Leveraging Preclinical Models To Inform Clinical Trial Strategy in SCN2A Developmental and Epileptic Encephalopathy

Oligonucleotides for CNS

June 7, 2023

Disclosure

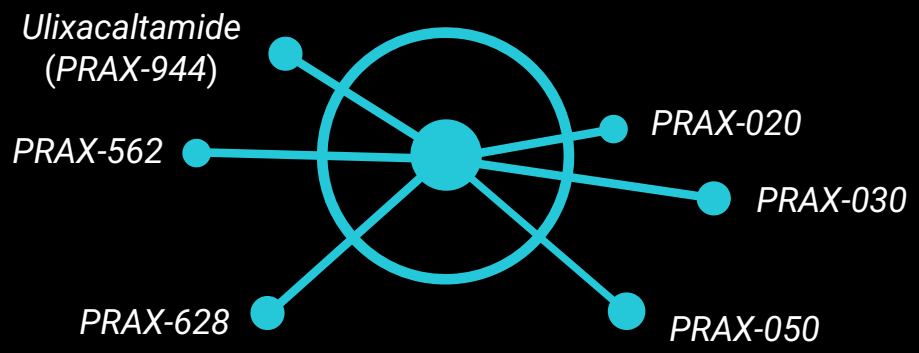
Kris Kahlig is a current employee of Praxis Precision Medicines and is a Praxis stockholder.

Developing Treatments Inspired By The Genetics of Epilepsy

ENABLED BY TWO PLATFORMS

CEREBRUM™

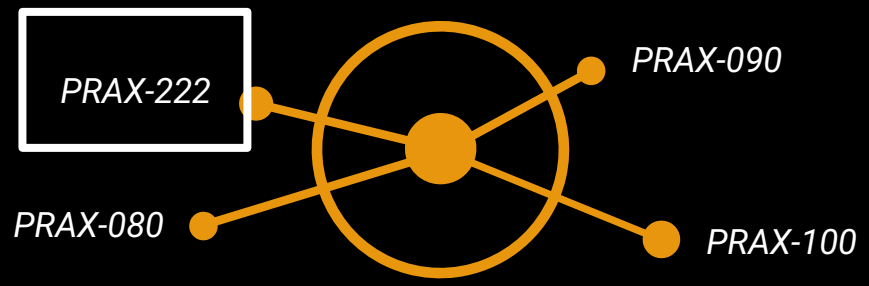
SMALL MOLECULE PLATFORM



Cerebrum™ utilizes deep understanding of neuronal excitability and neuronal networks and applies a series of computational and experimental tools to develop orally available precision therapies

SOLIDUS™

ANTISENSE OLIGONUCLEOTIDE (ASO) PLATFORM



Solidus™ is an efficient, targeted precision medicine discovery and development engine for ASOs anchored on proprietary, computational methodology

