Clinical and Electroencephalographic Characterization of Juvenile Myoclonic Epilepsy in Rhodesian Ridgebacks

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Meiner Familie

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ABBREVIATIONS

ACh	acetylcholine
ADAM	a-disintegrin-and-metalloproteinase
ADHD	attention deficit hyperactivity disorder
ADNFLE	autosomal dominant nocturnal frontal lobe epilepsy
AED	antiepileptic drug
a.m.	ante meridiem
ARSG	arylsulfatase G
BFJE	benign familial juvenile epilepsy
bp	base pair
BRD2	bromodomain-containing 2
CACNB4	calcium channel beta4 subunit
CAE	childhood absence epilepsy
cAMP	cyclic adenosine monophosphate
CASR	calcium channel sensor receptor
CBC	complete blood count
CHRNA2	cholinergic receptor nicotinic alpha2 subunit
CHRNA4	cholinergic receptor nicotinic alpha4 subunit
CHRNA7	cholinergic receptor nicotinic alpha7 subunit
CHRNB2	cholinergic receptor nicotinic beta2 subunit
CSF	cerebrospinal fluid
Cx-36	connexin 36
DIRAS	distinct subgroup of the Ras family
DRPLA	dentatorubral-pallidoluysian atrophy
EEG	electroencephalography
EFHC1	EF-hand domain containing one

e.g.	exempli gratia
EMG	electromyography
EPAC	exchange protein directly activated by cAMP
^{18}FDG	2-[18F] fluoro-2-deoxy-D-glucose
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
GABRA1	GABA receptor alpha one subunit
GABRD	GABA receptor delta subunit
GDP	guanosine diphosphate
GFP	green fluorescent protein
GRODs	granular osmiophilic deposits
GTCS	generalized tonic-clonic seizure
GTP	guanosine triphosphate
GTPase	guanosine triphosphatase
HE	haematoxylin and eosin
^{1}H-MRS	proton magnetic resonance spectroscopy
Hz	Hertz
i.e.	id est
IGE	idiopathic generalized epilepsy
ILS	intermittent light stimulation
IVETF	International Veterinary Epilepsy Task Force
JME	juvenile myoclonic epilepsy
KBr	potassium bromide
LAMP-1	lysosomal-associated membrane protein 1
LEV	levetiracetam
LGI protein	leucine-rich glioma-inactivated protein

ME2	malic enzyme 2
MEI	myoclonic epilepsy of infancy
MERRF	myoclonic epilepsy with ragged red fibres
MRI	magnetic resonance imaging
NAA	*N*-acetyl aspartate
nAChR	nicotinergic acetylcholine receptor
NCL	neuronal ceroid lipofuscinosis
NF- κB	nuclear factor κB
NFLE	nocturnal frontal lobe epilepsy
non-REM	non-rapid eye movement
PAS	periodic acid Schiff
PET	positron emission tomography
PME	progressive myoclonic epilepsy
PPR	photoparoxysmal response
PSWC	polyspike-wave complexes
Rap1A	Ras-related protein 1A
RBD	REM sleep behaviour disorder
REM	rapid eye movement
RR	Rhodesian Ridgeback
s	second
SAP	sphingolipid activator protein
SCMAS	subunit c of mitochondrial ATP synthase
SMA	supplementary motor area
SmgGDS	small GTP-binding protein GDP dissociation stimulator
SWC	spike-wave complexes
TPP1	tripeptidyl peptidase 1

I. INTRODUCTION

Dogs represent a promising large animal translational model for a number of human diseases. A wide range of spontaneous diseases in dogs have been shown to be advantageous for genetic, diagnostic, pathophysiological and therapeutic investigations of their human homologue diseases (DUAN, 2015; GRAHAM & SCHUURMAN, 2015; HICKS et al., 2017; ITO et al., 2014; PATTERSON, 2014; POTSCHKA et al., 2013; RECCHIA & LIONETTI, 2007; ROWELL et al., 2011; TRUVÉ et al., 2016; ZEISS, 2013). The usefulness of dog models for epilepsies, diabetes, cancers, as well as ocular and cardiac disorders in translational research has been shown (DUAN, 2015; GRAHAM & SCHUURMAN, 2015; HICKS et al., 2017; ITO et al., 2014; PATTERSON, 2014; POTSCHKA et al., 2013; RECCHIA & LIONETTI, 2007; ROWELL et al., 2011; TRUVÉ et al., 2016; ZEISS, 2013).

It has been postulated that dogs provide an exciting opportunity for the identification of epilepsy genes. Epilepsy is one of the most common chronic neurological diseases in humans and dogs (EKENSTEDT & OBERBAUER, 2013; VOLK, 2015). A strong genetic predisposition is suspected in many dog breeds with a high prevalence of idiopathic epilepsy (HÜLSMEYER et al., 2015). The genomic divergence between humans and dogs is less than that between humans and mice, and humans and dogs have roughly the same number of genes (POTSCHKA et al., 2013). Narrow gene pools and a high inbreeding coefficient in purebred dogs have reduced genetic heterogeneity compared with humans, and dog populations tend to express inherited monogenic diseases (KOSKINEN et al., 2015; LINDBLAD-TOH et al., 2005; POTSCHKA et al., 2013). Autosomal recessive disorders have been shown to have a substantially high prevalence because breeding programs frequently use only champion dogs, which are few in number and often carriers of mutations (MELVILLE et al., 2005). Although this is a concern with regard to the health of the breed population, it also facilitates gene discovery in this species (MELVILLE et al., 2005).

Dogs are also a valuable model for treatment studies. Rodent models, which are regularly used for the investigation of new drugs, are often criticized because they do not reflect clinical conditions accurately (POTSCHKA et al., 2013). Concerns include the restricted diversity of the genome and limited interindividual

pathophysiological differences (POTSCHKA et al., 2013). Dogs appear to be a promising model for the investigation of new treatment strategies, particularly in the field of epilepsy research. Rodent models are necessary for high-throughput screening of potential antiepileptic drugs (AED) (POTSCHKA et al., 2013). However, research in rodents mainly uses models of induced epilepsy that often do not reflect the clinical condition of spontaneously occurring epilepsies (PATTERSON, 2014). Moreover, a multifactorial mechanism is considered to underlie drug resistant epilepsy, which is likely better reflected by canine models with naturally occurring epilepsy (POTSCHKA et al., 2013).

The aim of this work was to provide a comprehensive clinical description of a novel genetic epilepsy in Rhodesian Ridgeback (RR) dogs. Wireless video-electroencephalography (EEG) in unsedated dogs was established as a clinical research tool to confirm the diagnosis of epilepsy, to recognize myoclonic and absence seizures, and to characterize the EEG features of this peculiar electroclinical syndrome. Photic stimulation was performed to investigate the reflex epileptic trait of the epilepsy syndrome. Segregation/pedigree analyses indicated a monogenic autosomal recessive disorder. Subsequent genetic analyses of cases and controls collected as part of this study were performed in collaboration with the University of Helsinki to identify the underlying genetic cause.

II. REVIEW OF THE LITERATURE

1. Canine genetic epilepsies

Epilepsy is the most common chronic neurological disease in dogs (VOLK, 2015). Idiopathic and structural epilepsy as well as epilepsy of unknown cause can be differentiated based on aetiology (BERENDT et al., 2015). Structural epilepsy can be caused by various lesions of the forebrain including vascular events, inflammation, trauma, congenital malformation, neoplasia or degenerative diseases (BERENDT et al., 2015). Idiopathic epilepsy can be further subdivided into (a) genetic epilepsy, when the causative gene has been identified, (b) suspected genetic epilepsy, when there is a family history or a high prevalence of epilepsy within a breed (>2%), and (c) epilepsy of unknown cause, when there is no apparent evidence of a structural process, but no disease mechanism can be identified (Figure 1) (BERENDT et al., 2015).

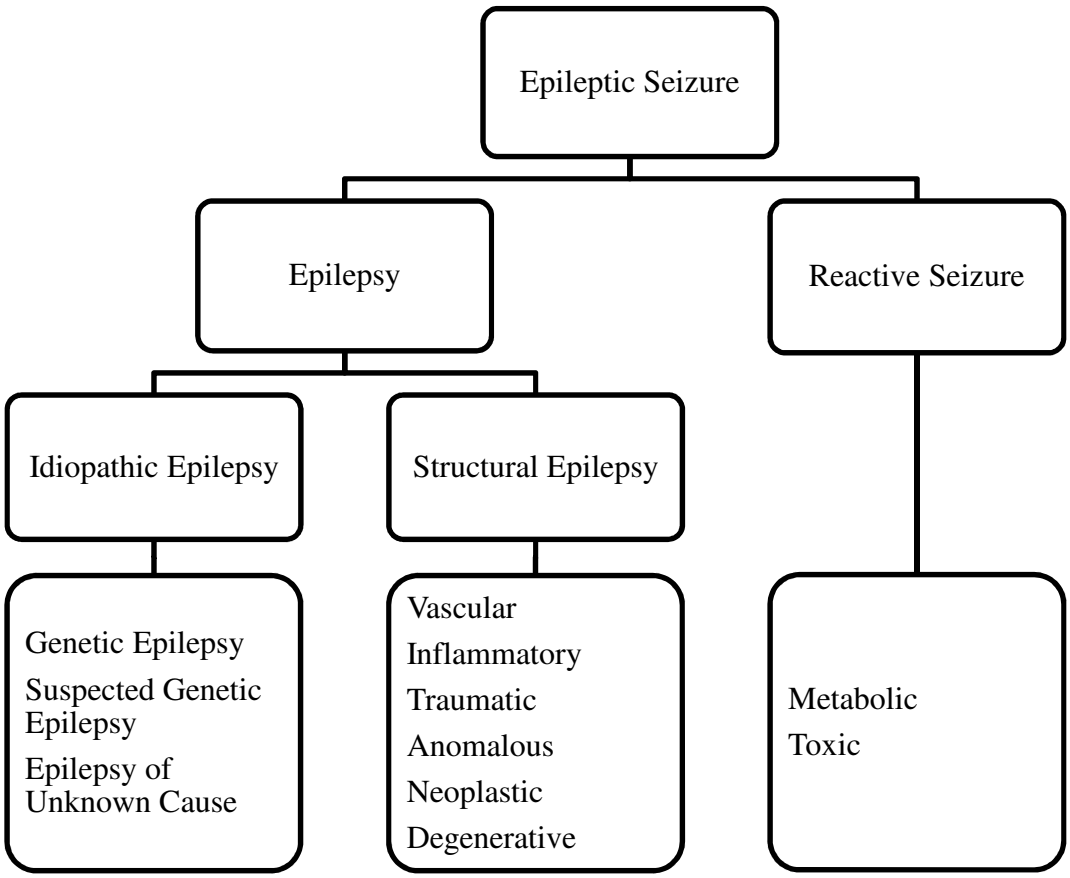

Figure 1: Current aetiological classification of epileptic seizures and epilepsy as proposed by the International Veterinary Epilepsy Task Force (BERENDT et al., 2015)

1.1. Structural epilepsies

1.1.1. Lafora disease

Lafora disease belongs to the group of progressive myoclonic epilepsies (PME). The condition has been described in the Miniature Wirehaired Dachshund, Beagle, Basset Hound, Miniature Poodle, Standard Poodle, Pointer and Corgi breeds (LOHI et al., 2005a; SCHOEMAN et al., 2002; SWAIN et al., 2017).

The discovery of the first epilepsy gene in Miniature Wirehaired Dachshunds with Lafora disease represented a milestone in veterinary epilepsy research (LOHI et al., 2005a). Together with identification of the genetic variant in the *EPM2B* gene the authors also provided a detailed description of the clinicopathological findings (LOHI et al., 2005a).

Affected dogs are presented with progressive myoclonic jerks that are bilaterally symmetrical and synchronous and involve mainly the face, neck, and thoracic limbs, causing retropulsion of the dog (GREDAL et al., 2003; HAJEK et al., 2016; LOHI et al., 2005a). Consciousness appears to be preserved during these episodes (GREDAL et al., 2003; HAJEK et al., 2016; SCHOEMAN et al., 2002). Myoclonus may occur spontaneously or in response to audiogenic and visual stimuli or sudden movements in the visual field (GREDAL et al., 2003; HAJEK et al., 2016; LOHI et al., 2005a; SCHOEMAN et al., 2002; SWAIN et al., 2017; WEBB et al., 2009). The frequency of myoclonus increases when the dogs are nervous or excited and diminishes when they are concentrating on a task (LOHI et al., 2005a). Age of onset varies from five months to twelve years, but dogs are usually presented at six to nine years of age (GREDAL et al., 2003; LOHI et al., 2005a; SWAIN et al., 2017; WEBB et al., 2009). In many dogs, the disease progresses to include generalized tonic-clonic seizures (GTCS), focal seizures, atonic attacks, ataxia, blindness, deafness, tremor, aggression, loss of house training and cognitive decline (HAJEK et al., 2016; LOHI et al., 2005a; SCHOEMAN et al., 2002; SWAIN et al., 2017; WEBB et al., 2009).

Neurological and physical examinations are otherwise unremarkable in affected dogs (WEBB et al., 2009). The results of haematological and cerebrospinal fluid (CSF) analyses are within normal limits (HAJEK et al., 2016; SCHOEMAN et al., 2002; WEBB et al., 2009). The results of magnetic resonance imaging (MRI) may be normal or reveal generalized ventricular dilation and cortical atrophy (LOHI et

al., 2005a; SWAIN et al., 2017; WEBB et al., 2009). Electromyography (EMG) shows abnormal spontaneous activity (fibrillation potentials, positive sharp waves) in the head and limb musculature (SCHOEMAN et al., 2002). Electroencephalography demonstrates bilateral synchronous polyspike-wave complexes (PSWC) and erratic myoclonus without an EEG correlate (GREDAL et al., 2003; LOHI et al., 2005a; SWAIN et al., 2017).

In humans, Lafora disease is caused by mutations in the *EPM1A* gene or the *NHLRC1* (formerly *EPM2B*) gene, which encode for the proteins laforin and malin, respectively (HAJEK et al., 2016; LOHI et al., 2005b). To date, only a variant in the *NHLRC1* gene has been associated with Lafora disease in veterinary medicine (HAJEK et al., 2016; LOHI et al., 2005a). In contrast to other species, the canine *NHLRC1* gene sequence harbours a two- or three-copy repeat of a twelve-nucleotide sequence (HAJEK et al., 2016; LOHI et al., 2005a). In affected dogs, this 12-bp repeat is massively expanded (HAJEK et al., 2016; LOHI et al., 2005a). The variant has been identified in a number of Miniature Wirehaired Dachshunds (19 to 26 copies), two Beagles (same expansion as is in Miniature Wirehaired Dachshunds) and one Basset Hound (14 copies) (HAJEK et al., 2016; LOHI et al., 2005a). Laforin is a glycogen phosphatase, whereas malin is an E3 ubiquitin ligase, that regulates laforin (HAJEK et al., 2016; WEBB et al., 2009). Loss of function of either leads to the accumulation of starch-like polyglucosans that are poorly branched and thus insoluble glycogen-like polysaccharides called Lafora bodies; they are pathognomonic for this eponymous disease (HAJEK et al., 2016; LOHI et al., 2005b; WEBB et al., 2009).

If genetic testing is not available, the gold standard for the diagnosis of Lafora disease is the combination of typical clinical findings and identification of the characteristic intraneural polyglucosan bodies (Lafora bodies) in central nervous system tissue (SCHOEMAN et al., 2002). Lafora bodies may also be found in peripheral tissues, such as sweat glands of the skin, striated musculature and liver (HAJEK et al., 2016; SCHOEMAN et al., 2002). However, the rate of false-negative results is high because of the variable content of Lafora bodies in these locations (HAJEK et al., 2016; SCHOEMAN et al., 2002). On light microscopy, Lafora bodies are round to oval and stain basophilic with haematoxylin and eosin (HE) stain. A characteristic feature of Lafora bodies is that they are periodic acid Schiff (PAS) positive and diastase resistant (LOHI et al., 2005a; SCHOEMAN et

al., 2002).

Treatment is limited to the management of myoclonus and seizures (HAJEK et al., 2016). Seizures may partially respond to AEDs, and various drugs including phenobarbital (PB), potassium bromide (KBr), levetiracetam (LEV), and gabapentin have been tried (GREDAL et al., 2003; HAJEK et al., 2016; WEBB et al., 2009). Although early studies suggested that dogs may benefit from an antioxidant-rich diet aimed at slowing disease progression, long-term observations failed to confirm this (HAJEK et al., 2016; SWAIN et al., 2017; WEBB et al., 2009).

Because the disease is fatal and the prognosis unfavourable, euthanasia is elected in most dogs when clinical signs have an unacceptable impact on the quality of life (WEBB et al., 2009).

1.1.2. Neuronal ceroid lipofuscinosis

The neuronal ceroid lipofuscinoses (NCL) belong to the group of PMEs although epileptic seizures are not a mandatory feature of this disease (DE SIQUEIRA, 2010; NITA et al., 2016; SHAHWAN et al., 2005). The NCLs are a heterogenous group of genetically determined, neurodegenerative, lysosomal storage disorders (FALLER et al., 2016; MOLE & COTMAN, 2015; NITA et al., 2016). A hallmark of the disease is the accumulation of autofluorescent storage material in neurons and other cell types (ASHWINI et al., 2016). Clinically, it is characterized by progressive cognitive and motor decline, vision loss, seizures, behavioural changes, and premature death (ASHWINI et al., 2016; KATZ et al., 2011). In addition to humans, NCL has been described in other species, including cats, cattle, ducks, sheep, ferrets, goats, horses, monkeys, parrots, pigs, mice, and many breeds of dogs (GILLIAM et al., 2015; KATZ et al., 2011; WEBER & PEARCE, 2013).

In human medicine the NCLs were first categorized according to the age of onset as infantile, late-infantile, juvenile, and adult (MOLE & COTMAN, 2015; NITA et al., 2016). Another classification system used the corresponding eponyms: Haltia-Santavuori disease, Jansky-Bielschowsky disease, Batten-Spielmeyer-Vogt disease, and Kufs disease (NITA et al., 2016). In the literature, “Batten disease” has been used for both juvenile NCL and the entire group of NCLs (NITA et al., 2016). Furthermore, human NCLs have been subdivided based on ultrastructural findings (HIRZ et al., 2017; NITA et al., 2016). A novel nomenclature was recently

recommended that took into account the involved gene and specific mutation, biochemical and clinical phenotype, ultrastructural findings, degree of functional disturbance, and other features (NITA et al., 2016).

The majority of human NCLs have an autosomal recessive mode of inheritance with two exceptions: adult onset NCL is caused by a mutation in *CLN4/DNAJC5* that has an autosomal dominant pattern of inheritance, and in one case report, a deletion in the *CLN8* gene was maternally transmitted (MOLE & COTMAN, 2015). To date, all canine cases described in veterinary medicine have an autosomal recessive mode of inheritance (KARLI et al., 2014). At least 14 different genes are associated with human NCL (NITA et al., 2016). Currently, 13 of these genes have been identified (*CLN1*-*CLN14*, with the exception of *CLN9* that refers to a predicted locus with an unidentified gene) (MOLE & COTMAN, 2015; NITA et al., 2016). Eight of these genes have been associated with canine NCLs in various breeds (Table 1) (HIRZ et al., 2017). Epileptic seizures have been reported to occur in association with five of these genes (*CLN5*, *CLN7*, *CLN8*, *TPP1*, *ATP13A2*) (ASHWINI et al., 2016; AWANO et al., 2006a; KOLICHESKI et al., 2017; MELVILLE et al., 2005; NAKAMOTO et al., 2011; TAYLOR & FARROW, 1992; WEBER & PEARCE, 2013; WÖHLKE et al., 2011; FALLER et al., 2016; FARIAS et al., 2011; GILLIAM et al., 2015; GUO et al., 2015, 2014; HIRZ et al., 2017; KARLI et al., 2014; KOLICHESKI et al., 2016). In addition, NCL has been described in numerous other dog breeds, namely the Cocker Spaniel, Dalmatian, Labrador Retriever, Miniature Schnauzer, Polish Owczarek Nizinny (Polish Lowland Sheepdog), Saluki, Japanese Retriever, Welsh Corgi, and Yugoslavian Shepherd (APPLEBY et al., 1982; BICHSEL & VANDEVELDE, 1982; GOEBEL et al., 1988; JOLLY et al., 1997; KARLI et al., 2014; MINATEL et al., 2000; NARFSTRÖM et al., 2007; ROSSMEISL et al., 2003; UMEMURA et al., 1985). Yet, the genetic background remains obscure in these breeds of dogs. A breed-specific variant in the *Arylsulfatase G* (*ARSG*) gene was shown to cause a late-onset form of NCL in American Staffordshire Terriers (ABITBOL et al., 2010). However, recent studies suggest that the disease caused by ARSG deficiency in American Staffordshire Terriers is instead a mucopolysaccharidosis (FALLER et al., 2016; GUO et al., 2015).

Although autofluorescent lysosomal storage bodies can be found not only in the CNS and retina but also in various other tissues, neurological signs are the

predominant clinical feature (Table 1) (FALLER et al., 2016; GILLIAM et al., 2015; HIRZ et al., 2017; SANDERS et al., 2011). Therefore, canine NCL is characterized by progressive motor impairment including ataxia, tetraparesis and hypermetria, cognitive decline with dementia, disorientation, and loss of housetraining and previously learned commands, and behavioural changes such as anxiety, nervousness and aggression (AWANO et al., 2006b, 2006a; FARIAS et al., 2011; GILLIAM et al., 2015; GUO et al., 2014; HIRZ et al., 2017; KOLICHESKI et al., 2016; ROSSMEISL et al., 2003; SANDERS et al., 2010). Myoclonic, focal, or generalized tonic-clonic seizures have been described in Dachshunds with CLN2, Australian Cattle dogs, Border Collies, and Golden Retrievers with CLN5, Chihuahuas and Chinese Crested dogs with CLN7, Australian Shepherds, Alpenländische Dachsbracke dogs and English Setters with CLN8, and Tibetan Terriers with CLN12 (ASHWINI et al., 2016; AWANO et al., 2006a; KOLICHESKI et al., 2017; MELVILLE et al., 2005; NAKAMOTO et al., 2011; TAYLOR & FARROW, 1992; WEBER & PEARCE, 2013; WÖHLKE et al., 2011; FALLER et al., 2016; FARIAS et al., 2011; GILLIAM et al., 2015; GUO et al., 2015, 2014; HIRZ et al., 2017; KARLI et al., 2014; KOLICHESKI et al., 2016). Other neurological signs include circling, pacing, vestibular syndrome, tremor, hallucinations, dysphagia, incontinence, insomnia, lethargy, and fatigue (ASHWINI et al., 2016; FARIAS et al., 2011; GUO et al., 2014; KOIE et al., 2004; KOLICHESKI et al., 2016, 2017; NARFSTRÖM et al., 2007; ROSSMEISL et al., 2003; SANDERS et al., 2010). A hallmark of NCL is progressive vision loss, which can be caused by lesions of the visual cortex (central blindness) or by an ongoing retinopathy (peripheral blindness) (ROSSMEISL et al., 2003).

Table 1: Genetics and clinical signs of canine NCL

Breed	Gene	Mutation	Onset in months	Clinical Signs						References
				Vision	Gait	Behaviour	Cognition	Seizures	Other	
Alpenländische Dachsbracke dog	*CLN8*	*CLN8:g.30,852,988_30,902,901del*	18-24	loss of vision	not reported	anxiety, aggression	disorientation	generalized tonic-clonic	bilateral ventral strabism	(HIRZ et al., 2017)
American Bulldog	*CTSD* (*CLN10*)	*CTSD:c.597 G>A*	11-36	not reported	ataxia, hypermetria	not reported	not reported	not reported	not reported	(AWANO et al., 2006b; EVANS et al., 2005)
Australian Cattle dog	*CLN5*	*CLN5:c.619 C>T*	6-19	loss of vision	ataxia, tetraparesis	anxiety, aggression	not reported	generalized tonic-clonic, focal, myoclonic, fly biting, trance-like episodes	circling, tremor, vestibular syndrome, lethargy	(KOLICHESKI et al., 2016)
Australian Shepherd	*CLN6*	*CLN6:c.829 T>C*	18	loss of vision	ataxia	anxiety	not reported	not reported	circling	(KATZ et al., 2011)
Australian Shepherd	*CLN8*	*CLN8:c.585 G>A*	8	loss of vision	ataxia	anxiety, aggression	loss of housetraining and learned commands	focal, fly biting, trance-like episodes	circling, pacing	(GUO et al., 2014)
Border Collie	*CLN5*	*CLN5:c.619 C>T*	15	loss of vision	ataxia	hyperactivity, aggression	dementia	fly biting, unclassified seizures	head tilt, halluci-nations	(MELVILLE et al., 2005; TAYLOR & FARROW, 1992)
Cane Corso	*PPT1* (*CLN1*)	*PPT1:c.124 + 1G>A*	8	loss of vision	ataxia, hypermetria	not reported	not reported	not reported	lethargy, vestibular syndrome	(KOLICHESKI et al., 2017)

Table 1: Genetics and clinical signs of canine NCL Cont.

Breed	Gene	Mutation	Onset in months	Clinical Signs						References
				Vision	Gait	Behaviour	Cognition	Seizures	Other	
Chihuahua	*MFSD8* (*CLN7*)	*MFSD8c.843delT*	12-18	loss of vision	ataxia	anxiety, hyperacusis, aggression	disorientation, cognitive impairment, loss of housetraining and learned commands	seizures	head tilt, difficulties in food prehension, circling	(ASHWINI et al., 2016; FALLER et al., 2016; NAKAMOTO et al., 2011)
Chinese Crested dog	*MFSD8* (*CLN7*)	*MFSD8c.843delT*	12	loss of vision	ataxia	anxiety, nervousness	disorientation	behavioural arrest	not reported	(GUO et al., 2015)
Dachs-hund	*PPT1* (*CLN1*)	*PPT1:c.736_737insC*	8-9	loss of vision	ataxia	behavioural changes	disorientation	not reported	weakness	(HIRZ et al., 2017; KOLICHESKI et al., 2017; SANDERS et al., 2010)
Dachs-hund	*TPP1* (*CLN2*)	*TPP1:c.325delC*	7-9	loss of vision	ataxia, hyper-metria	hyper-activity, aggression	mental dullness, loss of housetraining and learned commands, disorientation	myoclonic	vomiting, circling, weakness, head tremor	(AWANO et al., 2006a)
English Setter	*CLN8*	*CLN8:c.491T>C*	12-24	loss of vision	ataxia	not reported	cognitive impairment	unclassified seizures	not reported	(KARLI et al., 2014; KOLICHESKI et al., 2017; WEBER & PEARCE, 2013)
Golden Retriever	*CLN5*	*CLN5:c.934_935delAG*	13-17	loss of vision	ataxia	anxiety, aggression	loss of learned commands	generalized, focal, fly biting, trance-like episodes	pacing, circling, tremor	(GILLIAM et al., 2015)
Tibetan Terrier	*ATP13A2* (*CLN12*)	*ATP13A2:c.1623delG*	60-96	loss of vision	ataxia	anxiety, aggression, nervousness	loss of learned commands, disorientation	generalized tonic-clonic	not reported	(FARIAS et al., 2011; WEBER & PEARCE, 2013; WÖHLKE et al., 2011)

Age of onset depends on the breed and underlying gene mutation (HIRZ et al., 2017). In veterinary medicine, two forms are differentiated: the early or juvenile form, which has an approximate age at onset of 18 months and the late or adult form, which manifests at about 1.5 to nine years of age (KARLI et al., 2014). Age of onset is often underestimated by owners, who do not expect their dogs to develop neurological abnormalities (KOLICHESKI et al., 2017). For example, in Dachshunds with CLN2, owners reported an age of onset of seven to nine months (KOLICHESKI et al., 2017). In an experimental setting, however, animal technicians were able to identify affected dogs as early as five months of age (KOLICHESKI et al., 2017). Moreover, Sanders et al. demonstrated that in Dachshunds with CLN2, electroretinogram responses and cognitive function assessed using a reversal learning task were altered at the age of six months and clinical signs of motor and visual impairment became apparent only in a later stage of the disease (SANDERS et al., 2011).

A complete blood count (CBC), serum biochemical profile, pre- and postprandial serum bile acids, urinalysis, urine metabolic screen for inborn errors of metabolism, and CSF examination are within reference intervals (AWANO et al., 2006a; HIRZ et al., 2017; KOLICHESKI et al., 2016). Results of neurological examination reflect the involved areas of brain. Despite apparent visual deficits, absent menace response, and histologically confirmed retinal involvement, the pupillary light reflex may be normal, incomplete, or absent (ASHWINI et al., 2016; GUO et al., 2015; NARFSTRÖM et al., 2007). Ophthalmoscopic examination may be normal or reveal fundoscopic changes (GUO et al., 2015; NAKAMOTO et al., 2011; NARFSTRÖM et al., 2007). Likewise, electroretinography may show physiological or abnormal results (GUO et al., 2014, 2015; NARFSTRÖM et al., 2007). The results of MRI are consistent with generalized brain atrophy and reveal dilation of the ventricular system, thinning of the interthalamic adhesion, and widening of the cerebral and cerebellar sulci (GUO et al., 2014; HIRZ et al., 2017; KOIE et al., 2004; KOLICHESKI et al., 2016). An unexpected MRI finding in Chihuahuas with NCL is diffuse thickening of the meninges with marked contrast enhancement that represents collagenous tissue with mild infiltration of macrophages, lymphocytes, and plasma cells (NAKAMOTO et al., 2011). In one dog, EEG carried out during an episode of facial twitching that progressed to a GTCS revealed background slowing (7-9 Hz) and diffuse epileptiform discharges

(spike wave activity) (ROSSMEISL et al., 2003).

