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SCN8A Encephalopathy: Research Progress and Prospects

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Summary: On April 21st, 2015, the first *SCN8A* Encephalopathy Research Group convened in Washington, DC to assess current research into clinical and pathogenic features of the disorder and prepare an agenda for future research collaborations. The group was comprised of clinical and basic scientists and representatives of patient advocacy groups.

SCN8A encephalopathy is a rare disorder caused by *de novo* missense mutations of the sodium channel gene *SCN8A*, which encodes the neuronal sodium channel $Na_v1.6$. Since the initial description in 2012, approximately 140 affected individuals have been reported in publications or by *SCN8A* family groups. As a result, an understanding of the severe impact of *SCN8A* mutations is beginning to emerge.

Defining a genetic epilepsy syndrome goes beyond identification of molecular etiology. Topics discussed at this meeting included (1) comparison between mutations of *SCN8A* and the *SCN1A* mutations in Dravet Syndrome, (2) biophysical properties of the $Na_v1.6$ channel, (3) electrophysiological effects of patient mutations on channel

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properties, (4) cell and animal models of *SCN8A* encephalopathy, (5) drug screening strategies, (6) the phenotypic spectrum of *SCN8A* encephalopathy, and (7) efforts to develop a bioregistry. A panel discussion of gaps in bioregistry, biobanking, and clinical outcomes data was followed by a planning session for improved integration of clinical and basic science research.

Although *SCN8A* encephalopathy was identified only recently, there has been rapid progress in functional analysis and phenotypic classification. The focus is now shifting from identification of the underlying molecular cause to the development of strategies for drug screening and prioritized patient care.

Key words: encephalopathy, bioregistry, $Na_v1.6$, sodium channel, mutation, drug screening, SCN8A.

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Introduction

Epilepsy is a common pediatric neurological disorder, affecting up to 12 patients per 1,000.[1] Pharmacoresistant epilepsies make up 30% of cases and include epileptic encephalopathies (EEs). These severe disorders present in infancy or childhood and are characterized by multiple seizure types and significant developmental slowing and regression.[2] The frequent epileptic activity in EE is thought to contribute to cognitive and behavioral impairment. *SCN8A* encephalopathy is a newly defined EE caused by de novo mutations of the gene *SCN8A* encoding the sodium channel $Na_v1.6$ (OMIM #614558). Most cases result from *de novo* mutations[3] with the exception of 2 cases of inheritance from a mosaic parent [4; 5] The disorder typically presents with developmental epileptic encephalopathy within the first two years of life.

SCN8A is 1 of 9 human genes encoding voltage-gated sodium channel α subunits. Mutations of the related genes *SCN1A* and *SCN2A* are responsible for the EEs Dravet Syndrome (OMIM #606208) and *SCN2A* encephalopathy (OMIM #613721). *SCN1A*, *SCN2A,* and *SCN3A* are also implicated in a range of milder, self-limited neonatal and infantile epilepsy syndromes.[6-11] Targeted and genome-wide next generation sequencing have greatly increased the number of individuals identified with *SCN8A* encephalopathy, allowing researchers to prioritize functional studies and develop an understanding of the phenotypic spectrum.[12-14]

Mutations of *SCN1A* in patients with inherited epilepsy and the sporadic Dravet Syndrome were first identified in 2000 and 2001.[11; 15] Since then, a substantial body of knowledge regarding prognosis, comorbidities, optimal care, and quality of life has become available. In contrast, *SCN8A* encephalopathy was first identified in 2012, and an understanding of the severe impact of *SCN8A* mutations is just beginning to emerge.[16] Awareness of this need, fueled by devoted, caring, and highly informed families, led to the first *SCN8A* research and family advocacy group meeting in Washington, DC on April 21, 2015. The goal of the meeting was to review current knowledge and identify future needs for patient care groups and clinical investigators. Herein we discuss these efforts and future steps for the *SCN8A* community in

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advancing towards therapeutic trials and improved outcomes.

