Gene Therapy in Models of Severe Epilepsy due to Sodium Channelopathy

**dCas9-Based Scn1a Gene Activation Restores Inhibitory Interneuron Excitability and Attenuates Seizures in Dravet Syndrome Mice**

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Dravet syndrome (DS) is a severe epileptic encephalopathy caused mainly by heterozygous loss-of-function mutations of the SCN1A gene, indicating haploinsufficiency as the pathogenic mechanism. Here, we tested whether catalytically dead Cas9 (dCas9)-mediated Scn1a gene activation can rescue Scn1a haploinsufficiency in a mouse DS model and restore physiological levels of its gene product, the Nav1.1 voltage-gated sodium channel. We screened single guide RNAs (sgRNAs) for their ability to stimulate Scn1a transcription in association with the dCas9 activation system. We identified a specific sgRNA that increases Scn1a gene expression levels in cell lines and primary neurons with high specificity. Nav1.1 protein levels were augmented, as was the ability of wild-type immature GABAergic interneurons to fire action potentials. A similar enhancement of Scn1a transcription was achieved in mature DS interneurons, rescuing their ability to fire. To test the therapeutic potential of this approach, we delivered the Scn1a-dCas9 activation system to DS pups using adeno-associated viruses. Parvalbumin interneurons recovered their firing ability, and febrile seizures were significantly attenuated. Our results pave the way for exploiting dCas9-based gene activation as an effective and targeted approach to DS and other disorders resulting from altered gene dosage.

**Scn8a Antisense Oligonucleotide Is Protective in Mouse Models of SCN8A Encephalopathy and Dravet Syndrome**

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SCN8A encephalopathy is a developmental and epileptic encephalopathy caused by de novo gain-of-function mutations of sodium channel Nav 1.6 that result in neuronal hyperactivity. Affected individuals exhibit early-onset drug-resistant seizures, developmental delay, and cognitive impairment. This study was carried out to determine whether reducing the abundance of the Scn8a transcript with an antisense oligonucleotide (ASO) would delay seizure onset and prolong survival in a mouse model of SCN8A encephalopathy. Antisense oligonucleotide treatment was tested in a conditional mouse model with Cre-dependent expression of the pathogenic patient SCN8A mutation p.Arg1872Trp (R1872 W). This model exhibits early onset of seizures, rapid progression, and 100% penetrance. An Scn1a+/− haploinsufficient mouse model of Dravet syndrome was also treated. Antisense oligonucleotide was administered by intracerebroventricular injection at postnatal day 2, followed in some cases by stereotactic injection at postnatal day 30. We observed a dose-dependent increase in length of survival from 15 to 65 days in the Scn8a-R1872W/+ mice treated with ASO. Electroencephalographic recordings were normal prior to seizure onset. Weight gain and activity in an open field were unaffected, but treated mice were less active in a wheel running assay. A single treatment with Scn8a ASO extended survival of Dravet syndrome mice from 3 weeks to >5 months. Reduction of Scn8a transcript by 25% to 50% delayed seizure onset and lethality in mouse models of SCN8A encephalopathy and Dravet syndrome. Reduction of SCN8A transcript is a promising approach to treatment of intractable childhood epilepsies.

**Commentary**

The early infantile/developmental and epileptic encephalopathies are defined by onset of intractable epilepsy in early childhood associated with developmental delay/cognitive impairment and increased mortality. Novel treatments are desperately needed, and there is increasing interest in gene therapy approaches for this class of disorders, which have a genetic basis. Two recent studies report such attempts in experimental models of prominent epileptic encephalopathies: Dravet syndrome, due to deletion of pathogenic variants in SCN1A encoding the type 1 voltage-gated sodium (Na+) channel subunit...
Nav1.1, and SCN8A encephalopathy, due to pathogenic variants in SCN8A encoding Nav1.6.

As the genetic mechanism of Dravet syndrome is haploinsufficiency with heterozygous loss of function of the SCN1A gene, increasing the expression or activity of Nav1.1 subunit-containing Na⁺ channels is a logical approach to therapy. Colasante et al. used a CRISPR/dCas9 system to screen for guide RNAs that bind to specific regions of the mouse Scn1a promoter and that increase Scn1a gene expression. Briefly, CRISPR/Cas9 is the gene-editing technique whereby DNA sequences called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) facilitate targeting and subsequent destruction of DNA by the Cas (CRISPR-associated) proteins. The CRISPR/Cas9 system involves delivery of Cas9 along with a synthetic guide RNA (sgRNA) for specific editing of the genome. The CRISPR technique is highly relevant to the field of genetic epilepsy as dominant heterozygous pathogenic missense variation or small insertion/deletion is a major disease pathomechanism and such genetic lesions could be targeted for correction using this technique. Application of this technique in experimental animal model systems could provide important proof of principle of the potential utility of this or a related approach, and could demonstrate that features of a given epileptic encephalopathy can be treated or even prevented.

