LGI Proteins and Epilepsy in Human and Animals

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Leucine-rich glioma-inactivated (LGI) protein was first thought to have a suppressor effect in the formation of some cancers. Developments in physiology and medicine made it possible to characterize the function of the LGI protein family and its crucial role in different conditions more precisely. These proteins play an important role in synaptic transmission, and dysfunction may cause hyperexcitability. Genetic mutation of LGI1 was confirmed to be the cause of autosomal dominant lateral temporal lobe epilepsy in humans. The LGI2 mutation was identified in benign familial juvenile epilepsy in Lagotto Romagnolo (LR) dogs. Cats with familial spontaneous temporal lobe epilepsy have been reported, and the etiology might be associated with LGI protein family dysfunction. In addition, an autoimmune reaction against LGI1 was detected in humans and cats with limbic encephalitis. These advances prompted a review of LGI protein function and its role in different seizure disorders.

Key words: Autoimmune; Epilepsy; Genetic; LGI.

As a result of microbiological and genetic developments in recent decades, the etiology of different neurological disorders has become more clear. This progress also has improved our understanding of the various forms of epilepsy. The majority of congenital epilepsies are caused by mutations in genes that encode ion channels.\(^1\) The first epilepsy of humans not caused by an ion subunit-coding mutation, but with a confirmed genetic background, was autosomal dominant lateral temporal lobe epilepsy (ADLTE).\(^2\) The condition is caused by a mutation in a gene that codes a neuroprotein called LGI1. Leucine-rich glioma-inactivated protein (LGI1) was so named because it has a suppressor effect on glioblastomas.\(^3\) There are 4 different proteins in the LGI family, and LGI1 was the first identified and best investigated. Today, more than 30 different mutations are reported with some differences within the epileptic phenotype.\(^4\) The proteins LGI2, 3, and 4 also play roles in the central and peripheral nervous system.\(^5\)

The aim of our review was to summarize research on LGI proteins because these proteins have become relevant in veterinary medicine in recent years mainly in the field of epilepsy.

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Abbreviations:

- ADLTE: autosomal dominant lateral temporal lobe epilepsy
- ADAM: a disintegrin and metalloprotease
- AED: antiepileptic drugs
- AMPA: \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
- BFJE: benign familial juvenile epilepsy
- BS: Belgian Shepherd
- CASPR2: contactin-associated protein 2
- CNS: central nervous system
- EEG: electroencephalography
- EL: mouse epilepsy-like mouse
- EPTP: epitempin
- FBDS: faciobrachial dystonic seizures
- FEPSO: feline complex partial seizure with oro-facial involvement
- FLAIR: fluid attenuated inversion recovery
- FS: febrile seizures
- FMTLE: familial mesial temporal lobe epilepsy
- FSEC: familial spontaneous epileptic cats
- FTLE: familial temporal lobe epilepsy
- GAD: glutamic acid decarboxylase
- HS: hippocampal sclerosis
- IE: idiopathic epilepsy
- ISH: in situ hybridization
- LE: limbic encephalitis
- LGI: leucine-rich glioma-inactivated
- LR: Lagotto Romagnolo
- LRR: leucine-rich repeats
- MRI: magnetic resonance imaging
- NMDA: \(\alpha\)-methyl-D-aspartate
- PET: positron emission tomography
- PNS: peripheral nervous system
- PSD: postsynaptic density protein
- SNP: single nucleotide polymorphism
- VGKC: voltage-gated potassium channel

LGI Function

LGI1 is a neuronally secreted protein that contains 3 leucine-rich repeats (LRR) in the N-terminal region\(^3\) and epitempin (EPTP) repeats in the carboxyl half of the protein\(^6\) as protein–protein interaction domains. Epitempin repeats were only found in the LGI1 gene.\(^7\)

The protein LGI1 is multifunctional. It binds to the presynaptic voltage-gated potassium channel Kv1.1 (Kv
channel) and prevents Kv channel inactivation mediated by the β-subunit of the channel. Certain LGI1 mutants (typically nonsecreted mutants) fail to prevent channel inactivation, resulting in more rapidly closing channels, which extends presynaptic depolarization and leads to increased calcium influx. Consequently, neurotransmitter release is increased excessively, which may induce focal seizures. However, because the β-subunit acts from the intracellular side, it is not clear how secreted LGI1 can modulate the Kv channel.

In the brain, LGI1 also interacts at the presynaptic membrane with an ADAM (a disintegrin and metalloprotease) protein family member, the transmembrane protein ADAM23, and this interaction affects neurite outgrowth. LGI1 also is located postsynaptically and co-immunoprecipitates with the postsynaptic scaffolding protein PSD-95 (postsynaptic density protein). LGI1 does not interact with PSD-95 directly, but with the extracellular domain of the transmembrane protein ADAM22. ADAM22 binds to PSD-95. PSD-95 can bind to stargazin, which is a transmembrane regulatory subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), a non-NMDA (N-methyl-D-aspartate) type glutamate receptor (Fig 1). Incubation of hippocampal slices with LGI1 leads to an increase in the synaptic AMPA/NMDA ratio, which can be explained by the fact that stargazin and PSD95 both control the number of AMPARs at the synapses, and both interact indirectly with LGI1 via ADAM22. The glycosylphosphatidylinositol-anchored Nogo receptor 1 (NgR1), whose ligand Nogo inhibits axon outgrowth, also functions as a receptor for LGI1 and mediates LGI1-ADAM22 binding. The AMPAR-mediated synaptic transmission in the hippocampus is severely decreased when LGI1 is lacking.

