LGI Proteins and Epilepsy in Human and Animals

Pakozdy, A.

2015


http://hdl.handle.net/10138/166585
https://doi.org/10.1111/jvim.12610

Downloaded from Helda, University of Helsinki institutional repository.
This is an electronic reprint of the original article.
This reprint may differ from the original in pagination and typographic detail.
Please cite the original version.
LGI Proteins and Epilepsy in Human and Animals


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.

Abbreviations:

- ADLTE: autosomal dominant lateral temporal lobe epilepsy
- ADAM: a disintegrin and metalloprotease
- AED: antiepileptic drugs
- AMPA: α-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: N-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 neurally secreted protein that contains 3 leucine-rich repeats (LRR) in the N-terminal region6 and epitempin (EPTP) repeats in the carboxyl half of the protein6 as protein–protein interaction domains. Epitempin repeats were only found in the LGH1 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.8 Certain LGI1 mutants (typically nonsecreted mutants) fail to prevent channel inactivation,9,10 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 metallo-protease) protein family member, the transmembrane protein ADAM23, and this interaction affects neurite outgrowth.11,12

LGI1 also is located postsynaptically and co-immunoprecipitates with the postsynaptic scaffolding protein PSD-95 (postsynaptic density protein).13 LGI1 does not interact with PSD-95 directly, but with the extracellular domain of the transmembrane protein ADAM22. ADAM22 binds to PSD-95.13 PSD-95 can bind to stargazin, which is a transmembrane regulatory subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R).14 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.11 The AMPAR-mediated synaptic transmission in the hippocampus is severely decreased when LGI1 is lacking.12

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,12,15,16 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 strengthening the synapse and increasing neurotransmission.12 LGI1 also can weakly bind to ADAM11,17 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.18 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.18

The proteins LGI2, LGI3, and LGI4 all are neuro-nally secreted and act on ADAM family members as does LGI1.13 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.19 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 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 neurotogenesis.20 Outside of the brain, LGI3 also is expressed in adipose tissue and in pre-adipocytes and regulates adipogenesis through ADAM23.21 In human keratinocytes, LGI3,
the secretion of which is stimulated by ultraviolet B (UVB) irradiation, induces cell migration. An additional function of LGI3 in skin is promoting melanin synthesis. 

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. Interestingly, ADAM22 also is expressed in Schwann cells, and ADAM22-deficient mice have a similar phenotype with defects in peripheral nerve myelination. Binding of LGI4 to ADAM22 allows LGI4 to regulate PNS gliogenesis.

LGI Genes

The LGI gene family originated from 2 to 3 rounds of gene duplication in early vertebrate development. The LGI1 gene was first described in a comparison of neuronal tissue with a glioblastoma cell line (T98G). 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. 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. 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). 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.

LGI and Cancer

The observation of decreased or absent LGI1 expression in glioblastomas led to the hypothesis that LGI1-knockout animals would develop tumors of neural tissue. However, a study in LGI1+/− mice demonstrated only the onset of seizures as early as day 8. Until that time point, the animals developed similar to their wild-type or Lgi1+/− littermates, 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 fibrillar 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+/− littermates 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. In neuronal and nonneuronal tumor cell lines only infrequent expression of LGI1–4 mRNA of differing intensity was detected. Also, no correlation was found by comparing their expression in normal tissue and in tumors of the respective tissues. LGI3 had a dose- and time-dependent protective effect on keratinocytes exposed to UVB irradiation. 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. 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. 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. 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. 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 LGI1. Over 30 mutations in LGI1 that result in missense mutations, protein truncation, or internal deletions have been reported. 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 FTLE (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. 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. FMTLE with HS emerges at approximately 10 years of age with complex partial seizures or infrequently (CPS) or focal seizures or orofacial involvement (FEPSO), is commonly reported clinical signs of focal seizure phenomenology include ataxia, crawling, swaying, fearful behavior, excessive attention seeking, drooling, and nausea. 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. The mean age of seizure onset is between 3 and 4 years of age. 

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. Different modes of inheritance for IE in BS dogs have been suggested from incomplete 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. Homozygosity with respect to 2 separate single nucleotide polymorphisms (SNPs) within ADAM23 increased the risk for IE 7-fold. 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. ADAM23 recently was suggested also to be a potential major risk gene for IE in other dog breeds. Mutations in ADAM23 have not been found in epileptic human patients, but in a genomic cohort of IE dogs because ADAM23 interacts with 2 epilepsy-related proteins, LGI1 and LGI2. Furthermore, Adam23 knockout mice exhibit spontaneous seizures, and mice heterozygous for the Adam23 knockout gene have a lower seizure threshold.

Familial Epilepsy in Cats may be Associated with LGI Dysfunction

Compared with epilepsy in humans and dogs, idiopathic epilepsy in cats is less common 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. 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 oro-facial 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 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.

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). Furthermore, unilateral hippocampal atrophy without changes in signal intensity was observed on conventional and volumetric MRI (Fig 3). 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. 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, 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. 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). These patients have little or no response to immunotherapy.

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 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. Immunoreactions against these structures may cause only PNS clinical signs or only CNS clinical signs or combined clinical signs as observed in Morvan syndrome. Although LGI1 appears to be the most common antibody associated with LE, antibodies against glutamic acid decarboxylase (GAD), the AMPA receptor, and the GABA<sub>B</sub> receptor also have been associated with this syndrome. In addition, some patients with typical LE, who have CASPR2 antibodies or NMDA receptor antibodies, have been identified.

LGI-Antibody-Associated LE in Cats

Temporal lobe epilepsy is well known from the experimental research in cats, and there is growing evidence that it also occurs naturally in client-owned cats worldwide. The condition is characterized by complex partial seizures with orofacial involvement as well as FSEC, as mentioned above. Typical ictal clinical signs include episodic orofacial automatism with salivation, chewing, licking, facial twitching, motor arrest, vocalization, and mydriasis, referred to as feline complex partial seizure with orofacial involvement (FEPSEO). Behavioral changes also may occur. The etiology is usually undetermined, but neoplastic and vascular causes were identified or suspected in some cases. In many cats, acute cluster seizure episodes are observed, resembling LE in humans.

Very recently, additional investigations showed an association between FEPSEO 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

Fig 4. Scheme how antibody is binding to VGK-complex. The IgG binds to the complex but in fact to LGI1.

Fig 5. T1-weighted postcontrast MR image of a cat (female, 2 years-old) with seizures and LGI1 antibody-associated limbic encephalitis. Bilateral contrast enhancement on the hippocampal region is visible. (arrows are included only unilateral for better view, but contralateral are the same changes)
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.98 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 LGI 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**


**Acknowledgments**

The authors thank S. Kneissl and Zs. Demeter for their excellent help.

**Grant Support:** This study received financial support from the Vicerectorate for Research and International Relations of the University of Veterinary Medicine Vienna (grant number: P13011230).

**Conflict of Interest Declaration:** Authors disclose no conflict of interest. None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence the content of the paper.

**Off-label Antimicrobial Declaration:** Authors declare no off-label use of antimicrobials.

**References**


LGI Proteins and Epilepsy


