Structure-Function Properties in Sodium Channelopathies: Considerations for Targeted Therapy

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Biological Concepts in Human Sodium Channel Epilepsies and Their Relevance in Clinical Practice

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Objective: Voltage-gated sodium channels (SCNs) share similar amino acid sequence, structure, and function. Genetic variants in the 4 human brain-expressed SCN genes SCN1A/2A/3A/8A have been associated with heterogeneous epilepsy phenotypes and neurodevelopmental disorders. To better understand the biology of seizure susceptibility in SCN-related epilepsies, our aim was to determine similarities and differences between SCN disorders, allowing us to develop a broader perspective on precision treatment than on an individual gene level alone. Methods: We analyzed genotype–phenotype correlations in large SCN-patient cohorts and applied variant constraint analysis to identify severe SCN disease. We examined temporal patterns of human SCN expression and correlated functional data from in vitro studies with clinical phenotypes across different SCN disorders. Results: Comparing 865 epilepsy patients (504 SCN1A, 140 SCN2A, 171 SCN8A, 4 SCN3A, 46 copy number variation [CNV] cases) and analysis of 114 functional studies allowed us to identify common patterns of presentation. All 4 epilepsy-associated SCN genes demonstrated significant constraint in both protein truncating and missense variation when compared to other SCN genes. We observed that age at seizure onset is related to SCN gene expression over time. Individuals with gain-of-function SCN2A/3A/8A missense variants or CNV duplications share similar characteristics, most frequently present with early onset epilepsy (<3 months), and demonstrate good response to SCN blockers (SCBs). Direct comparison of corresponding SCN variants across different SCN subtypes illustrates that the functional effects of variants in corresponding channel locations are similar; however, their clinical manifestation differs, depending on their role in different types of neurons in which they are expressed. Significance: Variant function and location within one channel can serve as a surrogate for variant effects across related SCNs. Taking a broader view on precision treatment suggests that in those patients with a suspected underlying genetic epilepsy presenting with neonatal or early onset seizures (<3 months), SCBs should be considered.

Commentary

The human genome contains 10 paralogous sodium channel (SCN) genes encoding corresponding α subunits that exhibit a high degree of amino acid conservation ranging from 50% to 85%.[1] The *SCN1A, SCN2A, SCN3A,* or *SCN8A* brain expression is developmentally and regionally regulated and pathogenic variations underwrite a spectrum of human epilepsies but also nonepilepsy phenotypes of migraine, ataxia, and neurodevelopmental disorders. The increasing access to and utilization of clinical genetic testing has facilitated discovery of candidate SCN causal variants that has outpaced our ability for functional validation and thus confident determination of a structure-function relationship. This impacts not only variant classification but also therapeutic decisions. Many antiseizure medications block SCNs and it is important to know whether one is able to use them to a patient's benefit (eg, gain-of-function [GOF] variation in *SCN8A*-related epilepsy) or detriment (eg, loss-of-function [LOF] variants in *SCN1A*-related Dravet syndrome [DS]). Are there shared clinical, cartographic, and biophysical SCN properties that may inform a clinician about the likely functional impact of a suspected pathogenic variant in any of its members?

Brunklaus et al reviewed cases harboring either protein-truncating variants (PTV) or missense pathogenic variants in *SCN1A* (504), *SCN2A* (140), *SCN8A* (171), and *SCN3A* (4) genes.[2] The cohort of 865 patients also included 46 individuals affected by large (> 15 megabases long) copy number variants encompassing *SCN1/2/3A* or *SCN8A* genes. Only variants with expected large effect and absent in public databases were included. In order to clarify the apparent preponderance of

SCN1A/2A/3A/8A toward the association with epilepsy, the authors analyzed permissiveness of the human SCNs to pathogenic variation. All 4 SCN channels (1A/2A/3A/8A) showed strong evolutionary constraints and intolerance to both PTVs and missense variants in contrast to the more permissible *SCN4A/9A/10A/11A* channels. This feature, combined with individual channel developmental, regional, and cell specific brain expression patterns provide some rationale for the reported disease age of onset and epilepsy characteristics. In the manuscript, phenotypic correlations of the SCN cohort mirrored some of the previously published data; 97% of cases with *SCN1A* variants were affected by DS and the rest by a genetic epilepsy with febrile seizures plus syndrome (GEFS+). Dravet syndrome cases also harbored almost all of the PTVs (97.6%) and the functionally tested variants showed either a LOF (75% of variants) or a mixed effect. Over 96% of the *SCN1A* variant carriers developed epilepsy at or after 3 months, likely an effect of a DS-dominant patient group since the age of epilepsy onset in GEFS+ is more variable.[3] In contrast to their prior report, the authors did not observe a difference in the qualitative variant properties and the age of epilepsy onset.[4] This result likely reflects an enrichment of detrimental variants with large effect and underrepresentation of cases with the milder phenotypes.

