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Generalized myoclonic epilepsy with photosensitivity in juvenile dogs caused by a defective DIRAS family GTPase 1

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The clinical and electroencephalographic features of a canine generalized myoclonic epilepsy with photosensitivity and onset in young Rhodesian Ridgeback dogs (6 wk to 18 mo) are described. A fully penetrant recessive 4-bp deletion was identified in the DIRAS family GTPase 1 (*DIRAS1*) gene with an altered expression pattern of DIRAS1 protein in the affected brain. This neuronal *DIRAS1* gene with a proposed role in cholinergic transmission provides not only a candidate for human myoclonic epilepsy but also insights into the disease etiology, while establishing a spontaneous model for future intervention studies and functional characterization.

seizure | juvenile | canine | photosensitivity | Ras

Dogs provide physiologically relevant models of human disease. Aggressive breeding has resulted in a unique genetic architecture that facilitates gene discovery (1). Many breeds originate from a limited number of founder animals and the use of popular sires is a common practice. As a consequence, each breed represents an isolated population with high levels of phenotypic homogeneity, reduced genetic diversity, and enrichment of breed-specific disorders (2). Hundreds of naturally occurring canine conditions are analogous to human diseases, such as diabetes, cancers, epilepsies, eye diseases, autoimmune diseases, and monogenic diseases.

Epilepsy is the most common chronic neurological disease in dogs (3). A strong genetic background is suspected in many dog breeds with a high prevalence (4) and several genes have been discovered in both symptomatic and idiopathic epilepsy. Most of these genes represent orthologs to the corresponding human epilepsy genes, such the canine models for progressive myoclonic epilepsy, including *NHLRC1* in Lafora disease (5, 6) and *CLN1*, *CLN2*, *ATP13A2*, *CLN5*, *CLN6*, *CLN8*, and *MFSD8* in different types of neuronal ceroid lipofuscinosis (1, 7). Only two genes have been associated with idiopathic epilepsy in dogs, *ADAM23* and *LGI2* (8, 9).

In this study, we describe a unique model of genetic generalized epilepsy in Rhodesian Ridgeback (RR) dogs characterized by a young age of onset. The RR is an African dog breed, originating from Rhodesia, now Zimbabwe. The breed-defining characteristic is a dorsal ridge, caused by a lateral instead of caudal orientation of the hair in this region (10). The RR reflects a mixture of several European dog breeds and the local ridged Hottentot Khoi dog and was initially bred for lion hunting (10, 11). The presence of multiple affected dogs with a distinct phenotype in many litters proposed an inherited condition, which warranted us to embark a comprehensive study to describe the clinical features and find the genetic cause.

Results

Generalized Myoclonic Epilepsy with Photosensitivity in Young RR Dogs. Altogether, we studied 95 RR dogs, of which 24 (15 males, 9 females) shared a unique epilepsy phenotype of frequent myoclonic jerks/twitches, with an onset in young dogs (mean 6 mo; median 3.5 mo; range 6 wk–18 mo) as the outstanding feature. Eleven dogs were 5- to 18-mo-old (juvenile, adolescence) at age of

Significance

Comprehensive clinical, neurological, and genetic examinations characterized a generalized myoclonic epilepsy syndrome with photosensitivity in young Rhodesian Ridgeback dogs. The average age of onset of seizures was 6 mo. Genetic analyses revealed a defective DIRAS family GTPase 1 (*DIRAS1*) gene and protein. DIRAS1 is widely expressed in the brain and has been suggested to regulate acetylcholine release and play a role in neurodevelopment. This study reveals a candidate gene for human myoclonic epilepsies, and a translational model to further elucidate the role of DIRAS1 in neurotransmission and neurodevelopment, and its modulation as a therapeutic option in common epilepsy.

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Conflict of interest statement: H.L. is one of the owners of the Genoscoper Ltd., which provides the gene test developed based on the study.

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Data deposition: Whole-genome and exome sequences can be found in the BioSample database, https://www.ncbi.nlm.nih.gov/biosample (accession nos. SAMN06161402, SAMN06161403, SAMN06161404).

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onset, in 12 dogs onset was between 2 and 4 mo of age (corresponding to 2–10 y in humans, childhood), and in one dog it was at 6 wk (infantile). Photosensitivity was reported in eight dogs. The disease progressed to generalized tonic-clonic seizures (GTCS) in 38% of dogs within 6 mo (median; 1.5–29 mo) after onset of myoclonic seizures. Owners of three dogs (>8 y of age) reported that dogs retained normal cognition throughout life.