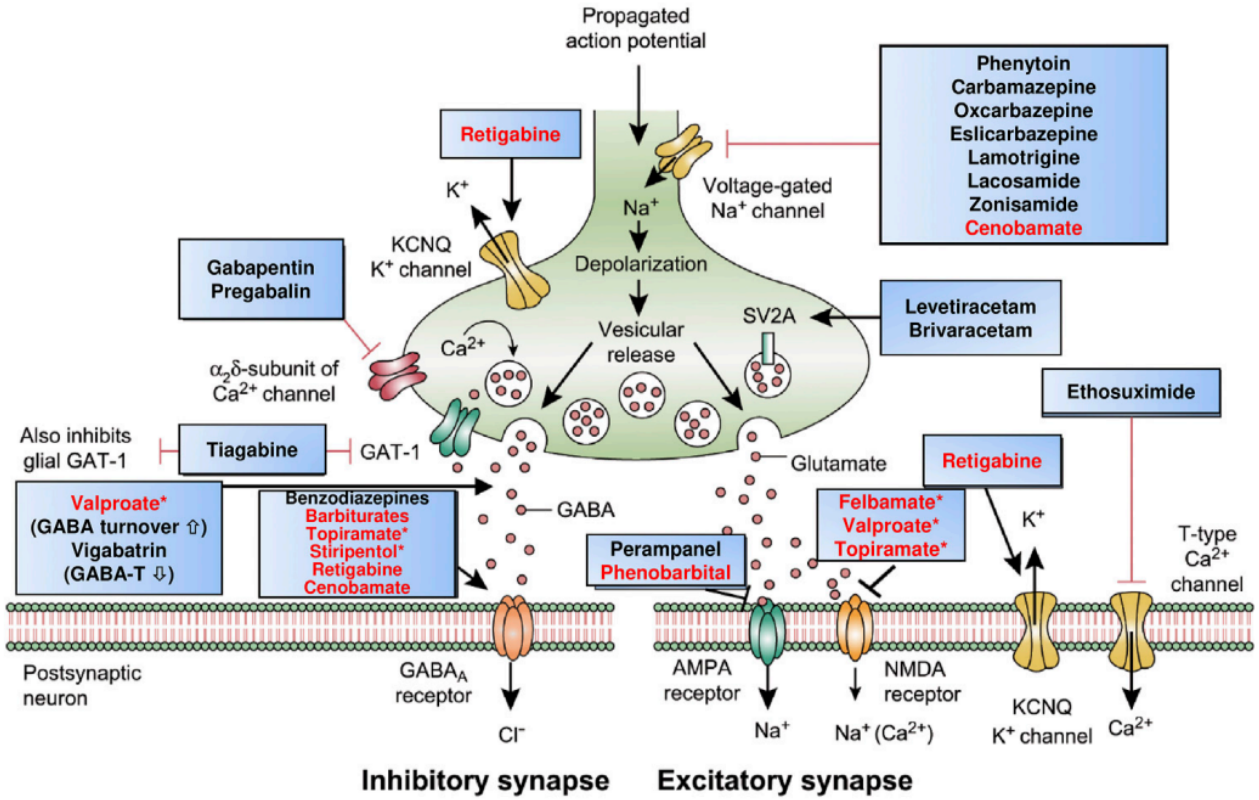
Genetics of Epilepsy

- Selection of an appropriate target for ASO modulation

SCN2A-DEE

- Model for evaluating therapeutic intervention
- Model for variant characterization

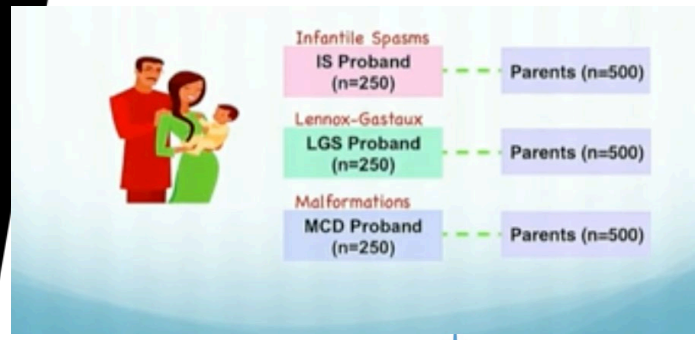
Current Anti-Seizure Medications (ASMs) Employ Small Molecules



- Current therapeutics build upon recognized targets and achieve incremental gains
- Small molecules and polypharmacy currently the mainstay ASMs
- New approaches needed for the 30% of patients that are resistant to current therapeutic options

Löscher, W. Single-Target Versus Multi-Target Drugs Versus Combinations of Drugs With Multiple Targets: Preclinical and Clinical Evidence for the Treatment or Prevention of Epilepsy. *Front Pharmacol* **12**, 730257 (2021).

