Received: 29 January 2019 | Revised: 6 March 2019 | Accepted: 6 March 2019

DOI: 10.1111/epi.14703

SUPPLEMENT ARTICLE

Epilepsia®

SCN8A encephalopathy: Mechanisms and models

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Funding information
NIH R01 NS034509; Dravet Syndrome Foundation

Summary

De novo mutations of the neuronal sodium channel *SCN8A* have been identified in approximately 2% of individuals with epileptic encephalopathy. These missense mutations alter the biophysical properties of sodium channel Nav1.6 in ways that lead to neuronal hyperexcitability. We generated two mouse models carrying patient mutations N1768D and R1872W to examine the effects on neuronal function in vivo. The conditional R1872W mutation is activated by expression of CRE recombinase, permitting characterization of the effects of the mutation on different classes of neurons and at different points in postnatal development. Preclinical drug testing in these mouse models provides support for several new therapies for this devastating disorder. In contrast with the gain-of-function mutations in epilepsy, mutations of *SCN8A* that result in partial or complete loss of function are associated with intellectual disability and other disorders.

KEYWORDS

encephalopathy, mouse model, mutation, *SCN8A*, sodium channel

1 | INTRODUCTION

The sodium channel gene *SCN8A* was first identified by molecular analysis of spontaneous neurological mutants in the mouse. Positional cloning and genome sequencing of mouse *Scn8a* from mutant lines identified two protein truncation mutations, a splice site mutation and a missense mutation, that were responsible for movement disorders ranging from tremor, ataxia, and dystonia to hind limb paralysis and lethality.[1–3] These spontaneous mouse mutations resulted in partial or complete loss of function of the sodium channel Nav1.6 and exhibited recessive inheritance and the absence of spontaneous tonic/clonic seizures.

The first human epileptogenic *SCN8A* mutation was described in 2012.[4] Michael Hammer at the University of Arizona carried out complete genome sequencing of two parents, an affected child, and an unaffected sibling. A de novo A>G mutation was identified in the affected daughter, resulting in the *SCN8A* missense mutation p.Asn1768Asp (N1768D).[4] To assess the pathogenicity of this mutation, we introduced it into a Nav1.6 cDNA and carried out functional evaluation in transfected ND7/23 cells. We observed a large increase in persistent current resulting in elevated firing of transfected neurons.[4] The causal role of the *SCN8A* mutation was confirmed in a knockin mouse model that recapitulated epilepsy and sudden death.[5]

The important role of *SCN8A* in epileptic encephalopathy was quickly confirmed in additional patients. Today, nearly 300 individuals with *SCN8A* encephalopathy have been reported in publications or described in databases maintained by family groups (eg, www.SCN8A.net). Like the first case, the subsequently identified mutations arose de novo in the affected children and resulted in missense substitutions of evolutionarily conserved amino acid residues that alter channel function.[6] The patient mutations are concentrated in transmembrane segments of the Nav1.6 channel protein, the inactivation gate, and the cytoplasmic C-terminal domain (Figure 1).

Functional studies of approximately 20 patient mutations have been carried out by expression in transfected cells, and two mutations have been studied in neurons of knockin mice. None of the *SCN8A* encephalopathy mutations results in protein truncation or loss of channel function. Rather, these are missense mutations that alter the biophysical properties of the channel, such as altered voltage dependence of channel activation or inactivation, delayed channel inactivation, or increased resurgent

or persistent current. Most mutations change more than one parameter, making it difficult to assign relative "severity." The most common changes are a hyperpolarized shift in voltage dependence of activation, impaired channel inactivation, and elevated persistent current.[6] Each of these lead to elevated neuronal firing, consistent with in vivo seizures.

Approximately one-third of the patient mutations in *SCN8A* encephalopathy occur at recurrent positions, many of which are located in CpG dinucleotides that are sites of elevated mutation rate in the human genome.[7] Arginine residues such as 1617 and 1872 are the sites of recurrent mutations with dramatic effects on channel function. For example, loss of the positive charge at Arg1872 due to substitution of any of three neutral amino acids results in destabilization of the inactive state of the channel, resulting in elevated neuronal excitability.[8]

We generated two in vivo mouse models expressing patient mutations, the original mutation Asn1768Asp (N1768D)[5] and the recurrent mutation Arg1872Trp (R1872W).[9] Both mice recapitulate the basic features of encephalopathy, with spontaneous seizures and premature lethality. These mice enabled us to probe the effect of the mutations on firing patterns of different classes of mutations, secondary effects on expression of other genes, effects of *Scn8a* on cardiac function, and responses to new drug therapies. The effects of strain background on seizure severity have also been examined. The features of the two mouse models are compared in Table 1.

