



GABA strikes down again in epilepsy

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Disruption of GABA transmission has been associated with different epilepsy syndromes according to the role of GABA as the major inhibitory neurotransmitter in the central nervous system (CNS) (1). In fact, mutations in several genes encoding GABA receptor subunits, including *GABRA1*, *GABRA6*, *GABRB2*, *GABRB3*, *GABRG2*, and *GABRD* have been identified in patients ranging from febrile seizures, idiopathic (or genetic) generalized epilepsy (GGE), up to severe epileptic encephalopathies, e.g., Dravet syndrome, infantile spasms/West syndrome, and Lennox-Gastaut syndrome (2). GABA receptors are ligand-gated anion channels with important effect on neuronal inhibition. Accordingly, mutations in GABA genes typically impair hyperpolarization of the receptor, thus leading to epilepsy. This effect is not simply caused by receptor gating abnormalities but rather mediated by multiple and complex mechanisms, including endoplasmic reticulum (ER)-associated degradation, nonsense-mediated mRNA decay, intracellular trafficking defects, and ER stress (3). However, despite the high heritability of many forms of epilepsy, the identification of pathogenetic variants has been slow over the years (4). Early linkage studies in families lead to the identification of mutations in several ion channel genes, e.g., *SCN1A*, *SCN1B*, *GABRG2*, and *GABRA1* (4). However, autosomal dominant families are relatively rare compared with most sporadic patients or families that typically show a complex inheritance patterns (4). A recent exome sequencing study (5) including 640 subjects affected by GGE failed to reveal major genetic cause of the disease but identified excess ultra-rare variation in well-

established epilepsy genes, supporting the link common and rare, severe epilepsies. Moreover, this case-control exome sequencing study revealed an excess of missense variants in genes encoding GABAA receptor subunits through in GGE patients and functional assessment of some of these variants showed loss-of-function mechanism for 4 out of 7 *GABRB2* and *GABRA5* variants.

Recently, a whole-genome sequencing study by Butler and colleagues (1) identified a *de novo* missense variant in *GABRA5* (c.880G>C, p.V294L) in an individual with drug-resistant epilepsy associated with intellectual disability. Then, the same authors sequenced of 279 patients and identified 19 rare changes in nine genes encoding for GABAA receptors, including a *de novo* missense variant in *GABRA2* (c.875C>A, p.T292K) and in *GABRB3* (c.902C>T, p.P301L) in two individuals with severe epilepsy and intellectual disability. The functional effects of the *GABRA5*, *GABRA2* and *GABRB3* missense changes were investigated by patch-clamp recordings in embryonic kidney 293T cells to explore their impact on the receptor function.

These experiments revealed a ten-fold increase in the GABA apparent affinity in the individual carrying the *GABRB3* mutation, probably due to the accumulation of more channels at desensitized state, which are removed from the pool of active receptors, leading in turn to reduced neuronal inhibition. The individual carrying this variant is unlikely to respond to conventional positive allosteric GABA A receptor modulators, e.g., benzodiazepines and barbiturates, since these drugs would further increase the number of desensitized receptors. The fact that the boy

went seizure-free at age 14 months with a combination of non-GABAergic drugs, namely zonisamide, levetiracetam, and oxcarbazepine, would further support this concept.

The *GABRA2* p.T292K variant produced a dysfunctional receptors that did not generate GABA-evoked currents, reduce channel expression at the cell surface and produce a channel tonically open even in absence of GABA. This basal leak currents suggests that the mutant channel is likely trapped in open state and this allow to pass indiscriminate GABA A receptor-mediated currents. As Cl concentration is higher in intracellular during early development when GABAergic synaptic development precedes glutamatergic development, this could promote the epileptogenic process (6) and it is reasonable to expect that this mutation confers the highest vulnerability during brain development (7). The receptors containing the p.P301L residue change in *GABRB3* are associated with a reduction in GABA apparent-affinity as well as GABA-evoked current amplitude.

Finally, Butler and colleagues did also identify 17 different heterozygous GABRA variants from individuals with epilepsy, three of which did not have functional effect and the remaining 13 were unlikely to be causative, even if not all the variants were functionally investigated.

Before this work, functional studies on *GABRA1* receptor had been previously performed by Johannesen and colleagues (8) who revealed a loss of function in four selected mutations, without a clear genotype-phenotype correlation. Likewise, Niturad and his group (9) showed that rare loss-of-function variants in *GABRA3* increase the risk for a varying combination of epilepsy, intellectual disability/developmental delay and dysmorphism. These data were confirmed by Møller and colleagues showing the broad phenotypic and functional spectrum associated with *GABRB3* variants (10).

In conclusion, this paper provides for the first time the functional demonstration that *de novo* *GABRA5* and *GABRA2* variants contribute to early-onset epilepsy and intellectual disability and does also support the concept that epilepsy is the result of impaired neuronal inhibition. Moreover, these data confirm the power of exome sequencing to identify rare variants in genes increasing the risk of epilepsy, although it is unlikely that all the variants confer disease risk. Collaborative, large studies should now replicate these findings as well as to identify the variants which are likely to be deleterious. This goal is crucial for interpreting variants in a clinically meaningful manner and to eventually provide families with accurate diagnosis and implementing effective therapies.

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Footnote

Conflicts of Interest: P Striano has served on a scientific advisory board or received honoraria from GW pharma, Kolfarma s.r.l., and Eisai pharma; he has received research support from the Italian Ministry of Health.

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