Clinical Aspects: Phenotype of SCN8A encephalopathy

The frequency of *SCN8A* mutations in patients with EE was measured in four recent studies that in combination identified 13 cases in 1157 EE patients.[4; 13; 17; 18] *SCN8A* mutations thus appear to account for a little more than 1% of EE. More than 140 individuals with EE known to have *SCN8A* mutations, including 50 published and 90 unpublished cases (SCN8A Family Group, personal communication, April 2015).[12; 13; 16; 18-28] The location within the $Na_v1.6$ channel protein of 31 published *SCN8A* mutations from 50 patients is shown in **Figure 1**. The number is rapidly growing with the inclusion of *SCN8A* in clinical epilepsy gene panels and the expanded use of whole exome sequencing for diagnostic evaluation of patients with genetic epilepsy syndromes.[3; 13; 29]

Within *SCN8A* encephalopathy, individuals have been diagnosed with syndromes including unclassified EE, Early Infantile EE, and Dravet-like presentation."[12; 13; 16; 18-28] The mean age of seizure onset for *SCN8A* encephalopathy is 4-5 months, with a range from the first day of life to 18 months, and *in utero* seizures may be part of the clinical spectrum.[12; 16; 19; 24-26] Tonic-clonic seizures are often seen at onset, and these are usually not triggered by fever (25 individuals reported).[12; 13; 16; 18-28] Most of the 50 patients in published series have multiple seizures types including tonic (21 individuals), absence seizures (10 individuals), myoclonic (10 individuals), focal (6 individuals), clonic (6 individuals), and epileptic spasms (6 individuals).[12; 13; 16; 18-28] In addition, 11 of the 50 individuals were reported to have convulsive or non-convulsive status epilepticus.[12; 13; 16; 18-28] EEG features include diffuse moderate to severe background slowing with focal or multifocal epileptiform abnormalities.[13] MRI brain studies are typically normal with a few reports of progressive cerebral atrophy.[13]

The majority of affected individuals have pharmacoresistant seizures and a mixed response to anti-epileptic drugs (AEDs).[13] Several individuals have had a positive response to sodium channel blocking drugs such as valproic acid, phenytoin, carbamazepine and oxcarbazepine.[13; 20; 30] Families have reported both positive and

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negative responses to some of the more widely used AEDs.

Although development of an infant with *SCN8A* encephalopathy may be delayed from birth, in many cases development is normal prior to seizure onset. After seizure onset, among 50 published cases[12; 13; 16; 18-28], development slowed in 29 individuals and regressed in 10 individuals. Intellectual disability was common, and ranged from mild (n=2) to moderate (n=15) or severe (n=23). Motor features included hypotonia (n=22), ataxia (n=13), dystonia (n=6), hyperreflexia (n=4), and choreoathetosis (n=4).[12; 13; 16; 18-28] Eleven individuals had no speech. In some cases, immobility leading to wheelchair dependence developed during disease progression (n=10). Sudden unexpected death in epilepsy (SUDEP) was reported in 5 individuals. Most of the published patients are in the first two decades of life.

Comparison of SCN8A encephalopathy with Dravet Syndrome (SCN1A) and SCN2A encephalopathy

Three recognized sodium channel gene EEs are caused by mutations in *SCN8A*, *SCN1A* (Dravet Syndrome), *SCN2A* and *SCN8A*.[11; 16; 31] The mean age of onset is similar in *SCN8A* encephalopathy and Dravet syndrome, but the variation of age of onset is broader in *SCN8A* encephalopathy (neonatal period to 18 months of age) compared with Dravet Syndrome (neonatal period to 12 months of age. Onset during the first week of life is frequently observed for *SCN2A* encephalopathy.[31]

While febrile seizures are the hallmark at presentation in the majority of infants with *SCN1A* mutations in Dravet syndrome, they are rare in *SCN8A* and *SCN2A* encephalopathies. Epileptic spasms are not a feature of Dravet syndrome but can occur in *SCN8A* and *SCN2A* encephalopathies. There is an important difference in response to treatment with sodium channel blockers. Patients with Dravet syndrome are well known to respond adversely to carbamazepine and phenytoin, for example, while these and other sodium channel blockers may be efficacious in *SCN8A* and *SCN2A* encephalopathies. EEG recordings in Dravet Syndrome exhibit generalized spike wave activity as well as multifocal discharges.[7] In contrast, *SCN8A* and *SCN2A* encephalopathy have predominantly focal and multifocal epileptiform discharges, and

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voltage attenuation during epileptic spasms.[31]