Key Points

- De novo gain-of-function mutations in *SCN8A* encephalopathy alter the biophysical properties of sodium channel $Na_v1.6$ and result in neuronal hyperexcitability
- Functional effects of pathogenic mutations include impaired channel inactivation, premature channel opening, and elevated persistent current
- Mouse lines expressing the *SCN8A* patient mutations p.Asn1768Asp and p.Arg1872Trp recapitulate the human disorder with spontaneous seizures and premature lethality
- Conditional expression of p.Arg1872Trp in excitatory forebrain neurons causes seizures and premature lethality, but expression in inhibitory neurons is nonpathogenic
- Loss-of-function variants of *SCN8A* are less severe clinically, resulting in isolated intellectual disability, myoclonus, or mild seizure disorders

2 | N1768D MOUSE MODEL OF *SCN8A* ENCEPHALOPATHY

We used targeting with a transcription activator–like effector nuclease (TALEN) endonuclease to knockin the patient mutation *SCN8A*-N1768D into exon 26 of the mouse *Scn8a* gene.[10] Spontaneous seizures were observed in heterozygous adults beginning at 2 months of age. Mice are highly sensitive to gain-of-function mutations of *Scn8a*, and the time from first observed seizure to death varies from 1 to 4 weeks depending on gene dosage (Figure 2).

Recordings from hippocampal neurons revealed spontaneous firing in subsets of CA1 and CA3 neurons (Figure 3).[11] Elevated activity was also observed in neurons from the medial entorhinal cortex that innervate the hippocampus.[12] Although Nav1.6 is expressed at a much lower level in cardiac myocytes, this expression is sufficient to generate cardiac arrythmia in the N1768D mice.[13,14]

Secondary effects on gene expression were investigated by sequence analysis of transcripts from three brain regions, forebrain, cerebellum, and hind brain.[15] Prior to seizure onset, we did not detect differences in transcript abundance between wild-type and mutant mice. Within 24 hours of the first observed seizure, there was elevation of transcripts associated with gliosis such as glial fibrillary acidic protein as well as neuropeptides previously associated with seizure susceptibility such as galanin and neuropeptide Y.[15] We also observed

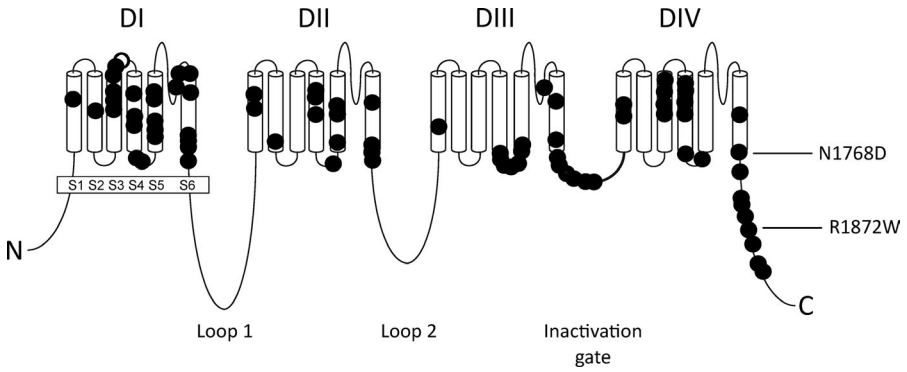

FIGURE 1 *SCN8A* mutations associated with epileptic encephalopathy. The protein structure of the four-domain sodium channel $Na_v1.6$ is represented; each symbol represents a patient mutation. The positions of the two mutations studied in mouse models are indicated

