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Vulnerabilities in a Dominant Receptor Subunit

AMPA Receptor GluA2 Subunit Defects Are a Cause of Neurodevelopmental Disorders

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4-amino-3-hydroxy-5-methyl-4isoxazolepropionic acid (AMPA) receptors (AMPARs) are tetrameric ligand–gated channels made up of combinations of GluA1-4 subunits encoded by *GRIA1-4* genes. GluA2 has an especially important role because, following posttranscriptional editing at the Q607 site, it renders heteromultimeric AMPARs Ca^{2+}-impermeable, with a linear relationship between current and transmembrane voltage. Here, we report heterozygous de novo *GRIA2* mutations in 28 unrelated patients with intellectual disability (ID) and neurodevelopmental abnormalities, including autism spectrum disorder (ASD), Rett syndrome-like features, and seizures or developmental epileptic encephalopathy (DEE). In functional expression studies, mutations lead to a decrease in agonist-evoked current mediated by mutant subunits compared to wild-type channels. When GluA2 subunits are coexpressed with GluA1, most *GRIA2* mutations cause a decreased current amplitude, and some also affect voltage rectification. Our results show that de novo variants in *GRIA2* can cause neurodevelopmental disorders, complementing evidence that other genetic causes of ID, ASD, and DEE also disrupt glutamatergic synaptic transmission.

A 1995 *Science* paper showed that genetic alteration in the *Gria2* gene (then known as *GluR-B* or *GluR2*) caused early-onset epilepsy and postnatal lethality in mice.[1] In the last sentence of that article, the authors speculated that human epilepsies may be caused by similar molecular defects. It took 25 years to convincingly verify this prediction, but a recent study by Salpietro et al. has identified 28 patients with neurodevelopmental disorders (NDDs) and heterozygous de novo variants in the *GRIA2* gene[2], which codes for a subunit of the 4-amino-3-hydroxy-5-methyl-4isoxazolepropionic acid (AMPA)–type, glutamate-gated ion channel. Twelve of these patients had either epilepsy or developmental epileptic encephalopathies, providing the first strong evidence that *GRIA2* variants cause human epilepsies.

Identification of new variants in a gene previously unknown to cause epilepsy is always noteworthy, but the rich history and fascinating biology of *Gria2* make this discovery even more impactful. The AMPARs are workhorses of the central nervous system that mediate the bulk of fast excitatory synaptic transmission. They are heteromeric tetramers composed of combinations of the 4 AMPAR subunits, GRIA1-GRIA4. GRIA2 is the most unique of the 4 because the presence of a GRIA2 subunit in the tetramer "dominates" its biophysical properties by changing the receptor from being Ca^{2+} permeable to Ca^{2+} impermeable, making its current–voltage relation (I/V) linear, and reducing channel conductance.[3] Most AMPARs in mature brains are GRIA2-containing and Ca^{2+} impermeable, with a notable exception being those on medial ganglionic eminence–derived inhibitory neurons.[4]

But the most intriguing aspect of GRIA2 is the mechanism through which it achieves its dominance. The difference is caused by a single amino acid change in a pore-lining position in GRIA2. This amino acid difference is not encoded in the DNA; it is posttranscriptionally modified by adenosine-to-inosine RNA editing,[5] a mechanism that can specifically alter a single codon in a messenger RNA and has been hypothesized to be a driving force in human brain evolution.[6] This regulation of glutamate receptor function by RNA editing provides a striking example of a mechanism to create receptor diversity in the nervous system.

The GRIA2 variants identified in this study included microdeletions, those generating premature stop codons, and loss of splice sites, all of which are predicted to cause loss of function. The majority of the variants were missense, however, and were positioned in various domains of the protein, including the extracellular amino terminal domain, the ligand-binding domain, and transmembrane domains. To assess the functional impact of the missense variants, the authors expressed the mutant proteins, either alone or with wild-type GRIA1, in HEK 293T cells in vitro. They then applied kainate, an agonist of AMPARs that does not cause the receptor to rapidly desensitize as glutamate does, and measured the current amplitudes and

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their relationship with the transmembrane voltage (I/V curve). Most variants tested reduced the amount of current through the receptor, and many made the I/V curve nonlinear, which would be expected if the variant caused a loss of surface expressed AMPARs incorporating the GRIA2 subunit. To follow-up on this, the authors assayed the amount of surface expression and found decreases caused by many of the variants. Thus, the overall picture is consistent with a loss of function of GRIA2-containing AMPA receptors via multiple molecular mechanisms, and the authors noted that there was no genotype–phenotype correlation.

One crucial physiological parameter of the receptor that the authors did not address was Ca^{2+} permeability. Because the incorporation of GRIA2 in the receptor renders it Ca^{2+} impermeable, loss of GRIA2 surface expression could increase Ca^{2+} permeability even while reducing the overall current density. Mice lacking the *Gria2* gene show a reduction in evoked glutamatergic transmission in the hippocampus but a 9-fold increase in Ca^{2+} permeability. These mice have learning deficits and increased mortality but no reported seizures.[7] Neurons from mice expressing unedited GRIA2 protein have AMPARs with increased Ca^{2+} permeability, neuronal degeneration in the hippocampus, seizures, and completely penetrant mortality,[1] suggesting that increasing Ca^{2+} permeability without decreasing receptor expression or activity may be even more detrimental. Interestingly, one of the variants described in this study was in the edited codon (R607G), and the patient has treatment-resistant, early-onset seizures. The expression of this variant in the HEK cells made the I/V curve highly nonlinear and reduced surface expression, but also presumably increased Ca^{2+} permeability. The authors noted that this and other variants reduced cell viability, which could be caused by excessive Ca^{2+} entry.

It is somewhat counterintuitive, perhaps, that loss of function in a gene that encodes for one of the major excitatory neurotransmitters in the brain can cause epilepsy. In fact, one of the more recently approved antiepileptic drugs, Perampanel, is a noncompetitive AMPA-type glutamate receptor antagonist. The fact that weakening of AMPAR-mediated currents can both treat and cause epilepsy demonstrates a complex relationship between AMPARs and seizure generation, and this complexity has already been addressed in the case of another AMPAR gene, *GRIA4*. *GRIA4* variants in humans also cause NDD and epilepsy,[8] and *Gria4* knockout mice have absence epilepsy.[9] Elegant studies in this animal model showed that synapse-specific weakening in the corticothalamic circuit leads to enhanced network synchrony via specific loss of feed-forward inhibition,[10] suggesting that cell type–specific expression patterns of the 4 AMPA receptor subunits or their interacting proteins may be key to a mechanistic understanding.

By uncovering novel variants in a gene that plays such a central and unique role in synaptic transmission and brain function, this study provides important information about the genetics underlying neurodevelopmental and seizure disorders, and will certainly inspire further investigation into the mechanisms linking *GRIA2* variants to altered neuron and network function and seizures. Studies similar to what has been done in *Gria4* knockout mice, but using animal models with the *GRIA2* variants identified in this study, will be necessary to understand cell- and circuit-specific mechanisms of seizure generation caused by *GRIA2* variants.

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