Missense Is No Nonsense for Epileptic Encephalopathies

De Novo GABRG2 Mutations Associated With Epileptic Encephalopathies.

Shen D, Hernandez CC, Shen W, Hu N, Poduri A, Shiedley B, Rotenberg A, Datta AN, Steffen Leiz, A, Patzer S, Boor R, Ramsey k, Goldberg E, Helbig I, Ortiz-Gonzalez XR, Lemke JR, Marsh ED, Macdonald RL. *Brain* 2017;140:49–67.

Epileptic encephalopathies are a devastating group of severe childhood onset epilepsies with medication-resistant seizures and poor developmental outcomes. Many epileptic encephalopathies have a genetic aetiology and are often associated with de novo mutations in genes mediating synaptic transmission, including $GABA_A$ receptor subunit genes. Recently, we performed next generation sequencing on patients with a spectrum of epileptic encephalopathy phenotypes, and we identified five novel (A106T, I107T, P282S, R323W and F343L) and one known (R323Q) de novo GABRG2 pathogenic variants (mutations) in eight patients. To gain insight into the molecular basis for how these mutations contribute to epileptic encephalopathies, we compared the effects of the mutations on the properties of recombinant α1β2γ2L $GABA_A$ receptors transiently expressed in HEK293T cells. Using a combination of patch clamp recording, immunoblotting, confocal imaging and structural modelling, we characterized the effects of these GABRG2 mutations on $GABA_A$ receptor biogenesis and channel function. Compared with wild-type α1β2γ2L receptors, $GABA_A$ receptors containing a mutant γ2 subunit had reduced cell surface expression with altered subunit stoichiometry or decreased GABA-evoked whole-cell current amplitudes, but with different levels of reduction. While a causal role of these mutations cannot be established directly from these results, the functional analysis together with the genetic information suggests that these GABRG2 variants may be major contributors to the epileptic encephalopathy phenotypes. Our study further expands the GABRG2 phenotypic spectrum and supports growing evidence that defects in GABAergic neurotransmission participate in the pathogenesis of genetic epilepsies including epileptic encephalopathies.

Commentary

Epileptic encephalopathies (EEs) are a group of devastating epilepsy syndromes characterized by severe seizures in early infancy and childhood. In general, they are associated with very poor prognoses, intellectual disabilities, and frequent, drug-resistant seizures. A number of particularly devastating infantile EEs exist, including Dravet syndrome, West syndrome, Ohtahara syndrome, Lennox-Gastaut syndrome, and myoclonic-astatic epilepsy. EEs, however, are often not easily categorized due to diverse EEG signatures, age of onset, seizure phenotype, and etiology. Through advances in applied genomics, including large-scale studies like Epi4K (1), the field has made great strides in identifying genes associated with EE. This has helped identify novel potential disease mechanisms and helped stratify patients into specific EE sub-syndromes. Identified human EE risk genes are linked to broad cellular functions, but the majority are related to neuronal and synaptic function (2). These include SCN1A, a sodium channel involved in inhibitory interneuron function (Dravet syndrome [3]); ARX, a transcription factor that is important to inhibitory interneuron maturation and migration (Ohtahara syndrome [4]); CDKL5, a gene important to neuronal signaling (Rett-like EEs [5]); and multiple GABA receptor subunits (6).

In an exciting recent study, Shen and colleagues identify a number of pathological missense mutations in the γ2 subunit of the $GABA_A$ receptor that appear to contribute to early onset EE (A106T, I107T, P282S, R323Q, R323W, and F343L). Interestingly, these mutations all come from patients with otherwise unclassified EEs (although a subset were eventually diagnosed with Lennox-Gastaut syndrome) who have epilepsy phenotypes diverse in their seizure semiology, EEG signatures, and antiepileptic drug responsiveness. The fact that mutations in the same gene manifest in a wide array of epilepsy phenotypes suggests that there may be functional differences in how each mutation affects $GABA_A$ receptor function. Using a beautiful combination of molecular modeling, biochemistry, and electrophysiology, this study identifies meaningful functional changes in all the γ2 mutations identified in this cohort of patients. Interestingly, the mutations cluster between the interface of the N-terminal and transmembrane domain of the receptor, an area important for the chloride channel function of the $GABA_A$ receptor. This suggests that these mutations may affect the $GABA_A$ receptor's affinity for GABA, its conductance, and its biophysical properties. Indeed, the authors report that in a recombinant expression system, all mutations reduce peak GABA-evoked currents and decrease GABA potency. A subset of γ2 mutations also resulted in increased zinc inhibition and

