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BENIGN FAMILIAL JUVENILE EPILEPSY IN
LAGOTTO ROMAGNOLO DOGS

Tarja Pääkkönen (Jokinen)

ACADEMIC DISSERTATION

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Helsinki 2016
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To My Family
ABSTRACT

Although the immature brain reportedly is more prone to seizure activity than the mature brain, there are no previous reports on well-defined juvenile epilepsy syndromes in dogs. This study describes a novel juvenile epilepsy syndrome in Lagotto Romagnolo (LR) dogs, namely benign familial juvenile epilepsy (BFJE).

We studied the clinical characteristics of this novel syndrome in 25 affected dogs, while healthy littermates of the affected dogs served as controls. The mean age at onset of focal seizures is 6 weeks, and spontaneous remission of seizures usually occurs by the age of 4 months. Between the seizure episodes, most of the affected puppies are neurologically normal, but puppies with the most severe seizure episodes exhibit some neurological deficits interictally. These deficits also resolve with remission of seizures. Interictal electroencephalography (EEG) shows focal abnormalities, including sharp waves and spikes, in most (88%) of the affected dogs.

Conventional imaging examinations, including magnetic resonance imaging (MRI), show no remarkable focal abnormalities in dogs with BFJE. Positron emission tomography (PET) is a nuclear neuroimaging modality that is able to detect abnormal metabolism in the epileptic focus of the brain. We investigated glucose metabolism of the brain in 6 affected and 5 control dogs using radiolabeled glucose, namely 2-[¹⁸F]fluoro-2-deoxy-D-glucose (FDG), as a tracer. In dogs with BFJE, FDG-PET shows areas of hypometabolism with good correspondence to focal EEG findings, thus supporting the area of abnormal metabolism being the epileptic zone.

Furthermore, we performed a follow-up study by utilizing two previously validated questionnaires on impulsivity and activity levels in dogs, and additionally, we telephone-interviewed the owners of the affected dogs. We evaluated the results based on the data collected for 25 dogs with a history of BFJE and 91 control dogs. We utilized principal component analysis to explore the factorial structure of the questionnaire. Although the life span of affected dogs seems to be comparable with that of healthy control dogs and recurrence of seizures after remission is rare, the dogs with a history of BFJE exhibit abnormalities in behavior reminiscent of attention deficit hyperactivity disorder (ADHD) in humans.

This study also reveals the mode of inheritance and the genetic defect behind BFJE. Based on pedigree analysis, we found that BFJE is inherited in a recessive Mendelian form. We further found that a mutation in the gene encoding for protein LGI2 is responsible for BFJE. LGI2, as well as LGI1, interacts with neuronal membrane proteins, namely ADAM22 and ADAM23, in synaptic transmission. LGI1, ADAM22, and ADAM23 have previously been shown to be important in the development of epilepsy, and this study reveals the importance of LGI2 in epileptogenesis of BFJE.
TIIVISTELMÄ

Vaikka epäkypsien aivojen tiedetään olevan täysikasvuisen yksilön aivoja alttiimpia kohtauksille, koirilla ei ole koskaan aiemmin raportoitu nuoruussiin epilepsiaoireyhtymää. Tämä tutkimus kuvaa uudenlaisen nuoruussiin epilepsiaoireyhtymän, benignin familiaalisen juveniilin epilepsian (BFJE), lagotto romagnolo -rotuisilla koirilla.


Perinteisillä kuvantamismenetelmissä, kuten magneettikuvauksella, ei nähdä merkittäviä muutoksia koirilla, joilla on BFJE. Positroniemissiotomografia (PET) on isotooppikuvantamismenetelmä, jonka avulla voidaan havaita aivojen epilepsiaakeskuksen epänormaali aineenvaihdunta. Tutkimme aivojen glukoosin aineenvaihduntaa 6 sairaan ja 5 kontrollikoiran avulla hyödyntäen merkkiaineena radioaktiivista glukoosiossa, 2-[18F]fluoro-2-deoksi-D-glukoosiossa (FDG). Koirilla, joilla on BFJE, havaitaan FDG-PET tutkimuksen avulla alentuneen aineenvaihdunnan alueita, jotka vastaavat hyvin paikallisia EEG -löydöksiä. Tämä tukee siten käsitystä, että epänormaalin aineenvaihdunnan alueet edustavat epilepsiaakeskusta.


Tämä tutkimus paljastaa myös BFJE:n periyttymismallin ja sairauden taustalla olevan geenimutaation. Totesimme sukutaulua analysoimalla BFJE:n periytymän reessivistä. Lisäksi selvitimme, että mutaatio LGI2 proteiinia koodaavassa geenissä aiheuttaa BFJE:n. LGI1 tavoin LGI2 on vuorovaikutuksessa hermosolujen solukalvoproteiinien, ADAM22 ja
ADAM23, kanssa hermosolujen välisessä tiedonvälityksessä. LGI1, ADAM22 ja ADAM23 on aiemmin todettu olevan tärkeitä epilepsian kehittymisessä, ja tämä tutkimus osoittaa LGI2 proteiinin merkityksen BFJE:n epileptogeenesissä.
ACKNOWLEDGMENTS

The work described here was mainly carried out at the Department of Equine and Small Animal Medicine and the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Helsinki, Finland. In addition, some of the dogs were examined at the Referral Neurology Hospital Aisti, Vantaa, Finland. Diagnostic imaging examinations were performed at Aisti, at the private human hospital Magneettimehiläinen, and at the Turku PET Centre. All collaboration is warmly acknowledged.

I am deeply grateful to Docent Liisa Metsähonkala, my supervisor, for supporting me through all of the projects. It has been a privilege to be guided by such a generous and esteemed clinician and scientist.

My sincere gratitude is also owed to my other supervisor, Professor Hannes Lohi, who has a contagious enthusiasm when it comes to inherited neurological diseases in dogs. Without his work on genes, this project could not have been completed.

I am grateful to Outi Vapaavuori, the director of these studies, for encouraging me to go on with the thesis during those times when I was not so sure where to go. I also thank her for all invaluable and numerous comments on the studies and the thesis.

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This thesis would never have been finished without Anna-Mariam Kiviranta, my own resident and now an ECVN diplomate, who has efficiently worked at the clinic while I’ve buried myself in my office with the thesis. I also thank our neurology nurse, Tiina Heinänen, for being such a wonderful person to work with and for always knowing what to do before anyone says a word.

I warmly thank my co-author Merja Haaparanta-Solin, who always took the time to inquire about the progress in the PET study. It was a great pleasure to work with her and all personnel at the Turku PET Centre.
I am grateful that these studies gave me the opportunity to work with all of my other co-authors from different fields, including Eija Seppälä to whom I sent all blood samples from Lagottos, Katriina Tiira, who helped me to investigate behavior in dogs, Pernilla Syrjä, the pathologist with a special interest in neuropathology, Anna Hielm-Björkman, who helped me with the statistics, and Lucy Bergamasco, who interpreted the electroencephalography.

Carol Ann Pelli is warmly thanked for editing the language of this thesis.

I thank my colleagues at the Department of Equine and Small Animal Medicine and the Veterinary Teaching Hospital, Faculty of Veterinary Medicine. The people who finished their thesis before me inspired me to finish mine. I am also grateful for the support of my friends outside the Faculty.

Lagotto dog owners and breeders have been extremely helpful, and I was touched by their willingness to share information with researchers.

My deepest thanks go to my mother for always encouraging me and saying that I can do anything I set my mind to do. I am also grateful to her for taking care of our children whenever needed. I warmly thank my sister Minna for patiently listening to my endless stories, although I think her main interests did not include epilepsy in dogs.

This thesis is dedicated to my dear family. I thank my beloved husband Pekka, who has suffered great neglect due to my work. Thank you for taking care of our children Jonni and Jessica, and for standing beside me through this long project. You mean everything to me.

Helsinki 29.9.2015

Tarja Pääkkönen
# CONTENTS

Abstract................................................................................................................................................. 4  
Tiivistelmä ............................................................................................................................................... 5
Acknowledgments..................................................................................................................................... 7
Contents............................................................................................................................................... 9 
List of original publications .................................................................................................................. 13
Abbreviations ........................................................................................................................................ 14

1 Introduction ......................................................................................................................................17
2 Review of the literature .................................................................................................................... 19
    2.1 Epilepsy; definitions and classifications in dogs and in humans..................................................... 19
        2.1.1 Classification of epilepsy based on seizure type ........................................................................ 20
        2.1.2 Etiologic classification of epilepsy ............................................................................................. 21
    2.2 Clinical signs of epileptic seizures ................................................................................................. 23
        2.2.1 Classification of ictal signs ........................................................................................................ 23
    2.3 Idiopathic epilepsy in dogs ......................................................................................................... 24
        2.3.1 Age at onset .................................................................................................................................. 24
        2.3.2 Seizure type ............................................................................................................................... 25
    2.4 Diagnosis of idiopathic epilepsy in dogs ....................................................................................... 26
        2.4.1 Neurological examination ........................................................................................................ 26
        2.4.2 Magnetic resonance imaging ................................................................................................... 27
        2.4.3 Cerebrospinal fluid .................................................................................................................... 28
        2.4.4 Electroencephalography ............................................................................................................ 29
    2.5 Genetic epilepsy in children ........................................................................................................... 30
        2.5.1 Benign epilepsy syndromes of childhood .................................................................................. 30
2.5.1.1 Electroencephalography in benign childhood epilepsies ................................................................. 31

2.5.2 Mechanisms contributing to the higher seizure susceptibility of the immature brain ................................................................. 31

2.6 Functional neuroimaging in epilepsy ................................................... 33

2.6.1 Functional neuroimaging in epilepsy of dogs .................................. 34

2.6.2 Analysis of FDG-PET data ................................................................. 34

2.7 Genetics of epilepsy ................................................................................ 35

2.7.1 General ................................................................................................. 35

2.7.2 Focal genetic epilepsies in children ................................................... 37

2.7.3 Genetics of epilepsy in dogs .............................................................. 38

2.8 Long-term effects and outcome of epilepsy ........................................ 38

2.8.1 Neurobehavioral comorbidities in humans ..................................... 38

2.8.1.1 Cognitive comorbidities ......................................................... 39

2.8.1.2 Psychiatric comorbidities .......................................................... 39

2.8.1.3 Social comorbidities.................................................................. 39

2.8.1.4 Etiology for comorbidities........................................................... 40

2.8.2 Neurobehavioral comorbidities in dogs ............................................ 41

2.8.3 Outcome of epilepsy ............................................................................ 41

3 Aims of the study ............................................................................................ 43

4 Materials and methods .................................................................................. 44

4.1 Ethical approval of study protocols ..................................................... 44

4.2 Dogs ........................................................................................................ 44

4.2.1 Affected dogs ...................................................................................... 44

4.2.2 Control dogs....................................................................................... 44

4.3 History and clinical and neurological examination (I) ....................... 45

4.4 Laboratory analyses (I,III) ................................................................. 45

4.5 Electrodiagnostics ................................................................................ 45
4.5.1 Electromyography (EMG) and brainstem auditory evoked response (BAER) (I) .............................................................. 45
4.5.2 Electroencephalography (EEG) (I, III) ................................................. 46
4.6 Diagnostic imaging .............................................................................. 46
  4.6.1 Magnetic resonance imaging (MRI) (I, III) ..................................... 46
  4.6.2 Positron emission tomography (PET) (III) .................................... 47
4.7 Genetics .............................................................................................. 48
  4.7.1 Pedigree analysis (I) ...................................................................... 48
  4.7.2 Detection of causative mutation (II) .............................................. 48
4.8 Behavioral questionnaire (IV) ............................................................. 49
4.9 Statistical analysis .............................................................................. 49
  4.9.1 Study I .......................................................................................... 49
  4.9.2 Study II ......................................................................................... 49
  4.9.3 Study III ....................................................................................... 50
  4.9.4 Study IV ....................................................................................... 50
5 Results .................................................................................................... 52
  5.1 History and clinical and neurological examination ......................... 52
    5.1.1 Seizure characteristics in affected dogs with juvenile-onset seizures ........................................................................ 52
    5.1.2 Seizure characteristics in affected dogs with adult-onset seizures ........................................................................ 53
    5.1.3 Physical and neurological examination ...................................... 53
  5.2 Laboratory analyses and electrodiagnostics ...................................... 53
  5.3 Diagnostic imaging ........................................................................... 54
    5.3.1 Magnetic resonance imaging (MRI) .......................................... 54
    5.3.2 FDG-PET ................................................................................... 54
  5.4 Genetics (I, II) ................................................................................ 57
  5.5 Long-term follow-up and neurobehavioral comorbidities (IV) ....... 59
6 Discussion .......................................................................................................................... 62
6.1 Recognition of a juvenile epilepsy syndrome in dogs .......................... 62
6.2 Focal abnormalities in glucose metabolism in canine focal idiopathic epilepsy ................................................................................................................. 63
6.3 BFJE is associated with long-lasting neurobehavioral comorbidities ......................................................................................................................... 64
6.4 Identification of causative mutation............................................................... 66
6.5 LGI proteins and epilepsy................................................................................ 67
6.6 Dogs serving as models for human epilepsy research............................ 69
6.7 Future aspects ........................................................................................................ 70
7 Conclusions .................................................................................................................. 71
References ......................................................................................................................... 72
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:


These publications are referred to in the text by their Roman numerals.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ADAM</td>
<td>a-disintegrin-and-metalloproteinase</td>
</tr>
<tr>
<td>ADHD</td>
<td>attention deficit hyperactivity disorder</td>
</tr>
<tr>
<td>ADLTE</td>
<td>autosomal dominant lateral temporal lobe epilepsy</td>
</tr>
<tr>
<td>AI</td>
<td>asymmetry index</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid</td>
</tr>
<tr>
<td>BAER</td>
<td>brainstem auditory evoked response</td>
</tr>
<tr>
<td>BCECTS</td>
<td>benign childhood epilepsy with centro-temporal spikes</td>
</tr>
<tr>
<td>BECTS</td>
<td>benign epilepsy with centro-temporal spikes</td>
</tr>
<tr>
<td>BFIE</td>
<td>benign familial infantile epilepsy</td>
</tr>
<tr>
<td>BFJE</td>
<td>benign familial juvenile epilepsy</td>
</tr>
<tr>
<td>BFNE</td>
<td>benign familial neonatal epilepsy</td>
</tr>
<tr>
<td>BFNIE</td>
<td>benign familial neonatal infantile epilepsy</td>
</tr>
<tr>
<td>BPEI</td>
<td>benign partial epilepsy in infancy</td>
</tr>
<tr>
<td>BRE</td>
<td>benign Rolandic epilepsy</td>
</tr>
<tr>
<td>CNV</td>
<td>copy number variation</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>computer tomography</td>
</tr>
<tr>
<td>DEPDC5</td>
<td>disheveled, Egl-10, and pleckstrin domain-containing protein 5</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalography</td>
</tr>
<tr>
<td>EMG</td>
<td>electromyography</td>
</tr>
<tr>
<td>EPMR</td>
<td>progressive epilepsy with mental retardation</td>
</tr>
<tr>
<td>FDG</td>
<td>2-[18F]fluoro-2-deoxy-D-glucose</td>
</tr>
<tr>
<td>FDH</td>
<td>Finnish Disease Heritage</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GWAS</td>
<td>genome wide association study</td>
</tr>
<tr>
<td>ICOE-G</td>
<td>idiopathic childhood occipital epilepsy of Gastaut</td>
</tr>
<tr>
<td>IE</td>
<td>idiopathic epilepsy</td>
</tr>
<tr>
<td>ILAE</td>
<td>International League Against Epilepsy</td>
</tr>
<tr>
<td>IVETF</td>
<td>International Veterinary Epilepsy Task Force</td>
</tr>
<tr>
<td>KA</td>
<td>kainic acid</td>
</tr>
<tr>
<td>LE</td>
<td>limbic encephalitis</td>
</tr>
<tr>
<td>LGI</td>
<td>leucine-rich glioma-inactivated</td>
</tr>
<tr>
<td>LR</td>
<td>Lagotto Romagnolo</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PCA</td>
<td>principal component analysis</td>
</tr>
<tr>
<td>PRRT2</td>
<td>proline-rich transmembrane protein 2</td>
</tr>
<tr>
<td>PS</td>
<td>Panayiotopoulos syndrome</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
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<tr>
<td>RBFOX</td>
<td>RNA binding forkhead box</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>SPECT</td>
<td>single photon emission computer tomography</td>
</tr>
<tr>
<td>SE</td>
<td>status epilepticus</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>SUV</td>
<td>standard uptake value</td>
</tr>
<tr>
<td>TNCC</td>
<td>total nucleated cell count</td>
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1 INTRODUCTION

Epilepsy is one of the most common neurological disorders in both humans and dogs (Podell et al., 1995, Raol et al., 2001, Chandler, 2006). Most dogs with recurrent seizures have no identifiable underlying cause and are diagnosed as having idiopathic epilepsy (IE) (Berendt and Gram, 1999, Chandler, 2006). The prevalence of IE has been reported to be 0.6% in a non-referral veterinary practice population (Kearsley-Fleet et al., 2013).

