

# Mass Spectrometry Identifies LGI1-Interacting Proteins that Are Involved in Synaptic Vesicle Function in the Human Brain

Padmaja Kunapuli · Geeng-Fu Jang · Latif Kazim · John K Cowell

Received: 9 March 2009 / Accepted: 2 April 2009 / Published online: 23 April 2009  
© Humana Press 2009

**Abstract** The LGI1 gene has been shown to predispose to epilepsy and influence cell invasion in glioma cells. To identify proteins that interact with LGI1 and gain a better understanding of its function, we have used co-immunoprecipitation (co-IP) of a secreted green fluorescent protein-tagged LGI1 protein combined with mass spectrometry to identify interacting partners from lysates prepared from human subcortical white matter. Proteins were recovered from polyacrylamide gels and analyzed using liquid chromatography coupled to tandem mass spectrometry. This analysis identified a range of proteins, but in particular synaptotagmin, synaptophysin, and syntaxin 1A. Each of these proteins is found associated with synaptic vesicles. These interactions were confirmed independently by co-IP and Western blotting and implicate LGI1 in synapse biology in neurons. Other vesicle-related proteins that were recovered by co-IP include clathrin heavy chain 1, syntaxin binding protein 1, and a disintegrin and metalloprotease 23. These observations support a role for LGI1 in synapse vesicle function in neurons.

**Keywords** LGI1 · Synapse proteins · Syntaxin 1A · Synaptophysin · Synaptotagmin · Mass spectroscopy

## Introduction

The LGI1 gene (Chernova et al. 1998) has been implicated in glioma cell invasion (Kunapuli et al. 2003), as well as in a predisposition to epilepsy (Kalachikov et al. 2002). Our recent studies have suggested that these different phenotypes, although involving glial cells and neurons respectively, may be facilitated through effects of LGI1 on the reorganization of the actin cytoskeleton, depending on the cell context (unpublished data). The suppression of cell movement and invasion in glioma cells is associated with the downregulation of the extracellular signal-regulated kinase (ERK) pathway (Kunapuli et al. 2004), where one of the consequences is the suppression of matrix metalloproteinase production. These cells also show increased stress fiber formation, a phenotype frequently associated with loss of migration/invasion. LGI1 is a secreted protein and has been shown to exert its effect on cell movement as an exogenous agent in glioma cells. In neurons, LGI1 is associated with the regulation of the KCNA1 (Kv1.1) sodium channel (Schulte et al. 2006) and interacts with the a disintegrin and metalloprotease (ADAM) 22/23 complex on the cell surface (Fukata et al. 2006; Sagane et al. 2008). These observations suggested that the function of LGI1 may be associated with channel function in this cell type.

The cell-specific functions of many proteins often depend on the partners with which they interact. As such, identifying the proteins present in specific complexes provides a more complete view of the function of that protein and how its modification can affect its participation in the complex. In many cases, therefore, identifying

---

**Electronic supplementary material** The online version of this article (doi:10.1007/s12031-009-9202-y) contains supplementary material, which is available to authorized users.

---

J. K. Cowell (✉)  
MCG Cancer Center, School of Medicine,  
Medical College of Georgia,  
1120 15th Street,  
Augusta, GA 30912, USA  
e-mail: jcowell@mccg.edu

P. Kunapuli · G.-F. Jang · L. Kazim  
Department of Cell Stress Biology, Roswell Park Cancer Institute,  
Buffalo, NY 14263, USA

G.-F. Jang · L. Kazim  
Proteomics Resource, Roswell Park Cancer Institute,  
Buffalo, NY 14263, USA

interacting partners for poorly characterized proteins can suggest novel functions. The yeast two hybrid (Y2H) assay has been a popular means of identifying protein–protein interactions, but only defines direct binding of protein partners. The proteins that are present in large complexes, however, may not all interact with each other and so, with a given bait, it is likely that not all proteins will be identified using Y2H. Immunoprecipitation, or tag-based recovery of proteins, on the other hand, targeting any member in the complex, should potentially co-immunoprecipitation (co-IP) all the other members and can therefore be much more informative in understanding protein function. With the frequent difficulty in obtaining highly specific antibodies for a given protein, adding tags such as green fluorescent protein (GFP) and FLAG to the bait protein provides a useful alternative, since specific high-affinity antibodies are available against these tags. The products of these IPs can then be identified using mass spectrometry-based methods.