Histological examination reveals a decrease in neuronal density in the cerebral cortex and granular layer of the cerebellum as well as a reduced number of Purkinje cells (FALLER et al., 2016; HIRZ et al., 2017; KOLICHESKI et al., 2017). The density of granule cells and neurons in the deep cerebellar nuclei may be decreased too (HIRZ et al., 2017; KOLICHESKI et al., 2017). The retina is markedly thinned and lacks readily identifiable retinal layers (ASHWINI et al., 2016). Autofluorescent storage material can be found throughout the CNS, but is most prevalent in the perinuclear area of neurons in the cerebral cortex, the granular layer of the cerebellum, the Purkinje cells, and the ganglion cells of the retina (HIRZ et al., 2017; KATZ et al., 2011; KOLICHESKI et al., 2017). Abundant deposits of intracellular lipid material may also be seen in the basal nuclei, hippocampus, thalamus, molecular layer of the cerebellum, brainstem, and spinal cord (ASHWINI et al., 2016; FALLER et al., 2016; WÖHLKE et al., 2011). These intracellular aggregates stain PAS positive, Sudan blue positive, Sudan black B positive, slightly positive with acid-fast staining, and dark blue with Luxol fast blue staining, and there is strong staining for lysosomal-associated membrane protein 1 (LAMP-1) (FALLER et al., 2016; HIRZ et al., 2017; NAKAMOTO et al., 2011). Lysosomal storage bodies can also be found in other organs and tissues such as the heart, duodenum, liver, enteric nervous system, and in macrophages in the spleen, lymph nodes, and lung (FALLER et al., 2016; HIRZ et al., 2017; KOLICHESKI et al., 2016).

Electron microscopic examination may reveal a distinct ultrastructural appearance of the accumulated material that includes granular osmiophilic deposits (GRODs), curvilinear profiles, rectilinear complexes, fingerprint profiles, and condensed or mixed forms (AWANO et al., 2006b; HIRZ et al., 2017; NITA et al., 2016). Traditionally, these ultrastructural changes have been used to subclassify human NCLs (HIRZ et al., 2017; NITA et al., 2016). However, the ultrastructural findings can be quite variable and more than one pattern may be found in many NCLs (NITA et al., 2016; O'BRIEN & KATZ, 2008). Moreover, the ultrastructural findings depend on the tissue examined (NITA et al., 2016).

In most types of NCL, the major component of the accumulated autofluorescent lipopigment is subunit c of mitochondrial adenosine triphosphate (ATP) synthase (SCMAS) (HIRZ et al., 2017; MELVILLE et al., 2005; MINATEL et al., 2000;

NARFSTRÖM et al., 2007). In contrast, sphingolipid activator proteins (SAPs or saposins) A and D are the principal constituents of the storage bodies in children with infantile NCL as well as in Miniature Schnauzers and Polish Lowland Sheepdogs (HIRZ et al., 2017; MELVILLE et al., 2005; MINATEL et al., 2000; NARFSTRÖM et al., 2007; PALMER et al., 1997).

Neuronal ceroid lipofuscinoses have an invariably fatal course, although the rate of disease progression can vary among breeds (ROSSMEISL et al., 2003). For example, the clinical course of CLN10 disease in American Bulldogs is less severe than in other breeds, possibly because of a residual cathepsin D enzymatic activity of 36% (AWANO et al., 2006b; KOLICHESKI et al., 2017).

To date, there is no efficient treatment to delay the progression of NCL (SANDERS et al., 2011, 2010; WEBER & PEARCE, 2013). However, several therapeutic strategies are currently under investigation (ASHWINI et al., 2016; KATZ et al., 2017, 2015; KOLICHESKI et al., 2017; SANDERS et al., 2011; TRACY et al., 2016; WHITING et al., 2016). Dachshunds that suffer from CLN2 disease caused by tripeptidyl peptidase 1 (TPP1) deficiency have been used as a model to investigate a variety of promising treatment approaches and this has paved the way for ongoing human trials (ASHWINI et al., 2016; KATZ et al., 2017, 2015; KOLICHESKI et al., 2017; SANDERS et al., 2011; TRACY et al., 2016; WHITING et al., 2016). A significant delay in the onset and progression of neurological signs and the extension of lifespan are achieved by periodic infusion of recombinant TPP1 into the CSF (KATZ et al., 2015; WHITING et al., 2016). The same effect is observed with *TPP1* gene transfer to the ependyma by a single administration of a gene vector to the CSF (KATZ et al., 2015; WHITING et al., 2016). However, these approaches address only the neurological system, although retinal ganglion cells may be selectively preserved (KATZ et al., 2017; WHITING et al., 2016). Indeed, patients that receive treatments that target the CNS may develop clinical signs that are related to extraneuronal lesions, for example cardiac dysfunction (ASHWINI et al., 2016; KATZ et al., 2017). Intravitreal stem cell therapy was shown to decelerate retinal pathology (TRACY et al., 2016). Systemic gene therapy or administration of TPP1 has been suggested as a treatment for extraneuronal disease (KATZ et al., 2017).

In breeds with a high carrier frequency, especially in those that have a later onset of NCL, genetic testing constitutes a valuable tool to decrease the number of

affected individuals. In a recent study, Kluth et al. showed that selective breeding based on genetic testing is a favourable method for the gradual, albeit slow reduction of carriers of the *ATP13A2* mutation in Tibetan Terriers (KLUTH et al., 2014).

1.2. Idiopathic epilepsies

1.2.1. Benign familial juvenile epilepsy in Lagotto Romagnolo dogs

Benign familial juvenile epilepsy (BFJE) in Lagotto Romagnolo dogs is a genetic focal epilepsy syndrome (JOKINEN et al., 2007; SEPPÄLÄ et al., 2011). Affected dogs are presented with focal seizures that are characterized by generalized tremor, stiffness, and ataxia with lateralization (JOKINEN et al., 2007). During these episodes, consciousness may be preserved or absent (JOKINEN et al., 2007). Seizures may occur at any time of the day and during activity as well as at rest (JOKINEN et al., 2007). Seizure frequency varies from multiple episodes per day to one event per week (JOKINEN et al., 2007). In the interictal period, some dogs may demonstrate ataxia and falling (JOKINEN et al., 2007). The mean age of onset is 6.3 weeks (five to nine weeks) (JOKINEN et al., 2007). In most cases, the disease resolves spontaneously at the age of ten weeks (7.5 to 13 weeks) (JOKINEN et al., 2007).

The results of physical examination are normal (JOKINEN et al., 2007). Neurological examination may reveal ataxia, hypermetria, tremor, postural reaction deficits, and a reduced menace response in some dogs (JOKINEN et al., 2007). Neurological deficits resolve with cessation of seizures (JOKINEN et al., 2007). The results of a serum biochemical profile, CBC, urinalysis, and CSF examination are within reference intervals (JOKINEN et al., 2007). Similarly, EMG, brainstem auditory evoked response, and MRI are unremarkable (JOKINEN et al., 2007). Histological examination reveals eosinophilic, intracytoplasmic inclusions, likely pseudo-Negri bodies, and mild Purkinje cell loss (JOKINEN et al., 2007). Interictal EEG under medetomidine sedation demonstrated focal epileptiform discharges (sharp waves and spikes) in the centrotemporal and occipital lobes in about 88% of affected puppies (JOKINEN et al., 2007; SEPPÄLÄ et al., 2011). 2-[18F] fluoro-2-deoxy-D-glucose (^{18}FDG)-positron emission tomography (PET)-MRI indicated hypometabolism in the parietal, temporal, and occipital cortices with EEG abnormalities in the same locations, suggesting that this may be the epileptogenic

focus in these dogs (JOKINEN et al., 2014).

Although Lagotto Romagnolo dogs with BFJE have a good long-term prognosis regarding seizure outcome, affected dogs may develop neurobehavioural comorbidities in adulthood (JOKINEN et al., 2015). Jokinen et al. demonstrated that dogs older than four years of age diagnosed with BFJE in remission had significantly higher inattention and excitability/impulsivity scores than appropriate controls (JOKINEN et al., 2015). The behavioural abnormalities seen in the Lagotto Romagnolo dogs were comparable to attention deficit hyperactivity disorder (ADHD) in humans, which is also a frequently observed comorbidity in human epilepsy syndromes (JOKINEN et al., 2015).

While the majority of dogs in the study of Jokinen et al. did not receive an antiepileptic medication, the authors recommended treating at least severely affected puppies to prevent seizure recurrence in adulthood (JOKINEN et al., 2007).

Benign familial juvenile epilepsy has been linked to a truncating mutation in the *LGI2* gene (c.1552A>T) (SEPPÄLÄ et al., 2011). The disease has an autosomal recessive mode of inheritance, although a 1.8% rate of non-penetrance and a 7% rate of disease through heterozygosity is observed (SEPPÄLÄ et al., 2011). Carrier frequency is as high as 32% (SEPPÄLÄ et al., 2011). LGI2 belongs to the leucine-rich glioma-inactivated (LGI) protein family, which consists of four members (LGI1-4) (SEPPÄLÄ et al., 2011). It is neuronally secreted and has a function similar to that of LGI1 (PAKOZDY et al., 2015). Both proteins interact with three different members of the a-disintegrin-and-metalloproteinase (ADAM) family (ADAM11, ADAM22, ADAM23) (PAKOZDY et al., 2015; SEPPÄLÄ et al., 2011). One of its functions is the co-assembly of postsynaptic ADAM22 and presynaptic ADAM23, pulling the pre- and postsynaptic membranes together, hence stabilizing the synapses and enhancing neurotransmission (PAKOZDY et al., 2015; SEPPÄLÄ et al., 2011). Truncation of either LGI1 or LGI2 prevents its secretion and interaction with ADAM proteins (SEPPÄLÄ et al., 2011). In human medicine, mutations in the *LGI1* gene are known to cause autosomal dominant lateral temporal lobe epilepsy starting at about ten years of age (SEPPÄLÄ et al., 2011). Moreover, limbic encephalitis in humans and cats is caused by auto-antibodies against LGI1 (PAKOZDY et al., 2015). LGI1 is mainly expressed at the end of the pruning phase and thereafter (PAKOZDY et al., 2015; SEPPÄLÄ et al.,

2011). In contrast, LGI2 expression levels are highest at birth and diminish towards the middle of neural pruning (PAKOZDY et al., 2015; SEPPÄLÄ et al., 2011). Therefore, it is possible that LGI1 takes over the function of LGI2 during pruning, which would explain the remitting course of BFJE (SEPPÄLÄ et al., 2011).

1.2.2. Genetic epilepsy in Boerboel dogs

The genetic background of a focal epilepsy syndrome has been identified in Boerboel dogs (STASSEN et al., 2013). This syndrome is characterized by epileptic seizures that involve spasms of the facial and limb musculature with impaired consciousness, vocalization, and anxiety (STASSEN et al., 2013). Age of onset is three months, and there are no abnormalities on diagnostic work-up (physical examination, CBC, serum biochemical profile, urinalysis, neurometabolic screening, MRI, and CSF) (STASSEN et al., 2013). Histological examination reveals that the neurons are partially filled with large vacuoles throughout the entire brain (STASSEN et al., 2013). Pedigree analysis points to an autosomal recessive mode of inheritance (STASSEN et al., 2013). A mutation in a neuronal gene, leading to a splicing deficiency was identified (STASSEN et al., 2013). This neuronal gene has not been associated with epilepsy to date (STASSEN et al., 2013). It encodes a mitochondrial membrane protein, the function of which has not yet been elucidated (STASSEN et al., 2013).

1.2.3. *ADAM23* – a risk factor for idiopathic epilepsy

The average prevalence of epilepsy of unknown origin in dogs is 0.62% but may be as high as 18% in certain breeds of dogs (KEARSLEY-FLEET et al., 2013; KOSKINEN et al., 2015; POTSCHKA et al., 2013). Together with familial based data and pedigree studies, this observation raises the suspicion of a genetic background (HÜLSMEYER et al., 2015; KOSKINEN et al., 2015). A breakthrough in understanding the genetics of idiopathic epilepsy was recently attained when a risk factor for idiopathic epilepsy in Belgian Shepherd dogs was identified in the *ADAM23* gene (SEPPÄLÄ et al., 2012). Belgian Shepherd dogs have a mild epilepsy phenotype and experience mainly focal seizures with secondary generalization but may also have primary generalized seizures and focal seizures without generalization (SEPPÄLÄ et al., 2012). The mean age of onset is approximately three years (SEPPÄLÄ et al., 2012). The results of neurological, haematological, and CSF examinations as well as MRI are unremarkable

(SEPPÄLÄ et al., 2012). Electroencephalography reveals epileptiform discharges (sharp waves, spikes, spike-wave complexes (SWC)) with variable foci (SEPPÄLÄ et al., 2012). Although the homozygous risk allele is common within the breed (22%) and suggests a rather low penetrance, there is a seven-fold increase in the risk of idiopathic epilepsy (SEPPÄLÄ et al., 2012).

Further studies that determined the impact of *ADAM23* in several dog breeds with idiopathic epilepsy confirmed a six-variant risk haplotype spanning a 28-kb region located between exon five and eleven in *ADAM23* (KOSKINEN et al., 2015, 2017). However, the precise causal variant still needs to be identified (KOSKINEN et al., 2015, 2017). Associations were identified in Whippet, Australian Shepherd, Kromfohrländer, Labrador Retriever, Finnish Spitz, Beagle, Belgian Shepherd, and Schipperke dogs (KOSKINEN et al., 2015, 2017). Although not statistically significant, this association had a similar trend in the Pyrenean Shepherd, Irish Setter and Miniature Pinscher breeds (KOSKINEN et al., 2017). The only breed in which there was no association with the haplotype was the Finnish Lapphund (KOSKINEN et al., 2017).

ADAM23 is a membrane-anchored protein, which is highly expressed in the CNS, especially by pyramidal cells of the cerebral cortex and hippocampus and the Purkinje cells of the cerebellum (KOSKINEN et al., 2015, 2017). It is involved in cellular adhesion and interacts with ADAM22 as well as with the two epilepsy-associated proteins LGI1 and LGI2 and it enhances neurite outgrowth and dendritic branching via its interaction with LGI1 (KOSKINEN et al., 2015, 2017; PAKOZDY et al., 2015; SEPPÄLÄ et al., 2011, 2012) Moreover, together with other proteins, ADAM23 builds a complex that draws the pre- and postsynaptic membranes together to increase neurotransmission (PAKOZDY et al., 2015; SEPPÄLÄ et al., 2012). Mouse models emphasize the epileptogenic potential of *ADAM23*, and a complete knockout of this gene leads to tonic-clonic seizures, other neurological deficits and premature death, and a reduced seizure threshold is observed in heterozygous animals (KOSKINEN et al., 2015; PAKOZDY et al., 2015).

2. Human juvenile myoclonic epilepsy

2.1. Clinical aspects

Juvenile myoclonic epilepsy (JME) is one of the most common forms of human idiopathic generalized epilepsies (IGE), accounting for 26.7% of IGE and 4.1% of all epilepsies (SERAFINI et al., 2013; YACUBIAN, 2017). The hallmarks of this epilepsy syndrome are myoclonic seizures that occur upon awakening and an early onset at around puberty (BAYKAN & WOLF, 2017; GENTON et al., 2013; WOLF et al., 2015). The mean age at onset is around 14 years and patients have normal intelligence (WELTY, 2006; YACUBIAN, 2017). There are no abnormalities on neurological examination, and routine brain imaging is unremarkable (ALFRADIQUE & VASCONCELOS, 2007; ANDERSON & HAMANDI, 2011; KOEPP et al., 2014; WANDSCHNEIDER et al., 2012; WELTY, 2006). It was thought that JME affected both genders equally, but recent studies have shown a female preponderance (ALFRADIQUE & VASCONCELOS, 2007; GENTON et al., 2013; YACUBIAN, 2017).

2.1.1. The triad of seizure types in JME

Three seizure types are observed in human JME, namely myoclonic seizures, GTCS, and absence seizures.

Myoclonic jerks are sudden, brief (1-2 s), and spontaneous without loss of consciousness (GENTON et al., 2013; WELTY, 2006; YACUBIAN, 2017). They predominate in the upper limbs, are bilateral, arrhythmic and irregular, and may be single or repetitive in arrhythmic clusters (GENTON et al., 2013; YACUBIAN, 2017). Myoclonic seizures are grossly symmetrical, but may be perceived as affecting the dominant side more, as the jerk will interfere with the activity executed by this hand (GENTON et al., 2013; WOLF et al., 2015). Nonetheless, praxis-induced reflex seizures may indeed predominate in the executive hand (GENTON et al., 2013; WOLF et al., 2015; YACUBIAN, 2017). Myoclonic jerks often make patients throw or drop objects (this is often misinterpreted as clumsiness by the patient), and less commonly make some patients fall (GENTON et al., 2013). Complaints associated with myoclonic seizures consist of slurred speech, sighs, walking difficulties, and nervousness of the limbs (WELTY, 2006).

Most patients (80-95%) continue to develop rare GTCS 1.3 to 3.3 years after onset

of myoclonic seizures (GENTON et al., 2013; WOLF et al., 2015). Generalized tonic-clonic seizures often follow a crescendo of myoclonic seizures and are actually clonic-tonic-clonic seizures (GENTON et al., 2013). Many JME patients may only seek medical advice after their first GTCS because myoclonic jerks are often overlooked or underestimated by patients and their families (GENTON et al., 2013; WOLF et al., 2015; YACUBIAN, 2017). In these cases, it is up to the physician to specifically ask about the occurrence of myoclonic jerks (GENTON et al., 2013).

The third seizure type encountered in about 30% of JME patients is absence seizures (NICOLSON & MARSON, 2010; WOLF et al., 2015; YACUBIAN, 2017). They are sporadic and brief and often neglected by patients due to incomplete interference with consciousness (GENTON et al., 2013; WOLF et al., 2015; YACUBIAN, 2017). Hence, this seizure type may only be discovered on long-term EEG recordings (GENTON et al., 2013). Interestingly, up to 15% of patients with childhood absence epilepsy (CAE) or juvenile absence epilepsy go on to develop JME (CAMFIELD et al., 2013; GENTON et al., 2013; WOLF et al., 2015).

2.1.2. Chronodependency and provocative factors

One of the most striking characteristics of JME is its chronodependency: the strict correlation between seizure occurrence and specific time of the day (ANDERSON & HAMANDI, 2011; KASTELEIJN-NOLST TRENITÉ et al., 2013a). Myoclonic jerks and GTCS occur almost exclusively within the first two hours after awakening, mostly in the morning, but to a lesser extent also after a nap or during an arousal at night (GENTON et al., 2013; NICOLSON & MARSON, 2010; YACUBIAN, 2017). This circumstance is more pronounced after provoked than after spontaneous awakening (GENTON et al., 2013; SERAFINI et al., 2013). Seizures may also occur in the evening relaxation period (GENTON et al., 2013; HASHEMIAGHDAM et al., 2015; WOLF et al., 2015; YACUBIAN, 2017). Chronodependency may be the explanation for the peculiar circadian pattern of activity in JME patients: they tend to have an evening type of circadian rhythm, being most active in the afternoon and evening, not feeling well before ten a.m. and going to bed late (GENTON et al., 2013; KASTELEIJN-NOLST TRENITÉ et al., 2013a).

A wide variety of provocative factors that are capable of triggering seizures in JME

patients have been described. The most frequent precipitating factors are sleep deprivation, stress, and alcohol (ALFRADIQUE & VASCONCELOS, 2007; ANDERSON & HAMANDI, 2011; GENTON et al., 2013; KASTELEIJN-NOLST TRENITÉ et al., 2013a; WELTY, 2006). Visual stimuli such as flashlights, television, and videogames can trigger seizures in 15% of patients (ALFRADIQUE & VASCONCELOS, 2007; GENTON et al., 2013; KASTELEIJN-NOLST TRENITÉ et al., 2013a). However, there is some discrepancy between the high proportion of patients exhibiting a photoparoxysmal response (PPR) on EEG with photic stimulation and the small number of patients that have clinical photosensitivity in daily life (GENTON et al., 2013). Other precipitants are fatigue, excitement/frustration, and fever (ALFRADIQUE & VASCONCELOS, 2007; KASTELEIJN-NOLST TRENITÉ et al., 2013a). In women menstrual cycle plays an important role (ALFRADIQUE & VASCONCELOS, 2007; GENTON et al., 2013; KASTELEIJN-NOLST TRENITÉ et al., 2013a). Other provocative factors are mental concentration, hand activities and complex finger movements, mental and written calculation, playing musical instruments, drawing, and specific types of music (ALFRADIQUE & VASCONCELOS, 2007; GENTON et al., 2013; KASTELEIJN-NOLST TRENITÉ et al., 2013a). Some of these aforementioned factors may be attributable to the phenomenon of praxis induction (BAYKAN & WOLF, 2017; KASTELEIJN-NOLST TRENITÉ et al., 2013a). Likewise, linguistic tasks such as reading are reported to generate perioral reflex myocloni (GENTON et al., 2013; KASTELEIJN-NOLST TRENITÉ et al., 2013a). Finally, hyperventilation can trigger absence seizures (KASTELEIJN-NOLST TRENITÉ et al., 2013a).

2.1.3. Diagnostic criteria and subsyndromes

Juvenile myoclonic epilepsy is a clinically and genetically heterogenous disorder (ANDERSON & HAMANDI, 2011; KOEPP et al., 2014). As a consequence, different diagnostic criteria have been used by scientists and clinicians from different countries, complicating comparison of studies and adequate recommendations for diagnosis and treatment regimens (KASTELEIJN-NOLST TRENITÉ et al., 2013b). Therefore, a group of international experts on JME collaborated to reach a consensus on the diagnosis and management of this epileptic syndrome (KASTELEIJN-NOLST TRENITÉ et al., 2013b). They proposed two groups of diagnostic criteria: one with fairly narrow requirements and the other with

more flexible requirements (KASTELEIJN-NOLST TRENITÉ et al., 2013b; WOLF et al., 2015). Class I criteria include myoclonic seizures without loss of consciousness occurring exclusively within two hours after awakening, with an ictal pattern of generalized high amplitude polyspikes or PSWC, an unremarkable EEG background, normal intelligence, and an age of onset between ten and 25 years (KASTELEIJN-NOLST TRENITÉ et al., 2013b; WOLF et al., 2015; YACUBIAN, 2017). Class II criteria comprise myoclonic seizures predominantly after awakening, a normal EEG background, generalized epileptiform discharges on EEG with or without concomitant myoclonic jerks and with some asymmetry permitted, no mental retardation or deterioration, and an age of onset between six and 25 years (KASTELEIJN-NOLST TRENITÉ et al., 2013b; WOLF et al., 2015; YACUBIAN, 2017). Furthermore, class II criteria encompass provocative factors such as sleep deprivation, stress, visual stimuli, praxis induction, and GTCS preceded by myoclonic seizures (KASTELEIJN-NOLST TRENITÉ et al., 2013b; WOLF et al., 2015; YACUBIAN, 2017).

Four subsyndromes can be distinguished in JME (BAYKAN et al., 2013; MARTÍNEZ-JUÁREZ et al., 2006; YACUBIAN, 2017). The largest number of patients make up the classic JME group (72%) (MARTÍNEZ-JUÁREZ et al., 2006). By definition, the first seizure type these patients experience is a myoclonic seizure or a GTCS (MARTÍNEZ-JUÁREZ et al., 2006; YACUBIAN, 2017). Absence seizures may emerge in the course of the disease, but they are not a mandatory feature (MARTÍNEZ-JUÁREZ et al., 2006). The second group is CAE evolving to JME (18%) (BAYKAN et al., 2013; YACUBIAN, 2017). Obviously in these patients the first seizure type observed are pyknoleptic seizures that start between the age of three to eleven years (MARTÍNEZ-JUÁREZ et al., 2006). This group is differentiated from JME with an adolescent onset (twelve years and older) of pyknoleptic seizures that accounts for 7% of JME patients (BAYKAN et al., 2013; MARTÍNEZ-JUÁREZ et al., 2006). The last group comprises patients with JME with astatic seizures, which is observed in 3% of cases (BAYKAN et al., 2013; MARTÍNEZ-JUÁREZ et al., 2006).

2.2. Reflex epileptic traits

Reflex epileptic seizures are defined as seizures that are triggered reproducibly and instantaneously by certain sensory or cognitive stimuli (BENICZKY et al., 2012). Four reflex epileptic traits can be distinguished in JME, including photosensitivity,

eye closure sensitivity, praxis induction, and orofacial reflex myocloni (BAYKAN & WOLF, 2017; BENICZKY et al., 2012; WOLF et al., 2015; YACUBIAN, 2017).

2.2.1. Photosensitivity

2.2.1.1. General aspects of photosensitivity

Photosensitivity is an abnormal visual sensitivity of the brain in reaction to external natural or artificial photic stimuli (LU et al., 2008). In the EEG it is expressed as a PPR (KASTELEIJN-NOLST TRENITÉ et al., 2013a; WALTZ et al., 1992). Video-EEG with photic stimulation or intermittent light stimulation (ILS) is used to investigate the occurrence of photosensitivity (VERROTTI et al., 2012). This photic stimulation can elicit no response, a physiologic response such as photic driving, or a PPR (SENEVIRATNE et al., 2012). Flickering light with a frequency of 15 to 20 Hz has the highest potential to provoke a PPR (MARTINS DA SILVA & LEAL, 2017). A variety of classification systems for PPR have been proposed, but in the current literature the classification system established by Waltz et al. prevails (PARRA et al., 2005; WALTZ et al., 1992). This system divides PPR into four grades (Table 2): grade one is characterized by spikes that are limited to the occipital lobe; grade two encompasses parieto-occipital spikes that are followed by a slow wave; grade three is characterized by discharges that also spread to the frontal regions; and grade four has generalized SWC or PSWC (POLEON & SZAFLARSKI, 2017; WALTZ et al., 1992).

Table 2: Classification of photoparoxysmal response

The four types of photoparoxysmal response	
1)	occipital spikes only
2)	parieto-occipital spikes followed by a biphasic slow wave
3)	parieto-occipital spikes followed by a biphasic wave with anterior extension
4)	generalized spike-wave- or polyspike-wave-complexes

Classification of photoparoxysmal response according to Waltz et al. (WALTZ et al., 1992).

A grade four PPR is observed significantly more frequently in patients with IGE and their relatives and has therefore been suggested as a biomarker for IGE (WALTZ et al., 1992). Photosensitivity is more common in children with a peak age of onset at about twelve years with a five-fold increased prevalence in seven- to 19-year-old individuals and it is 1.5 to two times more prevalent in females (POLEON & SZAFLARSKI, 2017; VERROTTI et al., 2012). The prevalence of PPR in healthy individuals ranges from 0.9 to 9% and varies among the different epilepsy syndromes (KOELEMAN et al., 2013; POLEON & SZAFLARSKI, 2017). Frequency estimations for JME range from 5 to 90% in contrast to a prevalence of 18% for CAE, 17% for Lennox-Gastaut syndrome, and 90% for Unverricht Lundborg disease and other PMEs (KASTELEIJN-NOLST TRENITÉ et al., 2013a; KOELEMAN et al., 2013; POLEON & SZAFLARSKI, 2017). It may be possible to explain this large discrepancy by variations in age, gender, treatment, and ILS protocols used (KASTELEIJN-NOLST TRENITÉ et al., 2013a; KOELEMAN et al., 2013; YACUBIAN, 2017). A standardized ILS protocol has been proposed for uniform evaluation of photosensitivity (KASTELEIJN-NOLST TRENITÉ et al., 2012). Treatment strategies include AEDs, e.g. valproate, lamotrigine and LEV, tinted or polarized glasses, darkened lenses, and avoidance of triggers (CRESPEL et al., 2013; KASTELEIJN-NOLST TRENITÉ et al., 2013a; POLEON & SZAFLARSKI, 2017). A multitude of studies provide strong evidence for a genetic component of PPR and many putative loci have been identified (VERROTTI et al., 2012). Nevertheless, the genetics of photosensitivity and its correlation to epilepsy are not entirely understood (VERROTTI et al., 2012).

