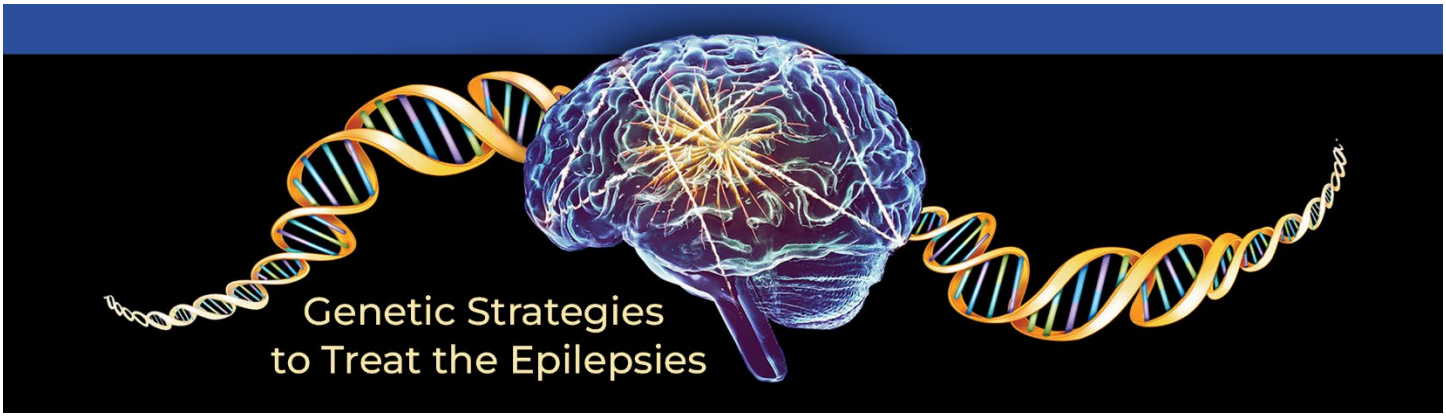

Pathogenic Variant in *ANKRD11* Identified in an Individual with Focal Cortical Dysplasia Type I

Ana Berglind[1], Meethila Gade[2], Sandya Krishna[3], Isha Asim[4,5], Abigail Freeman[4,5], Melodie Winawer [4,5], Manu Hegde [3,6], Cathryn R. Cadwell[6,7,8], Erin L. Heinzen[1,2]

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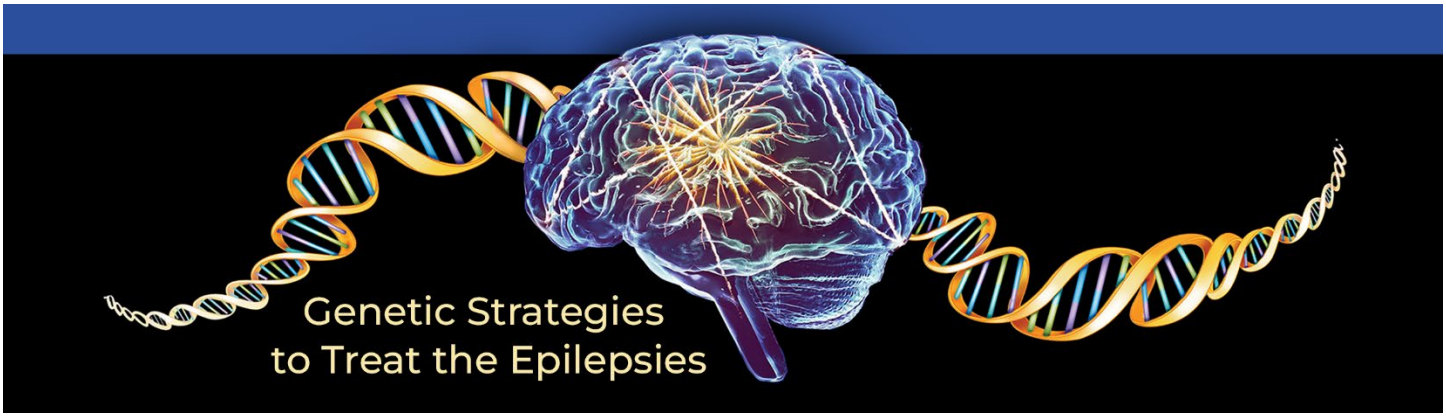
Cerebral cortex formation is achieved through a series of highly regulated processes. Disruptions in these processes can result in a range of cortical malformations, including focal cortical dysplasia (FCD). In this study, we sequenced individuals with intractable focal epilepsy who have undergone brain resection surgery to identify pathogenic variants that may contribute to disease risk. DNA isolated from resected tissue was analyzed in a cohort of 278 patients with radiographically lesional and non-lesional focal epilepsy, including hemimegalencephaly (n = 32), FCD type I and related phenotypes (n = 126), FCD type II (n = 98), or FCD type III (n = 22). High depth exome sequencing from a female with FCD type I revealed a germline mutation in *ANKDR11* c.2197C>T (p.R733X). The truncating mutation has been previously reported in individuals with inborn genetic diseases and KBG syndrome, a rare autosomal dominant genetic disorder characterized by distinctive craniofacial and skeletal malformations, intellectual disabilities, developmental delay, and seizures. Neuropathological review of resected tissue revealed disorganization of neurons in the deep cortical layers. Two additional *ANKRD11* variants [c.4604A>G (p.K1535R) and c.4147G>T (p.G1383C)] of unknown significance were identified in two additional cases with radiographically non-lesional focal epilepsy. *ANKRD11* has been shown to regulate neuronal migration during cortical development in mice but has not been seen in human tissue as these patients rarely undergo resections. These results support that *ANKRD11* could potentially be a novel FCD gene. Additional studies are required to evaluate the role of *ANKRD11* on neuronal activity and development.