Binding of LGI1 to ADAM22 and ADAM23 is mediated by the EPTP domain of LGI1. ADAM22 or ADAM23 knockout mice have a strong phenotypical overlap with LGI1 knockout mice, a phenotype that is characterized by spontaneous epilepsy and premature death. Interestingly, LGI1 leads to co-assembly of ADAM22 and ADAM23. These results suggest that LGI1 simultaneously binds presynaptic ADAM23 and postsynaptic ADAM22, pulling both the presynaptic membrane (containing voltage-gated potassium channel [VGKC] complexes) and the postsynaptic membrane (containing AMPA receptor scaffolds) together, thus stabilizing the synapse and increasing neurotransmission. LGI1 also can weakly bind to ADAM11, which is an essential protein for proper neuronal function.

In addition to its roles in synaptic transmission, LGI1 also has been proposed to regulate neuronal development. A role for LGI1 has been proposed in the maturation of glutamatergic synapses. Expression of mutant LGI1 (carrying the mutation 835delC, which is found in ADLTE and truncates the C-terminal EPTP domain) in mice arrests normal postnatal change in postsynaptic NMDA receptor (NMDAR) NR2 subunit composition, which is an important feature of synapse maturation. Furthermore, this mutant arrests normal postnatal down-regulation of presynaptic release probability, inhibits dendritic pruning and increases spine density leading to an enhanced excitatory transmission.

The proteins LGI2, LGI3, and LGI4 all are neuronally secreted and act on ADAM family members as does LGI1. Although LGI2 acts on the same receptors as LGI1, LGI2 expression levels are only high in the immediate postnatal period until halfway through neural pruning. In contrast, LGI1 is highly expressed during and after the later pruning phase. Thus, although both LGI1 and LGI2 seem to have similar roles in synaptic development, they act at different time points of postnatal nervous system development.

LGI3 is located near neuronal plasma membranes in the brain and colocalizes with endocytosis-associated proteins (eg, transferrin), exocytosis-associated proteins (eg, syntaxin-1) and lipid raft markers, such as flotillin-1, which forms scaffolds for membrane microdomains involved in trafficking. LGI3 colocalizes with both β-amyloid in astrocytes, is up-regulated by β-amyloid and promotes additional uptake of β-amyloid. In addition to its role in endocytosis and exocytosis, LGI3 is involved in neuronal differentiation and neuritogenesis. Outside of the brain, LGI3 also is expressed in adipose tissue and in pre-adipocytes and regulates adipogenesis through ADAM23. In human keratinocytes, LGI3,
the secretion of which is stimulated by ultraviolet B (UVB) irradiation, \(^2^2\) induces cell migration. \(^2^3\) An additional function of LGI3 in skin is promoting melanin synthesis.\(^2^4\)

LGI4 regulates myelination in the peripheral nervous system (PNS) and is secreted by Schwann cells. In claw paw mice, LGI4 is mutated and not secreted, resulting in a congenital hypomyelinating phenotype.\(^2^5\) Interestingly, ADAM22 also is expressed in Schwann cells,\(^2^6\) and ADAM22-deficient mice have a similar phenotype with defects in peripheral nerve myelination.\(^1^6\) Binding of LGI4 to ADAM22 allows LGI4 to regulate PNS gliogenesis.\(^2^7\)

**LGI Genes**

The LGI gene family originated from 2 to 3 rounds of gene duplication in early vertebrate development.\(^2^8\) The LGI1 gene was first described in a comparison of neuronal tissue with a glioblastoma cell line (T98G).\(^3\) The genes LGI1, 2, 3, and 4 are located on different chromosomes in the human, dog, and cat, and encode proteins that are secreted by glial and neuronal cells.\(^9\) The feline and the canine LGI1 gene products show 100% homology to their human counterpart. The other members of the LGI family also exhibit interspecies homologies of 94% to 98% at the protein level. Comparing the gene products of the LGI family, they exhibit approximately 40–50% sequence homology with one another and share functional similarities. Because of the very similar domain architecture of LGI1 and LGI2, mutations that are located at the same functional region affect phenotypically related forms of epilepsy.\(^2^9\) In contrast to their highly related architecture, their expression patterns within the central nervous system (CNS) overlap only weakly. In situ hybridization (ISH) with a single sagittal section of adult mouse brain identified low levels of diffuse staining throughout the brain for LGI1, 2, and 3 mRNA, and distinct localizations of intensive staining (ie, LGI4 mRNA expression was only found in two areas).\(^9\) These findings were confirmed by a comprehensive ISH study that generated a detailed map of the regional distribution of LGI transcripts in serial coronal sections.\(^3^0\)

**LGI and Cancer**

The observation of decreased or absent LGI1 expression in glioblastomas\(^3\) led to the hypothesis that LGI1-knockout animals would develop tumors of neural tissue. However, a study in LGI1\(^{-/-}\) mice\(^3^1\) demonstrated only the onset of seizures as early as day 8. Until that time point, the animals developed similar to their wild-type or Lgii\(^{+/-}\) littersmates, but at the onset of seizures, they lost weight and died by postnatal day 10–18. Epileptogenic alterations of the brain were assessed by immunohistochemistry. Among other epileptic markers, glial fibrillary acidic protein expression increased with the number of seizures, mainly in the hilus of the gyrus dentatus. However, no formation of tumors was found. The Lgi1\(^{+/-}\) littersmates behaved similar to the wild-type mice and reached the same age of >18 months without tumorigenesis. These animals were comparable to patients with ADLTE because starting at age 28 days seizures triggered by auditory stimuli were significantly more frequent than in wild-type animals.

