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Untangling the Dravet Syndrome Seizure Network: The Changing Face of a Rare Genetic Epilepsy

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Dravet syndrome (also known as Severe Myoclonic Epilepsy of Infancy) is a rare genetic epilepsy syndrome commonly associated with loss-of-function mutations in *SCN1A*, the gene encoding the α subunit of the voltage-gated sodium channel $Na_v1.1$, resulting in haploinsufficiency. Like other voltage-gated sodium channels, $Na_v1.1$ function contributes to the rising phase of the neuronal action potential; thus, the observation that loss-of-function mutations in this channel gene are associated with seizures has created a paradox for the field. Major work has been done to untangle this paradox during the past decade, resulting in the development of two distinct hypotheses to explain seizures in Dravet syndrome. Here, we review the history of these two hypotheses and speculate as to what the history of Dravet syndrome research might tell us about its future.

Dravet syndrome (DS, also known as Severe Myoclonic Epilepsy of Infancy or SMEI) is a genetic form of pediatric epilepsy that affects between 1 of 20,000 and 1 of 40,000 live births (1). Patients with DS typically experience their first seizure at 5 to 8 months of age. This is typically a clonic, generalized, or unilateral seizure triggered by fever. Within weeks to months, patients experience additional febrile seizures and then begin to exhibit temperature-independent seizures, including tonic, myoclonic, atypical absence, focal, generalized clonic, or generalized tonic-clonic seizures. Starting in the second year of life, many DS patients begin to show signs of other neurologic symptoms, including cognitive deficits (reviewed in [2]) and motor dysfunction. Cognitive dysfunction stabilizes (but in general does not improve) in the second half of the first decade of life, and seizures tend to decline into adolescence and adulthood (for a complete review of the clinical presentation of DS, see [1]).

The first mutations linked to DS were identified in 2001 in *SCN1A*, the gene encoding the α subunit of the voltage-gated sodium channel $Na_v1.1$ (3). To date, 70 to 80 percent of patients with DS have identified *SCN1A* mutations (4). Other genes have been implicated in DS by testing patients who do not have identifiable *SCN1A* mutations. These include *GABRG2* (5), *SCN1B* (6), and *SCN2A* (7). Several genes have been linked to seizure syndromes that are phenotypically very similar to DS, notably *PCDH19* (8) (a gene associated with Epilepsy in Females with Mental Retardation [EFMR]), and *SCN8A* (9). To further complicate the situation, it is becoming clear

that single gene mutations do not tell the whole story and that modifier genes play important roles in disease severity (10).

Studies of mutant *SCN1A* cDNAs from DS patients have shown that these mutations typically produce nonfunctional $Na_v1.1$ channels (11, 12). Approximately half of *SCN1A* patient mutations are truncations that are predicted to result in no protein from one allele. These studies, in addition to work suggesting that most DS *SCN1A* mutations arise de novo (3, 13–15) have led to a generally accepted idea that DS mainly arises from de novo *SCN1A* haploinsufficiency. However, there is a paradox that confronts this idea: How is it that reduced expression of a gene encoding an essential depolarizing current predisposes cortical networks to excitability and synchrony? This review aims to summarize the research community's attempts to untangle this paradox, and ultimately raises questions regarding what might be the most informative tools and questions to move the field forward.

The Interneuron Hypothesis

A major breakthrough in developing a theory for the pathophysiology of DS occurred with the development of the first mouse model of the disease by the Catterall group (16). This model, created through targeted, global deletion of the last coding exon from *Scn1a*, exhibited a strong seizure phenotype in both homozygous and heterozygous animals. These data were the first in vivo confirmation of *Scn1a* haploinsufficiency resulting in seizures. Patch clamp recordings in acutely dissociated hippocampal cells taken from postnatal day 14–16 (P14–16) *Scn1a*^{+/-} animals demonstrated that bipolar GABAergic neurons (but not glutamatergic pyramidal neurons) had a dramatic reduction in sodium current density. This loss in sodium current for GABAergic neurons limited their ability to increase firing frequency in response to injected current. Based

on these results, it was postulated that seizures in DS arise because selective defects in GABAergic interneurons produce a network that lacks sufficient inhibitory tone. This theory was named the “interneuron hypothesis.”

Not long after the development of the *Scn1a* last coding exon deletion mouse model, the Yamakawa group developed a human DS *SCN1A* mutation model with mice engineered with the mutation (*SCN1A*^{R1407X/+}) which implicated parvalbumin-positive interneurons in seizure pathogenesis (17). This group reported that Na_v1.1 was preferentially expressed in the axon initial segments of parvalbumin-positive interneurons, suggesting that this cell type might be preferentially affected by *SCN1A* haploinsufficiency. Indeed, trains of action potentials from parvalbumin-positive interneurons of the mice engineered with the mutation showed profound spike amplitude decrement as the spike train progressed. The results from this model suggested that hyperexcitability in DS might not result from dysfunction of GABAergic interneurons in general but rather dysfunction of the parvalbumin-positive population. These results were further supported when a parvalbumin-positive interneuron-specific *Scn1a*^{+/-} mouse was generated and found to have seizures (18).