TABLE 1 Features of two mouse models of *SCN8A* encephalopathy

	N1768D/+	R1872W/+
Patients	Ambulatory, verbal, SUDEP at 15 y	Nonambulatory, nonverbal
Channel biphysics	Impaired inactivation	More severely impaired inactivation
Mouse model	Knockin to *Scn8a* of single bp substitution	Knockin to *Scn8a* of floxed alternative exon
Onset in mouse	2-4 months of age	2 wk of age
Duration between onset and death	1-4 wk	<1 min to 24 h
Penetrance	50%-75%	100%

Note. The N1768D mouse was generated by transcription activator–like effector nuclease knockin of the first described patient mutation.[7] The R1872W mouse carries a conditional allele of a recurrent human mutation that leads to lethality in early childhood.[11] Onset is the age at first observed seizure. Duration is the interval between first observed seizure and death.

Abbreviation: SUDEP, sudden unexpected death in epilepsy.

a significant elevation of neuropeptide W. These changes did not occur in mutant mice that did not develop seizures. We did not observe compensatory up- or downregulation of transcripts encoding other voltage-gated sodium channels, including *Scn1a*, *Scn2a*, and *Scn3a*. The data suggest that there is not extensive remodeling of the brain via altered transcription prior to seizure onset.

3 | R1872W MOUSE MODEL OF *SCN8A* ENCEPHALOPATHY

We used the TALEN reagent to target exon 26 of mouse Scn8a to generate the R1872W mutant mouse line, but in this case the mutation is dependent on expression of CRE recombinase.[9] The targeting vector containing two copies of exon 26 is shown in Figure 4A. In the absence of CRE, the mice express a full diploid dose of Nav1.6 that contains wild-type exon 26a. Expression of CRE recombinase results in deletion of exon 26a and initiates heterozygous expression of exon 26b containing the R1872W mutation (Figure 4B).

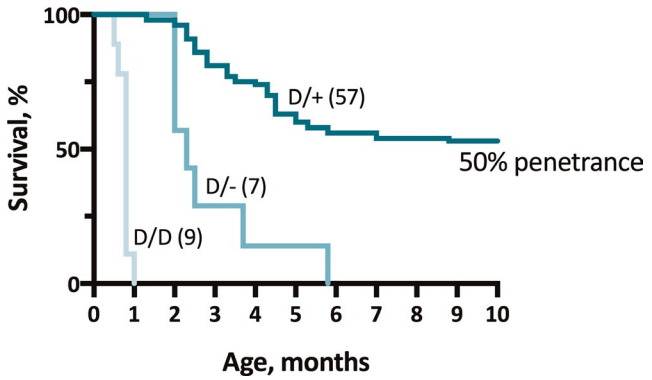

FIGURE 2 In vivo mouse model of the epileptic encephalopathy mutation *SCN8A*-N1768D. Knockin of the patient mutation[6] results in seizures and death in 50% of heterozygous D/+ animals. Combination with a null allele (D/−) or homozygosity of the mutant allele (D/D) exacerbates the phenotype, demonstrating partial protection by the wild-type allele in heterozygous animals[9]

We observed dramatic results after global activation of the mutant channel early in development by EIIA-CRE, with sudden onset of seizures at 14 days of age and death within 24 hours after the first observed seizures (Figure 5). Activation of R1872W specifically in excitatory neurons of the forebrain was sufficient to initiate seizures and death (Figure 5). Activation of R1872W exclusively in inhibitory neurons using GAD2 CRE or DLX5a CRE was not pathogenic (Figure 5). Expression of the mutant channel in the adult by tamoxifen treatment of mice expressing the CAG-CRE-ER also resulted in 100% penetrance of spontaneous seizures and sudden death, demonstrating the continued sensitivity of adult neurons and an apparent cell-autonomous effect of the mutant channel in the mature central nervous system (CNS).[9]

These results are consistent with the major role of Nav1.6 in the initiation of action potentials at the axon initial segment of excitatory neurons, and the elevated excitability of neurons expressing the mutant channel. This is in striking contrast with the Dravet syndrome mouse, in which inhibitory neurons appear to be the primary target responsible for seizure initiation due to reduced activity of *Scn1a*.[16]