Insertion of LGI1 into a glioblastoma cell line indicated a role of LGI1 in cell-matrix interactions and migratory processes in the CNS but involvement in glial tumor suppression could not be substantiated.\(^3^2\) In neuronal and nonneuronal tumor cell lines only infrequent expression of LGI1 mRNA of differing intensity was detected.\(^3^3\) Also, no correlation was found by comparing their expression in normal tissue and in tumors of the respective tissues.\(^3^3\) LGI3 had a dose- and time-dependent protective effect on keratinocytes exposed to UVB irradiation.\(^2^2\) Furthermore, LGI1 was identified as a suppressor that was down-regulated in tumor cells compared to adjacent normal tissue and additionally was significantly positively correlated with poorer prognosis and metastasis.\(^3^4\) These findings indicate an important but as yet unidentified role of the LGI family in tumorigenesis and demonstrate the potential of LGI1 for suppression of distinct tumors, but deficiency (as in some cases of epilepsy) does not imply the formation of tumors, especially within the CNS.

**Genetic Epilepsy in Humans Caused by LGI1 Mutation**

In the International League Against Epilepsy (ILAE) classification of 2010, many familial epilepsies were classified as electroclinical syndromes and arranged by age at onset.\(^3^7\) In the adolescence- to adult-onset group, familial temporal lobe epilepsy (FTLE) was divided into mesial and lateral forms. The lateral form of FTLE is known as ADLTE or autosomal dominant partial epilepsy with auditory features (ADPEAF), which is a benign epileptic syndrome with auditory (main symptom in 64% of patients), visual, olfactory, and other sensory ictal clinical signs.\(^3^8\) These seizures may be triggered by environmental noises or sounds. Many patients (90%) show secondary generalized tonic-clonic seizures. Intercital electroencephalography (EEG) in patients with ADLTE shows a normal pattern or mild abnormalities in the temporal region. In most ADLTE patients, there is no abnormality on conventional magnetic resonance imaging (MRI), but recent studies have found mild abnormalities in the lateral temporal cortex, and suggested malformation.\(^4^2\) Seizures of ADLTE are effectively treated with conventional antiepileptic drugs (AEDs) such as carbamazepine, phenytoin, and valproate.

Approximately 50% of ADLTE families and sporadic cases of lateral temporal lobe epilepsy with auditory features have mutations of LGII.\(^2^4\) Over 30 mutations in LGII that result in missense mutations, protein truncation, or internal deletions have been reported.\(^5\) The missense mutations tend to be distributed in the 5′ half (LRR) and the truncating mutations preferentially in the 3′ half (EPTP) of the gene. There is no clear correlation between genotypes and phenotypes.
Other forms of FTE (eg, mesial form or FMTLE) have been reported in over 20 families. FMTLE is divided into FMTLE without hippocampal sclerosis (HS) or febrile seizures (FS) and FMTLE with HS/FS.\(^{45}\) FMTLE without HS/FS is characterized by benign psychic and autonomic auras (déjà vu is most frequent) and complex partial seizures or infrequently generalizes secondarily.\(^{46,47}\) FMTLE with HS emerges at approximately 10 years of age with complex partial seizures or infrequently with FS.\(^{45}\) In families without HS, seizures begin at a mean age of 6.3 weeks and resolve by adulthood.\(^{55}\) Until now, only 1 well-defined genetic epilepsy mutation has been identified, namely LGI\(_2\) causing benign familial juvenile epilepsy (BFJE) in the LR breed,\(^{19}\) and ADAM23 predisposing to adult-onset epilepsy in the Belgian Shepherd (BS) breed.\(^{54}\)

Childhood epilepsies are common in human medicine, and the majority of these patients will become seizure-free by adulthood.\(^{55}\) Until now, only 1 well-defined juvenile epilepsy syndrome was reported in dogs.\(^{56}\) BFJE in LR dogs is characterized by focal-onset seizures beginning at a mean age of 6.3 weeks and resolving spontaneously by a mean age of 10 weeks. There is no sex predilection in the affected dogs. Seizures in BFJE are characterized by whole-body tremor, ataxia, and stiffness, and are sometimes associated with altered consciousness. The frequency and severity of seizures varies among affected individuals. The compound 2-[\(^{18}\)F]fluoro-2-deoxy-D-glucose (FDG) is widely used as a positron emission tomography (PET) tracer in human patients with epilepsy. A recent FDG-PET study showed focal cerebrocortical areas of hypometabolism interictally in dogs with BFJE. A good correspondence with EEG findings further supported the suggestion that the areas of focal parieto-occipitocortical hypometabolism represent the epileptogenic focus in the examined dogs.\(^{57}\)

The disease shows an autosomal recessive inheritance pattern, and a protein-truncating nonsense mutation in the LGI\(_2\) gene recently has been identified as a cause of BFJE.\(^{19}\) In a genetic study, LGI\(_2\) mutation also was screened in 114 dogs with adult-onset epilepsy and in 8 dogs with juvenile epilepsy (40 different breeds), but none was found to carry the BFJE mutation present in LR dogs.\(^{19}\)

