Yo GABA GABA! Convergent Mechanisms Driven by Gain-of-Function GABRD and Loss-of-Function SLC6A1 Variants Implicate Elevated GABAergic Tone in Generalized Epilepsies

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Gain-of-Function Variants in GABRD Reveal a Novel Pathway for Neurodevelopmental Disorders and Epilepsy
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A potential link between GABRD encoding the δ subunit of extrasynaptic GABAA receptors and neurodevelopmental disorders has largely been disregarded due to conflicting conclusions from early studies. However, we identified seven heterozygous missense GABRD variants in 10 patients with neurodevelopmental disorders and generalized epilepsy. One variant occurred in two sibs of healthy parents with presumed somatic mosaicism, another segregated with the disease in three affected family members, and the remaining five occurred de novo in sporadic patients. Electrophysiological measurements were used to determine the functional consequence of the seven missense δ subunit variants in receptor combinations of α1β3δ and α4β2δ GABAA receptors. This was accompanied by analysis of electro-clinical phenotypes of the affected individuals. We determined that five of the seven variants caused altered function of the resulting missense δ subunit variants in receptor combinations of α1β3δ and α4β2δ GABAA receptors. Surprisingly, four of the five variants led to gain-of-function effects, whereas one led to a loss-of-function effect. The stark differences between the gain-of-function and loss-of-function effects were mirrored by the clinical phenotypes. Six patients with gain-of-function variants shared common phenotypes: neurodevelopmental disorders with generalized epilepsy, behavioral issues, and various degrees of intellectual disability. Six patients with gain-of-function variants shared common phenotypes: neurodevelopmental disorders with behavioral issues, various degrees of intellectual disability, generalized epilepsy with atypical absences, and generalized myoclonic and/or bilateral tonic-clonic seizures. The EEG showed qualitative analogies among the different gain-of-function variant carriers consisting of focal slowing in the occipital regions often preceding irregular generalized epileptiform discharges, with frontal predominance. In contrast, the one patient carrying a loss-of-function variant had normal intelligence and no seizure history but has a diagnosis of autism spectrum disorder and suffering from elevated internalizing psychiatric symptoms. We hypothesize that increase in tonic GABA-evoked current levels mediated by δ-containing extrasynaptic GABAA receptors lead to abnormal neurotransmission, which represent a novel mechanism for severe neurodevelopmental disorders. In support of this, the electro-clinical findings for the gain-of-function GABRD variants resemble the phenotypic spectrum reported in patients with missense SLC6A1 (GABA uptake transporter) variants. This also indicates that the phenomenon of extrasynaptic receptor over-activity is observed in a broader range of patients with neurodevelopmental disorders, since SLC6A1 loss-of-function variants also lead to overactive extrasynaptic δ-containing GABAA receptors. These findings have implications when selecting potential treatment options, since a substantial portion of available anti-seizure medication act by enhancing GABAergic function either directly or indirectly, which could exacerbate symptoms in patients with gain-of-function GABRD variants.
Common Molecular Mechanisms of SLC6A1 Variant-Mediated Neurodevelopmental Disorders in Astrocytes and Neurons

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Solute carrier family 6 member 1 (SLC6A1) is abundantly expressed in the developing brain even before the CNS is formed. Its encoded GABA transporter 1 (GAT-1) is responsible for the reuptake of GABA into presynaptic neurons and glia, thereby modulating neurotransmission. GAT-1 is expressed globally in the brain, in both astrocytes and neurons. The GABA uptake function of GAT-1 in neurons cannot be compensated for by other GABA transporters, while the function in glia can be partially replaced by GABA transporter 3. Recently, many variants in SLC6A1 have been associated with a spectrum of epilepsy syndromes and neurodevelopmental disorders, including myoclonic atonic epilepsy, childhood absence epilepsy, autism, and intellectual disability, but the pathomechanisms associated with these phenotypes remain unclear.

Three individuals with potentially pathogenic GABRD variants were identified, for a total of ten individuals from eight unrelated families. In total, seven heterozygous missense variants in GABRD were identified and were predicted to be damaging by in silico prediction tools.

Unlike the strong evidence linking variants in α, β, and γ subunits with epilepsy, the association of variants in GABRD, encoding the δ subunit, with epilepsy has been disputed due to previous studies with conflicting results. Variants in GABRD were originally linked to susceptibility to generalized epilepsies and determined to be loss-of-function (LOF). The δ subunit is present in extrasynaptic and persynaptic GABA\(_A\)Rs that are responsible for mediating tonic inhibition. The predicted reduction in tonic inhibition contrasts with data from animal models suggesting that increased tonic inhibition is associated with seizures and may be required for absence seizures. Further analysis of GABRD variation in large cohorts of individuals with generalized epilepsy or neurodevelopmental disorder with epilepsy failed to find enrichment of GABRD variants in cases vs controls, leading the authors of those studies to suggest that GABRD may not be associated with epilepsy.

A new study from Ahring et al. screened 933 individuals with neurodevelopmental disorders and generalized epilepsy. Three individuals with potentially pathogenic GABRD variants were identified. The authors sought to identify additional individuals with epilepsy or neurodevelopmental disorders through international collaborations and GeneMatcher. From these efforts, an additional seven individuals were identified, for a total of ten individuals from eight unrelated families. In total, seven heterozygous missense variants in GABRD were identified and were predicted to be damaging by in silico prediction tools.