Previously, proteins that interact with LGI1 have been identified by chance, using other proteins as the bait (Fukata et al. 2006; Schulte et al. 2006). We have now isolated a GFP-tagged LGI1 protein from cells that stably secrete a functional protein (Head et al. 2007) and used co-IP/MS strategies to identify proteins in the complexes. Using this approach, we now demonstrate that LGI1 is found in complex with multiple protein partners many of which are known to be associated with synaptic vesicle function.

## Materials and Methods

### Gel Electrophoresis and Tryptic Digestion of the Protein Bands

Brain lysates were prepared in T-PER solution from Pierce. Briefly 1 g of brain tissue was minced and homogenized in 10 ml of the T-PER (containing 0.01% protease and 0.1% phosphatase inhibitor cocktails). These lysates were then filtered using cell strainers (Millipore) and centrifuged at 14,000 rpm for 20 min after which the supernatant was collected and the proteins concentration was determined using the Bio-rad protein assay. Lysates, containing 1 mg of the protein, were first precleared using rabbit pre-immune serum and then immunoprecipitated using an anti-GFP antibody (living colors from Clontech). After washing five times with 1× phosphate buffered saline, the IP proteins were eluted using sodium dodecyl sulfate (SDS)-sample buffer containing 2-mercaptoethanol at 100°C for 3 min. The immunoprecipitated samples were then analyzed using SDS-polyacrylamide gel electrophoresis (PAGE; 10%) on gels prepared using Bind-Silane (GE Healthcare) to adhere

the gel to a glass plate. After electrophoresis, the gel was stained with Deep Purple (GE Healthcare) and scanned using a Typhoon 9410 Imager (GE Healthcare). Protein bands of interest were excised either manually or with an Ettan SpotPicker (GE Healthcare) and placed in microtubes (0.6 ml, Axygen), prewashed with 18 MΩ·cm water (Mili-Q) and MeOH. In-gel digestion with trypsin was performed according to standard procedures routinely used in the Roswell Park Cancer Institute Proteomics Facility. Briefly, the gel pieces were destained with 50% acetonitrile (ACN)/100 mM ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>; 200 μL) for 30 min with constant mixing (MixMate, Eppendorf). After removal of the solution, the gel pieces were dehydrated with ACN (100 μL) for 15 min at room temperature (RT) and dried in a Speedvac concentrator (Eppendorf). The dried gel pieces were reduced with 10 mM dithiothreitol (Sigma)/100 mM NH<sub>4</sub>HCO<sub>3</sub> at RT for 45 min. The solution was removed, and the samples were alkylated with 50 mM iodoacetamide (Sigma)/100 mM NH<sub>4</sub>HCO<sub>3</sub> (200 μL) at RT in the dark for 30 min. After removal of the solution, the gel pieces were washed with of 100 mM NH<sub>4</sub>HCO<sub>3</sub> (200 μL) and incubated with 50% ACN/100 mM NH<sub>4</sub>HCO<sub>3</sub> (200 μL) at RT for 10 min. Gel pieces were dehydrated with ACN (100 μL), dried in a Speedvac concentrator, and digested with trypsin (Promega, 10 ng/μL in 10% ACN/40 mM NH<sub>4</sub>HCO<sub>3</sub>, 30–50 μL) at 37°C for 16 h. The digests were extracted twice with 50% ACN/0.1% trifluoroacetic acid (100 μL) at RT for 60 min with constant mixing. The extracts were pooled and dried in a Speedvac concentrator, and each sample was then reconstituted with 8 μL of 2% formic acid (FA).

### LC–MS/MS Analysis

The tryptic digests (4 μL of each sample) were analyzed by liquid chromatography–nanoelectrospray–tandem mass spectrometry (LC–ESI-MS/MS) using a nanoACQUITY UPLC (Waters) coupled through a nebulization-assisted nanospray ionization source to a Q-ToF Premier mass spectrometer (Waters/Micromass). The LC consisted of a trap column (Symmetry C18, 5 μ, 180 μ×20 mm, Waters), followed by separation on an analytical column (Atlantis C18, 3 μ, 100 μ×10 cm, Waters). Samples were loaded, trapped, and washed at a flow rate of 4 μL/min with 99% solvent A (0.1% FA)/1% solvent B (ACN containing 0.1% FA) for 5 min. Peptides were eluted with a gradient of 99% A/1% B to 65% A/35% B for 50 min at 0.4 μL/min, 10% A/90% B for 10 min at 0.6 μL/min, and then 10% A/90% B at 1.2 μL/min for 7 min. Throughout the gradient, the mass spectrometer was programmed (data dependent acquisition experiment, DDA) to monitor ions with *m/z* in the range of 350–1500, and only ions with charges between +2 to +4

were selected for MS/MS experiments using the preset DDA collision energy parameters.