Further evidence in favor of the interneuron hypothesis can be drawn from recent studies demonstrating that altering the balance of *Scn1a* expression in inhibitory versus excitatory neurons is sufficient to produce seizures. Forebrain GABAergic neuron-specific *Scn1a*^{+/-} mice (using the *Dlx1/2* promoter to drive Cre expression) have a robust seizure phenotype, suggesting that reduction of Na_v1.1 expression in this population of inhibitory neurons is sufficient to induce hyperexcitability (19). It was also shown that heterozygous *Scn1a* deletion in all inhibitory neurons (using a *VGAT*-Cre mouse line) produced a seizure phenotype that was more severe than that which was observed in the global *Scn1a* heterozygotes or in the excitatory neuron *Scn1a* heterozygotes (generated using the *EMX*-Cre mouse line) (20). Importantly, when these authors directly compared *Scn1a* heterozygous deletion in parvalbumin-positive interneurons with *Scn1a* heterozygous deletion in all inhibitory interneurons, they observed substantially higher mortality in the latter group, raising the possibility that parvalbumin-negative interneurons may play an as yet uncharacterized role in seizure pathogenesis for mouse models of DS.

Challenging the Interneuron Hypothesis: New Models, New Ideas

While the transgenic mouse has been the workhorse of all studies that support the interneuron hypothesis, work using other models has generated an alternative theory to explain seizures in DS. Recent data using human patient-derived induced pluripotent stem cell (iPSC) neurons argues that intrinsic hyperexcitability of both GABAergic and glutamatergic neurons might underlie seizure activity in DS. The first group to report this observation used a neuronal differentiation protocol that produced predominantly GABAergic (but also some glutamatergic) neurons that expressed forebrain markers. They found that both GABAergic and glutamatergic forebrain neurons from DS patients were hyperexcitable compared to nonepileptic controls, with increased sodium current density, increased rates of action potential firing in response to depo-

larizing current injection, and epileptic-like spontaneous activity (21). Another group used a differentiation protocol that produced predominantly cortical glutamatergic, but also some GABAergic, neurons. They too observed that DS patient-derived neurons were hyperexcitable (22). How might increased sodium current density in both excitatory and inhibitory neurons result in epilepsy? An analogy that may be helpful to consider here is the administration of a chemoconvulsant, such as kainic acid, that would increase the activity of both inhibitory and excitatory neurons with the net outcome of seizure generation. In the DS brain, it is proposed that increased excitability of both excitatory and inhibitory neurons would lead to network hyperexcitability or synchronization sufficient to produce seizures and cognitive dysfunction. Alternatively, neuronal hyperexcitability during embryonic brain development might lead to abnormal neuronal integration and result in network hyperexcitability and seizures.

The Liu et al. study using iPSC neurons hints at a novel theory of DS pathogenesis in which *SCN1A* haploinsufficiency results in a compensatory increase in sodium current, presumably through the expression of other voltage-gated sodium channels (21). The idea that *SCN1A* mutations in DS can produce changes in the expression of other sodium channel genes has been supported by several other studies. Acutely isolated cardiac myocytes from a DS mouse model engineered with the mutation show increased TTX-resistant sodium current density that is attributed to increased Na_v1.5 function. Although this change was observed in cardiac myocytes rather than neurons, it demonstrates that excitable cells have the capacity to increase the expression of other sodium currents in the face of *SCN1A* haploinsufficiency (23). Similarly, in the *Scn1b* null mouse model of DS, *Scn5a*/Nav1.5 expression is increased in the heart. Furthermore, *Scn1b* deletion dramatically decreased Na_v1.1 immunostaining in dentate granule cells from the outer leaflet but increased Na_v1.3 immunostaining in this brain area (24).

Interestingly, there is some indirect evidence to suggest that Na_v1.6 in particular may be a key player in DS pathogenesis. In the last coding exon *Scn1a*^{+/-} deletion mouse model of DS, *Scn8a* (encoding Na_v1.6) is a modifier of seizure pathogenesis. Here, *Scn1a*^{+/-} mice are more sensitive than WT mice to fluorothyl-induced seizures, while *Scn1a*^{+/-}/*Scn8*^{med-jo/+} mice have wild-type levels of sensitivity (26). Recently, a 15-year-old human patient was reported to have a seizure syndrome linked to a gain-of-function *SCN8A* mutation that shared many features with DS, including early-onset seizures, features of autism, intellectual disability, ataxia, and sudden unexplained death in epilepsy (SUDEP) (9).