Belgian Shepherd dogs suffer from a more commonly reported type of epilepsy. The prevalence of epilepsy has been estimated to be 9.5% in this breed.\(^{58}\) Epilepsy in BS dogs is dominated by focal-onset seizures with or without secondary generalization.\(^{54,58}\) The most commonly reported clinical signs of focal seizure phenomenology include ataxia, crawling, swaying, fearful behavior, excessive attention seeking, drooling, and nausea.\(^{54,58,59}\) In cases with secondary generalization, focal seizure phenomenology is followed by stiffening of the limbs and neck, muscle fasciculations, tremor, staring, drooling, and tonic-clonic convulsions.\(^{54}\) The mean age of seizure onset is between 3 and 4 years of age.\(^{54,58,60}\) and males and females are equally affected.\(^{60}\) A recent study found no significant decrease in the lifespan of affected dogs, a remission rate of 13.7% and a very low frequency of status epilepticus, suggesting that the epilepsy has a relatively mild course in this breed.\(^{61}\)

Different modes of inheritance for IE in BS dogs have been suggested from simple Mendelian to polygenic inheritance with a recessive gene of major influence having a substantial influence. A recent study found that variants at the ADAM23 locus on CFA37 increase the risk of IE in BS dogs.\(^{54}\) Homozygosity with respect to 2 separate single nucleotide polymorphisms (SNPs) within ADAM23 increased the risk for IE 7-fold.\(^{54}\) However, the risk haplotype also is common in unaffected BS, and the actual disease-causing mutation may lie in the vicinity of the risk locus.\(^{54}\) ADAM23 recently was suggested also to be a potential major risk gene for IE in other dog breeds.\(^{8}\) Mutations in ADAM23 have not been found in epileptic human patients, but it is a good candidate gene for IE because ADAM23 interacts with 2 epilepsy-related proteins, LGI1 and LGI2.\(^{12,17,19}\) Furthermore, Adam23 knockout mice exhibit spontaneous seizures, and mice heterozygous for the Adam23 knockout gene have a lower seizure threshold.\(^{66}\)

**Familial Epilepsy in Cats may be Associated with LGI Dysfunction**

Compared with epilepsy in humans and dogs, idiopathic epilepsy in cats is less common.\(^{62-64}\) and no reports identified genetic epilepsy until recently. In 2010, a familial form of spontaneous epilepsy was described in cats. So-called ‘familial spontaneous epileptic cats’ (FSECs), were identified in a closed colony of laboratory cats.\(^{65}\) From pedigree analysis, the phenotype of FSECs is inherited in an autosomal recessive manner. In addition, inbreeding of FSECs is successful (ie, it is not a lethal gene), and spontaneous recurrent seizures, EEG abnormalities, or both occur in F1 kittens. Clinically, FSECs have 2 seizure types: spontaneous limbic focal seizures with or without secondary generalization, and vestibular stimulation-induced generalized seizures. The former, so-called feline complex partial seizure with orofacial involvement (FEPSO), is the common seizure type in epileptic cats, which...
typically consists of attention behavior, arresting or gazing, lip-smacking, chewing, mydriasis, hypersalivation and facial twitching, and resembles the limbic kindling or kainate\textsuperscript{66} model in cats. The vestibular stimulation-induced generalized seizures are triggered by stimulation such as left-to-right swinging or rotating, similar to EL (epilepsy-like) mice, which is 1 of the genetic models of temporal lobe epilepsy.\textsuperscript{67} On scalp and deep EEG with video monitoring, FSECs show interictal discharges in the temporal region, and spontaneous clinical and subclinical seizures originating from the unilateral amygdala, hippocampus or both (Fig 2).\textsuperscript{68} Furthermore, unilateral hippocampal atrophy without changes in signal intensity was observed on conventional and volumetric MRI (Fig 3).\textsuperscript{69} All cats with FSECs show their first spontaneous seizure within 2 years of birth, but seizure frequency varies among individuals. Therefore, FSEC is a true and natural genetic model of temporal lobe epilepsy, especially FMTLE as mentioned above.

The causative gene of FSEC has not yet been identified. However, some mutations in the LGI and ADAM gene families in humans and dogs suggest that these genes may be associated with the pathophysiology of FSEC, as well as with limbic encephalitis (LE) in cats mentioned below.

**LGI1-Antibody-Associated Limbic Encephalitis in Human**

Limbic encephalitis is an autoimmune encephalopathy with predominant involvement of the limbic structures (hippocampus, amygdala, hypothalamus, and insular and cingulate cortex). LE typically occurs with a subacute onset and is more frequent in males. Typical clinical signs in humans are memory impairment, temporal lobe semiology seizures and psychiatric disturbances, and LE often is accompanied by hyponatremia.\textsuperscript{70} Many patients with LE have a specific seizure semiology of faciobrachial dystonic seizures (FBDS), which usually precede the onset of amnesia, temporal seizures and confusion,\textsuperscript{71} and patients do not respond to AEDs. FBDS are brief (a few seconds in duration), frequent (median, 50 per day) events that typically affect the arm and face. On T2/fluid attenuated inversion recovery (FLAIR) MRI, a high signal in the medial temporal lobe found is frequently.

The first LE cases described were associated with malignancies and a poor outcome.\textsuperscript{72} Antibodies in paraneoplastic LE are directed against intracellular proteins (ANNA, antineural nuclear antibody; CV2/CRMP5, collapsin response mediator protein; PNMA, paraneoplastic Ma, named according to the index patient).\textsuperscript{73,74} These patients have little or no response to immunotherapy.\textsuperscript{75}

An important development in the last decade has been the recognition of immunotherapy-responsive seizures and confusion,\textsuperscript{71} and patients do not respond to AEDs. FBDS are brief (a few seconds in duration), frequent (median, 50 per day) events that typically affect the arm and face. On T2/fluid attenuated inversion recovery (FLAIR) MRI, a high signal in the medial temporal lobe found is frequently.

