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One Size Doesn't Fit All: Variant-Specific Effects in SCN8A Encephalopathy

Variant-Specific Changes in Persistent or Resurgent Sodium Current in SCN8A-Related Epilepsy Patient-Derived Neurons

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Missense variants in the SCN8A voltage-gated sodium channel gene are linked to early infantile epileptic encephalopathy type 13, also known as SCN8A-related epilepsy. These patients exhibit a wide spectrum of intractable seizure types, severe developmental delay, movement disorders, and elevated risk of sudden unexpected death in epilepsy. The mechanisms by which SCN8A variants lead to epilepsy are poorly understood, although heterologous expression systems and mouse models have demonstrated altered sodium current properties. To investigate these mechanisms using a patient-specific model, we generated induced pluripotent stem cells from 3 patients with missense variants in SCN8A: p.R1872>L (patient 1); p.V1592>L (patient 2); and p.N1759>S (patient 3). Using small-molecule differentiation into excitatory neurons, induced pluripotent stem cell-derived neurons from all 3 patients displayed altered sodium currents. Patients 1 and 2 had elevated persistent current, while patient 3 had increased resurgent current compared to controls. Neurons from all 3 patients displayed shorter axon initial segment lengths compared to controls. Further analyses focused on one of the patients with increased persistent sodium current (patient 1) and the patient with increased resurgent current (patient 3). Excitatory cortical neurons from both patients had prolonged action potential repolarization. Using doxycycline-inducible expression of the neuronal transcription factors neurogenin 1 and 2 to synchronize differentiation of induced excitatory cortical-like neurons, we investigated network activity and response to pharmacotherapies. Both small-molecule differentiated and induced patient neurons displayed similar abnormalities in action potential repolarization. Patient-induced neurons showed increased burstiness that was sensitive to phenytoin, currently a standard treatment for SCN8A-related epilepsy patients, or riluzole, an FDA-approved drug used in amyotrophic lateral sclerosis and known to block persistent and resurgent sodium currents, at pharmacologically relevant concentrations. Patch clamp recordings showed that riluzole suppressed spontaneous firing and increased the action potential firing threshold of patient-derived neurons to more depolarized potentials. Two of the patients in this study were prescribed riluzole off-label. Patient 1 had a 50% reduction in seizure frequency. Patient 3 experienced an immediate and dramatic seizure reduction with months of seizure freedom. An additional patient with a SCN8A variant in domain IV of Nav1.6 (p.V1757>I) had a dramatic reduction in seizure frequency for several months after starting riluzole treatment, but then seizures recurred. Our results indicate that patient-specific neurons are useful for modeling SCN8A-related epilepsy and demonstrate SCN8A variant-specific mechanisms. Moreover, these findings suggest that patient-specific neuronal disease modeling offers a useful platform for discovering precision epilepsy therapies.

Commentary

SCN8A epileptic encephalopathy is a severe epilepsy syndrome in which patients manifest an array of symptoms including refractory seizures, cognitive, and motor impairment and have a substantial risk of sudden unexpected death in epilepsy.[1] Although transgenic mouse models of *SCN8A* encephalopathy have offered critical insights into mechanisms of disease and aided in the ongoing search for novel treatment strategies, there are important limitations to these models. For one, capturing the clinical diversity seen in patients with *SCN8A* encephalopathy in mouse models would require the generation of many mutation-specific transgenic mice strains, a costly and time-consuming process. Further, not all experimental findings in mice translate well to human patients. These limitations have contributed to increasing interest in generating and investigating patient-specific models of disease through the use of induced pluripotent stem cell (iPSC) technology to produce functional human neurons in culture.

In a recent report,[2] Tidball and colleagues generated cell culture lines for 3 *SCN8A* encephalopathy and 2 nonepileptic

control patients and compared voltage-gated sodium channel properties and corresponding cellular and network physiology. Neurons produced from 2 of the *SCN8A* encephalopathy patients exhibited an increased persistent sodium current, a type of "leak" sodium current that is a common occurrence in many of the gain-of-function *SCN8A* mutations as a result of disrupted inactivation of the channel.[3-5] Surprisingly, neurons produced from the third patient exhibited no change in the persistent current, but did have an elevated resurgent sodium current, a type of sodium current that inactivates more slowly, contributing to interspike depolarization and neuronal burst firing.[3,4] In recordings of neuronal excitability, *SCN8A* patient-derived neurons displayed widening of the action potential (AP) waveform, and in some cases, early afterdepolarization AP spikelets. The authors then cultured 2 of their patient-derived neuronal lines on multielectrode arrays to examine network function. Each *SCN8A* encephalopathy patient-derived neuron condition displayed unique differences from control neurons. For example, patient 1-derived neurons showed reduced weighted mean firing rates, while patient 3 showed elevated rates relative to control neurons. However, both conditions showed an overall increase in AP burstiness measured as AP burst duration and as the percent of total spikes occurring in bursts, consistent with epileptiform-like activity. These findings reveal that pathogenic neurons expressing *SCN8A* variants can differ physiologically from not only control neurons but also other *SCN8A* variants in a Venn-diagram-like fashion: Some functional differences are unique to a particular variant, while others are held in common between multiple *SCN8A* variants, but all culminating toward an end point of abnormal neuronal excitability. Identification of both general and patient-specific features in *SCN8A* encephalopathy is critical to meeting the goal of providing mechanistically informed patient-specific treatment approaches.