The International League Against Epilepsy (ILAE) classifies human epilepsies and defines epilepsy terminology based on current knowledge. The term epilepsy does not refer to a single disease, but instead to a group of disorders with several underlying causes that have in common an increased predisposition to cause epileptic seizures (Fisher et al., 2005). The newest human recommendation uses the following etiologic classification for epilepsy: genetic, structural/metabolic, and unknown cause instead of the terms idiopathic, symptomatic, and cryptogenic (Berg et al., 2010), which are still commonly used in veterinary medicine. On the other hand, ILAE has already suggested that six etiological classes could be used instead of three (www.ilae.org). These classes are genetic, structural, metabolic, immune, infectious, and unknown. Epilepsy is characterized by a persistent epileptogenic abnormality of the brain that is able to spontaneously generate paroxysmal abnormal activity (Engel, 2006). Epilepsy is also defined as a disease of the brain with an enduring predisposition to generate epileptic seizures, and thus, epilepsy exists in a patient who has had a seizure and whose brain demonstrates an enduring tendency to have recurrent seizures (Fisher et al., 2014). Seizure, on the other hand, refers to a transient occurrence of signs due to abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2005).

Transition from normal to epileptic activity can be viewed as a greater spread and neuronal recruitment secondary to a combination of enhanced connectivity and excitatory transmission, a failure of inhibitory mechanisms, and changes in intrinsic neuronal properties (Duncan et al., 2006). The pathophysiological background of epilepsy can be simplified and viewed as an imbalance between excitation and inhibition, and this imbalance can be due to increased activity of glutamate, the main excitatory neurotransmitter in the brain, or decreased activity of γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter (Raol et al., 2001). Glutamate and GABA act via ligand-gated ion channels involving sodium, calcium, potassium, and chloride, and the balance between excitatory and inhibitory neurotransmission is also significantly affected by voltage-gated ion channels (Patterson, 2013). Not surprisingly, mutations in these ion channels play an important role in the pathogenesis of genetic epilepsies because most of the currently known genes for human idiopathic epilepsies encode subunits of
ion channels (Turnbull et al., 2005, Reid et al., 2009). Most common genetic epilepsies in human medicine, however, are thought to have complex inheritance determined by multiple genes with possible environmental effects acting on inherited susceptibility (Cavalleri et al., 2007, Hildebrand et al., 2013). One susceptibility allele, namely ADAM23 has been identified to increase the risk for genetic epilepsy or IE in several dog breeds, but there are no previous reports about monogenic IEs of known genetic background in veterinary medicine (Seppälä et al., 2012, Koskinen et al., 2015).

The diagnosis of IE in animals is made by excluding other causes for seizures, including structural brain diseases and extracranial pathology. Magnetic resonance imaging (MRI) and computer tomography (CT) show no changes in canine patients with IE (Thomas, 2010). Nuclear medicine, including single photon emission computer tomography (SPECT) and positron emission tomography (PET) techniques, can detect abnormal areas of brain metabolism in patients with epilepsy (Lee et al., 2001, Goffin et al., 2008). These modalities are utilized in human epileptology, especially in the presurgical assessment of patients with refractory epilepsy (Goffin et al., 2008). No previous studies have reported the findings of PET in canine patients with epilepsy.

In humans, neurobehavioral abnormalities occur with some epilepsies and can be even more disturbing than the seizures themselves (Hermann et al., 2008, Hamiwka and Wirrell, 2009, Lin et al., 2012a, Lin et al., 2013). These include cognitive deterioration, mood disorders, anxiety disorders, and attention deficit hyperactivity disorder (Lin et al., 2012a). The cause of these abnormalities is currently unknown and may include seizure-related factors or a common etiologic background for both seizures and comorbidities (Lin et al., 2012a, Yoong, 2015). Only one study suggests behavioral changes with epilepsy to also be present in dogs (Shihab et al., 2011).

Studies in rodents have shown that the immature brain is more prone to seizures than the mature brain as a result of an imbalance between excitatory and inhibitory input (Raol et al., 2001). Idiopathic childhood epilepsies with benign outcomes are well-known and commonly encountered in human medicine, but surprisingly, no reports exist on benign juvenile epilepsy syndromes in dogs. This thesis describes the first benign familial juvenile epilepsy syndrome in dogs and its genetic cause. Also investigated are the use of nuclear imaging, namely FDG-PET, in dogs with epilepsy and the neurobehavioral comorbidities of epilepsy.
2 REVIEW OF THE LITERATURE

2.1 EPILEPSY; DEFINITIONS AND CLASSIFICATIONS IN DOGS AND IN HUMANS

The classification of epileptic seizures and epilepsy and the definition of the corresponding terminology are ongoing processes, and the International League Against Epilepsy (ILAE) has published several reports revising the terminology in human medicine (ILAE, 1981, ILAE, 1989, Blume et al., 2001, Engel, 2001, Engel, 2006, Berg et al., 2010, Fisher et al., 2014). In veterinary medicine, researchers have adopted human classifications and terminology, although several classifications for dogs have been suggested (Podell et al., 1995, Berendt and Gram, 1999, Licht et al., 2002, Thomas, 2010). Nevertheless, no formal and generally accepted classification scheme for seizures, epilepsy, or epileptic syndromes exists for dogs (Mariani, 2013). In 2014, a group of Veterinary Neurology Specialists and Non-specialists founded the International Veterinary Epilepsy Task Force (IVETF) in order to generate consensus statements on the key areas in the field of epilepsy corresponding to reports of the ILAE in human medicine (Volk, 2015). The IVETF group has very recently published consensus statements including epilepsy terminology and classifications in companion animals. Whether these statements will be generally accepted in veterinary medicine remains unknown.

In human medicine, an epileptic seizure is defined as a transient occurrence of signs due to abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2005), and this definition is also commonly used in veterinary medicine. Epilepsy, on the other hand, is defined as a disease of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiologic, cognitive, psychological, and social consequences of this condition (Fisher et al., 2005, Fisher et al., 2014). Previously, epilepsy was also defined in humans as a chronic neurological condition characterized by recurrent epileptic seizures (Blume et al., 2001), and the definition of epilepsy in veterinary medicine is concordant with this earlier human definition (Thomas, 2010, Mariani, 2013, Berendt et al., 2015). However, in human medicine the more recent definition has questioned the requirement for two unprovoked seizures and takes into account circumstances with a high risk for future seizures after the first unprovoked seizure (Fisher et al., 2014).

In humans, an epileptic syndrome is defined and distinguished from epileptic seizure and epilepsy. Each syndrome can be characterized not only according to seizure type, but also according to numerous other features, including age at onset, cognitive and developmental antecedents and consequences, motor and sensory examinations, electroencephalography
(EEG) features, provoking or triggering factors, and patterns of seizure occurrence with respect to sleep (Berg et al., 2010).

The term benign has been used in human medicine to refer to an epilepsy syndrome with a high likelihood of spontaneous remission at a predictable age. The latest recommendation suggests the term self-limited instead of benign due to the misleading character of the word benign because of increasing knowledge about comorbidities of epilepsy, including cognitive, behavioral, and psychiatric disorders, as well as mortality (Berg et al., 2010). The nomenclature of the previously named syndromes has to date remained unchanged.

In addition to epilepsy, several other diseases can cause paroxysmal signs or symptoms (Benbadis, 2009). Paroxysmal movement disorders and episodic ataxias are characterized by paroxysmal attacks of abnormal postures or movements, with no disturbance of consciousness (Fernandez-Alvarez and Perez-Duenas, 2013). Ambulatory EEG can differentiate between non-epileptic conditions and epilepsy by documenting the absence of EEG changes in non-epileptic events (Benbadis, 2009).

2.1.1 CLASSIFICATION OF EPILEPSY BASED ON SEIZURE TYPE

In human medicine, the classification of epilepsy based on seizure type is continuously developed by ILAE. In veterinary medicine, the corresponding classification follows the recommendations in human medicine, but with a delay and lack of consensus because there is no official organization such as ILAE to define epilepsy terminology for animals although the IVETF has recently made an effort to offer guidelines for veterinary medicine.

Seizure types can be divided into self-limited and continuous seizures (Engel, 2001). Self-limited epileptic seizure types can be further divided into generalized and focal seizures (Engel, 2006). In human medicine, by the newest definition generalized seizures originate at some point within the rapidly engaging bilaterally distributed networks, which can include both cortical and subcortical structures (Berg et al., 2010). Focal seizures in the newest terminology originate within networks limited to one hemisphere and may also originate in subcortical structures (Berg et al., 2010). In both, generalized and focal seizures, it was thus recognized that seizures can also originate from subcortical structures (Berg et al., 2010). Still, it has been emphasized that the term focal does not mean that the epileptogenic region is a small, well-defined locus, but instead focal seizures are almost always due to diffuse and sometimes widespread areas of cerebral dysfunction (Engel, 2001).

In human medicine, the former division of focal seizures into simple and complex categories based on the level of consciousness, with simple complex seizures having preserved consciousness and complex focal seizures having impaired consciousness (ILAE, 1981), was abandoned in the latest report (Berg et al., 2010). Consciousness refers to the degree of awareness or
responsiveness of the patient to external stimuli (ILAE, 1981). Still, it was deemed important to report impaired consciousness when observed during focal seizures (Berg et al., 2010). Additionally, the term secondarily generalized was abandoned and recommended to be replaced by the description “evolving to bilateral, convulsive seizure” when seen in connection with a focal seizure (Berg et al., 2010).

In dogs, seizures have usually been classified as simple or complex partial/focal with possible secondary generalization, or generalized according to the human terminology described in 1981 (Berendt and Gram, 1999, Licht et al., 2002, Thomas, 2010). Recently, others have suggested that seizures could be classified based on the latest human recommendations as only focal and generalized seizures (Mariani, 2013) or based on the proposal in 2015, as focal, generalized, and focal epileptic seizures evolving into generalized epileptic seizures (Berendt et al., 2015). In veterinary medicine, focal seizures are determined by the newest proposal such that they are characterized by lateralized and/or regional signs and may originate in subcortical structures (Berendt et al., 2015). Focal epileptic seizures can present with focal motor phenomena including facial twitches or repeated rhythmic jerks of one extremity, with parasympathetic and epigastric components including hypersalivation or vomiting, or with episodic change in behavior including restlessness or abnormal attention seeking (Berendt et al., 2015). Generalized seizures are characterized by involvement of both sides of the body, thus involving both cerebral hemispheres (Berendt et al., 2015). The newest proposal suggested that generalized seizures may occur alone or evolve from a focal epileptic seizure (Berendt et al., 2015). In a generalized seizure, consciousness must be lost if the seizure activity lasts at least 30 seconds (Licht et al., 2002).

Continuous seizure types also include generalized and focal status epilepticus (Engel, 2001). In veterinary medicine status epilepticus (SE) has been defined as a seizure that persists for longer than five minutes or recurrent seizures without recovery of normal consciousness between the seizure episodes (Mariani, 2013, Berendt et al., 2015).

### 2.1.2 Etiologic Classification of Epilepsy
Epilepsy can also be classified based on the etiology. In concordance with seizure type classification, the etiologic classification of epilepsy in veterinary medicine follows human guidelines defined by ILAE. However, the classifications used by different researchers in veterinary medicine vary.

In humans, the newest ILAE report recommends the use of the following etiologic classification: genetic, structural/metabolic and unknown cause (Berg et al., 2010). Genetic epilepsy is a direct result of a known or presumed genetic defect, with seizures being the core symptom. In structural/metabolic epilepsy, a separate structural or metabolic condition or disease exists that has been demonstrated to be associated with a substantially increased risk of
developing epilepsy. Structural lesions comprise acquired disorders including sequelae to stroke, trauma, or infection or may be of genetic origin such as tuberous sclerosis and many malformations of cortical development. However, there is a separate disorder interposed between the genetic defect and the epilepsy. Epilepsies of unknown cause are the most poorly understood, but account for at least one-third of all epilepsies. The term unknown cause means that the nature of the underlying cause remains obscure (Berg et al., 2010). Reactive seizures are not considered as epilepsy and occur in association with transient systemic perturbation such as intercurrent illness, sleep loss, or emotional stress (Blume et al., 2001).

The following etiologic classification concordant with the earlier human classification is commonly used in veterinary medicine: idiopathic with normal diagnostic workup and familial predisposition, symptomatic caused by a known intracranial disorder, and cryptogenic epilepsy or probable symptomatic with an underlying symptomatic cause suspected but not confirmed (Podell et al., 1995, Berendt and Gram, 1999, Smith et al., 2008, Thomas, 2010). Idiopathic epilepsy (IE) in dogs has been defined as recurrent seizures for which no underlying brain abnormality can be identified (Knowles, 1998). Reactive epileptic seizures represent an additional form to this classification and are a reaction of the normal brain to extracranial metabolic or toxic insults (Podell et al., 1995), but it has been suggested that this not be considered as true epilepsy (Chandler, 2006).

Others have proposed that the latest human classification, comprising genetic, structural, and unknown epilepsy, could be adopted also in veterinary medicine (Mariani, 2013). Genetic epilepsy (synonyms primary, inherited, idiopathic) is a direct result of a known or suspected genetic defect, with the seizures being the core sign of the disorder, and without identifiable brain lesion or other neurologic signs, and an age-dependent onset (Mariani 2013). On the other hand, the proposal by IVETF in 2015 retained the term idiopathic instead of genetic and further divided idiopathic into three subclasses, namely idiopathic – genetic, idiopathic – suspected genetic, and idiopathic – unknown cause (Berendt et al., 2015). Structural epilepsy (synonyms symptomatic, secondary) is a result of an identifiable structural lesion of the brain, including vascular, inflammatory/infectious, traumatic, anomalous, neoplastic and degenerative diseases, and this can also be an inherited disorder (Mariani, 2013, Berendt et al., 2015). In unknown epilepsy (synonyms cryptogenic, probably symptomatic, for some idiopathic), the underlying cause remains obscure (Mariani, 2013). The researchers suggested also that seizures caused by extracranial disorders (metabolic conditions like hypoglycemia or electrolyte abnormalities, or toxins) should not be considered as epilepsy, but should be termed as reactive seizures (Mariani, 2013).
2.2 CLINICAL SIGNS OF EPILEPTIC SEIZURES

The epileptic seizure is described as a sequence of events: the prodrome, the preictal phase or the aura, the ictus, and the postictal phase. It is important to be aware of these characteristic stages, as it may help to differentiate epileptic seizures from non-epileptic ones (Pakozdy et al., 2014).

Prodrome is a preictal phenomenon with subjective or objective clinical alteration (e.g., ill-localized sensation or agitation) that may precede the seizure, but is not part of it (Blume et al., 2001). In dogs, if recognized, it may last hours to days, but at least one hour, during which time the dog will usually express restlessness, attention-seeking, or anxious behavior (Berendt and Gram, 1999, Licht et al., 2002). A prodrome reflects a preictal phenomenon with increased excitability of an epileptogenic focus or of the entire brain, and it must be distinguished from an aura, which lasts from seconds to a few minutes (Berendt and Gram, 1999).

An aura is an ictal subjective phenomenon that may precede an observable seizure, but if present alone is regarded as a sensory seizure (Blume et al., 2001). Sensory in this context is defined as a perceptual experience not caused by an appropriate stimulus from the environment and includes, for example, abnormal pins and needles sensations, with visual, olfactory, and auditory hallucinations/sensations perceived as warning signs (Berendt and Gram, 1999, Blume et al., 2001, Berendt et al., 2004). Due to the nature of aura being a subjective initial feeling of the ictal event, it is difficult or even impossible to differentiate it from the prodrome without ictal EEG in animals (Mariani, 2013, Pakozdy et al., 2014). The recent proposal of IVETF suggested that the term aura should not be used in veterinary medicine, but the signs occurring as the first indication of seizure activity and appearing seconds to minutes before an observable seizure should be referred to as a focal seizure onset (Berendt et al., 2015).

Ictus is a sudden neurological event and refers to the epileptic seizure itself (Blume et al., 2001). The clinical signs of ictus include motor, autonomic, and behavioral signs usually lasting up to two minutes (Berendt et al., 2004).

Postictal phenomenon is a transient clinical abnormality in the function of the central nervous system after the ictus has ceased (Blume et al., 2001). In dogs, the postictal phase, if present, is characterized by reorientation with or without deep sleep and reportedly lasts up to 30 minutes (Berendt and Gram, 1999).

2.2.1 CLASSIFICATION OF ICTAL SIGNS

The ictal semiology, or ictal signs, depends on the location of the abnormal electrical discharge in the brain (Pakozdy et al., 2014). Seizures can involve sensory, motor, and autonomic functions, and additionally changes in consciousness and neurobehavioral signs can be seen (Pakozdy et al., 2014,
Packer et al., 2015). In human medicine, detailed reports of seizure semiology are essential for proper management of patients with epilepsy, for classification of the epilepsy syndrome, and for differentiation between epileptic and non-epileptic events. However, interobserver reliability of seizure semiology observation has been shown to be relatively low in humans as well as in dogs and cats (Bleasel et al., 1997, Packer et al., 2015).

During epileptic seizures dogs and humans usually express signs or symptoms from more than one group (i.e., sensory, motor, autonomic, changes in consciousness), although in most cases the signs from one group predominate (Berendt et al., 2004, Noachtar and Peters, 2009). Seizures are accordingly classified based on the predominant sign. Thus, for example, seizures with motor phenomena as their main manifestations are called motor seizures (Noachtar and Peters, 2009). Motor signs such as head turning, head tremor, and abnormal limb movements are commonly seen in dogs with focal or generalized seizures (Licht et al., 2002, Berendt et al., 2004). Autonomic signs including salivation, urination, defecation, vomiting, and pupillary dilatation, and behavioral signs including anxiety, restlessness and aggression are easily and commonly recognized in dogs (Licht et al., 2002, Berendt et al., 2004). Somatosensory signs, such as abnormal “pins and needles” sensations, visual, auditory, or olfactory hallucinations/sensations are subjective symptoms reported in humans without objective signs that could be documented by an observer (Noachtar and Peters, 2009). These are difficult to witness in dogs (Berendt et al., 2004). Impairment of consciousness may also be difficult to objectively interpret in a canine patient (Berendt et al., 2004, Parker et al., 2015).

