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No Pain, No Gain of Function: Epilepsy-Associated Variants in SCN2A Defy Classification

Epilepsy-Associated *SCN2A* (NaV1.2) Variants Exhibit Diverse and Complex Functional Properties

Thompson CH, Potet F, Abramova TV, DeKeyser JM, Ghabra NF, Vanoye CG, Millichap JJ, George AL. *J Gen Physiol*. 2023;155(10):e202313375. doi:10.1085/jgp.202313375. PMID: 37578743; PMCID: PMC10424433

Pathogenic variants in neuronal voltage-gated sodium (NaV) channel genes including *SCN2A*, which encodes NaV1.2, are frequently discovered in neurodevelopmental disorders with and without epilepsy. *SCN2A* is also a high confidence risk gene for autism spectrum disorder (ASD) and nonsyndromic intellectual disability (ID). Previous work to determine the functional consequences of *SCN2A* variants yielded a paradigm in which predominantly gain-of-function (GoF) variants cause epilepsy whereas loss-of-function (LoF) variants are associated with ASD and ID. However, this framework is based on a limited number of functional studies conducted under heterogenous experimental conditions whereas most disease-associated *SCN2A* variants have not been functionally annotated. We determined the functional properties of more than 30 *SCN2A* variants using automated patch clamp recording to assess the analytical validity of this approach and to examine whether a binary classification of variant dysfunction is evident in a larger cohort studied under uniform conditions. We studied 28 disease-associated variants and 4 common population variants using two distinct alternatively spliced forms of NaV1.2 that were heterologously expressed in HEK293 T cells. Multiple biophysical parameters were assessed on 5,858 individual cells. We found that automated patch clamp recording provided a valid high throughput method to ascertain detailed functional properties of NaV1.2 variants with concordant findings for a subset of variants that were previously studied using manual patch clamp. Additionally, many epilepsy-associated variants in our study exhibited complex patterns of gain- and loss-of-function properties that are difficult to classify overall by a simple binary scheme. The higher throughput achievable with automated patch clamp enables study of a larger number of variants, greater standardization of recording conditions, freedom from operator bias, and enhanced experimental rigor valuable for accurate assessment of NaV channel variant dysfunction. Together, this approach will enhance our ability to discern relationships between variant channel dysfunction and neurodevelopmental disorders.

Commentary

Over the past 20 years, advances in molecular genetic testing has identified variants in dozens of ion channel genes associated with epilepsy, and thousands of individual variants have been characterized using patch clamp electrophysiology in heterologous experimental systems such as Xenopus oocytes or human embryonic kidney (HEK) cells. Results are typically classified using what is likely an oversimplistic dichotomy as "loss" of function (LoF; due to a decrease in impairment of channel activity) or "gain" of function (GoF; increased/enhanced channel activity) based on the effect of the identified variant on the recorded current. However, the link between diverse alterations in biophysical parameters, impact on neuronal and circuit function, and specific clinical phenotype, remains incompletely understood. Expanded access to genetic testing and the emerging promise of targeted therapies highlights the need for more scalable high-throughput methods of variant analysis.[1] The automated patch-clamp technique allows characterization of key biophysical properties of hundreds of cells and hence multiple variants simultaneously and hence represents an important advance in the epilepsy field.[2]

Thompson et al[3] aimed to clarify the key biophysical abnormalities in Na^+ currents (I_{Na}) due to epilepsy-linked variants in the gene *SCN2A* using such high-throughput electrophysiology. *SCN2A* encodes the voltage-gated Na^+ channel subunit $Na_v1.2$, known to be important for the generation and forward and backpropagation of action potentials including in excitatory glutamatergic neurons in the cerebral cortex[4] and is highly expressed during development. Variation in *SCN2A* is associated with a spectrum of neurodevelopmental impairment including autism spectrum disorder (ASD), self-limited neonatal epilepsy, and developmental and

epileptic encephalopathy (DEE). Prior electrophysiological characterization via manual patch clamp has shown that LoF variants correlate with ASD while GoF variants are associated with DEE.[5] However, this inference is made based on a limited number of studies and perhaps some oversimplification of the results.

Thompson et al[3] instead characterized 30+ *SCN2A* variants associated with severe early-onset epilepsy versus late-onset epilepsy as well as rare population variants of uncertain significance and channels containing wild-type $Na_v1.2$, in both neonatal and adult isoforms across a detailed range of biophysical properties using the high throughput automated patch-clamp technique. To do so, the authors first validated the method by characterizing previously reported variants recorded manually, to show similar or near-identical results. The study was facilitated by development of a highly efficient protocol for cell transfection as well as use of a dual 384-well automated electrophysiology system.