Development of Splice-switching Antisense Oligonucleotides to Treat SCN8A Epilepsy

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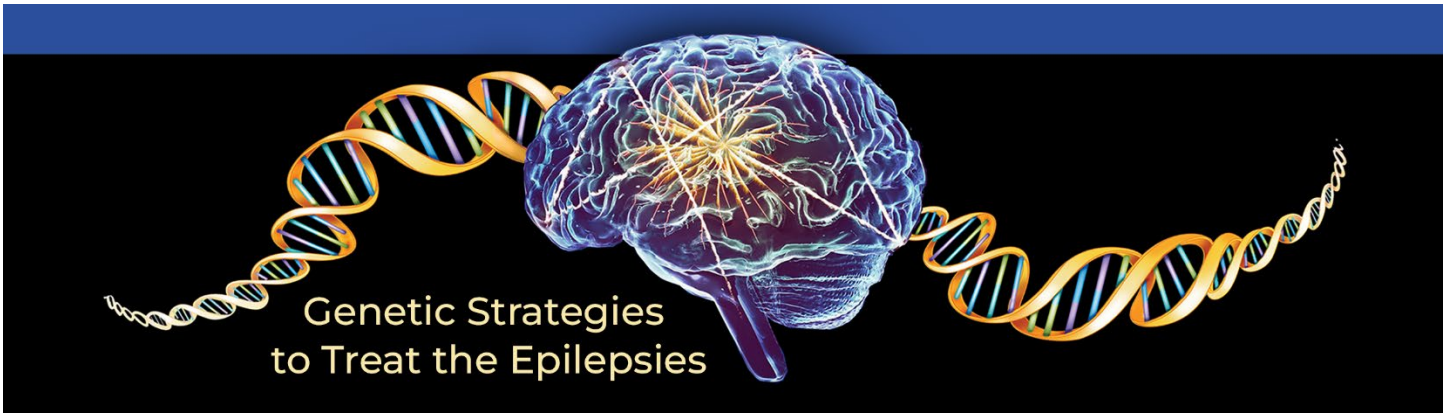
Mutations in the SCN8A gene coding for the Nav1.6 sodium channel are responsible for up to 1% of developmental epileptic encephalopathy (DEE). SCN8A undergoes developmentally-regulated alternative splicing, yielding a neonatal isoform, exon 5N, or an adult isoform, exon 5A. There are 40 patients with pathogenic mutations in the mutually exclusive 5N or 5A exon, and we hypothesize that developing splice-switching antisense oligonucleotides (ASOs) inducing a switch from the mutated exon to a corrected exon could offer a novel therapeutic approach for SCN8A patients, correcting Nav1.6 protein, reducing seizures and promoting development.