Myoclonic jerks were described by the owners as severe startling or even resembling an electric shock. Preceding alterations in behavior were not observed. Myoclonic twitches mainly occurred when the animals were in a recumbent position and relaxed, drowsy, or in the first stages of sleep, and with the eyes either closed or open. Occasionally twitches occurred also when the dogs were sitting, standing, or walking (Movie S1). No autonomic signs occurred during the myoclonic seizures. Based on video review, myoclonic jerks were predominantly confined to the trunk, proximal limb musculature (especially the thoracic limbs), cervical musculature producing nodding movements of the head (Movie S2), and the face (masticatory muscles resulting in chewing movements, eyelid and ear twitches). Myoclonic jerks would often be limited to or start at one side of the body; however, a consistent side predilection could not be detected. Intensity varied between events and individual dogs. Some muscle contractions were rather subtle, with just a small range of motion, whereas others were very vigorous and at times made the dogs jump into the air or dash against the floor, wall, or furniture. Although a single event lasted less than 1 s, twitches often occurred in series as repetitive myoclonic muscle contractions. GTCS were also frequently preceded by a series of myoclonic twitches. Some dogs appeared confused or scared following the episodes and seemed to be very agitated after the events, rising up and wandering around restlessly. Hence, sleep appeared impaired in these dogs. Dogs were normal between events. Owners reported daily (87%) or almost daily (13%; every second to third day) occurrence of myoclonic twitches with a frequency of up to 150 twitches per day. Up to 50 jerks per hour were recorded with EEG in some dogs. Two dogs showed increased myoclonic jerks during heat (cases 2 and 11). In three siblings (cases 8, 9, and 10) and another dog (case 6) onset of myoclonus was observed 2 d after vaccination. Onset of GTCS appeared to be temporally related to vaccination in another two dogs (cases 6 and 7).

Diagnostic investigations (Table S1) failed to identify any consistent structural abnormalities. A few dogs had potential brain abnormalities on neuroimaging evaluations that may be incidental findings, such as ventricular asymmetry (12) (Table S2). Twenty-one RRs with myoclonic epilepsy were treated with a variety of antiepileptic drugs (AEDs: phenobarbital, potassium bromide, primidone, levetiracetam, clonazepam, imepitoin; monotherapy or combination) in adequate dosages and with serum concentrations (phenobarbital: mean 28.6 mg/L; potassium bromide: mean 1,353 mg/L) within therapeutic range (13). Levetiracetam, which is also an effective drug for juvenile myoclonic epilepsy (JME) in humans (14), and potassium bromide seemed to be the most effective based on response of dog owners.

By the time of submission, three dogs were euthanized at 9 mo, 2 y, and 5 y of age because of poor seizure control; three dogs died from causes unrelated to the epilepsy and one died for unknown reasons (Table S2). One dog was available for postmortem examination (case 2) that ruled out extracranial pathologies. In this single brain, histology showed postictal changes only including mild clustered neuronal hypereosinophilia in lateral geniculate nucleus and pyramidal cell layers of the neocortex. Histoarchitectural changes, dysmorphic neurons, and reactive gliosis were not evident. The remaining dogs were alive without any evidence of mental or cognitive decline.