### 2.3 IDIOPATHIC EPILEPSY IN DOGS

Epilepsy is often stated to be the most common chronic neurologic disease in dogs. Researchers have reported that in 60-70% of dogs with seizures no structural or metabolic cause for epilepsy was found (Berendt and Gram, 1999, Zimmermann et al., 2009, Armasu et al., 2014) thus IE recorded as the most common cause for canine epilepsy. Researchers have reported the prevalence of IE in a non-referral dog population to be 0.62%, although breed-specific studies report higher prevalences (Kearsley-Fleet et al., 2013).

#### 2.3.1 AGE AT ONSET

The typical age at onset of seizures in IE is suggested to vary from 6 months or 1 year to 5 or 6 years (Smith et al., 2008, Zimmermann et al., 2009, De Risio et al., 2015a), but several studies have reported dogs having their first seizures at a much older age. In Vizslas with IE, 14% of dogs were older than 4 years, and in Springer Spaniels 20% were between 5 and 6 years of age at first seizure episode (Patterson et al., 2003, Patterson et al., 2005). A recent
study revealed that 35% of dogs over 5 years of age at seizure onset had IE, and IE has also been described in dogs with seizure onset after 10 years of age (Schwartz et al., 2013, Ghormley et al., 2015). Nevertheless, a recent study showed that dogs with IE had their first seizure at a mean age of 39.8 months, while dogs with a structural brain lesion had theirs at a mean age of 90.9 months, and that the risk for an intracranial lesion as the cause of epileptic seizures increased with each additional year of age at seizure onset (Armasu et al., 2014).

Some researchers have suggested that the dogs with seizure onset before the age of one year would be more likely to have a structural brain lesion (Podell et al., 1995). However, later studies have reported that most dogs with seizure onset before the age of one year have IE, and with only 20% having a structural brain lesion (Arrol et al., 2012, Armasu et al., 2014). There are no reports about benign juvenile epilepsies in dogs comparable with the benign epilepsies of childhood described in humans.

2.3.2 SEIZURE TYPE

Older reports have considered generalized seizures to be typical for IE (Schwartz-Porche, 1994) and focal seizures indicative of symptomatic epilepsy (Berendt and Gram, 1999). Other studies, especially later ones, have nevertheless shown that focal seizures can also be associated with IE (Jaggy and Bernardini, 1998, Patterson et al., 2003, 2005, Licht et al., 2007). A recent study found similar prevalences of generalized seizures in dogs with IE and in dogs with asymmetrical intracranial lesions, and additionally, some of the dogs with IE had focal seizures (Armasu et al., 2014). Thus, in IE both focal and generalized seizures are possible, and seizure type alone should not be used to establish the underlying etiology (Armasu et al., 2014).

Cluster seizures seem to predominate in dogs with structural brain lesions, with >60% of dogs having a structural brain lesion exhibiting cluster seizures (Thomas, 2010, Schwartz et al., 2011, Armasu et al., 2014). Thus, single seizures rather than cluster seizures would be more likely in dogs with IE (Armasu et al., 2014). One study reported that SE was 1.57 times more likely if the cause for the seizures was secondary or reactive epilepsy rather than IE (Platt and Haag, 2002). On the other hand, another study found that 59% of dogs diagnosed with IE had episodes of SE (Saito et al., 2001). Dogs with reactive seizures due to intoxication have been reported to possess a significantly higher risk of developing SE, particularly as a first manifestation of a seizure disorder (Zimmermann et al., 2009). Dogs with IE had a reduced risk of developing SE, and particularly SE as a first seizure, relative to dogs with symptomatic epilepsy or reactive seizures (Zimmermann et al., 2009).
2.4 DIAGNOSIS OF IDIOPATHIC EPILEPSY IN DOGS

In humans, the diagnosis of epilepsy remains clinical and is based on the probability after assessing the whole individual and includes seizure description, subject's age and comorbidities, EEG changes, and finally brain imaging (Duncan et al., 2006). In concordance with human medicine, there is no test to confirm IE in dogs, and the diagnosis is based on exclusion of other possible etiologic factors, including reactive seizures and structural epilepsy (Pakozdy et al., 2014, De Risio et al., 2015b). Careful history-taking is important and includes recognition of age of onset, possible stages of epileptic seizure, and presence of autonomic signs such as urination or salivation (Pakozdy et al., 2014). Clinical and neurological examinations should be performed, with interictal neurological abnormalities other than postictal signs being indicative of structural brain disease (Pakozdy et al., 2014). Complete blood cell count and serum biochemical analysis are performed in patients presented for epileptic seizures to exclude reactive seizures (Kearsley-Fleet et al., 2013).

The neurological examination findings determine the need for further investigations, including magnetic resonance imaging (MRI). The inclusion of such factors as age of seizure onset and abnormalities detected in neurological examination results in highly predictive models for intracranial lesions (Armasu et al., 2014) that could help in decision-making about the need for advanced imaging. Animals that exhibit no neurological deficits interictally are usually considered to have a low risk for an intracranial disorder (Smith et al., 2008). Dogs with abnormal interictal neurological examination findings are 16.5 times more likely to have an asymmetrical structural intracranial lesion and 12.5 times more likely to have a symmetrical structural lesion (Armasu et al., 2014).

Neurological examination has been reported to be a good predictor of an abnormal MRI scan in epileptic dogs aged less than 6 years (Smith et al., 2008). In dogs with epilepsy and normal interictal neurological examination,
only 2.2% of dogs aged less than 6 years exhibited significant MRI abnormalities, but in dogs older than 6 years the proportion of dogs with MRI abnormalities was 26.7% (Smith et al., 2008). Another study suggested that the combination of findings in neurological examination and results of cerebrospinal fluid (CSF) analysis are useful in predicting the benefit of MRI scan, as only 6% of dogs with normal neurological examination and normal CSF analysis had abnormal MRI (Bush et al., 2002). These investigators suggested that age was not a significant predictor for the benefit of MRI, as in their dogs with normal interictal neurological examination and aged less than 6 years 23% had brain lesions on their MRI, in comparison to dogs aged 6 years or older in which 22% had changes in MRI (Bush et al., 2002). This discrepancy between the two above-mentioned studies might be due to geographical differences since the latter study was performed in the USA, where the risk for infectious diseases is higher (Smith et al., 2008).

Nevertheless, the predictive value of neurological examination findings may be lower for animals with later seizure onset. One study investigating dogs with seizure onset at ≥ 7 years of age found that 59% of dogs with a normal interictal neurological examination had an underlying CNS disease to which the seizures were attributed (Schwartz et al., 2013). A recent study also reported lower sensitivity and specificity for neurological examination in predicting intracranial lesion in older dogs. This study investigating dogs with seizure onset at or after 5 years of age found that dogs with abnormal neurological examination findings had an abnormal MRI or CSF analysis results in 79% of cases, and in dogs with normal neurological examination the proportion with abnormal findings in the aforementioned examinations was 45% (Ghormley et al., 2015). Accordingly, a complete neurological work-up has been recommended at least for all dogs with late-onset seizures (Schwart et al., 2013, Ghormley et al., 2015) as well as for dogs with medically refractory epilepsy (Knowles, 1998, Moore, 2013).

For dogs with seizure onset before one year of age, the predictive value of neurological examination findings may be good, as one additional study found that only in 5.3% of dogs with the first seizure episode under the age of one year and normal neurological examination interictally had an underlying cause identified using MRI and CSF analysis (Arrol et al., 2012). Thus, especially in a situation where referral is difficult, IE could be diagnosed with reasonable confidence without advanced imaging in a clinically normal juvenile dog (Stalin, 2013).

### 2.4.2 MAGNETIC RESONANCE IMAGING

Ideally, brain MRI is a vital part of a work-up for a patient presented due to seizures and is necessary to make a diagnosis of IE (Chandler, 2006, Moore, 2013). For practical and financial reasons, the use of MRI in veterinary patients with epilepsy may not be high, with one study reporting that only 2.2% of dogs with suspected IE had received an MRI examination during the
Review of the literature

study period (Kearsley-Fleet, 2013). A recent IVETF proposal recommended that MRI be performed on dogs with seizure onset either <6 months or >6 years, ictal neurological abnormalities, SE or cluster seizure as a first seizure, a previous presumptive diagnosis of IE, and drug resistance with one antiepileptic drug titrated to the highest tolerable level (De Risio et al., 2015b).

Low-field MRI with a 0.2-Tesla MRI machine is still in common use in veterinary patients and could reduce the ability to detect intracranial lesions relative to high-field MRI (Smith et al., 2008, Ghormley et al., 2015). This is likely to have an impact on detecting subtle lesions, including cortical dysplasia, in dogs, although the clinical aspect might currently be unimportant since surgical treatment of epilepsy in dogs is seldom considered (Smith et al., 2008). One should also appreciate the possible occurrence of reversible T2 hyperintense lesions in MRI following seizures (Mellema et al., 1999, Viitmaa et al., 2006). Additionally, the prevalence of lateral ventricle asymmetry is approximately 40% in dogs and is considered to be an incidental finding (Pivetta et al., 2013). Administration of intravenous contrast media is highly unlikely to detect a lesion in cases with normal neurological examination and normal precontrast MRI, but may be indicated in animals with persistent neurological deficits even though precontrast MRI shows no abnormalities (Ives et al., 2014).

Researchers have reported that in dogs with seizures and aged between 1.5 and 202 months no underlying cause was identified in MRI examination in 63.9% of cases. Of the dogs under investigation, 2.7% had reportedly a symmetrical structural lesion and 33.4% an asymmetrical structural lesion of the brain detected in MRI (Armasu et al., 2014). Another study found a structural brain disease as an etiology for epileptic seizures in 38.3% of dogs, and of these cases, inflammatory brain disease was detected in 36.7%, cerebral tumor in 47.8%, and another unspecified cause in 15.6% (Zimmermann et al., 2009). Others investigated MRI findings in dogs with seizure onset after the age of 5 years; 35% of the cases were diagnosed as having IE, 49% had neoplasia as an underlying etiology for seizures, and in the rest of the cases an inflammatory disease, hydrocephalus, infarction, or cyst was diagnosed (Ghormley et al., 2015). Furthermore, researchers reported in dogs with seizure onset after 7 years that no underlying abnormality was detected in MRI in 21%, neoplasia was detected in 57%, cerebrovascular accident in 10%, encephalitis in 5%, and degenerative central nervous system disease in 2% of the dogs. The cause was undetermined in 6% of the cases investigated (Schwartz et al., 2013).

2.4.3 CEREBROSPINAL FLUID

Demonstrating normal CSF test results is important in excluding especially infectious and inflammatory causes for seizures (Goncalves et al., 2010). Researchers have suggested that combined results of CSF analysis and
neurologic examination are useful in predicting whether MRI results will be abnormal; MRI results were abnormal for 43% of dogs with abnormal CSF analysis and normal neurological examination, but only for 6% of dogs with normal CSF analysis and normal neurologic examination (Bush et al., 2002). Only 4% of dogs with CSF protein concentrations exceeding 35 mg/dl had normal MRI results (Bush et al., 2002). On the other hand, the authors pointed out that it remained uncertain whether the time interval between the last seizure episode and CSF collection has an effect on CSF protein concentrations in dogs (Bush et al., 2002). Another study reported a mild increase in total protein content in the CSF of some dogs presented for seizures (Schwartz et al., 2013). The authors suggested this to represent the postictal change reported in humans (Schwartz et al., 2013). Another study reported a significant association between total nucleated cell count (TNCC) and time between the last seizure and CSF collection (i.e., the longer the time interval, the lower the TNCC), suggesting that seizures possibly can cause changes in CSF cell count (Goncalves et al., 2010). On the other hand, these researchers found no similar association with regard to CSF protein concentration.

2.4.4 ELECTROENCEPHALOGRAPHY

Electroencephalography (EEG) is an important, noninvasive diagnostic tool for human patients with a history of seizures (Flink et al., 2002). The purpose of the EEG recording is to detect interictal or ictal activity and to localize the region of abnormal activity (Flink et al., 2002). In clinical practice, the hallmark of human epilepsy is interictal epileptic activity, including spikes, sharp waves, and spike-wave discharges (Duncan et al., 2006). The sensitivity of a single EEG examination may be low and can be increased with either repeated EEG recordings or sleep deprivation (Renzel et al., 2015). Researchers have reported better sensitivity of EEG after sleep deprivation in patients with primary generalized epilepsies than in patients with focal epilepsies (64% vs. 17%) (Renzel et al., 2015). The specificity of EEG is high in adults, but may be lower in children, with up to 9% of normal children exhibiting epileptiform activity in EEG (Holmes, 2003, Renzel et al., 2015). Ictal electroencephalogram can be used to diagnose the type of epileptic disorder and differentiate epileptic from non-epileptic paroxysms in humans (Fernandez-Alvarez, 1998, Paolicchi, 2002, Holmes, 2003).

In dogs, EEG is only infrequently used in diagnostics of epilepsy and only few data exist on the typical EEG findings in different types of epilepsies in dogs. Visual analysis of interictal EEG may have low value in the diagnosis of IE because recent reports reveal interictal epileptic activity in less than 30% of dogs with IE (Jeserevics et al., 2007, Brauer et al., 2012, Pakozdy et al., 2012). Although video EEG monitoring would be the preferred method in diagnosis and classification of seizures, such methods are not widely available in veterinary medicine (Packer et al., 2015). A recent study showed...
Review of the literature

relatively low levels of interobserver agreement in canine paroxysmal event
semiology and classification (Packer et al., 2015), highlighting the need for
more objective assessment methods. While ictal EEG is conducted only
infrequently in veterinary medicine, it is necessary for objective confirmation
that a paroxysmal event represents an epileptic seizure episode (Poma et al.,
2010, Pakozdy et al., 2014).

2.5 GENETIC EPILEPSY IN CHILDREN

2.5.1 BENIGN EPILEPSY SYNDROMES OF CHILDHOOD

Approximately half of the epilepsies with onset in childhood are either
genetic epilepsies or epilepsies of unknown origin, and the rest of the cases
represent structural/metabolic epilepsies (Sillanpää and Shinnar, 2010).
Childhood epilepsy syndromes with presumed genetic etiology and benign
nature represent 5-20% of the epilepsies with onset in childhood (Sillanpää

In humans, several benign epilepsy syndromes of childhood have been
described. Three epilepsy syndromes of the first year of life have been
delineated with predominant focal seizures, benign outcome, and autosomal
dominant inheritance, and these include benign familial neonatal epilepsy
(BFNE), benign familial neonatal-infantile epilepsy (BFNIE), and benign
familial infantile epilepsy (BFIE) (Berg et al., 2010, Mulley et al., 2011, Zara
et al., 2013). They are characterized by similar clinical features, but have an
ascending average age at seizure onset, with seizures appearing at the mean
age of 2-3 days in BFNE, at 11 weeks in BFNIE, and at 6 months in BFIE
(Mulley et al., 2011). Typical seizures are brief, focal motor manifestations
with eye and head deviation, and subsequent tonic and clonic movements,
staring and apnea (Zara et al., 2013). There is usually spontaneous remission
of seizures within the first year of life (Deprez et al., 2009). Benign partial
epilepsy in infancy (BPEI) represents a non-familial form of BFIE (Vigevano,
2005, Yamamoto et al., 2015).

Benign childhood epilepsies with focal origin comprise three identifiable
electroclinical syndromes recognized by ILAE and include benign Rolandic
epilepsy (BRE, i.e., benign childhood epilepsy with centrotemporal spikes
(BCECTS) or benign epilepsy with centrotemporal spikes (BECTS)),
Panayiotopoulos syndrome (PS), and idiopathic childhood occipital epilepsy
of Gastaut (ICOE-G) (Engel, 2006, Panayitopoulos et al., 2008). Physical,
mental, and laboratory examinations as well as brain imaging other than
EEG are normal in these benign childhood epilepsies so they are idiopathic in
origin (Panayiotopoulos, 1993). The most common childhood genetic focal
epilepsy is BRE (Park et al., 2015). In BRE, the age of onset ranges from 1 to
14 years and remission occurs within 2-4 years from onset and before the age
of 16 years (Holmes, 2003, Panayiotopoulos et al., 2008). BRE is
characterized by focal seizures consisting of unilateral facial sensory-motor symptoms, oro-pharyngeal signs, speech arrest, and hypersalivation, with centrotemporal spikes accelerating in sleep in EEG being the hallmark of this syndrome (Guerrini and Pellacani, 2012). In PS, the age of onset varies between 2 and 8 years, with seizures remitting usually within 1-2 years (Caraballo et al., 2000, Panayiotopoulos, 1989). PS is characterized by seizures that are often prolonged, with predominantly autonomic symptoms, and by an EEG that shows shifting and/or multiple foci with occipital predominance in sleep (Ferrie et al., 2006). ICOE-G is less common, the age of onset is at 6 years, and the prognosis is unclear, but current data indicate that remission of seizures occurs in 50-60% of patients within 2-4 years of onset (Panayiotopoulos, 2008, Guerrini and Pellacani, 2012). In ICOE-G, seizures primarily manifest with visual hallucinations, and interictal EEG shows occipital paroxysms (Panayiotopoulos, 2008, Guerrini and Pellacani, 2012).