First, we developed a novel mouse model of Ser217Pro mutation in the 5N exon in C57BL/6 mice, a pathogenic gain of function mutation. The heterozygous S217P(+/-) mutation in mice induces early onset seizures and an average life expectancy of 19 days. Using EEG we were able to capture multiple seizures occurring in the S217P(+/-) mice. Second, we designed ASOs that could induce the exclusion of a mutated exon 5N and inclusion of the non-mutated SCN8A exon 5A. We identified several ASOs able to induce the exclusion of *Scn8a* exon 5N and increased inclusion of *Scn8a* exon 5A in neuroblastoma cell lines ND7/23 (mouse) and SH-SY5Y (human). These ASOs were also able to induce splice switching when cultured with primary neurons from P0 mice. Experiments are ongoing to test the ASOs in the S217P(+/-), evaluating effects on seizures and survival. These ASOs are the first treatment strategy with the goal of correcting a mutated protein for patients with SCN8A DEE.



Unraveling the Molecular Basis of Hemimegalencephaly with Genotype Informed Single-cell RNA Sequencing

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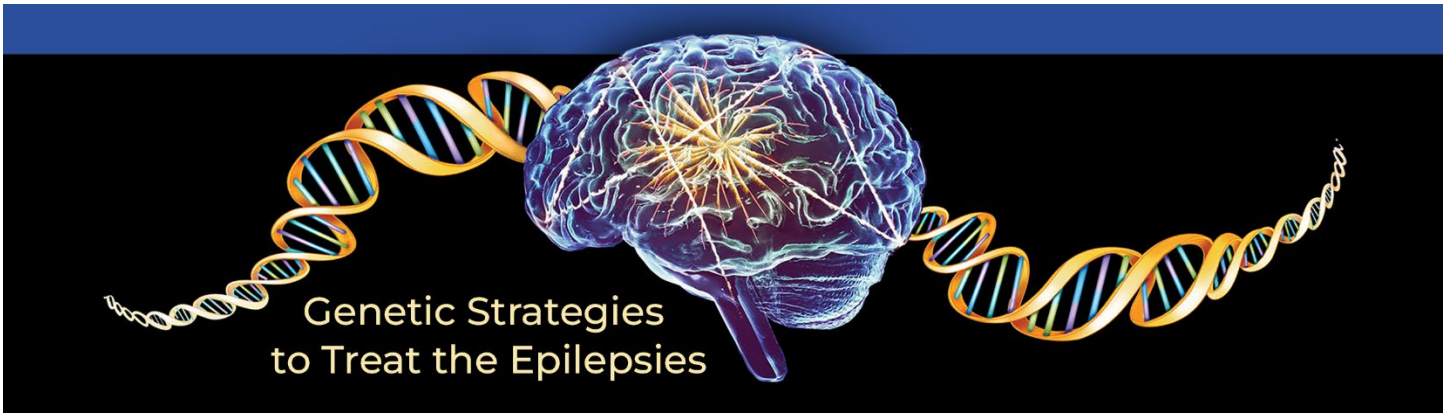
Hemimegalencephaly (HMEG) is a developmental malformation associated with post-zygotically acquired variants (somatic variants) in the PI3K/AKT/mTOR signaling pathway. Mosaic surgically resected tissue from individuals with disease-causing somatic variants can provide valuable insights into disease mechanisms and cell type involvement. We employed a novel methodology, Somatic Mosaic Sequencing (SoMoSeq), to investigate cell type- and mutation-specific gene expression associated with a recurrent pathogenic somatic variant in HMEG (*PIK3CA*: E545K). Our objectives were to delineate the spectrum of *PIK3CA* variant burden across different cell types in individuals with *PIK3CA* HMEG and to understand cell type-specific transcriptomic signatures linked to variant status. We conducted SoMoSeq on approximately 1,500 nuclei extracted from two individuals with HMEG, with variant allele frequencies of 14% and 25%, respectively, alongside two neurotypical controls. Subsequently, 96 nuclei positive and negative for the variants (N=384) were sequenced at a depth of 8-10M reads/nuclei. Analysis of cell type-specific variant burden, utilizing de-novo clustering and data integration approaches, revealed the presence of the variant across all eight major cell types, including microglia, suggesting an early embryonic origin of the variant. Additionally, we observed an overall upregulation of the mTOR signaling pathway in mutation-positive nuclei compared to mutation-negative nuclei from cases and controls. Differential gene expression analysis, investigating the autonomous and non-cell autonomous effects of the variant in different cell types using a mixed effects model, is currently underway. This proof-of-concept study demonstrates the utility of SoMoSeq in dissecting the complexity of HMEG at the single-cell level and shedding light on its pathophysiological underpinnings.