2.2.1.2. Photosensitivity in JME

Juvenile myoclonic epilepsy has one of the strongest associations with photosensitivity among all epilepsies (GUERRINI & GENTON, 2004). Most studies report a prevalence of 30 to 50%, but in one study by Appleton et al., it was as high as 90% with more intense and prolonged stimulation (ALFRADIQUE & VASCONCELOS, 2007; ANDERSON & HAMANDI, 2011; APPLETON et al., 2000; BAYKAN & WOLF, 2017; NICOLSON & MARSON, 2010; SERAFINI et al., 2013; WELTY, 2006; WOLF et al., 2015). Although electroencephalographic paroxysmal responses upon photic stimulation are common, the prevalence of clinical response to visual stimuli in daily life is lower (BAYKAN & WOLF, 2017; GENTON et al., 2013). Similar to other epilepsy syndromes, photosensitivity is

more prevalent in adolescent JME patients (KASTELEIJN-NOLST TRENITÉ et al., 2013a). Females are more often affected (48%) than males (26%) (SERAFINI et al., 2013). Other factors influencing photosensitivity in JME are current AED treatment, sleep deprivation, time of stimulation (more pronounced in the morning), and stimulation methodology, e.g. luminance, flicker frequency, amount of stimulated retina, and duration of stimulation (ANDERSON & HAMANDI, 2011; BAYKAN & WOLF, 2017; KASTELEIJN-NOLST TRENITÉ et al., 2013a; SERAFINI et al., 2013). The high prevalence of 90% described by Appleton et al. raises the question of whether photosensitivity is really an endophenotype of JME or whether there is a common mechanism for photosensitivity and ictogenesis in JME (APPLETON et al., 2000; BAYKAN & WOLF, 2017).

2.2.2. Eye closure sensitivity

Eye closure sensitivity is defined as the occurrence of occipitally accentuated 3 Hz spike and wave discharges within 2 s after eye closure (BAYKAN & WOLF, 2017; WOLF et al., 2015; YACUBIAN, 2017). The clinical pendant is eyelid myoclonia with or without absence seizures (BAYKAN & WOLF, 2017; WOLF et al., 2015). There is some overlap with photosensitivity, however it should be pointed out that the two entities are not identical (BAYKAN & WOLF, 2017; WOLF et al., 2015). Eye closure sensitivity is a core feature of Jeavons syndrome (eyelid myoclonia with absences), but can also be observed in 15-20% of JME patients (BAYKAN & WOLF, 2017; WOLF et al., 2015; YACUBIAN, 2017). Interestingly, only slow (spontaneous or voluntary) eye closure elicits eyelid myoclonia (BAYKAN & WOLF, 2017; DA CONCEIÇÃO et al., 2015; WOLF et al., 2015). In contrast, eye closure sensitivity is not observed with spontaneous or reflex blinking, suggesting an interaction between the supplementary motor area (SMA) and the visual system as the origin of this reflex epileptic trait (BAYKAN & WOLF, 2017; DA CONCEIÇÃO et al., 2015; WOLF et al., 2015). A functional MRI (fMRI) study by Vaudano et al. found an activation of the occipital cortex at eye closure followed by an activation of the SMA area 3 s later during eyelid myoclonia, supporting the hypothesis of the involvement of these two areas in ictogenesis (BAYKAN & WOLF, 2017; VAUDANO et al., 2014; WOLF et al., 2015).

2.2.3. Praxis induction

Praxis induction is defined as epileptic seizures that are precipitated by complex,

visuomotor tasks involving decision-making (BAYKAN & WOLF, 2017; WOLF et al., 2015; YACUBIAN, 2017). Myoclonic seizures elicited by praxis induction typically predominate in the active limb (BAYKAN & WOLF, 2017). This phenomenon is almost exclusively found in JME patients with a prevalence ranging from 30 to 50% (BAYKAN & WOLF, 2017). An fMRI study by Vollmar et al. revealed insights into the pathomechanism of this reflex epileptic trait (VOLLMAR et al., 2011). Study participants were confronted with a frontal lobe task using visuomotor coordination (VOLLMAR et al., 2011; WOLF et al., 2015). Performance did not differ between patients and controls (VOLLMAR et al., 2011; WOLF et al., 2015). However, JME patients showed an increased coactivation of the primary motor cortex and SMA as well as an increased functional connectivity between these areas and the frontoparietal cognitive networks and an impaired deactivation of the default mode network (VOLLMAR et al., 2011; WOLF et al., 2015). The authors concluded that these findings may explain how cognitive activity can provoke myoclonic jerks in JME patients (VOLLMAR et al., 2011; WOLF et al., 2015). Although praxis induction is only observed in a subgroup of JME patients, the aforementioned study suggests that the underlying pathomechanism is a feature of JME patients in general, and individuals with praxis induction exhibit only a higher degree of this mechanism (BAYKAN & WOLF, 2017; VOLLMAR et al., 2011). Moreover, it was proposed that patients with praxis induction may also represent a more severe subtype of JME because this reflex epileptic trait is a strong negative prognostic factor regarding response to AED treatment (BAYKAN & WOLF, 2017; GUARANHA et al., 2011; UCHIDA et al., 2015).

2.2.4. Orofacial reflex myocloni

Orofacial reflex myocloni are small, lightning-like myocloni in the perioral muscles, jaw, tongue, and throat, triggered by language-related activities such as reading and talking (BAYKAN & WOLF, 2017; SALEK-HADDADI et al., 2009; WOLF et al., 2015; YACUBIAN, 2017; YACUBIAN & WOLF, 2015). It is the hallmark of primary reading epilepsy, but also represents a reflex epileptic trait in 25 to 30% of JME patients (BAYKAN & WOLF, 2017; SALEK-HADDADI et al., 2009; WOLF et al., 2015; YACUBIAN & WOLF, 2015). It is attributable to hyperactivation of the network that is required for linguistic communication (BAYKAN & WOLF, 2017; YACUBIAN, 2017; YACUBIAN & WOLF, 2015).

The observation that emotional or difficult tasks increase provocation and myoclonic jerks occur with some delay suggests that a certain number of neurons must be activated within cortico-reticular and cortico-cortical circuitries to elicit a myoclonic response (SALEK-HADDADI et al., 2009; WOLF et al., 2015).

2.3. Electroencephalographic findings

Typical EEG changes can be detected in 75 to 85% of JME patients on routine EEG examination, although a prevalence of only 54% has been reported in one study (GENTON et al., 1995; SERAFINI et al., 2013). Prolonged recordings or activation procedures such as photic stimulation or sleep deprivation may assist in the identification of paroxysmal EEG discharges (ALFRADIQUE & VASCONCELOS, 2007; SERAFINI et al., 2013; WELTY, 2006). Since epileptiform activity follows a circadian distribution similar to myoclonic seizures, EEGs performed in the morning hours are more likely to be diagnostic compared to those performed in the afternoon (SERAFINI et al., 2013). Electroencephalographic recording during sleep may also be beneficial because epileptic discharges increase at sleep onset, during sleep arousals, especially when provoked, and during the transition phase (one hour prior and half an hour after awakening) (BAYKAN & WOLF, 2017; SERAFINI et al., 2013).

The EEG of patients with JME reveals a normal background rhythm (ALFRADIQUE & VASCONCELOS, 2007; BAYKAN & WOLF, 2017; SERAFINI et al., 2013). Characteristic interictal findings consist of 3-6 Hz generalized bilateral synchronous, but not necessarily symmetrical, PSWC with a frontocentral accentuation (ALFRADIQUE & VASCONCELOS, 2007; ANDERSON & HAMANDI, 2011; BAYKAN & WOLF, 2017; KOEPP et al., 2014; NICOLSON & MARSON, 2010; SERAFINI et al., 2013; WELTY, 2006). Other interictal findings include single spikes, generalized spike-wave discharges, and irregular SWC, or diffuse or intermittent slowing (ALFRADIQUE & VASCONCELOS, 2007; SERAFINI et al., 2013). Asymmetric EEG changes and focal discharges, such as focal spikes, sharp waves, slow waves, or focal onset of generalized seizures are observed in 6 to 48% and may erroneously lead to a diagnosis of a focal epilepsy syndrome (ANDERSON & HAMANDI, 2011; BAYKAN & WOLF, 2017; KOEPP et al., 2014; SERAFINI et al., 2013; WELTY, 2006). Asymmetric and focal epileptiform discharges may remain stable for years, but more commonly switch hemisphere, even during the same recording

(BAYKAN & WOLF, 2017; SERAFINI et al., 2013). Myoclonic jerks are associated with PSWC that consist of runs of 10-27 Hz spikes followed by 3-4 Hz slow waves with a frontocentral maximum (ALFRADIQUE & VASCONCELOS, 2007; KOEPP et al., 2014; SERAFINI et al., 2013; WANDSCHNEIDER et al., 2012; WOLF et al., 2015; YACUBIAN, 2017). The number of spikes in the PSWC ranges from five to 20 and is correlated with severity rather than duration of myoclonic seizures (ALFRADIQUE & VASCONCELOS, 2007; SERAFINI et al., 2013). Other ictal patterns include 3-5 Hz generalized, irregular SWC, slow sharp waves, and groups of slow waves, sharp waves, or spikes (ALFRADIQUE & VASCONCELOS, 2007; SOUSA et al., 2005). Absence seizures are characterized by 3-4 Hz SWC (PANAYIOTOPOULOS et al., 1989). This pattern may be similar to the one observed in CAE or may be preceded or superimposed by single/double/triple or multiple spikes (PANAYIOTOPOULOS et al., 1989; SERAFINI et al., 2013).

2.4. Treatment, neuropsychological aspects, and outcome

Juvenile myoclonic epilepsy has been considered a highly treatable disorder with seizure freedom being achieved in 80 to 96% of patients (ALFRADIQUE & VASCONCELOS, 2007; BAYKAN & WOLF, 2017; KOEPP et al., 2014). However, due to a high relapse rate of about 90%, JME has been considered a lifelong disorder requiring AED treatment throughout life (BAYKAN & WOLF, 2017; KOEPP et al., 2014; WELTY, 2006). Recent studies have cast doubt on both of these paradigms since long-term surveys demonstrated remission in only two thirds of patients and up to 30% were seizure free and off AEDs (BAYKAN & WOLF, 2017; BAYKAN et al., 2013; KOEPP et al., 2014; NICOLSON & MARSON, 2010; YACUBIAN, 2017). Treatment is based on appropriate AEDs, avoidance of precipitating factors, such as fatigue, sleep deprivation, provoked awakening, alcohol, and photic stimuli, and management of co-pathologies (ALFRADIQUE & VASCONCELOS, 2007; CRESPEL et al., 2013; WELTY, 2006; YACUBIAN, 2017). The first choice AED is valproic acid, which has an efficacy of up to 90% (ALFRADIQUE & VASCONCELOS, 2007; BAYKAN & WOLF, 2017; BAYKAN et al., 2013; WELTY, 2006; YACUBIAN, 2017). However, due to its high teratogenic potential, the use of valproic acid is discouraged in women of childbearing age (BAYKAN & WOLF, 2017; WELTY, 2006; YACUBIAN, 2017). Valuable alternatives include LEV, lamotrigine,

topiramate, and zonisamide (ALFRADIQUE & VASCONCELOS, 2007; CRESPEL et al., 2013; HASHEMIAGHDAM et al., 2015; NOACHTAR et al., 2008; WELTY, 2006). In contrast, some AEDs may aggravate myoclonic seizures, especially sodium channel blockers, such as phenytoin, oxcarbazepine, carbamazepine, and lamotrigine, but also gabapentin, pregabalin, tiagabine, and vigabatrin (ALFRADIQUE & VASCONCELOS, 2007; CRESPEL et al., 2013; KOEPP et al., 2014; YACUBIAN, 2017).

Early descriptions of JME depicted specific personality traits, such as emotional instability, forgetfulness, unreliability, poor planning, rapid and frequent mood changes, impulsivity, and indifference (ANDERSON & HAMANDI, 2011; BAYKAN & WOLF, 2017). Psychiatric disorders are encountered in 26.5 to 47% of JME patients, particularly anxiety, mood, and cluster B personality disorders (ALFRADIQUE & VASCONCELOS, 2007; BAYKAN & WOLF, 2017; DE ARAUJO FILHO & YACUBIAN, 2013; KOEPP et al., 2014). The risk for psychiatric comorbidities appears to be increased in patients with praxis induction or eye closure sensitivity/photosensitivity (BAYKAN & WOLF, 2017). Neuropsychological problems do not seem to reflect a genetic variation but rather a higher grade of frontal lobe impairment (BAYKAN & WOLF, 2017).

Despite good response to AED treatment, 74% of patients have at least one major unfavourable social outcome, including depression, failure to complete high school, unemployment, alcohol or substance abuse, divorce, unplanned pregnancy, abortion, social isolation, and even criminal records (BAYKAN et al., 2013; KOEPP et al., 2014; WOLF et al., 2015; YACUBIAN, 2017). In addition to other factors, circadian dysrhythmia may contribute markedly to poor social outcome (SCHMITZ et al., 2013; WOLF et al., 2015). Negative prognostic factors influencing outcome of JME comprise a combination of all three seizure types, more than one AED, non-classical JME such as CAE evolving to JME, psychiatric comorbidities, delay in diagnosis, focal EEG abnormalities, long disease duration, and expression of reflex epileptic traits (BAYKAN et al., 2013; KOEPP et al., 2014; YACUBIAN, 2017).

2.5. Genetics

Twin and family studies suggest that JME is a heritable IGE syndrome (PELJTO et al., 2014; VADLAMUDI et al., 2014; ZIFKIN et al., 2005). The risk of epilepsy is

increased in first-degree relatives of JME patients and a positive family history is encountered in 40% of affected individuals (KOEPP et al., 2014; WELTY, 2006). Despite this strong genetic background and heritability estimates of up to 70%, the underlying genetic variants are still unknown in over 90% of cases, emphasizing the hypothesis of JME as a spectrum disorder with extensive genetic heterogeneity (BAYKAN & WOLF, 2017; DELGADO-ESCUETA et al., 2013; VADLAMUDI et al., 2014; WOLF et al., 2015). In only a small subgroup (1-2%) of patients is the disease attributable to a single gene defect, whereas in the majority of cases, a complex oligo- or polygenic mode of inheritance is suspected (KOEPP et al., 2014; WOLF et al., 2015). To date, five Mendelian genes, specifically *calcium channel beta4 subunit* (*CACNB4*), *calcium channel sensor receptor* (*CASR*), *gamma-aminobutyric acid* (*GABA*) *receptor alpha one subunit* (*GABRA1*), *GABA receptor delta subunit* (*GABRD*), and *EF-hand domain containing one* (*EFHC1*), and three single nucleotide polymorphisms in three genes, namely *bromodomain-containing 2* (*BRD2*), *connexin 36* (*Cx-36*), and *malic enzyme 2* (*ME2*) have been identified (BAYKAN & WOLF, 2017; DELGADO-ESCUETA et al., 2013; WOLF et al., 2015). Moreover, copy number variants at 15q13.3, 15q11.2, and 16p13.11 have been associated with JME (HELBIG et al., 2013; KOEPP et al., 2014). The microdeletion at 15q13.3 contains *cholinergic receptor nicotinic alpha7 subunit* (*CHRNA7*), coding for the α7 subunit of the nicotinergic acetylcholine (ACh) receptor (nAChR), as prime candidate gene (HELBIG et al., 2009). This microdeletion implicates a 50-fold increased risk for IGE (WOLF et al., 2015). Interestingly, the same copy number variants are also associated with a broad spectrum of neuropsychiatric diseases, suggesting a major role of neurodevelopmental mechanisms in the pathogenesis of JME (BAYKAN & WOLF, 2017; HELBIG et al., 2013; WOLF et al., 2015).

2.6. Pathophysiology – insights from advanced neuroimaging

Although no structural abnormalities are found using standard MRI or computed tomography, advanced neuroimaging techniques have identified subtle functional and structural changes in JME patients (ALFRADIQUE & VASCONCELOS, 2007; ANDERSON & HAMANDI, 2011; BAYKAN & WOLF, 2017; KOEPP et al., 2013, 2014; WANDSCHNEIDER et al., 2012).

Quantitative and voxel-based MRI studies reveal increased grey matter volumes affecting primarily orbitofrontal and superior mesial areas close to the SMA,

increased frontal CSF, and reduced occipital grey matter concentrations in patients with photosensitivity (ANDERSON & HAMANDI, 2011; KOEPP et al., 2013, 2014; WANDSCHNEIDER et al., 2012; WOLF et al., 2015). Moreover, there is a decrease of grey matter volume of the thalamus that is correlated with age, disease duration, as well as personality traits (ANDERSON & HAMANDI, 2011; WOLF et al., 2015). Interestingly, these changes are evident within twelve months of disease onset (KOEPP et al., 2013, 2014; WANDSCHNEIDER et al., 2012). Therefore, it is assumed that seizures and neurocognitive abnormalities are a consequence of an altered thalamo-frontocortical circuitry (KOEPP et al., 2013, 2014; WANDSCHNEIDER et al., 2012).

The use of ^{18}FDG- and $H_2{}^{15}O$-PET has identified increased metabolic activity during generalized SWC in the thalamus (ANDERSON & HAMANDI, 2011; KOEPP et al., 2014). In a study utilizing a visual working memory task, JME patients performed worse than controls, and ^{18}FDG-PET showed glucose hypometabolism in the ventral premotor cortex, the caudate nucleus, and dorsolateral prefrontal cortex at resting state, suggesting frontal lobe dysfunction (ANDERSON & HAMANDI, 2011; KOEPP et al., 2013, 2014; WANDSCHNEIDER et al., 2012; WOLF et al., 2015). Increased ^{18}FDG uptake in the lateral orbital and medial temporal area presumably represents a compensatory process for frontal lobe impairment (KOEPP et al., 2013; WANDSCHNEIDER et al., 2012). Results of PET studies support the hypothesis of a dysfunctional thalamo-frontocortical network leading to seizures and decreased cognitive performance (WANDSCHNEIDER et al., 2012).

Combined EEG and fMRI studies have shown thalamic and mesial frontal activation several seconds before generalized spike wave activity, followed by deactivation of the association cortex including frontal, parietal, and cingulate areas, the so-called default mode network (ANDERSON & HAMANDI, 2011; KOEPP et al., 2014; WOLF et al., 2015). Another fMRI study that used a working memory task revealed a coactivation of the primary motor cortex and the SMA, reduced deactivation of the default mode network, and increased functional connectivity between the primary motor cortex, SMA, and frontoparietal cognitive networks (KOEPP et al., 2013, 2014; VOLLMAR et al., 2011; WANDSCHNEIDER et al., 2012; WOLF et al., 2015; YACUBIAN, 2017). These observations provide an explanation for the cognitive triggering of myoclonic

seizures (KOEPP et al., 2013; VOLLMAR et al., 2011; WANDSCHNEIDER et al., 2012).

Increased structural connectivity between the motor cortex, SMA, and prefrontal cortex can also be seen with diffusion tensor tractography (KOEPP et al., 2013; WANDSCHNEIDER et al., 2012). These findings are similar to those obtained by fMRI and strengthen the theory of an altered network enabling ictogenesis by cognitive effort (WANDSCHNEIDER et al., 2012).

Proton magnetic resonance spectroscopy (^{1}H-MRS) reveals reduced *N*-acetyl aspartate (NAA) concentrations in the prefrontal cortex and thalamus and in the occipital lobe in patients with photosensitivity (BAYKAN & WOLF, 2017; KOEPP et al., 2013, 2014; WANDSCHNEIDER et al., 2012; WOLF et al., 2015). *N*-acetyl aspartate is a neuron-specific metabolite and reduced levels are considered a marker of neuronal loss or dysfunction (ANDERSON & HAMANDI, 2011; KOEPP et al., 2014; WANDSCHNEIDER et al., 2012). Similar to progressive thalamic volume loss observed with voxel-based morphometry, ^{1}H-MRS thalamic changes are progressive as well (ANDERSON & HAMANDI, 2011; KOEPP et al., 2014). Decreased NAA concentrations in the thalamus and frontal lobe are negatively correlated with age and disease duration (ANDERSON & HAMANDI, 2011; KOEPP et al., 2013). Moreover, the degree of neuronal dysfunction is associated with epilepsy severity and more substantial personality disorders (ANDERSON & HAMANDI, 2011).

In summary, advanced neuroimaging studies show structural and functional thalamic and frontal lobe abnormalities as well as altered connectivity between the motor cortex, SMA, and prefrontal cortex (ANDERSON & HAMANDI, 2011; BAYKAN & WOLF, 2017; KOEPP et al., 2013, 2014; WANDSCHNEIDER et al., 2012). These changes are consistent with the frontal accentuation of EEG paroxysms and neuropsychological profiles and provide an explanation for the seizure-provoking mechanisms in JME (ANDERSON & HAMANDI, 2011; KOEPP et al., 2013). This thalamo-frontocortical network dysfunction challenges the dogma of JME as a generalized epilepsy syndrome, and reclassification to a frontal lobe variant of a multi-regional, thalamocortical network epilepsy has been proposed (ANDERSON & HAMANDI, 2011; KOEPP et al., 2013).

III. PUBLICATIONS

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

The following manuscript entitled “Generalized myoclonic epilepsy with photosensitivity in juvenile dogs caused by a defective DIRAS family GTPase 1” has been accepted for publication in the “Proceedings of the National Academy of Sciences of the United States of America” (PNAS) on January 25, 2017 (received for review September 7, 2016).

The publication comprises a shared first-authorship of Franziska Wieländer (LMU Munich, clinical and electroencephalographic part, pedigree analysis and collection of blood samples of cases and controls for genetic analyses) and Riika Sarviaho (University of Helsinki, genetic part) and a shared last authorship of Andrea Fischer (LMU Munich) and Hannes Lohi (University of Helsinki).

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

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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 GTPase1 (*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.

Key words: seizure, juvenile, canine, photosensitivity, Ras

Abstract

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.

Introduction

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 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 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. 2D), 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. 2A), including the *DIRAS1* gene (Fig. 2C). The critical region was further split into a 300-kb and an 890-kb region (Fig. 2B) 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 56.3Mb (Fig. 2B). 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. 2D), 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. 3A).

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 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. 3B), 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 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 points 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 bio-rhythmicity 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 movement 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 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.

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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Supporting Information

SI Materials and Methods

Study Cohorts. Twenty-four RR cases were identified. Four study sites (Veterinary Hospital Trier, Trier, Germany; Veterinary Practice Bathen-Noethen, Cologne, Germany; Department of Small Animal Medicine and Surgery, University of Veterinary Medicine, Hannover, Germany; Clinic of Small Animal Medicine, LMU Munich, Germany) participated in recruitment of the initial cohort. Additional cases were recruited via call for study participants on the homepage of LMU Munich and referral by diplomates of the European College of Veterinary Neurology. Inclusion criteria were clinical observation of myoclonic jerks on video recordings or observation at one of the study sites and completion of an extensive online questionnaire (semse.vetmed.uni-muenchen.de/umfragen/index.php?sid=%2018469&lang=de) or personal interview. Dogs with other types of muscle contractions (e.g., tremor, fasciculations) or GTCS or focal seizures as the only manifestation of epilepsy were excluded. Dogs without video documentation of myoclonic jerks with insufficiently completed questionnaires were denied study participation.

Altogether, 538 EDTA-blood and tissue samples were collected from privately owned RRs in Germany, Finland, and 11 other countries (Table S6). The German RR study cohort included blood samples of 19 affected RRs displaying the generalized myoclonic epilepsy phenotype, 11 unaffected relatives (dam and sire of two litters, four littermates, one additional dam and her sister, one cousin) and 18 controls (13 healthy RRs, 4 RRs with idiopathic GTCS, 2 RRs with other types of muscle twitches). Genotyping analysis included also a cohort of 965 epileptic dogs from 12 other breeds from Finland (Table S6). The samples were stored at −20 °C until genomic DNA was extracted using a semiautomated Chemagen extraction robot (PerkinElmer Chemagen Technologie) or the Nucleon Bacc2 kit (GE Healthcare). DNA concentration was determined either with the NanoDrop ND-1000 UV/Vis Spectrophotometer or Qubit 3.0 Fluorometer (Thermo Fisher Scientific). 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 “Institutional Review Board” of the Clinic of Small Animal Medicine (LMU Munich, Germany 28.05.13).

Neurodiagnostic Investigation. All dogs underwent a clinical and neurological examination by a veterinary neurologist. Laboratory investigations comprised complete blood cell count and serum biochemistry profile (alanine aminotransferase, alkaline phosphatase, total bilirubin, urea, creatinine, total protein, albumin, glucose, sodium, potassium, chloride, calcium, phosphate), fasted venous ammonia (14 cases), fasted and postprandial bile acids (7 cases), creatine kinase activity (8 cases), ionized calcium (4 cases), coagulation profile (PT, PTT, TT; 1 case), and thyroid panel (total T4, free T4, thyroid stimulating hormone; 7 cases). AED serum concentration measurements (IDEXX Laboratories) were accomplished in six dogs treated with phenobarbital and four dogs treated with potassium bromide. Neurometabolic screening comprised urinary organic acids, oligosaccharides, mucopolysaccharides, and urinary and serum amino acids in 10 cases and 1 related control RR in specialized laboratories (Children's Hospital Frankfurt, Germany; Biocontrol, Labor Ingelheim, Ingelheim, Germany; Comparative Biochemical Genetics Laboratory Veterinary Unit, University of California, San Diego, CA; Metabolic Genetics Screening Laboratory, School of Veterinary Medicine, University of Philadelphia, PA). Skin biopsy from one affected dog was immersed in 2.5% glutaraldehyde and was screened for ultrastructural evidence of metabolic disorders by electronmicroscopy (ZM906, Zeiss). Advanced imaging through MRI (LMU Munich: 1.5 T Magnetom Symphony Syngo MR Siemens; University of Leipzig: Philips MR Ingenia 3T with Omega HP Gradient; Philips Medical Systems; various other high- and low-field MR machines) or CT was undertaken in 12 and 7 dogs, respectively. CSF for analysis was obtained following sterile preparation by atlantooccipital puncture with the dogs under general anesthesia in 17 cases.

EEG. Awake ambulatory wireless video-EEG was conducted in 17 RR cases and 11 RR control dogs (10 healthy RRs and 1 RR with idiopathic epilepsy with GTCS) using a Trackit MK3 EEG/Polygraphy Recorder with video (Lifelines Neurodiagnostic Systems) and Persyst 12 software (Persyst Development Corporation and Micromed Morpheus Home LTM ambulatory EEG recorder (Micromed Neurodiagnostic)). Because the episodes in question emerged primarily when the dogs were relaxed, recordings were performed in a quiet environment where the dogs had the possibility to lie down in a comfortable position. EEG was recorded routinely using 15 subdermal stainless steel needle electrodes (F3, Fz, F4,

F7, F8, C3, Cz, C4, O1, Pz, O2, T1, T2, Ref, Neut; NatusEurope, product number 019-475800). Electrode placement was as described by Pellegrino and Sica (52) for mesencephalic dogs, but modified for our demands: we placed subdermal needles at the anterior canthus of the base of the ear for the temporal loci (T3, T4) and the reference more caudally between the medial canthi of the eyes both as proposed by James et al. (53). We did not use the frontopolar leads, but added two additional electrodes 1- to 1.5-cm dorsolateral of the lateral canthi of the eyes (F7, F8). After positioning, all electrodes were fixed in place with medical tape (Leukoplast). Subsequently, the recorder - as well as the remaining wires - were attached to a harness on the dog's back via cohesive bandages (Co-Flex). In one dog, seven subdermal electrodes were used, including two frontal (F3, F4), one central (V), and two occipital electrodes (O1, O2), as well as a reference at the dorsum of the nose (Ref) and a ground in the neck (Neut). Three cases and one control required sedation for electrode placement (0.015 mg/kg dexmedetomidine, reversal with 0.15 mg/kg atipamezole). In one case and one control, video-EEG recording was undertaken upon awakening from general anesthesia. Impedance was kept under 10 kΩ in most cases (up to 20 kΩ in rare cases). In six cases and four controls, an additional video-EEG with photic stimulation was conducted at the end of the EEG study (Micromed Morpheus Home LTM ambulatory EEG recorder, Micromed Neurodiagnostic). A lamp with circular reflector and a viewing distance of 30 cm was used. Stimulation was conducted for 10 s followed by a rest of 5 s per each flash frequency. The following flash frequencies (in Hz) were used in this order: 1–6–11– 18–7–12–16–4–25–10–17–9–14–3.

GWAS. Genotyping of 10 affected and 18 unaffected RRs from the German study cohort (Fig. S2) was performed using Illumina's Canine HD array (173 k). The genotype data were filtered with a SNP call rate of >95%, array call rate of >95%, minor allele frequency of >5% and by using a Hardy–Weinberg equilibrium of $P \leq 0.00005$. After frequency and genotyping pruning (with a window size of 50, a step of five SNPs, and an r^2 threshold of 0.5) no individual dogs were removed and 110,492 SNPs remained for analysis. A case-control association test (GWAS) was performed by PLINK (50) and by Ped-GWAS, using Mendel software (51). Two cases and four controls were excluded in PLINK analysis because of relatedness. After Ped-GWAS quality control analysis, 107,852 SNPs were included in the analysis.