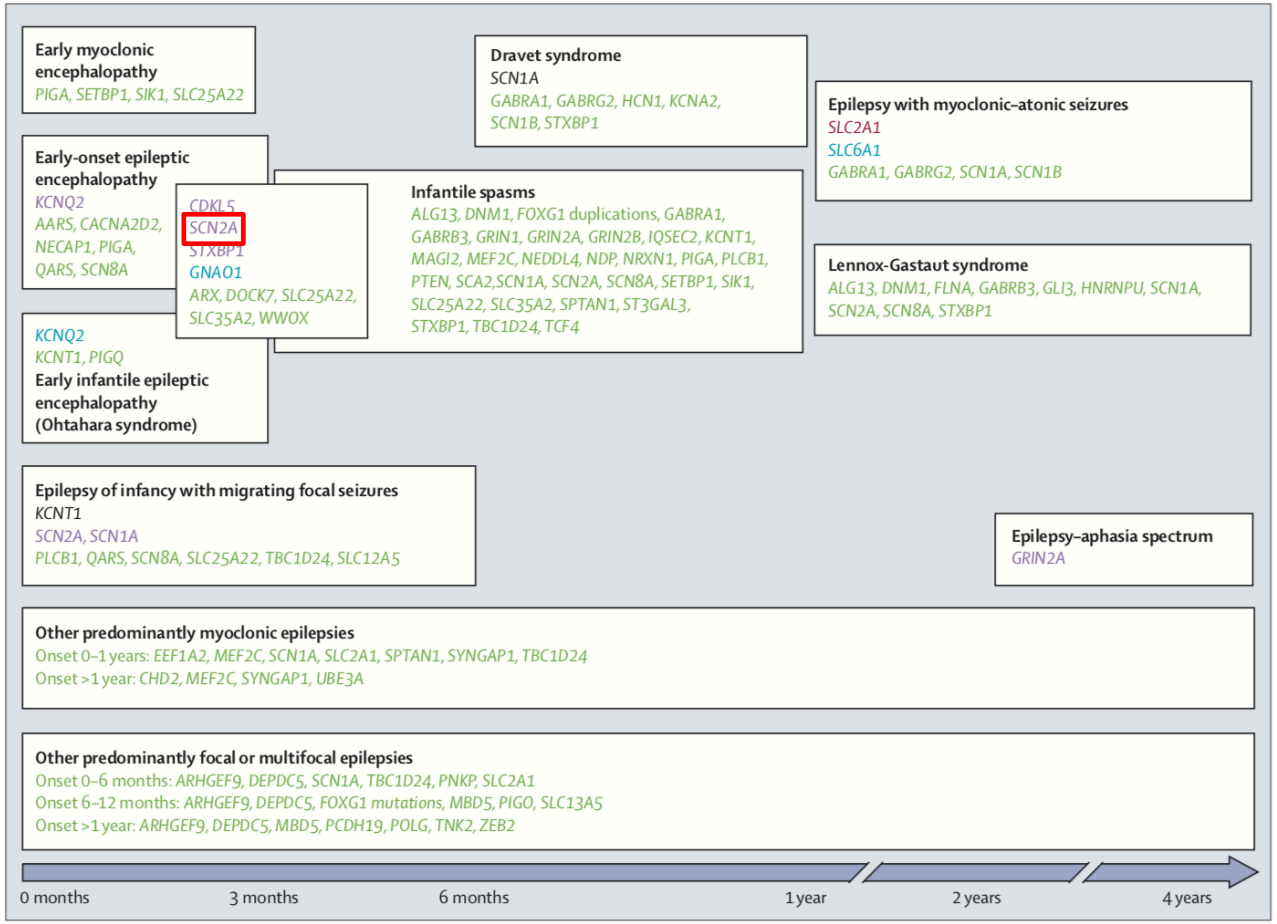
Developmental and Epileptic Encephalopathies (DEEs) are Caused by Genetic Variation