Survival is approximately 4 weeks longer in mice expressing the Emx1-CRE compared with the ubiquitously expressed EIIA-CRE. This may be explained by the expression of the EIIA-CRE in many cells that do not express Emx1-CRE, including neurons in the brainstem that regulate respiration and cardiac function, and cardiac myocytes that express $Na_v1.6$ at a low level.[13,14]

4 | PRECLINICAL DRUG TESTING IN *SCN8A* MUTANT MICE

Existing antiepileptic drugs are inadequate for seizure control in the majority of children with epileptic

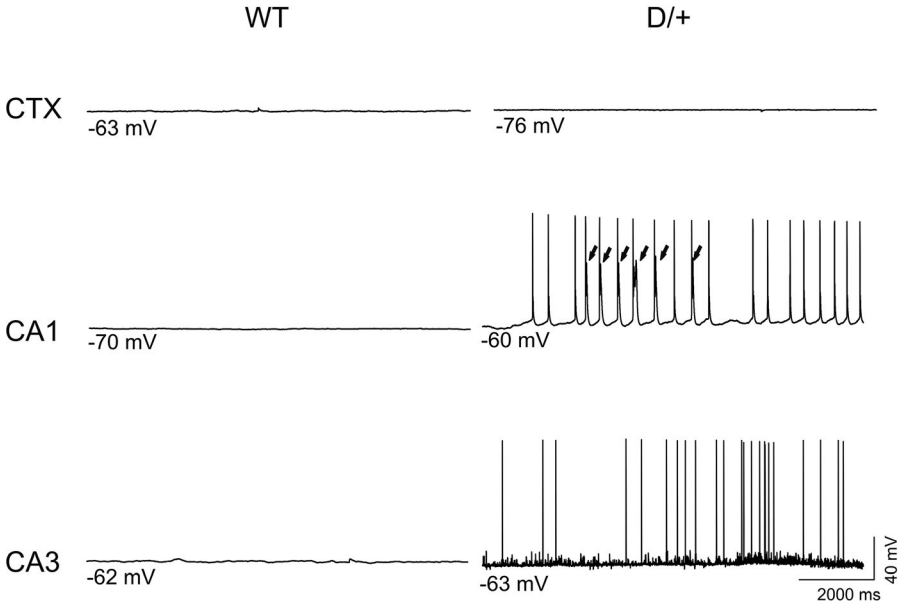

FIGURE 3 Spontaneous firing of hippocampal neurons in brain slices from N1768D mice.[13] CTX, cortex; WT, wild type

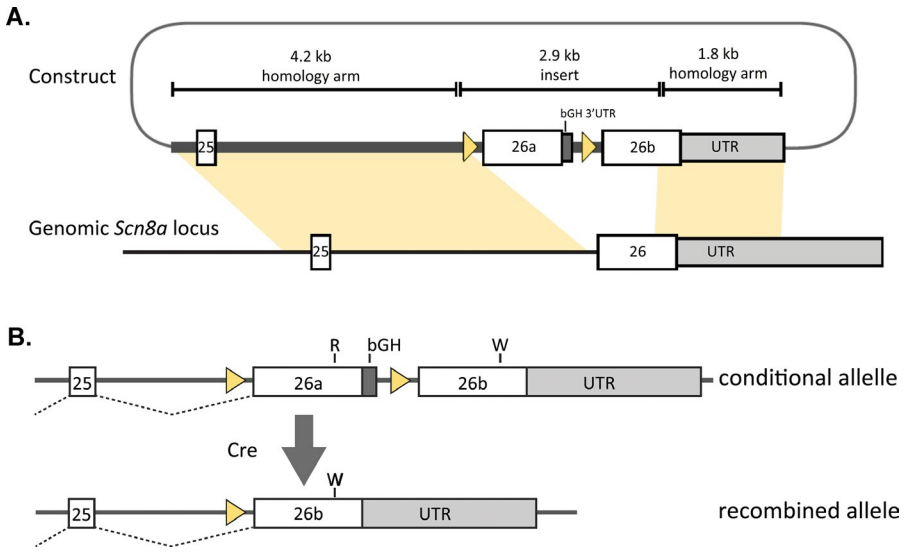

FIGURE 4 The conditional mouse mutation *SCN8A*-R1872W. A, Two copies of the final coding exon 26 of *Scn8a* were inserted into the endogenous gene. B, In the absence of CRE recombinase, the wild-type exon 26a is expressed. Deletion of exon 26a by CRE initiates expression of the mutant exon 26b.[11] UTR, untranslated region; bGH, bovine growth hormone 3'UTR; R, arginine 1872; W, tryptophan 1872