Funding: This study was supported by R01NS094596



Interneuron-specific AAV-mediated Gene Replacement Therapy in a Mouse Model of Dravet Syndrome

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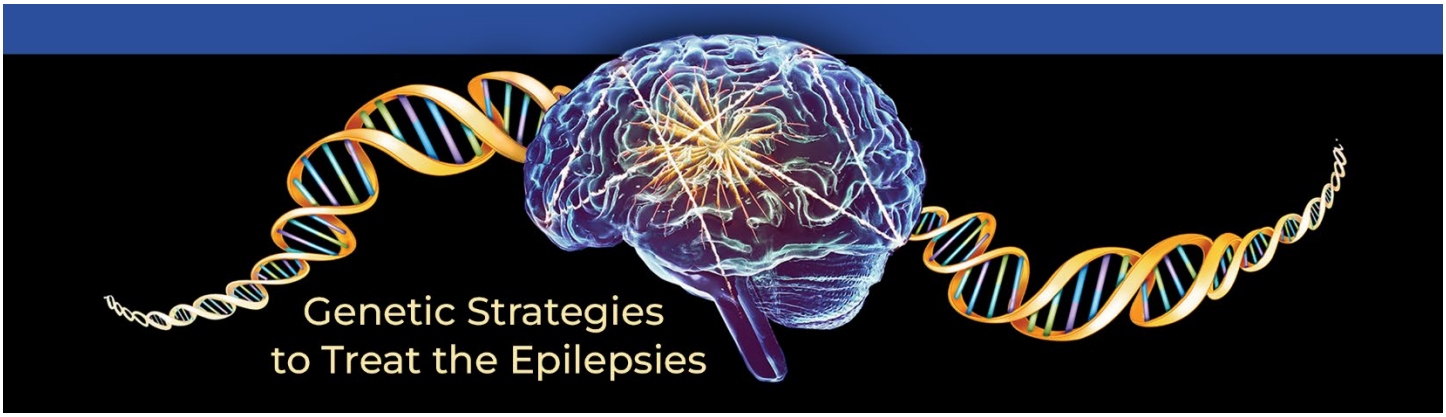
Dravet syndrome (DS) is a devastating developmental epileptic encephalopathy marked by treatment-resistant seizures, developmental delay, intellectual disability, motor deficits, and a 10-20% rate of premature death. Most DS patients harbor loss-of-function mutations in one copy of *SCN1A*, which has been associated with inhibitory neuron dysfunction. Here we developed an interneuron-targeting AAV *SCN1A* gene replacement therapy using cell class-specific enhancers. The therapeutic *SCN1A* transgene was split in two segments and delivered using the dual vector approach to circumvent AAV packaging limitations and the split-intein technology was used to reconstitute the full-length gene product post-translationally. Split-intein *SCN1A* constructs generated by this approach produced full-length and functional NaV1.1 channel proteins in HEK293 cells and in brain cells *in vivo*. After packaging into enhancer-AAVs and administering to mice, immunohistochemical analyses showed telencephalic GABAergic interneuron-specific and dose-dependent transgene biodistribution. These vectors conferred strong dose-dependent protection against postnatal mortality and seizures in two DS mouse models carrying independent loss-of-function alleles of *Scn1a,* at two independent research sites, supporting the robustness of this approach. No mortality or toxicity was observed in wild-type mice injected with single vectors expressing either the N-terminal or C-terminal halves of *SCN1A*, or the dual vector system targeting interneurons. In contrast, nonselective neuronal targeting of *SCN1A* conferred less rescue against mortality and presented substantial preweaning lethality. These findings demonstrate proof-of-concept that interneuron-specific AAV-mediated *SCN1A* gene replacement is sufficient for significant rescue in DS mouse models and suggest it could be an effective therapeutic approach for patients with DS.