EPGP
EPILEPSY PHENOME/GENOME PROJECT

epi4K
GENE DISCOVERY IN EPILEPSY

Targets

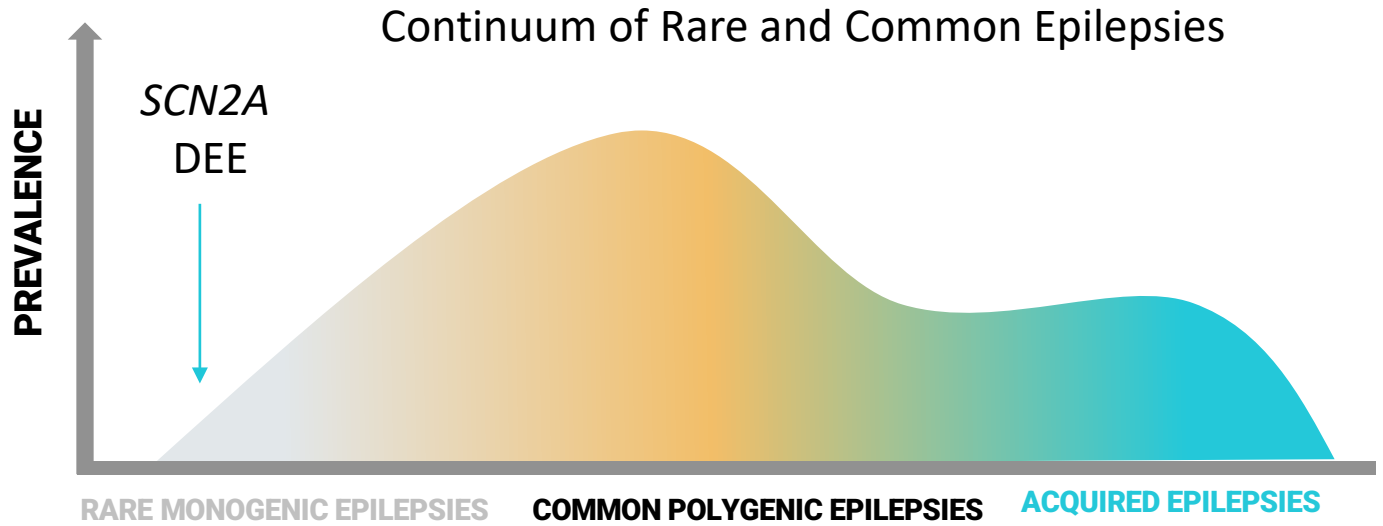


McTague et al, *Lancet Neurol* (2015)

- DEEs now know to be rare, often catastrophic diseases
- Advances in genetics providing mechanistic understanding and new targets for DEEs
- Oligonucleotides represent novel approach to modulate targets with promise of disease modification

Insights from Rare DEEs will Inform on More Common Epilepsies

- Advances in treating rare monogenetic epilepsy will impact treatment of common forms of epilepsies
- Genetics of epilepsy
  - Revealed novel targets
  - Refocused efforts on validated targets



Challenge now is how to safely modulate target

SCN2A as example

Genetics of Epilepsy

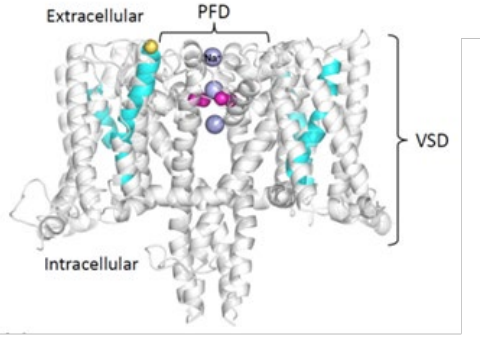
- Selection of an appropriate target for ASO modulation