encephalopathy. The availability of mouse mutants expressing patient mutations has made it possible to evaluate new therapeutic approaches. The polycyclic sodium channel modulator GS967/Prax330 acts to reduce persistent current.[17] $Na_v1.6$ contributes to persistent current in CNS neurons, as demonstrated by the reduced persistent current in hippocampal CA1 neurons,[18] cerebellar granule cells,[19] and trigeminal mesencephalic neurons[20] from *Scn8a* null mice. Administration of GS967/Prax330 in the food to *Scn8a* N1768D/+ mice rescues lethality and reduces the incidence of spontaneous seizures by >50%[21] (Figure 6). The compound is also effective in mice with mutations of *Scn1a* and *Scn2a,*[17,22] possibly an indirect consequence of its direct effect on wildtype Nav1.6. Phase 1 clinical trials of this compound are in progress.

A series of compounds with preferential effects on $Na_v1.6$ developed by Xenon Pharmaceuticals are in phase 1 clinical trials. Antisense oligonucleotides that reduce transcript abundance are a potential third route to protection against gain-of-function mutations in *SCN8A*[23]; this approach may be applicable to *SCN8A* encephalopathy and is testable in the mutant mice.

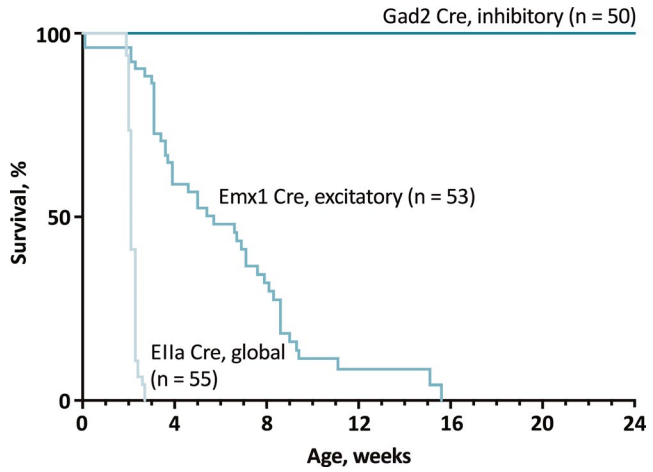

FIGURE 5 Predominant role of excitatory neurons in *SCN8A* encephalopathy in the conditional R1872W mouse. Global preimplantation activation of the mutant allele by EIIA-CRE results in seizure onset at 2 weeks of age followed rapidly by lethality. Activation of the mutant allele in excitatory forebrain neurons by Emx1 CRE also results in 100% penetrance of seizures and death, approximately 4 weeks later than the EIIA-CRE. In contrast, activation by the GAD2 CRE in inhibitory neurons had no detectable pathogenicity[11]

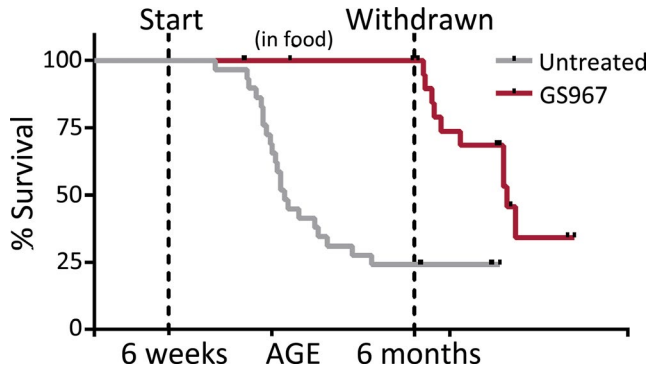

FIGURE 6 Rescue of *Scn8a*-N1768D mice by GS967/Prax330. The polycyclic drug GS967/Prax330[18] was incorporated into pelleted food and fed to the heterozygous mutant mice from the age of 1.5 months (before seizure onset) to 6 months. The mice were protected against seizures and neurotoxicity until removal of the drug.[19] $P < .0001$ (log-rank). Disease penetrance was higher than in Figure 2; this represents experimental variability within the same mouse line