URGenT: The NINDS Approach to Therapeutic Development for Ultra-Rare Neurological Disorders

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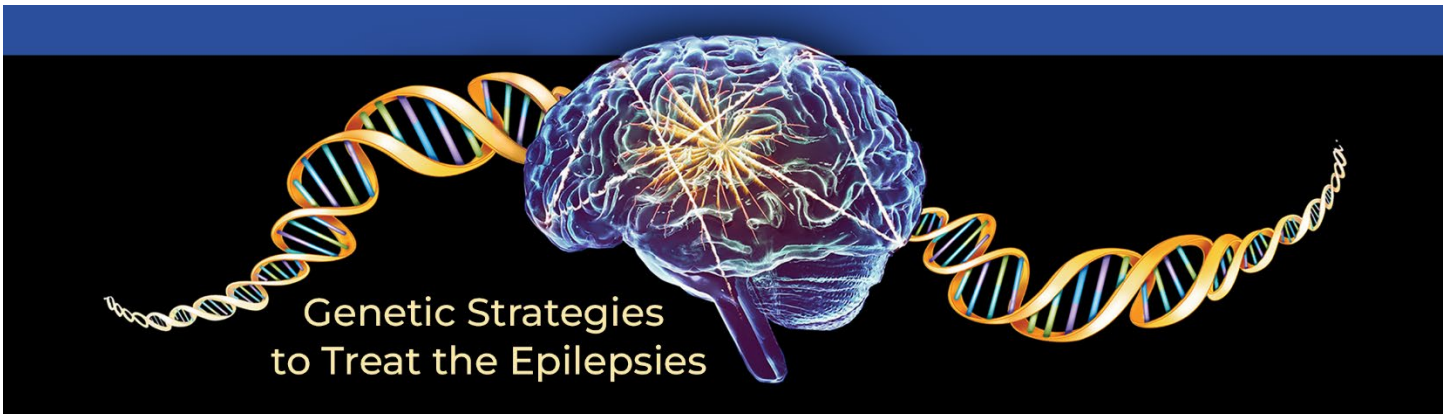
The National Institutes of Health reports that nearly 7,000 rare diseases affect 4-6% of the world's population. Ultra-rare diseases affect substantially fewer people, less than 6000 in the US. With approximately 80% of rare diseases that have an identified genetic origin, gene-targeted treatments will require academia, industry, patients and families, and other stakeholders to think in new ways that will allow more efficient and speedy processes to bring these treatments to the clinic.

The Ultra-Rare Gene-based Therapy (URGenT) network was launched by the National Institute of Neurological Disorders and Stroke (NINDS) in 2021 to support the late-stage pre-clinical development of state-of-the-art gene-based therapies for ultra-rare neurological and neuromuscular diseases (PAR-22-028 & PAR-22-030). Building on this program, on March 1, 2024, NINDS published a Research Opportunity Announcements (ROA) - to invite applications for URGenT clinical trials (OTA-24-011 & OTA-24-012) through the Network for Excellence in Neuroscience Clinical Trials (NeuroNEXT) with a Gene Therapy Consortium (GTC), launched on March 5, 2024, to provide guidance on potential challenges related to safety and efficacy research, regulatory approval, and business processes that may be unique for gene-based therapy trials.

The URGenT network has established R&D contracts with CROs and over 25 consultants to deliver assistance at different stages of the awarded projects including manufacturing, IND-enabling studies, and IND submission. This proposed NINDS model aims to accelerate the development of gene-based therapeutics for ultra-rare diseases, standardizing data and resource sharing across diseases and enhance the efficiency and accessibility of therapies development for ultra-rare diseases.