Ambulatory Wireless Video EEG Defines the Electroclinical Syndrome. Simultaneous video and EEG recordings documented the epileptic origin of the events in 82% of examined cases (Table S2). EEG was recorded for prolonged times (>1 h, 13 recording leads) in 17 affected RRs displaying myoclonic twitches, and 11 breed-matched controls (10 healthy RRs, 1 RR with idiopathic epilepsy with GTCS). Background activity was appropriate to state in all dogs (15). Myoclonic twitches of variable intensity occurred in all but two cases during EEG recording. The characteristic ictal pattern was generalized 4–5 Hz spike-and-wave complexes (SWC) (Fig. 1 *A* and *B*) or polyspike-wave complexes (PSWC) during the initial phase, with a predominantly fronto-central maximum that often switched between different leads over both hemispheres, and occasionally generalized with a time lag. Another ictal pattern comprised biphasic spikes and paroxysmal bursts consisting of 7- to 8-Hz spikes that at times again were followed by SWC and an occasional occurrence of focal activity (Fig. S1). In some dogs, myoclonic activity was consistent with onset of ictal discharges, whereas in others myoclonic twitches were preceded by a crescendo of EEG paroxysms. Not all motor activity was accompanied by EEG paroxysms, but myoclonic jerks appeared identical and muscle artifact may have obscured the EEG correlate on some occasions. Affected RRs displayed also epileptiform discharges comprising ictal spikes or interictal 4- to 5-Hz SWC (Fig. S1). Furthermore, some dogs intermittently displayed rhythmical 4- to 5-Hz slowing that at times morphed into SWC accompanied by myoclonic jerks. During EEG recording, myoclonic twitches emerged predominantly during quiet rest, drowsiness, or slow-wave sleep. In some dogs, single episodes were recorded while awake and even less often while standing. Similarly, EEG paroxysms emerged with higher frequency when the dogs were less alert. The effect of sleep deprivation was not assessed. For the EEG, instead of sleep deprivation, we encouraged the dogs to nap. Unremarkable EEG recordings were obtained from control dogs.

Photosensitivity Is a Feature of Generalized Myoclonic Epilepsy in RR Dogs. Visually induced seizures were reported in 8 of 23 (35%; confidence interval 95%: 18.7–55.2%) RRs with generalized

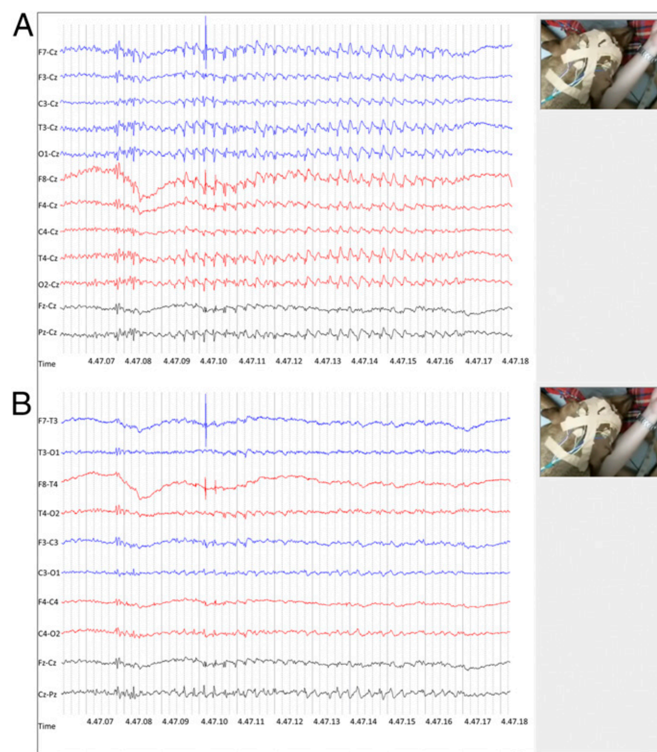

Fig. 1. Ictal EEG. LFF: 1 s; HFF: 70 Hz. (*A* and *B*) Minor head and eyelid twitches were accompanied by 4-Hz spike-and-wave complexes with a central maximum (*A*: Cz referential montage; *B*: bipolar montage). EEG is also presented as online supporting material (Fig. S1 and Movie S4).

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myoclonic epilepsy (Table S2). These were described as myoclonic seizures triggered by visual stimuli, such as light flashes, sudden incidence of light when opening the shutters in the morning, or sunlight interrupted by trees while walking through the forest. A videotape was provided where each photic stimulus (produced by photoflashes) was followed by myoclonic jerks (Movie S3). Upon video-EEG recording with photic stimulation in six affected RRs, four dogs (66%) displayed photoconvulsive responses time-locked with the onset of the photic stimulus (Table S2 and Movie S4). Video-EEG with photic stimulation did not reveal any abnormalities in clinically healthy RR controls, including three heterozygous carriers of the gene deletion. Besides light, noise was also a triggering factor in three siblings (cases 8–10).