All three sodium channel epileptic encephalopathies have a high mortality rate of approximately 10 to 15% by age 20, based on published reports. The rate of SUDEP in the sodium channel encephalopathies seems to be higher than in other disorders such as *PCDH19* encephalopathy.[7; 31]

As discussed below, most *SCN8A* mutations in EE are missense mutations that cause increased $Na_v1.6$ channel activity. The same is true for *SCN2A* mutations in EE. In contrast, most *SCN1A* mutations in Dravet syndrome result in reduced $Na_v1.1$ activity. This fundamental difference in mechanism is likely to explain why sodium channel blockers can be effective for some patients with *SCN8A* encephalopathy, who have an excess of *SCN8A* channel activity, but may exacerbate seizures in Dravet Syndrome patients with a deficiency of *SCN1A* channel activity. This difference in drug response is one important reason to prioritize early genetic testing, since the results directly influence patient management.

Characteristics of sodium channel $Na_v1.6$ encoded by SCN8A

SCN8A encodes the sodium channel α subunit $Na_v1.6$, the current-conducting component of a complex that also contains modulatory β subunits.[32] As a member of the voltage-gated sodium (Na_v) channel family, $Na_v1.6$ has the typical structure with four homologous domains (DI-DIV) each containing six transmembrane segments (S1-S6) (**Figure 1**). Voltage-sensitivity is provided by positively charged arginine and histidine residues in the four S4 transmembrane segments. The channel "fast-inactivates" through a hinged-lid mechanism (internal DIII-DIV linker) that occludes the intracellular mouth of the pore (composed of the S5-S6 segments of all four domains). A second, less well-defined "slow" inactivation mechanism may involve a collapse of the outer mouth of the pore.[33] The unique properties of $Na_v1.6$ were recently reviewed.[29] $Na_v1.6$ is predominantly expressed in neurons, and is one of the most, if not the most, abundant sodium channels in the CNS. There is also a low level of expression in heart.[34-36] *SCN8A* transcripts are readily detected in prenatal brain. Adult levels of

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expression and splicing patterns are reached by 1 month in the mouse [37;38] and between 1 and 9 years of age in human.[39] Expression of $Na_v1.6$ is widespread throughout the central and peripheral nervous system in both excitatory and inhibitory neurons.[29; 37] An important aspect of $Na_v1.6$ biology is its concentration at two specialized membrane domains, the axon initial segment (the site of action potential initiation) and the node of Ranvier (the site of action potential regeneration during axonal saltatory conduction).[40-46] These subcellular localizations of $Na_v1.6$, and its variable expression in different types of neurons, contribute to the unique features of *SCN8A* channelopathies.

Many biophysical properties of $Na_v1.6$ resemble the other neuronal Na_v channels, including voltage-dependence and kinetics of inactivation and recovery.[29] However, $Na_v1.6$ uniquely generates higher levels of persistent and resurgent current, both of which contribute to repetitive neuronal firing.[47; 48] A working hypothesis for the mechanism of increased seizure susceptibility resulting from *SCN8A* gain-of-function mutations is that mutant $Na_v1.6$ mediates elevated transient, persistent, and/or resurgent sodium current. This hypothesis is supported by the functional analysis described in a later section, which has demonstrated that many disease-linked mutations directly increase channel activity. This hypothesis is also consistent with studies in the mouse indicating that reduced *Scn8a* activity appears to reduce seizure susceptibility.[49] One logical approach to developing new treatments for *SCN8A* epilepsies is therefore to identify drugs that specifically block $Na_v1.6$. The extensive conservation between $Na_v1.6$ and the other Na_v channels has made this a challenging undertaking.

