lgi-1 gene

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ABSTRACT

Lgi-1 is a gene that codes for LGI1, commonly known as epitempin, a protein in which disruptions of have shown links to autosomal dominant lateral temporal epilepsy. This is a seizure disorder that is characterized by auditory signs followed by partial seizures. Little is known about the protein but numerous studies isolating the protein to understand the structure and function have been done. LGI-1 protein is a 60-kDa secreted protein, with a N-terminal leucine-rich repeat (LRR) domain and a C-terminal epitempin-repeat (EPTP) domain. These repeat domains are of vital importance in the role LGI-1 plays in connecting receptors ADAM-22 and ADAM-23 in the presynaptic and postsynaptic cleft, respectively. Ultimately the protein has shown to have two primary functions: (i) the regulation of AMPAR-mediated synaptic transmission and (ii) presynaptic potassium ion channels mechanics. This allows for a promising epilepsy therapeutic target in the future.

INTRODUCTION

The gene *lgi*-1, a member of the secreted leucine-rich repeat (LRR) superfamily, may play a role in the regulation in the activity of voltage-gated potassium channels and thus the regulation of the growth of neurons (LGI1, n.d.). This gene codes for the Leucine-rich, glioma inactivated 1 protein that is involved in the control of cell proliferation, cell migration and neurogenesis. A common name for this LGI1 protein is epitempin (G.H., n.d.), however despite the available research results the precise role of this protein in the brain remains uncertain. Mutations in the gene help with gaining an understanding of what the normal function of the protein is. Since it is a neuronal protein, LGI1 may have a potential influence on synaptic function and therefore an interest in the study of it is increasing.

Found in *Homo Sapiens*, mutations in this gene have shown to result in autosomal dominant lateral temporal epilepsy. Autosomal dominant lateral temporal epilepsy is a seizure disorder that is characterized by auditory signs followed by partial seizures. A study using samples from two families affected by this order demonstrates that the disease is of heterogeneity (Morante-Redolat et al., 2002). This is made evident by results that show that there were samples that lacked mutations in the LGI-1 gene even though the individuals expressed phenotypes consistent with this type of epilepsy. Additionally the protein has been recognized as being the ligand of the trans-membrane protein receptor ADAM22, which also causes seizures when mutated. The gene that codes for this protein receptor, *adam*-22, is a member of a family of genes that have been implicated in a variety of processes including neurogenesis and is thus highly expressed in the brain (ADAM22, n.d.). A study conducted in 2018 provided comprehensive results that support the notion that the LGI1-ADAM22 complex has a primary role in the trans-synaptic machinery for precise synaptic transmission (Yamagata et al., 2018).

By isolating the LGI-1 protein, it can be studied to gain a more comprehensive understanding of the structure and function. For example it is known that the LGI-1 protein is a 60-kDa secreted protein, with a N-terminal leucine-rich repeat (LRR) domain and a C-terminal epitempin-repeat (EPTP) domain. This is shown in Figure 1.0, where the purple boxes represent the LRR domain and the orange boxes represent the EPTP domains (Yamagata et al., 2018). Studying the gene allows for further understanding of the processes it is involved in, and studying the effect of mutations in this gene can contribute to improving treatments and potential preventative measures.

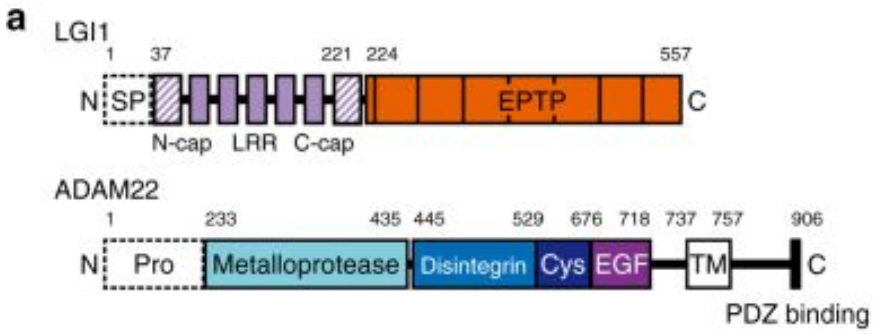

Figure 1.0: Domain Ranges of *lgi*-1 and *adam*-22 retrieved from Yamagata et al., 2018

MATERIALS AND METHODS

None.