Genetic Analyses Reveal a 4-bp Deletion Mutation in *DIRAS1*. The pedigree established around the affected dogs suggested an autosomal recessive inheritance (Fig. S2). To identify the genetic cause of the generalized myoclonic epilepsy in RRs, we combined a genome wide association study (GWAS) and next-generation sequencing analyses using whole-exome (WES) and whole-genome (WGS) resequencing. Assuming a recessive mode of inheritance, the WES analysis of two unrelated cases against 169 exomes from nonepileptic dogs (Table S3) resulted in a group of 10 variants, of which 6 were in the predicted coding regions (Table S4). Only one nonsynonymous variant was found, a 4-bp deletion in the exon 2 of the *DIRAS1* gene (c.564_567delAGAC; gene structure according to the Broad Institute CanFam3 Improved Annotation Data v1) (Fig. 2*D*), resulting in a frameshift and a stop loss (Fig. S3). A GWAS and haplotype analysis in 10 RR cases and 18 RR controls supported the WES study by identifying the best-associated region ($P = 0.977 \times 10^{-5}$) (Table S5) in a 1.6-Mb region (55,597,243–57,195,857) at chromosome 20 (Fig. 2*A*), including the *DIRAS1* gene (Fig. 2*C*). The critical region was further split into a 300-kb and an 890-kb region (Fig. 2*B*) by a 400-kb recombination in one of the cases. WGS of one epileptic dog also identified the c.564_567delAGAC deletion in *DIRAS1*, but it was absent from 99 control whole genomes (Table S3). Structural variation analysis in the WGS data were performed within the original 1.6-Mb associated region of the epileptic RR dog. Only one 35-kb duplication (56,210,949–56,246,523) was found in the region; however, it resided outside of the 890-kb disease-associated haploblock, which starts at 56.3 Mb (Fig. 2*B*). In addition, the duplication was present in several nonepileptic control dogs within our 99 dogs WGS data (Table S3), excluding it as an epilepsy candidate in RRs.

The genotyping of the *DIRAS1* deletion in 14 clinically verified RR cases and 26 controls revealed a homozygous mutant genotype in all cases, a heterozygous genotype in the obligate carriers, and the homozygous wild-type genotype in controls (Fig. 2*D*), indicating a complete segregation of the deletion allele with the disease. Genotyping additional 498 RRs from 13 countries indicated a carrier frequency of ∼15% (Table S6). To investigate the breed and epilepsy specificity of the deletion, we genotyped an additional 965 epileptic dogs in 12 breeds, but did not find any carriers, indicating that the mutation is specific to generalized myoclonic epilepsy in RRs. Collectively, these results strongly suggest that the deletion in the coding region of *DIRAS1* causes the generalized myoclonic epilepsy in the breed.

Altered Intracellular Expression Pattern of Mutant DIRAS1. The expression pattern of the *DIRAS1* transcript is poorly characterized and suggested to be limited to the brain and heart (16). We amplified the transcript in 28 canine tissues, including 12 brain regions, the spinal cord, and 15 peripheral tissues, and found abundant expression in all brain regions, whereas the pattern was more limited and variable in extra neural tissues (Fig. S4). The possible developmental expression pattern of *DIRAS1* was also investigated in the frontal cortices at six different time points: 2, 5, and 23 mo and 4, 5, and 9 y. The results indicate increased expression until adulthood (Fig. 3*A*).

The 4-bp deletion resides at the end of the *DIRAS1* coding region, resulting in a frameshift at the C-terminal end of the predicted protein (Fig. S3). The last 10 amino acids of DIRAS1 change causing a stop loss, which is followed by 104 extra amino acids. The only functional domain, RAS, remains intact, but the protein has additional low complexity regions toward its C terminus (Fig. S3), likely rendering the mutated protein functionally altered. The effect of the deletion mutation on the stability