Alternative splicing and rare introns of SCN8A

The *SCN8A* gene contains two pairs of alternatively spliced exons that encode transmembrane segments S3 and S4 of domain I and domain III.[38] Both of these mutually exclusive exon pairs contain one neonatal (N) and one adult (A) exon. In domain 1, the alternative exons differ by a single amino acid. In DIII, the neonatal exon contains an in-frame stop codon that results in protein truncation. Expression of the adult exon with the open reading frame is highly restricted to neurons[38; 39] and a low

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level in heart,[35] while transcripts containing the in-frame stop codon are widely expressed at a low level in non-neuronal tissues. Another unusual feature of the *SCN8A* gene is the presence of two minor-class introns whose splice sites begin with an AT dinucleotide and end with AC, rather than the major-class GT and AG.[50] These non-consensus splice sites influence the degree of exon skipping that results from mutations in nearby splice sites.[50]

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Patient Mutations in SCN8A encephalopathy

Patient mutations of *SCN8A* arise *de novo* in the affected individual and result in a single amino acid substitution rather than protein truncation. Mutations of *SCN1A* in Dravet syndrome also arise *de novo*, but 60% of mutations cause protein truncation and loss of function. Without functional studies the effects of amino acid substitutions are not obvious. Software algorithms such as PhyloP,[51] SIFT,[52] and PolyPhen-2[53] provide estimates of pathogenicity based on evolutionary conservation of the substituted amino acid and the chemical relationship between the original amino acid and its replacement. Nonetheless, reliable predictions regarding the biophysical consequences of amino acid substitutions are not yet possible. Functional comparisons between wild type and mutant Na_v channels can be made using electrophysiological patch-clamp experiments, but these require extensive individualized laboratory investigation. Ten missense mutations of *SCN8A* have thus far been functionally evaluated for their effect on $Na_v1.6$ channel activity,[3; 12; 16; 22; 29;54] and eight resulted in elevated channel activity. Since sodium channels are involved in initiation and propagation of action potentials, increased sodium channel activity in excitatory neurons can lead to central hyperactivity, the hallmark of seizures. Three distinct functional changes leading to elevated channel activity in the mutated channels are illustrated in Figure 2: premature channel opening, impaired channel closing, and increased persistent current.[3; 12; 16; 22; 29; 54] These are classified as "gain-of-function" effects because they produce new channel properties not seen in the wild-type channel. (This is in distinction to "loss of function" mutations that reduce activity, often by protein truncation, as in Dravet Syndrome.)

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With the small number of mutations analyzed to date, no clear correlation between phenotypic severity and genetic mutation has emerged. Patients with the identical genetic variant can present with phenotypes of different severity, demonstrating an important role of genetic background and, possibly, environment, in influencing the clinical outcome. For example, the mutation p.Arg1617Gln has been identified in 5 unrelated patients. This mutation replaces a positively charged arginine residue with an uncharged glutamine residue in transmembrane segment 4 of Domain IV. Functional analysis demonstrated impaired inactivation,[54] as predicted by the loss of a gating charge in the S4 segment of the domain IV voltage sensor, a region known to influence fast inactivation.[3; 55] The age of seizure onset among the 5 patients with this mutation varied from 3 months to 12 months, the ability to sit without assistance was achieved between 8 and 24 months of age, and the EEG patterns and responses to medication were heterogeneous.[3] This kind of phenotypic heterogeneity has been observed in other genetic epilepsies, e.g. mutations of the potassium channel KCNT1.[56]

The *SCN8A* gene contains several hot spots for recurrent mutations (indicated in Figure 1). The 50 published cases include 19 recurrent mutations each identified in 2 to 5 unrelated individuals. Analysis of patients with recurrent mutations provides an opportunity for analysis of the contribution of genetic background to clinical outcome and future identification of modifier genes.

Mutations of SCN8A can cause other less severe disorders

One inherited *SCN8A* mutation with loss of channel function due to protein truncation resulted in moderate intellectual disability without seizures in four related heterozygous carriers.[57] The proband in this family had ataxic gait and cerebellar hypoplasia. A mosaic, *de novo* intragenic deletion of *SCN8A* spanning exons 2 to 14 was identified in an individual with intellectual disability and absence seizures, but no

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convulsive seizures. The inherited *SCN8A* variant p.Glu1483Lys was described in three unrelated families with benign infantile seizures, paroxysmal dyskinesis, and normal cognition.[58] Thus missense mutations of *SCN8A* can result in less severe disorders than EE.