### 2.5.1.1 Electroencephalography in benign childhood epilepsies

The interictal EEG may be abnormal in patients with BFNE, but there are no characteristic diagnostic features (Park et al., 2015). In BFNIE, the interictal EEG is reportedly normal in over half of the patients (Heron et al., 2002). In BFIE, the interictal EEG is usually normal, but interictal EEG performed during a cluster of seizures shows lateralized slow waves and spikes in the occipito-parietal areas (Vigevano, 2005). In patients with BRE, interictal EEG shows a classic pattern consisting of centrotemporal spikes often activated by drowsiness or non-REM sleep, and only rarely do children with BRE have a normal EEG (Panayiotopoulos et al., 2008). In PS, interictal EEG shows shifting or multiple foci with high-amplitude sharp and slow wave complexes and occipital predominance (Ferrie et al., 2006). These EEG abnormalities are accentuated by sleep (Ferrie et al., 2006). Ten percent of patients with PS may show normal awake EEG, but abnormalities are almost always detected at sleep EEG or serial EEG examinations (Ferrie et al., 2006). The interictal EEG in patients with ICOE-G shows occipital paroxysms (Gastaut, 1982). Some patients may exhibit only random occipital spikes, others may have occipital spikes only at sleep EEG, and some may have consistently normal EEG (Panayiotopoulos, 1999).

### 2.5.2 MECHANISMS CONTRIBUTING TO THE HIGHER SEIZURE SUSCEPTIBILITY OF THE IMMATURE BRAIN

Experimental studies in rodents have shown that immature animals are more susceptible to seizures than mature animals (Raol et al., 2001). Clinically also infants and children are much more prone to have seizures than adults, with
the highest seizure incidence occurring during the first year of life (Raol et al., 2001). This is suggested to stem from increased excitation and decreased inhibition in the developing brain (Holmes et al., 2002). The factors contributing to increased seizure susceptibility of the immature brain contribute to the epileptogenesis in both structural/metabolic and genetic causes of epilepsy.

Several features of normal development may contribute to increased seizure susceptibility of the immature brain. Glutamate acts as the main excitatory transmitter of the adult brain and following its release derives its action via two types of receptors, ionotropic and metabotropic. These ionotropic receptors are further subdivided into N-methyl-D-aspartate (NMDA) and non-NMDA receptors, including alpha-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid (AMPA) and kainic acid (KA) receptors (Raol et al., 2001). γ-aminobutyric acid (GABA), by contrast, acts as the major inhibitory neurotransmitter in the mature brain and exerts its effect via two major classes of receptors, namely GABA_A and GABA_B (Raol et al., 2001, Holmes et al., 2002). Although glutamate acts as the primary excitatory neurotransmitter in the mature brain, GABA mediates much of the excitatory neurotransmission in the immature brain (Rivera et al., 1999, Rivera et al., 2005, Dehorter et al., 2012, Ben-Ari, 2014). This profound change in the function of GABA arises in part from alterations in the chloride ion reversal potential mediated by changes in expression of chloride transporter proteins responsible for extrusion of Cl^- from neurons (Rivera et al., 1999, Dehorter et al., 2012, Ben-Ari, 2014). In the mature brain, neurons maintain a low intracellular Cl^- concentration, which is required for classical hyperpolarizing inhibition mediated by GABA_A receptors (Kaila, 1994). In the immature brain with high intracellular Cl^-, activation of GABA_A channels is associated with a Cl^- efflux and depolarization. This removes the voltage-dependent Mg^{2+} block from NMDA channels, resulting in NMDA receptor activation (Raol et al., 2001, Dehorter et al., 2012). Additionally, GABA_A receptors are functionally more active than NMDA and AMPA receptors at this time of life, providing a net excitatory drive in the developing brain (Holmes et al., 2002, Levene, 2002).

Other major inhibitory systems, including the postsynaptic GABA_B, also have a delayed maturation, contributing to a higher excitability (Holmes et al., 2002, Dehorter et al., 2012). Several other factors in normal development contribute to hyperexcitability of the immature brain, making it more prone to development of seizure activity. These factors include increased density of NMDA receptors, differences in NMDA receptor subunit composition, and longer receptor opening, with all of these factors enhancing NMDA-mediated excitation in immature neurons, thus favoring hyperexcitability (Raol et al., 2001, Heinrichs and Seyfried, 2006, Sánchez Fernández and Loddenkemper, 2014). AMPA receptors without GluA2 subunit are typically expressed in the immature brain, leading to an increased permeability to Ca^{2+}, and thus, contributing to lower seizure threshold (Sánchez Fernández and
Loddenkemper, 2014). In conclusion, the depolarizing effect of GABA, the delayed maturation of other forms of postsynaptic inhibition, the subunit composition of neurotransmission receptors in the immature brain, and an overabundance of excitatory synapses makes the immature brain more prone to seizure activity (Holmes et al., 2002, Sánchez Fernández and Loddenkemper, 2014).

2.6 FUNCTIONAL NEUROIMAGING IN EPILEPSY

Cerebral blood flow and metabolism change according to the course of the seizure within the epileptogenic zone (i.e., the ictal onset area) (Chiron, 2013). In general, during an epileptic seizure cerebral glucose metabolism and blood flow in the epileptogenic focus are increased (Chiron, 2013, Stanescu et al., 2013). Ictal hyperperfusion is followed by postictal hypoperfusion and cerebral blood flow remains at this very low level for several minutes before returning to the interictal level (i.e., interictal hyperperfusion and hypometabolism) (Chiron, 2013, Stanescu et al., 2013). These changes can be investigated with interictal positron emission tomography (PET) detecting changes in brain metabolism or peri-ictal (i.e., either ictal or immediately postictal) single photon emission computer tomography (SPECT) reflecting cerebral blood flow.

Positron emission tomography utilizes tracer compounds that are labeled with specific positron-emitting isotopes, and the imaging technique is based on the determination of the source and concentration of emission (Spencer, 1994). Glucose metabolism provides most of the ATP required for brain function, and thus, glucose metabolism is tightly connected to neuronal activity (Varrone et al., 2009). Changes in neuronal activity due to different disease processes cause changes in glucose metabolism, and these changes can be visualized with PET utilizing 2-[¹⁸F]fluoro-2-deoxy-D-glucose (FDG) as a tracer (Varrone et al., 2009). FDG is a glucose analog that is taken up into cells and phosphorylated by hexokinase. Unlike glucose, however, FDG does not metabolize further, but accumulates in the cell, and thus, can represent the regional metabolic rate of glucose consumption of a given tissue (Sokoloff et al., 1977).

In SPECT, the tracers used are gamma emitters. A labeled tracer is trapped in the small vessels of the brain. Due to slow elimination over several hours, the tracer continues to generate radioactivity, which is measured and localized using tomographic cameras (Chiron, 2013).

In human medicine, patients with medically intractable epilepsy can be treated with neurosurgical resection if the patient has a focal epileptogenic abnormality that is safe to remove (Rubi et al., 2011, Stanescu et al., 2013). MRI can be non-lesional or discordant despite localizing signs on seizure semiology and EEG (Rubi et al., 2011). Focal cortical dysplasia is one of the most common causes of intractable epilepsy in children and may not be
detected with MRI (Sisodiya, 2000, Tassi et al., 2002). In these cases functional neuroimaging, including PET utilizing FDG as a tracer and peri-ictal SPECT, can help to detect the area of abnormal metabolism to be resected (Rubi et al., 2011, Chiron, 2013, Stanescu et al., 2013). This is in contrast to generalized seizures where FDG-PET is unable to detect focal hypometabolism (Theodore et al., 1985).

Ictal SPECT is the best localizing method for temporal lobe epilepsy in adults, with sensitivity reaching 97% in ictal studies and 75% in post-ictal studies (Chiron, 2013). Interictal FDG-PET reaches a sensitivity of about 70% (Rubi et al., 2011, Chiron, 2013). In extratemporal lobe epilepsies, the sensitivity of ictal SPECT and FDG-PET is lower, and in children extratemporal epilepsies are twice as common as temporal epilepsies (Rubi et al., 2011, Chiron, 2013, Stanescu, 2013). Ictal SPECT in children reportedly reveals a hyperperfused area consistent with the presurgical work-up in about 75% of patients (Chiron, 2013).

### 2.6.1 FUNCTIONAL NEUROIMAGING IN EPILEPSY OF DOGS

Little data exist on functional neuroimaging in epileptic dogs. One study investigated interictal SPECT in dogs with epilepsy, but did not detect areas of cortical hypoperfusion when epileptic dogs were compared as a group with healthy controls (Martlé et al., 2009). Another study investigated FDG-PET in dogs with IE (Viitmaa et al., 2014). The researchers detected abnormalities in cerebral glucose uptake in 82% of epileptic dogs and concluded that FDG-PET may be a useful tool for dogs with focal epilepsy.

### 2.6.2 ANALYSIS OF FDG-PET DATA

Several methods exist to assess the images received from FDG-PET studies. Qualitative analysis comprises the visual assessment of images for areas of hypo- or sometimes hypermetabolism (Engel et al., 1982). It is commonly performed in clinical practice, but the results may be dependent on the examiner’s expertise (Kim et al., 2002).

Because knowledge of an individual’s clinical data may bias the interpretation of subtle changes, researchers have developed different methods for more objective assessment of FDG-PET images (Henry et al., 1993). A pixel-wise comparison of the patient’s image to an age-matched database can be performed in an automated way, thus providing an objective evaluation of potential abnormalities in brain glucose metabolism (Goffin et al., 2008). An asymmetry analysis is based on the detection of differences between the hemispheres, with a 10% cut-off threshold usually used to identify areas of decreased glucose metabolism (Benedek et al., 2006). An advantage of an asymmetry-based analysis is that it is less sensitive to global metabolic changes, including brain maturation (Benedek et al., 2006). Techniques comparing the activity of regions of interest (ROIs) between
homologous regions in the right and left hemispheres, or with the same regions in normal controls share a similar problem, with possibly different gyral and sulcal morphologies making it difficult to determine the exact location of homologous regions in the contralateral hemisphere or in normal controls (Joo et al., 2005).

Researchers have suggested that, instead of qualitative analysis, quantitative analysis of FDG-PET images should increase the sensitivity, accuracy, and reliability of detecting regional metabolic dysfunction (Henry et al., 1993). An absolute quantification method of FDG-PET studies requires dynamic image acquisition and serial arterial blood sampling, which is complex and time-consuming, therefore being impractical in most clinical settings (Goffin et al., 2008, Kim et al., 2010). When arterial blood samples cannot be obtained, standardized uptake values (SUVs) can serve as an alternative method for analyzing the cerebral glucose uptake (Suhonen-Polvi et al., 1995). SUV is the most commonly used semi-quantitative parameter in FDG-PET studies and is defined by ROI concentration of tracer per injected dose of normalized patient body weight (Suhonen-Polvi et al., 1995, Kim et al., 2010).

2.7 GENETICS OF EPILEPSY

2.7.1 GENERAL

Epilepsy is a common disorder in humans, and in approximately 20-30% of cases there is a clear acquired cause (e.g., head trauma, cerebrovascular disease), but in the remainder of cases genetic factors may play a role (Hauser et al., 1993, Jallon et al., 2001, Hildebrand et al., 2013, Petrovski and Kwan, 2013). A high heritability for epilepsy has been suggested based on twin studies (Hemminki et al., 2006, Petrovski and Kwan, 2013). A large study calculated the standardized risk ratio (SIR) for affected sibling pairs by comparing them with those whose siblings had no epilepsy (Hemminki et al., 2006). The risk was highest among 0- to 4-year-old children with an SIR of 6.82. In epidemiological studies, focal genetic epilepsy syndromes represent 9% and generalized genetic epilepsy syndromes 11% of all patients with epilepsy onset in childhood (Sillanpää et al., 1999). Additionally, genes have been identified in structural/metabolic epilepsies with Mendelian inheritance, with seizures being a sign or symptom of a more widespread central nervous system disorder. Examples include genes underlying malformations of cortical development, Lafora disease, and neuronal ceroid lipofuscinoses (Ottman et al., 2010).

Most of the currently known genes for human monogenic epilepsies encode ion channel subunits. These include voltage-gated (K⁺, Na⁺, Ca²⁺, and Cl⁻ channels) and ligand-gated channels (nicotinic acetylcholine and GABA_A receptors) that regulate neuronal excitability (Turnbull et al., 2005,
Review of the literature

Reid et al., 2009). Still, single-gene epilepsies, including recessive and dominant Mendelian epilepsy genes, represent the minority of genetic epilepsies (Hildebrand et al., 2013). Even though monogenic epilepsies are rare, they reveal the importance of ion channels as major determinants of epileptic phenotype (Reid et al., 2009, Ottman et al., 2010, Klassen et al., 2011). Instead of monogenic epilepsies, most genetic epilepsies are thought to have polygenic inheritance determined by multiple genes with or without environmental influences (Cavalleri et al., 2007, Hildebrand et al., 2013, Petrovski and Kwan, 2013, Mirza et al., 2015). Common genetic epilepsies in humans show complex inheritance, but to date only a small fraction of the susceptibility genes have been identified (Ottman et al., 2010, Hildebrand et al., 2013, Petrovski and Kwan, 2013, Mirza et al., 2015). Thus, it remains open whether “channelopathies” are relevant also in the most common epilepsies with complex inheritance (Reid et al., 2009). Possible genetic mechanisms behind these epilepsies include contribution of susceptibility alleles, de novo mutations, and copy number variations (CNVs) (Hildebrand et al., 2013).

A single susceptibility gene variant is typically not sufficient to cause epilepsy, and single variants of strong effect or a combination of multiple variants with weaker effects may determine susceptibility to epilepsy (Cavalleri et al., 2007, Petrovski and Kwan, 2013). The type and pattern of genetic variants might be more important than the number of variants (mutational load hypothesis) and whether they are common or rare, in conferring susceptibility to epilepsy (Klassen et al., 2011). This inheritance pattern can explain sporadic epilepsy cases with no family history of seizures (Ottman et al., 2010, Hildebrand et al., 2013). Researchers have suggested heterogeneous configurations of susceptibility loci to be associated with different epilepsy subtypes (Hempelmann et al., 2006, Petrovski and Kwan, 2013). Epilepsy with complex inheritance is suggested to arise in an individual when meiotic reshuffling produces by chance a combination of susceptibility alleles with sufficient effect to push neuronal hyperexcitability over the seizure threshold (Mulley et al., 2005).

A substantial contribution of de novo mutations to common epilepsies has also been suggested, although their role has been proven only in a group of severe childhood epilepsy disorders (i.e., epileptic encephalopathies) (Helbig et al., 2008, Hildebrand et al., 2013, Epi4K Consortium et al., 2013). Most de novo mutations are suggested to occur in the parenteral gametes, more often the father’s, but also in the very early embryo or later in embryonic or fetal development (Hildebrand et al., 2013). A germ line mutation is present in all cells of the body. In case a mutation occurs later during embryonic development, it leads to an uneven distribution of the mutation, or somatic mosaicism (Ottman et al., 2010). Mutations confined to the brain or neuroectodermal tissues due to somatic mosaicism might also play a role in the etiology of the more common epilepsies, but evidence for this is still lacking (Hildebrand et al., 2013).
CNVs are common even in healthy individuals and include both deletions and duplications of DNA sequences in the genome of humans and other mammals (Redon et al., 2006). CNVs can cause monogenic disease (familial or de novo) or act as risk alleles (Hildebrand et al., 2013, Petrovski and Kwan, 2013).

Other factors contributing to the complexity in inheritance of epilepsy are variable expressivity, reduced penetrance, and genetic heterogeneity (Ottman et al., 2010, Petrovski and Kwan, 2013, Mirza et al., 2015). Due to variable expressivity, the clinical epilepsy phenotype may vary widely among patients carrying the same mutation. Modifier genes and environmental factors are likely sources for variable expressivity (Helbig et al., 2008, Ottman et al., 2010, Petrovski and Kwan, 2013). Penetrance describes the likelihood of developing epilepsy in an individual with a mutation in a disease-causing gene (Ottman et al., 2010). Reduced penetrance may complicate the relationship between genotype and phenotype. Furthermore, mutations have been found in different genes causing the same syndrome, with these genes often encoding different subunits of the same ion channel (Ottman et al., 2010, Grinton et al., 2015). This genetic heterogeneity also complicates the relationship between genotype and phenotype.

2.7.2 FOCAL GENETIC EPILEPSIES IN CHILDREN
As stated previously, incidence rates of epilepsy in children are highest during the first year of life, with epilepsy most frequently resulting from structural defect in the brain (Deprez et al., 2009). Some infants have epilepsy for which no underlying etiology can be identified, except for a genetic predisposition (Deprez et al., 2009). The three familial focal epilepsy syndromes in the first year of life, namely BFNE, BFNIE, and BFIE, have autosomal dominant inheritance with high penetrance (Deprez et al., 2009). Most families with BFNE have mutations in the gene encoding the voltage-gated potassium channel subunit KCNQ2 (Helbig et al., 2008, Grinton et al., 2015). Mutations in the voltage-gated sodium channel subunit SCN2A have been identified in most families with BFNIE (Helbig et al., 2008, Grinton et al., 2015). A mutation in a gene encoding proline-rich transmembrane protein 2 (PRRT2) is reported to be responsible for 70% of cases with BFIE (Zara et al., 2013).