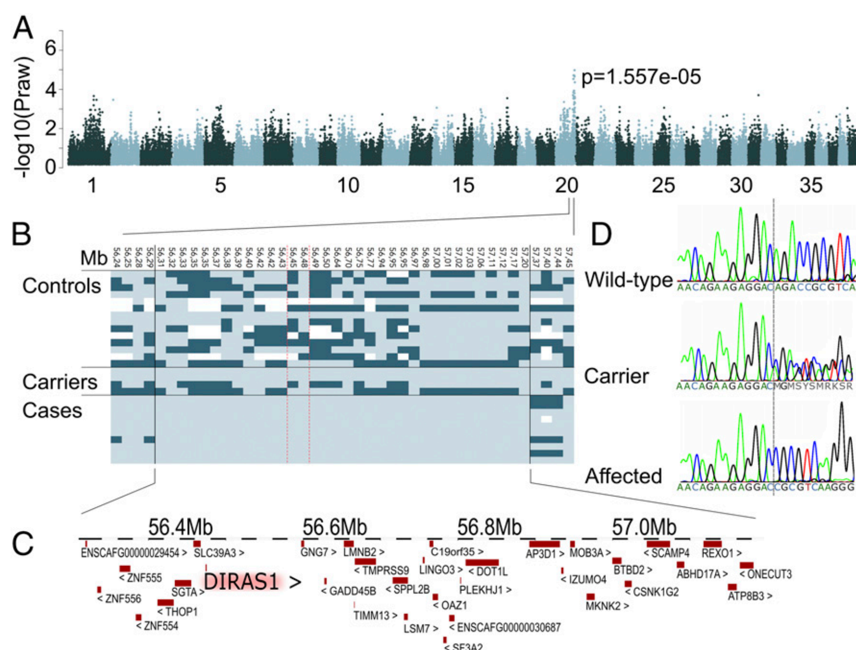

Fig. 2. GWAS. (*A*) Manhattan plot indicates best *P* values at chromosome 20. (*B*) An 890-kb haplotype is shared by cases. (*C*) The associated region contains 33 genes including *DIRAS1*. (*D*) Chromatograms of an affected, carrier, and wild-type dog indicate the c.564_567delAGAC variant.

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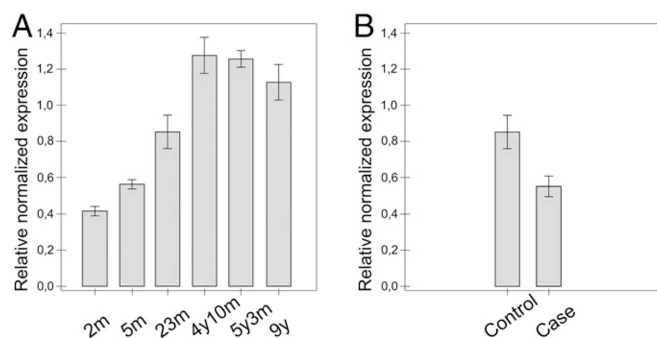

Fig. 3. *DIRAS1* expression. (*A*) The increase in the expression of the *DIRAS1* transcript by age was observed when comparing six different age points in the frontal cortex (the ages of 2 mo, 5 mo, 23 mo, 4 y and 10 mo, 5 y and 3 mo, and 9 y, $n = 1$ in each). (*B*) The stability of the *DIRAS1* transcript was studied in the frontal cortices of age-matched (24- and 23-mo-old) case (RR, $n = 1$) and control (Great Dane, $n = 1$) dogs by a quantitative PCR. The result suggests a modest decrease in the stability of the mutant transcript. The error bars refer to variance in experimental triplicates (SD < 0.13 in each). *YWHAZ* and *GADPH* were used as loading controls in quantitative PCR.

of the *DIRAS1* transcript was investigated by quantitative PCR in the frontal cortices between the age-matched 2-y-old case and control dogs. The result suggested only a modest decrease in the case (Fig. 3*B*), which also agrees with an unremarkable change in the semiquantitative PCR (Fig. S4).

Immunolabeling revealed abundant expression of DIRAS1 antigen throughout the brain (brainstem, cerebellum, and prosencephalon), including the cholinergic basal forebrain nuclei, depicted in Fig. 4. The intracellular expression pattern of wild-type and mutant DIRAS1 somewhat differed between the single affected dog and control dogs. Distinctive nuclear and membranous pattern observed in the control dogs (RRs and non-RRs) (Fig. 4 *A* and *C*) had changed to advanced diffuse staining of nerve cell somata in the affected RR (Fig. 4 *B* and *D*). These results suggest that there is a persistent expression of the mutated DIRAS1 protein with an altered intracellular localization.

Discussion

This study characterizes a breed-specific generalized myoclonic epilepsy with an early onset. Results from genetic and functional studies suggest that the epilepsy is caused by a 4-bp deletion in the coding region of the *DIRAS1* gene, resulting in a frameshift and a stop loss. We found abundant expression of *DIRAS1* throughout the canine brain with a difference in subcellular expression patterns between wild-type and mutant proteins. Although mammalian functions are unknown, previous studies suggest that DIRAS1 is needed for acetylcholine transmission at neuromuscular junctions in *Caenorhabditis elegans* (17) and neuronal development in zebrafish (18). Therefore, this canine DIRAS1 defect provides not only a candidate gene for generalized myoclonic epilepsies but also insights to the disease etiology, while establishing a spontaneous model for preclinical studies and functional characterization.