RESULTS

A leucine-rich repeat (LRR) domain is a protein that forms a α/β horseshoe fold (Kobe and Deisenhofer, 1994). It is made up of 20-30 repeating amino acid sequences that are folded together to form a solenoid protein domain. These repeating amino acid sequences are rich in the hydrophobic amino acid leucine and while this is a remarkable feature, leucine-rich motifs have been identified in many functionally-unrelated proteins (Rothberg et al., 1990).

At least 42 *lgi*-1 mutations have been identified as of 2018. Of these 42 mutations, 28 are missense mutations that are found in both the LRR and EPTP domains found in the *lgi*-1 gene. These mutations shown in Table 1.0, retrieved from a structural-based study on the LGI1-ADAM22 complex, have shown to be directly linked to autosomal dominant lateral temporal epilepsy (Yamagata et al., 2018). From these missense mutations 24 cause a change to Alanine, 2 to Cysteine and 2 to Glutamine. Alanine is a non-polar hydrophobic amino acid, and Glutamine and Cysteine are polar and uncharged.

LGI1 Mutagenesis		
Primer Name	**Primer Sequence (5' to 3')**	**Description**
hLGI1 F256A -F	ATGTAGTCATCGCTCAGCCTGCT ACTGGAAAATGCATTTTCC	forward primer to mutate human LGI1 Phe256 to Alanine
hLGI1 F256A -R	GGAAAATGCATTTTCCAGTAGC AGGCTGAGCGATGACTACAT	reverse primer to mutate human LGI1 Phe256 to Alanine
hLGI1 V284A -F	CATTACAGGCACATCCACTGCA GTATGCAAGCCTATAGTCA	forward primer to mutate human LGI1 Val284 to Alanine
hLGI1 V284A -R	TGACTATAGGCTTGCATACTGC AGTGGATGTGCCTGTAATG	reverse primer to mutate human LGI1 Val284 to Alanine
hLGI1 L302A -F	TCTATGTTATTGTGGCCCAGGCG TTTGGTGGCTCTCACATCTA	forward primer to mutate human LGI1 Leu302 to Alanine
hLGI1 L302A -R	TAGATGTGAGAGCCACCAAACG CCTGGGCCACAATAACATAGA	reverse primer to mutate human LGI1 Leu302 to Alanine
hLGI1 R330A -F	ATATTGAAATTCTCAAAATCGC AAAACCCAATGACATTGAAAC	forward primer to mutate human LGI1 Arg330 to Alanine
hLGI1 R330A -R	GTTTCAATGTCATTGGGTTTTGC GATTTTGAGAATTTCAATAT	reverse primer to mutate human LGI1 Arg330 to Alanine
hLGI1 K331A -F	TTGAAATTCTCAAAATCCGAGC ACCCAATGACATTGAAACAT	forward primer to mutate human LGI1 Lys331 to Alanine
hLGI1 K331A -R	ATGTTTCAATGTCATTGGGTGCT CGGATTTTGAGAATTTCAA	reverse primer to mutate human LGI1 Lys331 to Alanine
hLGI1 K330A_K331A -F	ATATTGAAATTCTCAAAATCGC AGCACCCAATGACATTGAAACA T	forward primer to mutate human LGI1 Arg330 to Alanine and Lys331 to Alanine
hLGI1 K330A_K331A -R	ATGTTTCAATGTCATTGGGTGCT GCGATTTTGAGAATTTCAATAT	reverse primer to mutate human LGI1 Arg330 to Alanine and Lys331 to Alanine
hLGI1 K353A -F	TTGTTGTTGCTGACAGTTCAGCA GCTGGTTTTACTACCATTT	forward primer to mutate human LGI1 Lys353 to Alanine
hLGI1 K353A -R	AAATGGTAGTAAAACCAGCTGC TGAACTGTCAGCAACAACAA	reverse primer to mutate human LGI1 Lys353 to Alanine
hLGI1 R378A -F	AATCCTTACACGCGTGGTACGC GGACACTGATGTGGAATATC	forward primer to mutate human LGI1 Arg378 to Alanine
hLGI1 R378A -R	GATATTCCACATCAGTGTCCGC GTACCACGCGTGTAAGGATT	reverse primer to mutate human LGI1 Arg378 to Alanine
hLGI1 Y433A -F	TTCCTAACATGGAGGATGTGGC CGCAGTGAAGCACTTCTCAG	forward primer to mutate human LGI1 Tyr433 to Alanine
hLGI1 Y433A -R	CTGAGAAGTGCTTCACTGCGGC CACATCCTCCATGTTAGGAA	reverse primer to mutate human LGI1 Tyr433 to Alanine
hLGI1 M477A -F	GGATGCCATCGCGAGGATCCGC GGTGTTCCAGCCTCTTCAAA	forward primer to mutate human LGI1 Met477 to Alanine
hLGI1 M477A -R	TTTGAAGAGGCTGGAACACCGC GGATCCTCGCGATGGCATCC	reverse primer to mutate human LGI1 Met477 to Alanine
hLGI1 R474Q -F	AGAGGATGCCATCGCGAGGATC CATGGTGTT	forward primer to mutate human LGI1 Arg474 to Glutamine
hLGI1 R474Q -R	AACACCATGGATCCTCGCGATG GCATCCTCT	reverse primer to mutate human LGI1 Arg474 to Glutamine
hLGI1 R407C -F	TCTAGTAGTTCCCAGTGTCCTGT AATTTATC	forward primer to mutate human LGI1 Arg407 to Cysteine
hLGI1 R407C -R	GATAAATTACAGGACACTGGGA ACTACTAGA	reverse primer to mutate human LGI1 Arg407 to Cysteine