Epilepsy Currents, Vol. 17, No. 3 (May/June) 2017 pp. 171–173

altered activation and decay kinetics. The vast majority of the reported changes are consistent with decreased inhibitory function, linking reduced inhibitory drive and EE. The report includes a fascinating depth of analysis of variant-specific changes in GABA receptor function, which the reader is encouraged to pursue. It should also be noted that the genetics of epilepsy are far from simple, and it is not unusual for damaging mutations to result in a gamut of epilepsy phenotypes or no epilepsy phenotype at all (7). With that in mind, there may be more complexity to the phenotypic variation in patients with γ2 mutations beyond variant-specific changes in $GABA_A$ receptor function. For example, patient-specific genomic variation may have significant impact on how γ2 mutations manifest.

To fully consider the scope of the functional changes associated with each variant, the authors also examined the effects of γ2 variants on GABA receptor trafficking and surface expression. They found that γ2 missense mutations led to increased intracellular retention and decreased surface expression of the $GABA_A$ receptor. Previous studies by this group have shown that a nonsense mutation in the γ2 receptor (Q390X) causes significant retention in the endoplasmic reticulum, failure of GABA receptors to reach the cell surface, and even protein-accumulation induced cell death (8). This mutation is also associated with EE, further linking γ2 dysfunction with severe, early onset epilepsy. Previous studies have linked γ2 missense mutations to altered protein trafficking (9, 10) but had not yet established the link to EE. Showing that EE-associated missense mutations also affect trafficking and surface expression of the GABA receptor sheds new light on how subtle changes in protein structure and function can cause functional deficits. This may not be entirely surprising to the reader, as the intracellular trafficking of the GABA receptor is highly regulated by receptor phosphorylation and multiple intracellular signaling cascades (11). Any perturbation of this system likely has multiple downstream effects.

The current study by Shen et al. is especially noteworthy because they identify *missense* mutations associated with EE. Previous studies linking γ2 variants to EE have largely focused on nonsense variants (Q390X and Q40X, Dravet syndrome [12]), which lead to truncated protein products or no protein expression at all. The γ2 missense variants have previously been linked to less severe epilepsy syndromes, such as febrile seizures and childhood absence epilepsy (R82Q, R117G), as well as the more severe GEFS+ (K328M) (13). The current study sets in place a new understanding of how more subtle genetic variants can link to very severe epilepsies.

The next step in understanding how these γ2 variants lead to EE is attempting to place their functional changes within the developing brain. The subunit composition of the GABA receptor contributes to its function, localization, and pharmacologic sensitivity. We know that the γ2 subunit is part of the most common isoform of the GABA receptor, α1β2γ2, indicating a broad importance of the γ2 subunit. The γ2 subunit is also predominantly found in synapses rather than being extrasynaptic. This underscores its importance in maintaining network-level inhibition and suggests that mutation-induced changes in GABA receptor kinetics may play a role in in disease pathology. Molecular modeling presented in this study demonstrates that these mutations likely affect how other subunits function in the GABA pentamer function, broadening the potential disease mechanisms beyond γ2. Because of the developmental nature of EE, an interesting question is raised about the role of GABAergic inhibition. We know that GABAergic neurotransmission, while inhibitory in the adult brain, is excitatory in the immature brain. Disrupting this developmental GABAergic excitation can disrupt network maturation and later inhibitory network maturation (14, 15). Therefore, mutations in γ2 may contribute to EE phenotypes due to disrupted developmental GABAergic excitation (and subsequent network reorganization) and dysfunction GABAergic inhibition in the more mature brain. As massively parallel sequencing becomes more common and economical, the epilepsy research community will continue to identify novel variants linked to epilepsy. Studies similar to that of Shen et al. will be essential to understanding how variants link to changes in protein function and subsequently lead to disease. In addition, new information about the structural and functional characterization of mutant ion channels and receptors may allow for more selective drug targeting to create improved and more specific epilepsy therapies.