Distinguishing between pathogenic and nonpathogenic missense variants is a major challenge in interpretation of genetic test results for *SCN8A*. While most de novo patient mutations are likely to be pathogenic, it is not always the case. For example, the *de novo* missense mutation p.Asp58Asn in the cytoplasmic N-terminus of *SCN8A* did not differ from wildtype channel in functional tests and may be a nonpathogenic bystander.[19] Several patients have *de novo* variants that are also represented in exome databases at low allele frequencies and may be nonpathogenic. Other missense mutations affect amino acid residues that are not well conserved during evolution, suggesting that they may be non-deleterious. Thus identification of a *de novo SCN8A* variant in a patient should be followed up with expert interpretation.

iPSC derived neuron models of SCN1A and SCN8A epilepsies

An efficient platform for development of precision therapy based on the electrophysiological impact of individual mutations may come from induced pluripotent stem cells (iPSCs), reprogrammed from patient derived skin or blood cells. The generation of neurons from iPSCs has been used in Dravet syndrome to characterize sodium current density using whole-cell voltage- and current-clamp recordings.[59-61] The use of iPSCs provides a robust modeling tool, permitting the physiological properties of multiple cell types with identical genotype to be examined. Study of different mutations may yield insight into the influence of a single mutation in different cell types.[3; 54; 60] In iPSCs, the mutant channels are expressed in cells with the precise genetic background of the patient, which affords functional analyses of unparalleled physiological accuracy. CRISPR/Cas gene editing can be used to generate isogenic control lines with the mutation corrected for comparison. The technique is not without challenges, however, and independent studies have produced different outcomes.[59-61] Nonetheless, iPSC disease models constitute, at present, the most native and flexible drug testing platform.

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iPSCs can also be differentiated into cardiac myocytes, permitting analysis of pathogenic mechanisms that may contribute to SUDEP in *SCN8A* encephalopathy.

Strategies to screen for effective therapies for SCN8A encephalopathy

Existing *SCN8A* cell and mouse models provide an opportunity for early screening *in vitro* to be followed by *in vivo* testing based on appropriate evidence. Generation of *Scn8a* mutations in zebrafish may provide another model applicable to drug screening, as was done for *SCN1A* to model Dravet syndrome.[62; 63] The NIH Anticonvulsant Drug Development Program at the University of Utah provides a low-throughput but rigorous testing program to narrow down drug selection, accounting for efficacy as well as toxicity and safety issues.[64] All AEDs that have advanced to clinical trials have passed through this program since its inception in 1975. Surveying libraries of US Food and Drug Administration approved compounds may provide an expedited opportunity for effective and approved therapies for *SCN8A* diseases.

Modeling SCN8A mutations in the mouse

Mouse models are useful for understanding pathogenic mechanisms as well as evaluation of new treatments emerging from cell-based screening programs. A mouse model carrying the first published patient mutation of *SCN8A* (p.Asn1768Asp) has been described.[65] These mice recapitulate the seizures, EEG abnormalities, and premature death that were observed in the original patient (Figure 3).[14; 16] This mutation causes impaired channel inactivation, increased persistent current, and elevated channel activity.[16] In addition to hyperexcitable neurons, the *Scn8a* mutant mice display abnormal firing of ventricular myocytes, suggesting that cardiac arrhythmia may contribute to SUDEP in *SCN8A* encephalopathy (Frasier et al, manuscript in review). These mice and additional lines with other patient mutations will be important for pre-clinical testing of current and novel therapies. Correlating biophysical abnormalities of *SCN8A* mutants with *in vivo* responses may eventually provide personalized recommendations for treatment of newly diagnosed patients. Many fundamental questions can be addressed with mouse models, such as the impact of gain-of-function

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SCN8A mutations on various classes of neurons and on inhibitory versus general circuits.

Gaps in bioregistry, biobanking, and clinical outcome information that must be filled to become "trial ready"

Building on the emerging molecular understanding of *SCN8A* encephalopathy, there is urgent need to develop clinical platforms for testing the efficacy of interventions. To be "trial ready" for assessing therapies for *SCN8A* encephalopathy, more data on the natural history of the disorder are needed, including a better understanding of the phenotypic spectrum. A registry of mutations and the associated clinical outcomes will be essential. Comprehensive clinical data will be needed, including data regarding seizure phenotypes, developmental delay, developmental regression, movement disorders, other co-morbidities, age of onset of later features, hospitalization rate, efficacy of anti-epileptic and other medications, and survival. In combination with genomic studies, such a comprehensive database would also facilitate the systematic identification of modifier genes and pharmacogenetic interactions. Three important areas for development were discussed at the April 2015 meeting: bioregistry, biobanking, and documentation of clinical outcomes.

