

GLUTAMATE RECEPTORS: FINALLY FINGERED IN INHERITED EPILEPSY?

Epilepsy-Related Ligand/Receptor Complex LGI1 and ADAM22 Regulate Synaptic Transmission. Fukata Y, Adesnik H, Iwanaga T, Brecht DS, Nicoll RA, Fukata M. *Science* 2006;313(5794):1792–1795. Abnormally synchronized synaptic transmission in the brain causes epilepsy. Most inherited forms of epilepsy result from mutations in ion channels. However, one form of epilepsy, autosomal dominant partial epilepsy with auditory features (ADPEAF), is characterized by mutations in a secreted neuronal protein, LGI1. We show that ADAM22, a transmembrane protein that when mutated itself causes seizure, serves as a receptor for LGI1. LGI1 enhances AMPA receptor-mediated synaptic transmission in hippocampal slices. The mutated form of LGI1 fails to bind to ADAM22. ADAM22 is anchored to the postsynaptic density by cytoskeletal scaffolds containing stargazin. These studies in rat brain indicate possible avenues for understanding human epilepsy.

COMMENTARY

The role of ion channelopathy in inherited epilepsy continues to expand as new gene mutations underlying epilepsy syndromes are identified. Since the first study describing the linkage of autosomal dominant nocturnal frontal lobe epilepsy with the mutation of a gene encoding the nicotinic acetylcholine receptor (1), the number of epilepsy syndromes linked to single gene mutations has grown dramatically (2). If we consider the pure human epilepsy syndromes that lack other neurological or nonneurological phenotypes (e.g., excluding tuberous sclerosis and similar syndromes with associated cortical dysplasia and other pathologic features), it is remarkable that the identified genes have almost invariably encoded ion channels, whether voltage-gated or ligand-gated. In the voltage-gated channel category, dysfunctional sodium, potassium, calcium, and chloride channels have all been linked to inherited epilepsy, while GABA_A and nicotinic acetylcholine receptors have been implicated among ligand-gated channels. (Curiously, the major excitatory glutamate-gated channels, AMPA and NMDA, have been absent from this list—but read on.) These findings reinforce the primary role of ion channel dysfunction in inherited epilepsy—a compelling pathogenic mechanism for what had been an idiopathic disease.

However, this almost perfect correspondence of inherited epilepsy and channelopathy has been marred by one notable outlier: the syndrome of autosomal dominant partial epilepsy with auditory features (ADPEAF). This syndrome is relatively rare but unmistakable when encountered in the clinic. Patients typically have secondarily generalized seizures that are preceded by unusual auditory auras (3). The aura may consist either of unformed sounds, such as a “machinery-like” whine that gradually increases in intensity before the convulsion, or of recognizable music or voices. Onset is typically in the teens or 20s, and the

seizures are usually relatively easily controlled with medication. Spontaneous remission of seizures often occurs in later years. Inheritance is autosomal dominant with incomplete penetrance. The gene implicated in ADPEAF is the leucine-rich, glioma-inactivated 1 (*LGI1*) locus (4), which was initially described to be homozygously deleted in a subset of cerebral gliomas, suggesting that its product functions as a tumor suppressor.

While the link between the *LGI1* mutation and ADPEAF appears to break the one-to-one correspondence between ion channelopathy and epilepsy, several recent studies have delineated functions of the LGI1 protein that are unrelated to its putative tumor suppressor action. LGI1 is part of a family of genes, *LGI1-4*, also known as *epitempin*. Analysis of their protein structures suggests that they lack the transmembrane domains typical of ion channels. Rather, the structures predict a secreted protein, and in vitro evidence shows that LGI1 and other family members are secreted when exogenously expressed (5). Typical *LGI1* mutations seen in ADPEAF would be predicted to cause truncation of the expressed protein and do in fact reduce their secretion or their extracellular stability. Thus, the mutations seen in ADPEAF would be expected to produce a loss-of-function of the LGI1 protein.