hLGI1 R470A -F	CCTCGTTCCAGGATATTCAGGC GATGCCATCGCGAGGATCCA	forward primer to mutate human LGI1 Arg470 to Alanine
hLGI1 R470A -R	TGGATCCTCGCGATGGCATCGC CTGAATATCCTGGAACGAGG	reverse primer to mutate human LGI1 Arg470 to Alanine
hLGI1 R76A -F	ATCCTTTGTGGCATCTGGTTTT	forward primer to mutate human LGI1 Arg76 to Alanine
hLGI1 R76A -R	AAAACCAGATGCCACAAAGGAT	reverse primer to mutate human LGI1 Arg76 to Alanine

Table 1.0: Adapted table of the mutations found on the *lgi*-1 gene, retrieved from Yamagata et al., 2018

Another study looked at the effect of disrupting the LGI1-linked synaptic complex by experimenting on mice that had their *lgi*-1 disrupted (Fukata et al., 2010). Western blotting was done to ensure that the LGI-1 protein was absent. Initially the mice were born with no anatomical defects however all homozygous null LGI1-/- mice began to show growth failure starting on day 14 as shown in Table 1.1. These LGI-/- mice showed recurring spontaneous generalized seizures that were preceded with sudden wild running and jumping, followed by limb clonus and tonic limb extension. The images of the mice in Figure 1.1 display the epileptic behaviours of LGI1-/- mice on postnatal day 17. The image on the right is blurry, indicating the spontaneous generalized seizure. This is followed by a full tonic extension as is shown in the second picture to the left. By the third week almost all LGI1-/- were suddenly dying and none survived more than 25 days after birth.