Towards the Development of Potential Treatments for *SCN8A*-derived Epilepsy

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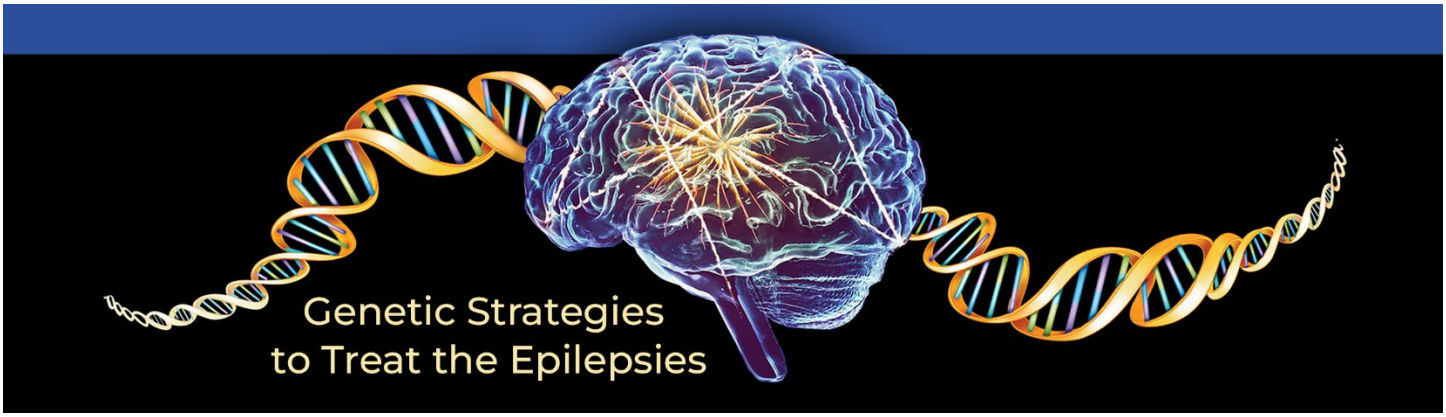
De novo SCN8A mutations have been identified in patients with catastrophic, treatment-resistant childhood epilepsy, and in patients with autism, intellectual disability and developmental delay but with less severe epilepsy. Currently, it is unclear which treatments are the most efficacious for these patients, underscoring the need to identify and develop better treatments for *SCN8*A-derived epilepsy. In an effort to identify drugs that could be potentially beneficial in *SCN8A*-associated epilepsy, we have tested multiple compounds in a mouse line expressing the human *SCN8A* R1620L mutation. We previously showed that heterozygous mutants (RL/+) exhibit increased seizure susceptibility and a range of behavioral abnormalities, including learning and memory deficits and abnormal social behavior.

We have evaluated the ability of amitriptyline, nilvadipine, carvedilol, cannabidiol (CBD), and Huperzine A to increase resistance to 6 Hz or pentylenetetrazole (PTZ)-induced seizures in wild-type CF1 mice and RL/+ mutant mice. We also evaluated the effects of fenfluramine administration, which was recently associated with a 60-90% decrease in seizure frequency in three patients with *SCN8A*-associated epilepsy. Carvedilol (20 mg/kg) and oxcarbazepine (15-20 mg/kg) both provided protection against 6 Hz seizures in approximately 50% of mice. We also found a dose-dependent increase in seizure protection with CBD treatment. Notably, we were able to achieve complete protection against 6 Hz seizures with Huperzine A (1 mg/kg) and a higher dose of oxcarbazepine (50 mg/kg). Taken together, these results identified several compounds that might be potentially beneficial in patients with *SCN8A* mutations.



Advancing Gene Correction Therapy for *SCN2A* Genetic Epilepsies with CRISPR Prime Editing