What Makes a Good Model of DS: Insights from the Clinic

The two competing theories that have emerged to explain seizures in DS have been developed using different model systems. The interneuron hypothesis has relied entirely on work done in mouse models, while the more recent data that challenge the interneuron hypothesis have been generated using human patient-derived iPSC neurons. This discrepancy raises the possibility that disagreement between these two theories results from differences in the model systems themselves. Assessing the validity of these theories requires us to take what is

known about the human disease and to determine the extent to which the models that have been put forward reflect it in a functionally relevant manner.

Careful consideration of the interneuron hypothesis reveals a number of shortcomings when one tries to superimpose experimental findings from the mouse models with what is known clinically about DS. Much of the data supporting the interneuron hypothesis have been generated with a focus on the hippocampus. Although the hippocampus may be involved in some seizures that are seen in DS patients, it has limited relevance for the myoclonic seizures of DS, which are likely to be of cortical or thalamocortical origin (27). Because patients present with an array of different seizure types, it may be necessary to be more precise with our definitions of seizure pathophysiology in DS and acknowledge the possibility that different processes across different circuits may individually account for the genesis of different seizure types in this disease. Beyond this issue, it is worth noting that a great deal of the data in mouse models implicating GABAergic interneuron dysfunction in DS demonstrate only that *Scn1a* disruption in GABAergic interneurons is *sufficient* to produce seizures. This is not surprising, since one would expect targeted sodium channel deletion in inhibitory neurons to result in hyperexcitability. With these issues not yet addressed, it remains unclear to what extent insights from these models are functionally relevant in the seizures that human DS patients experience.

An important consideration is that the phenotypic severity of DS mouse models is highly dependent on genetic background and age of the animals at analysis. For example, Kearney and colleagues (29) demonstrated recently that *Scn1a*^{+/-} mice exhibit strain-dependent seizure severity and survival. Mice on strain 129S6/SvEvTac (129.*Scn1a*^{+/-}) showed no overt phenotype and normal survival compared with mice bred to C57BL/6J (F1.*Scn1a*^{+/-}) that had severe epilepsy and premature lethality. Patch clamp recording of hippocampal neurons from P21–24 129.*Scn1a*^{+/-} or F1.*Scn1a*^{+/-} mice showed that sodium current density was lower in GABAergic interneurons from F1.*Scn1a*^{+/-} mice compared to wild-type, while on the 129 strain there was no difference in GABAergic interneuron sodium current density between 129.*Scn1a*^{+/-} mice and wild-type. In contrast, and similar to the iPSC neuron model of DS, sodium current density was elevated in pyramidal neurons from both 129.*Scn1a*^{+/-} and F1.*Scn1a*^{+/-} mice. These authors also observed age-dependent differences in pyramidal neuron sodium current density between wild-type and *Scn1a*^{+/-} animals. Interestingly, the observed elevated sodium current density in excitatory pyramidal neurons at P21–24 correlated with age-dependent onset of lethality in F1.*Scn1a*^{+/-} mice. This is in contrast to the study by the Catterall group (16), described above, which analyzed neurons from P14–16 mice.

The hypothesis that intrinsic hyperexcitability is a primary driver of seizures in DS also has its shortcomings, in large part because the data supporting this hypothesis were generated using iPSC neurons. Importantly, while these data have demonstrated a cell autonomous phenotype, there is not yet a connection between intrinsic cellular hyperexcitability in iPSC neurons and clinically relevant seizure behavior that we know is dependent on neuronal circuits. Beyond that, the relatively

novel and untested nature of iPSC neuron models is itself an issue: The iPSC technology has existed only since 2006, and functional studies in neurons derived from iPSCs are even newer. It is worth noting that iPSCs have been used to successfully investigate Timothy syndrome, another developmental epilepsy. Studies of iPSCs from Timothy syndrome patients that were differentiated to follow a cortical specification showed activity-dependent changes in dendrite structure that were mirrored in rat and mouse models of the disease (28). This work in Timothy syndrome is proof-of-principle that differentiated iPSC neurons can be used to model at least some types of developmental epilepsies, with the added value of mutations being expressed and realized in their endogenous cellular context. Further study is required to see whether the DS iPSC models are relevant to the same extent.

Conclusion

DS is a catastrophic developmental epilepsy whose seizure pathogenesis remains poorly understood, although several lines of research are developing promising potential explanations for seizures. DS research in many ways reflects the trends in research on other neurodevelopmental diseases, with stem cell technologies providing a novel platform for investigating conditions that had previously been studied only in rodent models. DS research also demonstrates that there is great power in employing multiple model systems to investigate a developmental process. The future of DS research is likely to rely on a marriage of old and new, with different model systems being leveraged for their strengths in order to develop robust theories of pathogenesis. For example, recent advances using zebrafish and fly models of epilepsy may provide deeper insights into epilepsy mechanisms and with that, the development of novel therapeutics. With an array of powerful tools at its disposal, the field stands poised to make substantial progress in uncovering epilepsy mechanisms and moving towards novel therapies to treat a complicated and devastating condition.

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