Mutation of the nuclease domain of Cas9 creates a nuclease-deficient Cas9 (dCas9), which interacts with and modulates transcription of a target site rather than inducing gene cleavage and editing. Colasante et al successfully identified a single sgRNA that, in combination with dCas9, significantly enhanced Scn1a expression in heterologous cells. This was specific to Scn1a as determined by RNAseq, with no effect on expression of other genes (including other Na⁺ channels). This ingenious approach allows for widespread upregulation of Na⁺ channel expression in brain via transduction with adeno-associated viral (AAV) vectors, which otherwise cannot accommodate a gene the size of a Na⁺ channel α subunit (which is >6 kb). Using this strategy, Scn1a expression was increased 2-fold in cultured Gad67-expressing mouse neurons, which showed increased firing rates relative to neurons transduced with a control system. The authors then showed that transduction of cultured neurons from Dravet syndrome (Scn1a⁺/−) mice with Scn1a.dCas9 produced a 3.5-fold increase in Scn1a expression relative to control, and this led to increased Nav1.1 expression in Scn1a⁺/− (but not Scn1a⁺/+ ) neurons.

Nav1.1 expression in the cerebral cortex is more prominent in GABAergic inhibitory interneurons than in excitatory pyramidal cells. Colasante et al delivered an AAV9-based Scn1a.dCas9 system targeted to GABAergic interneurons using an mDix5/6 promoter via intracerebroventricular injection into neonatal Scn1a⁻/⁻ mouse pups. Acute brain slices were prepared at postnatal day (P) 21 to 28, and electrophysiological recordings demonstrated that deficits in action potential generation in parvalbumin-expressing fast-spiking GABAergic interneurons (PV-INS)—known to be dysfunctional in Scn1a⁻/− mice—were rescued by the Scn1a.dCas9 system. Recent data suggest that PV-INS in Scn1a⁺/− mice that survive to P35 or beyond recover normal intrinsic excitability and in vivo 2-photon calcium imaging of PV-INS in awake, behaving Scn1a⁻/− mice shows that activity during quiet wakefulness and running behavior is similar to wild-type, supporting the conclusion that PV-INS excitability normalizes over time. These data raise the question as to whether increasing Nav1.1 expression exclusively in PV-INS in a chronic phase of Dravet syndrome would actually be efficacious at all, as these neurons seem to have at least partially “fixed themselves.” Colasante et al performed a range of assays in Scn1a⁺/− mice at 1 month of age and found an increase in the temperature threshold for seizure induction (ie, seizures occurred at higher temperature), lowering efficacy, and not just PV-INS, and hence, the observed in vivo effects cannot necessarily be attributed to changes in PV-INS excitability.

Finally, intraventricular injections of P0 pups with AAA.Scn1a.dCas9 under control of a pan-neuronal promoter led to an increase in (higher/improved) seizure threshold at P14 to 17 using an assay that combined lipopolysaccharide-induced fever and kainic acid administration. This experiment is an attempt to prevent disease, rather than treat existing disease. The result is interesting, although again the authors did not report the long-term effect of this manipulation on the development and severity of epilepsy or on mortality, which would be of great interest.

In a separate paper, Lenk et al. use synthetic oligonucleotides (antisense oligonucleotides [ASOs])—an RNA-based gene-directed therapy—to modify disease pathogenesis in an experimental mouse model of SCN8A encephalopathy. Anti-sense oligonucleotides are short oligonucleotides engineered to specifically bind to a unique target RNA sequence and manipulate the levels of messenger RNA (mRNA) transcripts that encode specific target proteins via degradation or block of protein translation. In this case, Lenk et al target Scn8a in a mouse model that conditionally expresses a Scn8a-p.R1872 W missense variant and exhibits normal development prior to seizure onset at P14 to 16 followed by death within 24 hours. Na⁺ channels containing Nav1.6 subunits harboring the p.R1872 W missense variant exhibit so-called gain of channel function with impaired inactivation, such that decreasing Scn8a mRNA might be expected to lead to lower Nav1.6 protein expression (the ASO is not specific to the p.R1872W variant and decreases both wild-type and variant transcripts, but not other Na⁺ channel transcripts). However, heterozygous loss-of-function variants in or deletion of SCN8A produce mild intellectual disability with or without ataxia in humans, while an Scn8a null mutation in mouse is lethal; hence, marked reduction in Scn8a transcript could be deleterious and the authors titrate use of the ASO to achieve modest and reproducible reductions in Scn8a mRNA and in turn Nav1.6 protein.