Common human idiopathic generalized epilepsies recognized by the International League Against Epilepsy include childhood absence epilepsy, epilepsy with myoclonic absences, epilepsy with myoclonic atonic seizures, epilepsy with GTCS alone, juvenile absence epilepsy, myoclonic epilepsy in infancy, and JME (19). Generalized myoclonic epilepsy in RR dogs reveals important parallels to JME, which is one of the most common forms of epilepsy in humans (14, 20–23). As in humans, jerks are bilateral, arrhythmic, at times asymmetric, and predominate upon the upper limbs and trunk (20, 21), whereby additional nodding movements of the head were present in some RRs. EEG recordings revealed a pattern found in human JME patients: SW or PSW discharges with a fronto-central accentuation and a normal background activity with an occasional occurrence of focal activity, EEG asymmetries switching sides, and diffuse or intermittent slowing (22, 24, 25). An important characteristic shared by human JME and generalized myoclonic epilepsy in RRs is the manifestation with photosensitivity, particularly as JME has one of the strongest associations with photosensitivity among all epilepsies (26, 27). Phenotypic heterogeneity was apparent because not all dogs were photosensitive, which may reflect influences of age, sex, or individual genetic background of the dogs. In humans, photosensitivity is an age-dependent phenomenon and is more prevalent in children with a peak age of onset about 12 y (27). There is also strong evidence for a genetic component of photoparoxysmal response (PPR) in humans and many loci have been identified (27–30). Thus, affected RRs provide another spontaneous large animal model to investigate the neural mechanisms of photosensitivity (31, 32).

However, there are also a number of differentiating characteristics and phenotypic heterogeneity: the low prevalence of GTCS (JME 80–95%; RRs 38%), the absence of absence seizures (although this seizure type might be difficult to recognize in dogs), and

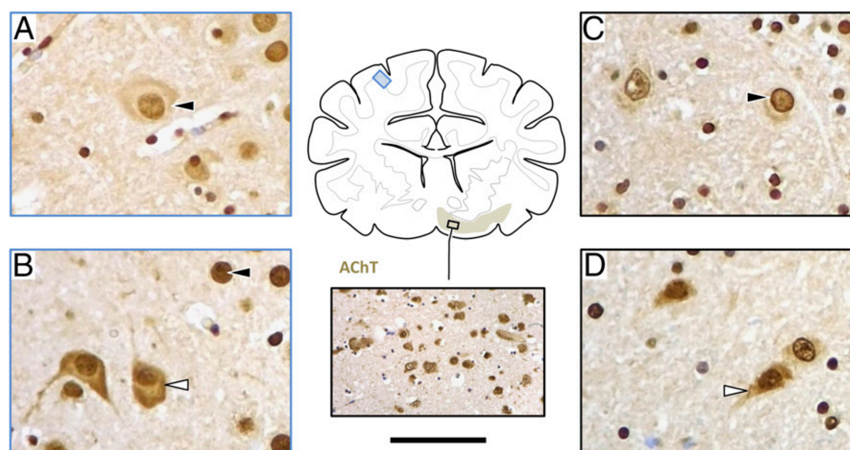

Fig. 4. Immunohistochemical *DIRAS1* expression. Wild-type RRs show predominantly nuclear staining (black arrowhead) as seen in the parietal cortex (*A*; blue frame) and cholinergic forebrain nuclei (*C*; black frame). With *DIRAS1* mutation (*B* and *D*) protein expression is abundant and there is a more diffuse staining of nerve cell perikarya (white arrowhead) in all brain regions, including the brainstem. Figure shows expression in parietal cortex (*B*; blue frame), and forebrain nuclei (*D*; black frame). Cholinergic target areas were confirmed by staining for the vesicular acetylcholine transporter (AChT), as demonstrated in the *Inset*. (Scale bar: *A*–*D*, 35 μm; inlet AChT, 150 μm.)