Benign childhood epilepsies of focal origin comprise three identifiable electroclinical syndromes including BRE, PS and ICOE-G (Engel, 2006, Panayitopoulos et al., 2008). Researchers have suggested that these three syndromes may be linked together by a genetically determined, functional derangement of systemic brain maturation (Panayitopoulos et al., 2008). Although the majority of the underlying genetics remains elusive, several risk-conferring genes for BRE have been identified recently (Reinthaler et al., 2015). Mutations in GRIN2A, a subunit of the excitatory glutamate receptor NMDA, represent a major risk factor for BRE (Lemke et al., 2013, Lesca et
al., 2013, Carvill et al., 2013). In a small number of patients, mutations of RBFOX1, RBFOX3, DEPCD5, and GABA\(_\alpha\)-R genes are involved (Lal et al., 2013, Lal et al., 2014, Reinthaler et al., 2015). The RBFOX genes encode neuron-specific splicing factors predicted to regulate neuronal splicing networks (Lal et al., 2013). DEPCD5 encodes the disheveled, Egl-10, and pleckstrin domain-containing protein 5 and GABA\(_\alpha\)-R GABA type A receptor (Lal et al., 2014, Reinthaler et al., 2015).

2.7.3 GENETICS OF EPILEPSY IN DOGS
Several mutated genes have been identified in structural/metabolic epilepsies in dogs, and these include a mutation causing Lafora disease, mutations causing neuronal ceroid lipofuscinoses, and a mutation causing spinocerebellar ataxia with myokymia or seizures, or both (Ekenstedt and Oberbauer, 2013, Gilliam et al., 2014). IE is suspected or confirmed to have a genetic basis in several dog breeds (Ekenstedt and Oberbauer, 2013). The epilepsy phenotype varies in different breeds, but also within a breed, according to the age at onset and seizure characteristics indicating a complex inheritance pattern of IE also in dogs (Ekenstedt and Oberbauer, 2013). On the other hand, unexpected heterogeneity and overlap have also been observed, with several different mutations causing a similar epilepsy phenotype in humans (Hildebrand et al., 2013).

Despite the suggestion of a genetic cause for epilepsy for many dog breeds, only one gene underlying canine IE has been identified in addition to that here. A recent study reported that variants at the ADAM23 locus increase the risk of IE in Belgian Shepherd dogs (Seppälä et al., 2012). Studies suggest ADAM23 also to be a potential major risk gene for IE in several other dog breeds (Koskinen et al., 2015).

2.8 LONG-TERM EFFECTS AND OUTCOME OF EPILEPSY

2.8.1 NEUROBEHAVIORAL COMORBIDITIES IN HUMANS
Neurobehavioral comorbidities, such as psychiatric, cognitive, and social deficits, are evident in children and adults with epilepsy (Hermann et al., 2008, Lin et al., 2012a). Different epilepsy syndromes may pose different risks for comorbidities, and the mechanisms underlying them appear to be different between focal and generalized epilepsies (Parisi et al., 2010). Even children with “benign” epilepsy syndromes - with studies mostly reporting comorbidities of BRE – which have a good prognosis and remission of seizures before adulthood, may have cognitive and behavioral deficits (Verrotti et al., 2002, Vinayan et al., 2005, Tovia et al., 2011, Terra et al., 2013). Cognitive impairment, neuropsychiatric problems, and social
difficulties can even be more disabling than the seizures themselves in people with epilepsy (Hamiwka and Wirrell, 2009).

2.8.1.1 Cognitive comorbidities
Epilepsy may be associated with cognitive dysfunction such as memory, attention, or processing difficulties (Holmes, 2015). Evidence of subnormal global cognitive function has been reported in one-fourth of children with epilepsy (Berg et al., 2008). Children with BRE often have an intelligence quotient within the normal range, but they may exhibit language delay, learning disabilities, and academic problems (Nicolai et al., 2006). These deficits improve over time when seizures remit. Little is known about the other genetic focal epilepsies in children.

2.8.1.2 Psychiatric comorbidities
Psychiatric comorbidities that show a higher prevalence in patients with epilepsy include mood disorders, anxiety disorders, attention deficit hyperactivity disorder (ADHD), and other psychiatric disorders (Lin et al., 2012a). Behavioral problems are nearly five times more common in children with epilepsy than in the general population (Terra et al., 2013).

ADHD is characterized by inattention, impulsivity, and hyperactivity. The diagnosis of ADHD requires the presence of 6 of 9 specific behavioral and functional symptoms of inattention or hyperactivity/impulsivity for a duration of at least 6 months, with onset before the age of 7 years (Kaufmann et al., 2009). ADHD and epilepsy have a particularly high comorbidity in children, and 20-40% of children with epilepsy have clinical ADHD in comparison with the general pediatric population, with 3-7% suffering from ADHD (Dunn et al., 2003, Thome-Souza et al., 2004, Dunn et al., 2005, Kaufmann et al., 2009). Studies report that 31-65% of children with BRE have ADHD (Tovia et al., 2011, Kim et al., 2014).

2.8.1.3 Social comorbidities
The psychological and social impact of epilepsy is more pronounced in epilepsy than in most other chronic diseases in humans (Hamiwka and Wirrell, 2009). The diagnosis of epilepsy and the lack of certainty where and when a seizure may occur can result in, for example, social withdrawal and isolation (de Souza and Salgado, 2006, Hamiwka and Wirrell, 2009). The social impact of epilepsy might be less important in dogs (Preston et. al., 2013).
2.8.1.4 Etiology for comorbidities

Despite the knowledge that people with epilepsy have a high prevalence of neurobehavioral comorbidities relative to the general population, the underlying mechanisms remain largely obscure (Yoong, 2015). Studies have suggested several underlying factors responsible for comorbidities in epilepsy: etiology of the seizures, seizure-related factors, abundant interictal epileptic activity, adverse effects of antiepileptic drugs, and psychosocial factors (Hermann et al., 2008, Hamiwka and Wirrell, 2009, Parisi et al., 2010, Rudzinski and Meador, 2013). Comorbid symptoms may be directly related to epilepsy and its possible underlying inherent brain abnormality including structural abnormalities (Hamiwka and Wirrell, 2009). Common biological mechanisms exist between various comorbidities and epilepsy such as abnormalities of the neurotransmitter pathways involving serotonin, norepinephrine, dopamine, glutamate, and GABA (Hamiwka and Wirrell, 2009).

Seizure-related factors that may play a role include seizure type, frequency, and severity, duration of epilepsy, and age at seizure onset (Schubert, 2005, Hermann et al., 2008, Hamiwka and Wirrell, 2009, Rudzinski and Meador, 2013). Frequent or prolonged seizures may result in long-lasting sequelae, especially in the developing brain, although there is no clear consensus about the effects of seizure frequency or the degree of seizure control and the severity or presence of neurobehavioral comorbidities (Helmstaedter et al., 2003, Schubert, 2005, Caplan et al., 2008, Hamiwka and Wirrell, 2009, Pineda et al., 2014). Early onset of seizures is reportedly associated with a higher risk of comorbidities (Hamiwka and Wirrell, 2009, Lin et al., 2012a). Ictal and interictal noxious factors including pathological neuronal network activity might exhaust the capacity of the brain to restore its homeostasis. Consequently, the longer the duration of refractory epilepsy, the higher the risk of cognitive impairment (Jokeit and Ebner, 1999, Elger et al., 2004). Electroencephalographic abnormalities without clinical seizures might affect attention and cognitive function, although the magnitude of this effect remains controversial (Schubert, 2005). Antiepileptic drugs are known to be associated with the development of changes in behavior (Terra et al., 2013). On the other hand, researchers have suggested that, excluding phenobarbital, gabapentin, and topiramate, most antiepileptic drugs seem not to adversely affect attention and behavior in therapeutic doses (Schubert, 2005).

Many of the comorbidities are present at or even before seizure onset (Lin et al., 2012a). While seizures may play a role, researchers have suggested a bidirectional relationship or a common underlying abnormality between epilepsy and comorbidities (Lin et al., 2012a, Yoong, 2015). Although the majority of children with epilepsy show no visible abnormalities in routine neuroimaging (Berg et al., 2000, Hermann et al., 2006), substantial evidence indicates that childhood-onset epilepsy is associated with an abnormal prospective pattern of brain development (Hutchinson et al., 2010, Pulsipher
et al., 2011, Tosun et al., 2011, Lin et al., 2012b) and that these structural or microstructural abnormalities in cortical and subcortical structures are linked to neurobehavioral comorbidities (Daley et al., 2007, Caplan et al., 2008, Pulsipher et al., 2009, O’Muircheartaigh et al., 2011, Vollmar et al., 2011, Lin et al., 2012b, Lin et al., 2013).

2.8.2 NEUROBEHAVIORAL COMORBIDITIES IN DOGS
The importance of neurobehavioral comorbidities in dogs with epilepsy is largely unknown. To date only one study has specifically investigated neurobehavioral comorbidities of IE in dogs (Shihab et al., 2011). The investigators reported that 71% of dogs with IE had behavioral changes visible with the development of epilepsy and suggested that these behavioral changes are comparable to anxiety disorders in humans. The study also reported changes in behavior depending on the medication status. Another study investigating epilepsy in the Italian Spinone breed also reported changes in behavior of dogs at IE onset, and these behavioral abnormalities included abnormal perception and anxiety (DeRisio et al., 2015a).

2.8.3 OUTCOME OF EPILEPSY
In humans, epilepsy is considered to be resolved in cases who either have remained seizure-free for the last 10 years and off antiepileptic drugs for at least the last 5 years or had an age-dependent epilepsy syndrome but are now beyond the applicable age (Fisher et al., 2014). A significant portion of patients with onset of epilepsy in childhood will become seizure-free by adulthood, with an overall remission rate of 64% (Sillanpää et al., 1998). Evidence suggests that antiepileptic treatment can be discontinued in children with focal epilepsy after two seizure-free years (Strozzi et al., 2015), and in Rolandic epilepsy one year of treatment has been recommended (Braathen et al., 1996). Although chronic epilepsy is associated with increased mortality, patients whose epilepsy goes into remission show no higher risk of death (Sillanpää et al., 1998). Some researchers have suggested that seizures in the immature brain could result in subsequent lowering of the seizure threshold (Holmes et al., 1998, Holmes et al., 2002). Drug-resistant epilepsy in both adults and children compromises the quality of life and results in morbidity and premature mortality (Duncan et al., 2006, Sillanpää and Shinnar, 2010).

In dogs, only one study has assessed the remission rates of juvenile epilepsy (defined by seizures occurring before the age of one year), and remission rates in that study were substantially lower (22%) than those reported in children (Arrol et al., 2012). The researchers suggested that this difference may result from the selection of dogs that were too old to be comparable with children concerning brain maturational stage (Arrol et al., 2012). The general remission rate of epilepsy in dogs with a median age of
two years at seizure onset is reportedly 13–15% (Berendt et al. 2007, Fredso et al., 2014).

Others have suggested that dogs with epilepsy have a shorter life span than dogs in the general population (Proschowsky et al., 2003, Berendt et al., 2007), but these studies investigated epilepsy in general, not IE alone. A life span similar to dogs in general has been reported in a study focusing on IE (Fredso et al., 2014), although genetic factors might cause variability between the severity of IE in different breeds (Casal et al., 2006, Hülsmeyer et al., 2010, Gullov et al., 2012, Weissl et al., 2012, DeRisio et al., 2015a). Studies report shorter survival times, especially with a severe clinical course of IE (Hülsmeyer et al., 2010, Weissl et al., 2012, DeRisio et al., 2015a). Breeds kept solely for companionship have reportedly longer survival times than breeds kept for dual-purposes, such as hunting, indicating that pet dogs are more likely to be treated for their epilepsy (Heske et al., 2014). A study investigating the long-term outcome of juvenile epilepsies in dogs found no association between age at seizure onset and survival rate (Arrol et al., 2012). However, a history of status epilepticus shortened dog’s survival time (Arrol et al., 2012).

The impact of epilepsy on the quality of life of a dog is currently unclear (Wessman et al., 2014). In one study investigating IE and the impact of its treatment on dogs, 48% of dog owners described a decrease in their dog’s quality of life (Chang et al., 2006). This lower quality of life was attributed to the side-effects of antiepileptic medications, inadequate seizure control, and behavioral changes (Chang et al., 2006).
Aims of the study

3 AIMS OF THE STUDY

This study originates from the clinical examination of two Lagotto Romagnolo litters introduced by local breeders. Several affected dogs suffering from episodic signs were found in both litters. We hypothesized the presence of a novel inherited neurological syndrome, a form of benign juvenile epilepsy, in the breed and embarked on a large collaborative study to characterize its clinical features and genetic cause with the following specific aims:

1. To characterize the clinical picture of benign familial juvenile epilepsy in Lagotto Romagnolo dogs (I).

2. To describe typical findings in magnetic resonance imaging, electroencephalography, and 2-\[^{18}\text{F}\]\text{fluoro-2-deoxy-D-glucose} – positron emission tomography in Lagotto Romagnolo dogs with juvenile epilepsy (I, III).

3. To determine the mode of inheritance and the causative mutation of affected dogs (I, II).

4. To determine the long-term course of the disease and to assess possible neurobehavioral comorbidities of juvenile epilepsy in Lagotto Romagnolo dogs (IV).
4 MATERIALS AND METHODS

4.1 ETHICAL APPROVAL OF STUDY PROTOCOLS

All studies were approved by the Ethics Committee on Animal Trials at the University of Helsinki, Finland.

4.2 DOGS

4.2.1 AFFECTED DOGS
Lagotto Romagnolo (LR) puppies (n=25) with neurological signs primarily of an episodic nature and suspected to be focal onset seizure episodes were included in Study I. The dogs were presented to us at the Veterinary Teaching Hospital or the Referral Neurology Hospital Aisti by breeders or dog owners.

The genetic study (II) investigated dogs (n=28) from Study I, and, based on a retrospective questionnaire, additional affected dogs were also included. Moreover, we included adult LR dogs with focal seizures and similar clinical findings to Studies I and II in order to determine whether these dogs represented the same epilepsy syndrome with a different age of onset of seizures (Study I n=3, Study II n=5). In Study II, we also investigated clinically diagnosed juvenile epilepsy cases (n=8) from other breeds, namely Barbets, Collies, and German Shepherd dogs, and adult-onset epilepsy cases (n=114) from 40 different breeds.

In Study III, the FDG-PET study, we investigated cerebral glucose uptake in the brain of LR dogs with juvenile (n=6) or adult-onset epilepsy (n=2) from Study I.

To evaluate long-term outcome (IV), we recruited 25 affected dogs from Study I or II by contacting the dogs’ owners by email or phone and asking them to complete an online questionnaire.

4.2.2 CONTROL DOGS
In Study I, 33 littermates of affected LR puppies served as controls.

In Study II, the control dogs (n=112) consisted of healthy littermates, their parents, and, based on the retrospective questionnaire-based phenotype information, additional healthy control LR dogs.

In Study III, volunteer healthy control LR dogs (n=5) at different ages were examined.
Materials and methods

For Study IV, we recruited control LR dogs (n=91) with no history of epilepsy via email, breeders, or the LR breed club.

The numbers of dogs investigated by different diagnostic methods are presented in Table 1.

4.3 HISTORY AND CLINICAL AND NEUROLOGICAL EXAMINATION (I)

Age at onset, course of disease, character and frequency of seizure episodes, interictal signs, possible exposure to toxins, and vaccination history were recorded. Clinical and neurological examinations were performed at the Veterinary Teaching Hospital of the University of Helsinki or at the Referral Neurology Hospital Aisti. The neurological examination included observation of mentation, behavior, posture, and gait; postural reaction testing including proprioception, hopping, wheelbarrow, and extensor postural thrust; testing of spinal reflexes including myotatic and withdrawal reflexes; and evaluation of cranial nerves. We video-recorded the neurological examination for almost all dogs and encouraged the owners of affected dogs to videotape the seizures.

4.4 LABORATORY ANALYSES (I,III)

We performed the following laboratory analyses at the Central Laboratory of the Department of Equine and Small Animal Medicine, University of Helsinki (I): complete blood cell count (CBC), serum biochemistry analysis (sodium, potassium, calcium, magnesium, glucose, total protein, albumin, cholesterol, creatinine, urea, creatine kinase, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase), and urinalysis. Cerebrospinal fluid (CSF), collected by cisternal puncture, was analyzed for total cell count, cytology, and protein content.

Plasma glucose was analyzed by glucose/glucose oxidase method with the Analox GM7 glucose analyzer (Analow Instruments Limited, London, UK) at the Turku PET Centre (III).

4.5 ELECTRODIAGNOSTICS

4.5.1 ELECTROMYOGRAPHY (EMG) AND BRAINSTEM AUDITORY EVOKED RESPONSE (BAER) (I)

In electromyography (EMG), we recorded intramuscular potentials from one side of the body (fore- and hindlimb, paraspinal muscles) under sedation
with medetomidine and butorphanol, and administered propofol intravenously as needed. We performed brainstem auditory evoked response (BAER) under the same sedation as EMG. Alternating click stimuli at the 90 decibel sound pressure level (dB SPL) were delivered through earplugs; a masking noise of 50 dB SPL was applied to the contralateral ear. Between 500 and 2000 clicks were averaged and each recording was repeated twice.

4.5.2 ELECTROENCEPHALOGRAPHY (EEG) (I, III)
For electroencephalography (EEG), dogs were sedated with medetomidine (0.04 mg/kg intramuscularly, IM). Dogs who resisted needle placement after 15 min of initial sedation received an additional dosage of medetomidine (0.02 mg/kg, IM). Dogs were placed in sternal recumbency and subcutaneous needle electrodes were inserted over the calvaria. A 17-channel reference montage (F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2; reference: on the nose; ground: caudally to the external occipital protuberance). The total recording time was 20 min. All of the EEG recordings were visually examined by an experienced EEG specialist (L. Bergamasco). Spikes and sharp waves as well as focal abnormalities of the background were registered. Evaluation of EEG recordings was not blinded.