#### Database Search and Peptide and Protein Identification

MS/MS spectra were processed and transformed to the PKL file format using Proteinlynx Global Server v2.2 (Waters/Micromass) and the default parameters of MaxEnt3 (Waters/Micromass). The PKL files were used to search the *Homo sapiens* subset of the Swiss-Prot database (release 54.7, containing 18,117 sequences) using a locally installed version of MASCOT (Matrix Science, v 2.2.0). The search parameters were as follows: trypsin as the proteolytic enzyme with two possible missed cleavages, carboxyamido-methylation of cysteine as a fixed modification, oxidation of methionine as a variable modification, the allowable mass error was 100 ppm for peptides, and 100 mDa for fragment ions; peptide charge was set to 2+ and 3+; the instrument was set to electrospray ionization quadrupole time of flight. The Mascot default significance threshold of  $p < 0.05$  for assignments was used in the searches and a minimum of two unique peptides were used as a criteria for a match.

#### Immunoprecipitations and Western Blotting

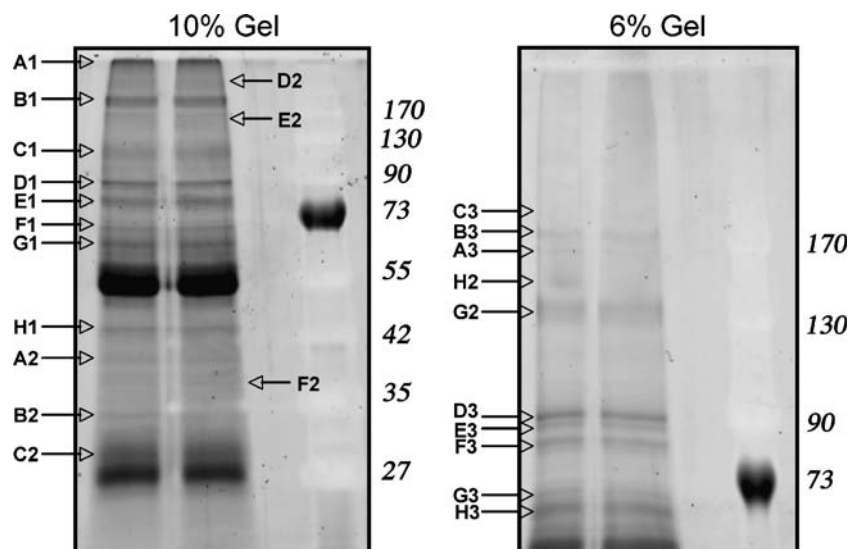
Brain lysates were immunoprecipitated using the living colors anti-GFP antibody as described above. Anti-synaptotagmin (1:1,000) anti-synaptophysin (1:1,000) and anti-syntaxin (1:250) were used for reverse immunoprecipitation after preclearing with respective control antibodies. Following immunoprecipitation, proteins were Western blotted and probed with monoclonal antibody to GFP (Covance 1:5,000), synaptotagmin and synaptophysin (1:1,000), and syntaxin (1:500). These antibodies were obtained from Labvision (Neomarkers).

## Results

We have previously described a 293 cell line that constitutively expresses and secretes an LGI1 protein tagged with GFP at the C-terminal end (Head et al. 2007). This secreted LGI1 protein has also been shown to suppress ERK activation (Kunapuli et al. 2004) when applied to various cell types demonstrating biological activity (unpublished data). Thus, we anticipated that this functional protein would also bind to its endogenous partner proteins in co-IP pull-down assays. The supernatant from the GFP–LGI1 expressing cells was collected and concentrated and then mixed with proteins from lysates of human brain tissue isolated from subcortical white matter. An anti-GFP polyclonal antibody was then used to IP LGI1 and its interacting proteins, which were then analyzed by SDS-PAGE (Fig. 1). The gels were stained with Deep Purple and specific bands were excised and digested with trypsin. The peptides were then analyzed using LC–MS/MS. The same procedure was repeated with IPs from cells expressing GFP alone as a control, where no additional protein bands were seen on SDS-PAGE (data not shown).