5 | SUMMARY OF MOUSE MODELS

Investigation of two mouse models has clearly demonstrated that gain-of-function mutations of *SCN8A* are sufficient to generate severe seizures and a lethal phenotype. Neurons from mutant mice exhibit hyperactivity, with greater severity and earlier lethality in the R1872W mutant. These mice have been used for preclinical evaluation of new drugs, accelerating progress to clinical trials. Analysis of the conditional R1872W mutant mouse demonstrated the specific role of excitatory neurons in *SCN8A* encephalopathy, in contrast to the key role of inhibitory neurons in Dravet syndrome. The susceptibility of the adult nervous system to activation of the R1872W allele observed in the conditional mouse model indicates that life-long treatment will be required to protect against the effects of gain-of-function *SCN8A* alleles in the CNS.

6 | EXPANDING SPECTRUM OF *SCN8A* DISORDERS

The addition of *SCN8A* into gene testing panels and the increase in exome sequencing is resulting in the association of *SCN8A* with a broader spectrum of clinical conditions,[24–27] and this growth is likely to continue. Protein truncation mutations and missense mutations resulting in complete loss of channel activity have been identified in individuals with intellectual disability unaccompanied by seizures.[26,28] Partial loss of channel activity was identified in a family with the *SCN8A* mutation that segregated with isolated myoclonus.[27] We anticipate the future discovery of many more mutations in patients with movement disorders like those seen in the spontaneous mouse *Scn8a* mutants, such as ataxia, dystonia, and essential tremor.[29]

ACKNOWLEDGMENTS

Our work on *SCN8A* has been supported by NIH R01 NS034509 and by the Dravet Syndrome Foundation, the Wishes for Elliott Foundation, and the Cute Syndrome Foundation. I thank Dr Jacy Wagnon for helpful discussions.

DISCLOSURE

The author has no conflict of interest to disclose. I confirm that I have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

REFERENCES

1. Burgess DL, Kohrman DC, Galt J, et al. Mutation of a new sodium channel gene, SCN8A, in the mouse mutant 'motor endplate disease'. Nat Genet. 1995;10(4):461–5.
2. Kohrman DC, Harris JB, Meisler MH. Mutation detection in the med and medJ alleles of the sodium channel SCN8A. Unusual splicing due to a minor class AT-AC intron. J Biol Chem. 1996;271:17576–81.
3. Kohrman DC, Smith MR, Goldin AL, Harris J, Meisler MH. A missense mutation in the sodium channel Scn8a is responsible for cerebellar ataxia in the mouse mutant jolting. J Neurosci. 1996;16:5993–9.