4.6 DIAGNOSTIC IMAGING

4.6.1 MAGNETIC RESONANCE IMAGING (MRI) (I, III)
We performed magnetic resonance imaging (MRI) examinations using 1.5T Siemens Magnetom Symphony (Siemens AG, Medizinische Technik, Germany) at a private hospital for humans (Mehiläinen) (I), 1.5T Philips Gyrosan Intera CV Nova Dual (Philips Medical Systems, Best, the Netherlands) at the Turku PET Centre (I, III), or Vet-MR 0.2 T equipment (Esaote, Genova, Italy) at the Referral Neurology Hospital Aisti (I).

The dogs were positioned in sternal recumbency under general anesthesia maintained with propofol (0.5-1 mg/kg) when performing MRI with the 1.5T MRI machine and with 1.5-2.0% isoflurane when performing MRI with the 0.2T MRI machine. With the 1.5T MRI, T2-weighted images were obtained on at least two planes (sagittal, transverse, or dorsal), and multiplanar reconstructions (MPR; with reconstructions as T1-weighted images) before and after contrast (gadopentetate dimeglumine, Magnevist 469 mg/mL inject., Schering AG, Berlin, Germany, 0.1 mmol/kg) were obtained, and sagittal, transverse, and dorsal planes were examined with a 3 mm slice thickness. With 0.2T MRI, T1-weighted images were examined in at least two planes (usually transverse and sagittal) before and after intravenous contrast (gadopentetate dimeglumine) administration. T2-weighted images in 4 mm
slice thickness were also examined in at least two planes (usually transverse and dorsal).

4.6.2 POSITRON EMISSION TOMOGRAPHY (PET) (III)
We performed positron emission tomography (PET) examinations with 2-\[^{18}\text{F}\]fluoro-2-deoxy-D-glucose (FDG) as a tracer at the Turku PET Centre. The dogs fasted at least 8 h prior to the study. We sedated the dogs with medetomidine 0.03 mg/kg and butorphanol 0.2 mg/kg IM injection 15 min before administering FDG in order to prevent excitation. Approximately 10 min later, we placed an intravenous catheter in the cephalic vein and collected a blood sample in order to measure the blood glucose level. Fifteen minutes before the PET scan, the dogs received an additional injection of midazolame 0.2 mg/kg and medetomidine 0.015 mg/kg IM. We injected on average 48 ± 17 MBq, corresponding to 4.5 ± 1.3 MBq/kg of FDG, intravenously 55 min before the emission scan began.

PET imaging was performed using a dedicated, high-resolution brain PET scanner (ECAT HRRT, Siemens Medical Solutions, Knoxville, TN, USA) with minimal spatial resolution of 2.5 mm in the reconstructed images in the 10 cm field of view. The dogs were positioned in ventral recumbency using head fixation foam pads to restrict head motion. Data were collected for 40 min in list mode format. After each emission scan, a 5 min transmission scan was performed for attenuation correction. True events were normalized, corrected for attenuation and scatter and then reconstructed by the iterative ordered subsets expectation maximization 3D algorithm. Images were reconstructed into a volume of 256 x 256 x 207 cubic voxels of size 1.81 mm\(^3\).

Visual analysis of PET images was performed by three independent and experienced evaluators using non-commercial image analysis software (Vinci 2.56, Max-Planck-Institute for Neurological Research, Cologne, Germany). Zones of hypometabolism or hypermetabolism using the basal ganglia as a reference region were identified in the FDG tomographic images displayed as transversal, dorsal, and sagittal planes. Focal abnormalities were defined as focal or lateralized areas of decreased or increased radioactivity that could be recognized on three or more adjacent slices. Findings were considered significant if at least two of the three evaluators detected them. Also the degree of agreement was assessed with full agreement referring to cases where either all three evaluators detected the same area of abnormal glucose uptake or none of them detected abnormal areas of glucose uptake; 2/3 agreement referring to cases where two of the three evaluators detected the same area of abnormal glucose uptake or no abnormalities in glucose uptake; and all evaluators showing disagreement referring to cases where either all three evaluators detected different areas of abnormal glucose uptake or two evaluators detected different areas of glucose uptake and one evaluator detected no areas of abnormal glucose uptake. Evaluators were unaware of the dogs’ healthy status.
Additionally, we performed a semiquantitative analysis. Irregular regions of interest (ROI) were manually drawn on the transverse PET slices by using the Imadeus software (version 1.50, Forima Inc., Turku, Finland) and applying individual corresponding 3D MRI slices as an anatomical reference. ROIs were drawn on several cortical regions (frontal, temporal, parietal, occipital), the hippocampus, the basal ganglia, the thalamus, the caudal colliculus, the cerebellum, the cerebellar vermis, and white matter (to the area of the internal capsule) with special emphasis on maximizing the symmetry of ROIs. ROIs were drawn on several adjacent slices on the corresponding brain areas. Calculations of the standard uptake values (SUVs) normalized with injected FDG dose (kBq) / body weight (g) were based on the activity concentration of the entire ROI. Relative SUV values with SUV values normalized against the white matter value for the same dog were calculated for each ROI in order to exclude variations in individual general SUV level of each dog. Relative SUV values of different areas of the brain were compared between epileptic and control dogs. Asymmetry indices (AI; [(activity concentration of ROI on the left side – activity concentration of ROI on the corresponding area on the right side) / [(activity concentration of ROI on the left side + activity concentration of ROI on the right side) / 2] / x 100%)] were calculated to estimate lateralization; an AI greater than 10% was considered significant.

4.7 GENETICS

4.7.1 PEDIGREE ANALYSIS (I)
We generated a multigenerational pedigree and counted the segregation frequency based on the proportion of affected dogs of all dogs in the pedigree. We also looked for a possible common ancestor and whether the affected dogs are related. Furthermore, we investigated the sex ratio of affected dogs.

4.7.2 DETECTION OF CAUSATIVE MUTATION (II)
We collected EDTA-blood samples from affected and healthy control dogs for the genetic study. DNA was isolated with a Qiagen DNA isolation kit and samples were stored at Dr. Lohi’s laboratory in the dog DNA bank.

We performed a single-nucleotide polymorphism (SNP) genome-wide association study (GWAS) with DNA from affected dogs and their unaffected littermates. After discovering homozygous SNPs in all affected dogs and none of the controls, we investigated this region further. We sequenced the suspected mutated gene in the affected and control dogs. We genotyped an additional cohort of affected and control dogs for the suspected SNP, for the suspected mutation, and for three additional SNPs from the homozygous
region. Additionally, we included in the study population adult-onset epilepsy LR cases. Finally, we investigated the presence of the mutation in other breeds by sequencing juvenile and adult-onset epilepsy cases from different breeds.

The suspected pathological sequence change was predicted to be a truncating mutation. The consequences of the mutation were analyzed by various methods, including transcript analyses, cell cultures, transfections, Western blotting, and cell-surface binding assays. Moreover, we investigated the developmental expression of the \( Lgi2 \) transcript using brains of mice at different ages.

### 4.8 BEHAVIORAL QUESTIONNAIRE (IV)

To determine the long-term outcome of LR dogs with a history of seizure episodes experienced during puppyhood, we created an open-access questionnaire. The questionnaire included 77 questions, of which 46 inquired about details of the dog's background and daily routines, and 31 were related to the dogs' activity, impulsivity, and inattention behavior. Questions concerning the dog's activity, impulsivity, and inattention behavior were drawn from two previously published studies on impulsivity and activity levels in dogs (Vas et al., 2007, Wright et al., 2011).

To determine the current status of the dog, and especially the presence of possible neurological symptoms or behavioral problems after remission of seizures, the owners who completed the online questionnaire also participated in a telephone interview.

The length of the follow-up period for the affected dogs was defined as the time between the last seizure episode in puppyhood and the time of the telephone interview. The information in our database on carrier status for the \( LGI2 \) c.1552A>T (p.K518X) mutation responsible for seizures during puppyhood was checked for both affected and control dogs.

### 4.9 STATISTICAL ANALYSIS

#### 4.9.1 STUDY I

We calculated the mean value and range for age at onset and disappearance of neurological signs. We calculated the segregation frequency by dividing the number of affected dogs by the total number of dogs in the pedigree.

#### 4.9.2 STUDY II

The SNP association analysis was performed with PLINK software with the criteria of MAF <0.05, call rate >75%, and <25% of missing genotypes in
Materials and methods

individual dogs. Odds ratios were calculated using conditional maximum likelihood estimation and the corresponding 95% confidence intervals were calculated by using the Fisher exact test. The calculations were done with R statistical software package.

4.9.3 STUDY III
We applied descriptive statistics to summarize the data by health status groups. We also calculated 95% confidence intervals of mean values. Normality assumptions were checked with Normal QQ-plots and the Shapiro-Wilks test of normality. We investigated any difference among the relative SUV values of the health status groups with a one-way ANOVA model.

Health status was divided into three groups: control, epileptic [juvenile] (both with ongoing seizures and recovered dogs), and epileptic [adult]. In all of the fitted ANOVA models, Tukey’s HSD test was used in the pair-wise comparisons as a post-hoc test, taking into account the multiplicity issues in the conducted comparisons. We assessed any difference among regions of the brain with the one-way ANOVA model. The analysis was performed separately for adults and juveniles. Statistical analyses for this study were performed at 4Pharma Ltd with the SAS® System for Windows (version 9.2, SAS Institute Inc., Cary, NC, USA).

4.9.4 STUDY IV
We used the t-test for independent samples to compare the distribution of age and sex between affected and control dog groups. Principal Component Analysis (PCA) with varimax rotation and Kaiser normalization was conducted to explore the factorial structure of the questionnaire. The questionnaire items were grouped into factors with eigenvalue > 1. Variables with loadings <0.40 were excluded from the factor descriptions. We also considered the biological interpretation of the factors. We calculated the individual factor scores for each dog as the sum of the scores from individual items included in that factor. Comparisons between the scores for created factors between the affected and control groups were performed with independent samples t-test, and all tests were two-sided.

In addition, we divided the affected group into two subgroups: those with mild form (defined as history of less than five seizures altogether; focal seizures only) and those with severe form (more than five seizures altogether). We used an independent samples t-test to compare the factor scores between these subgroups. Statistical analyses were performed with SPSS statistical program (version 21, IBM, Armonk, NY, USA).

A p-value <0.05 was considered statistically significant in all studies performed.
Table 1  Number of puppies with juvenile-onset epilepsy, control Lagotto Romagnolo (LR) dogs, and adult-onset epilepsy LR dogs examined with each diagnostic method.

<table>
<thead>
<tr>
<th>Examination</th>
<th>Affected LR puppies</th>
<th>Control LR dogs</th>
<th>Adult-onset epilepsy LR dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological examination (I)</td>
<td>21</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Telephone interview for historic assessment (I)</td>
<td>4</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>CBC and serum biochemistry (I)</td>
<td>17</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Urinalysis (I)</td>
<td>15</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CSF (I)</td>
<td>16</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>EMG (I)</td>
<td>15</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>BAER (I)</td>
<td>11</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>EEG (I, III)</td>
<td>16</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>MRI (I, III)</td>
<td>18</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>FDG-PET (III)</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Pedigree analysis (I)</td>
<td>25</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>SNP GWAS (II)</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Gene sequencing for suspected SNP, for suspected mutation, and for three additional SNPs (II)</td>
<td>28</td>
<td>112</td>
<td>5</td>
</tr>
<tr>
<td>Follow-up study</td>
<td>25</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

CBC=cell blood count, CSF=cerebrospinal fluid, EMG=electromyography, BAER=brainstem auditory evoked response, EEG=electroencephalography, MRI=magnetic resonance imaging, FDG-PET=[18F]fluoro-2-deoxy-D-glucose – positron emission tomography, SNP=single nucleotide polymorphism, GWAS=genome wide association study.
5 RESULTS

5.1 HISTORY AND CLINICAL AND NEUROLOGICAL EXAMINATION

5.1.1 SEIZURE CHARACTERISTICS IN AFFECTED DOGS WITH JUVENILE-ONSET SEIZURES

Twenty-five of 58 studied LR dogs exhibited neurological signs, primarily of an episodic nature, as puppies. Fifteen of the 25 affected dogs were females. Episodes were interpreted as focal onset seizures due to clinical findings in the first puppies examined. The frequency of the seizure episodes varied from multiple episodes per day to one episode per week. Individual seizures lasted from ten seconds to a few minutes. Seizures were independent of the time of day, with some seizures occurring during sleep and others during exercise. Seizures were characterized by generalized tremor, ataxia, and stiffness. During seizure episodes most of the dogs appeared conscious (i.e., responded to speech), but especially those in lateral recumbency during episodes failed to respond normally and had either impaired or lost consciousness. With repeated analysis of videos of seizures provided by the dog owners, we were able to detect lateralizing signs instead of just the whole-body tremor described by owners.

Seizures began at a mean age of 6.3 (range 5-9) weeks and the last seizures were apparent at a mean age of 10 (range 7.5-13) weeks. Most of the puppies were normal between seizures, but some puppies exhibited ataxia or falling reported by the breeders or owners. Clinical signs varied between puppies and in litters with multiple affected puppies; some exhibited more severe seizures and interictal signs. Otherwise puppies were bright and healthy.

Only one of the 25 affected puppies received antiepileptic medication (phenobarbital 2 mg/kg twice per day) from the age of seven weeks due to focal seizures and remained seizure-free until the medication was discontinued at the age of 11 weeks. At the age of 13 weeks, the dog experienced two more seizures and the medication was re-administered, and afterwards the puppy experienced only one mild seizure. The medication was gradually discontinued after one month and no subsequent seizures were noted.
Results

5.1.2 SEIZURE CHARACTERISTICS IN AFFECTED DOGS WITH ADULT-ONSET SEIZURES

One of the dogs with adult-onset seizures began to exhibit focal seizures characterized by head tremor (movement of the head in horizontal plane) at the age of one year and seven months. This dog was from a litter with two puppies exhibiting seizures as puppies. One adult dog came to its present owner at the age of six months and began to exhibit seizures characterized by whole-body tremor as an adult. The pedigree for this dog was unavailable, as was information about possible seizures in puppyhood in littermates. The third adult dog included in this study came from a litter with no history of seizures during puppyhood. This dog experienced three focal seizures characterized by horizontal head tremor during two days at the age of two years and one month. At that point, phenobarbital was started (2 mg/kg twice a day), and the seizures became milder and less frequent.

5.1.3 PHYSICAL AND NEUROLOGICAL EXAMINATION

Physical examination was normal in the affected and control LR puppies. Abnormal findings in interictal neurological examination were present in ten affected puppies. These abnormalities included generalized ataxia (n=7) and hypermetria (n=5), intention tremor (n=3), tremor (n=3), decreased postural reactions in all limbs, or in hindlimbs only (n=8), and bilaterally decreased menace reaction in all dogs (considered normal for puppies). Mental status appeared normal in all puppies. The severity of neurological abnormalities varied between puppies from mild changes to severe ataxia and falling. Puppies with the most severe seizures also exhibited the most remarkable findings in the neurological examination. Nine of ten puppies with interictal abnormalities were re-examined until no changes were present in the neurological examination. Interictal neurological deficits usually disappeared earlier than seizures. All three adult dogs studied had normal physical and neurological examinations.

5.2 LABORATORY ANALYSES AND ELECTRODIAGNOSTICS

Hematologic and biochemical analyses, urinalysis, and CSF examinations revealed no remarkable changes in any of the examined dogs.

EMG and BAER recording appeared normal in all dogs examined.

Interictal EEG analysis indicated epileptiform activity in 14 affected LR puppies (focal abnormal activity in 13 and generalized activity in 1). These abnormalities included sharp waves and spikes (Figure 1). Two affected puppies showed normal EEG recording. EEG was performed on 12 puppies while still experiencing seizures (including one affected puppy with normal EEG recording). In four puppies, EEG was performed 1-4 (mean 2.1) weeks
after the last seizure episode. EEG of the five healthy littermates studied exhibited abnormalities (sharp waves) in one dog and was normal in four dogs. Two of the three adult dogs studied showed epileptiform activity in EEG recording.

![Figure 1](image.png)  
**Figure 1** EEGs for two Lagotto Romagnolo puppies. Both puppies exhibited focal seizures and focal epileptiform activity in interictal EEG (paper speed 15 mm/s, 1 s/division). (A) Sharp waves (amplitude: 200 μV/cm) and (B) spikes (amplitude: 100 μV/cm) are visible in these EEG recordings.

### 5.3 DIAGNOSTIC IMAGING

#### 5.3.1 MAGNETIC RESONANCE IMAGING (MRI)

No significant findings appeared in the MRI images of the examined puppies. MRIs were also normal in all adult dogs examined.

#### 5.3.2 FDG-PET

The affected dog group consisted of three dogs with juvenile epilepsy and ongoing seizures (PET examination performed at the age of 8, 10.5, and 10.5 weeks), three dogs with history of juvenile seizures but upon PET examination the seizures had spontaneously resolved (PET examination
performed at 8, 19, and 46 weeks after the last seizure episode at the age of 17, 28, and 55 weeks, respectively), and two dogs with adult-onset epilepsy and focal seizures (PET examination performed at the age of 89 and 312 weeks). Five control dogs were examined at the age of 8 (a littermate of a sick dog), 104, 182, 260, and 312 weeks.