The authors rescued the increase in persistent sodium current observed in one of the patient mutations (patient #2: V1592L) by deleting the pathogenic allele using CRISPR gene-editing techniques. Resulting electrophysiological recordings revealed an unexpected increase in the transient sodium current amplitude even though these neurons were hemizygous for *SCN8A* expression, as well as an expected reduction in the persistent current to control neuron levels. A crucial parameter not tested by the authors was the impact of deleting the pathogenic allele on neuronal excitability and epileptiform activity, especially considering the increase in transient sodium current detected.

Tidball and colleagues went on to demonstrate how these patient-specific physiological alterations might be utilized to inform patient-specific treatment approaches by comparing the antiseizure medication (ASM) phenytoin, and riluzole, an approved treatment for amyotrophic lateral sclerosis known to attenuate persistent and resurgent sodium currents,[6,7] but not typically prescribed to epilepsy patients. At clinically relevant concentrations, both ASMs reduced the aberrant network burstiness observed in *SCN8A* encephalopathy patient-derived neurons without significantly altering the control neuron network bursts. However, riluzole was more effective in reducing burst spikes and mean firing rates than phenytoin possibly due to its greater preference for inhibiting persistent and resurgent sodium currents, the 2 types of sodium channel current that were elevated in the *SCN8A* patient-derived neurons.

Considering riluzole's increased efficacy in the *in vitro* assays relative to phenytoin, the authors reasoned that riluzole might be more effective than phenytoin in treating seizures in patients whose iPSCs were used in the study. Tidball and colleagues explored this possibility, as patients 1 and 3 along with an additional *SCN8A* encephalopathy patient elected to begin riluzole treatment after becoming refractory to phenytoin and other ASMs. Patient 1 experienced a $\sim 50\%$ reduction in seizure frequency with riluzole treatment, while patient 3 experienced strong seizure reduction in response to riluzole as an add-on therapy to high-dose levetiracetam. The additional patient experienced an initial reduction in seizure frequency with riluzole, but unfortunately this was short lived with a return to pretreatment baseline within 4 months of treatment. Despite the encouraging initial response to riluzole, all patients elected to discontinue treatment due to the appearance of side effects (excessive sleepiness, urinary tract infections, etc) or loss of efficacy. Nevertheless, the fact that these patients did respond to riluzole is highly encouraging.

The results of this study are impactful for a number of reasons. They provide a degree of agreement between reports using transgenic mouse lines and human models of *SCN8A* encephalopathy. Similar to this study, neuronal recordings from transgenic mouse models of *SCN8A* encephalopathy have also identified elevations in persistent and resurgent sodium currents as well as aberrant AP waveforms and burstiness as potentially important manifestations of the disease. Furthermore, preferential inhibition of the persistent and/or resurgent sodium currents was able to correct aberrant AP waveforms, reduce seizure frequency, and extend survival in the mice.[3,4,8,9] Although riluzole ultimately did not succeed to convey long-term treatment for seizures in the 3 patients, its initially strong efficacy encourages ongoing efforts to develop preferential inhibitors of persistent and resurgent sodium currents to treat *SCN8A* patients. Yet, riluzole treatment was far from completely successful, and gaining a full appreciation of the mechanisms of why it failed will be informative regarding the potential therapeutic benefit of other preferential inhibitors of persistent and resurgent sodium currents. Of note, riluzole affects a number of channels and receptors besides sodium channels and these additional unwanted actions could possibly account for why riluzole's beneficial effects were short lasting and side effects were intolerable to patients. The development of sodium channel selective compounds with increased preference for inhibiting persistent and resurgent sodium channel currents may be the solution, providing both efficacy and tolerability. These congruent findings in both mouse and human models ultimately serves to increase the confidence in both systems as valid and complementary approaches to understand and treat *SCN8A* encephalopathy. Nonetheless, patient-specific cell culture models of *SCN8A* epilepsy have the advantage over

mouse models since they allow rapid investigation of mutation-specific physiological mechanisms of disease and provide a unique ASM screening platform that has the potential to identify which ASMs may provide the greatest protection against variant-specific seizure phenotypes.

SCN8A encephalopathy is spectral in its clinical presentation: Some patients have some language abilities, can walk independently, and can go weeks between seizures whereas others have daily seizures, are unable to speak, and are non-ambulatory.[10] Theoretically, the wide range of the disease is due in part to variant-specific effects on voltage-gated sodium channel biophysics and neuronal physiology. However, with few transgenic mouse models of *SCN8A* epilepsy currently available, gaining detailed variant-specific insight into disease mechanism has been strongly limited. Here, Tidball and colleagues make an important conceptual step in experimentally demonstrating that distinct *SCN8A* patient-derived variants have unique biophysical defects which ultimately give rise to unique physiological signatures which can be treated differently depending on the particular mechanism of sodium channel biophysical dysfunction. Future studies using variant-specific characterization and selective pharmacology will be critical, alongside ongoing validation approaches utilizing transgenic mouse models, to deliver on the hopes and promises of precision medicine to treat *SCN8A* encephalopathy.

Eric R. Wengert and Manoj K. Patel

ORCID iD

Manoj K. Patel https://orcid.org/0000-0003-3591-6716

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