Resequencing. Dog exome libraries for two German RR cases were generated from standard indexed Illumina libraries using a custom Roche/Nimblegen solution-based capture library (120705_CF3_Uppsala_Broad_EZ_HX1) according to the protocol described previously by Elvers et al. (54). The sequencing data were analyzed using our in-house scripts as previously described (55). The average sequencing coverage varied between 25–35× per sample and revealed on average ~108,909 variants per sample. The two affected dogs shared 30,506 homozygous variants; however, filtering under a recessive model against 169 additional exomes from other nonaffected breeds available (Table S3) resulted in a total of 10 case-specific homozygous variants (Table S4), of which 6 variants were in the predicted coding regions (1 indel, 5 synonymous). SnpEff (56) was used to predict the pathogenicity of the coding variants in the CanFam 3.1 annotation. One RR case was whole genome-sequenced in the Science for Life Laboratory in Stockholm, Sweden, as described previously (57). The RR whole genome (~25×) was filtered against 99 additional whole genomes from other nonaffected breeds available (Table S3). Variants in the 890-kb critical region at chromosome 20 were investigated, revealing the previously identified mutation in the case, although none of the other dogs carried it. The presence of the candidate mutation was inspected visually in the BAM file using the Integrative Genomics Viewer. Structural variation analysis in the associated region was performed using DELLY v0.6.7 to detect possible case-specific deletions or duplications in the WGS data of an epileptic RR against 99 nonepileptic control genomes including two RRs (Table S3). The detected sites were merged and regenotyped across all samples using DELLY v0.7.5. Genotyped copy number variants were required to have both split-read (SR) and paired-end (PE) support (SR > 1 and PE > 5 or SR > 5 and PE > 1) to retain a high-confidence call set.

Sanger Sequencing and TaqMan Genotyping. The identified candidate variant (chr20:g.56’474’668_56’474’671delAGAC) was first validated by a standard PCR followed by Sanger sequencing in 33 German samples, including 12 cases from the initial study cohort. Forward (ACAATGTCAAGGAGCTCTTCC) and reverse (GCTCACATGAGGACGCATT) primers were designed to amplify the mutation region using AmpliTaq Gold 360 Mastermix (Applied Biosystems, Life Technologies) or Biotools’ DNA Polymerase. The sequencing reactions were then performed on an ABI 3730 capillary sequencer (Applied Biosystems, Life

Technologies), after treatment with exonuclease I and shrimp alkaline phosphatase. The Sanger sequence data were analyzed using Sequencher 5.1 (GeneCodes). For a larger mutation screening in additional samples (Table S6), a TaqMan assay (Applied Biosystems) was developed (probes: GGAACATGAGCCTCAACATCGAT and GGACGCATTTGCCCTTGAC) and reactions were run by the Bio-Rad's CFX96 Touch Real-Time PCR Detection System instrumentation according to the manufacturer's instructions.

Sequence Alignment. The CanFam 3.1 assembly was used for all analyses. All numbering within the canine *DIRAS1* gene correspond to the accessions XM_005633100.2 and XP_005633157.1. For the annotation of the gene and the identified risk variant, we have used Broad Institute CanFam3 Improved Annotation Data v1. The sequence alignments of the normal and mutated DIRAS1 proteins were constructed by using the Clustal Omega tool (www.ebi.ac.uk/Tools/msa/clustalo/). The aligned protein sequences were derived from the Entrez Protein database (https://www.ncbi.nlm.nih.gov/protein). The domain structures were investigated by SMART (smart.embl-heidelberg.de/).

Gene Expression. Fresh postmortem samples were collected from seven dogs. Twenty-eight different tissues were collected from one Finnish epilepsy-free Belgian Shepherd dog (for the full list, see Fig. S4) killed at the age of 4 y and 10 mo. The frontal cortex was collected from five other epilepsy-free control dogs (one 2-mo-old Wire Fox Terrier, one 5-mo-old Labrador Retriever, two Great Danes aged 23 mo and 5 y and 3 mo, one 9-y-old Saluki) and one case RR. The 24-mo-old female RR case was killed because of severe seizures nonresponsive to treatment with phenobarbital and the control dogs because of nonepilepsy- related disorders or behavior by owners' consent. Samples were harvested immediately after killing and snap-frozen in liquid nitrogen before storage in −80 °C. Total RNA was extracted from the canine tissues by using the RNeasy Mini Kit (Qiagen), and sample concentrations were measured by using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific). The High Capacity RNA-to-cDNA Kit (Applied Biosystems, Life Technologies) was then used to reverse-transcribe equal amounts of total RNA into cDNA. In both semiquantitative and quantitative PCR, the canine *DIRAS1* transcript was amplified and sequenced by using a primer pair (TGAAGATGGACGTCATACTGCTT and GAACACCACCACTCGGTAGTC) that was designed to span over exon–intron boundaries to control for genomic DNA

contamination. Semiquantitative PCR was performed in three different cycles (27, 32, and 37 cycles) before visualization of the amplicons on a 2.5% (wt/vol) agarose gel (100 V, 2 h). *GAPDH* was used as a loading control. Quantitative PCR was performed for triplicates of each sample using Bio-Rad's CFX96 Touch Real- Time PCR Detection System instrumentation according to the manufacturer's instructions. *YWHAZ* and *GAPDH* were used as loading controls. The relative normalized expression pattern was calculated with Bio-Rad's CFX Manager Software. The SE between triplicates was below 0.13 in each studied sample.

Immunohistochemistry. Tissue studies were conducted on the brain of one affected RR (case 2) donated by the owner for postmortem examination. The animal underwent routine autopsy on 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. Identical sections were obtained and examined from three unaffected (two dogs without seizures and one with seizures secondary to acute hemorrhagic stroke) RRs admitted for diagnostic reasons unrelated to the purpose of this study. pAB were directed at DIRAS1 protein (rabbit polyclonal, PA5-26409, Fisher Scientific) and Vesicular Acetylcholine Transporter (VAChT, rabbit polyclonal, Ab68984, Abcam). Anti-DIRAS1 polyclonal antibody was developed against synthetic peptide between 109 and 136 amino acids from the central region of human DIRAS1. This epitope has 100% amino acid identity with the corresponding canine epitope. Therefore, sequence homology scale renders specific cross-reaction of the antibody very likely. Before staining, the slides underwent microwave-based antigen demasking in citrate buffer. Slides were incubated with pAB (DIRAS1 1:500; VAChT 1:100) for 18 h at 4 °C. Subsequent staining used polymer technology (IMPRESS Linaris) and a diaminobenzidine tetrahydrochloride staining kit.

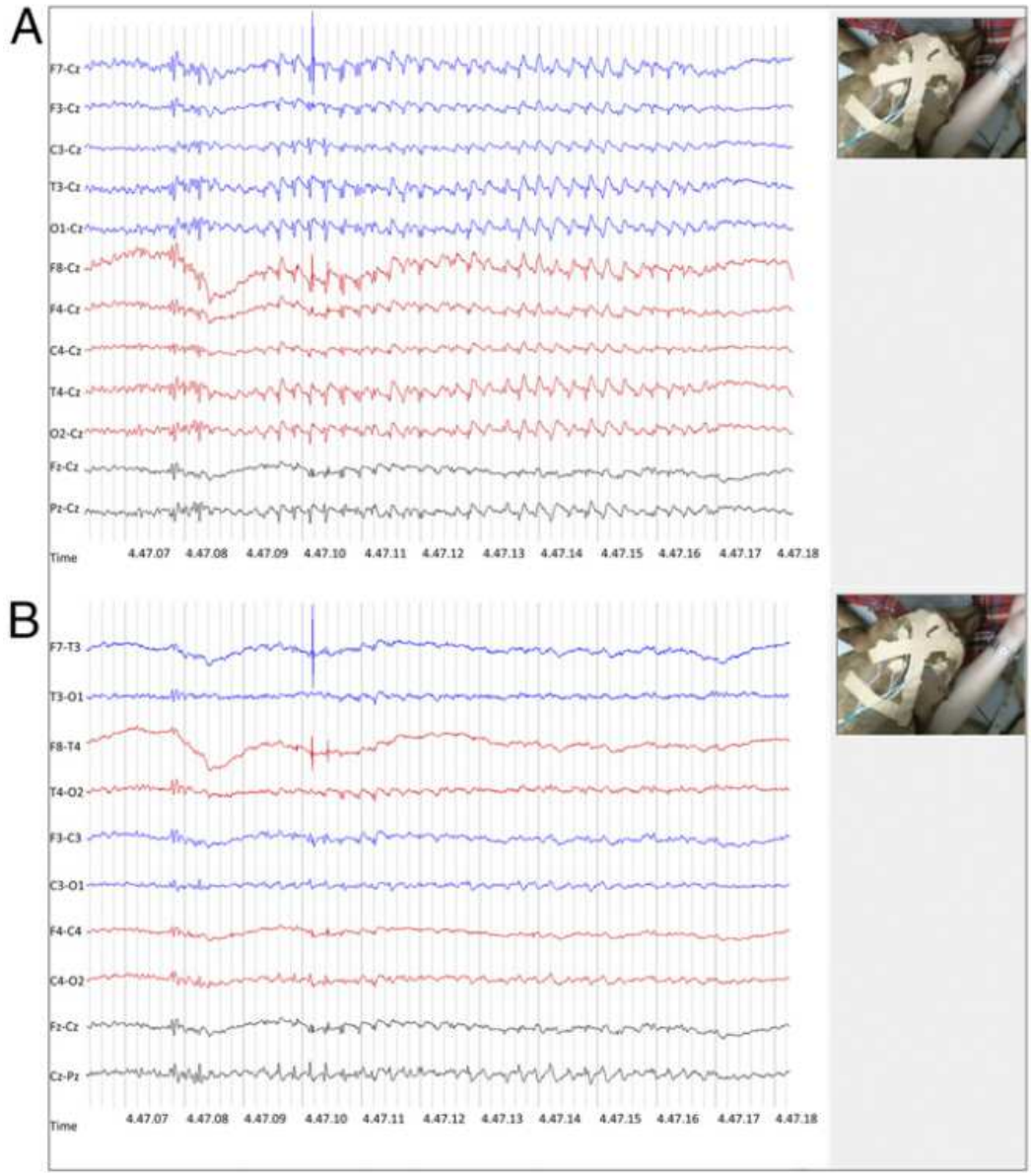

Fig. 1. Ictal EEG. LFF: 1 s; HFF: 70 Hz. (*A* and *B*) Minor head and eyelid twitches were accompanied by 4-Hz spike-and-wave complexes with a central maximum (*A*: Cz referential montage; *B*: bipolar montage).

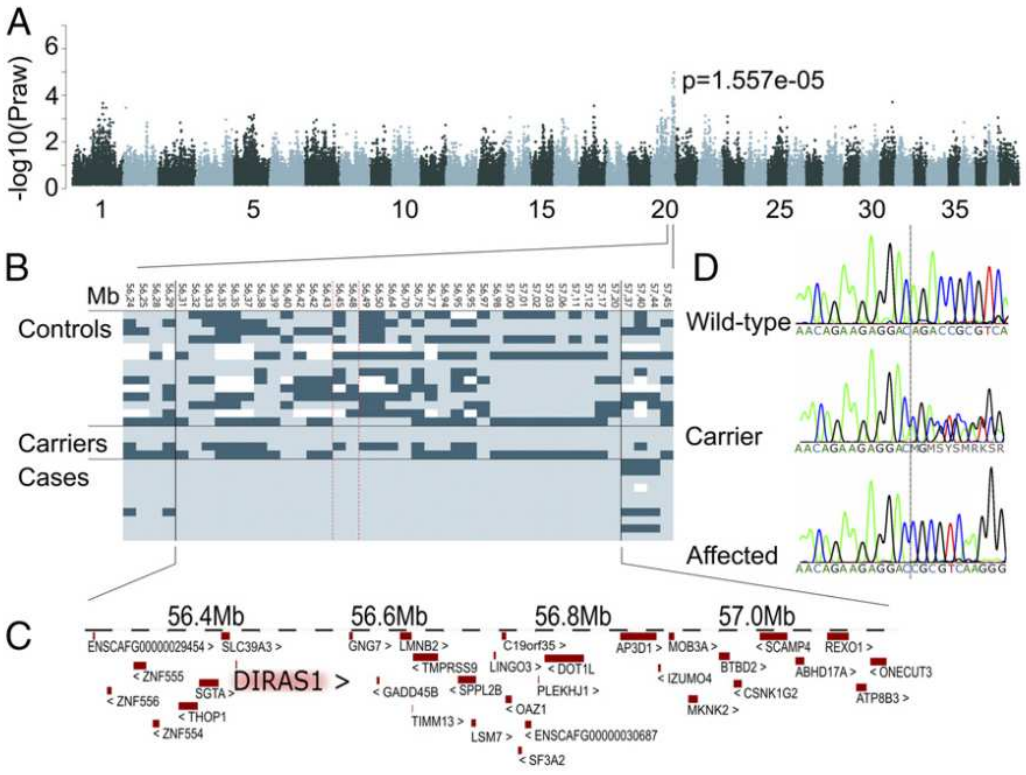

Fig. 2. GWAS. (*A*) Manhattan plot indicates best *P* values at chromosome 20. (*B*) An 890-kb haplotype is shared by cases. (*C*) The associated region contains 33 genes including *DIRAS1*. (*D*) Chromatograms of an affected, carrier, and wild-type dog indicate the c.564_567delAGAC variant.

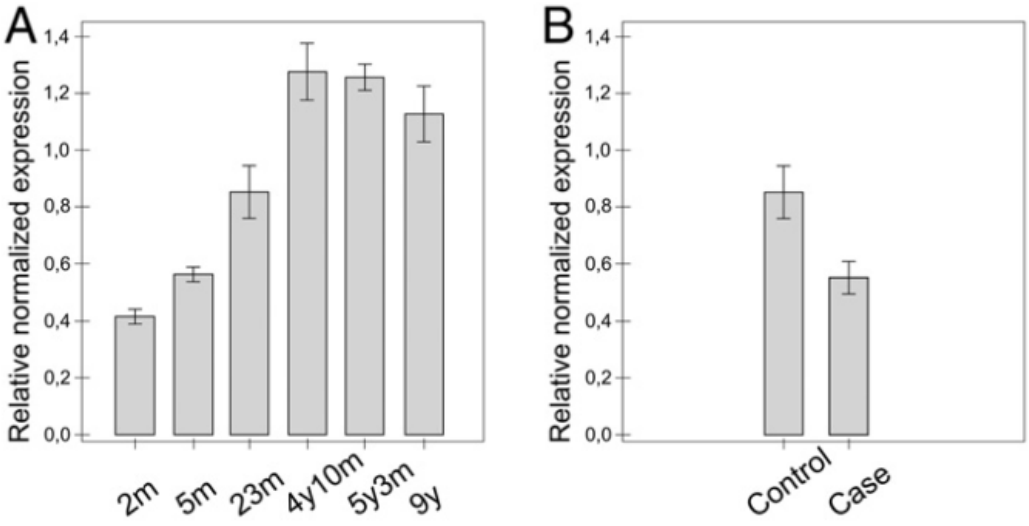

Fig. 3. *DIRAS1* expression. (*A*) The increase in the expression of the *DIRAS1* transcript by age was observed when comparing six different age points in the frontal cortex (the ages of 2 mo, 5 mo, 23 mo, 4 y and 10 mo, 5 y and 3 mo, and 9 y, $n = 1$ in each). (*B*) The stability of the *DIRAS1* transcript 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.

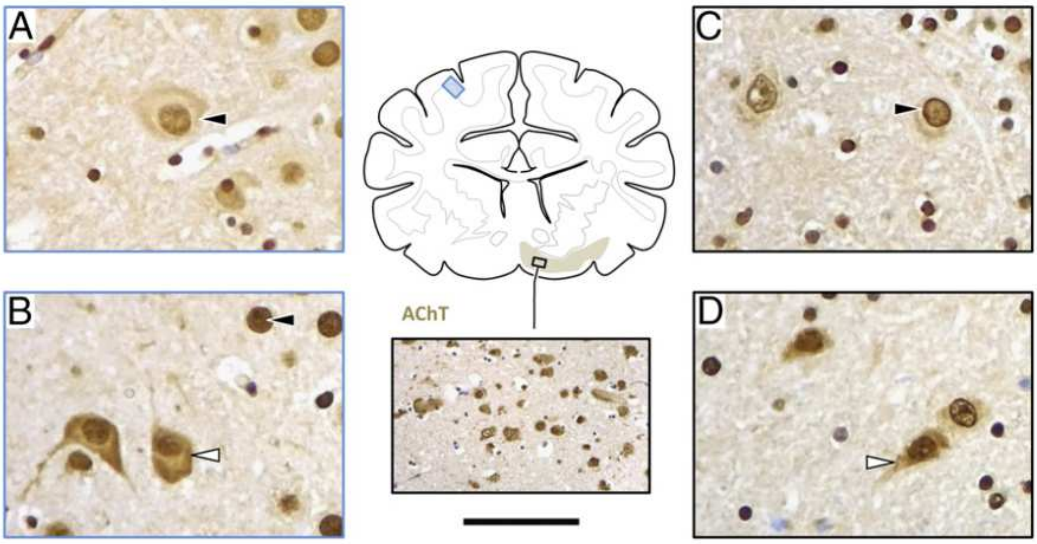

Fig. 4. Immunohistochemical *DIRAS1* expression. Wild-type RRs show predominantly nuclear staining (black arrowhead) as seen in the parietal cortex (*A*; blue frame) and cholinergic forebrain nuclei (*C*; black frame). With *DIRAS1* mutation (*B* and *D*) protein expression is abundant and there is a 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*. (Scalebar: *A–D*,35 µm; inlet AChT, 150 µm).

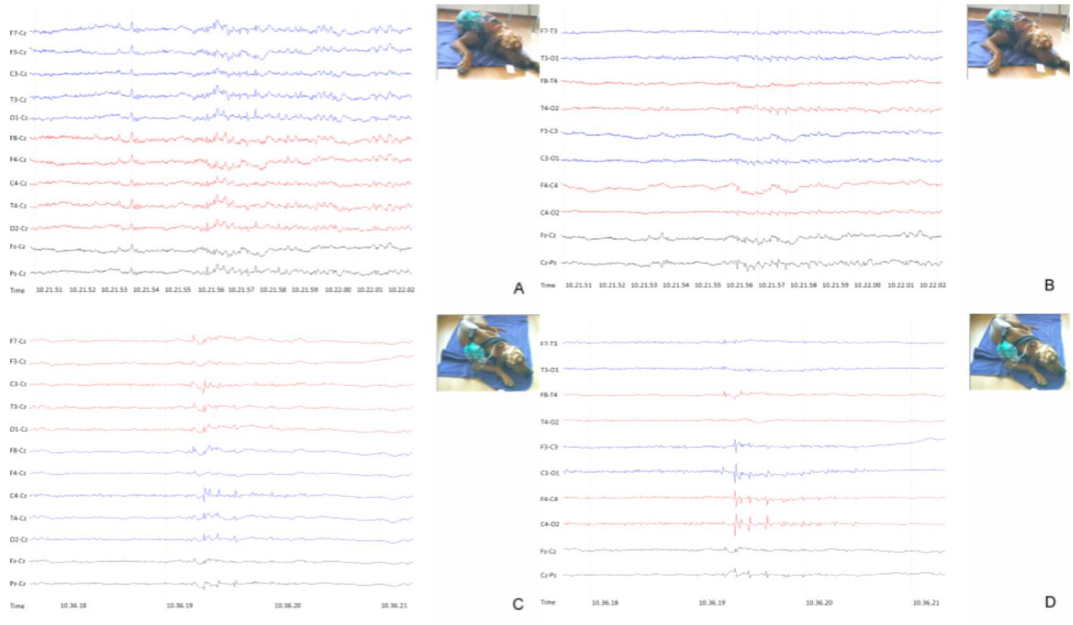

Fig. S1. Video-EEG with interictal SWC and ictal spikes. LFF: 1 s; HFF: 70 Hz. (*A* and *B*) These 4-Hz spike-and-wave complexes were not accompanied by any visible movements (*A*: Cz referential montage; *B*: bipolar montage). (*C* and *D*) Ictal spikes (*C*: Cz referential montage; *D*: bipolar montage).

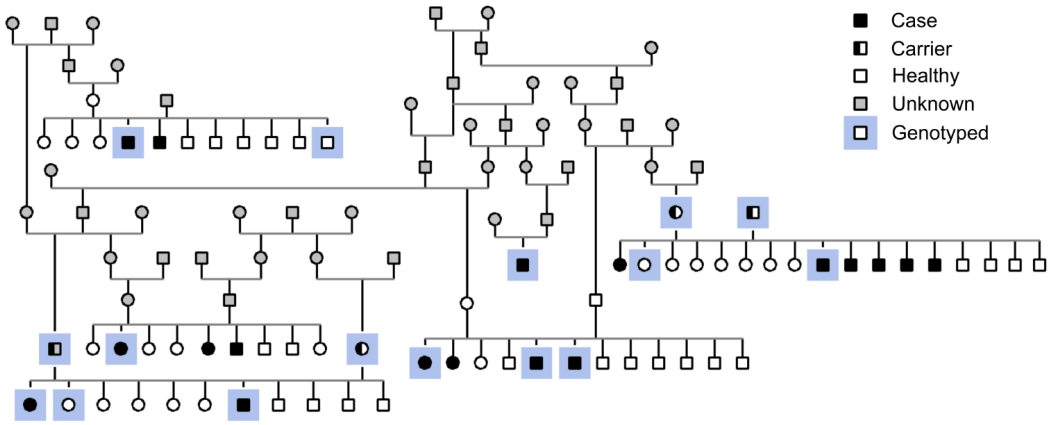

Fig. S2. Pedigree. An example of a RR pedigree with generalized myoclonic epilepsy, suggesting a recessive mode of inheritance.

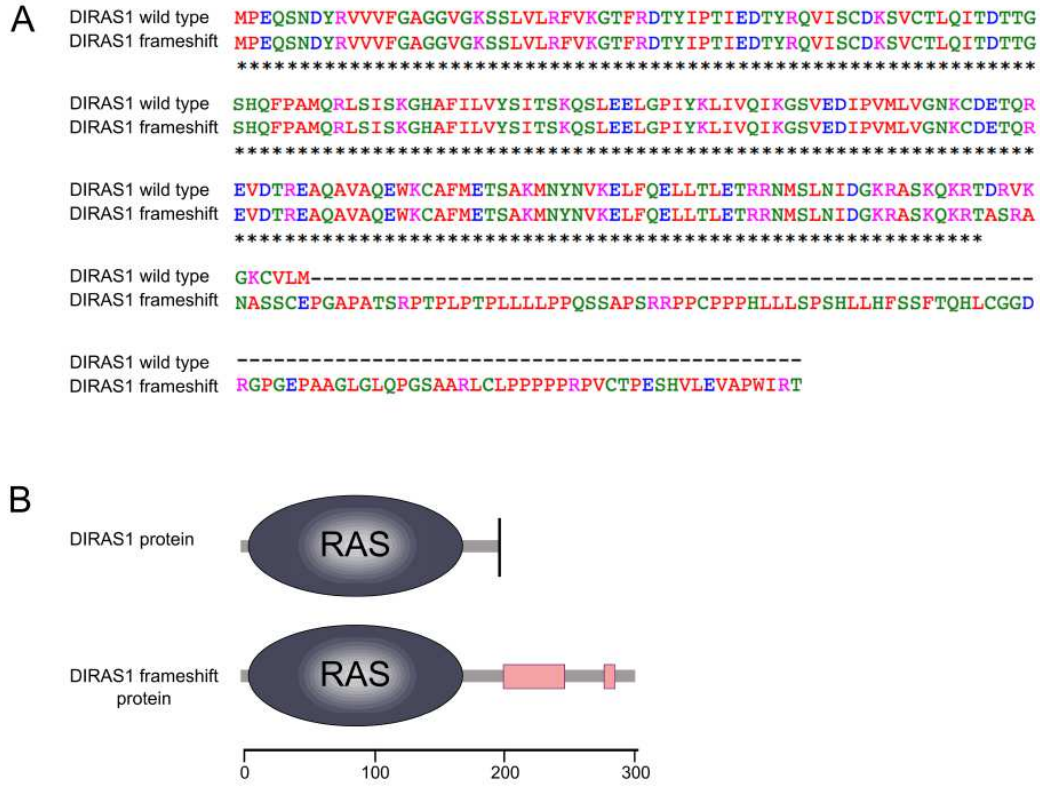

Fig. S3. Sequence comparison. (*A*) Sequence alignment of the normal and mutated DIRAS1 protein was constructed to assess the effect of the c.564_567delAGAC frameshift. The variant changes the last 10 amino acids of the normally 198 amino acids-long protein and produces a stop loss leading to a protein 104 amino acids longer than the wild-type. The Ras domain remains intact. (*B*) Schematic overview.

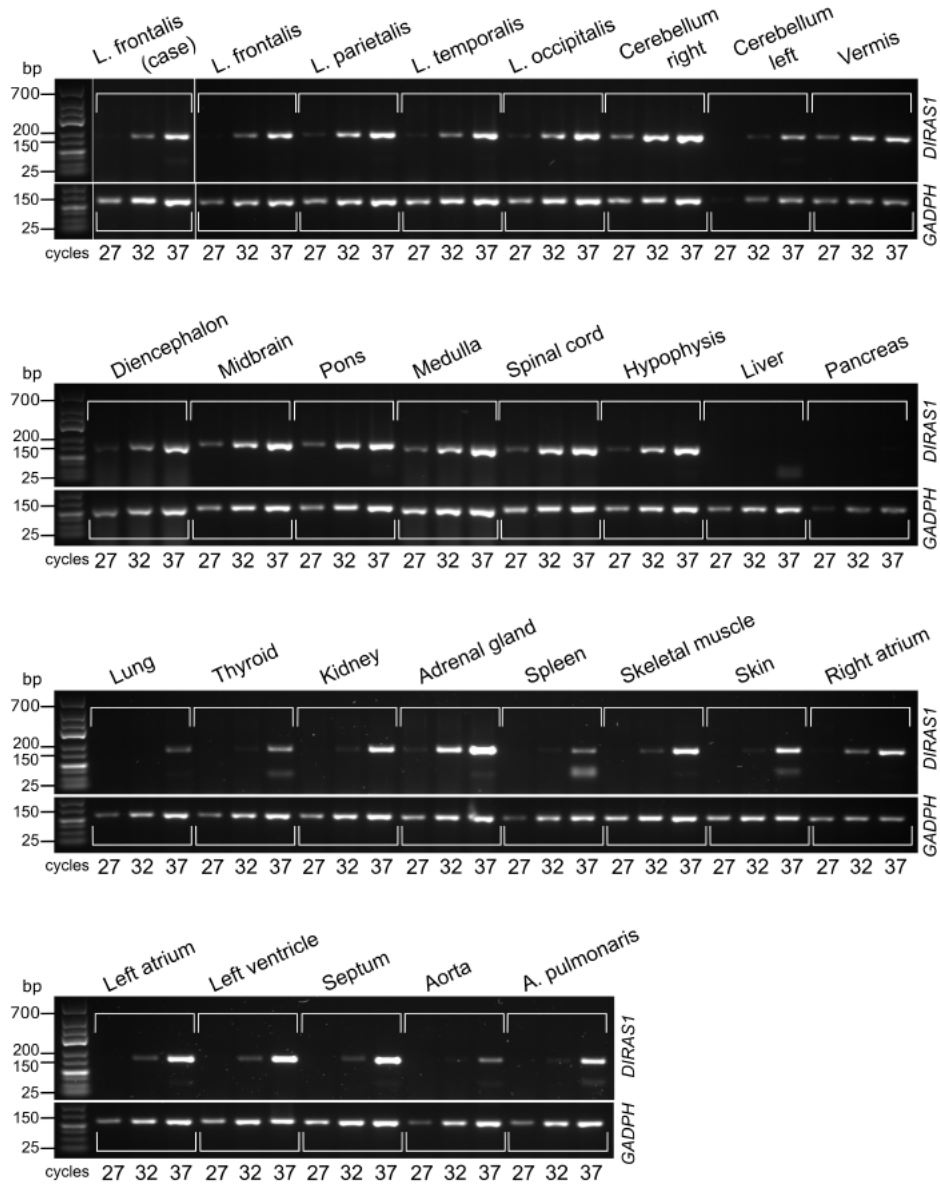

Fig. S4. *DIRAS1* expression pattern. Expression profiling of the *DIRAS1* transcript across 28 tissues indicates abundant pattern in the different regions of the brain but more limited pattern is found in nonneural tissues. The stability of the *DIRAS1* transcript is not markedly altered in the frontal lobes of a case (RR) and a control (Belgian Shepherd) dog by a semiquantitative PCR using *GADPH* as a loading control. A, arteria; L, lobus.