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An important development in the last decade has been the recognition of immunotherapy-responsive
The antibodies initially were thought to be against VGKC. In LE, however, the antibodies are only very rarely directed against the VGKCs themselves, but are usually directed against other extracellular or cell surface proteins, which are tightly associated with VGKCs and have a role in the regulation of neuronal excitability (Fig 4). These proteins are LGI1, contactin-associated protein 2 (CASPR2) and contactin-2. Almost all patients with LE and FBDS have anti-LGI1 antibodies. Faciobrachial dystonic seizures often precede anti-LGI1 antibody production.

Evidence also indicates that autoimmune encephalitis may progress to adult-onset HS and evolve into temporal lobe epilepsy and immunotherapy often has been used with success. Immunotherapy decreased serum VGKC-complex antibody concentration and produced significant functional benefits; improvements were greatest in patients that received steroids early. During immunotherapy, sequential serum antibody concentrations appear to correlate well with the clinical features, suggesting that the antibodies are causative. Indeed, in experiments with mice, application of anti-LGI1 antibodies to rat hippocampal slices produced synaptic hyperexcitability, which was mimicked by alpha-dendrotoxin, a selective blocker of Kv1-VGKCs. These findings were the first evidence that IgG may decrease VGKC function at CNS synapses and has a direct effect on channel kinetics. CASPR2 and contactin-2 are expressed in both central and peripheral nervous system neurons.

In many cats, acute cluster seizure episodes are observed, resembling LE in humans. Very recently, additional investigations showed an association between FEPSO and antibodies against VGKC-complexes/LGI1. In a prospective study, increased concentrations of antibodies directed against VGK and LGI1 were detected in cats in the acute stage of the disease. Five of 14 (36%) cats had VGKC antibody concentrations above the reference concentration for positivity (100 pmol/L), whereas no increased antibody concentrations could be found in the 19 control cats, suggesting that the detected immunoglobulins are associated with the condition. Analysis of sera from cats in remission showed that the antibody titer had returned to within the reference range. The study suggests that autoimmune LE might be common in cats, and that the target of the immunoreaction is the VGKC complex associated with LGI1. CASPR2 and GAD antibodies could not be detected. Unfortunately, in this study, EEG and MRI were not performed regularly and are only available for a single reported cat. The
examined cat exhibited temporal lobe seizures, and MRI showed bilateral temporal lobe changes (Fig 5), whereas rhythmic positive spike activity with focal onset was detected by EEG. Increased VGKC-complex antibody concentrations also were found.97

Histologically, cats with increased VGKC-complex antibodies showed lesions in the hippocampus with mild T-cell infiltrates, but strong complement (C9neo) deposition and IgG infiltration. In both, human and feline brains, massive neurodegeneration and acute cell death predominated. The alterations were accompanied by mild-to-moderate astrogliosis and activation of microglial cells. In particular, the presence of complement strongly resembles what is observed in VGKC encephalitis in humans and separates FEPSO cats from cats with other epileptic conditions.99 An additional interesting finding was that a concurrent neoplasm (pulmonary adenoma) was found only in 1 cat, suggesting that paraneoplastic origin is exceptional but may occur. In other cases, nonparaneoplastic etiology was presumed. In another post mortem study, 9 of 70 epileptic cats were found to have LE, but serology was not performed.99

Conclusion

The LGI1 protein family has many functions, the best characterized of which is the neuronal function of LGI1. This protein influences potassium channel function, synaptic development of glutamate receptors and regulates synaptic transmission together with ADAM22 and ADAM23 proteins. Genetic and immune-mediated dysfunction may cause neuronal hyperexcitability and lead to epilepsy in laboratory animals, humans, dogs, and cats. Veterinarians should be aware of the LGI protein family members and disorders caused by their dysfunction.

Footnote

* Koskinen LLE, Seppälä EH, Arumilli M, et al Dogs as an Animal Model for Idiopathic Epilepsy: Identification of Common Risk Variants in the ADAM23 Gene. Conference Proceedings of European Society of Human Genetics -meeting, Milan, Italy, 2014.

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References

1. D’Adamo MC, Cataruzzeno L, Di GG, et al. K(+) Channelopathy: Progress in the neurobiology of potassium channels and epilepsy. Front Cell Neurosci 2013;7:134.

2. Kalachikov S, Evgrafov O, Ross B, et al. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. Nat Genet 2002;30:335–341.

3. Chernova OB, Somerville RP, Cowell JK. A novel gene, LGI1, from 10q24 is rearranged and downregulated in malignant brain tumors. Oncogene 1998;17:2873–2881.

4. Ho YY, Ionia A. Domain-dependent clustering and genotype-phenotype analysis of LGI1 mutations in ADPEAF. Neurology 2012;78:563–568.

5. Kegel L, Aunin E, Meijer D, Bingham MR. LGI proteins in the nervous system. ASN Neuro 2013;5:167–181.

6. Staub E, Perez-Tur J, Siebert R, et al. The novel EPTP repeat defines a superfamily of proteins implicated in epileptic disorders. Trends Biochem Sci 2002;27:441–444.

7. Scheel H, Torniuk S, Hofmann K. A common protein interaction domain links two recently identified epilepsy genes. Hum Mol Genet 2002;11:1757–1762.

8. Schulte U, Thumfart JO, Klocker N, et al. The epilepsy-linked LGI1 protein assembles into presynaptic KV1 channels and inhibits inactivation by Kvbeta1. Neuro 2006;49:697–706.

9. Senechal KR, Thaller C, Noebels JL. ADPEAF mutations reduce levels of secreted LGI1, a putative tumor suppressor protein linked to epilepsy. Hum Mol Genet 2005;14:1613–1620.