Blood glucose levels were within the reference range in all but one dog with mild hypoglycemia. Table 2 provides a detailed description of abnormal FDG-PET findings and correspondence to EEG recordings. In the affected group, visual analysis of the scans with a special focus on the asymmetric metabolic pattern revealed areas of hypometabolism in all three LR dogs with juvenile epilepsy and ongoing seizures (Figure 2). PET was also abnormal in two of the three additional dogs in the epileptic group with a history of juvenile epilepsy, but with PET examination performed after spontaneous recovery from seizures. Two dogs with adult-onset epilepsy had a normal PET scan. Three control dogs had normal PET scans, but two showed hypometabolic areas. One of these normal control dogs with abnormal PET visual analysis was the littermate of an affected puppy. The three evaluators agreed fully in 30.8% of the cases (4/13), showed 2/3 agreement in 61.6% of the cases (8/13), and showed disagreement in 7.7% of the cases (1/13).

The three evaluators agreed on the abnormality in all three LR dogs with juvenile epilepsy and ongoing seizures and in the one dog with a history of juvenile epilepsy but 19 weeks since its last seizure. In the rest of the dogs, which exhibited a significant change in glucose uptake, two of the three evaluators agreed.

Electroencephalography results were available for four dogs exhibiting changes in cerebral glucose uptake (two dogs with juvenile epilepsy and ongoing seizures, and two dogs with history of juvenile epilepsy but recovered from seizures). In three of them, EEG showed changes in the same areas where PET revealed cortical hypometabolism (Table 2). AIs showed no significant differences in affected or control dogs.

In semiquantitative analysis, affected dogs showed no different relative SUV values in any of the examined brain areas compared with control dogs.
Results

Table 2  **PET visual analyses in dogs with abnormal findings and correspondence to EEG abnormalities.**

<table>
<thead>
<tr>
<th>Study subject</th>
<th>EEG findings</th>
<th>FDG-PET findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR dog with juvenile epilepsy (ongoing seizures)</td>
<td>Normal EEG (performed immediately after a seizure episode)</td>
<td>Left occipital lobe wide hypometabolic area</td>
</tr>
<tr>
<td>LR dog with juvenile epilepsy (ongoing seizures)</td>
<td>Sharp waves on the left parietal-occipital brain areas</td>
<td>Left occipital lobe hypometabolic area</td>
</tr>
<tr>
<td>LR dog with juvenile epilepsy (ongoing seizures)</td>
<td>No EEG</td>
<td>Left temporal lobe hypometabolic area</td>
</tr>
<tr>
<td>LR dog with juvenile epilepsy (seizures in remission)</td>
<td>Sharp waves on the right parietal-occipital brain areas</td>
<td>Right parietal lobe hypometabolic area, right occipital lobe hypometabolic area</td>
</tr>
<tr>
<td>LR dog with juvenile epilepsy (seizures in remission)</td>
<td>Sharp waves on the right parietal-occipital brain areas</td>
<td>Right parietal lobe hypermetabolic area</td>
</tr>
<tr>
<td>Control dog</td>
<td>No EEG</td>
<td>Left occipital lobe hypometabolic area</td>
</tr>
<tr>
<td>Control dog</td>
<td>No EEG</td>
<td>Left temporal lobe hypometabolic area</td>
</tr>
</tbody>
</table>

Figure 2  **FDG-PET images from an eight-week-old puppy with juvenile epilepsy showing an area of hypometabolism in the left occipital lobe.** Images appear in transverse and dorsal planes. The cross localizers indicate the area of hypometabolism. The color bar represents different radioactivity levels [kBq/mL].
5.4 GENETICS (I, II)

The multigenerational pedigree generated based on the studied dogs included 115 dogs, 25 (21.7%) of which were reportedly affected with an observed segregation frequency of 0.43 (25/58). All affected puppies were related, but had no single common ancestor. Male and female dogs seemed equally affected, 43.5% (10/23) and 44.1% (15/34), respectively. Disease segregation suggested autosomal recessive inheritance. The pedigree structure of the affected dogs of Study I is presented in Figure 3.

Figure 3  Pedigree of Lagotto Romagnolo puppies with juvenile epilepsy. All 25 affected puppies representing both sexes were born to unaffected parents, consistent with an autosomal recessive mode of inheritance. Square=male; circle=female; shaded symbol=affected dog.

The SNP genome-wide association study (11 affected dogs and 11 unaffected littermates) revealed a very strong association in the region of chromosome 3 (CFA3), peaking at the marker at the base-pair 89159216 (P_{raw} 0.000035; P_{genomewide} 0.08). No significant association existed at any other genomic locus, the next best association being over 100-fold less significant. Genotype analysis around the 89159216 SNP revealed a 1.7 Mb block of homozygous SNPs between markers at 87.3 Mb and 89.0 Mb in affected dogs.
and none of control dogs. This region contains nine genes, including LGI2. Sequencing LGI2 revealed an exonic homozygous protein-truncating sequence change, c.1552A>T (p.K518X), in all affected dogs, but none of the control dogs. Genotyping an additional cohort (140 dogs) for the 89159216 SNP, for the LGI2 c.1552 sequence change, and for three additional SNPs from the homozygous region revealed extremely high associations including \( P_{\text{raw}} 4.47 \times 10^{-16} \) at 89159216 and \( P_{\text{raw}} 1.05 \times 10^{-23} \) (the highest association) at LGI2 c.1552. These results strongly suggest that LGI2 c.1552A>T (p.K518X) is the mutation causing juvenile epilepsy in LR dogs.

Segregation of the sequence change in the pedigree was studied next. Of the 28 affected dogs, 26 (93%) were homozygous for LGI2 c.1552T (p.518X) (i.e. homozygous for the nonsense codon), two were heterozygous (7%), and none were homozygous for the wild-type A nucleotide. The two affected dogs that were heterozygous were also heterozygous for the 13 SNP haplotype around the LGI2 locus, and we found no evidence for compound heterozygosity, as all other variants in the gene were synonymous. These results suggest that if the LGI2 c.1552A>T (p.K518X) change is the disease-causing mutation, it can, in a minority of cases, cause the juvenile epilepsy heterozygously. We additionally screened an independent set of 36 sporadic LR dogs and found three homozygous for c.1552T, 14 heterozygous (39%), and 19 wild-type. All three dogs homozygous for c.1552T had the syndrome, as did one of the carriers (7%), appearing to confirm the 7% rate of disease through heterozygosity, assuming that LGI2 c.1552A>T (p.K518X) is the causative mutation.

Among the 112 unaffected dogs of the 140 genotyped, 69 were homozygous for the wild-type A nucleotide, 41 were heterozygous, and two, 1.8%, were homozygous for c.1552T (OR=532, 95%CI: 95.0-5747.1 and \( p=1.05 \times 10^{-23} \)). The latter two may be incorrectly specified as unaffected as clinical information on many of the dogs in the pedigree was obtained through retrospective questionnaires, and the breeder may have missed seizures, as the epilepsy in some of the cases was mild. Alternatively, these two cases may represent incomplete penetrance.

Four of the five adult-onset epileptic LR dogs were genotypically wild-types. One of the cases was homozygous for the juvenile epilepsy mutation. The puppyhood history of this dog was unclear, and this dog might have both juvenile epilepsy and adult-onset epilepsy. The LGI2 c.1552A>T mutation is breed-specific; none of the adult-onset or juvenile epilepsy cases from other breeds investigated carried the mutant allele present in LR dogs. The coding regions and splice sites were also screened for additional variants in the LGI2 gene in the two heterozygous affected dogs and dogs representing other breeds with either juvenile or adult-onset seizures. Although several variants were found, none of them appeared to be disease-causing.

The c.1552A>T (p.K518X) sequence change did not prevent LGI2 mRNA expression, as RT-PCR experiments showed no mRNA reduction. LGI2 c.1552A>T (p.K518X) truncation was shown to prevent LGI2 secretion with
Results

Western blot experiments. With immunofluorescent cell surface-binding assays, we showed that wt LGI2 was secreted and then bound ADAM22, ADAM23, and ADAM 11 expressed on the cell surface, but that truncated LGI2 was not secreted and did not bind the ADAMs. The developmental expression studies revealed that Lgi2 expression changes over time such that expression in the forebrain of mice was highest at birth and during neural network construction phase, but declined to half by midway through the pruning phase.

5.5 LONG-TERM FOLLOW-UP AND NEUROBEHAVIORAL COMORBIDITIES (IV)

Because all of the dogs with a history of juvenile epilepsy were more than four years old, we excluded all control dogs younger than four years. The mean age was 7.4 (range 4.6-10.7) years and 6.4 (range 4.0-12.6) years in the affected and control groups, respectively. There was a significant difference in the age of the groups (p = 0.033); the control dogs were younger than affected dogs. The affected group comprised 11 male and 14 female dogs, and the control group, 41 male and 50 female dogs. The distribution of sexes did not differ significantly between the groups (p = 0.926). Twenty affected dogs were homozygous for the LGI2 mutation, and three of the phenotypically affected dogs were heterozygote carriers based on the gene test; two affected dogs were not genetically tested but were included in the study. The three heterozygote carriers of the LGI2 mutation had had seizures similar to those in the juvenile epilepsy. Clinical examinations performed on all three dogs excluded other seizure-causing diseases, so the dogs were also included in the study. Three affected dogs had been euthanized prior to the questionnaire study: one dog due to cerebellar cortical degeneration at the age of 11 years, one dog due to cancer at the age of 6 years, and one dog at the age of 8.5 years due to a heart problem. The three dogs had died 5 months, 3.5 years and 2.5 years before the study, respectively. Of the 25 dogs in the affected group, 22 were still alive at the time of the study.

The mean follow-up period for dogs in the affected group was 7.1 (range 4.75–10.75) years. Of the 25 dogs with a history of juvenile epilepsy, 24 experienced no further seizures after the remission of epilepsy by the age of four months; thus, the remission rate for juvenile epilepsy was 96% (24/25). One dog with a history of juvenile epilepsy had had one seizure episode at the age of eight months. Twenty-four dogs showed no other neurological signs after the remission of epileptic seizures, while one dog testing genetically homozygous for the LGI2 p.K518X mutation showed a slowly progressive generalized ataxia since puppyhood and was euthanized at the age of 11 years; pathological examination confirmed cerebellar cortical degeneration. During the telephone interview the owners of four dogs with a history of juvenile epilepsy reported behavioral changes, including hyperactivity (2
Results

dogs), separation anxiety (1 dog), and aggressiveness (1 dog). The owners of 21 dogs reported no changes in their dogs’ behavior.

Principal component analysis of the 31 questionnaire items revealed seven factors (Table 3). Analysis comparing the factor scores between the affected and the control groups revealed differences in two factors. The affected dogs had significantly higher scores for factors 3 Inattention (p = 0.003) and 4 Excitability/Impulsivity (p = 0.021) than did the control group. Differences for the two factors remained significant, even after excluding the three heterozygous dogs from the affected group (p = 0.002; p = 0.016, respectively). Since the dogs in the control group were younger than those in the affected group, we also compared the behavior of dogs with a history of juvenile epilepsy (n=25) with that of control dogs aged over five years (n = 58), as in this case, the ages of the groups no longer differed significantly. We nevertheless found similar results; factors 3 (p = 0.003) and 4 (p = 0.015) showed significantly higher scores in the affected group than in the control group.

Table 3  Results of Principal Component Analysis.

<table>
<thead>
<tr>
<th>Item</th>
<th>Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor 1: Low self-control</strong></td>
<td></td>
</tr>
<tr>
<td>(K11) It is likely to react hastily, and that’s why it is failing tasks</td>
<td>0.708</td>
</tr>
<tr>
<td>(K12) Its attention can be easily distracted</td>
<td>0.696</td>
</tr>
<tr>
<td>(K6) It fidgets all the time</td>
<td>0.694</td>
</tr>
<tr>
<td>(K13) It cannot wait, as it has no self-control</td>
<td>0.574</td>
</tr>
<tr>
<td>(K5) It cannot be quiet; it cannot be easily calmed</td>
<td>0.561</td>
</tr>
<tr>
<td><strong>Factor 2: Impulsivity</strong></td>
<td></td>
</tr>
<tr>
<td>(Q7) My dog does not think before it acts (e.g., it would steal food without first looking to see if someone is watching).</td>
<td>0.709</td>
</tr>
<tr>
<td>-(Q14) My dog appears to have a lot of control over how it responds.</td>
<td>-0.675</td>
</tr>
<tr>
<td>(Q17) My dog is not very patient.</td>
<td>0.653</td>
</tr>
<tr>
<td>(Q8) My dog can be very persistent (e.g., it will continue to do something even if it knows it will get punished or told off).</td>
<td>0.635</td>
</tr>
<tr>
<td>-(Q10) My dog is easy to train.</td>
<td>-0.449</td>
</tr>
<tr>
<td><strong>Factor 3: Inattention</strong></td>
<td></td>
</tr>
<tr>
<td>(K1) Your dog has a difficult time learning, because it is careless or other things can easily attract its attention</td>
<td>0.725</td>
</tr>
<tr>
<td>(K3) It’s difficult for it to concentrate on a task or play</td>
<td>0.646</td>
</tr>
<tr>
<td>(K4) It leaves from its place when it should stay</td>
<td>0.613</td>
</tr>
<tr>
<td>(K7) It seems that it doesn’t listen even if it knows that someone is speaking to it</td>
<td>0.526</td>
</tr>
<tr>
<td><strong>Factor 4: Excitability/Impulsivity</strong></td>
<td></td>
</tr>
<tr>
<td>(Q1) My dog shows extreme physical signs when excited (e.g., drooling, panting, raising hackles, urination, licking lips, widening of eyes).</td>
<td>0.784</td>
</tr>
<tr>
<td>(Q2) When my dog gets very excited, it can lead to fixed repetitive behavior (i.e.,</td>
<td>0.631</td>
</tr>
</tbody>
</table>
Results

<table>
<thead>
<tr>
<th>Factor 5: Reactivity</th>
<th>Factor 6: Short attention</th>
<th>Factor 7: Aggressiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Q15) My dog is very interested in new things and new places.</td>
<td>(K2) It's easy to attract its attention, but it loses its interest soon</td>
<td>(Q5) My dog becomes aggressive (e.g., growls, snarls, snaps, bites) when excited.</td>
</tr>
<tr>
<td>(Q16) My dog reacts very quickly.</td>
<td>(Q12) My dog takes a long time to lose interest in new things.</td>
<td>(Q9) My dog may become aggressive (e.g., growl, snarl, snap, bite) if frustrated with something.</td>
</tr>
</tbody>
</table>

Factor loadings of questionnaire items; 31 items from 2 questionnaires grouped into 7 factors with an eigenvalue > 1 and of biological importance. Two sets of questions, labeled K (Vas et. al., 2007) and Q (Wright et. al., 2011), have been validated previously.
6 DISCUSSION

Epilepsy is one of the most common chronic neurological disorders in childhood, with prevalence estimates of 0.5-1% in children under 16 years of age (Camfield et al., 1996). Furthermore, several benign epilepsy syndromes of childhood are recognized (Engel et al., 2006, Panayiotopoulos et al., 2008, Berg et al., 2010, Mulley et al., 2011, Zara et al., 2013). This thesis describes the first juvenile epilepsy syndrome in dogs, namely benign familial juvenile epilepsy (BFJE). We report the clinical characteristics of this novel neurological syndrome, diagnostic imaging, including FDG-PET, findings in affected dogs, mode of inheritance, causative mutation, and finally, long-term outcome, including a description of neurobehavioral comorbidities.

6.1 RECOGNITION OF A JUVENILE EPILEPSY SYNDROME IN DOGS

It remains an open question why there are no previous reports about juvenile epilepsy syndromes in dogs, although studies in rodents and in humans show that the immature brain is more prone than the mature brain to seizure activity (Raol et al., 2001). If the seizures appear before weaning when puppies are still under the breeder’s care, the breeders may select euthanasia of sick puppies without further examinations. On the other hand, seizures may be infrequent or mild, as was the case in some of our affected dogs. Thus, mild seizure episodes might go unnoticed. In some cases, also veterinarians might recommend the wait-and-see approach for juvenile seizure episodes and in case of benign epilepsy syndrome with spontaneous remission of seizures affected dogs could be handled as normal ones.

Among LR dog breeders, it was known that LR puppies may manifest tremor episodically, but this resolves with time. Luckily, there were breeders willing to investigate this “benign tremor” further, enabling discovery of a novel epilepsy syndrome in veterinary medicine. During the genetic study we also included dogs of other breeds with seizure episodes as puppies showing that juvenile epilepsy also seems to occur in other breeds.

The appearance of seizures in LR dogs with BFJE was mainly predominated by whole-body tremor. This might, if it appears in isolated cases, make it difficult to interpret them as seizures and to distinguish them from, for example, paroxysmal movement disorders. Ictal EEG could be valuable in these cases to confirm the epileptic nature of the episodes (Fernandez-Alvarez, 1998, Paolicchi, 2002, Holmes, 2003). Unfortunately, we were not able to perform ictal EEG studies, but affected LR dogs exhibited changes, including sharp waves and spikes, in interictal EEG, and some of the dogs showed decreased responsiveness during the episodes. Additionally,
blood examinations showed no abnormalities and MRI was non-lesional. Thus, episodes in affected LR dogs were interpreted as epileptic seizures. The seizures were further classified as focal seizures mainly because most of the dogs appeared conscious during the seizures. The analysis of videos of the seizures provided valuable information. This supports the use of videos in analysis of seizure episodes despite some studies showing low interobserver validity in interpretation of seizure characteristics based on the analysis of videos (Bleasel et al., 1997, Packer et al., 2015).