4. Veeramah KR, O'Brien JE, Meisler MH, et al. De novo pathogenic SCN8A mutation identified by whole-genome sequencing of a family quartet affected by infantile epileptic encephalopathy and SUDEP. Am J Hum Genet. 2012;90:502–10.
5. Wagnon JL, Korn MJ, Parent R, et al. Convulsive seizures and SUDEP in a mouse model of SCN8A epileptic encephalopathy. Hum Mol Genet. 2015;24:506–15.
6. Meisler MH, Helman G, Hammer MF, et al. SCN8A encephalopathy: research progress and prospects. Epilepsia. 2016;57:1027–35.
7. Wagnon JL, Meisler MH. Recurrent and non-recurrent mutations of SCN8A in epileptic encephalopathy. Front Neurol. 2015;6:104.
8. Wagnon JL, Barker BS, Hounshell JA, et al. Pathogenic mechanism of recurrent mutations of SCN8A in epileptic encephalopathy. Ann Clin Transl Neurol. 2016;3:114–23.
9. Bunton-Stasyshyn RKA, Wagnon JL, Wengert ER, et al. Prominent role of forebrain excitatory neurons in SCN8A encephalopathy. Brain. 2019;142(2):362–75.
10. Jones JM, Meisler MH. Modeling human epilepsy by TALEN targeting of mouse sodium channel SCN8A. Genesis. 2014;52:141–8.
11. Lopez-Santiago LF, Yuan Y, Wagnon JL, et al. Neuronal hyperexcitability in a mouse model of SCN8A encephalopathy. Proc Natl Acad Sci U S A. 2017;114:2383–8.
12. Ottolini M, Barker BS, Gaykema RP, Meisler MH, Patel MK. Aberrant sodium channel currents and hyperexcitability of medial entorhinal cortex neurons in a mouse model of SCN8A encephalopathy. J Neurosci. 2017;37:7643–55.
13. Noujaim SF, Kaur K, Milstein M, et al. A null mutation of the neuronal sodium channel NaV1.6 disrupts action potential propagation and excitation-contraction coupling in the mouse heart. FASEB J. 2012;26:63–72.
14. Frasier CR, Wagnon JL, Bao Y, et al. Cardiac arrhythmia in a mouse model of SCN8A epileptic encephalopathy. Proc Natl Acad Sci U S A. 2016;113:12838–43.
15. Sprissler RS, Wagnon JL, Bunton-Stasyshyn RK, Meisler MH, Hammer MF. Altered gene expression profile in a mouse model of SCN8A epilepsy reveals reactive astrocytosis in response to seizures. Exp Neurol. 2016;288:134–41.
16. Yu FH, Mantegazza M, Westenbroek RE, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat Neurosci. 2006;9:1142–9.
17. Anderson LL, Thompson CH, Hawkins NA, et al. Antiepileptic activity of preferential inhibitors of persistent sodium current. Epilepsia. 2014;55:1274–83.
18. Royeck M, Horstmann MT, Remy S, Reitze M, Yaari Y, Beck H. Role of axonal NaV1.6 sodium channels in action potential initiation of CA1 pyramidal neurons. J Neurophysiol. 2008;100:2361–80.
19. Osorio N, Cathala L, Meisler MH, Crest M, Magistretti J, Delmas P. Persistent Nav1.6 current at axon initial segments tunes spike timing of cerebellar granule cells. J Physiol. 2010;588(Pt 4):651–70.
20. Enomoto A, Han JM, Hsiao CF, Chandler SH. Sodium currents in mesencephalic trigeminal neurons from Nav1.6 null mice. J Neurophysiol. 2007;98:710–9.
21. Baker EM, Thompson CH, Hawkins NA, et al. The novel sodium channel modulator GS-458967 (GS967) is an effective treatment in a mouse model of SCN8A encephalopathy. Epilepsia. 2018;59:1166–76.
22. Anderson LL, Hawkins NA, Thompson CH, Kearney JA, George AL. Unexpected efficacy of a novel sodium channel modulator in Dravet syndrome. Sci Rep. 2017;7:1682.
23. Roovers J, De Jonghe P, Weckhuysen S. The therapeutic potential of RNA regulation in neurological disorders. Expert Opin Ther Targets. 2018;22:1017–28.
24. Larsen J, Carvill GL, Gardella E, et al. The phenotypic spectrum of SCN8A encephalopathy. Neurology. 2015;84:480–9.
25. Gardella E, Becker F, Moller RS, et al. Benign infantile seizures and paroxysmal dyskinesia caused by an SCN8A mutation. Ann Neurol. 2016;79:428–36.
26. Wagnon JL, Barker BS, Ottolini M, et al. Loss-of-function mutations of SCN8A in intellectual disability without seizures. Neurol Genet. 2017;3:e170.
27. Wagnon JL, Mencacci NE, Barker BS, et al. Partial loss-of-function of sodium channel SCN8A in familial isolated myoclonus. Hum Mutat. 2018;39:965–9.
28. Trudeau MM, Dalton JC, Day JW, Ranum LP, Meisler MH. Heterozygosity for a protein truncation mutation of sodium channel SCN8A in a patient with cerebellar atrophy, ataxia and mental retardation. J Med Genet. 2006;43:527–30.
29. Sharkey LM, Jones JM, Hedera P, Meisler MH. Evaluation of SCN8A as a candidate gene for autosomal dominant essential tremor. Parkinsonism Relat Disord. 2009;15:321–3.

How to cite this article: Meisler M. *SCN8A* encephalopathy: Mechanisms and models. *Epilepsia*. 2019;60(S3):S86–S91. https://doi.org/10.1111/epi.14703