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Beyond Dravet Syndrome: Characterization of a Novel, More Severe SCN1A-Linked Epileptic Encephalopathy

Not All *SCN1A* Epileptic Encephalopathies Are Dravet Syndrome: Early Profound Thr226Met Phenotype

Sadleir LG, Mountier EI, Gill D, et al. *Neurology*. 2017;89(10):1035-1042.

Objective: To define a distinct *SCN1A* developmental and epileptic encephalopathy with early onset, profound impairment, and movement disorder. Methods: A case series of 9 children were identified with a profound developmental and epileptic encephalopathy and *SCN1A* mutation. Results: We identified 9 children 3 to 12 years of age; 7 were male. Seizure onset was at 6 to 12 weeks with hemiclonic seizures, bilateral tonic-clonic seizures, or spasms. All children had profound developmental impairment and were nonverbal and nonambulatory, and 7 of 9 required a gastrostomy. A hyperkinetic movement disorder occurred in all and was characterized by dystonia and choreoathetosis with prominent oral dyskinesia and onset from 2 to 20 months of age. Eight had a recurrent missense *SCN1A* mutation, p.Thr226Met. The remaining child had the missense mutation p.Pro1345Ser. The mutation arose de novo in 8 of 9; for the remaining case, the mother was negative and the father was unavailable. Conclusions: Here, we present a phenotype-genotype correlation for *SCN1A*. We describe a distinct *SCN1A* phenotype, early infantile *SCN1A* encephalopathy, which is readily distinguishable from the well-recognized entities of Dravet syndrome and genetic epilepsy with febrile seizures plus. This disorder has an earlier age at onset, profound developmental impairment, and a distinctive hyperkinetic movement disorder, setting it apart from Dravet syndrome. Remarkably, 8 of 9 children had the recurrent missense mutation p.Thr226Met.

***SCN1A* Gain of Function in Early Infantile Encephalopathy**

Berecki G, Bryson A, Terhag J, et al. *Ann Neurol*. 2019; 85:514-525.

Objective: To elucidate the biophysical basis underlying the distinct and severe clinical presentation in patients with the recurrent missense SCN1A variant, p.Thr226Met. Patients with this variant show a well-defined genotype–phenotype correlation and present with developmental and early infantile epileptic encephalopathy that is far more severe than typical SCN1A Dravet syndrome. Methods: Whole cell patch clamp and dynamic action potential clamp were used to study T226M Nav 1.1 channels expressed in mammalian cells. Computational modeling was used to explore the neuronal scale mechanisms that account for altered action potential firing. Results: T226M channels exhibited hyperpolarizing shifts of the activation and inactivation curves and enhanced fast inactivation. Dynamic action potential clamp hybrid simulation showed that model neurons containing T226M conductance displayed a left shift in rheobase relative to control. At current stimulation levels that produced repetitive action potential firing in control model neurons, depolarization block and cessation of action potential firing occurred in T226M model neurons. Fully computationally simulated neuron models recapitulated the findings from dynamic action potential clamp and showed that heterozygous T226M models were also more susceptible to depolarization block. Interpretation: From a biophysical perspective, the T226M mutation produces gain of function. Somewhat paradoxically, our data suggest that this gain of function would cause interneurons to more readily develop depolarization block. This "functional dominant negative" interaction would produce a more profound disinhibition than seen with haploinsufficiency that is typical of Dravet syndrome and could readily explain the more severe phenotype of patients with T226M mutation.

Commentary

Dravet syndrome (DS) is a severe and intractable developmental and epileptic encephalopathy (DEE) that typically presents in the first year of life with intractable seizures, cognitive, and motor impairments, developmental delays, and increased risk for sudden unexpected death in epilepsy.[1,2] Dravet syndrome

has been considered the most severe *SCN1A*-linked DEE, caused primarily by heterozygous, de novo, truncation, or missense variants that lead to *SCN1A* haploinsufficiency and consequent $Na_v1.1$ loss-of-function (LOF).[3,4] One of the most critical decisions for physicians and scientists working with patients with DS is whether the best course of action is to treat the genotype or the phenotype. There is a large diversity of *SCN1A* variants and patient symptomology associated with DS—making it difficult for scientists to extrapolate findings from animal model-based research to the entire *SCN1A*-DS patient population, as well as for physicians to create standardized and successful treatment regimens for *SCN1A*-DS patients. Recent studies have focused on a recurrent, missense *SCN1A* variant, Nav1.1-p.Thr226Met (T226M). This particular variant, which is associated with a far more profound clinical phenotype than typical DS, represents a new class of early infantile epileptic encephalopathy (EIEE) located even beyond DS on the classical severity spectrum of *SCN1A*-linked disorders.[5] Encouragingly, research regarding the aberrant mechanisms linked to this recurring variant might produce therapies that positively impact the lives of children presenting with this new class of EIEE.