Table S1. Clinical protocols

RR dog	on site observation of myoclonic seizures	video documen-tation	completed questionnaire	pedigree available	neuro-imaging	CSF	neuro-metabolic screening[1]	EEG	EEG with ILS
case 1	yes	yes	no	no	MRI	yes	yes	yes	no
case 2	yes	yes	no	yes	MRI	yes	yes	yes[2]	no
case 3	yes	yes	no	no	CT	yes	yes	no	no
case 4	yes	yes	yes	no	CT	yes	yes	yes	no
case 5	yes	no	yes	no	CT, MRI	yes	no	no	no
case 6	yes	yes	yes	yes	-	yes	yes	yes	no
case 7	yes	no	no	no	MRI	yes	no	no	no
case 8	yes	no	no	yes	MRI	yes	yes	no	no
case 9	yes	yes	yes	yes	-	no	no	yes	no
case 10	yes	yes	yes	yes	-	no	no	yes	no
case 11	yes	yes	yes	yes	-	no	yes	yes	no
case 12	yes	yes	yes	yes	-	no	yes	yes	no
case 13	yes	yes	yes	yes	MRI	yes	yes	yes[3]	yes
case 14	no[4]	no[4]	yes	yes	CT	yes	no	no	no
case 15	yes	yes	yes	no	MRI	yes	no	yes[3]	no
case 16	yes	yes	yes	yes	CT	yes	no	no	no
case 17	yes	yes	yes	no	MRI	yes	no	yes[3]	no
case 18	yes	yes	yes	no	MRI	yes	yes	no	no
case 19	yes	yes	yes	yes	MRI	yes	no	yes	no
case 20	yes	yes	yes	yes	-	no	no	yes	yes
case 21	yes	yes	no	yes	-	no	no	yes	yes
case 22	no	yes	no	yes	-	no	no	yes	yes
case 23	yes	yes	yes	yes	CT, MRI	yes	no	yes	yes
case 24	yes	yes	yes	yes	CT, MRI	yes	no	yes	yes
control 1	-	-	-	no	-	-	no	yes	no
control 2	-	-	-	no	-	-	yes	yes[3]	no
control 3	-	-	-	no	-	-	no	yes	no
control 4	-	-	-	no	-	-	no	yes	no
control 5	-	-	-	no	-	-	no	yes	no
control 6	-	-	-	no	-	-	no	yes	no
control 7	-	-	-	no	-	-	no	yes	yes
control 8	-	-	-	yes	-	-	no	yes	yes
control 9	-	-	-	yes	-	-	no	yes	yes
control 10	-	-	-	yes	-	-	no	yes	yes
control 11	-	no	yes	yes	MRI	yes	no	yes[2]	no

Summary of the used diagnostic investigation protocols.

ILS = intermittent light stimulation. [1]neurometabolic screening: urinary amino acids and urinary organic acids; case 2, 6 and 13 also serum amino acids. [2]recording

upon awakening from general anesthesia (isoflurane, propofol). [3]sedation for electrode placement with 0.015 mg/kg dexmedetomidine, reversal with 0.15 mg/kg atipamezole. [4]this dog was included despite lack of onsite observation or video documentation of myoclonic twitches, as one sibling (dog 13) was affected as well and those two dogs were possessed by the same owner.

Table S2. Clinical and EEG data

RR	sex	geno-type	onset myoclonic seizures	onset GTCS	clinical photo-sensi-tivity	neurologic examination	brain imaging	CSF	neuro-metabolic screen	ictal EEG findings	interictal EEG findings	EEG with ILS	outcome (observation time)
case 1	f	dd	18 m	-	no	spinal pain	T1 and T2 hypointensity in the transition area of the frontal lobe and the olfactory bulb (MRI)	wnl	wnl	no myoclonic jerks during recording[i]	wnl	-	alive
case 2	f	dd	17 m	1 y 11 m	no	PL proprio-ceptive deficits, mild lumbosacral pain	wnl (MRI)	wnl[a]	serum alanine elevated	generalized 4-5 Hz SWC, at times epileptogenic discharges precede the myoclonic jerks	generalized 4-5 Hz SWC	-	dead (euthanised due to poor seizure control; nercropsy wnl)
case 3	m	-	7 m	-	-	wnl	wnl (CT)	wnl	results not available, reported wnl	-[g]	-	-	dead
case 4	m	dd	10 w	-	no	abraded claws of all limbs	asymmetric lateral ventricles[e] (CT)	wnl	wnl	generalized 4 Hz SWC with anterior maximum, at times preceded by 5 Hz slowing, at times polyspikes	generalized 4 Hz SWC with anterior maximum	-	alive

Table S2. Cont.

RR	sex	geno-type	onset myoclonic seizures	onset GTCS	clinical photo-sensi-tivity	neurologic exam-ination	brain imaging	CSF	neuro-metabolic screen	ictal EEG findings	interictal EEG findings	EEG with ILS	outcome (observation time)
case 5	m	-	3 m	1 y 5 m	no	inconsisten t menace OS	asymmetric lateral ventricles[e] (CT, MRI)	wnl	-	-[g]	-	-	dead (ana-plasmosis, AIHA, ARF)
case 6	m	dd	5 m	8 m	no	wnl	-	wnl	wnl	myoclonic jerks without clear EEG correlate[c]	wnl	-	dead (ruptured splenic tumour)
case 7	f	-	11 m	1 y 5 m	no	wnl	wnl (MRI)	wnl	-	-[g]	-	-	dead (foreign body, renal tumor)
case 8	f	-	10 w	-	yes	wnl	wnl (MRI)	wnl	wnl	-[g]	-	-	dead (euthanised due to poor seizure control)
case 9	f	dd	10 w	-	yes	wnl	-	-	-	right side dominant (C4, T4, F8, Cz, C3) 5-6 Hz biphasic spikes that approach a 4-5 Hz SWC morphology at points; events generalize, occasionally with a time lag, and often with independent spikes occurring at different channels	right side dominant spikes and 4-5 Hz SWC (C4, T4, F8, Cz, C3), at times spikes in C4/T4 and C3 do not generalize	-	alive

Table S2. Cont.

RR	sex	geno-type	onset myoclonic seizures	onset GTCS	clinical photo-sensi-tivity	neurologic exam-ination	brain imaging	CSF	neuro-metabolic screen	ictal EEG findings	interictal EEG findings	EEG with ILS	outcome (observation time)
case 10	m	dd	10 w	-	yes	wnl	-	-	-	single spikes or polyspikes	single spikes with anterior maximum	-	alive
case 11	f	dd	14 m	-	yes	wnl	-	-	wnl	4-5 Hz (occasionally 3 Hz) generalized SWC with anterior maximum, at times preceded by 4 Hz slowing, bursts of 7-8 Hz polyspikes	generalized 4 Hz SWC with anterior maximum	-	alive
case 12	m	dd	9 w	2 y 7 m	no	wnl	-	-	smear in urinary organic acids (qualitative analysis)	singlet or douplet SW	wnl	-	alive
case 13	m	dd	3 m	4.5 m	yes	wnl	lateral ventricle asymmetry[e] and mild meningeal contrast enhancement (MRI)	wnl	wnl	generalized 4-5 Hz SWC with anterior maximum, at times SW or slowing precede the myoclonic jerks	generalized 4 Hz SWC with anterior maximum	photoconvulsive responses with 4 Hz generalized SWC (stimulation frequencies 8-14 Hz)	alive

Table S2. Cont.

RR	sex	geno-type	onset myoclonic seizures	onset GTCS	clinical photo-sensi-tivity	neurologic exam-ination	brain imaging	CSF	neuro-metabolic screen	ictal EEG findings	interictal EEG findings	EEG with ILS	outcome (observation time)
case 14	m	-	6 m	-	no	wnl	wnl (CT)	wnl	-	-[g]	-	-	dead (euthanised due to poor seizure control)
case 15	m	dd	8 m	-	no	wnl	wnl (MRI)	wnl	-	generalized 4 Hz SWC or single spikes, central maximum, at times switching side, at times jerks are preceded by a crescendo of epileptogenic discharges, occasionally myoclonic jerks without clear EEG correlate[d]	generalized 4 Hz SWC or single spikes, central maximum, at times switching side	-	alive
case 16	m	dd	11 m	-	no	wnl	wnl (CT)	wnl	-	-[g]	-	-	alive
case 17	m	dd	6 m	-	no	wnl	wnl (MRI)	wnl	-	single spikes, occasionally myoclonic jerks without clear EEG correlate[d]	single spikes	-	alive
case 18	f	dd	6 m	8 m	no	wnl	slight reduction of brain volume (MRI)[f]	wnl	wnl[b]	-[h]	-	-	alive
case 19	m	dd	6 w	7 m	no	wnl	wnl (MRI)	wnl	-	irregular generalized 4 Hz SWC, generalized 4 Hz slowing with anterior maximum switching between different leads, at times emerging into SWC, occasionally myoclonic jerks without clear EEG correlate[d]	irregular generalized 4 Hz SWC with anterior maximum switching between different leads, generalized 4 Hz slowing, at times emerging into SWC	-	alive

Table S2. Cont.

RR	sex	geno-type	onset myoclonic seizures	onset GTCS	clinical photo-sensi-tivity	neurologic exam-ination	brain imaging	CSF	neuro-metabolic screen	ictal EEG findings	interictal EEG findings	EEG with ILS	outcome (observation time)
case 20	m	dd	8 w	8 m	yes	wnl	-	-	-	single spikes and SW	single spikes and SW	photoconvulsive responses with 4 Hz generalized SWC with occipital maximum (stimulation frequencies 6-17 Hz)	alive
case 21	f	dd	4 m	-	no	wnl	-	-	-	single spikes	wnl	no PPR	alive
case 22	f	dd	10 w	-	no	wnl	-	-	-	no myoclonic jerks during recording[i]	wnl	no PPR	alive
case 23	m	dd	10 w	-	yes	wnl	wnl (CT, MRI)	wnl	-	polyspikes and SW	wnl	photoconvulsive responses with 4 Hz generalized SWC with occipital maximum (stimulation frequencies 3-4 Hz)	alive
case 24	m	dd	8 w	-	yes	wnl	wnl (CT, MRI)	wnl	-	no myoclonic jerks during recording without activation procedures[i]	no epileptogenic discharges during recording without activation procedures	photoconvulsive responses with 4 Hz generalized SWC with occipital maximum (stimulation frequencies 6-10 Hz)	alive

Table S2. Cont.

RR	sex	genotype	onset myoclonic seizures	onset GTCS	clinical photo-sensitivity	neurologic exam-ination	brain imaging	CSF	neuro-metabolic screen	ictal EEG findings	interictal EEG findings	EEG with ILS	outcome (observation time)
control 1	f	DD	-	-	-	wnl	-	-	-	-	wnl	-	alive
control 2	m	Dd	-	-	-	wnl	-	-	wnl	-	wnl	-	alive
control 3	m	DD	-	-	-	wnl	-	-	-	-	wnl	-	alive
control 4	m	DD	-	-	-	wnl	-	-	-	-	wnl	-	alive
control 5	f	DD	-	-	-	wnl	-	-	-	-	wnl	-	alive
control 6	f	-	-	-	-	wnl	-	-	-	-	wnl	-	alive
control 7	m	-	-	-	-	wnl	-	-	-	-	wnl	no PPR	alive
control 8	f	Dd	-	-	-	wnl	-	-	-	-	wnl	no PPR	alive
control 9	f	Dd	-	-	-	wnl	-	-	-	-	wnl	no PPR	alive
control 10	f	Dd	-	-	-	wnl	-	-	-	-	wnl	no PPR	alive
control 11	m	DD	-	1 y 10 m	no	wnl	wnl (MRI)	wnl	-	-	wnl	-	alive

Table S2. Cont.

Summary of all clinical and EEG data of generalized myoclonic epilepsy in 24 RR dogs (cohort used for clinical characterization).

AIHA: autoimmune hemolytic anemia; ARF: acute renal failure; CT computed tomographic imaging; DD: wildtype genotype; Dd: heterozygous DIRAS1 mutation (carrier); dd: homozygous DIRAS1 mutation; GTCS: generalized tonic-clonic seizures; ILS: intermittent light stimulation; MRI: magnetic resonance imaging; OS: left eye; PPR: photoparoxysmal response; SW: spike-and-wave; SWC: spike-and-wave complexes; wnl: within normal limits. [a]mild increase in monocytes and banded neutrophils in cytospin preparations, normal total nucleated cell count, normal protein; [b]mild elevation in amino acids cystathionine and homocysteine; organic acids wnl; [c]EEG correlate was obscured by muscle artifact; [d]EEG correlate was occasionally obscured by muscle artifact; [e]may be clinically irrelevant as ventricle asymmetry is seen in 38% of healthy dogs and 44% of dogs with idiopathic epilepsy (15); [f]unremarkable on repeat 3T MRI; [g]unavailable for EEG; [h]non-diagnostic EEG (panting); [i]treated with AED.

Table S3. Breeds with NGS data

Breed	Whole exome	Whole genome
Akita	1	-
Alaskan Malamute	5	1
American Hairless Terrier	4	-
American Staffordshire Terrier	-	1
Australian Cattle dog	-	2
Australian Kelpie	11	-
Australian Terrier	13	-
Beagle	-	1
Bearded Collie	-	7
Belgian Shepherd	16	-
Berger Blanc Suisse	-	1
Bichon Frisé	4	-
Border Collie	8	25
Boston Terrier	1	-
Boxer	4	-
Central Asian Shepherd dog	-	1
Chihuahua	2	-
Coton de Tulear	2	-
Dachshund	-	1
Dalmatian dog	-	1
Dandie Dinmont Terrier	-	1
Doberman Pinscher	9	3
Elo	-	1
English Bulldog	1	-
Entlebucher Sennenhund	-	8
Eurasier	-	2
Finnish Hound	3	-
Finnish Lapphund	6	1
Finnish Spitz	6	-
Fox Terrier	4	-
French Bulldog	1	1
German Pointer	3	-
German Shepherd	1	1
German Wirehaired	-	1
Great Dane	16	-
Irish Soft-Coated Wheaten Terrier	4	1
Irish Terrier	-	1

Table S3. Cont.

Breed	Whole exome	Whole genome
Italian Greyhound	2	1
Karelian Beardog	6	1
King Charles Spaniel	2	-
Kromfohrländer	-	1
Kuvasz	3	-
Labrador Retriever	-	3
Lagotto Romagnolo	1	4
Lancashire heeler	1	-
Landseer	-	2
Leonberger	-	1
Malinois	-	2
Miniature Schnauzer	2	-
Mixed breed	-	1
Newfoundland Dog	4	-
Norwegian Lundehund	1	-
Parson Russel Terrier	1	-
Perro de Agua Español	-	1
Pinscher	1	-
Pomeranian	-	1
Pyrenean Shepherd	1	-
Rhodesian Ridgeback (cases)	2	1
Rhodesian Ridgeback	-	2
Rottweiler	2	2
Saluki	2	1
Samoyed	1	-
Schnauzer	5	-
Shetland Sheepdog	3	-
Siberian Husky (not purebred)	-	3
Sloughi	-	3
Swedish Vallhund	2	2
Welsh Springer Spaniel	-	2
West Highland White Terrier	-	1
Whippet	4	1
White Shepherd	-	1
Yorkshire Terrier	-	1
Total	171	100

A summary of the breeds with whole exome and genome data.

Table S4. Candidate mutations

Chr	Position (bp)	Reference	Alternative	Gene name	Function (Ensembl)	Exonic function (Ensembl)	Effect (snpEff)	Impact (snpEff)
16	1855004	G	A	Y_RNA	down-stream	.	inter-genic	modifier
X	41819512	G	A	WDR13	splicing	.	down-stream	modifier
16	2006387	G	A	CUL1	splicing	.	intron	modifier
3	85098858	C	CTTTAAATGTAACATTTTGTTTTTTTGTTTTTTTTTTT	SEPSECS	intronic	.	intron	modifier
20	56474664	GGACAGAC	GGAC	DIRAS1	exonic	frame-shift deletion	frame-shift	high
2	77117099	C	T	WNT4	exonic	synony-mous SNV	synony-mous coding	low
X	57124177	C	T	CDX4	exonic	synony-mous SNV	synony-mous coding	low
X	60408672	G	T	TAF9B	exonic	synony-mous SNV	synony-mous coding	low
16	2023357	G	A	CUL1	exonic	synony-mous SNV	synony-mous coding	low
X	55708156	C	T	ENSCAFG00000017103_11	exonic	synony-mous SNV	synony-mous coding	low

A summary of 10 case-specific variants identified in the whole exome sequencing analysis after filtering against 169 control exomes.

Table S5. GWAS hits

Chr	Predictor name	Position (bp)	*P* value	SNP model or adjusted *P* value
Ped-GWAS			Marginal *P* value	SNP model
20	BICF2G630446654	53461931	0.97687E-05	Add
20	BICF2P1269879	52411334	0.97687E-05	Add
20	TIGRP2P277323_RS	53534730	0.10225E-04	Add
5	BICF2S23234898	44292103	0.21872E-04	Add
5	BICF2P419745	44230649	0.21872E-04	Add
5	BICF2G630187408	44061256	0.21872E-04	Add
20	TIGRP2P278055_RS	55666391	0.37939E-04	Add
20	TIGRP2P278052_RS	55661675	0.37939E-04	Add
20	BICF2S23636929	55653830	0.37939E-04	Add
20	BICF2P1342156	55615324	0.45206E-04	Add
PLINK			Unadjusted *P* value	Adjusted *P* value
20	BICF2S23636929	55653830	1.557e-05	1.557e-05
20	TIGRP2P278052_RS8588937	55661675	1.557e-05	1.557e-05
20	TIGRP2P278055_RS8819835	55666391	1.557e-05	1.557e-05
20	BICF2G630445927	54625801	2.834e-05	2.834e-05
20	BICF2G630447057	52856293	2.834e-05	2.834e-05
20	BICF2G630445375	55637322	3.219e-05	3.219e-05
20	BICF2G630445416	55621611	3.219e-05	3.219e-05
20	BICF2P1342156	55615324	3.219e-05	3.219e-05
20	BICF2G630446654	53461931	4.32e-05	4.32e-05
20	BICF2P1269879	52411334	4.32e-05	4.32e-05

Top 10 SNPs identified by Ped-GWAS and PLINK in the GWAS.

Table S6. Genotyped dogs: Epidemiological investigation

Breed	Country	Wild-type	Carrier	Mutation homozygote	Total
Rhodesian Ridgeback	Czech Republic	31	3	-	34
Rhodesian Ridgeback	Denmark	5	-	-	5
Rhodesian Ridgeback	Finland	189	32	-	221
Rhodesian Ridgeback	Germany	46	15	18	79
Rhodesian Ridgeback	Hungary	2	1	-	3
Rhodesian Ridgeback	Latvia	6	2	-	8
Rhodesian Ridgeback	Poland	4	-	-	4
Rhodesian Ridgeback	Russia	24	11	-	35
Rhodesian Ridgeback	Slovakia	11	-	-	11
Rhodesian Ridgeback	South-Africa	3	-	-	3
Rhodesian Ridgeback	Spain	-	-	1	1
Rhodesian Ridgeback	Sweden	2	-	1	3
Rhodesian Ridgeback	Switzerland	111	19	-	130
Rhodesian Ridgeback	Unknown	1	-	-	1
Total		435	83	20	538
Percent		80,9	15,4	3,7	100
Percent (Germany excluded)		84,7	14,8	0,4	100
Australian Shepherd		45	-	-	45
Belgian Shepherd		175	-	-	175
Border Collie		88	-	-	88
Border Terrier		92	-	-	92
Finnish Spitz		158	-	-	158
Kromfohrländer		44	-	-	44
Lagotto Romagnolo		46	-	-	46
Miniature Pinscher		45	-	-	45
Pyrenean Shepherd		22	-	-	22
Saluki		78	-	-	78
Schipperke		120	-	-	120
Whippet		52	-	-	52
Total		965			965

A summary of the breeds and dogs genotyped for the *DIRAS1* mutation.

2. Absence seizures as a feature of juvenile myoclonic epilepsy in Rhodesian Ridgeback dogs

The following manuscript entitled “Absence seizures as a feature of juvenile myoclonic epilepsy in Rhodesian Ridgeback dogs” has been accepted for publication in the “Journal of Veterinary Internal Medicine” (JVIM) on October 31, 2017 (received for review August 17, 2017).

Absence seizures as a feature of juvenile myoclonic epilepsy in Rhodesian Ridgeback dogs

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Short title: Absence Seizures in a Rhodesian Ridgeback

Keywords: Canine; *DIRAS1*; Electroencephalography (EEG); Wireless video-EEG

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Data were collected at the Centre for Clinical Veterinary Medicine, LMU Munich, Munich, Germany, and the Department of Small Animal Medicine and Surgery, University of Veterinary Medicine, Hannover, Germany.

Part of this study was presented as an oral presentation, "Absence Seizures in a Rhodesian Ridgeback with Juvenile Myoclonic Epilepsy," at the 30th Annual Symposium of ESVN-ECVN in Helsinki, Finland.

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Footnote: [a]Micromed BQ Home LTM 2, Micromed s.p.A. Via Giotto 2, 31201 Mogliano Veneto (Treviso), Italy.

Abbreviations:

AED	antiepileptic drug
EEG	electroencephalography
JME	juvenile myoclonic epilepsy
MRI	magnetic resonance imaging
PSWC	polyspike-wave complexes
RR	Rhodesian Ridgeback
SWC	spike-and-wave complexes

Abstract

Myoclonic epilepsy in Rhodesian Ridgeback (RR) dogs is characterized by myoclonic seizures occurring mainly during relaxation periods, a juvenile age of onset and generalized tonic-clonic seizures in one-third of patients. An 8-month-old female intact RR was presented for myoclonic seizures and staring episodes that both started at 10 weeks of age. Testing for the *DIRAS1* variant indicated a homozygous mutant genotype. Unsedated wireless video-electroencephalography (EEG) identified frequent, bilaterally synchronous, generalized 4 Hz spike-and-wave complexes (SWC) during the staring episodes in addition to the characteristic myoclonic seizures with generalized 4–5 Hz SWC or 4–5 Hz slowing. Photic stimulation did not evoke a photoparoxysmal response. Repeat video-EEG 2 months after initiation of levetiracetam treatment disclosed a >95% decrease in frequency of myoclonic seizures, and absence seizures were no longer evident. Absence seizures represent another seizure type in juvenile myoclonic epilepsy (JME) in RR dogs, which reinforces its parallels to JME in humans.

A novel genetic myoclonic epilepsy in juvenile Rhodesian Ridgeback (RR) dogs, characterized by vigorous myoclonic seizures that occur mainly during relaxation periods, recently has been described.[1] More than one-third of affected dogs develop generalized tonic-clonic seizures in the course of the disease, and 35% are reported to be photosensitive.[1] The mean age of onset is 6 months (range, 6 weeks–1.5 years).[1] Wireless video-electroencephalography (EEG) in unsedated dogs was used as a tool to investigate the spontaneous and recurrent epileptic nature and to characterize the EEG features of the electroclinical syndrome.[1] Ambulatory video-EEG confirmed the epileptic origin of the myoclonic twitches.[1] Typically, affected dogs show generalized 4–5 Hz spike-and-wave complexes (SWC) and polyspike-wave complexes (PSWC) with a fronto-central maximum.[1] Genetic analyses identified a fully penetrant autosomal recessive 4-bp truncating deletion mutation in the *DIRAS1* gene, which is suggested to play a role in ACh release.[1,2]

Myoclonic epilepsy in RRs has important parallels to juvenile myoclonic epilepsy (JME) in humans, including juvenile onset, myoclonic seizures as the predominant seizure type with propagation to generalized tonic-clonic seizures, similar EEG characteristics and photosensitivity.[3-6] In humans with JME however, apart from myoclonic seizures and generalized tonic-clonic seizures (80–95% of patients), a third seizure type, namely absence seizures (approximately 30% of patients), is reported.[7,8] In the following case report, we describe the occurrence of absence seizures in a RR dog diagnosed with JME, completing the triad of seizure types observed in humans with JME.

An 8-month-old female intact RR was presented for multiple episodes of unresponsiveness, staring into space without any visible purposeful movement. Additionally, the dog experienced myoclonic jerks that occurred mainly at rest. Age of onset of both entities was 10 weeks with myoclonic seizures being observed a few days earlier than the staring episodes. At the beginning, myoclonic jerks manifested as nodding movements of the head, but became more vigorous in the course of the disease. At time of presentation, myoclonic seizures were said to resemble an electric shock such that the dog would jump into the air. Photosensitivity was not reported. Prior treatment with imepitoin (12.6 mg/kg PO q12h) decreased the intensity but not the frequency of the myoclonic seizures and did not influence the frequent staring episodes. According to the owner, the dog's

behavior was normal between episodes, and the dog did not show learning difficulties or any developmental delay.

Results of physical and neurologic examinations, as well as CBC, serum biochemistry profile, plasma ammonia concentrations, and abdominal ultrasound examination were unremarkable. Testing for the *DIRAS1* variant identified the homozygous mutant genotype (c.564_567delAGAC). Unsedated wireless video-EEG with synchronized video recording was performed using an ambulatory EEG recorder[a] in a quiet environment in which the dog was encouraged to lie down, because the episodes in question were reportedly more likely to occur at rest. Fifteen subdermal stainless-steel needle electrodes were used (F3, Fz, F4, F7, F8, C3, Cz, C4, O1, Pz, O2, T3, T4, Ref, Neut). Electrodes were placed as described previously and could be placed without any sedation.[1] Impedance was kept <10 kOhm. Time of recording was approximately 2 hours. At the end of the EEG study, photic stimulation was performed, utilizing a lamp with circular reflector and a viewing distance of 30 cm. The following flash frequencies (in Hz) were used in this order: 1 – 6 – 11 – 18 – 7 – 12 – 16 – 4 – 25 – 10 – 17 – 9 – 14 – 3, employing a period of 10 seconds of stimulation followed by a rest of 5 seconds per flash frequency.

Frequent episodes occurred, where the dog stared into the space and did not respond to any stimulus presented by us. Video-EEG confirmed the occurrence of generalized 4 Hz SWC associated with the staring events (Fig 1). During these episodes, the dog occasionally would lower the neck and appear as if the dog were about to buckle (Video S1; 14:12:30 hours). The dog's behavior was normal before (Video S2; 13:54:58 hours) and after the staring events (Video S3; 14:13:11 hours). The dog also showed several vigorous myoclonic seizures (213 jerks in the first hour of recording) that were characterized by twitches of the face, cervical, and proximal limb musculature or the trunk, accompanied by generalized 4–5 Hz SWC with a central maximum (Fig 2, Video S4; 14:13:52 hours) or 4–5 Hz slowing, and sporadic single spikes. During photic stimulation, some myoclonic seizures occurred, but seizure frequency did not change and the myoclonic seizures were not associated in time with the photic stimuli. Therefore, the dog was not classified as photosensitive. A diagnosis of JME in RRs with occurrence of myoclonic seizures and absence seizures was made.

A 20-minute wireless video-EEG with subsequent photic stimulation was performed on 5 healthy relatives with known genotype (mother [heterozygous], 2 sisters [both wild type], 2 brothers [1 wild type, 1 heterozygous]), and 1 affected littermate (male, homozygous for the DIRAS1 variant, 1 hour of recording). Healthy controls had normal EEG and no photosensitivity. The affected brother had myoclonic twitches that started at 9 weeks of age. Generalized tonic-clonic seizures or absence seizures were not observed by the owner. The EEG demonstrated myoclonic seizures associated with polyspikes or PSWC, no absence seizures, and no photoparoxysmal response upon photic stimulation.

Treatment with levetiracetam (24 mg/kg PO q8h) was initiated, and imepitoin was tapered. Owners were asked to keep a seizure diary. To ensure punctual drug administration, the owners used an automated dispensing machine that was filled with the pills embedded in treats. Owners reported a substantial decrease in seizure frequency and intensity, from multiple violent myoclonic jerks per day to 1 mild myoclonic twitch per week and a complete cessation of absence seizures.

Two months after treatment onset with levetiracetam, a follow-up EEG was performed. One-hour unsedated video-EEG disclosed only 6 mild and hardly visible myoclonic seizures (97.2% decrease in seizure frequency compared to the initial recording), and absence seizures were no longer recorded.

Discussion

In human medicine, JME is a common type of idiopathic generalized epilepsy, accounting for 4.1% of all epilepsies and 26.7% of idiopathic generalized epilepsies.[4] Juvenile myoclonic epilepsy usually begins between 12 and 18 years of age, with a mean age of onset of approximately 14 years.[9] Myoclonic jerks usually occur after awakening, are bilateral, arrhythmic, and predominate on the upper limbs, making the patients drop or throw objects.[3] Most patients with JME continue to develop rare generalized tonic-clonic seizures, which often are preceded by a cluster of myoclonic jerks, and approximately 30% experience absence seizures.[7,8] Photosensitivity is seen in another 30% of patients.[10] Electroencephalography identifies generalized and irregular SWC and PSWC with a fronto-central accentuation.[4,11] Complete seizure control is achieved in the majority of patients, but relapse rate after antiepileptic drug (AED) withdrawal may be up to 91%.[12,13] Despite a strong genetic background with positive family history in at least 40% of patients and several genetic loci associated with this syndrome, no unique pathogenic mechanism has yet been identified.[9,14]

Juvenile myoclonic epilepsy in humans and dogs shares a wide range of similar characteristics such as myoclonic seizures, generalized tonic-clonic seizures, ictal and interictal EEG features, and photosensitivity. The disease occurs in juvenile patients in both species, but onset may be much earlier in a substantial portion of dogs, with some being as young as 6 weeks of age.[1] In the current case report, we describe the manifestation of absence seizures in a RR dog suffering from JME, highlighting another important parallel to JME in humans.