Commentary

Aberrant neuronal excitability is a basic mechanism underlying epilepsy. Many molecules influence excitability in the brain, including excitatory and inhibitory neurotransmitters. The major inhibitory neurotransmitter in the brain is γ-aminobutyric acid (GABA), which exerts its function via ionotropic GABAA and metabotropic GABAB receptors. This commentary focuses on the GABAA receptor (GABA\(_A\),R). Altered GABA\(_A\)R signaling is associated with many neurological, neurodevelopmental, and neuropsychiatric disorders, including epilepsy, autism spectrum disorder (ASD), intellectual disability (ID), and schizophrenia. GABA\(_A\)Rs are pentameric proteins, and the most common subtype contains two α subunits, two β subunits, and one γ2 or δ subunit. Variants in many of the genes encoding GABA\(_A\),R subunits have been identified in a broad range of rare and common seizure disorders, including generalized epilepsy with febrile seizures plus (GEFS+), genetic generalized epilepsy (GGE), childhood absence epilepsy (CAE), juvenile myoclonic epilepsy (JME), and developmental and epileptic encephalopathy (DEE). Typically, these variants result in loss-of-function GABA\(_A\),R subunits, including GABRA1, GABRB3, and GABRG2 encoding α, β, and γ subunits, respectively. In addition, GABA\(_A\),R gene variants can act as genetic modifiers of epilepsy in animal models, with reduced expression of Gabra2, coding the α subunit, resulting in more severe seizure phenotypes.
tools. In one family, the variant segregated with the epilepsy phenotype, while the remaining variants occurred de novo.

Functional analysis of the seven variants was carried out using voltage-clamp electrophysiology in transfected *Xenopus laevis* oocytes. In contrast to the previous reports linking *GABRD* to epilepsy, functional analysis revealed that four epilepsy-associated variants cause gain-of-function (GOF) effects that increased tonic GABAergic tone. One variant, identified in an individual with ASD without ID or seizures, resulted in LOF effects. The remaining two variants showed no difference from wildtype δ subunits. The authors took an extra step to analyze two additional *GABRD* variants found in the gnomAD database, which would be expected to be non-pathogenic. Indeed, they found no difference between these two population variants and wildtype, concluding that the five variants that showed significant deviations from wildtype are indeed likely to be pathogenic in those individuals.

The electro-clinical phenotype of the individuals with GOF *GABRD* variants included abnormal EEG and atypical absences, generalized myoclonic, and generalized tonic-clonic seizures. The authors noted a phenotypic overlap between the GOF *GABRD* individuals and those with variants in *SLC6A1*, which encodes the GABA transporter GAT-1. Variants in *SLC6A1* have been identified in many types of epilepsy, including myotonic atonic epilepsy and childhood absence epilepsy, in addition to neurodevelopmental disorders such as ASD and ID. In a separate study, Mermer et al. investigated the molecular mechanism underlying *SLC6A1*-related neurodevelopmental disorders. They found that variants in *SLC6A1* resulted in LOF effects including reduced GAT-1 protein, reduced surface expression of GAT-1, and reduced GABA uptake. Reduced activity of GAT-1 results in increased tonic current amplitude and increased tonic GABAergic tone, similar to the result found for the GOF *GABRD* variants. Thus, variants resulting in aberrant activity of different components mediating tonic inhibition via the GABAergic signaling pathway give rise to similar neurological phenotypes. These findings underscore the importance of understanding the molecular pathways underlying neuronal excitability, which can help explain how GOF and LOF genetic variants in that pathway can both lead to seizures. Similarly, genetic evidence can inform our understanding of pathways involved in the etiology of epilepsy and guide functional studies to elucidate molecular mechanisms.

In particular, data from these two genetic studies combined with data from animal models suggests that increased tonic inhibition is involved in the etiology of absence seizures. Additional functional characterization of absence epilepsy-associated variants of *GABRD* and *SLC6A1* is needed to investigate this mechanistic association. For example, the impact of these variants on action potential generation and network excitability could be studied using induced pluripotent stem cell (ipsc)-derived neuronal cultures or brain organoids. Mouse models harboring these variants would allow detailed electrophysiological examination of cortico-thalamic networks and could serve as pre-clinical models for testing of drugs that modulate GABAergic tone.

Many anti-seizure medications attempt to enhance GABAergic signaling. However, drugs that increase GABAergic tone may exacerbate seizures in individuals with GOF *GABRD* or LOF *SLC6A1* variants. These studies, which suggest that elevated GABAergic tone underlies seizures in these patients, indicate that reduction of tonic GABAergic signaling may be therapeutic in these individuals. Antisense oligonucleotides (ASOs) that target mRNA to reduce gene expression are promising treatments for genetic epilepsies. Reduction of δ-subunit containing GABAA Rs by targeting *GABRD* with an ASO could be a therapeutic strategy for GOF *GABRD* variants. Similarly, gene therapy via adeno-associated virus (AAV) delivery of *SLC6A1* could elevate GABA uptake and rescue GABAergic tone caused by LOF *SLC6A1* variants.

Overall, more genetic and molecular evidence is needed to (1) establish a definitive relationship between *GABRD* and epilepsy and (2) determine genotype-phenotype correlations in *GABRD* and *SLC6A1* epilepsy. With only four rare missense variants in the Ahring paper, more genetic and functional data are needed to establish whether GOF variants in *GABRD* are pathogenic and how they produce alterations in GABAergic signaling in different regions of the brain to generate seizures. In addition, the LOF variant of *GABRD* that was associated with ASD suggests that a phenotypic spectrum may emerge for *GABRD* variants similar to that seen for other ion channel-related neurodevelopmental disorders. For *SLC6A1*, Mermer and colleagues were not able to establish a correlation between reduced GABA uptake and phenotypic severity. It will be important to identify functional parameters that may underlie the relationship between GAT-1 activity and phenotypic severity in these patients to inform development of effective treatments.

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