	LGI1 genotype body weight (g)		
Postnatal day	$^{+/+}$	$^{+/-}$	$^{-/-}$
P10	5.76 ± 0.89	5.51 ± 1.17 ($P = 0.429$)	5.01 ± 1.04 ($P = 0.031$)
P12	6.07 ± 0.94	6.11 ± 1.41 ($P = 0.437$)	5.51 ± 0.99 ($P = 0.014$)
P14	6.69 ± 0.53	6.83 ± 0.89 ($P = 0.545$)	5.82 ± 0.96 ($P = 0.015$)
P17	7.77 ± 1.29	7.70 ± 1.01 ($P = 0.866$)	5.29 ± 0.99 ($P < 0.001$)

$^{+/+}$ Wild type;
$^{+/-}$ heterozygote; $^{-/-}$ homozygote,

Table 1.1: Growth Failure of LGI1-/- Mice At Postnatal Third Week, retrieved from Fukata et al., 2010

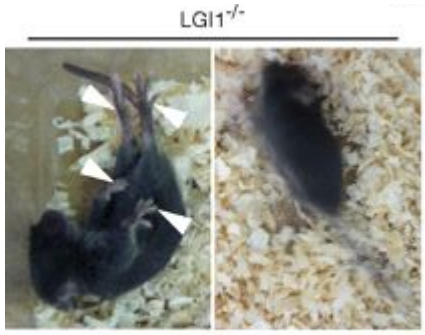

Figure 1.1: Epileptic Behaviours of LGI1-/- Mice , retrieved from Fukata et al., 2010

The study data ultimately showed that a complete loss of LGI-1 in mice (LGI`-/-) resulted in specific lethal epilepsy, and the heterozygous mutation (LGI1+/-) caused an increase in seizure susceptibility compared to homozygous LGI1(+/+). Figure 1.2 displays the increased seizure score for the (LGI1+/-) sample in relation to the normally functioning (LGI1+/+) sample. In addition to linking the disruption of the complex to abnormal synaptic transmission and epilepsy, the study was able to identify the two major LGI-1 receptors in the brain named ADAM22 and ADAM23. Furthermore the study proposed that LGI-1 is an antiepileptogenic secreted protein that connects pre- and postsynaptic protein complexes for finely tuned synaptic transmission.

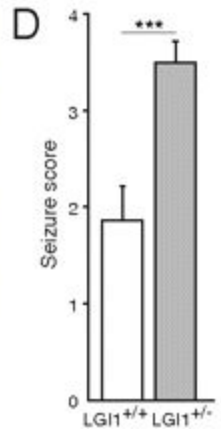

Figure 1.2: Growth Failure of LGI1-/- Mice At Postnatal Third Week, retrieved from Fukata et al., 2010

DISCUSSION

Using various studies of different aspects of the gene, it can be concluded that the LGI-1 protein has two primary functions that have been supported with data. First is the regulation of AMPAR-mediated synaptic transmission. A second potential function of the LGI-1 protein is involved with presynaptic potassium ion channels. One of the studies proposed a novel type of transsynaptic protein interaction in which the protein LGI-1 is secreted extracellularly in the synaptic cleft, which then binds the presynaptic ADAM-23 protein to the postsynaptic ADAM-22 (Fukata et al., 2006). The EPTP domain of the LGI-1 gene is what is directly responsible for the ADAM-22 connection. What makes LGI-1 unique is that the strength of the connection is dependent on the amount of LGI-1 secreted and the lack of information on any other protein that shares the mechanism suggests that LGI-1 may potentially be a major determinant of brain excitation.

Current treatments of most types of epilepsy include medications, diet, surgery, devices or a combination of any. Current anticonvulsant medication function by either decreasing excitation or enhancing inhibition to help reduce and prevent seizures. They do this by targeting the electrical activity in ion channels, or by altering the chemical transmission between neurons such as GABA and Glutamate (Neurology, n.d.). Ultimately, extensive knowledge in the LGI1/ADAMs ligand-receptor complex will allow for an exciting therapeutic target for human epilepsy in the future.

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