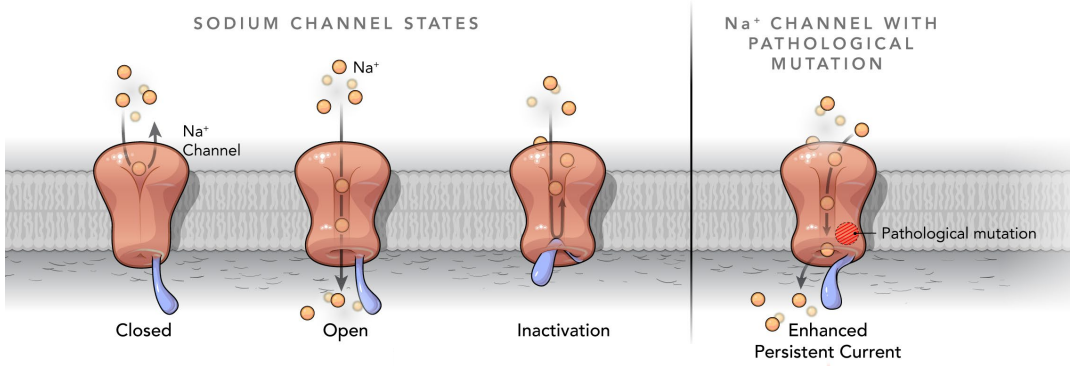
SCN2A-DEE

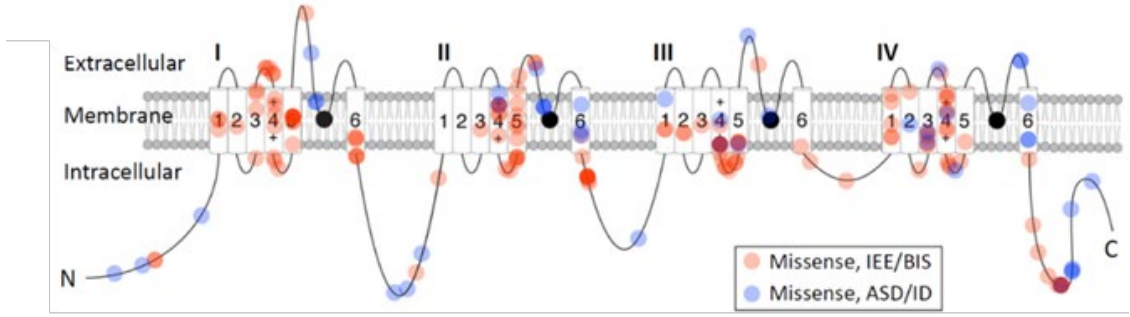
- Model for evaluating therapeutic intervention
- Model for variant characterization

Variants in *SCN2A* are a Common Cause of Genetic Disease

- *SCN2A* encodes $Na_V1.2$
- Dominantly expressed in the cortical excitatory neurons
- Localized in the proximal AIS
- Commonly associated with neurological and psychiatric disorders

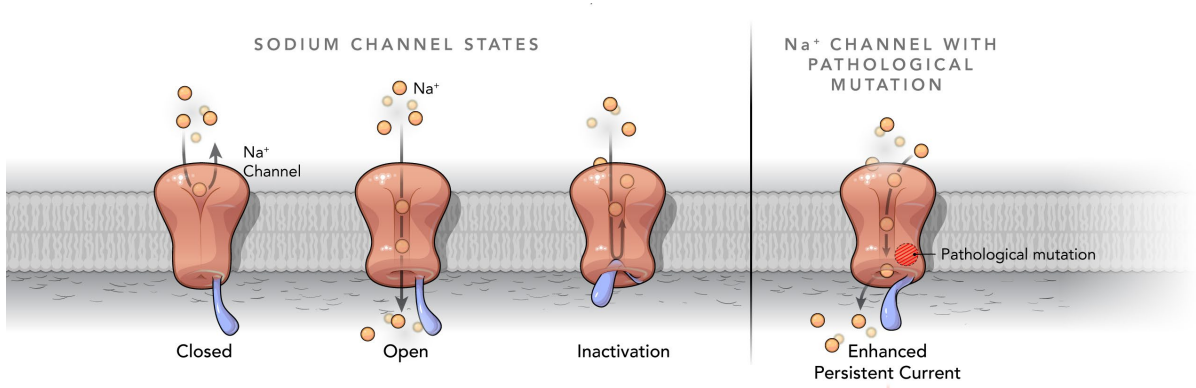






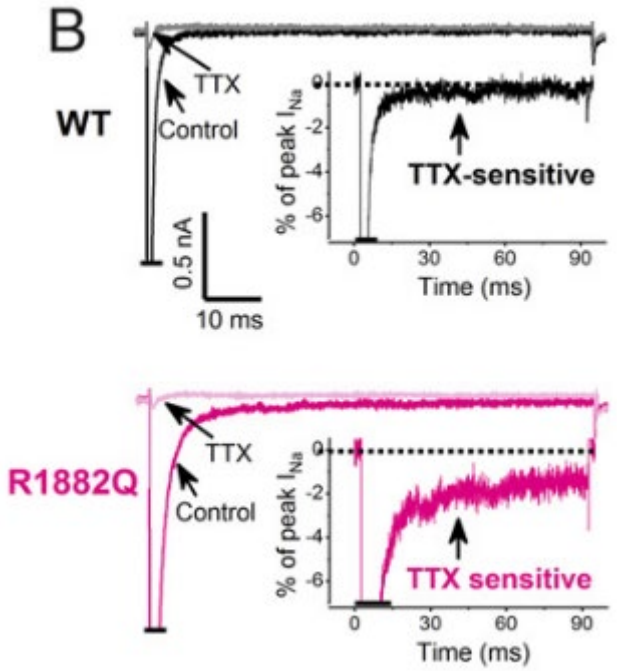
S. J. Sanders *et al.*, *Trends Neurosci.* **41**, 442–456 (2018).