10. Nobile C, Michelucci R, Andreauza S, et al. LGI1 mutations in autosomal dominant and sporadic lateral temporal epilepsy. Hum Mutat 2009;30:530–536.

11. Thomas R, Favell K, Morante-Redolat J, et al. LGI1 is a nogo receptor 1 ligand that antagonizes myelin-based growth inhibition. J Neurosci 2010;30:6607–6612.

12. Fukata Y, Lovero KL, Iwanaga T, et al. Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy. Proc Natl Acad Sci U S A 2010;107:3799–3804.

13. Fukata Y, Adesnik H, Iwanaga T, et al. Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission. Science 2006;313:1792–1795.

14. Schnell E, Szemere M, Karimzadegan S, et al. Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. Proc Natl Acad Sci U S A 2002;99:13902–13907.

15. Owuor K, Harel NY, Englot DJ, et al. LGI1-associated epilepsy through altered ADAM23-dependent neuronal morphology. Mol Cell Neurosci 2008;42:448–457.

16. Sagane K, Hayakawa K, Kai J, et al. Ataxia and peripheral nerve hypomyelination in ADAM22-deficient mice. BMC Neurosci 2005;6:33.

17. Sagane K, Ishihama Y, Sugimoto H, LG11 and LGI14 bind to ADAM22, ADAM23 and ADAM11. J Biol Chem 2008;4:387–396.

18. Zhou YD, Lee S, Jin Z, et al. Arrested maturation of excitatory synapses in autosomal dominant lateral temporal lobe epilepsy. Nat Med 2009;15:1208–1214.

19. Seppala EH, Jokinen TS, Fukata M, et al. LGI2 truncation causes a remitting focal epilepsy in dogs. PLoS Genet 2011;7: e1002194.

20. Park WJ, Lim YY, Kwon NS, et al. Leucine-rich glioma inactivated 3 induces neurite outgrowth through AKT and focal adhesion kinase. Neurochem Res 2010;35:789–796.
21. Kim HA, Park WJ, Jeong HS, et al. Leucine-rich glioma inactivated 3 regulates adipogenesis through ADAM23. Biochim Biophys Acta 2012;1821:914–922.

22. Lee SH, Jeong YM, Kim SY, et al. Ultraviolet B-induced LGI3 secretion protects human keratinocytes. Exp Dermatol 2012;21:716–718.

23. Jeong YM, Park WJ, Kim MK, et al. Leucine-rich glioma inactivated 3 promotes HaCaT keratinocyte migration. Wound Repair Regen 2013;21:634–640.

24. Jeong HS, Jeong YM, Kim J, et al. Leucine-rich glioma inactivated 3 is a melanogenic cytokine in human skin. Exp Dermatol 2014;23:600–602.

25. Berringham JR Jr, Shearin H, Pennington J, et al. The claw paw mutation reveals a role for Lgi4 in peripheral nerve development. Nat Neurosci 2006;9:76–84.

26. Ozkaynak A, Abello G, Jaegle M, et al. Adam22 is a major neuronal receptor for Lgi4-mediated schwann cell signaling. J Neurosci 2010;30:3857–3864.

27. Nishino J, Saunders TL, Sagane K, Morrison SJ. Lgi4 promotes the proliferation and differentiation of Gial lineage cells throughout the developing peripheral nervous system. J Neurosci 2010;30:15228–15240.

28. Dehal P, Boore JL. Two rounds of whole genome duplication in the ancestral vertebrate. PLoS Biol 2005;3:e314.

29. Limviphuvadh V, Chua LL, Rahim RA, et al. Similarity of molecular phenotype between known epilepsy gene LGI1 and disease candidate gene LG12. BMC Biochem 2010;11:39.

30. Herranz-Perez V, Olucha-Bordonau FE, Morante-Redolat JM, Perez-Tur J. Regional distribution of the leucine-rich-glioma inactivated (LGI1) gene family transcripts in the adult mouse brain. Brain Res 2010;1307:177–194.

31. Chabrol E, Navarro V, Provenzano G, et al. Electroclinical characterization of epileptic seizures in leucine-rich, glioma-inactivated 1-deficient mice. Brain 2010;133:2749–2762.

32. Piepoli T, Jakupoglu C, Gu W, et al. Expression studies in gliomas and Glial cells do not support a tumor suppressor role for LGI1. Neuro Oncol 2006;8:96–108.

33. Rossi MR, Huntoon K, Cowell JK. Differential expression of the LGI and SLT families of genes in human cancer cells. Gene 2005;356:85–90.

34. Zhu YH, Liu H, Zhang LY, et al. Downregulation of LGI1 promotes tumor metastasis in esophageal squamous cell carcinoma. Carcinogenesis 2014;35:1154–1161.

35. Head K, Gong S, Joseph S, et al. Defining the expression pattern of the LGI1 gene in BAC transgenic mice. Mamm Genome 2007;18:328–337.

36. Cowell JK, Head K, Kunapuli P, et al. Inactivation of LGI1 expression accompanies early stage hyperplasia of prostate epithelium in the TRAMP murine model of prostate cancer. Exp Mol Pathol 2010;88:77–81.

37. Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsy: Report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia 2010;51:676–685.

38. Ottman R, Ottman R, Hauser WA, et al. Localization of a gene for partial epilepsy to chromosome 10q. Nat Genet 1995;10:56–60.

39. Poza JJ, Saenz A, Martinez-Gil A, et al. Autosomal Dominant lateral temporal epilepsy: Clinical and genetic study of a large basque pedigree linked to chromosome 10q. Ann Neurol 1999;45:182–188.