The gel excisions were performed strategically to avoid the heavy (58 kD) and light (18 kD) chain bands from the IgG molecules used in the IP. Proteins identified by MS analysis are shown in Table 1, relative to their band location. Bands D1, E1, and F1 (Fig. 1) contained predominantly LGI1 and so only one entry is shown in Table 1. Bands G1 and H3 were the same band but derived from different concentration gels. We also generated IPs from the supernatant of the 293-LGI1 cells as a control which showed no additional protein bands on the gel. For the most part, the size of the proteins identified coincided with their expected size on the gel. Peptide coverage of the identified proteins ranged from 3% to 55% (Table 1), and representative examples of the tandem

**Figure 1** SDS-PAGE analysis of proteins co-immunoprecipitated using anti-GFP. Samples were run in duplicate (lanes 1 and 2) on 10% and 6% gels. Bands, as indicated (arrows), were excised, in gel digested with trypsin and analyzed by LC–MS/MS. Marker lanes are included on the right with sizes in kilo Daltons in each case, where the 73-kDa dark bands are seen with the others in silhouette

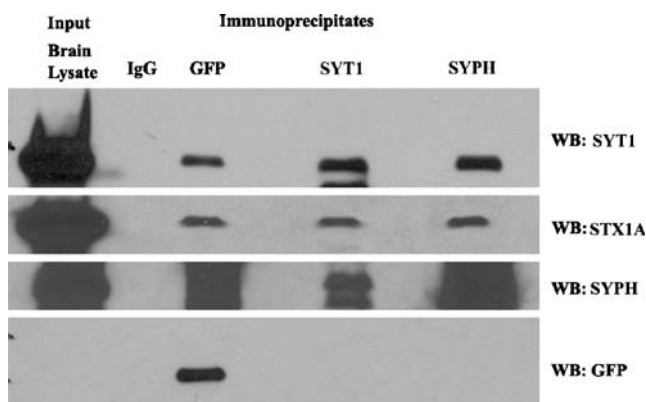


**Table 1** Proteins identified by LC–MS/MS of excised and trypsin-digested gel bands

Gel band	Accession no.	Entry	Primary (citable) accession numbers	Description	Mascot score	MW	Unique peptides matched	Coverage (%)
D1	O95970	LGII_HUMAN	O95970	Leucine-rich glioma-inactivated protein 1 precursor	368	64,519	23	44
H1	P60709	ACTB_HUMAN	P60709	Actin, cytoplasmic 1	269	42,052	15	55
	P14136	GFAP_HUMAN	P14136	Glial fibrillary acidic protein	183	49,907	9	18
A2	P08247	SYPH_HUMAN	P08247	Synaptophysin	49	34,109	2	7
B2	P21796	VDAC1_HUMAN	P21796	Voltage-dependent anion-selective channel protein 1	70	30,868	2	8
	P08758	ANXA5_HUMAN	P08758	Annexin A5	58	35,971	3	10
	Q16623	STX1A_HUMAN	Q16623	Syntaxin-1A	43	33,174	2	9
	P51674	GPM6A_HUMAN	P51674	Neuronal membrane glycoprotein M6-a	32	31,930	3	10
E2	P05023	AT1A1_HUMAN	P05023	Sodium/potassium-transporting ATPase subunit alpha-1 precursor	174	114,135	20	24
	Q00610	CLH1_HUMAN	Q00610	Clathrin heavy chain 1	150	193,260	20	13
F2	Q96A08	H2B1A_HUMAN	Q96A08	Histone H2B type 1-A	25	14,159	2	12
G2	P01857	IGHG1_HUMAN	P01857	Ig gamma-2 chain C region	123	36,596	6	18
A3	Q00610	CLH1_HUMAN	Q00610	Clathrin heavy chain 1	176	193,260	30	21
H3	P21579	SYT1_HUMAN	P21579	Synaptotagmin-1	76	47,885	5	12
	P61764	STXB1_HUMAN	P61764	Syntaxin-binding protein 1	29	67,925	2	3

The gel bands are numbered as shown in Fig. 1. The entry names and the primary (citable) accession numbers (UniProtKB) are provided along with a brief description. The Mascot scores were obtained from Mascot searches (Matrix Science). The number of unique peptides found by LC–MS/MS for each protein and the protein coverage are also reported. Each gel band analysis yielded several significant protein matches. Duplicates were removed and from this list, only those proteins which were considered significant in the context of the tissue (brain) being analyzed are reported. See text for experimental details

MS spectra of peptides from key proteins interacting with LGII are shown in Fig. 2 and in [Supplementary Figures S1–S3](#). From these analyses, it was immediately obvious that many proteins involved in the process of synaptic vesicle function were identified.