The International League Against Epilepsy (ILAE) defines absence seizures as "sudden cessation of activity with a brief pause and staring, sometimes with eye fluttering and head nodding or other automatic behaviors" with an obligatory EEG pattern of generalized spike waves.[15] This definition holds true for the behavior and EEG pattern observed in the dog described here.

Several theories have been advanced to explain the pathophysiology of absence seizures.[16] The initial hypothesis suggests that generalized SWC are created by a pacemaker in the brainstem and diencephalon (centrencephalic theory), whereas the "thalamic clock theory" assumes a pacemaker in the reticular thalamic nucleus.[16,17]

The “corticoreticular theory”, proposing a thalamocortical network for the generation of generalized seizures and SWC, has attracted much attention.[16,17] Although this concept postulates that the SWC seen in absence seizures are a consequence of the transformation of sleep spindles in the hyperexcitable cortex, another study concluded that, although both spike-wave discharges and sleep spindles arise from the cortico-thalamo-cortical network, the initiation site of the activity is different with sleep spindles emerging from the thalamus and spike-wave discharges from the cortex (cortical focus theory).[16,18,19]

To our knowledge, only 1 other case report describes absence seizures with myoclonic features in a dog.[20] An 8-month-old Chihuahua was presented for staring episodes associated with head and nose twitching.[20] Diagnostic evaluation (neurologic examination and blood evaluation, magnetic resonance imaging [MRI]) was unremarkable.[20] Long-term video-EEG identified generalized bilaterally synchronous 4 Hz SWC time-locked to the clinical events.[20] However, the prevalence of absence seizures in dogs may be highly underestimated. In 1 study, owners perceived a generalized tonic-clonic seizure to be more harmful than a focal seizure and were less likely to report a focal seizure to their veterinarian.[21] Similarly, owners and veterinarians might underestimate the impact of absence seizures or not even identify those episodes as being epileptic. Some patients with absence seizures may go on to develop generalized tonic-clonic seizures. Therefore, the clinician should specifically ask about the occurrence of absence seizures and educate the owner about the semiology of this seizure type. Moreover, a definitive diagnosis of absence seizures requires confirmation by EEG. In the majority of studies in which EEG was used as a diagnostic tool, dogs were sedated or even anesthetized.[22-27] This technique makes the diagnosis of absence seizures challenging, because level of alertness or consciousness, a key factor for the diagnosis of absence seizures, cannot be assessed. Consequently, EEG should be performed in unsedated dogs to enable the diagnosis of absence seizures.[28]

The dog in our study exhibited typical absence seizures characterized by impaired consciousness in association with generalized SWC with a frequency of 4 Hz. In human medicine, absence seizures are considered as transient impairment of consciousness time-locked to generalized 3 Hz spike-wave discharges.[29] In the current understanding, however, typical absence seizures that occur in association with idiopathic generalized epilepsies are characterized by generalized spike- or

polyspike-wave discharges with a frequency of >2.5 Hz (mainly 3-4.5 Hz) similar to those observed in the dog of our study.[19,30] In contrast, atypical absence seizures are observed in human patients in the context of cognitive-related conditions and symptomatic epilepsies (eg, Lennox-Gastaut syndrome) and are characterized by lower frequencies.[19,30] Furthermore, the frequency of spike-wave discharges in absence seizures varies with different types of epilepsy syndromes in humans.[29] In JME, absence seizures with 3–4 Hz spike-wave discharges are described similar to the 4 Hz SWC in the dog described here and the Chihuahua with absence seizures with myoclonic features.[20,29]

Short elimination half-life time (90-120 minutes) prevents the use of valproic acid in dogs, which is the first choice AED in humans with JME, reaching effectiveness of 85-90%.[9] Therefore, other AEDs with an appropriate pharmacokinetic profile are used in dogs. Levetiracetam is another effective drug to treat JME in humans and is superior to phenobarbital for the treatment of myoclonic seizures in cats with feline audiogenic reflex seizures.[31,32] For the RR in this report, treatment with imepitoin resulted only in mild reduction in intensity of myoclonic seizures, but frequency of myoclonic and absence seizures did not change. In contrast, much improvement of intensity and frequency of myoclonic seizures and complete cessation of absence seizures were achieved by levetiracetam treatment. Therefore, levetiracetam may be considered for the treatment of myoclonic seizures as well as absence seizures in veterinary medicine. However, more studies are warranted to confirm our observations.

Our study had several limitations. Because there is currently only 1 case with proven absence seizures in a RR with JME, occurrence of this seizure type could just be a coincidental finding. However, humans with idiopathic generalized epilepsies can present with various generalized seizure types such as generalized tonic-clonic, tonic, clonic, atonic, myoclonic, or absence seizures.[33] Therefore, absence seizures indeed can be a feature of JME in RRs. Moreover, several owners of RRs with JME that were presented to our clinics reported that their dogs were experiencing staring episodes. Although confirmation by video-EEG was not attempted in those dogs, these observations suggest that absence seizures might be a more common seizure type in RRs with JME than expected. In the present case, absence seizures were confirmed by EEG at the age of 8 months, whereas for the controls (1 affected and 4 healthy littermates), EEG was performed at the age of 10

months. Therefore, absence seizures could have been an age-related phenomenon that vanished over time. However, in contrast to the current case, owners of controls never observed staring episodes in their dogs. Brain imaging was not performed in the RR of this case report. However, the neurologic examination was normal and the dog tested positive for the DIRAS1 variant. In the previous study, RRs with JME had normal MRI findings.[1] Furthermore, the RR of this report had an EEG pattern of generalized epileptic discharges and no focal discharges. Taking these points together, we considered structural brain disease very unlikely.

The current case report describes the occurrence of absence seizures in a RR diagnosed with JME. Our findings expand the spectrum of JME to include a third seizure type and highlight the potential to use this dog breed as a large animal translational model for the investigation of pathophysiologic, therapeutic, and genetic aspects of JME in humans. Furthermore, the usefulness of unsedated wireless video-EEG for the diagnosis of seizure types and electroclinical syndromes with staring episodes is emphasized.

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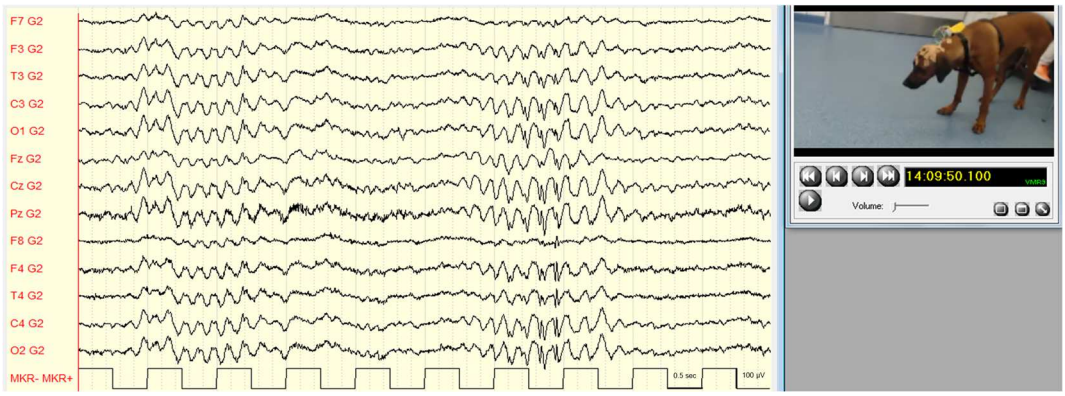

Fig 1. Absence seizure with generalized 4 Hz spike-and-wave complexes. Referential montage (G2=Ref). Low pass filter: 70 Hz; high pass filter: 0.53 Hz; gain: 150µV/cm.

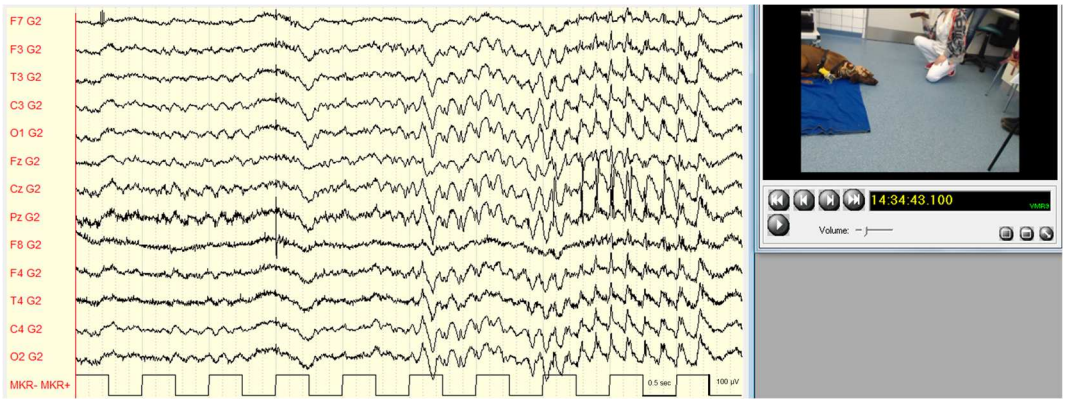

Fig 2. Myoclonic seizure with generalized 4–5 Hz spike-and-wave complexes. Referential montage (G2=Ref). Low pass filter: 70 Hz; high pass filter: 0.53 Hz; gain: 150µV/cm.

IV. DISCUSSION

The present study provides a detailed clinical and electroencephalographic description of a novel genetic epilepsy in dogs. The epilepsy was classified as a generalized idiopathic epilepsy, and a novel epilepsy gene *DIRAS1* was discovered based on the clinical investigations described here. Wireless video-EEG in unsedated dogs was established as a novel diagnostic tool to prove the epileptic origin of involuntary movements and behavioural paroxysms. This method also allowed characterization of canine epilepsies at a level that enabled comparison to human epilepsy syndromes. The main goals achieved in this study were (1) the diagnosis and description of new seizure types previously underreported in dogs, namely myoclonic seizures and absence seizures, which were identified in association with JME in RRs, and (2) the comparison of canine and human IGEs at a syndromic level, which requires a complete description of the electroclinical syndrome including EEG manifestations. The introduction of wireless video-EEG into veterinary diagnostics and the recognition of currently underrecognized types of generalized seizures will likely influence the future approach to the diagnosis of seizures and epilepsy in dogs and to the comparison of canine and human epilepsies to enhance translational research.

1. Classification of Juvenile Myoclonic Epilepsy in Rhodesian Ridgebacks

1.1. Veterinary epilepsy classification system

The present work provides a detailed description of a novel electroclinical syndrome in RRs. A structural cause was ruled out by comprehensive diagnostic investigations, EEG confirmed the generalized nature of the epileptic seizures, and genetic analyses identified the underlying variant in the *DIRAS1* gene.

In 2015, the International Veterinary Epilepsy Task Force (IVETF) proposed a classification system for epilepsy in dogs (BERENDT et al., 2015). An aetiological classification system distinguishes between idiopathic and structural epilepsy as well as epilepsy of unknown cause (BERENDT et al., 2015). Idiopathic epilepsy is further divided into (a) genetic epilepsy, (b) suspected genetic epilepsy, and (c) epilepsy of unknown cause (BERENDT et al., 2015). Moreover, the semiological

classification differentiates focal and generalized epileptic seizures, and focal epileptic seizures with propagation to generalized epileptic seizures (BERENDT et al., 2015).

By applying these veterinary guidelines, JME in RRs can therefore be classified as an idiopathic genetic epilepsy with generalized epileptic seizures.

1.2. Human epilepsy classification system

Based on the human classification scheme of the International League Against Epilepsy (ILAE), RRs with JME have a generalized seizure onset, a generalized epilepsy, and the epilepsy syndrome of JME in RRs with a genetic aetiology.

In human medicine, epilepsy classification represents a dynamic process. Since the introduction of a classification system in the early 1960s, the ILAE has made a number of additional proposals, mirroring the increase in knowledge about epilepsy and its underlying mechanisms (SCHEFFER et al., 2017). The current 2017 ILAE Seizure Classification comprises three levels (SCHEFFER et al., 2017). In the first level, a diagnosis of seizure type (focal, generalized, or unknown seizure onset) is made (SCHEFFER et al., 2017). In the next level, the type of epilepsy is determined, including focal, generalized, combined generalized and focal epilepsy, and an unknown epilepsy group (SCHEFFER et al., 2017). The last level involves the identification of the epilepsy syndrome (SCHEFFER et al., 2017). An epilepsy syndrome is defined as a combination of several characteristics such as seizure type, electroencephalographic characteristics, and brain imaging findings (SCHEFFER et al., 2017). Additionally, similarities in age of onset, triggering factors, chronodependency, response to treatment, and prognosis are often encountered (SCHEFFER et al., 2017). An aetiological diagnosis should be attempted at each of the three levels and includes the following groups: structural, genetic, infectious, metabolic, immune, and unknown (Figure 2) (SCHEFFER et al., 2017).

Currently, very few epilepsy syndromes have been described in dogs, probably because of the lack of solid EEG data. Juvenile myoclonic epilepsy in RRs is therefore one of the first electroclinical syndromes described in dogs.

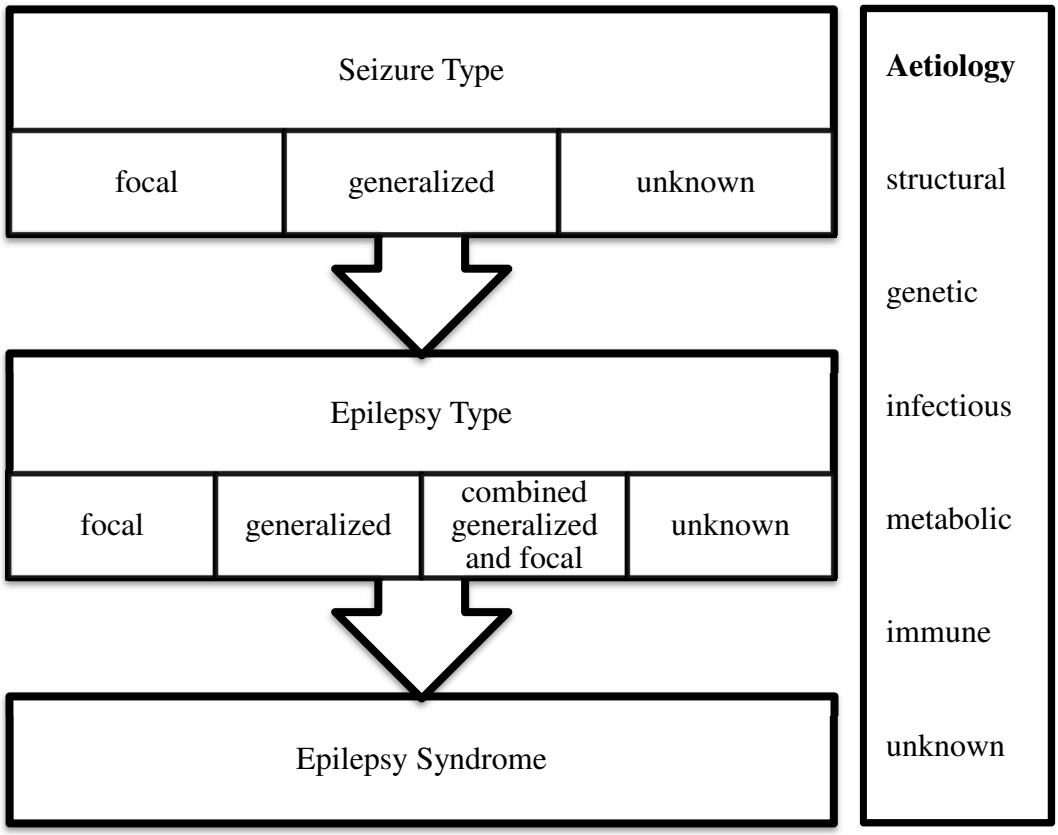

Figure 2: 2017 ILAE Seizure Classification (SCHEFFER et al., 2017)

2. Clinical aspects

2.1. New seizures types

The present work introduces two new seizure types that have been underrecognized in veterinary medicine. The main feature of JME in RRs is myoclonic seizures, and a subset of dogs also has absence seizures. In contrast, the current veterinary literature focuses on focal and generalized tonic-clonic seizures (BERENDT et al., 2015). Other seizure types such as absence seizures have been neglected or in the case of myoclonic seizures only been regarded in the context of Lafora disease and NCLs, but hardly ever in association with idiopathic epilepsies (AWANO et al., 2006a; BERENDT et al., 2015; KOLICHESKI et al., 2016; LOHI et al., 2005a; LOWRIE & GAROSI, 2017; LOWRIE et al., 2016; POMA et al., 2010). The present study shows that the spectrum of seizure types in dogs is much broader than expected. Loss of consciousness may not be a mandatory feature of generalized seizures, as seen with generalized myoclonic seizures (BERENDT et al., 2015). On the other hand, generalized seizures can present solely as an impaired level of

consciousness without a motor component, as in the case of absence seizures (POMA et al., 2010). The new technique of wireless video-EEG in unsedated dogs facilitated the diagnosis of these seizure types in RRs with JME. Moreover, a focal origin could be ruled out because EEG constitutes a valuable tool to differentiate focal and generalized seizures and to determine the epileptogenic focus. This novel diagnostic method will presumably aid in the identification of further seizures types that have not yet been recognized in dogs.

2.1.1. Myoclonic seizures

One of the main goals of this study was to clarify whether or not the myoclonic jerks had an epileptic origin. This was achieved with the help of wireless video-EEG in unsedated dogs - a new diagnostic approach. The epileptic nature of the myoclonic seizures was confirmed in 82% of the dogs examined. Myoclonic seizures were typically characterized by generalized 4-5 Hz SWC or PSWC with a frontocentral maximum. Therefore, wireless video-EEG confirmed the diagnosis of a generalized epilepsy syndrome and revealed an EEG pattern similar to that observed in human JME, highlighting the parallels between these two electroclinical syndromes. The diagnosis of a myoclonic epilepsy syndrome also had important implications for the affected dogs. It enabled early treatment of the epilepsy, selection of AEDs that were effective for this seizure type, and classification of the epilepsy onset.

To date, there has been a paucity of descriptions of epileptic myoclonus in the veterinary literature and most have been limited to Lafora disease in dogs. It is therefore likely that the results of this study will change the approach to myoclonus. The current veterinary literature suggests subdividing myoclonus into epileptic (cortical) and non-epileptic myoclonus similar to classification schemes used in human medicine (LOWRIE & GAROSI, 2017). Non-epileptic myoclonus can have a subcortical (brainstem), spinal, or peripheral origin (LOWRIE & GAROSI, 2017). In a recent review, Lowrie et al. suggested using the occurrence of concomitant GTCS to distinguish epileptic myoclonus from non-epileptic myoclonus (LOWRIE & GAROSI, 2017). However, the results of the present EEG study challenge this approach because only a third of the 24 affected RRs developed GTCS in the course of the disease. Thus, when this classification scheme is applied to the RR cohort of the present study, 16 dogs (and all dogs at the time of initial presentation) would have been erroneously diagnosed with a non-epileptic myoclonus. Therefore, the

use of wireless video-EEG in unsedated dogs is strongly encouraged to distinguish epileptic from non-epileptic myoclonus.

In veterinary medicine, myoclonic seizures are a fairly underrecognized seizure type. In the literature, they are mainly described in the context of structural epilepsies such as Lafora disease and NCLs, but reports of idiopathic myoclonic epilepsy are scarce (AWANO et al., 2006a; BERENDT et al., 2015; KOLICHESKI et al., 2016; LOHI et al., 2005b; LOWRIE & GAROSI, 2017; LOWRIE et al., 2016). A current review of myoclonus in dogs only mentions myoclonic seizures in Lafora disease and NCLs, and myoclonic epilepsy of unknown origin in older dogs (LOWRIE & GAROSI, 2017). The latter is characterized by myoclonic seizures with a geriatric age of onset and unremarkable initial neurological examination and brain imaging, but a precise description, EEG details, and histological data are not provided (LOWRIE & GAROSI, 2017). However, as the dogs develop cognitive decline and other neurological signs in the course of the disease, the authors propose that this epilepsy syndrome is again part of a neurodegenerative brain disease (LOWRIE & GAROSI, 2017). Myoclonic seizures as a feature of idiopathic epilepsy are not mentioned in this review (LOWRIE & GAROSI, 2017). Similarly, the IVETF lists myoclonic seizures as a type of generalized epileptic seizures, but states that generalized epileptic seizures in dogs and cats present predominantly as GTCS and only rarely as myoclonic or atonic seizures (BERENDT et al., 2015). In contrast, the present study demonstrated myoclonic seizures as the main seizure type in an idiopathic genetic epilepsy syndrome.

In the present study, the owners of two affected RRs that tested homozygous for the *DIRAS1* variant stated that their dogs had never experienced clinical signs. However, during EEG recording, the dogs showed several subtle myoclonic jerks, and EEG proved their epileptic nature (unpublished observation). It is therefore very likely that myoclonic seizures are often dismissed by owners and veterinarians, especially when they are subtle, and myoclonic epilepsy is probably a widely underestimated seizure entity. Some dogs with myoclonic or absence seizures may go on to develop GTCS, which then prompt the owner to seek veterinary advice. Similarly, many humans with JME only consult a physician after their first GTCS because myoclonic jerks are often overlooked or underrated by patients and their families (GENTON et al., 2013; WOLF et al., 2015; YACUBIAN, 2017). In these cases, it is up to the clinician to specifically ask about the previous occurrence of

other seizure types. Otherwise determination of age of onset will be biased. These findings concerning neglected seizure types should be taken into consideration when reviewing the current literature, and age of onset should be evaluated with caution due to the possible bias that occurs when age of onset is determined by the first GTCS and other preceding subtle seizure types are disregarded.

2.1.2. Absence seizures

The results of the present study provided further proof of the existence of absence seizures in dogs. Furthermore, the findings suggested that this seizure type may be underrecognized in dogs because EEG is not routinely used as a diagnostic tool in cases with epilepsy. Specifically, the practice of performing EEG only in sedated or anesthetized dogs has virtually excluded the detection of absence seizures in dogs to date because their recognition requires an ictal EEG in awake dogs during the episodes in question.

The initial description of JME in RRs did not include the occurrence of absence seizures. However, as the number of affected dogs referred to our clinics increased, we became increasingly aware of this peculiar seizure type and finally managed to confirm it by EEG in one RR. This dog had frequent staring episodes that were associated with 4 Hz SWC, and EEG was essential for recognition of this seizure type. Although there is currently only one proven case of absence seizures in a RR with JME, owner descriptions suggest a higher prevalence of this seizure type.

The above-mentioned dog had absence seizures characterized by multiple episodes of unresponsiveness and freezing or staring in association with generalized SWC that had a frequency of 4 Hz. In human medicine, absence seizures were traditionally considered as transient impairment of consciousness associated with generalized SWC with a frequency of 3 Hz (CORTEZ et al., 2016; PANAYIOTOPOULOS et al., 1989). However, the current understanding is that typical absence seizures are characterized by generalized SWC or PSWC with a frequency of >2.5 Hz (mainly 3-4.5 Hz) (CORTEZ et al., 2016; PANAYIOTOPOULOS, 2008). Moreover, in typical absence seizures in humans, frequency often varies during one event, with a fast 3.5-4.5 Hz opening phase, followed by a gradual slowing to 2.5-3.5 Hz (DRURY & HENRY, 1993; PANAYIOTOPOULOS, 2008; PANAYIOTOPOULOS et al., 1989). Furthermore, frequency of spike-wave discharges in human absence seizures varies with the

different types of epilepsy syndrome (PANAYIOTOPOULOS et al., 1989). In CAE, for example, SWC have a frequency of 2.5-3.5 Hz, whereas in juvenile absence epilepsy, the frequency is often faster than 3 Hz, and in JME, absence seizures with 3-4 Hz spike-wave discharges are described (PANAYIOTOPOULOS et al., 1989). Finally, in a case report of a Chihuahua with absence seizures with myoclonic features, SWC with a frequency of 4 Hz were described (POMA et al., 2010).

To date in veterinary medicine, absence seizures have attracted little attention. In the IVETF consensus report on the definition, classification, and terminology of epilepsy, this seizure type is not even mentioned (BERENDT et al., 2015). Based on the results of the present study it is possible that absence seizures are widely underdiagnosed and underrecognized. There is currently only one other case report describing absence seizures with myoclonic features; continuous video EEG monitoring was used to document absence seizures in a Chihuahua dog similar to the methodology used in the present study (POMA et al., 2010). Packer et al. showed that owners perceive a GTCS to be more harmful than a focal seizure and are less likely to report a focal seizure to their veterinarian (PACKER et al., 2017). Similarly, it is possible that owners and veterinarians underestimate the impact of absence seizures or are unable to identify these episodes as being epileptic. Thus, for the phenotyping of canine epilepsies, the clinician should specifically include questions about the occurrence of absence seizures in history taking and educate owners about the semiology of this seizure type. Moreover, EEG should be performed in unsedated dogs suspected of having absence seizures to confirm their diagnosis.

2.2. Electroencephalography

In the present study, wireless video-EEG was established as a novel tool to investigate the epileptic nature of the paroxysmal events and to further characterize the electroclinical syndrome. Electroencephalography enabled the characterization of two new previously underreported seizure types in dogs, namely myoclonic seizures and absence seizures. Furthermore, EEG proved the generalized onset of the seizures and excluded focal seizures. Critical for this assessment was the use of an extended electrode montage with 15 electrodes in most dogs, which increased spatial resolution and therefore allowed exclusion of a focal origin. Lastly, by performing EEG with simultaneous video recordings in unsedated dogs, ictal and

interictal EEG changes could be differentiated and specifically characterized and it could be determined whether the onset of myoclonic activity was consistent with the onset of ictal EEG discharges or whether motor manifestations were preceded by a crescendo of EEG paroxysms.

In order to verify these findings, EEG was also carried out in other related and unrelated control dogs, and recordings were compared to those from affected RRs. In addition, EEG recordings were reviewed by a neuropediatrician (G. Kluger), a neurophysiologist (M. Cortez), and a veterinary expert (F. James).

The IVETF states that "the use of EEG in veterinary medicine is currently of questionable routine clinical value" (BERENDT et al., 2015). However, the present study demonstrates that EEG can be a valuable tool to investigate the epileptic nature of paroxysmal events of unknown aetiology and to further characterize the EEG pattern of specific electroclinical syndromes. Juvenile myoclonic epilepsy in RRs proved particularly useful for recording ictal events due to the frequent occurrence of the myoclonus. Previous studies of canine EEG usually employed an interictal short-term EEG in sedated or even anesthetized dogs (BERENDT et al., 1999; BRAUER et al., 2012a; JAGGY & BERNARDINI, 1998; JESEREVICS et al., 2007; JOKINEN et al., 2007; WRZOSEK et al., 2017). In contrast, the aim of the present study was not only to demonstrate interictal epileptiform discharges but also to capture the paroxysms occurring during an ictal event on EEG. Since most of the RRs with JME experience multiple myoclonic seizures per day, this goal was achieved in the majority of cases. Electroencephalography revealed epileptiform discharges in 82% of the dogs examined. It appears logical that ictal EEG recordings are more easily achieved when events occur on a daily base or on multiple occasions during the day, which is the case in many RRs with JME. It may be much more difficult to achieve ictal EEG recordings in epilepsies with rare seizure events. It may also be more difficult to achieve readable EEG recordings if vigorous convulsions occur due to muscle activity obscuring the EEG signal. In these situations, EEG diagnosis may rely solely on interictal recordings demonstrating epileptiform discharges. This approach has been most commonly used in veterinary medicine with short-term interictal EEG in sedated dogs. Alternatively, more long-term EEG monitoring would be needed to capture the ictal events. In the current study, a definitive diagnosis could not be made via EEG in only three dogs; two dogs were on AED medication and seizure-free at the time of

recording, and muscle artefact was superimposed on the EEG correlate and therefore inconclusive in the other dog. Other veterinary EEG studies report success rates of 20 to 86%, and in humans, a diagnosis can be made in 58% of cases (JAMES et al., 2017; MCGONIGAL et al., 2004). The comparatively high diagnostic yield of the present study is likely due to the high frequency of myoclonic seizures. Electroencephalographic records from the present study were also part of another study that investigated the likelihood of EEG leading to a diagnosis (JAMES et al., 2017). Video-EEG was considered diagnostic when ictal or interictal epileptiform discharges were detected or EEG records during an event were unremarkable (JAMES et al., 2017). That study demonstrated that the likelihood of achieving a diagnosis via EEG improved as the frequency of the episodes increased (JAMES et al., 2017).

