Malvern, PA

Acknowledgments

We thank Robert Wayne of the University of California Los Angeles, Matt Huntelman of the Translational Genomics Research Institute, Natasha Olby, Kate Meurs, and Josh Stern of North Carolina State University, and Paula Henthorn of the University of Pennsylvania for providing genome sequence data for use as controls. We also thank Cathryn Mellersh and Oliver Forman of the Wellcome Trust Centre for Human Genetics, University of Oxford, for providing us with DNA from RGTs with a homozygous *CAPN1*

mutation. Finally, we thank all the veterinarians, breeders, and dog owners who contributed clinical information and DNA samples for the study and the breed clubs that helped to organize those efforts. The work was supported by the Jack Russell Terrier Club of America and the University of Missouri (Mizzou Advantage Program).

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

References

1. Hartley WJ, Palmer AC. Ataxia in Jack Russell Terriers. Acta Neuropathol 1973;26:71–74.

2. Bhatti SF, Vanhaesebrouck AE, Van S, et al. Myokymia and neuromyotonia in 37 Jack Russell Terriers. Vet J 2011;189:284–288.

3. Simpson K, Eminaga S, Cherubini GB. Hereditary ataxia in Jack Russell Terriers in the UK. Vet Rec 2012;170:548.

4. Vanhaesebrouck AE, Van Soens I, Poncelet L, et al. Clinical and electrophysiological characterization of myokymia and neuromyotonia in Jack Russell Terriers. J Vet Intern Med 2010;24:882–889.

5. Wessmann A, Goedde T, Fischer A, et al. Hereditary ataxia in the Jack Russell Terrier—Clinical and genetic investigations. J Vet Intern Med 2004;18:515–521.

6. Van Ham L, Bhatti S, Polis I, et al. 'Continuous muscle fibre activity' in six dogs with episodic myokymia, stiffness and collapse. Veterinary Record 2004;155:769–774.

7. Browne DL, Gancher ST, Nutt JG, et al. Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. Nat Genet 1994;8:136–140.

8. Dedek K, Kunath B, Kananura C, et al. Myokymia and neonatal epilepsy caused by a mutation in the voltage sensor of the KCNQ2 K^+ channel. Proc Natl Acad Sci USA 2001;98:12272–12277.

9. Van Poucke M, Vanhaesebrouck AE, Peelman LJ, et al. Experimental validation of in silico predicted KCNA1, KCNA2, KCNA6 and KCNQ2 genes for association studies of peripheral nerve hyperexcitability syndrome in Jack Russell Terriers. Neuromuscul Disord 2012;22:556–565.

10. Forman OP, De RL, Mellersh CS. Missense mutation in CAPN1 is associated with spinocerebellar ataxia in the Parson Russell Terrier dog breed. PLoS One 2013;8:e64627.

11. Zimin AV, Marcais G, Puiu D, et al. The MaSuRCA genome assembler. Bioinformatics 2013;29:2669–2677.

12. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7:248–249.

13. Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 1999;14:143–149.

14. Sims MH, Moore RE. Auditory-evoked response in the clinically normal dog: Early latency components. Am J Vet Res 1984;45:2019–2027.

15. Walker TJ, Redding RW, Braund KG. Motor nerve conduction velocity and latency in the dog. Am J Vet Res 1979;40:1433–1439.

16. Gutman GA, Chandy KG, Adelman JP, et al. International Union of Pharmacology. XLI. Compendium of voltage-gated ion channels: Potassium channels. Pharmacol Rev 2003;55:583–586.

17. Djukic B, Casper KB, Philpot BD, et al. Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. J Neurosci 2007;27:11354–11365.

18. Hibino H, Inanobe A, Furutani K, et al. Inwardly rectifying potassium channels: Their structure, function, and physiological roles. Physiol Rev 2010;90:291–366.

19. Neusch C, Rozengurt N, Jacobs RE, et al. Kir4.1 potassium channel subunit is crucial for oligodendrocyte development and in vivo myelination. J Neurosci 2001;21:5429–5438.

20. Scholl UI, Choi M, Liu T, et al. Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME syndrome) caused by mutations in KCNJ10. Proc Natl Acad Sci USA 2009;106:5842–5847.

21. Bockenhauer D, Feather S, Stanescu HC, et al. Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCNJ10 mutations. N Engl J Med 2009;360:1960–1970.

22. Vanhaesebrouck AE, Bhatti SFM, Franklin RJM, et al. Myokymia and neuromyotonia in veterinary medicine: A comparison with peripheral nerve hyperexcitability syndrome in humans. Vet J 2013;197:153–162.

23. Ando M, Takeuchi S. Immunological identification of an inward rectifier K^+ channel (Kir4.1) in the intermediate cell (melanocyte) of the cochlear stria vascularis of gerbils and rats. Cell Tissue Res 1999;298:179–183.

24. Rozengurt N, Lopez I, Chiu CS, et al. Time course of inner ear degeneration and deafness in mice lacking the Kir4.1 potassium channel subunit. Hear Res 2003;177:71–80.

25. Kaji M, Shoji H. Myokymia. Nihon Rinsho 1993;51:2866–2870.

26. Vit JP, Ohara PT, Bhargava A, et al. Silencing the Kir4.1 potassium channel subunit in satellite glial cells of the rat trigeminal ganglion results in pain-like behavior in the absence of nerve injury. J Neurosci 2008;28:4161–4171.

27. Bandulik S, Schmidt K, Bockenhauer D, et al. The salt-wasting phenotype of EAST syndrome, a disease with multifaceted symptoms linked to the KCNJ10 K^+ channel. Pflugers Arch 2011;461:423–435.

28. Kofuji P, Ceelen P, Zahs KR, et al. Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: Phenotypic impact in retina. J Neurosci 2000;20:5733–5740.

29. Thompson DA, Feather S, Stanescu HC, et al. Altered electroretinograms in patients with KCNJ10 mutations and EAST syndrome. J Physiol 2011;589:7–9.

30. Cross JH, Arora R, Heckemann RA, et al. Neurological features of epilepsy, ataxia, sensorineural deafness, tubulopathy syndrome. Dev Med Child Neurol 2013;55:846–856.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Unique homozygous coding variants.

Table S2. Alignment of amino acids sequences in *KCNJ10* orthologs predicted by 20 codons either side of canine KCNJ10:p.209I.

Video S1. Gait of the Jack Russell Terriers that had whole-genome sequencing performed.