Epilepsy variants in *SCN2A* can increase $Na_V1.2$ Activity



Persistent I_{Na} is a common feature of $Na_V1.2$ Gain of Function (GoF)
Other forms of GoF also observed

Early Onset DEE Variant R1882Q
Causes $Na_V1.2$ GoF Including Persistent I_{Na}

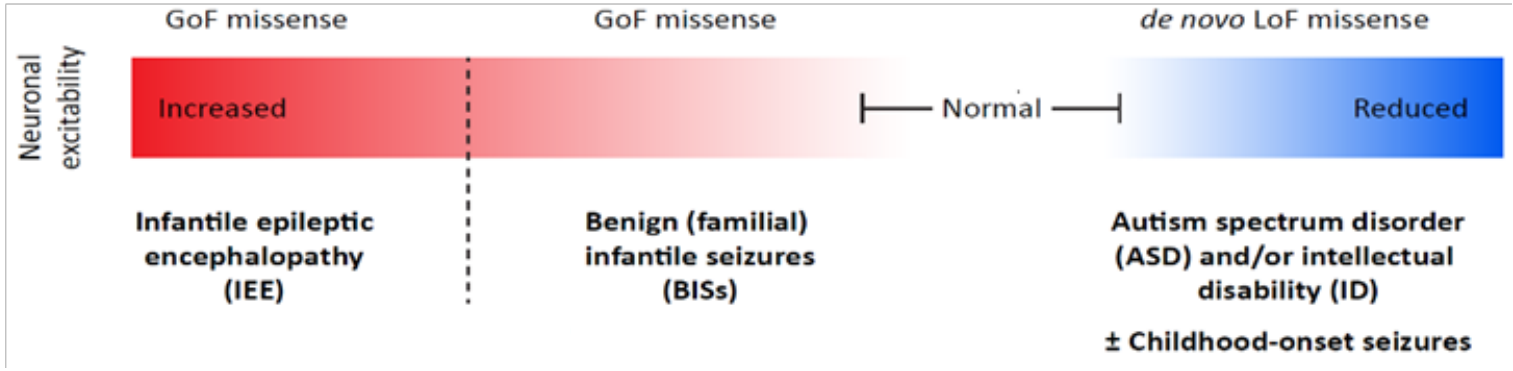


G. Berecki *et al.*, *Proceedings of the National Academy of Sciences*. 115, E5516–E5525 (2018).

SCN2A ($Na_V1.2$) Pathophysiology Reflects both Gain-of-Function and Loss-of-Function

Onset < 3 months of age -> predicts GoF

Onset > 3 months of age -> predicts LoF



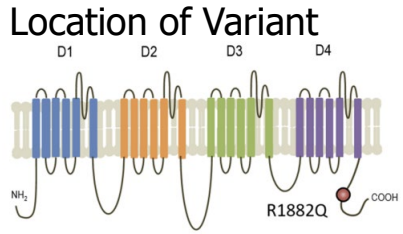
S. J. Sanders *et al.*, *Trends Neurosci.* **41**, 442–456 (2018).

Potential Therapeutic Approach
Selective reduction in $Na_V1.2$ function

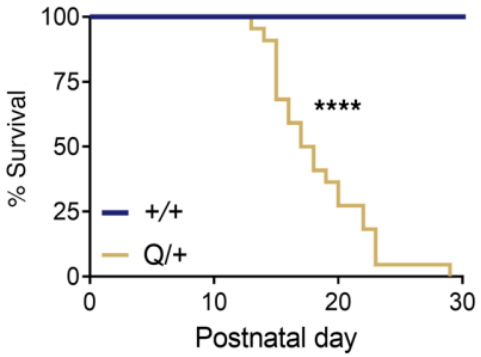
Potential Therapeutic Approach
Selective increase in $Na_V1.2$ function

An ASO selectively downregulating SCN2A could be a significant therapeutic advance

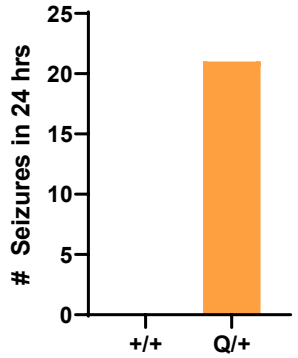
Mouse Model of *SCN2A*-DEE: $Na_V1.2$-R1882Q (Q/+)