Compared with SCN1A variants, most carriers of the *SCN2A* pathogenic variants were affected by developmental and epileptic encephalopathy (DEE; 50%) while benign or unclassified epilepsies were present in 19% and 14%, respectively. Variant properties seemed to influence age of onset that followed a bimodal distribution. The GOF variants cosegregated with epilepsy occurring in the neonatal period while LOF variants were present in patients with neurodevelopmental disorders, including autism and a later age at epilepsy onset. The group of *SCN3A* deficient carriers was too small to make inferences about variant properties and resultant neurological phenotype. However, GOF mechanisms seemed to predispose to epilepsy. This finding was upheld by a recently published collection of 22 patients carrying *SCN3A* pathogenic variants. It showed that 76% of cases developed a treatment-resistant epilepsy and a severe or profound developmental delay in the first year of life (median onset, 2 weeks), 79% manifested malformations of cortical development (MCD), and 91% of pathogenic variants and all variants associated with MCD showed a GOF effects.[5] Among 171 patients affected by *SCN8A* pathogenic variation, 64% developed DEE while the rest had either benign or unclassified epilepsies (11%) or an intermediate epilepsy phenotype (25%). Patients with PTVs and LOF variants showed a later age of epilepsy onset as compared to those with GOF missense variants. This is an interesting finding since prior experimental and clinical research indicated that epilepsy phenotype was restricted to *SCN8A* GOF variants while LOF variation resulted in developmental delay, autism, and intellectual disability *without* seizures.[6] However, a case report of EE due to a deleterious heterozygous *SCN8A* LOF variant and experimental findings in the Scn8a-null mouse support findings by Brunklaus et al and suggest that *SCN8A* gene LOF is not an absolute exclusion for an appearance of epilepsy.[7] The detailed correlative analysis of structural and biophysical variant properties in selected SCN-related epilepsies prompted Brunklaus et al to further examine functional and clinical expression of variants shared among the channel genes. Out of 109 pathogenic, functionally assessed variants, there were 8 variant pairs with a shared missense change in 2 paralogous SCNs, SCN1A/2A (3 pairs), SCN1A/8A (4 pairs), SCN2A/8A (1 pair). Interestingly, the resultant functional consequences were similar between channels and variants occurring in the S5-6 pore loop region exhibited LOF effects regardless of the channel.

In all, the work by Brunklaus et al delivers an update and a synthesis of existing clinical, structural, and functional knowledge pertaining to epilepsy-related SCNs. It is a novel approach examining shared properties as well as unique and potentially discriminating features of individual channels and corresponding pathogenic variants. The authors have uncovered some common structural and functional patterns that seem to be reflected in clinical phenotypes thus advancing our broader understanding of the SCN gene family relevant to epilepsy and neurodevelopmental disorders. Results of the study tie together developmental channel biology with a disease and age-based expressivity and with a disease severity. The manuscript delivers an interesting probabilistic evaluation of SCN-based variants of unknown clinical significance as illustrated in the reported high likelihood of GOF variants in early onset epileptic encephalopathy due to either *SCN2A* (88%) or *SCN8A* (100%) mutations. This has implications for a timely initiation of appropriate therapy, such as an avoidance of SCN blocking agents in DS-related LOF variation in order to avoid further blockage of the remaining functional Nav1.1 channels preferentially expressed on the inhibitory neurons. On the contrary, SCN blockers may be a preferential treatment in SCN8A-related epilepsy due to GOF pathogenic variants where they seem to block the overactive Nav1.6 channel of the excitatory neurons.[8,9] Results also have a potential implications for a drug development and design of clinical trials which are challenging in monogenic epilepsies. It will be important to replicate these results as functional data and pathogenic variants in SCNs accrue overtime. This study focused on the presumed most severe variants which led to a preferential selection of patients at the extremes of the phenotypic spectrum. It will be interesting to see whether less strict variant constraints and epilepsy phenotypes linked to a different gene family will afford similar results. Thus, at least for now, we will need more, not less, variant specific functional data to improve future *in silico* predictions.

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