**Figure 2** Western blot analysis of immunoprecipitates obtained using anti-IgG, anti-GFP, anti-synaptotagmin, and anti-synaptophysin (*lanes 2 through 4*, respectively). Whole brain lysates (*lane 1*) and immunoprecipitates from *lanes 2–4* were immunoprobed with anti-GFP, anti-synaptotagmin, anti-syntaxin, and anti-synaptophysin as indicated. See text for details

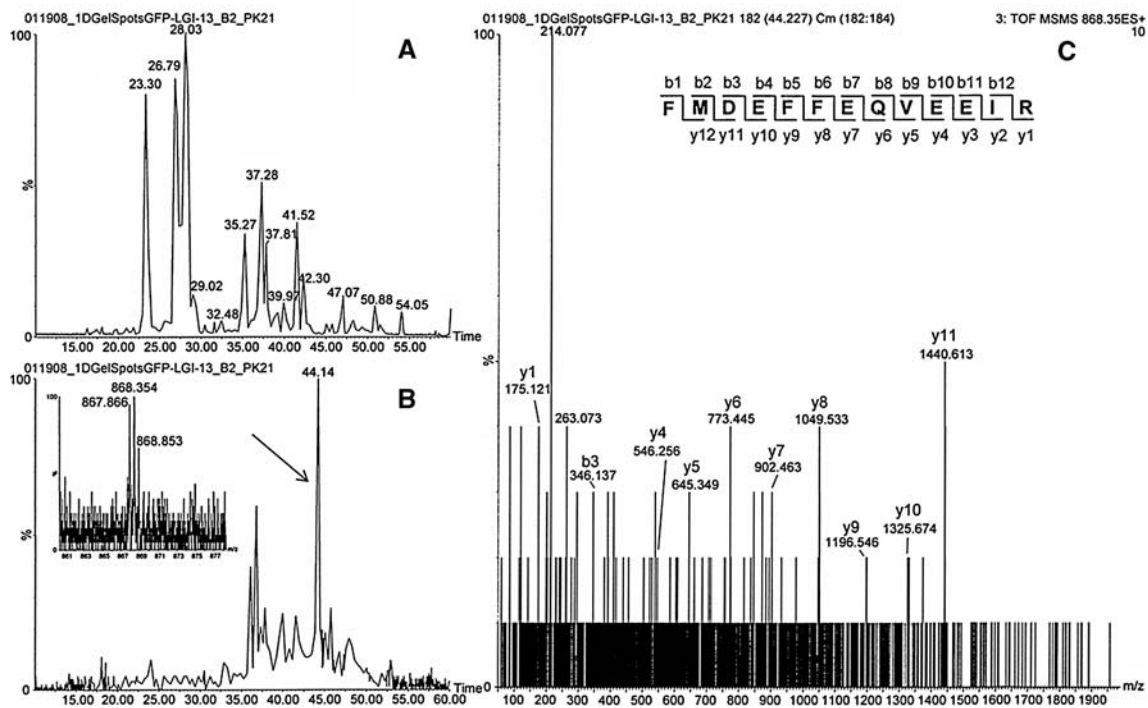
To verify specific interactions related to synaptic vesicle biology, we chose proteins for which well-characterized antibodies were available, specifically, synaptophysin (SYPH), synaptotagmin (SYT1), and syntaxin (STX1A). In these studies, the LGII–GFP protein was recovered by IP as outlined above and co-IP proteins were resolved on PAGs.

The input lysate from normal brain samples identifies the abundant presence of all proteins except GFP, as expected, using Western blotting (Fig. 3). Using anti-GFP, these co-IPs were shown to contain SYPH, SYT1, and STX1A, confirming the MS observations. When whole brain lysates were used for the reverse IPs for the three synapse-related proteins were performed, the presence of all three proteins were again observed in the same complex as expected, since each antibody is capable of bringing down the entire complex. In the absence of an LGII antibody, the presence of the endogenous protein in the complex could not be assayed.