But what is that function? The current paper by Fukata et al. (6) discovers a role for LGI1 that completes the link between ADPEAF and channelopathy. The investigators started by screening for proteins associated with the postsynaptic density protein-95 (PSD-95). As its name implies, PSD-95 is a major constituent of the neuronal membrane area juxtaposed to the synaptic cleft on the postsynaptic side. It functions as a backbone for a variety of synaptic proteins (including glutamate-gated ion channels), their regulatory subunits, and downstream signaling molecules. When the authors isolated PSD-95 from neuronal membranes, they principally found three tightly associated proteins: LGI1, stargazin, and ADAM22. Stargazin is a protein that mediates insertion of AMPA receptors into the postsynaptic membrane by anchoring them to PSD-95 (7); interestingly, it is mutated in the stargazer mouse strain with absence epilepsy and ataxia. ADAM22 is a member of a large

family of transmembrane proteins, and it too is tied to PSD-95 on its intracellular end but also traverses the membrane to protrude into the extracellular space, possibly functioning as a cell adhesion molecule. Fukata and colleagues demonstrated that LGI1 binds to the extracellular portion of ADAM22; this binding in turn appears to increase the number of AMPA receptors inserted into the postsynaptic membrane, augmenting excitatory neurotransmission. Loss of LGI1 function, as seen in ADPEAF, would thus be expected to reduce glutamatergic neurotransmission via AMPA receptors.

The results of Fukata et al. provide a molecular mechanism for the genetic defect seen in ADPEAF. This exciting finding potentially adds glutamate receptor dysfunction to the list of human epileptic channelopathies and strengthens the association between inherited epilepsy and ion channelopathy. But as may be expected for a result this novel, more questions are generated than can be immediately answered. Loss of LGI1 function as would occur in ADPEAF would be predicted to reduce synaptic AMPA receptors, much as mutant stargazin does in epileptic mice, but this hypothesis remains to be proven, and doing so may depend on the generation of mice with *LGII* deletion. Why the defects in AMPA receptor trafficking seen (or predicted) in *stargazin* and *LGII* mutations would produce epilepsy is not immediately clear—much less why they would cause such disparate forms of epilepsy in mice (generalized seizures) versus humans (focal onset seizures). And, considering that the distributions of LGI1 and its partner-in-crime ADAM22 appear widespread throughout the cortex (among other structures), why does the *LGII* mutation in ADPEAF cause seizures with such apparently focal onset in lateral temporal neocortex? Finally, the demonstration of a biological mechanism is of course not the proof that it is sufficient to cause the disease phenotype. An additional interaction has been proposed for LGI1 in the modulation of Kv1.1 channels (8). As the loss of these voltage-gated channels has been associated with epilepsy in animal models, this finding too might be a plausible mechanism in human epilepsy. Confirmation of the biological relevance of these mechanisms in epilepsy almost certainly will require further work using animal models.

The present work, nonetheless, is important for delving into the molecular roots of neuronal excitability to discover the causes of human epilepsy. That this path of investigation again leads to ion channelopathy suggests that ion channel dysfunction is the primary basis of inherited human epilepsy syndromes. One might wonder whether such channelopathy mechanisms will be found to underlie the various acquired forms of epilepsy as well.

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References

1. Steinlein OK, Mulley JC, Propping P, Wallace RH, Phillips HA, Sutherland GR, Scheffer IE, Berkovic SF. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet* 1995;11:201–203.
2. Hirose S. A new paradigm of channelopathy in epilepsy syndromes: intracellular trafficking abnormality of channel molecules. *Epilepsy Res* 2006;70(suppl 1):S206–217.
3. Winawer MR, Ottman R, Hauser WA, Pedley TA. Autosomal dominant partial epilepsy with auditory features: defining the phenotype. *Neurology* 2000;54:2173–2176.
4. Kalachikov S, Eygrafov O, Ross B, Winawer M, Barker-Cummings C, Martinelli Boneschi F, Choi C, Morozov P, Das K, Teplitskaya E, Yu A, Cayanis E, Penchaszadeh G, Kottmann AH, Pedley TA, Hauser WA, Ottman R, Gilliam TC. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nat Genet* 2002;30:335–341.
5. Senechal KR, Thaller C, Noebels JL. ADPEAF mutations reduce levels of secreted LGI1, a putative tumor suppressor protein linked to epilepsy. *Hum Mol Genet* 2005;14:1613–1620.
6. Fukata Y, Adesnik H, Iwanaga T, Brecht DS, Nicoll RA, Fukata M. Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission. *Science* 2006;313:1792–1795.
7. Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, Brecht DS, Nicoll RA. Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* 2000;408:936–943.
8. Schulte U, Thumfart JO, Klocker N, Sailer CA, Bildl W, Biniössek M, Dehn D, Deller T, Eble S, Abbas K, Wangler T, Knaus HG, Fakler B. The epilepsy-linked Lgi1 protein assembles into presynaptic Kv1 channels and inhibits inactivation by Kvbeta1. *Neuron* 2006;49:697–706.