In their 2017 *Neurology* article, Sadleir et al describe the clinical presentation of this more severe *SCN1A*-linked EE—denoted "early infantile *SCN1A* encephalopathy" by the authors. This group identified 8 unrelated patients with an identical, presumed de novo (one patient's father was of unknown genotype) missense mutation resulting from c.677C>T in *SCN1A* exon 5. A ninth unrelated patient, with the de novo *SCN1A* missense mutation p.Pro1345Ser (c.4033C>T), was also included in the study due to the similarities in symptomology to the T226M patients—thus illustrating that early infantile *SCN1A* encephalopathy can arise from more than one particular variant.

Early infantile *SCN1A* encephalopathy can be distinguished from traditional DS in several ways, which are outlined by Sadleir et al. As its name suggests, early infantile *SCN1A* encephalopathy has an earlier age of onset than DS, with seizures arising at an average of 9 weeks of age and a movement disorder phenotype also appearing at as early as 9 weeks of age. Phenotypically, early infantile *SCN1A* encephalopathy is associated with more profound developmental impairments than DS; all patients included in the study were nonambulatory, and the majority required feeding tubes. Additionally, early infantile *SCN1A* encephalopathy presents with hyperkinetic movement disorders—including choreoathetosis, dystonia, myoclonus, and perioral hyperkinesia—and epileptic spasms, neither of which are typically seen in patients with DS. While hyperkinetic movements are not characteristic of *SCN1A*-linked DS, similar movement disorders are associated with *SCN2A*- and *SCN8A*-linked EIEEs; this overlap in symptomology led Sadleir et al to speculate that the early infantile *SCN1A* encephalopathy, like *SCN2A*- and *SCN8A*-linked EIEEs, may be associated with a gain-of-function (GOF) variant—a theory that was addressed by Berecki et al in their 2019 *Annals of Neurology* manuscript.

Berecki et al used Chinese hamster ovary (CHO) cells transfected with $Na_v1.1$-p.T226M to assess the biophysical changes in sodium current associated with the variant. In CHO cells, the variant was observed to cause negative shifts in the voltage-dependence of activation and inactivation of $Na_v1.1$-expressed sodium current, meaning that the mutant channel opens and closes at more hyperpolarized voltages compared to wild-type $Na_v1.1$—interpretable in terms of both GOF and LOF. A potential confound is that CHO cells are known to express endogenous $Na_v1.2$, which might modulate the effects of the transfected T226M variant.[6] Physiologically, $Na_v1.1$ channels are expressed in high densities at the axon initial segment (AIS) of fast-spiking GABAergic parvalbumin-positive (PV+) interneurons, which are thought to be critical for maintaining homeostatic excitability in the brain; furthermore, these PV+ interneurons exhibit hypoexcitability in mouse models of *SCN1A*-DS.[7,8]

To determine what effect the hyperpolarizing shifts in sodium current observed with $Na_v1.1$-p.T226M expression might have on action potential (AP) generation, Berecki et al carried out "hybrid neuron" experiments, in which they performed dynamic AP clamp on transfected CHO cells. This technique enabled the authors to model effects of the *SCN1A* variant in the presence of other in silico sodium and potassium conductances, thus attempting to mimic conditions within the AIS of PV+ interneurons. Interestingly, Berecki et al observed that, compared to wild-type, the $Na_v1.1$-p.T226M CHO-hybrid neurons exhibited increased AP frequency at low stimulus intensities followed by a marked reduction in AP frequency at higher stimulus intensities, again showing GOF and LOF. In their final experiment, Berecki et al used a computational model of a PV+ cortical interneuron to examine the effects of in silico expression of homozygous and heterozygous $Na_v1.1$-p.T226M variants. Hyperexcitability at low stimuli was not observed in the computational model in either condition; however, depolarization block was observed in homozygous $Na_v1.1$-p.T226M model neurons at high intensity stimuli, consistent with a loss of excitability during periods of high-frequency firing. Importantly, depolarization block was also seen in the heterozygous model, where $Na_v1.1$-p.T226M variants constituted 50% of the sodium conductance—thus more accurately modeling the physiological expression of the variant in early infantile *SCN1A* encephalopathy patients. Overall, the authors interpret their results to suggest that $Na_v1.1$-p.T226M produces a mixed biophysical, GOF-LOF phenotype that enhances AP firing at low levels of synaptic input and collapses AP firing at higher levels of synaptic input.