1. Bioregistry. Creation of a centralized registry would facilitate the early stages of research into innovative care for *SCN8A*-related disorders, and it will be important to identify long-term support for database maintenance and moderation. A community website hosting a patient-reported registry, modeled on the PCORI funded *Rare Epilepsy Network* developed for other genetic encephalopathies[66], is under development at the University of Arizona (www.SCN8A.net). This site provides information tailored to the interests of three groups: families, health care providers, and researchers. Features include the ability to determine whether an *SCN8A* variant has been previously reported, a directory of physicians who have treated patients with

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SCN8A mutations, and a private forum for families to ask questions and interact with each other. The website includes information about scientific advances in *SCN8A* research, clinical tools developed for other early-onset epileptic encephalopathies,[67] and links to groups such as CURE (http://www.cureepilepsy.org). A feature under development is a patient-reported registry that will allow participants to provide consent online and to fill out an extensive questionnaire designed specifically for *SCN8A*-related disorders. New information on clinical features that are shared among children with *SCN8A* mutations has already emerged. Eventually, the data will include a complete curated list of all known *SCN8A* variants, pathogenic or of unknown pathogenicity, with cross-reference to clinical information from individuals carrying those mutations. This data will benefit the physicians treating patients whose molecular test detects a potentially pathogenic *SCN8A* alteration.

Development of a patient registry will also be key to the systematic evaluation of responses of *SCN8A* encephalopathy patients to standard AEDs. To go beyond anecdotal reports, it will be necessary to combine detailed information for a cohort of patients, including clinical status prior to treatment, with precise data on dosage, timing of drug administration, and clinical impact. The frequent use of poly therapy, or combinations of AEDs, remains a confounding feature in sorting out the efficacy of specific AEDs. Lessons may be learned from a recent effort to assess AED effectiveness in a cohort of 58 patients with PCDH19 mutations based on retrospective reports of caregivers.[68] With the expansion of early genetic testing, it may become possible in the future to carry out prospective studies of sequential monotherapy that could provide more definitive data.

The benefits of crowd-sourcing for this rare disorder are becoming clear, with the number of patient-reported mutations (n=140) now exceeding those in the published literature (n-50). However, a disadvantage of patient reported registries is the lack of data from medical records. It may become possible for patients/caregivers to request their records and upload them to a website or send them to the registry. In studies of very rare conditions, highly motivated participants may enroll in multiple studies or

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registries being conducted by different investigators. Results from these studies may appear to be confirmatory when in fact they are derived from overlapping patient populations.

2. Biobanking. Biobanking of patient samples with standardized sample collection is another high priority for advancing understanding and therapy for *SCN8A* encephalopathy. In combination with an online registry, collection of high quality specimens with confirmed *SCN8A* mutations will facilitate the development of genotype/phenotype correlations. Modeling with patient-derived cells, by reprogramming of skin cells and peripheral blood monocytes, has already provided insight into the pathogenic roles of $Na_v1.6$. In the circumstance of SUDEP, mechanisms will be better investigated by banking tissue, fibroblast cultures and DNA. As with all biobanking, quality control and making specimens available to the research community will be critical.

3. Clinical outcomes. Effective recording of clinical outcomes will require better definitions and methods of assessment. To advance the quality of patient self-reporting in clinical research and practice, the National Institutes of Health (NIH) has developed PROMIS, the *Patient-Reported Outcomes Measurement Information System*. This initiative is developing new ways to measure patient-reported outcomes (PROs) that impact quality-of-life such as pain, fatigue, physical functioning, emotional distress, and social role participation. Work is needed to develop additional PROs specific to epilepsy. In one such study, Berg and collaborators investigated the outcomes most highly valued by parents of children with epilepsy and found that highest priority was given to seizure freedom and improved cognition.[69; 70] Epilepsy patient support groups are also developing approaches to monitoring outcomes.

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Conclusions

Previous experience with Dravet syndrome has demonstrated that understanding a genetic epilepsy syndrome requires more than identification of the molecular etiology.