A major concern in dogs is muscle artifact that obscures the EEG. Therefore, in the majority of veterinary studies in which EEG was used as a diagnostic tool, dogs were sedated or even anesthetized (BERENDT et al., 1999; BRAUER et al., 2012a; JAGGY & BERNARDINI, 1998; JESEREVICS et al., 2007; JOKINEN et al., 2007; WRZOSEK et al., 2017). Electroencephalography in unsedated dogs was regarded unfeasible because the patients were considered to be uncooperative and the EEG inconclusive due to movement and muscle artefacts, especially those of the large temporal muscle that covers the skull in dogs (JESEREVICS et al., 2007). However, in the present study, it was shown that the dogs were cooperative, and despite muscle and movement artefacts, a certain proportion of the recordings were free of artefacts. In addition, unsedated video-EEG is not only less invasive than the traditional method, but it may also have a higher diagnostic success rate. The last resort in treating a dog with status epilepticus is inducing general anaesthesia to suppress epileptic brain activity. Hence, it can be assumed that general anaesthesia will also reduce interictal epileptiform discharges during EEG recording. Therefore, it could be speculated that EEG in unsedated dogs will increase the diagnostic success rate even if no ictal recordings are obtained. Finally, sedation or general anaesthesia prohibits the assessment of consciousness; responsiveness and level of consciousness are important aspects for the characterization of distinct types of epilepsy and a key factor in the diagnosis of absence seizures.

In the author’s experience, dogs required approximately 20 minutes to become

accustomed to the procedure of EEG recording, but then started to relax and most fell asleep. Therefore, after the initial 20 minutes, muscle and movement artefacts largely vanished and recordings were much more conclusive. The fact that the majority of previous studies used short-time EEGs with a duration of only 20 minutes may explain their low diagnostic yield (BRAUER et al., 2012a; JESEREVICS et al., 2007; JOKINEN et al., 2007). Moreover, several studies used different drugs, for example lidocaine or rocuronium, to reduce muscle artefact (BRAUER et al., 2012b; WARD et al., 2016). However, it is possible that prolongation of time of recording makes such treatments unnecessary. Consequently, the results of the present study strongly recommend expanding the time of recording to at least one hour.

A major concern was the use of a standardized montage in canine EEG. In human epileptology, there is an internationally recognized protocol for standardized electrode positioning called the 10-20 system. Veterinary medicine lacks such a standardized protocol, which complicates comparison of the results of different studies. In addition, many studies use a montage that contains a small number of electrodes, which cover only a small percentage of the cerebral cortex because the electrical activity captured by one electrode has a surface diameter of only 1-2 cm (BRAUER et al., 2012b, 2012a, 2011; HOLLIDAY & WILLIAMS, 1999). Therefore, an electrode positioning protocol with a larger number of electrodes is necessary to differentiate focal from generalized epileptic seizures, to exclude a focal onset of generalized epileptiform discharges, and to determine the epileptogenic zone. An extended electrode placement protocol that used 15 electrodes and was easy to apply in unsedated dogs was employed in the present study. This protocol was initially introduced by Pellegrino et al. and thereafter modified by James et al. for use in ambulatory EEG and long-term recordings (JAMES et al., 2011; PELLEGRINO, 2004). The present study demonstrates the feasibility of this method in a large cohort of dogs for the electroencephalographic description of a novel canine epilepsy syndrome. Nevertheless, this electrode placement protocol requires further validation, and the exact anatomic correlations of the electrode positions need to be determined, a process that was beyond the scope of this study.

The present study illustrates that long-term wireless video-EEG in unsedated dogs is a novel and feasible technique for the investigation of frequent paroxysmal events

of unknown etiology and the classification and investigation of epilepsy syndromes in dogs for comparison to human epilepsy syndromes. Furthermore, the study revealed that in some circumstances this method is crucial, especially for the diagnosis of absence and myoclonic seizures. Further research is needed to determine the diagnostic success of EEG with rare events. Thus, with the help of EEG, researchers can obtain an expanded view of seizure activity occurring in the canine brain. The use of EEG can also identify more episodes of epileptic activity compared with observation and registration of GTCS alone. Further research on expansion and standardization of this technique in dogs with spontaneous naturally occurring epilepsy is warranted.

2.3. Photosensitivity

The present study demonstrated that reflex epileptic traits occur in dogs with IGE. In veterinary medicine, photosensitivity is a rather neglected and understudied phenomenon. Only in the context of structural epilepsies such as Lafora disease are seizures triggered by visual stimuli occasionally reported; even then, this reflex epileptic trait is only described and not called by its name – photosensitivity (HAJEK et al., 2016; LOHI et al., 2005a; SCHOEMAN et al., 2002; WEBB et al., 2009). Moreover, although current neurological evaluation rarely uses EEG as a diagnostic tool, photic stimulation, as an activation method, is used even less. The present study shows that photosensitivity can constitute an important feature of an idiopathic epilepsy syndrome and demonstrates the diagnostic value of photic stimulation. Furthermore, a detailed description of an appropriate ILS protocol is provided.

In human medicine, photic stimulation is a long-established activation technique to enhance the diagnostic yield of EEG. Interestingly, photosensitivity has currently become a main focus of interest in human epilepsy research. Not only does it have therapeutic consequences, but it is considered a biomarker for IGE, an endophenotype with prognostic impact and is used as a model to study the efficacy of new AEDs (KASTELEIJN-NOLST TRENITÉ et al., 1996; WALTZ et al., 1992; YACUBIAN, 2017).

In the initial study cohort, clinical photosensitivity was observed in 35% of affected RRs. Subsequently, EEG with ILS was performed in six affected dogs; four dogs (66%) had a grade four PPR with photoconvulsions. Photic stimulation did not

evoke a PPR in one unrelated (wildtype) and three related (all heterozygous) healthy control dogs. Although the prevalence of clinical and electroencephalographic photosensitivity seems to be divergent, the small number of dogs examined by EEG with ILS should be taken into consideration. In a subsequent study setting that focussed on the confirmation of absence seizures, two more affected RRs and members of their family were examined by EEG with photic stimulation. Neither of the affected RRs nor their healthy relatives showed clinical or electroencephalographic photosensitivity. In human JME, there is some discrepancy between the number of patients that show clinical photosensitivity in daily life (15%) and the number of affected individuals that exhibit a PPR on EEG upon photic stimulation (30-90%) (ALFRADIQUE & VASCONCELOS, 2007; ANDERSON & HAMANDI, 2011; APPLETON et al., 2000; BAYKAN & WOLF, 2017; GENTON et al., 2013; KASTELEIJN-NOLST TRENITÉ et al., 2013a; NICOLSON & MARSON, 2010; SERAFINI et al., 2013; WELTY, 2006; WOLF et al., 2015). Current data suggest a similar distribution pattern for JME in RRs. However, the prevalence rates may change when a larger number of dogs are evaluated. In contrast to JME in humans, clinical photosensitivity was predictive of the results of EEG with photic stimulation in RRs. Therefore, all affected RRs that were clinically photosensitive also showed a PPR upon photic stimulation, and individuals that did not show myoclonic seizures following visual stimuli did not have a PPR on EEG.

According to Waltz et al., four grades of PPR can be distinguished (WALTZ et al., 1992). Interestingly, photosensitivity in RRs with JME follows an all-or-none law: either the dogs do not show PPR or they exhibit a grade four PPR with photoconvulsions. Therefore, photosensitivity could constitute an endophenotype of JME in RRs and it appears to be closely linked to the *DIRAS1* mutation. Further studies should investigate, whether this reflex epileptic trait has an influence on outcome and prognosis of affected individuals.

Photic stimulation is an activation technique that is rarely used in veterinary medicine. Brauer et al. used two activation techniques, namely photic stimulation and hyperventilation, to investigate their ability to increase the diagnostic yield of short-time EEG (BRAUER et al., 2012b, 2011, 2012a). Dogs and cats, suffering from idiopathic or symptomatic (structural) epilepsy as well as healthy controls were examined under propofol anaesthesia (BRAUER et al., 2012b, 2011, 2012a).

Photic stimulation did not evoke a PPR in any of the examined individuals (BRAUER et al., 2012b, 2011, 2012a). Therefore, the use of the two activation techniques was discouraged as both methods had no influence on the diagnostic value of short-time EEG under propofol anesthesia (BRAUER et al., 2012b, 2011, 2012a). In a recent study, dogs with a variety of epileptic encephalopathies were examined by EEG with photic stimulation under medetomidine sedation (WRZOSEK et al., 2017). Although ILS raised the number of spikes and sharp waves in dogs with meningoencephalitis of unknown origin and in dogs with portosystemic shunt, and dogs with idiopathic epilepsy had more spike waves following photic stimulation, total frequency of epileptiform discharges was not significantly affected by ILS (WRZOSEK et al., 2017). In contrast, the present study showed that a PPR was observed in 66% of the examined cases and one dog that exhibited an initially normal EEG had a grade four PPR with photoconvulsions only upon photic stimulation.

The results of the present study demonstrate that it is essential to identify photosensitivity because it has important therapeutic consequences. Owners can be advised to avoid having visual stimuli, such as camera flashes, sudden light changes, and television, in the presence of affected dogs. Routine inclusion of photic stimulation in EEG recording protocols appears to be warranted. Furthermore, the gene discovery indicates an important pathway for future investigations of the mechanisms of photosensitivity.

2.4. Age of onset

Age of onset is an important clinical aspect for the classification of epilepsy syndromes (SCHEFFER et al., 2017). However, determination of seizure onset largely relies on owner observation. In the current study, many dogs had a history of mild myoclonic twitches that then progressed to more violent myoclonic seizures. Therefore, there may have been some delay between true seizure onset and the age of onset reported by the owners if initial subtle twitches were not noticed. In fact, two owners did not know that their dogs were suffering from mild myoclonic seizures until proven otherwise by video-EEG. Consequently, age of onset may be biased, and JME in RRs may have a younger age of onset than expected. Early EEG examinations should be done in dogs that test homozygous for the *DIRAS1* variant to clarify the actual onset of seizures.

Juvenile myoclonic epilepsy in RRs has a mean age of onset of six months and a median age of onset of 3.5 months. Because the literature on human epilepsy syndromes refers to the mean age of onset, the mean value was also used in RRs with JME for comparison with other human epilepsies. However, median age of onset may better reflect the true age of onset. Studies investigating the developmental stages in dogs, especially the differences between the various breeds and comparison to child development, are scarce. In general, the development of locomotor skills precedes that of social skills in dogs, whereas primary socialization precedes locomotor development in humans (SCOTT & FULLER, 1965). In puppies, the neonatal period is followed by the socialization period (until the age of twelve weeks), the juvenile period (twelve weeks - six months or longer), and adolescence (~ six months - one to two years depending on the breed) (SCOTT & FULLER, 1965; SERPELL & DUFFY, 2016). Therefore, 14 dogs in the present study had a juvenile or adolescent age of onset. However, ten dogs were between six and ten weeks of age when they first had a myoclonic seizure. This observation may challenge the classification of a juvenile epilepsy syndrome. Nevertheless, some variance in age of onset is also encountered in human epilepsy syndromes. The introduction of two groups of diagnostic criteria in human JME mirrors this clinical heterogeneity (KASTELEIJN-NOLST TRENITÉ et al., 2013b; WOLF et al., 2015). Class I criteria are quite narrow and require an age of onset of ten to 25 years, whereas class II criteria are more flexible and allow an age of onset of six to 25 years (KASTELEIJN-NOLST TRENITÉ et al., 2013b; WOLF et al., 2015; YACUBIAN, 2017). Therefore, an age of onset during childhood does not exclude the diagnosis of JME in humans.

Further studies using early video-EEG examinations should investigate the real age of onset of JME in RRs, which is possibly younger than current data suggest. However, JME in RRs could still constitute a valuable translational model for JME in humans. In many well-recognized animal models of human disease some inconsistencies exist. For example, Lafora disease in humans and dogs is characterized by myoclonus, progressive neurological decline, and the same genetic background (DE SIQUEIRA, 2010; LOHI et al., 2005a; SHAHWAN et al., 2005). However, age of onset differs greatly: children are as young as twelve to 17 years of age, whereas Lafora disease occurs in older dogs between six and nine years of age (LOHI et al., 2005a; SHAHWAN et al., 2005). Moreover, in dogs, the disease

seems to be less progressive compared with humans (LOHI et al., 2005a; SHAHWAN et al., 2005). Nevertheless, Lafora disease and other PMEs such as NCLs are frequently and successfully used as large animal translational models (ASHWINI et al., 2016; KATZ et al., 2017, 2015; KOLICHESKI et al., 2017; SANDERS et al., 2011; TRACY et al., 2016; WHITING et al., 2016).

2.5. Diagnostic investigations

In the present study, diagnostic investigations were consistent with idiopathic epilepsy and failed to identify a structural cause for the epilepsy. Physical and neurological examinations as well as blood and CSF analyses were unremarkable in the dogs.

Neurometabolic screening revealed mild abnormalities in three affected dogs (elevated serum alanine; smear in urinary organic acids in the qualitative analysis; mild elevation in amino acids cystathionine and homocysteine). However, these findings were inconsistent and neurometabolic screening was normal for the remaining dogs. Therefore, a neurometabolic disorder was considered unlikely as a cause for JME in RRs.

Brain imaging was performed in the majority of dogs (16 dogs). Diagnostic imaging failed to identify gross or consistent abnormalities, and all findings were indicative of idiopathic epilepsy. However, imaging of epilepsy still has important drawbacks. Firstly, computed tomography was carried out in few dogs of this study cohort and is considered an inadequate method for the investigation of epileptic seizures due to lack of detail. Secondly, recent recommendations by the IVETF suggest using imaging protocols that focus on the detection of changes in the hippocampus (RUSBRIDGE et al., 2015). Lastly, a wide variation in imaging quality and protocols were used in this study, ranging from CT and low-field 0.5T MRI to 1.5T and 3T MRI with IVETF-suggested protocols (RUSBRIDGE et al., 2015). Brain imaging revealed minor abnormalities of questionable significance in five dogs. Findings included ventricle asymmetry (three dogs), mild meningeal contrast enhancement, suspected mild brain atrophy, and T1 and T2 hypointensity in the transition area of the frontal lobe and the olfactory bulb in one dog each. Ventricle asymmetry has been described as an incidental finding in dogs because it can also be frequently found in dogs without epileptic seizures (PIVETTA et al., 2013). Likewise, a small amount of meningeal contrast enhancement is often

present in healthy dogs (JOSLYN et al., 2011). In one dog, slight brain atrophy was suspected on initial imaging, but follow-up examination using 3T PET-MRI ruled this out. The focal findings observed in one RR may very well have been an epileptogenic focus. Although an effect of this structural abnormality on seizure phenotype, such as lowering seizure threshold, cannot be excluded, the EEG was clearly indicative of a generalized and not a focal epilepsy syndrome.

One limitation of the present study was that necropsy and detailed post-mortem examination of the brain were done in only one dog. In this dog, post-mortem examination revealed postictal changes only and structural epilepsy was excluded. Most importantly, there was no evidence of Lafora bodies, thus excluding Lafora disease in these dogs. The results of brain MRI were unremarkable in the remaining dogs, and long-time follow-up revealed no signs of neurological or cognitive deterioration. These observations suggest that JME in RRs is truly a generalized idiopathic epilepsy syndrome. Nevertheless, histologic examination of further affected dogs would be desirable.

2.6. Seizure frequency

The results of the present study show that data on seizure frequency should be interpreted carefully. The frequency of GTCS, myoclonic, and absence seizures is based on owner observations. However, for several reasons, seizure frequency may be highly underestimated. On the one hand, few owners can directly observe their dogs 24 hours a day and therefore, it is possible that owners do not notice every epileptic seizure. On the other hand, the manifestation of myoclonic seizures may be very variable, ranging from very subtle twitches of the face (for example jerks of the eyelids, ears, or cheeks) to pronounced jerks that affect the whole body. Mild myoclonic twitches in particular can be easily overlooked or disregarded by the owner. It is particularly difficult to recognize absence seizures because they are by definition a short period of loss of consciousness without motor activity. Therefore, the frequency of absence seizures may be easily underestimated, or this type of seizure may be overlooked completely. For accurate quantification of seizure frequency, telemetry and/or long-term video-EEG or invasive EEG monitoring is needed. Current research focusses on development of seizure recognition and seizure alert technology in humans, and invasive long-term EEG monitoring has been successfully applied to dogs in a research setting (DAVIS et al., 2016; HOWBERT et al., 2014).

2.7. Influence of heat and neutering status

Dog owners always question the effect of oestrus (heat) and sex hormones on the manifestations of epilepsy. The recurrent oestrus-associated deterioration observed in three bitches in the present study may be similar to the effect that sex steroid hormones have on seizures in women. A twofold increase in average daily seizure frequency in relation to the menstrual cycle is defined as catamenial epilepsy in women (HERZOG, 1997). This phenomenon is attributed to the neuroactive properties of steroid hormones (HERZOG, 1997). Although the potential of oestrogens to facilitate or inhibit seizures is controversial, it is well-recognized that progesterone has an anticonvulsant effect in humans and there is a positive correlation between oestrogen/progesterone ratio and seizure frequency (BACKSTROM, 1976; HERZOG, 1997; REDDY, 2009; VELÍŠKOVÁ, 2006). Further studies are needed to determine whether this finding is specific for the syndrome in RRs with JME, reflects the general effect of sex hormones on epilepsy in dogs, or is just an incidental finding (VAN MEERVENNE et al., 2014).

The effect of castration on the course of an epilepsy has not been clarified. It is interesting that in some RRs, a reduction of seizure frequency after neutering was reported by the owners. While some studies could not demonstrate a correlation between castration and epilepsy of unknown cause or the incidence of cluster seizures, others showed an increased risk for cluster seizures in castrated dogs and an increased incidence of epilepsy of unknown cause in male neutered dogs (KEARSLEY-FLEET et al., 2013; MONTEIRO et al., 2012; PACKER et al., 2016; SHORT et al., 2011).

Similar to oestrus, the interaction of sexually intact dogs with their conspecifics results in stress for many dogs. In the present study, most owners stated that stress was a potent triggering factor for their dog's epilepsy. It is therefore possible that the increased stress level alone led to an increased seizure frequency and amelioration after castration is attributable to the elimination of those stress peaks, which was proposed by Berendt et al. (BERENDT et al., 2008).

2.8. Treatment and outcome

Comparison of response to treatment was challenging because a multitude of different treatment regimens were used by the referring veterinarians. This applied in particular to the initial phase of the study, when reasonable differentials for the

sleep-associated twitches included sleep disorders and paroxysmal dyskinesias in addition to epileptic seizures. To date, the best results were achieved with LEV and KBr. However, carefully planned and executed studies with a larger number of dogs are necessary to confirm this observation. Valproic acid is the drug of choice in humans with JME and provides a seizure control rate of up to 90% (CRESPEL et al., 2013; WELTY, 2006). However, valproic acid has a very short elimination half-life in dogs, and other AEDs with a better pharmacokinetic profile must be used in this species. Levetiracetam is another effective drug to treat JME in humans and is the first-line treatment for women of childbearing age (CRESPEL et al., 2013; NOACHTAR et al., 2008). Furthermore, in a prospective randomized open-label study, Lowrie et al. showed that LEV is superior to PB for the treatment of myoclonic seizures in cats with feline audiogenic reflex seizures (LOWRIE et al., 2017). These findings support the preliminary observations on the beneficial effects of LEV in RRs with JME.

One limitation of the present study was that evaluation of AED efficacy was based mainly on the results of owner-based seizure monitoring and therefore relied on owner compliance. In one dog however, owner observations were confirmed by a follow-up EEG with synchronized video recording. In this case, seizure frequency could be objectively compared to the initial recording and revealed a reduction of myoclonic seizures of 97.2%.

Furthermore, owners stated that their dogs had no learning difficulties or developmental delays and retained normal cognition throughout life. Although the follow-up time of some dogs was more than eight years, which is almost the life expectancy of an RR, validated questionnaires and objective learning tasks would be desirable for assessing cognitive impairment or behavioural abnormalities. In addition, because of the small size of the cohort in the present study, behavioural changes or cognitive impairment in a subset of RRs with JME could not definitely be excluded.

In the present study, preliminary observations suggest that when treatment with LEV or KBr was initiated in the early phase of the disease, dogs were more likely to become seizure free or at least experience a marked reduction in seizure frequency than when treatment was delayed. Dogs that had a major delay in the start of treatment with LEV or KBr or were treated with AEDs other than LEV or KBr appeared to have a higher risk for treatment resistance. The potential benefit

of starting treatment promptly requires further study. The identification of the gene variant causing JME in RRs and the widespread availability of genetic testing for the *DIRAS1* variant raises another question: would a dog with a homozygous mutant genotype benefit from prophylactic treatment, i.e. a treatment that starts before clinical manifestation of epileptic seizures? In other words, would prophylactic treatment have a positive effect on the course of the disease or even prevent the dog from experiencing seizures? In recent years, a multitude of studies investigated the potential antiepileptogenic properties of LEV and a variety of other AEDs, with controversial results (BRANDT et al., 2007; HEUERMANN & CHETKOVICH, 2016; KLEE et al., 2015; OHNO et al., 2010; SUGAYA et al., 2010; VINOGRADOVA & VAN RIJN, 2008). Whether LEV has an antiepileptogenic effect remains to be seen, and further studies are warranted to determine whether starting treatment early in the course of JME in RRs is beneficial.

3. *DIRAS1*

Based on the genetic investigations of the epilepsy cohort identified in the present study, a causal variant in a new epilepsy gene was identified. *DIRAS1* is widely expressed in the mammalian brain and has not been associated with epilepsy thus far. The encoded protein belongs to the family of guanosine triphosphatases (GTPase), whereby its GTPase activity is low and it predominantly remains in its guanosine triphosphate (GTP)-bound state (COLICELLI, 2004; KONTANI et al., 2002). The biological function of DIRAS1 in mammals is poorly understood.

A recent study investigated the role of *diras1a* and *diras1b* in a zebrafish model. The authors could show, that the two genes are predominantly expressed in the brain and retina ganglion cells (YEH & HSU, 2016). Transfection of mouse Neuro-2a cell lines with green fluorescent protein (GFP)-*diras1a* or GFP-*diras1b* revealed that zebrafish *diras1a* and *diras1b* may promote neurite outgrowth of these cells (YEH & HSU, 2016). Furthermore, in a model of developing zebrafish, in which knockdown of *diras1a* and/or *diras1b* was attempted by morpholino antisense oligonucleotides, reduced axon guidance and a decreased number of trigeminal ganglia were observed (YEH & HSU, 2016).

Tada et al. used *Caenorhabditis elegans* to further elucidate the function of DIRAS1, and showed that *drn1* (homologue to *DIRAS1*) mutants obtained some resistance to aldicarb treatment (TADA et al., 2012). Aldicarb is an

acetylcholinesterase inhibitor that causes hypercontraction of muscles and paralysis due to insufficient breakdown of ACh (TADA et al., 2012). Furthermore, the aldicarb-hypersensitive phenotype caused by gain of function of *gsa-1* was partially reversed in *drn1* mutants (TADA et al., 2012). In addition, the investigators showed that the DIRAS1 and exchange protein directly activated by cyclic adenosine monophosphate (cAMP) (EPAC) orthologues colocalize at the presynaptic membranes and are involved in the regulation of presynaptic ACh release at neuromuscular junctions.

The understanding of the role of DIRAS1 in cholinergic neurotransmission and epilepsy remains an important area of study. Nicotinergic cholinergic activity influences brain excitability and cognition, regulates the excitatory/inhibitory switch of GABA during neuronal development, stimulates glutamate release from thalamocortical terminals, controls GABA release onto pyramidal neurons, and maintains non-REM sleep by low levels of ACh whereby cholinergic stimulation is associated with microarousals in this sleep stage (BECCHETTI et al., 2015; LIU et al., 2006). Mutations in the nAChR subunits *cholinergic receptor nicotinic alpha4 subunit* (*CHRNA4*)*, cholinergic receptor nicotinic alpha2 subunit* (*CHRNA2*)*,* and *cholinergic receptor nicotinic beta2 subunit* (*CHRNB2*) are associated with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) and sporadic nocturnal frontal lobe epilepsy (NFLE) (CONTI et al., 2015). Several models for the pathogenic mechanism have been postulated (COMBI et al., 2004; RAGGENBASS & BERTRAND, 2002). As a common trait, either an increased ACh sensitivity or a shift in the proportion of low-sensitivity and high-sensitivity receptors has been proposed (FERINI-STRAMBI et al., 2012). Other mechanisms are an imbalance between excitation and inhibition enhancing and synchronizing spontaneous oscillations in thalamocortical circuits, and abnormal formation of neuronal circuits and alteration of network assembly in the developing brain leading to epilepsy (MANFREDI et al., 2009; RAGGENBASS & BERTRAND, 2002). A mutation in *PRIMA1* encoding for a transmembrane protein that anchors acetylcholinesterase is associated with autosomal recessive NFLE, probably due to overstimulation of nAChRs (HILDEBRAND et al., 2015). *CHRNA7* coding for the α7 subunit of the nAChR is also a potential candidate gene for JME in humans (HELBIG et al., 2009). Therefore, abnormal DIRAS1 function could alter cholinergic neurotransmission or facilitate neurodevelopmental defects resulting in

myoclonic epilepsy.

Interestingly, *DIRAS2* has recently been associated with ADHD and co-morbid impulsive disorders in humans (REIF et al., 2011). Attention deficit hyperactivity disorder has been associated with BFJE in Lagotto Romagnolo dogs and is a frequently observed comorbidity in human epilepsy syndromes (JOKINEN et al., 2015). It would therefore be interesting to determine whether a similar condition occurs in RRs with JME.

Other studies investigating the function of DIRAS1 proposed that it plays an important role in oncogenesis. It has been shown that *DIRAS1* is down-regulated in cultured glioma cells and primary glioblastoma samples (LIGON et al., 1997). Thus, it was postulated that an altered expression and distribution pattern of *DIRAS1* may promote malignant progression of human glioblastomas (LIGON et al., 1997). Down-regulation of *DIRAS1* was also observed in 50% of human patients with oesophageal squamous cell carcinoma, where it was significantly associated with advanced stage of disease, metastasis, and a lower overall three-year survival rate (ZHU et al., 2013). *DIRAS1* and *DIRAS2* are also down-regulated in ovarian cancer and are correlated with a reduced disease-free interval and overall survival time (SUTTON et al., 2018). Finally, the promotor region of *DIRAS1* was methylated in 47% of human specimens of primary colorectal cancer, whereas no methylation was found in healthy control tissue (ZHENG et al., 2017). This methylation led to a diminished expression of *DIRAS1* and was significantly associated with an advanced tumour stage and a reduced survival time (ZHENG et al., 2017). Bergom et al. showed that DIRAS1 binds to small GTP-binding protein guanosine diphosphate (GDP) dissociation stimulator (SmgGDS), a protein that promotes the activation of several oncogenic GTPases (BERGOM et al., 2016). Via this binding, DIRAS1 prevents SmgGDS from binding to oncogenic small GTPases, such as K-Ras4B, RhoA, and Ras-related protein 1A (Rap1A) (BERGOM et al., 2016). Furthermore, DIRAS1 suppresses basal nuclear factor κB (NF-κB) activation in glioblastoma and other tumour cell lines (BERGOM et al., 2016). Therefore, it was suggested that *DIRAS1* plays a role as a tumour suppressor gene (BERGOM et al., 2016; ELLIS et al., 2002; ZHENG et al., 2017).

At the time of publication, seven of the 24 dogs from the initial study cohort had died. Of the seven dogs, two were euthanized because of neoplasia; one was diagnosed with a renal tumour at the age of four years, and the other died from a

ruptured splenic tumour at six years of age. Another dog from this population died due to a splenic tumour at ten years of age, and one other was euthanized at two years of age because of lymphoma, which was diagnosed histologically (unpublished data). Future studies should therefore focus on the prevalence of tumours in RRs that carry the *DIRAS1* variant to determine whether this subpopulation is at increased risk of developing neoplastic disease.

4. Comparison with human epilepsy syndromes

The present study proposes that generalized myoclonic epilepsy in RRs shares many common features with JME in humans, which is the most common human IGE. Initial reports on the symptoms of RRs with JME consisted of sleep-associated jerks with an early age of onset. Therefore, initial differentials for this breed-specific syndrome not only included myoclonic seizures but also paroxysmal dyskinesias, non-epileptic myoclonus, and parasomnias. Consequently, a wide range of human and canine disorders were considered and evaluated for common features, outlined in the following part of the discussion.