by Antonella Pirone, Ph.D. and Chris Dulla, Ph.D

References

1. Epi4K Consortium; Epilepsy Phenome/Genome Project, Allen AS, Berkovic SF, Cossette P, Delanty N, Dlugos D, Eichler EE, Epstein MP, Glauser T, Goldstein DB, Han Y, Heinzen EL, Hitomi Y, Howell KB, Johnson MR, Kuzniecky R, Lowenstein DH, Lu YF, Madou MR, Marson AG, Mefford HC, Esmaeeli Nieh S, O'Brien TJ, Ottman R, Petrovski S, Poduri A, Ruzzo EK, Scheffer IE, Sherr EH, Yuskaitis CJ, Abou-Khalil B, Alldredge BK, Bautista JF, Berkovic SF, Boro A, Cascino GD, Consalvo D, Crumrine P, Devinsky O, Dlugos D, Epstein MP, Fiol M, Fountain NB, French J, Friedman D, Geller EB, Glauser T, Glynn S, Haut SR, Hayward J, Helmers SL, Joshi S, Kanner A, Kirsch HE, Knowlton RC, Kossoff EH, Kuperman R, Kuzniecky R, Lowenstein DH, McGuire SM, Motika PV, Novotny EJ, Ottman R, Paolicchi JM, Parent JM, Park K, Poduri A, Scheffer IE, Shellhaas RA, Sherr EH, Shih JJ, Singh R, Sirven J, Smith MC, Sullivan J, Lin Thio L, Venkat A, Vining EP, Von Allmen GK, Weisenberg JL, Widdess-Walsh P, Winawer MR.. De novo mutations in epileptic encephalopathies. *Nature.* 2013;501:217–221.
2. Macdonald RL, Kang, JQ, Gallagher MJ. GABAa receptor subunit mutations and genetic epilepsies. In *Jasper's Basic Mechanisms of the Epilepsies* (Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, eds.). 4th ed. New York: Oxford University Press; 2012:740–749.
3. Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, Sadleir LG, Andermann E, Gill D, Farrell K, Connolly M, Stanley T, Harbord M, Andermann F, Wang J, Batish SD, Jones JG, Seltzer WK, Gardner A; Infantile Epileptic Encephalopathy Referral Consortium, Sutherland G, Berkovic SF, Mulley JC, Scheffer IE. The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain.* 2007;130:843–852.
4. Kato M, Saitoh S, Kamei A, Shiraishi H, Ueda Y, Akasaka M, Tohyama J, Akasaka N, Hayasaka K. A longer polyalanine expansion mutation in the ARX gene causes early infantile epileptic encephalopathy with suppression-burst pattern (Ohtahara syndrome). *Am J Hum Genet.* 2007;81:361–366.

5. Weaving LS, Christodoulou J, Williamson SL, Friend KL, McKenzie OLD, Archer H, Evans J, Clarke A, Pelka GJ, Tam PPL, Watson C, Lahooti H, Ellaway CJ, Bennetts B, Leonard H, Gécz J. Mutations of CDKL5 cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. *Am J Hum Genet.* 2004;75:1079–1093.
6. Macdonald RL, Kang JQ, Gallagher MJ. Mutations in $GABA_A$ receptor subunits associated with genetic epilepsies. *J Physiol.* 2010;588:1861–1869.
7. Klassen T. Davis C, Goldman A, Burgess D, Chen T, Wheeler D, McPherson J, Bourquin T, Lewis L, Villasana D, Morgan M, Muzny D, Gibbs R, Noebels J. Exome sequencing of ion channel genes reveals complex profiles confounding personal risk assessment in epilepsy. *Cell.* 2011;145:1036–1048.
8. Kang JQ, Shen W, Zhou C, Xu D, Macdonald RL. The human epilepsy mutation GABRG2(Q390X) causes chronic subunit accumulation and neurodegeneration. *Nat Neurosci.* 2015;18:988–996
9. Huang X, Tian M, Hernandez CC, Hu N, Macdonald RL. The GABRG2 nonsense mutation, Q40X, associated with Dravet syndrome activated NMD and generated a truncated subunit that was partially rescued by aminoglycoside-induced stop codon read-through. *Neurobiol Dis.* 2012;48:115–123.
10. Todd E, Gurba KN, Botzolakis EJ, Stanic AK, Macdonald RL. GABAA receptor biogenesis is impaired by the gamma2 subunit febrile seizure-associated mutation, GABRG2(R177G). *Neurobiol Dis.* 2015;69:215–224
11. Jacob TC, Moss SJ, Jurd R. GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci.* 2008;9:331–343.
12. Harkin LA, Bowser DN, Dibbens LM, Singh R, Phillips F, Wallace RH, Richards MC, Williams DA, Mulley JC.. Truncation of the $GABA_A$-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am J Hum Genet.* 2002;70:530–536.
13. Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, Berkovic SF. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet.* 2001;28:49–52.
14. Bortone D, Polleux F. KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner. *Neuron.* 2009;62:53–71.
15. Zechel S, Nakagawa Y, Ibanez CF. Thalamo-cortical axons regulate the radial dispersion of neocortical GABAergic interneurons. *eLife.* 2016;5. doi:10.7554/eLife.20770.