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Lenk et al. engineered an ASO that targets the proximal 3′-untranslated region of mouse Scn8α, which decreased Scn8α transcript in cultured neurons. Scn8α ASO delivered via intracerebroventricular injection at P2 decreased Scn8α transcript levels (presumably both wild-type and variant) to approximately 50% of control levels without affecting Scn1a levels, and reduced corresponding Nav1.6 protein. Treatment prolonged survival and markedly delayed epilepsy onset in 100% of treated mice in a dose-dependent manner with no effect of control ASO; however, all treated mice eventually exhibited epilepsy and premature death. Interestingly, survival could be further prolonged by a “booster,” although such mice did all eventually die, suggesting that there is not a critical period or developmental window of dysfunction beyond which suppression of variant Scn8α might no longer be required. This work in an experimental animal model provides strong preclinical support for the conclusion that chronic administration of an intrathecal ASO could be a potential therapeutic option for patients with SCN8A encephalopathy.

Prior work has shown that decreased expression of Scn8a can ameliorate the phenotype of Scn1a+/− mutant mice, suggesting the perhaps counterintuitive idea that an epileptogenic heterozygous loss of function of Scn1a can be balanced by a parallel loss of Scn8a. This and related work provided rationale for testing the impact of Scn8a ASO in Scnα+/− mice, and quite surprisingly, all treated mice survived beyond 5 months of age. How exactly this might occur is not clear. There may be upregulation of Scn8a and/or other Na+ channel transcripts in excitatory neurons and/or GABAergic interneurons in Scn1a+/− mice, which could be compensatory or epiphenomenal or could perhaps be contributing to or causing persistent impairment in Scn1a+/− mice and in human patients with Dravet syndrome. So, there is theoretical and now experimental support for targeting SCN8A as a treatment for Dravet syndrome as well. It would be quite interesting to perform a negative control and test the Scn8a ASO in another mouse model of epilepsy, such as a genetic potassium channelopathy or chronic-acquired temporal lobe epilepsy after brain injury. Perhaps Scn8a ASO would be a potential treatment for many types of epilepsy beyond Na+ channelopathies?

A concern as we envision clinical implementation of ASO therapy is that the SCN8A (or any) transcript reduction needed to produce a meaningful effect might be variant-specific. For example, electrophysiological recordings of Na+ channels containing Nav1.6-p.R1872W variant subunits exhibit increased slowly inactivating (“persistent”) current and impaired fast inactivation, but other epilepsy-associated pathogenic variants may be more severe and require suppression of transcript levels to below 50% to achieve an antiseizure effect. It would be interesting to test the extent to which older mice can tolerate more aggressive targeting. Since the mean age of onset of SCN8A encephalopathy is in early infancy with the first clinical feature often being seizures, it will be important to determine what the effect of an ASO-based (or any gene therapy) approach might be after seizures have emerged.

In summary, there is accumulating experimental support from model systems for further development toward gene therapy-like approaches for the epileptic encephalopathies. This class of disorders is severe and otherwise largely untreatable. Yet, the proximate cause is known and due in many cases to a single pathogenic missense variant, providing a clear therapeutic target. Dravet syndrome and perhaps to a lesser extent SCN8A encephalopathy represent well-defined clinical populations. However, such approaches need further refinement to improve delivery, achieve widespread or brain-wide expression, and prolong biological activity of the therapeutic agent and its effects, while demonstrating safety with maintained efficacy.

However, major questions remain. When during the disease course must such agents be administered to achieve a meaningful effect? Which cell type(s) and brain area(s) should be targeted? What are the appropriate output measures to monitor efficacy? There is still much to be learned about the basic biology of SCN1A and SCN8A, including the key cell type(s) and brain regions expressing these genes, the functional roles of such channels, the impact of pathogenic variants on ion channel and neuronal function, the mechanisms whereby alteration of neuronal function leads to seizures, epilepsy and neurodevelopmental disability, and how these processes evolve over time during brain development. While there are certainly challenges ahead, these reports are part of a growing literature that gives us reason for optimism.

By Ethan M. Goldberg

ORCID iD
Ethan M. Goldberg MD, PhD https://orcid.org/0000-0002-7404-735X

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