## Discussion

Mutations in the LGII gene have been reported (Kalachikov et al. 2002) in affected family members with the rare form of autosomal dominant partial epilepsy with auditory features

**Syntaxin: Chromatograms, MS and MS/MS Spectra of  $m/z$  867.86,  $[M+2H]^{+2}$ ,  
FMDEFFEQVEEIR, MASCOT Score 43 (9.21 ppm)**



**Fig. 3** A representative chromatogram, with MS and MS/MS spectra of a peptide from syntaxin,  $m/z$ 867.86,  $[M+2H]^{+2}$ , FMDEFFEQVEEIR. The procedures for processing the immunoprecipitated sample by 1D SDS-PAGE, in-gel trypsinization of the protein bands, LC-MS/MS, and database search for peptide and protein identification are described in the text. The base peak intensities and extracted ion current

chromatograms are shown in **a** and **b**, respectively, and the *inserts* in **b** show the MS spectra of the peptides. The MS/MS spectra and de novo sequencing indicating the *b* and *y* ions that were identified are shown in **c**. The MASCOT scores and peptide mass tolerances (shown in *parentheses*) are also reported

(ADPEAF). Epilepsy has been related to the maintenance of excitatory synapse signaling and many other genes predisposing to different types of epilepsy have been shown to have specific channel functions in neurons (Gutierrez-Delgado and Serratos 2004; Hirose et al. 2005). LGI1 was the first reported epilepsy-related gene that did not apparently have a specific channel function (Chernova et al. 1998), although recently it was suggested that LGI1 interacts with KCNA1, a voltage-gated potassium presynaptic channel (Schulte et al. 2006). KCNA1 modulates synaptic transmission in central nervous system neurons and LGI1 selectively prevents *N*-type inactivation mediated by the  $Kv\beta 1$  subunit (Schulte et al. 2006). These observations suggested that LGI1 is related to the epileptic activity, possibly through abnormally synchronized synaptic transmission.

LGI1 has also been shown to interact with ADAM22 (Fukata et al. 2006) as well as other members of the ADAM family (ADAM 11 and ADAM23) of extracellular proteins (Sagane et al. 2008), which are potential receptors for LGI1. Disruption of ADAM22 in mice results in seizures (Sagane et al. 2005). Similarly, disruption of ADAM23 leads to early death associated with tremor (Mitchell et al. 2001). ADAM23 showed the strongest interaction with

LGI1 within this family of proteins (Sagane et al. 2008). We also identified ADAM23 in our assays, although only through a single peptide (the cutoff in our study was set a two peptides) but with a series of *y* ions that confirmed the sequence (see Supplemental figures). The Mascot score for this single peptide was 41 (Supplemental Figure 1), which indicates identity and confirms the interaction with this protein. ADAM 22/23 is anchored to the postsynaptic cytoskeletal scaffolds that contain the stargazin protein (CACNG1) which also interacts with the postsynaptic density-95 (PSD95) protein, a scaffolding protein associated with excitatory synapses which plays a critical role in synaptogenesis and synaptic plasticity. PSD95 was also shown to be in a complex with LGI1 (Fukata et al. 2006). PSD95 interacts with various synaptic proteins which help organize  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors and cell adhesion molecules at synapses (Ko and Kim 2007). This association of LGI1 with specific receptors may also be related to its function in glioma cells, where cell invasion and motility is restricted in the presence of LGI1 (Kunapuli et al. 2004). The data described above clearly implicates LGI1 in the process of synapse biology, indirectly through the identification of

LGII in complexes with these proteins. By using LGII as the bait in pull-down assays, however, we have now been able to expand the repertoire of proteins that are present in complex with it, many of which are related to synaptic function. It should be noted, however, that we specifically isolated the dominant bands that were seen on the acrylamide gel, and so this may not have identified all of the proteins that interact with LGII, although the proof of principle means that we can extend these studies to the interband regions in the future as well as other cell types where LGII has been shown to be expressed (Head et al. 2007).