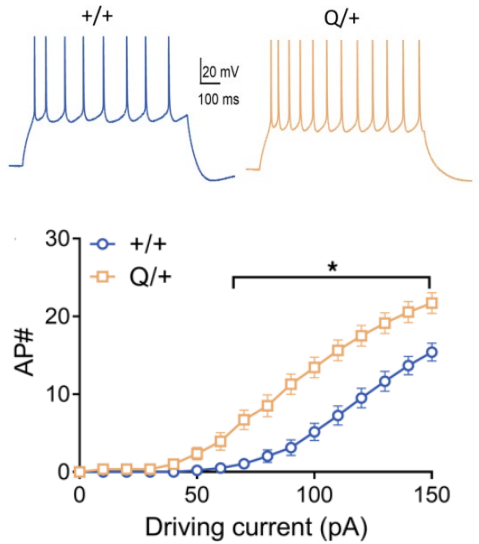
Premature Lethality



Spontaneous Seizure



Neuronal Hyperexcitability

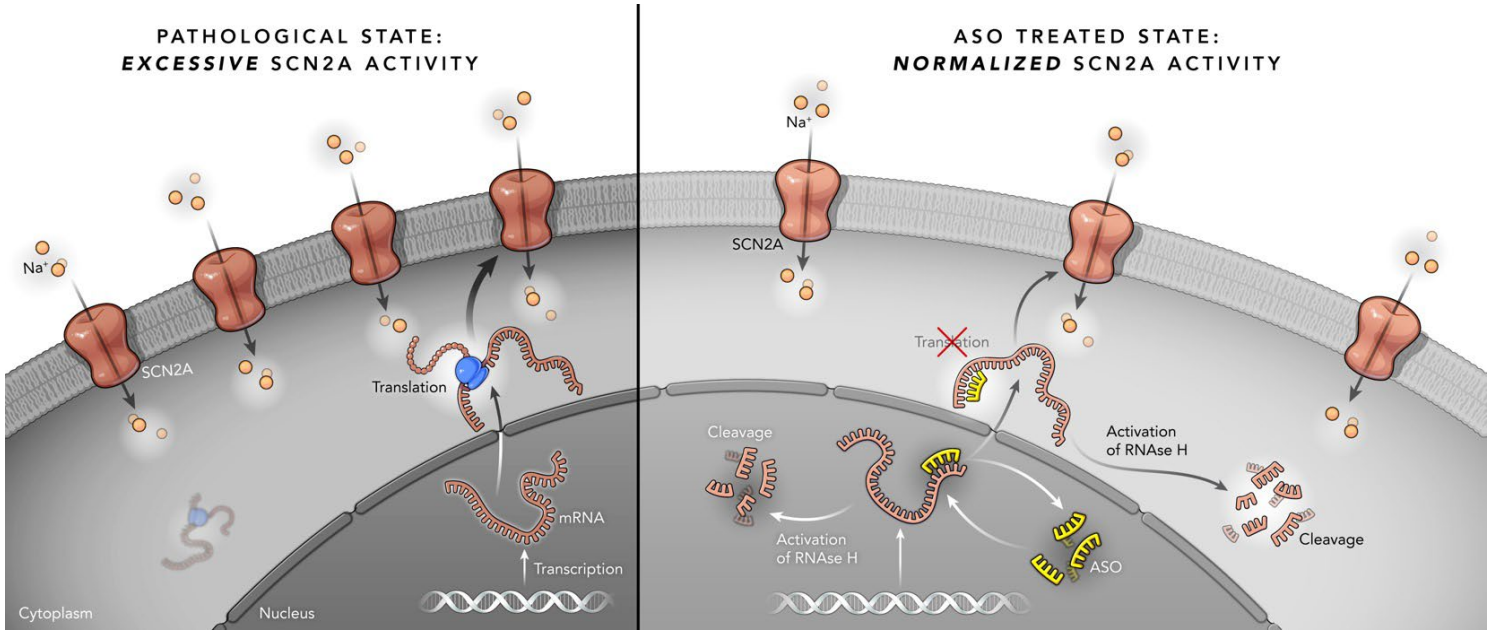


The *SCN2A*-DEE mouse model exhibits premature lethality due to spontaneous seizure driven by neuronal hyperexcitability

Li, M. *et al. J Clin Invest* **131**, e152079 (2021).