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a variable age of onset, with several dogs showing a relatively early onset (6–10 wk) in the socialization period (6–12 wk) and others during the juvenile period (starting at 12 wk) and adolescence up to 18 mo, when behavioral maturation tends to reach adult values in the dogs (33–35). Differences between dog breeds exist and differences in the order of development of social and motor skills between dogs and humans have been encountered (34). Thus, early age of onset may still be in line with human genetic generalized epilepsy syndromes, such as JME, in which 25% may have absence—and not myoclonic—seizures in childhood. People with JME also have 2- to 3-Hz and 4- to 6-Hz interictal epileptic discharges, and most have polyspikes (22). EEG failed to demonstrate clear ictal discharges in association with myoclonus on some occasions. Although it was considered that EEG was obscured by muscle artifact of the myoclonus, myoclonic behavior needs to be monitored with telemetry for further investigations. There is also a possibility that the generator for myoclonic seizures is not superficial, rather subcortical. EEG will not be able to detect deep neuronal function if the generator is at the brainstem level. Although photosensitivity was observed in 35% of dogs, the prevalence of photosensitivity based upon the EEG studies appears to be higher (66%) than in humans and, in humans, the photoparoxysmal and photoconvulsive responses are maximal fronto-centrally and not occipitally. There is a high prevalence of MRI findings, which is not typical for human JME. We acknowledge that the presence of MRI findings point toward a symptomatic etiology; however, there were no consistent findings. Ventricle asymmetry is also frequently present in dogs without epileptic seizures, and thus may be clinically not relevant (12). Similarly, a small amount of meningeal enhancement is consistently demonstrated in normal dogs (36). However, we cannot exclude that some of the structural abnormalities interacted with the phenotype: for example, lowered seizure threshold on both hemispheres. The EEG phenotype is consistent with generalized myoclonic epilepsy, and certainly not focal epilepsy. Finally, human JME is characterized by strong chronodependency, with myoclonic jerks and GTCS in the morning after awakening or during relaxation periods in the evening (37). Although generalized myoclonic epilepsy in RRs also shows a strong association with the sleep–wake cycle, myoclonic twitches and EEG discharges appeared predominantly in the relaxed state, at rest, or during the first stages of sleep, mirroring subtypes of JME (38). The observed differences may reflect species-specific differences in biorhythmicity and sleep regulation, or may indicate parallels to other genetic sleep-associated epilepsies, such as myoclonic epilepsy in infancy, which sometimes progresses to JME (39), or autosomal dominant nocturnal frontal lobe epilepsy (NFLE) (40).

DIRAS1 is a novel epilepsy gene with a robust expression pattern in the CNS tissues. It is part of the Ras family of small GTPases, which have been linked to many cellular signaling pathways in cell growth and differentiation, synaptic plasticity, learning, and memory (41–43). DIRAS1 and DIRAS2 form a biochemically and functionally distinct branch of Ras GTPases, which are characterized by a fast guanidine–nucleotide exchange rate (16). The biological function of DIRAS1 in mammals is poorly characterized. DIRAS1 has been suggested to function as a tumor suppressor in glioblastoma and other tumor cell lines through the inhibition of Ras-mediated transformation, altered NF-κB transcription activity, diminished ERK1/2 and MAPK signaling, and antagonization of pro-oncogenic small Ras GTPases (44). Studies in *C. elegans* have demonstrated that the DIRAS1 and exchange protein directly activated by cAMP (EPAC) orthologs colocalize at the presynaptic membranes and are needed for the maintenance of normal presynaptic acetylcholine release at neuromuscular junctions (17). DIRAS1 was also suggested to play a role in cell migration, neurite outgrowth, and dendrite architecture in the developing nervous system of a zebrafish model (18).

Understanding the role and mechanisms of DIRAS1 in cholinergic neurotransmission and epilepsy remains an important task. Nicotinergic cholinergic activity influences brain excitability and cognition, regulates the excitatory/inhibitory switch of GABA during neuronal development (45), stimulates glutamate release from thalamocortical terminals, controls GABA release onto pyramidal neurons, and maintains nonrapid eye movment sleep by low levels of acetylcholine, whereby cholinergic stimulation is associated with microarousals in this sleep stage (46). Mutations in nicotinergic acetylcholine receptor (nAChR) subunits *CHRNA4*, *CHRNA2*, and *CHRNB2* are associated with autosomal dominant NFLE and sporadic NFLE (47). *CHRNA7* coding for the α7 subunit of the nAChR is also a potential candidate gene for JME in humans (48). Abnormal DIRAS1 function could alter cholinergic neurotransmission or formation of neuronal circuits and network assembly in the developing brain resulting in myoclonic epilepsy and photosensitivity. This canine model establishes a prime resource to address these questions and mechanisms in future experiments, including mutation-specific–induced neuronal cultures.