The demonstration here that LGII is found in complex with synaptophysin (SYPH) and synaptotagmin (SYT1) further implicates LGII in synapse function. The process of synaptic transmission involves the release of neurotransmitters in response to influxes of calcium. SYPH is one of the major synaptic vesicle membrane proteins that mediates this process. SYT is also central to the process of synapse transmission in inhibitory as well as excitatory neurons (Chapman 2008). Neuronal exocytosis is mediated by the vesicular SNARE, synaptobrevin, and the membrane SNAREs syntaxin and SNAP25. Membrane fusion is catalyzed by  $Ca^{2+}$  at synapses and SYT I acts as the sensor that regulated the fusion process. The presence of LGII in this complex containing SYT suggests that it may also have an important role in synapse transmission. SYT functions to synchronize and accelerate  $Ca^{2+}$ -triggered membrane fusion and any influence of LGII in this process could affect efficient synapse transmitter release at the membrane which may be related to seizures.

Syntaxin 1A (STX1A), which we also identified in complex with LGII, binds to SYT and is also intimately involved with synapse transmitter release as a calcium receptor associated with exocytosis (Fernández-Chacón et al. 2002). STX1A also binds to KCNA1 (Fili et al. 2001) and has recently been shown to interact with LGI3 (Park et al. 2008). Taken together, the confirmed finding of the presence of LGII with various synapse related protein complexes firmly positions LGII in a role in this process and disruption of some aspect of this association may be related to ADPEAF.

Interestingly, although STX1A is involved in synapse function, it also functions more widely in the vesicle fusion process and is not specifically a neuronal protein. Syntaxin is found in a complex with other proteins, such as SNAP25, which is associated with vesicle exocytosis in the islets of Langerhans (Takahashi et al. 2004) for example. The Clathrin heavy chain 1 protein, which we also found in complex with LGII, is also more generally involved with vesicle trafficking (Qualmann and Kessels 2002). It is interesting that in our survey of the cellular expression pattern of LGII in bacterial artificial chromosome trans-

genic mice (Head et al. 2007), we demonstrated that the prostate epithelium and pancreatic islet cells also express high levels of LGII, suggesting that this protein may have a wider function in secretory cells than hitherto suggested from the focused studies so far of its role in neuronal function based on its association with epilepsy.

Several proteins associated with synapse function were identified by the MS analysis as interacting with LGII, and although they have not specifically been analyzed using Western blotting to confirm the interactions, the associated Mascot scores demonstrate identity. Thus, the syntaxin binding protein 1 (STXBP1) is essential for synaptic vesicle release, and mutations in this gene predispose to infantile epileptic encephalopathy (Saitou et al. 2008). STXBP1 also appears to play a positive role in insulin secretion from the islet cells in the pancreas (Tomas et al. 2008). In the same way, the neuronal membrane glycoprotein 6A also appears to play an important role in synapse formation as well as neurite/filopodium outgrowth (Alfonso et al. 2005). Taken together, the proteins identified in our pull-down experiments indicate a role for LGII in synapse function and possibly in a wider role for vesicle formation and trafficking in a variety of cell types.

**Acknowledgments** We are grateful to Rama-Dey Rao and Kyoung-Soo Choi for assistance with the proteomics analysis. This work was supported by grant CA from the National Institutes of Health. The authors have no conflicts of interest.

## References

- Alfonso, J., Fernández, M. E., Cooper, B., Flugge, G., & Frasch, A. C. (2005). The stress-regulated protein M6a is a key modulator for neurite outgrowth and filopodium/spine formation. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 17196–17201. doi:10.1073/pnas.0504262102.
- Chapman, E. R. (2008). How does synaptotagmin trigger neurotransmitter release? *Annual Review of Biochemistry*, *77*, 615–641. doi:10.1146/annurev.biochem.77.062005.101135.
- Chernova, O. B., Somerville, R. P. T., & Cowell, J. K. (1998). A novel gene, LGII, from 10q24 is rearranged and downregulated in malignant brain tumors. *Oncogene*, *17*, 2873–2881. doi:10.1038/sj.onc.1202481.
- Fernández-Chacón, R., Shin, O. H., Königstorfer, A., et al. (2002). Structure/function analysis of  $Ca^{2+}$  binding to the C2A domain of synaptotagmin 1. *The Journal of Neuroscience*, *22*, 8438–8446.
- Fili, O., Michaelievski, I., Bledi, Y., Chikvashvili, D., Singer-Lahat, D., Boshwitz, H., et al. (2001). Direct interaction of a brain voltage-gated  $K^+$  channel with syntaxin 1A: functional impact on channel gating. *The Journal of Neuroscience*, *21*, 1964–1974.
- Fukata, Y., Adesnik, H., Iwanaga, T., Bredt, D. S., Nicoll, R. A., & Fukata, M. (2006). Epilepsy-related ligand/receptor complex LGII and ADAM22 regulate synaptic transmission. *Science*, *313*, 1792–1795. doi:10.1126/science.1129947.

