LGI Proteins and Epilepsy in Human and Animals

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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 \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BEJE 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 orofacial 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 \(\text{Kv}1.1\) (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. 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 neurotrophically 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 neuronal 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 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,
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The secretion of which is stimulated by ultraviolet B (UVB) irradiation,\textsuperscript{22} induces cell migration.\textsuperscript{23} An additional function of LGI3 in skin is promoting melanin synthesis.\textsuperscript{24}

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.\textsuperscript{25} Interestingly, ADAM22 also is expressed in Schwann cells,\textsuperscript{26} and ADAM22-deficient mice have a similar phenotype with defects in peripheral nerve myelination.\textsuperscript{16} Binding of LGI4 to ADAM22 allows LGI4 to regulate PNS gliogenesis.\textsuperscript{27}

**LGI Genes**

The LGI gene family originated from 2 to 3 rounds of gene duplication in early vertebrate development.\textsuperscript{28} The LGI1 gene was first described in a comparison of neuronal tissue with a glioblastoma cell line (T98G).\textsuperscript{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.\textsuperscript{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.\textsuperscript{29} 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).\textsuperscript{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.\textsuperscript{30}

**LGI and Cancer**

The observation of decreased or absent LGI1 expression in glioblastomas\textsuperscript{3} led to the hypothesis that LGI1-knockout animals would develop tumors of neural tissue. However, a study in LGI1\textsuperscript{+/−} mice\textsuperscript{31} 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\textsuperscript{+/−} 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\textsuperscript{+/−} 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 LG11 into a glioblastoma cell line indicated a role of LG11 in cell-matrix interactions and migratory processes in the CNS but involvement in glial tumor suppression could not be substantiated.\textsuperscript{32} In neuronal and nonneuronal tumor cell lines only infrequent expression of LGI1 mRNA of differing intensity was detected.\textsuperscript{33} Also, no correlation was found by comparing their expression in normal tissue and in tumors of the respective tissues.\textsuperscript{33} LGI3 had a dose- and time-dependent protective effect on keratinocytes exposed to UVB irradiation.\textsuperscript{22} 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.\textsuperscript{34–36} 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.\textsuperscript{37} 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.\textsuperscript{38–41} 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.\textsuperscript{42,43} 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.\textsuperscript{2,44} Over 30 mutations in LGI1 that result in missense mutations, protein truncation, or internal deletions have been reported.\textsuperscript{5,10} 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. FMTLE without HS/FS is characterized by benign psychic and autonomic auras (để bị run) and can be associated with simple partia  

The disease shows an autosomal recessive inheritance pattern, and a protein-truncating nonsense mutation in the LGI2 gene recently has been identified as a cause of BFJE. In a genetic study, LGI2 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.  

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. Epilepsy in BS dogs is dominated by focal-onset seizures with or without secondary generalization. The most 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. and males and females are equally affected. 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 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. 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 it is a good candidate gene because ADAM23 interacts with 2 epilepsy-related proteins, LG11 and LG12. 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 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 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 VGKCs. 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.

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. 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 FEPSE 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.
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

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