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Careful clinical and electrophysiological phenotyping will be required to reveal the consequences of specific *SCN8A* mutations and to personalize AED choice for patients. In parallel with continuing research on disease mechanisms, the development of robust natural history and outcome measures will be essential to evaluating targeted therapeutics. This data will ultimately reveal whether developmental outcomes can be affected by early intervention and informed choice of AED. With the availability of a mouse model of *SCN8A* encephalopathy and additional models in development, there should be a concerted effort to test the clinically available drugs to identify the agents most likely to be successful in clinical trials. Mouse models can also address fundamental questions such as the impact of a gain-of-function mutation of $Na_v1.6$ on inhibitory neuronal circuits.

Further efforts will be enhanced by the development of an interface between patients, clinicians, and researchers. Biobanking, and partnership with established epilepsy and *SCN8A* advocacy groups will be important steps. Although *SCN8A* encephalopathy has only recently been discovered, important findings from functional studies and phenotypic classification has already shifted the focus from mutation identification to functional analysis and the development of strategies for drug screening. These important steps have implications for all parties invested in *SCN8A* encephalopathy, as we work towards reducing the uncertainty that comes with this diagnosis, often after a prolonged diagnostic odyssey.

Key Points:

- *SCN8A* encephalopathy was first identified in 2012 and an understanding of the severe impact of *SCN8A* mutations is beginning to emerge
- *SCN8A* mutations appear to account for approximately 1% of epileptic encephalopathies overall, with more than 140 individuals identified to date
- Distinctive properties of the sodium channel $Na_v1.6$ include a higher level of persistent and resurgent currents and localization at the AIS and nodes of Ranvier.
- Distinguishing between pathogenic and nonpathogenic variants is a major challenge for interpretation of missense mutations of *SCN8A*

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- Rapid progress in functional studies and phenotypic classification has focused attention on the development of strategies for drug screening

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MHM, GH, AV, and IES coordinated the meeting and preparation of the manuscript. All authors contributed to the writing and review of the manuscript. JH, GC, JB, and HS represented the voice of patient advocacy groups. VW, BF, and RS represented the National Institutes of Health.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure:

None of the authors has any conflict of interest to disclose. The *SCN8A* Encephalopathy Meeting was sponsored by the Wishes for Elliott: Advancing *SCN8A* Research family group.

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Figure Legends

Figure 1. Locations of missense mutations in *SCN8A* encephalopathy. The $Na_v1.6$ channel encoded by *SCN8A* is composed of four homologous domains (DI to DIV) each containing six transmembrane segments (S1 to S6). The channel also contains intracellular N- terminal and C-terminal domains, two large intracellular loops, and a small intracellular loop between domain III and domain IV that functions as the inactivation gate. Thirty-one published *de novo* mutations that were identified in 50 unrelated patients are shown. Pathogenic mutations are concentrated in transmembrane segments and in the N- and C-terminal domains. Black symbols, one patient; blue symbols, recurrent mutations found in multiple patients (Adapted from ref. 28).

Figure 2. Effects of gain-of-function mutations in *SCN8A* in patients with epileptic encephalopathy. (A) The Thr767Ile substitution in transmembrane segment S1 of domain II causes a hyperpolarizing shift in the voltage-dependence of activation, resulting in premature channel opening (ref. 20). (B). Three mutations of Arg1872 in the cytoplasmic C-terminal domain remove a critical positive charge resulting in delayed channel inactivation (ref. 53). (C) The substitution Asn1768Asp in transmembrane segment S6 of domain IV results in an increase in persistent sodium current that facilitates repetitive firing (ref.13).

Figure 3. A mouse model of *SCN8A* encephalopathy generated by knock-in of the patient mutation p.Asn1768Asp (N1768D). Approximately 40% of the heterozygous D/+ mice develop abnormal EEGs and seizures leading to premature death before 6 months of age. Homozygous D/D mice and hemizygous D/- mice are more severely affected. The number of mice in each group is shown in parentheses (adapted from ref.11).