40. Winawer MR, Ottman R, Hauser WA, Pedley TA. Autosomal dominant partial epilepsy with auditory features: Defining the phenotype. Neurology 2000;54:2173–2176.

41. Michelucci R, Pasini E, Nobile C. Lateral temporal lobe epilepsies: Clinical and genetic features. Epilepsia 2009;50(Suppl 5):52–54.

42. Kobayashi E, Santos NF, Torres FR, et al. Magnetic resonance imaging abnormalities in familial temporal lobe epilepsy with auditory auras. Arch Neurol 2003;60:1546–1551.

43. Tessa C, Michelucci R, Nobile C, et al. Structural anomaly of left lateral temporal lobe in epilepsy due to mutated LGI1. Neurology 2007;69:1298–1300.

44. Ottman R, Winawer MR, Kalachikov S, et al. LGI1 mutations in autosomal dominant partial epilepsy with auditory features. Neurology 2004;62:1120–1126.

45. Gambardella A, Labate A, Giallonardo A, Aguglia U. Familial mesial temporal lobe epilepsies: Clinical and genetic features. Epilepsia 2009;50(Suppl 5):55–57.

46. Berkovic SF, McIntosh A, Howell RA, et al. Familial temporal lobe epilepsy: A common disorder identified in twins. Ann Neurol 1996;40:227–235.

47. Gambardella A, Messina D, Le PE, et al. Familial temporal lobe epilepsy autosomal dominant inheritance in a large pedigree from Southern Italy. Epilepsy Res 2006;38:127–132.

48. Berkovic SF, Izzillo P, McMahon JM, et al. LGI1 mutations in temporal lobe epilepsies. Neurology 2004;62:1115–1119.

49. Maurer-Morelli CV, Secolin R, Marchesini RB, et al. THE SCN2A gene is not a likely candidate for familial mesial temporal lobe epilepsy. Epilepsy Res 2006;71:233–236.

50. Maurer-Morelli CV, Marchesini RB, Secolin R, et al. Linkage study of voltage-gated potassium channels in familial mesial temporal lobe epilepsy. Arq Neuropsiquiatr 2007;65:20–23.

51. Baulac S, Picard F, Herman A, et al. Evidence for digenic inheritance in a family with both febrile convulsions and temporal lobe epilepsy implicating chromosomes 18pter and 1q25-q31. Ann Neurol 2001;49:786–792.

52. Claes L, Audenaert D, Deprez L, et al. Novel locus on chromosome 12q22-q23.3 responsible for familial temporal lobe epilepsy associated with febrile seizures. J Med Genet 2004;41:710–714.

53. Fanciulli M, Di BC, Egeo G, et al. Suggestive linkage of familial mesial temporal lobe epilepsy to chromosome 3q26. Epilepsia 2014;108:232–240.

54. Seppala EH, Koskien LL, Gullov CH, et al. Identification of a novel idiopathic epilepsy locus in belgian shepherd dogs. PLoS ONE 2012;7:e33549.

55. Sillanpaa M, Jalava M, Kaleva O, Shinnar S. Long-term prognosis of seizures with onset in childhood. N Engl J Med 1998;338:1715–1722.

56. Jokinen TS, Metsalonnkala L, Bergamasclo L, et al. Benign familial juvenile epilepsy in lagotto romagnolo dogs. J Vet Intern Med 2007;21:464–471.

57. Jokinen TS, Haaparanta-Solin M, Viitmaa R, et al. FDG-Pet in healthy and epileptic lagotto romagnolo dogs and changes in brain glucose uptake with age. Vet Radiol Ultrasound 2014;55:331–341.

58. Berends M, Gullov CH, Christensen SL, et al. Prevalence and characteristics of epilepsy in the Belgian shepherd variants groenendael and tervueren born in Denmark 1995-2004. Acta Vet Scand 2008;50:51.

59. Berends M, Gullov CH, Fredholm M. Focal epilepsy in the belgian shepherd: Evidence for simple mendelian inheritance. J Small Anim Pract 2009;50:655–661.

60. Oberbauer AM, Belanger JM, Grossman DI, et al. Genome-wide linkage scan for loci associated with epilepsy in Belgian shepherd dogs. BMC Genet 2010;11:35.

61. Gullov CH, Toft N, Berends M. A longitudinal study of survival in Belgian shepherds with genetic epilepsy. J Vet Intern Med 2012;26:1115–1120.

62. Smith BK, Dewey CW. The seizure cat. Diagnostic work-up and therapy. J Feline Med Surg 2009;11:385–394.
63. Wahle AM, Bruhschwein A, Matiasek K, et al. Clinical characterization of epilepsy of unknown cause in cats. J Vet Intern Med 2014;28:182–188.

64. Pakozdy A, Halasz P, Klang A. Epilepsy in cats: Theory and practice. J Vet Intern Med 2014;28:255–263.

65. Kuwabara T, Hasegawa D, Ogawa F, et al. A familial spontaneous epileptic feline strain: A novel model of idiopathic/genetic epilepsy. Epilepsia Res 2010;92:85–88.

66. Tanaka T, Tanaka S, Fujita T, et al. Experimental complex partial seizures induced by a microinjection of kainic acid into limbic structures. Prog Neurobiol 1992;38:317–334.

67. King JT Jr, LaMotte CC. El mouse as a model of focal epilepsy: A review. Epilepsia 1989;30:257–265.

68. Hasegawa D, Mizoguchi S, Kuwabara T, et al. Electroencephalographic features of familial spontaneous epileptic cats. Epilepsia Res 2014;108:1018–1025.