Voltage-gated Na^+ channels can be characterized along a set of biophysical properties. Thompson et al[3] measured current density; voltage-dependence of activation and voltage-dependence of steady-state inactivation, as well as window current; kinetics of activation and fast and slow inactivation; recovery from inactivation and persistent (non-inactivating) current. All variants were classified as LoF or GoF versus wild-type across each individual parameter. The main finding was that many epilepsy-associated variants exhibited currents that could not be easily classified as LoF or GoF. For example, of the 22 variants associated with early-onset epilepsy, 15 exhibited normal current density (measured as pA/pF normalized to cell size/capacitance), 6 showed decreased current density (LoF), while only 1 showed increased current density (GoF). Among the 6 late-onset variants, 2 had normal current density, 3 exhibited decreased current density, while 1 showed increased current density. One population variant showed decreased current density. For the voltage dependence of activation (the relationship between membrane voltage and activation of the channel), 3 early-onset variants showed a left/hyperpolarized shift in the voltage at half-maximal activation ($V_{1/2}$) associated with GoF, while 3 showed a right/depolarized shift associated with LoF. Similarly diverse findings occurred across a comprehensive range of analyses. Overall, few variants could be easily/consistently assigned to LoF or GoF across the spectrum of parameters, and these were typically variants that exhibited complete LoF with no current at all (eg, *SCN2A*-p.F978L). Most variants were associated with a mix of gain and loss of function properties with a differential impact of splice variant on this categorization in a subset of cases. This result is beautifully illustrated in a figure at the end of the paper that is well worth looking at and perhaps printing out for easy reference. Of the 22 early-onset variants, only 7 displayed consistent findings across parameters with regard to loss versus gain of function, 2 of which appeared to be LoF. No late-onset variants showed pure GoF, instead showing either LoF or mixed GoF/LoF.

What explains the apparent discrepancies between the findings of Thompson et al[3] and what is now almost established dogma with regards to the LoF/GoF dichotomy in *SCN2A*-related disorders? One possibility is a dissociation between the impact of a disease-associated variant on channel function in heterologous systems versus neurons, not further examined by Thompson et al[3] but which can be investigated using dynamic clamp, human-induced pluripotent stem cell technology, mouse models, and other approaches. It is also possible that such variants impact more than biophysical properties, perhaps altering subcellular localization or interaction with other associated proteins in the Na+ channel macromolecular complex (including potential Nav-Nav interactions).[6]

Limitations of the Thompson et al[3] work are otherwise minor. First, certain more subtle types of channel dysfunction might be missed using the automated approach, such as changes in resurgent current or induction of gating pore currents.[7] Second, the quality of the recordings obtained with manual patch-clamp—including low access resistance (important for obtaining optimal voltage control) and compensation of series resistance and cell capacitance—may not be similarly obtained via automated patch clamp. The authors note this and suggest that the slower inactivation kinetics observed via automated relative to manual patch clamp may be attributed to the inferior compensation of series resistance. This limitation was addressed via use of internal controls (wild-type and population variants) as such errors would be applied equally across variants.

In conclusion, variants in *SCN2A* are responsible for a spectrum of neurological disease including ASD and epilepsy of varying severity. Such conditions are triggered by the alterations of the voltage- and time-dependent biophysical properties of Nav1.2-containing Na^+ channels that can individually be categorized as LoF or GoF. This distinction is clinically important as epilepsy associated with GoF variants may be more responsive to anti-seizure medications with a mechanism of action of Na^+ channel blockade. While it remains the case that early-onset variants tend to show GoF features while late-onset variants show LoF, most epilepsy-associated variants show mixed LoF/GoF, and clinically defined categories align imperfectly with the impact of underlying variants on any single biophysical property. Hence, the authors elegantly demonstrate how the GoF versus LoF paradigm is an oversimplification as it applies to *SCN2A* epilepsy and in fact difficult to implement following detailed biophysical analysis of disease-associated variants. This finding was facilitated by use of the automated patch clamp technique capable of analyzing variants at scale, a method which is increasingly important given the vast number of variants in ion channel and neurotransmitter receptor genes identified in the clinic. This type of approach will most certainly continue to increase our understanding of the key drivers linking ion channel dysfunction and epilepsy, thereby enhancing variant classification and diagnostic and prognostic accuracy, and informing development of novel therapies. The study by Thompson et al[3] is a pure gain for the epilepsy field, and it's your loss if you don't take a look at it.

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Declaration of Conflicting Interests

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