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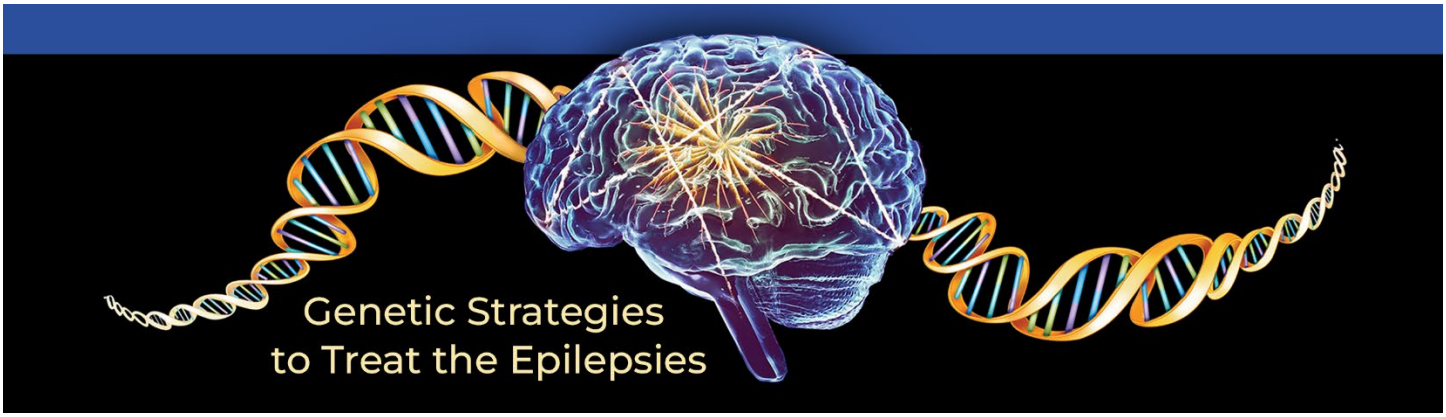
Genetic mutation in *SCN2A* has been found to be one of the leading causes of monogenic epilepsy. *SCN2A* encodes the voltage-gated sodium channel Nav1.2, predominantly expressed in principal neurons including excitatory neurons of the cortex to mediate neuronal action potential firing in the brain. *SCN2A* mutations often time leads to severe drug-resistant seizures but unfortunately there is no cure for these genetic epilepsies. We have established human induced pluripotent stem cells (iPSCs) derived neuron and brain organoid models to understand how *SCN2A* genetic mutation found in patients with epilepsy affects functions and molecular profiles of human neurons. To treat genetic disorder, genetic medicine including advanced Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Prime Editing, holds enormous promise. We have been optimized Prime Editing in a blue-green fluorescent protein (BFP-GFP) iPSCs system, which allows us to reach an exciting, close to 100% single nucleotide editing efficiency in iPSCs. We are now working on correcting seizure related *SCN2A* mutations in iPSCs based model systems with the ultimate goal of developing genetic interventions for monogenic epilepsies.



Effective Down-regulation of Sodium Channel *Scn8a* by Three Genetic Methods

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DEEs (Developmental and Epileptic Encephalopathies) are rare epileptic disorders characterized by refractory seizures, developmental delay, and impaired movement. *De novo* missense mutations of *SCN8A* have been identified in several hundred individuals with DEE. *SCN8A* encodes the voltage-gated sodium channel $Na_v1.6$. The most common pathogenic mechanisms are gain-of-function mutations causing premature channel opening or impaired channel inactivation. We treated mouse models of DEE using three genetic therapies: antisense oligonucleotide (ASO) and shRNA to reduce *Scn8a* transcripts, and CRISPR-i to inactivate the pathogenic allele.

Antisense oligonucleotides (ASO): Intracerebroventricular (ICV) injection of an *Scn8a*-ASO reduced abundance of the $Scn8a^{R1872W}$ transcript to 50% of normal level. Repeated administration, prior to seizure onset or after seizure onset, reduced seizures and rescued survival of N1768D mice. The ASO also extended survival of $Scn1a^{+/-}$ Dravet mice.

Allele-specific knock-out by CRISPR/Cas9. Allele-specific sgRNAs targeting the patient *Scn8a* mutation N1768D were administered to mice with a germline copy of Cas9. ICV injection generated indels in the mutant allele but not in the wildtype allele. The $Scn8a^{N1768D}$ allele was inactivated in approximately 25% of mutant transcripts representing 25% of neurons throughout the brain. This editing efficiency was sufficient to rescue seizures and lethality.

Short hairpin RNA (shRNA): A single ICV administration of the AAV10-*Scn8a*-shRNA prevented seizures and lethality in *Scn8a*-DEE mice. shRNA treatment also rescues *Scn1a*+/- Dravet mice. Stereotactic administration of AAV10-*Scn8a*-shRNA to the hippocampal dentate gyrus achieved partial rescue of survival, but administration to CA1 plus CA3 regions was not effective.