69. Mizoguchi S, Hasegawa D, Kuwabara T, et al. Magnetic resonance volumetry of the hippocampus in familial spontaneous epileptic cats. Epilepsie Res 2014;108:1940–1944.

70. Bień CG, Urbach H, Schramm J, et al. Limbic encephalitis as a precipitating event in adult-onset temporal lobe epilepsy. Neurology 2007;69:1236–1244.

71. Irani SR, Buckley C, Vincent A, et al. Immunotherapy-responsive seizure-like episodes with potassium channel antibodies. Neurology 2008;71:1647–1648.

72. Brierley JB, Corsellis JAN, Hierons R, Nevin S. Subacute encephalitis of later adult life mainly affecting the limbic areas. Brain 1960;83:357–369.

73. Vincent A, Lily O, Paluce J. Pathogenic autoantibodies to neuronal proteins in neurological disorders. J Neuroimmunol 1999;100:169–180.

74. Vincent A, Lang B, Kleopa KA. Autoimmune channelopathies and related neurological disorders. Neuron 2006;52:123–138.

75. Dalmaj, J, Rosenfeld MR. Paraneoplastic syndromes of the CNS. Lancet Neurol 2009;7:327–340.

76. Buckley C, Oger J, Clover L, et al. Potassium channel antibodies in two patients with reversible limbic encephalitis. Ann Neurol 2001;50:73–78.

77. Vincent A, Buckley C, Schott JM, et al. Potassium channel antibody-associated encephalitis: A potentially immunotheraphy-responsive form of limbic encephalitis. Brain 2004;127:701–712.

78. Thieben MJ, Lennon VA, Boeve BF, et al. Potentially reversible autoimmune limbic encephalitis with neuronal potassium channel antibody. Neurology 2004;62:1177–1182.

79. Irani SR, Alexander S, Waters P, et al. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactive-1 protein and contactin-associated protein-2 in limbic encephalitis, morvan’s syndrome and acquired neurnyotonia. Brain 2010;133:2748–2749.

80. Lai M, Huijbers MG, Lancaster E, et al. Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: A case series. Lancet Neurol 2009;7:776–785.

81. Irani SR, Michell AW, Lang B, et al. Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. Ann Neurol 2011;69:892–900.

82. Wieser S, Kelemen A, Barsi P, et al. Pilomotor seizures and status in non-paraneoplastic limbic encephalitis. Epileptic Disord 2005;7:205–211.

83. Graus F, Saiz A, Lai M, et al. Neuronal surface antigen antibodies in limbic encephalitis: Clinical-immunologic associations. Neurology 2008;71:930–936.

84. Lalic T, Pettingill P, Vincent A, Capogna M. Human limbic encephalitis serum enhances hippocampal mossy fiber-CA3 pyramidal cell synaptic transmission. Epilepsia 2011;52:121–131.

85. Malter MP, Helmstaedter C, Urbach H, et al. Antibodies to glutamic acid decarboxylase define a form of limbic encephali- tis. Ann Neurol 2010;67:470–478.

86. Lai M, Hughes EG, Peng X, et al. AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. Ann Neurol 2009;65:424–434.

87. Lancaster E, Lai M, Peng X, et al. Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: Case series and characterisation of the antigen. Lancet Neurol 2010;9:67–76.

88. Zandi MS, Irani SR, Follows G, et al. Limbic encephalitis associated with antibodies to the NMDA receptor in h Hodgkin lymphoma. Neurology 2009;73:2039–2040.

89. Wada JA, Sata M. Generalized convulsive seizures induced by daily electrical stimulation of the amygdala in cats. Correlative electrographic and behavioral features. Neurology 1974;24:565–574.

90. Sato M. Hippocampal seizure and secondary epileptogene- sis in the “kindled” cat preparations. Folia Psychiatr Neurol Jpn 1975;29:239–250.

91. Pakozdy A, Gruber A, Kneissl S, et al. Complex partial cluster seizures in cats with orofacial involvement. J Feline Med Surg 2011;13:687–693.

92. Marioni-Henry K, Monteiro R, Behr S. Complex partial orofacial seizures in english cats. Vet Rec 2012;170:471.

93. Vanhaesebrouck AE, Posch B, Baker S, et al. Temporal lobe epilepsy in a cat with a pyriform lobe oligodendroglioma and hippocampal necrosis. J Feline Med Surg 2012;14:932–937.

94. Gelberg HB. Sudden behavior change in a cat. Vet Pathol 2013;50:1156–1157.

95. Tamura M, Hasegawa D, Uchida K, et al. Feline anaplastic oligodendroglioma: Long-term remission through radiation therapy and chemotherapy. J Feline Med Surg 2013;15:1137–1140.

96. Pakozdy A, Halasz P, Klang A, et al. Suspected limbic encephalitis and seizures in cats associated with Voltage-Gated Potassium Channel (VGKC) complex antibody. J Vet Intern Med 2013;27:212–214.

97. Pakozdy A, Glantschnigg U, Leschnik M, et al. EEG-confirmed epileptic activity in a cat with VGKC-complex/LGI1 anti- body-associated limbic encephalitis. Epileptic Disord 2014;16:116–120.

98. Klang A, Schmidt P, Kneissl S, et al. IgG and complement deposition and neuronal loss in cats and humans with epilepsy and voltage-gated potassium channel complex antibodies. J Neuro- pathol Exp Neurol 2014;73:403–413.

99. Wagner E, Rosati M, Molin J, et al. Hippocampal sclerosis in feline epilepsy. Brain Pathol 2014;24:607–619.