4.1. Juvenile Myoclonic Epilepsy

Juvenile myoclonic epilepsy in humans is a primary generalized epilepsy syndrome with multiple associated gene mutations and a mean age of onset of 14 years (WELTY, 2006). Typically, people present with myoclonic seizures without loss of consciousness (GENTON et al., 2013). Myoclonic jerks usually occur after awakening, are bilateral and arrhythmic, and predominate in the upper limbs, making the patients drop or throw objects (GENTON et al., 2013). Seizures are often precipitated by sleep deprivation, stress, and alcohol consumption (GENTON et al., 2013; KASTELEIJN-NOLST TRENITÉ et al., 2013a). The majority of patients (80-95%) develop rare GTCS after a mean delay of 1.3 to 3.3 years (GENTON et al., 2013; WOLF et al., 2015). Often GTCS are preceded by a cluster of myoclonic jerks (GENTON et al., 2013). A smaller number also present with absence seizures (NICOLSON & MARSON, 2010; WOLF et al., 2015; YACUBIAN, 2017). Neurological examination and brain imaging are normal, and there are no intellectual deficits (WELTY, 2006). Electroencephalography typically reveals generalized and irregular SWC and PSWC, often with a frontocentral accentuation (BAYKAN & WOLF, 2017; KOEPP et al., 2014; SERAFINI et al., 2013). Electroencephalographic paroxysmal responses upon photic stimulation are

common (30%), although the prevalence of clinical response to visual stimuli in daily life is lower (BAYKAN & WOLF, 2017; GENTON et al., 2013). Therefore, of all epilepsies, JME has one of the strongest associations with photosensitivity (GUERRINI & GENTON, 2004).

There are striking similarities between JME in humans and RRs: the prevailing seizure type is myoclonic seizures with propagation to GTCS in a subgroup of patients (humans: 80-95%; RRs: 38%) (GENTON et al., 2013; WOLF et al., 2015). These GTCS are often preceded by a series of myoclonic seizures in both species. Whereas absence seizures are encountered in 30% of human patients, to date there is only one proven case of this seizure type in RRs (NICOLSON & MARSON, 2010; WOLF et al., 2015; YACUBIAN, 2017). Nevertheless, owner observations suggest a higher prevalence of absence seizures in canine JME. Mean age of onset occurs around puberty in humans and dogs (humans: 14 years; RRs: six months), although some dogs were as young as six weeks of age when they experienced their first myoclonic seizures (WELTY, 2006). Seizures follow a circadian rhythm in humans and dogs. In human patients, myoclonic seizures and GTCS occur predominantly within the first two hours after awakening, although another peek during relaxation periods in the evening is encountered in some patients (KASTELEIJN-NOLST TRENITÉ et al., 2013b). In RRs, myoclonic seizures mainly occur when the animals are in a recumbent position and relaxed, drowsy, or in the first stages of sleep. Neurological examination and routine brain imaging are normal in both species (ANDERSON & HAMANDI, 2011). Moreover, JME in humans and dogs share similar EEG characteristics. Both epilepsy syndromes are characterized by SWC or PSWC with a frontocentral accentuation (BAYKAN & WOLF, 2017; KOEPP et al., 2014; SERAFINI et al., 2013). Photosensitivity is a striking feature of JME in humans and dogs. A PPR upon photic stimulation is observed in 30-50% of human patients (up to 90% with prolonged stimulation), although clinical photosensitivity is only encountered in 15% (ALFRADIQUE & VASCONCELOS, 2007; ANDERSON & HAMANDI, 2011; APPLETON et al., 2000; BAYKAN & WOLF, 2017; GENTON et al., 2013; NICOLSON & MARSON, 2010; SERAFINI et al., 2013; WELTY, 2006; WOLF et al., 2015). In contrast, all dogs that showed a PPR upon photic stimulation, also had photosensitivity in daily life (35%). Short elimination half-life (90-120 min) and hepatotoxicity prevent the use of valproic acid in dogs, which is the first choice

AED in humans with JME, having an efficacy of up to 85-90% (ALFRADIQUE & VASCONCELOS, 2007; BAYKAN & WOLF, 2017; BAYKAN et al., 2013; WELTY, 2006; YACUBIAN, 2017). Therefore, other AEDs with an appropriate pharmacokinetic profile have been used in dogs. The best results were achieved with LEV, which is also an effective drug for JME in humans, and KBr was also effective (NOACHTAR et al., 2008). Although JME in humans is a genetically heterogenous disorder, one associated microdeletion at 15q13.3 encompasses *CHRNA7* as the prime candidate gene (HELBIG et al., 2009). This gene codes for the α7 subunit of the nAChR (HELBIG et al., 2009). Juvenile myoclonic epilepsy in RRs is caused by a deletion mutation in *DIRAS1*, which has been proposed to play a role in presynaptic ACh release. Therefore, the pathomechanism of both diseases may implicate abnormal cholinergic neurotransmission.

4.2. Myoclonic Epilepsy of Infancy

Myoclonic epilepsy of infancy (MEI) is an idiopathic generalized epilepsy syndrome that occurs in children with normal development in the first three years of life (CARABALLO et al., 2013). Infants have brief, spontaneous and/or reflex, generalized myoclonic seizures during wakefulness and drowsiness, most notably in the upper limbs and head (CARABALLO et al., 2013; DARRA et al., 2006). Electroencephalography reveals ictal and interictal generalized SWC, polyspikes, and PSWC, and some patients are photosensitive (CARABALLO et al., 2013; ZUBERI & O'REGAN, 2006). Other seizure types do not occur (CARABALLO et al., 2013). Prognosis is good in terms of seizure control, but about one third of patients develop neuropsychological problems, and in rare cases other epilepsy syndromes may develop after remission of myoclonic seizures (CARABALLO et al., 2013).

A syndrome comparable to MEI in humans has not been observed in dogs to date. Juvenile myoclonic epilepsy in RRs shares many features with MEI, including EEG findings, photosensitivity, and otherwise normal development. Although the mean age of onset occurs in juvenile RRs, a substantial number of dogs have a much earlier onset, mirroring the infantile age of onset observed in MEI. However, a large proportion of RRs develop other seizure types such as GTCS or absence seizures in addition to myoclonic seizures. Furthermore, remission has not been observed in any of the affected RRs, and thus it remains a lifelong epilepsy.

4.3. Nocturnal Frontal Lobe Epilepsy

Nocturnal frontal lobe epilepsy is an idiopathic focal epilepsy syndrome originating in the frontal lobe (FERINI-STRAMBI et al., 2012; NOBILI et al., 2014). Seizures occur predominantly during non-rapid eye movement (non-REM) sleep, especially during light (stage two) sleep (PROVINI et al., 1999). They tend to coincide with K-complexes and often recur in a quasi-periodic rhythm at a rate similar to that of K-complexes (PROVINI et al., 1999). A third of patients occasionally experience seizures during daytime wakefulness, and another third have rare secondarily generalized seizures (PROVINI et al., 2000a). The syndrome is characterized by a wide spectrum of clinical features of variable duration and complexity (NOBILI et al., 2014). Three subtypes, which are considered different aspects of one heterogeneous syndrome, are distinguished: (a) paroxysmal arousals: brief events with a duration of less than 20 s manifesting as abrupt arousals accompanied by stereotyped and often dystonic movements; (b) nocturnal paroxysmal dystonia: episodes, lasting up to two minutes, with wide, dyskinetic, dystonic, or even ballistic movements; and (c) episodic nocturnal wanderings: events of up to three minutes duration characterized by paroxysmal, agitated, and stereotyped ambulation and vocalization (HALÁSZ et al., 2012; PROVINI et al., 1999, 2000a, 2000b). Consciousness is often preserved throughout the attacks (SCHEFFER, 2000). Nocturnal frontal lobe epilepsy has a mean age of onset of 14 years and occurs predominantly in males (PROVINI et al., 1999). Most patients benefit from carbamazepine, although about one third are drug resistant (NOBILI et al., 2014; PROVINI et al., 1999). There is a positive family history in 25% of patients (FERINI-STRAMBI et al., 2012). Autosomal dominant nocturnal frontal lobe epilepsy is a familial variant of NFLE. It has an autosomal dominant mode of inheritance and does not differ from the sporadic form of NFLE with respect to clinical and electroencephalographic findings (PROVINI et al., 1999). To date, several mutations in a total of six genes have been identified in the context of ADNFLE; three of the genes are associated with the neuronal nAChR (NOBILI et al., 2014). The diagnosis of NFLE is often challenging because the clinical symptoms can be mistaken for non-REM sleep parasomnias (PROVINI et al., 1999). This is further complicated by the fact that interictal wake and sleep EEG as well as ictal EEG are normal in about half of the patients, and only 8% show clear-cut epileptic activity (spikes or SWC) on ictal EEG (PROVINI et al., 1999).

To date, there are no reports of a disorder comparable to NFLE in dogs. The results of the present study revealed an epileptic disorder that was predominantly sleep-associated and in which some dogs experienced rare episodes during daytime wakefulness similar to the diurnal pattern observed in NFLE. Some RRs developed GTCS, and in addition, disease onset occurred in juvenile dogs. The genetic background was identified with a proposed involvement of presynaptic ACh release. However, the disease has an autosomal recessive mode of inheritance. Moreover, the type of movement encountered in the dogs in the present study was myoclonic rather than dystonic, and there was no gender predisposition. Another issue is that an electroencephalographic correlate comprising ictal and interictal generalized SWC was found in a great majority of the dogs examined.

4.4. REM sleep behaviour disorder

Rapid eye movement (REM) sleep behaviour disorder (RBD) is not an epilepsy syndrome but instead a sleep disorder. Nevertheless, it is given consideration in this discussion because of its association with sleep and myoclonus-like properties.

Rapid eye movement sleep behaviour disorder is a parasomnia characterized by a dream enactment behaviour during REM sleep comprising abnormal motor behaviour, abnormal vocalization, and an altered dream mentation (BOEVE, 2010). Motor activity is often very violent and manifests in humans as punching, kicking, beating, biting, flailing, running, or jumping out of bed (BOEVE, 2010; IRANZO et al., 2009). In healthy individuals, there is minimal skeletal muscle activity during REM sleep due to activation of inhibitory neurons in the pons and medulla oblongata, which inhibit lower motor neuron ventral horn cells in the brainstem and spinal cord (BOEVE et al., 2007; SCHUBERT et al., 2011). In RBD, skeletal muscle atonia during REM sleep is lost leading to the above-mentioned vigorous limb movements (BOEVE et al., 2007). Because dream enactment behaviour resembles other diseases, such as obstructive sleep apnoea, somnambulism, and nocturnal epilepsy, most experts agree that a polysomnography is required for the clinical diagnosis of RBD and to prove the presence of REM sleep without atonia and the absence of epileptiform activity on EEG (BOEVE et al., 2007). In human medicine, men between the age of 50 and 80 years are overrepresented, and RBD is often associated with neurodegenerative diseases including Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy (BOEVE, 2010, 2013). The majority of patients respond well to clonazepam and/or melatonin (BOEVE,

2010).

Rapid eye movement sleep behaviour disorder has been reported in dogs as well (BUSH et al., 2004; SCHUBERT et al., 2011). Violent limb movements, chewing, teeth grinding, biting, growling, howling, and barking were the presenting features (SCHUBERT et al., 2011). A gender predisposition has not been detected to date (BUSH et al., 2004; SCHUBERT et al., 2011). Schubert et al. proposed that two different forms of RBD occur in dogs: a form that has a juvenile onset (two years of age or less) and a form that has an adult onset (three years of age and older), with 71% having a juvenile onset in that study (SCHUBERT et al., 2011). In contrast to humans, dogs do not appear to benefit from clonazepam or melatonin, but KBr and tricyclic antidepressants have been shown to be effective (BUSH et al., 2004; SCHUBERT et al., 2011).

The RRs in the present study also experienced vigorous limb movements, which were associated predominantly with sleep. Unfortunately, a polysomnography, which would require inclusion of EMG recordings, was not used. Therefore, a clear distinction between the different sleep stages and an exclusion of the presence of REM sleep without atonia was not possible. However, those episodes were observed in all dogs during drowsiness as well as in awake dogs that were in lateral or sternal recumbency or even in a standing position, making a sole parasomnia unlikely. Moreover, the epileptic nature of the events was confirmed by EEG. Some RRs responded very well to KBr similar to the dogs in the study of Schubert et al., but as KBr is an AED, the effect seen in the RRs of the present study can be explained by the anticonvulsant properties of KBr (SCHUBERT et al., 2011).

4.5. Progressive myoclonic epilepsies

In human beings, the PMEs are an uncommon and heterogeneous group of inherited neurodegenerative disorders that are resistant to treatment, leading to a debilitating course and poor outcome (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). Common features are myoclonic seizures, other types of seizures, particularly GTCS, and progressive neurological dysfunction, especially dementia and ataxia (DE SIQUEIRA, 2010; SHAHWAN et al., 2005; SHIELDS, 2004). Most patients with PMEs present in late childhood or adolescence, and with the exception of myoclonic epilepsy with ragged red fibres (MERRF), dentatorubral-pallidoluysian atrophy (DRPLA), and some forms of NCLs (see below) have an autosomal

recessive mode of inheritance (SHIELDS, 2004). Electroencephalography may be normal at the beginning of the disease, but as the disease progresses, background slowing and epileptiform discharges become apparent in most cases (SHAHWAN et al., 2005). In addition to other rare forms of this syndrome, there are six main causes of PME (DE SIQUEIRA, 2010).

The most common one is Unverricht-Lundborg disease, an autosomal recessive disorder associated with mutations in *CSTB* encoding cystatin B (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). Myoclonus is stimulus-sensitive (visual, auditory, and proprioceptive) and prevails upon awakening (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). Electroencephalography shows a high sensitivity to photic stimulation (SHAHWAN et al., 2005). The disease is progressive but seems to stabilize after age 40, and life expectancy is not overly shortened (DE SIQUEIRA, 2010; SHAHWAN et al., 2005; SHIELDS, 2004).

Lafora disease has an autosomal recessive mode of inheritance and is caused by mutations in the genes *EPM2A* or *NHLRC1* (formerly *EPM2B*) (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). Apart from myoclonus and other seizure types, patients typically show occipital seizures with transient blindness and visual hallucinations, and photosensitivity is a common feature (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). As there is rapid and severe progression of the disease, most patients die within ten years after onset (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). Diagnosis can be established by demonstration of the pathognomonic Lafora bodies, which are PAS-positive polyglucosan inclusions found in neurons and other tissues, especially the excretory ducts of eccrine sweat glands (CONRY, 2002; DE SIQUEIRA, 2010; SHAHWAN et al., 2005).

Myoclonic epilepsy with ragged red fibres is transmitted by mitochondrial inheritance (SHAHWAN et al., 2005). In addition to the typical signs of PME, myopathy, neuropathy, hearing loss, and optic atrophy are often present (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). The course of the disease may be slowly or rapidly progressive (CONRY, 2002; LEPPIK, 2003). Muscle biopsy reveals ragged red fibres in over 90% of patients (SHAHWAN et al., 2005).

The NCLs are a group of neurodegenerative disorders (LEPPIK, 2003). A characteristic finding is the accumulation of autofluorescent ceroid lipofuscin (lipopigments) in different tissues (DE SIQUEIRA, 2010). To date, 14 genes have

been associated with NCL, and 13 genes have already been identified (*CLN1-CLN8* and *CL10-CLN14*) (MOLE & COTMAN, 2015; NITA et al., 2016). The great majority of NCLs have an autosomal recessive mode of inheritance (MOLE & COTMAN, 2015). Loss of vision occurs in most cases (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). The clinical course is characterized by rapid deterioration and premature death (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). Detection of intracellular inclusions by electron microscopy in eccrine secretory cells and conjunctival, muscle, or rectal mucosa biopsy specimens is used to confirm the diagnosis (DE SIQUEIRA, 2010; SHAHWAN et al., 2005).

Sialidosis type I and type II are autosomal recessive disorders that belong to the group of lysosomal storage disorders and are related to mutations in the *NEU1* gene causing a deficiency of alpha-N-acetyl neuraminidase-1 (CACIOTTI et al., 2009; SHAHWAN et al., 2005). Patients show pure action and intention myoclonus, evolution of visual failure, and a slow clinical progression without mental deterioration (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). Fundoscopy reveals characteristic cherry-red spots in the macula (DE SIQUEIRA, 2010). Evaluation of urine for sialyloligosaccharides and demonstrating a deficiency of neuraminidase activity in leucocytes or cultured fibroblasts are used to diagnose this disorder (LEPPIK, 2003; SHAHWAN et al., 2005).

The autosomal dominant disorder DRPLA is caused by an unstable expansion of CAG repeats of a gene at 12p13.31 and can be divided into three clinical forms: a choreoathetoid, a pseudo-Huntington, and a PME form (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). Patients under the age of 20 usually present with the PME form (DE SIQUEIRA, 2010; SHAHWAN et al., 2005). Magnetic resonance imaging reveals atrophy of the cerebellum and brainstem, and pathological examination shows degeneration of the rubral and dentato-pallidoluysian systems (SHAHWAN et al., 2005).

The main difference between JME in RRs and the PMEs is the lack of a progressive course of disease; dogs in the present study did not develop neurological dysfunction such as ataxia or have cognitive decline. In addition, Lafora disease and NCL were ruled out based on the absence of PAS-positive inclusion bodies or NCL specific autofluorescent inclusion bodies on post-mortem or skin biopsy evaluation. Similarly, MERRF was ruled out because of the lack of ragged red fibres in muscle biopsy samples as well as the absence of any neuro- or myopathy

and visual or auditory deficits. Furthermore, unremarkable ophthalmic examination and metabolic screening for oligosaccharides did not support a diagnosis of sialidosis in the RRs of the present study. A diagnosis of DRLPA was also considered unlikely because there was no ataxia, dementia, or progressive course of disease, and the results of MRI and pathological examination were unremarkable.

In summary, all of the above-mentioned syndromes share some features with JME in RRs. However, RBD, a parasomnia, could be ruled out because affected dogs also have myoclonic twitches during wakefulness in a standing position and EEG clearly demonstrated the epileptic nature of the episodes in question. Similarly, NFLE was considered unlikely because EEG was indicative of a generalized and not a focal epilepsy syndrome. Myoclonic epilepsy of infancy shares many similarities with JME in RRs. However, MEI was considered less likely because the majority of dogs had a juvenile age of onset, developed other seizure types besides myoclonic seizures, and lacked a remitting course of disease. The PMEs were excluded because of the lack of a progressive course of disease and structural alterations in brain imaging and necropsy. In contrast, JME in humans reveals striking similarities to JME in RRs. They both have a juvenile age of onset, myoclonic seizures as the prevailing seizure type with progression to generalized tonic-clonic or absence seizures, similar EEG features, photosensitivity in a substantial portion of patients, and good response to LEV treatment. Therefore, JME in RRs is considered a valuable translational model for the investigation of clinical, genetic, and therapeutic aspects of human JME, as well as photosensitivity.

V. PERSPECTIVE

The present study shows that long-term wireless video-EEG in unsedated dogs is a feasible and valuable technique to investigate the epileptic background of paroxysmal events and to further characterize the EEG patterns of specific epilepsy syndromes. This novel method was used to confirm the diagnosis of myoclonic seizures and absence seizures in RRs with JME, two seizure types that were previously underrecognized in dogs with idiopathic epilepsy. The current veterinary literature focuses on focal and generalized tonic-clonic seizures. Other seizure types have been neglected. The present study determined that the spectrum of seizure types in dogs is much broader than expected. In the future, the use of wireless video-EEG in unsedated dogs will enable the diagnosis of myoclonic and absence epilepsy and facilitate the identification of new seizures types that have not yet been recognized in dogs.

There are laboratories that now offer genetic testing for the *DIRAS1* variant, which facilitates the development of breeding programs aimed at preventing this disease. It is thus conceivable that the disease will vanish with time and the percentage of heterozygous carriers will decrease. Therefore, the findings of the present study may help to improve the health of the RR population.

In human medicine, the treatment regimen for epilepsy is determined by the specific seizure type and epilepsy syndrome. Treatment based on this type of differentiation does not exist in veterinary neurology, where a small number of veterinary licensed products are used for all types of epilepsy syndromes. Therefore, future treatment studies should focus on the specific epilepsy syndrome to evaluate the efficacy of a particular AED. Moreover, veterinarians should attempt to make a definitive diagnosis of the precise type of epilepsy and choose an appropriate AED.

Genetic analyses of the cohort in the present study were done in a joint approach with researchers at the University of Helsinki and identified a fully penetrant 4-bp deletion mutation in the *DIRAS1* gene with a proposed role in presynaptic ACh release (TADA et al., 2012). It was shown that important parallels exist between JME in RRs and human JME, which is one of the most common types of IGEs in people (SERAFINI et al., 2013). Common features include young age of onset, myoclonic seizures as the predominant seizure type with propagation to generalized

tonic-clonic or absence seizures, and similarities in EEG characteristics, photosensitivity, and response to LEV treatment. The findings of the present study provide evidence for a role of *DIRAS1* in epilepsy and a base to further elucidate the function of this gene and the contribution of cholinergic neurotransmission in myoclonic epilepsies. Moreover, RRs may represent a valuable large animal translational model for clinical, therapeutic. and genetic aspects of human epilepsies and photosensitivity.

The present study gave rise to a number of subsequent investigations. The study by Waltz. et al. served as a model to further investigate the impact and genetics of photosensitivity in RRs with JME (WALTZ et al., 1992). With that purpose in mind, affected dogs and their families and relatives will be examined by video-EEG with photic stimulation. Furthermore, two different approaches will be used to generate translational animal models. In addition to a transgenic mouse line, morpholinos will be used to produce a knockout of the *diras1a* and/or *diras1b* gene in zebrafish larvae. These two models will then be used to further investigate the function of *DIRAS1* and to validate the efficacy of a variety of well-established as well as novel AEDs. Moreover, these models will be used to study the role of *DIRAS1* as a tumour-suppressor gene. The results of these studies will hopefully contribute to better understanding and treatment of JME in humans and dogs.

VI. SUMMARY

Dogs represent a promising large animal translational model for human disease. The aim of the present study was to provide a detailed clinical and electroencephalographic description of a novel myoclonic epilepsy syndrome in Rhodesian Ridgeback (RR) dogs and to investigate the underlying genetic cause. The following were required for inclusion of dogs in the study: on-site observation of myoclonic jerks or video recordings of seizure activity that were available for review, the results of neurological examination, and data collected from owner interview or completion of an online questionnaire. Extensive diagnostic work-up (blood examination, magnetic resonance imaging and/or computed tomography, cerebrospinal fluid examination, urine neurometabolic screening) was offered. Wireless video-electroencephalography (EEG) in unsedated dogs was established as a tool to investigate the epileptic nature of the myoclonic jerks and to characterize the EEG features of the electroclinical syndrome. Recordings were a minimum of one hour in length. Electroencephalography with photic stimulation was used in a subset of dogs. Pedigree analyses were conducted, and blood samples were collected for subsequent genetic analyses in collaboration with the University of Helsinki to identify the underlying genetic cause.

Ninety-five RRs were evaluated, and 24 met the inclusion criteria. These dogs shared a common phenotype. The hallmark of the epilepsy was vigorous myoclonic seizures that occurred mainly during periods of relaxation. Age of onset varied from six weeks to 1.5 years (mean six months). In more than one third of the dogs, the disease progressed to include generalized tonic-clonic seizures (GTCS), and a third of the dogs were clinically photosensitive. Another feature of juvenile myoclonic epilepsy (JME) in RRs was absence seizures. The results of magnetic resonance imaging of the brain and post-mortem examination were unremarkable and served to rule out a structural epilepsy. Ambulatory video-EEG confirmed the epileptic origin of the myoclonic twitches. Typically, affected dogs showed myoclonic seizures accompanied by generalized 4-5 Hz spike-wave complexes (SWC) and polyspike-wave complexes with a frontocentral maximum. Absence seizures were associated with generalized 4 Hz SWC. Two thirds of the dogs examined showed photoconvulsions upon photic stimulation. The best treatment results were achieved with levetiracetam or potassium bromide. Pedigree analyses were consistent with

an autosomal recessive mode of inheritance. Genetic analyses identified a fully penetrant recessive 4-bp truncating deletion mutation in the *DIRAS1* gene, which is involved in presynaptic acetylcholine release (TADA et al., 2012).

Wireless video-EEG in unsedated dogs is a novel, feasible, and valuable technique for the investigation of paroxysmal events of unknown aetiology in veterinary clinics. This diagnostic method will presumably influence the approach to diagnosis of canine epilepsy and facilitate the identification of further seizure types that have not yet been recognized in dogs. Furthermore, JME in RRs has important parallels to JME in humans, including young age of onset, myoclonic seizures as the predominant seizure type with propagation to GTCS or absence seizures, and similar EEG characteristics, photosensitivity, and response to treatment with levetiracetam. Comparison of spontaneous canine and human epilepsies offers a unique opportunity to study the clinical, genetic, and therapeutic aspects of epilepsies and photosensitivity in a translational setting.

VII. ZUSAMMENFASSUNG

Hunde stellen ein vielversprechendes translationales Tiermodell für Erkrankungen des Menschen dar. Ziel der vorliegenden Arbeit war es, ein neues myoklonisches Epilepsiesyndrom bei Rhodesian Ridgeback (RR) Hunden klinisch und elektroenzephalographisch zu charakterisieren und die zugrundeliegende genetische Ursache zu identifizieren. Einschlusskriterien waren direkte Beobachtung der myoklonischen Zuckungen oder zur Auswertung verfügbare Videoaufzeichnungen, eine neurologische Untersuchung und ein persönliches Interview oder die Beantwortung eines Online-Fragebogens. Eine umfangreiche Diagnostik (Blutuntersuchung, Magnetresonanztomographie, neurometabolisches Screening des Urins) wurde angeboten. Die kabellose Video-Elektroenzephalographie (EEG) wurde als Untersuchungsmethode bei unsedierten Hunden etabliert, um den epileptischen Ursprung der Myoklonien zu untersuchen und das EEG der erkrankten Hunde im Detail zu beschreiben. Die Dauer einer EEG-Untersuchung betrug mindestens eine Stunde. Im Anschluss wurde bei einem Teil der RRs eine Photostimulation durchgeführt. Die Stammbäume der betroffenen RRs wurden analysiert und Blutproben für nachfolgende genetische Analysen in Zusammenarbeit mit der Universität Helsinki gesammelt.

Die Krankengeschichten, Online-Fragebögen und Videoaufzeichnungen von 95 RRs wurden ausgewertet, von denen 24 RRs die Einschlusskriterien erfüllten und einen gemeinsamen Phänotyp aufwiesen. Hauptcharakteristikum der Erkrankung waren heftige Myoklonien, die überwiegend in Ruhephasen auftraten. Der Krankheitsbeginn lag zwischen sechs Wochen und 1,5 Jahren (sechs Monate im Durchschnitt). Mehr als ein Drittel der Hunde entwickelte im Verlauf auch generalisierte tonisch-klonische Anfälle. Photosensibilität wurde bei einem Drittel der Hunde beschrieben. Ein weiteres Merkmal der juvenilen myoklonischen Epilepsie (JME) des RRs waren Absence Anfälle. Die unauffälligen Befunde der Kernspintomographie und pathologischen Untersuchung des Gehirns schlossen eine strukturelle Epilepsie aus. Das ambulatorische Video-EEG bestätigte den epileptischen Ursprung der Myoklonien. Betroffene Hunde zeigten im EEG generalisierte 4-5 Hz Spike-Wave Komplexe und Polyspike-Wave Komplexe mit einem frontozentralen Maximum während der myoklonischen Anfälle. Absencen waren mit generalisierten 4 Hz Spike-Wave Komplexen assoziiert. Bei der

Photostimulation zeigten zwei Drittel der untersuchten Hunde Photokonvulsionen. Die besten Behandlungserfolge wurden mit Levetiracetam oder Kaliumbromid erzielt. Die Stammbaumanalysen wiesen auf eine autosomal-rezessive Erkrankung hin. Die genetischen Analysen identifizierten eine vollständig penetrante, rezessive, vier basenpaargroße trunkierende Deletionsmutation im *DIRAS1* Gen, welches eine Rolle bei der präsynaptischen Freisetzung von Acetylcholin spielen soll (TADA et al., 2012).

Das kabellose Video-EEG bei unsedierten Hunden erwies sich als eine praktikable und wertvolle Technik zur Klärung des epileptischen Ursprungs von paroxysmalen Episoden unbekannter Ursache. Diese Methode kann die Vorgehensweise bei der Diagnostik von Epilepsien des Hundes beeinflussen und die Identifizierung von epileptischen Anfällen ermöglichen. Die JME des RRs weist wichtige Parallelen zur JME des Menschen auf, darunter der frühe Krankheitsbeginn, das Auftreten von myoklonischen Anfällen als vorwiegendem Anfallstyp mit einem Fortschreiten zu generalisierten tonisch-klonischen Anfällen oder Absence Anfällen, ähnliche EEG-Muster, Photosensibilität und gutes Ansprechen auf eine Therapie mit Levetiracetam. Der Vergleich zwischen Epilepsien des Hundes und des Menschen ermöglicht es in einer translationalen Herangehensweise, klinische, genetische und therapeutische Aspekte von Epilepsien und Photosensibilität zu untersuchen.

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