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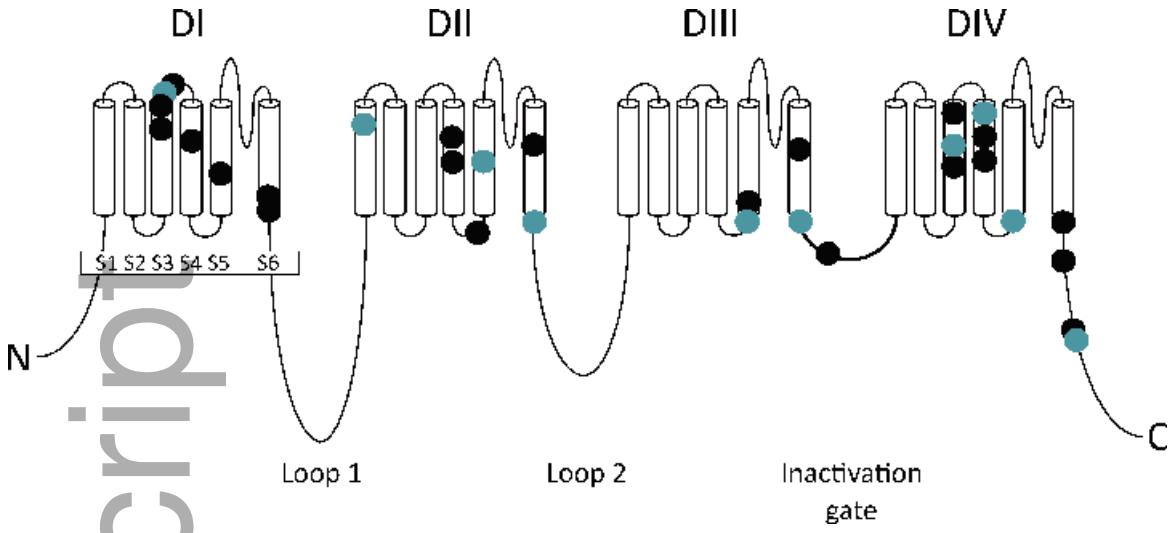

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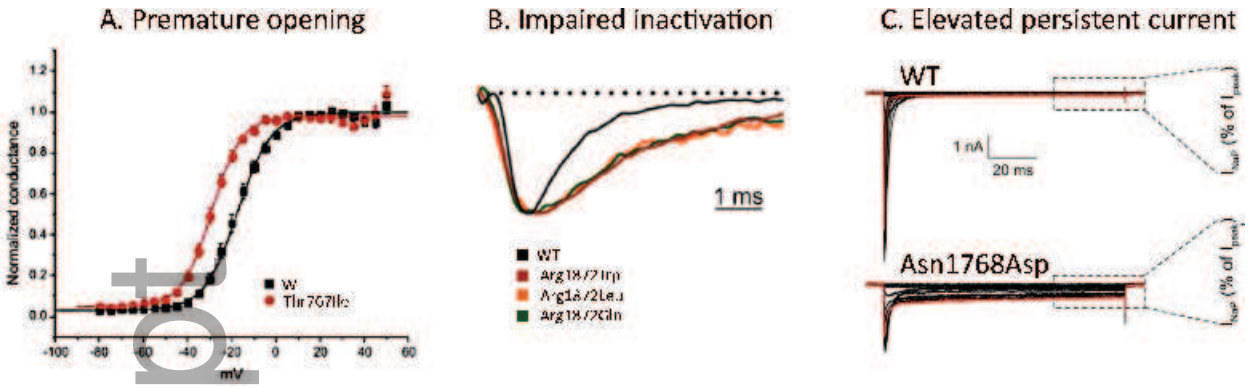

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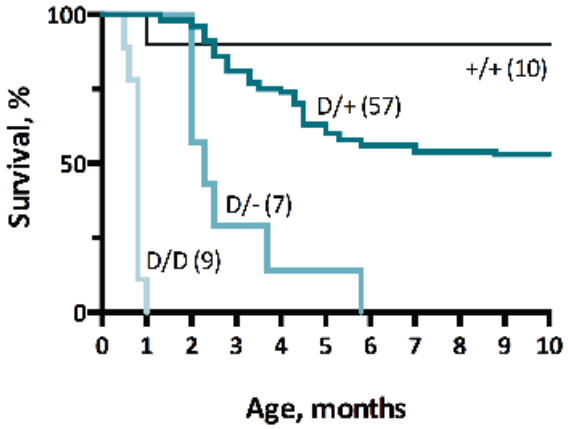

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