Some of the puppies with the most severe seizure episodes exhibited interictal neurological, mainly cerebellar, signs. Still, BFJE was classified as an idiopathic epilepsy, although this might be debated. Based on the ILAE 1989 terminology, idiopathic epilepsy was defined as a disorder with no underlying cause and a presumed genetic etiology, which fits with BFJE. In the ILAE 2001 report, idiopathic epilepsy was defined as only epilepsy with no underlying brain lesion or other neurologic signs or symptoms, and presumed to be genetic and usually age-dependent (Engel, 2001). Based on this terminology, other neurologic signs would not be part of an idiopathic epilepsy syndrome. On the other hand, according to the latest ILAE classification, the term “genetic epilepsy” refers to known genetic etiology and is not dependent on seizure type or other neurological symptoms (Berg et al., 2010). Thus, based on the newest human classification, BFJE would be classified as a genetic epilepsy. Additionally, the recent IVETF recommendation defines IE in dogs as epilepsy with proven or suspected genetic background (Hülsmeyer et al., 2015).

### 6.2 FOCAL ABNORMALITIES IN GLUCOSE METABOLISM IN CANINE FOCAL IDIOPATHIC EPILEPSY

FDG-PET is commonly performed in humans with drug-resistant epilepsy in order to determine the seizure focus to be resected in epilepsy surgery, especially in patients with MRI-negative epilepsy (Spencer, 1994). In dogs, surgical treatment of epilepsy is seldom performed (Smith et al., 2008), explaining the scant knowledge about FDG-PET in epileptic dogs. Our findings showed that also dogs with focal seizures exhibit abnormal focal glucose metabolism visualized with FDG-PET.

A study of non-lesional refractory childhood epilepsy has reported that 58.1% of patients with a hypometabolic focal region in PET had hemispherically concordant changes in EEG, and of these, 38.7% were also focally concordant (Rubí et al., 2011). FDG-PET and EEG showed concordant findings in 3 of 4 dogs with BFJE and a focal region of abnormal glucose metabolism detected with FDG-PET. This finding strengthens the hypothesis that FDG-PET can also be used in dogs to detect the seizure focus. Our
Results suggest that FDG-PET could also be applicable for dogs for whom epilepsy surgery was considered a treatment option.

Dogs with BFJE exhibited focal cerebrocortical areas of hypometabolism interictally. The focal findings were most often in the posterior regions (temporo-occipital and parietal, but not frontal). These findings may represent a transient focal abnormality in the cortical metabolism, which might disappear during the course of epilepsy. Transient focal abnormalities of cortical metabolism have been described in severe infantile epilepsy in humans (Metsähonkala et al., 2002).

The areas of hypometabolism were detected by visual analysis, but not by asymmetry analysis or by comparison of relative SUVs. This discrepancy may stem from the small number of dogs examined and also the way ROIs are drawn. Furthermore, when utilizing relative SUVs, we compared BFJE dogs to control dogs as groups. Different animals may possess areas of hypometabolism in different cortical regions and changes in individual animals might have gone undetected if the epileptic animals were only compared as a group with healthy controls and not analyzed individually.

Researchers have developed different methods to replace visual analysis of individual images to avoid bias caused by knowledge of an individual’s clinical data. These include a comparison of the patient’s image to an age-matched database, asymmetry analysis, and analysis of SUVs (Suhonen-Polvi et al., 1995, Benedek et al., 2006, Goffin et al., 2008). Still, based on our results visual analysis of FDG-PET images should be performed also in dogs with epilepsy and even when semiquantitative methods are utilized.

6.3 BFJE IS ASSOCIATED WITH LONG-LASTING NEUROBEHAVIORAL COMORBIDITIES

The clinical picture of LR dogs with BFJE with seizures appearing at the mean age of 6 weeks and remitting spontaneously by the age of 4 months with a remission rate of 96% in our study population corresponds well with that of benign childhood epilepsies reported in human epilepsy (Panayiotopoulos, 2008, Mulley et al., 2011, Guerrini and Pellacani, 2012, Zara et al., 2013). Different studies report variable survival times in dogs with IE, with survival depending on the severity of the seizures (Hülsmeyer et al., 2010, Weissl et al., 2012, De Risio et al., 2015a). We detected no signs of shortened life expectancy; the dogs in the BFJE group were rather old and seemed to have experienced no premature death due to epilepsy-related factors during the mean 7.1-year follow-up.

Neurobehavioral comorbidities are evident in children and adults with epilepsy (Hermann et al., 2008, Lin et al., 2012a). ADHD and epilepsy have a particularly high comorbidity in children (Dunn et al., 2003, Thome-Souza et al., 2004, Dunn et al., 2005). Although neurobehavioral comorbidities are well-known in human medicine, only one previous study has investigated
neurobehavioral comorbidities of epilepsy in dogs (Shihab et al., 2011). The researchers reported behavior changes with the development of epilepsy in 71% of dogs with IE (Shihab et al., 2011). Despite the excellent long-term outcome of seizures, dogs with a history of BFJE showed a decreased ability to concentrate as well as increased excitability or impulsivity, which can be considered comparable to behavioral abnormalities seen in ADHD patients. The questionnaire study was not blinded and the owners of the affected dogs knew that their dogs had a history of BFJE and that the study was investigating behavioral abnormalities in dogs with epilepsy. Still, the telephone interview did not reveal behavioral abnormalities in most of the affected dogs, while the questionnaire study did, making our findings about behavioral abnormalities in dogs with a history of BFJE more reliable. Due to homogeneity of the population of dogs examined (i.e., breed, specific epilepsy syndrome, no ongoing seizures, no antiepileptic drugs), we avoided many confounding factors, and thus, this study provides unique information about the importance of neurobehavioral comorbidities in dogs.

Researchers have reached no clear consensus about the effects of seizure-related factors on the development and severity of neurobehavioral comorbidities (Helmstaedter et al., 2003, Schubert, 2005, Caplan et al., 2008, Hamiwka and Wirrell, 2009, Lin et al., 2012a). Many of the comorbidities are present at or even before seizure onset, suggesting a bidirectional relationship or a common underlying etiology for epilepsy and comorbidities (Lin et al., 2012a, Yoong, 2015). Moreover, a recent study in children with active epilepsy showed no association between epilepsy variables (e.g., age of onset, seizure frequency) and behavioral problems. Researchers thus concluded that neurobehavioral abnormalities seen are not caused by seizures per se, but that a common neurobiological factor underlies seizures and neurobehavioral comorbidities (Reilly et al., 2014). Reports of neurobehavioral comorbidities in benign childhood epilepsies concern mainly BRE. There is evidence that cognitive problems in patients with BRE disappear over time (Nicolai et al., 2006, Metz-Lutz and Filippini, 2006). However, it remains an open question whether cognitive and behavioral problems are dependent on the frequency of centrotemporal spikes in BRE or whether both are markers of a more “severe” BRE phenotype (Vannest et al., 2015). We detected no correlation between the severity of any behavioral abnormalities and the frequency or severity of seizures. Moreover, we found behavioral abnormalities even after a long seizure-free period. Thus, our findings support the hypothesis that there is a common underlying etiology for seizures and comorbidities.

Researchers have suggested that the LGI1 mutation could have an impact on other diseases of synaptic connectivity (Kegel et al., 2013). Others have described hyperactive behavior in a family with autosomal dominant lateral temporal lobe epilepsy (ADLTE) resulting from a mutated LGI1 (Berghuis et al., 2013). LGI2 is important for initial synapse formation during central nervous system development and may also have an impact on other diseases...
Discussion

of synaptic connectivity, thus offering a plausible explanation for ADHD-like behavioral changes in dogs with a history of BFJE.

6.4 IDENTIFICATION OF CAUSATIVE MUTATION

BFJE represents the first monogenic IE in dogs in which the causative mutation has been revealed. Only one susceptibility allele, namely ADAM23, has been identified in multiple breeds to increase the risk for IE with a complex inheritance pattern (Seppälä et al., 2012, Koskinen et al., 2015). Identification of genes behind epilepsies can be beneficial for both research and clinical practice. In research, identified genes can reveal the processes underlying seizure susceptibility, which may lead to the development of new treatments or even ways of preventing epileptogenesis (Ottman et al., 2010). In clinical practice, genetic testing can clarify the diagnosis or predict the onset of epilepsy for those at risk due to family history (Ottman et al., 2010).

GWAS with SNP chips is used in order to associate common variants with a certain disease phenotype (Helbig et al., 2008). GWAS generates an enormous amount of data susceptible to false-positive associations, thus requiring multiple testing and confirmation with independent samples (Helbig et al., 2008). Functional studies are obligatory in genetic research in order to relate a putative risk variant to a disease-relevant biological effect because a highly associated variant may not be causative, but may lie close to the actual disease-causing mutation (Helbig et al., 2008). Despite the high association we found in GWAS, we further confirmed the genetic causation with genetic testing in independent populations and with functional studies.

We also investigated three adult-onset epileptic LR dogs with similar ictal semiology to the affected puppies. We showed that apart from BFJE syndrome adult-onset epilepsy with focal seizures is present in the LR breed but is not associated with the BFJE mutation. Thus, this study confirmed for the first time two genetically distinct idiopathic epilepsies in the same breed. This study also showed that the clinical phenotype of BFJE varies between affected individuals in seizure frequency, severity of seizure episodes, and interictal neurological signs. Researchers have suggested that modifier genes and environmental factors may be sources for variable expressivity, and thus, the variable clinical epilepsy phenotype (Helbig et al., 2008, Ottman et al., 2010).

We also tested dogs representing several other breeds and with either juvenile or adult-onset seizures for the BFJE mutation in LGI2 or for additional variants in the same gene, but found no association with disease phenotype.
6.5 LGI PROTEINS AND EPILEPSY

The LGI family consists of four known proteins, one of which, namely LGI1, has previously been reported to be mutated in a known epilepsy syndrome, namely ADLTE (Ho et al., 2012). This thesis shows the role of LGI2 in the pathogenesis of BFJE. Most human epilepsy genes identified to date encode structural components of ion channels regulating neuronal excitability (Baulac and Baulac, 2010). However, ADLTE and, as shown in this thesis, also BFJE are characterized by mutations in secreted neuronal proteins LGI1 and LGI2 (Nobile et al., 2009, II).

Most ADLTE mutations inhibit LGI1 secretion (Senechal et al., 2005, de Bellescize et al., 2009, Nobile et al., 2009, Limviphuvadh et al., 2010, Di Bonaventura et al., 2011, Striano et al., 2011). We showed that also the Lgi2 truncating mutation causing BFJE prevents secretion. The clinical epilepsy phenotype thus likely arises from loss of protein function. Still, the exact mechanism for how mutations in LGI1 or LGI2 lead to epilepsy remains largely unknown.

At least no gross abnormalities exist in brain routine histopathology of Lgi1 knockout mice and rats (Yu et al., 2010, Chabrol et al., 2010, Baulac et al., 2012). The major hypothesis of LGI1 and epileptogenesis has been that LGI1 is involved in the control of synaptic strength at excitatory synapses by forming a bridge between presynaptic ADAM23 and postsynaptic ADAM22 (Figure 4), and then the impairment of LGI-mediated signaling leads to epilepsy (Fukata et al., 2010, Kusuzawa et al., 2012). Developmental actions of LGI1 on dendritic, synaptic, and axon maturation may also contribute to epileptogenesis (Zhou et al., 2009, Chabrol et al., 2010, Zhou et al., 2012). Recently, it has also been suggested that LGI1 might be responsible for developmental inhibitory interneuron anomalies, which could lead to seizures by altering the excitation/inhibition balance (Kusuzawa et al., 2012).

LGI1 antibodies are present in limbic encephalitis (LE) and seizures, highlighting the importance of LGI1 in epileptic disorders and for proper functioning of vertebrate synapses. On the other hand, the phenotype of patients with limbic encephalitis and LGI1 antibodies formed as a part of subacute immune response differs from patients with ADLTE and mutated LGI1 (Lai et al., 2010). Accordingly, in the ADLTE the symptoms may reflect disruption of both developing and mature synapses, whereas only mature synapses are attacked in the LE (Kegel et al., 2013). This further supports the hypothesis that the role of LGI1 is important for both maintenance of synapses and neuronal development.
Mice and rats heterozygous for the Lgi1 gene mutation and mice heterozygous for the Adam23 mutation show decreased seizure threshold (Owuor et al., 2009, Chabrol et al., 2010, Fukata et al., 2010, Baulac et al., 2012). Furthermore, Adam22 and Adam23 knockout mice as well as Lgi1 knockout mice and rats show a similar severe epileptic phenotype and premature death (Sagane et al., 2005, Owuor et al., 2009, Chabrol et al., 2010, Fukata et al., 2010, Yu et al., 2010, Baulac et al., 2012). This suggests that the amount of the LGI1/ADAM22/ADAM23 complex determines seizure susceptibility (Fukata et al., 2010).

We showed that LGI2 binds the same ADAM substrates following secretion as LGI1 and that the BFJE mutation prevents secretion and ADAM protein interaction. Based on our studies, we suggest that LGI2 has a similar function at synaptic maturation to LGI1, but at a different time-point during postnatal nervous system development. Postnatal mammalian brain development consists of three phases: the initial construction phase of the primary neural network (zero to two years of age in humans, estimated zero to one to two months in dogs), the second phase of pruning characterized by selection of the most useful neurons, synapses, and dendrites (two to ten years of age in humans, estimated two to four months in dogs), and the final phase comprising the remainder of life, during which synapse numbers remain stable (Huttenlocher, 1990, Watson et al., 2006, II). We showed that
**Discussion**

$LG{i}_2$ is highly expressed in the forebrain during the first phase of postnatal development (i.e., the construction phase) and diminishes and plateaus during the network pruning phase, unlike $LG{i}_1$, which is expressed in the latter part of pruning and beyond. Thus, we suggest that LGI2 acts during the network construction phase to ensure that the network will not seize during the pruning phase. LGI1 would then act during the pruning phase to ensure an electrically stable network for the rest of the animal’s life, and would also be capable of compensating defects introduced earlier by possible LGI2 deficiency, thus explaining the remittance of seizures in BFJE.

### 6.6 DOGS SERVING AS MODELS FOR HUMAN EPILEPSY RESEARCH

Researchers have suggested that human epilepsy researchers may benefit from canine epilepsy research, with dogs serving as a model for naturally occurring epilepsy with a clinical manifestation of seizures comparable to that of humans (Berendt et al., 2004, Patterson, 2014). Dogs could also serve as a model organism in genetic research for the discovery of the molecular mechanisms of epilepsy or other genetic diseases (Ekenstedt and Oberbauer, 2013). Dog populations are relatively inbred which facilitates genetic research. The situation in inbred dog populations is comparable to Finnish Disease Heritage (FSH) with a group of rare monogenic, mostly autosomal recessive disorders markedly overrepresented in Finland due to national and regional isolation (Norio, 2003). Dogs could therefore provide a relevant model for human genetic epilepsies (Ekenstedt and Oberbauer, 2013). However, in many instances, just the opposite is the case, with canine research following a few steps behind human research.

Our study found a new epilepsy locus, namely LGI2, which interacts, like LGI1, in synaptic transmission with proteins of the ADAM family, namely ADAM22 and ADAM23, recently found to be important for the development of epilepsy. Our findings support the idea of this complex being important for the development of epilepsy and give new insight also into human epilepsy research. In human medicine, the most common genetic epilepsies have a complex inheritance pattern, and a recessive mode of inheritance of epilepsies is rare (Cavalleri et al., 2007, Hildebrand et al., 2013). Two autosomal recessive epilepsies are included in FSH, namely Unverricht-Lundborg disease, which is the most common single cause for progressive myoclonus epilepsy, and progressive epilepsy with mental retardation (EPMR) (Ranta et al., 1999, Lehesjoki, 2003). Due to inbreeding, the mode of inheritance in dogs might be simpler compared to the most common genetic epilepsies in humans, namely autosomal recessive. Studies in dogs are still likely to yield valuable information about genes important for development of epilepsy.
6.7 FUTURE ASPECTS

Utilization of several modalities and cooperation between clinical veterinary medicine, basic veterinary sciences, genetics, and human medicine are invaluable. Without collaboration between the different disciplines and multimodality investigations, we would have not been able to reveal all of the fascinating aspects of BFJE. Future studies aim at investigating juvenile epilepsies in other dog breeds, other neurological diseases in LR dogs, the utilization of video EEG in dogs with paroxysmal signs, and finally, more specifically, the action of LGI2 in the central nervous system.
Based on these studies, the following conclusions can be drawn:

1. A novel juvenile epilepsy syndrome, namely benign familial juvenile epilepsy (BFJE), exists in LR dogs and is characterized by focal seizures beginning at the mean age of 6 weeks and spontaneous remission by the age of 4 months.

2. MRI shows no changes in dogs with BFJE but focal, most often posterior, cerebral hypometabolism can be detected with interictal FDG-PET, in accordance with interictal EEG findings, confirming that the detected area of hypometabolism represents the epileptic focus.

3. BFJE is inherited in an autosomal recessive manner and is caused by mutated LGI2. LGI2 is expressed highly in the forebrain during the construction phase until halfway through pruning, unlike LGI1, which is expressed in the latter part of pruning and beyond. This LGI2 to LGI1 transition serves as a model for remission of juvenile epilepsies.

4. Dogs with a history of BFJE have excellent seizure outcome, but exhibit changes in behavior comparable to ADHD in humans. These neurobehavioral comorbidities are most likely due to a common antecedent underlying factor for both seizures and comorbid conditions.
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Benign Familial Juvenile Epilepsy in Lagotto Romagnolo Dogs

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