Though these initial experiments serve as an excellent starting-point for examining the effects of this recurrent variant on $Na_v1.1$ functionality, we must be cautious when applying findings in CHO cells, even under dynamic clamp, to the intricate physiology of PV+ interneurons in vivo. In addition to the voltage-gated sodium channels $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$, voltage-gated potassium channels K_v1 and K_v3 are also critical to proper PV+ interneuron function.[8] K_v3 channels exhibit a high threshold of activation, fast activation, and fast deactivation, while K_v1 channels possess a lower activation threshold and much slower gating

kinetics.[8] This article does not extend the authors' previously published dynamic clamp model to include these characteristic PV+ interneuron channels, which may limit the relevance to fast spiking neuron physiology.[9] Ionic conductances that are present in neurons in vivo, but not included in the CHO-hybrid neuron model, likely account for the hyperexcitability observed in the CHO-hybrid cells but not in the computational model.

Another important consideration when determining how *SCN1A* variants affect neuronal excitability is that physiological sodium channel density in PV+ interneuron axons is 1.5 to 2.5 times higher than necessary for reliable AP generation and propagation.[10] Thus, defects in sodium channel function may be more critical for the speed of AP propagation than overt AP failure under physiological conditions. Recent work investigating in vivo excitability defects in an *SCN1A*-DS model demonstrated the importance of such considerations, as PV+ interneuron firing rates in vivo were not overtly altered even though PV+ interneuron excitability was observed to be impaired in acute brain slices at high current injections.[7]

Dynamic clamp can provide a more physiological context to studies performed in heterologous systems. However, the lack of morphological complexity, neuronal membrane properties, complexities of channel trafficking, signaling pathways regulating channel function, and interactions of the ion channel milieu present in intact neurons complicates interpretations of heterologous expression of $Na_v1.1$ GOF or LOF phenotypes on physiological PV+ interneuron firing rates. Future experiments in patient-derived iPSC neuron or CRISPR-Cas9-generated knock-in mouse models are also necessary to determine the effect of the $Na_v1.1$-p.T226M variant on channel trafficking to the plasma membrane and localization to the AIS, as it is plausible that the variant may reduce cell surface expression of $Na_v1.1$ within the cortex, thereby rendering it an overall LOF phenotype. Along these lines, previous work has shown that the β1 subunit of voltage-gated sodium channels can rescue conductance in protein folding-defective DS variants, suggesting that β1 subunits play a role in modulating the cell-surface expression and function of some $Na_v1.1$ variants.[11] However, the authors did not co-express β1 subunits in the CHO cells used in these experiments. Future work should also explore the effect of the variant on other $Na_v1.1$-expressing neuron populations affected in DS. Work in human iPSCs shows that pyramidal and bipolar neurons from DS patients exhibit increased sodium current density as well as spontaneous, repetitive firing at resting membrane potential.[12]

The dual-action, GOF and LOF, phenotype proposed by Berecki et al predicts a collapse in the activity of fast-spiking interneurons that are thought to mediate feedforward and feedback inhibition in the cortex. This could induce a severe excitation-inhibition imbalance in neuronal networks, which may explain the more severe phenotype observed in early infantile *SCN1A* encephalopathy patients. The authors suggest that $Na_v1.1$-p.T226M patients may benefit from antiepileptic drugs that reduce sodium current, which are relatively contraindicated for patients with traditional DS but serve as standard of care for patients with GOF *SCN8A* EIEEs. Sadleir et al describe one patient with comorbid $Na_v1.1$-p.T226M and a predicted LOF *SCN9A* variant who exhibited less severe developmental delays and lacked the distinctive movement disorder observed in the other $Na_v1.1$-p.T226M patients.[13] Perhaps a more selective reduction of $Na_v1.7$ activity would prove to be therapeutic in other early infantile *SCN1A* encephalopathy patients. A thorough understanding of the effects of the variants on neurons in vivo and the generation of human and animal models will be critical to determining the potential efficacy of these treatments.

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