- Gutierrez-Delgado, E., & Serratos, J. M. (2004). Genetics of the epilepsies. *Current Opinion in Neurology*, *17*, 147–153. doi:10.1097/00019052-200404000-00011.
- Head, K., Gong, S., Joseph, S., et al. (2007). The expression pattern of the LGI1 gene in tissues and organs from BAC transgenic mice demonstrate a neuronal and glial expression pattern as well as other distinct cell types in the adult animal. *Mammalian Genome*, *18*, 328–337. doi:10.1007/s00335-007-9024-6.
- Hirose, S., Mitsudome, A., Okada, M., et al. (2005). Genetics of idiopathic epilepsies. *Epilepsia*, *46*(Suppl 1), 38–43. doi:10.1111/j.0013-9580.2005.461011.x.
- Kalachikov, S., Evgrafov, O., Ross, B., et al. (2002). Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nature Genetics*, *30*, 335–341. doi:10.1038/ng832.
- Ko, J., & Kim, E. (2007). Leucine-rich repeat proteins of synapses. *Journal of Neuroscience Research*, *85*, 2824–2832. doi:10.1002/jnr.21306.
- Kunapuli, P., Chitta, K. S., & Cowell, J. K. (2003). Suppression of the cell proliferation and invasion phenotypes in glioma cells by the LGI1 gene. *Oncogene*, *22*, 3985–3991. doi:10.1038/sj.onc.1206584.
- Kunapuli, P., Kasyapa, C., Hawthorn, L., & Cowell, J. K. (2004). LGI1, a putative tumor metastasis suppressor gene, controls in vitro invasiveness and expression of matrix metalloproteinases in glioma cells through the Erk1/2 pathway. *The Journal of Biological Chemistry*, *279*, 23151–23157. doi:10.1074/jbc.M314192200.
- Mitchell, K. J., Pinson, K. I., Kelly, O. G., et al. (2001). Functional analysis of secreted and transmembrane proteins critical to mouse development. *Nature Genetics*, *28*, 241–249. doi:10.1038/90074.
- Park, W. J., Lee, S. E., Kwon, N. S., Baek, K. J., Kim, D. S., & Yun, H. Y. (2008). Leucine-rich glioma inactivated 3 associates with syntaxin 1. *Neuroscience Letters*, *444*, 240–244. doi:10.1016/j.neulet.2008.08.044.
- Qualmann, B., & Kessels, M. M. (2002). Endocytosis and the cytoskeleton. *International Review of Cytology*, *220*, 93–114. doi:10.1016/S0074-7696(02)20004-2.
- Sagane, K., Hayakawa, K., Kai, J., et al. (2005). Ataxia and peripheral nerve hypomyelination in ADAM22-deficient mice. *BMC Neurosciences*, *6*, 33.
- Sagane, K., Ishihama, Y., & Sugimoto, H. (2008). LGI1 and LGI4 bind to ADAM22, ADAM23 and ADAM11. *International Journal of Biological Sciences*, *4*, 387–396.
- Saito, H., Kato, M., Mizuguchi, T., et al. (2008). De novo mutations in the gene encoding STXBPI (MUNC18-1) cause early infantile epileptic encephalopathy. *Nature Genetics*, *40*, 782–788. doi:10.1038/ng.150.
- Schulte, U., Thumfart, J. O., Klöcker, N., et al. (2006). The epilepsy-linked Lgi1 protein assembles into presynaptic Kv1 channels and inhibits inactivation by Kvbeta1. *Neuron*, *49*, 697–706. doi:10.1016/j.neuron.2006.01.033.
- Takahashi, N., Hatakeyama, H., Okado, H., Miwa, A., Kishimoto, T., Kojima, T., et al. (2004). Sequential exocytosis of insulin granules is associated with redistribution of SNAP25. *The Journal of Cell Biology*, *165*, 255–262. doi:10.1083/jcb.200312033.
- Tomas, A., Meda, P., Regazzi, R., Pessin, J. E., & Halban, P. A. (2008). Munc 18-1 and granuphilin collaborate during insulin granule exocytosis. *Traffic (Copenhagen, Denmark)*, *9*, 813–832. doi:10.1111/j.1600-0854.2008.00709.x.