In summary, careful clinical and genetic studies identified a candidate gene for one of the most common forms of human epilepsy with a postulated function in cholinergic neurotransmission. While inspecting the gene in human myoclonic and epilepsy cohorts for risk variants, future functional studies should identify the DIRAS1-mediated mechanisms in neurotransmission and provide drug targets for common epilepsies.

Materials and Methods

Study Cohorts. Twenty-four RR cases were identified (Table S2). Inclusion criteria were clinical observation of myoclonic jerks on video recordings or observation at one of the study sites and completion of an online questionnaire or an interview. Altogether, 538 EDTA-blood and tissue samples were collected from privately owned RRs in Germany, Finland, and 11 other countries (Table S6). A cohort of 965 epileptic dogs from 12 other breeds from Finland was included (Table S6). Sample collection was ethically approved by the Animal Ethics Committee of State Provincial Office of Southern Finland, Hämeenlinna, Finland (ESAVI/6054/04.10.03/2012), "Cantonal Committee for Animal Experiments" (Canton of Bern; permit 23/10), and the German Animal Welfare Act. Further details are provided in *SI Materials and Methods*.

Neurodiagnostic Investigation. All RR cases underwent a clinical, neurological, and laboratory examination. Structural epilepsy was excluded by imaging through MRI in 12 RR cases and postmortem examination of 1 dog. Additional investigations comprising cerebrospinal fluid (CSF) analysis, neurometabolic screening, imaging through CT, skin biopsy, and AED serum concentration measurements were performed for a number of studied dogs. Further details are provided in *SI Materials and Methods*.

EEG. Awake ambulatory wireless video-EEG was conducted in 17 RR cases and 11 RR control dogs. Recordings were performed in a quiet environment, with dogs encouraged to lie down. EEG was recorded routinely using 15 (7 in one dog) subdermal needle electrodes. In six cases and four controls an additional video-EEG with photic stimulation was conducted at the end of the EEG study. Further details are provided in *SI Materials and Methods*.

Postmortem Examination. Postmortem examination was conducted on one affected RR. The animal underwent routine autopsy in which the brain was removed in toto and trimmed according to standardized algorithms (49). Relevant brain areas (prosencephalon, cerebellum, brainstem) were sampled and histologically evaluated using neurohistological standard stains on paraffin sections.

GWAS. Genotyping of 10 affected RRs from the initial study cohort and 18 unaffected RRs was performed. The genotype data were filtered and frequency and genotyping pruned. A case-control association test was performed by PLINK (50) and by Mendel software's Ped-GWAS (51). Further details are provided in *SI Materials and Methods*.

Resequencing. Dog exome libraries for two German RR cases were generated. The sequencing data were analyzed and filtered under a recessive model against 169 additional exomes (Table S3). The pathogenicity of the coding variants was predicted in the CanFam 3.1 annotation. One RR case was whole-genome sequenced and filtered against 99 additional whole genomes (Table S3) and the presence of the candidate mutation was inspected visually. Further details are provided in *SI Materials and Methods*.

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Sanger Sequencing and TaqMan Genotyping. The identified candidate variant was validated by a standard PCR followed by Sanger sequencing in 33 German RR samples, including 12 cases from the initial study cohort. For a larger mutation screening in additional samples (Table S6), a TaqMan assay was run. Further details are provided in *SI Materials and Methods*.

Gene Expression. Fresh postmortem samples were collected (for the full list, see Fig. 3 and Fig. S4) from one case and six control dogs. RNA was extracted and reverse-transcribed into cDNA. The canine *DIRAS1* transcript was amplified and sequenced. Semiquantitative and quantitative PCRs were performed. Further details are provided in *SI Materials and Methods*.

Immunohistochemistry. Tissue studies were conducted on the brains (prosencephalon, cerebellum, brainstem) of one RR case and three control RRs (LMU Munich neuropathology brain archive). Primary antibodies (pAB) were directed at DIRAS1 and the vesicular acetylcholine transporter. The slides were antigen-demasked, incubated with pAB, and stained using polymer technology and a diaminobenzidine tetrahydrochloride. Further details are provided in *SI Materials and Methods*.

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