Proof of Concept Efficacy Using Mouse Directed ASO to Knockdown mRNA

Mouse SCN2A targeting gapmer ASO (5-10-5 MOE)

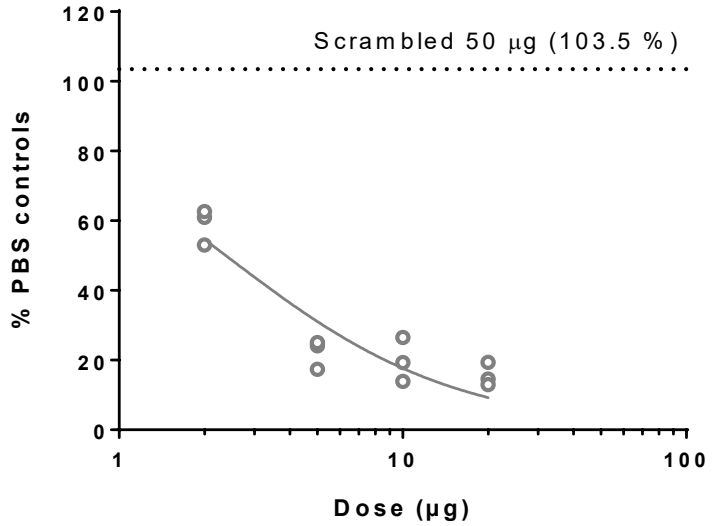


Therapeutic goal: Normalize $Na_V1.2$ activity by reducing *SCN2A* mRNA

Li, M. *et al. J Clin Invest* **131**, e152079 (2021).

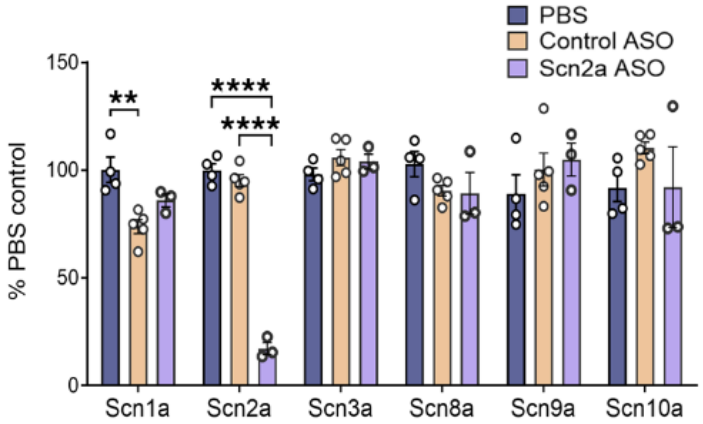
Mouse Scn2a ASO Selectively Reduces Scn2a Expression

Potent Knockdown of Scn2a mRNA in Mouse Cortex Dosing at P1



Age @ ICV	ED_{50} (µg)	ED_{80} (µg)
P1	2.0	8.7

Selective Knockdown of Scn2a mRNA at ED_{80}

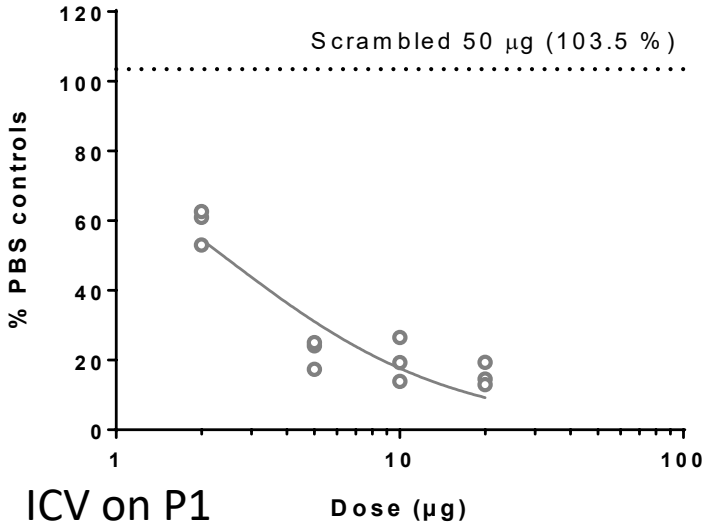


**p<0.01
****p<0.0001
All experiments conducted with SCN2A R1882Q mouse model

Li, M. *et al. J Clin Invest* **131**, e152079 (2021).

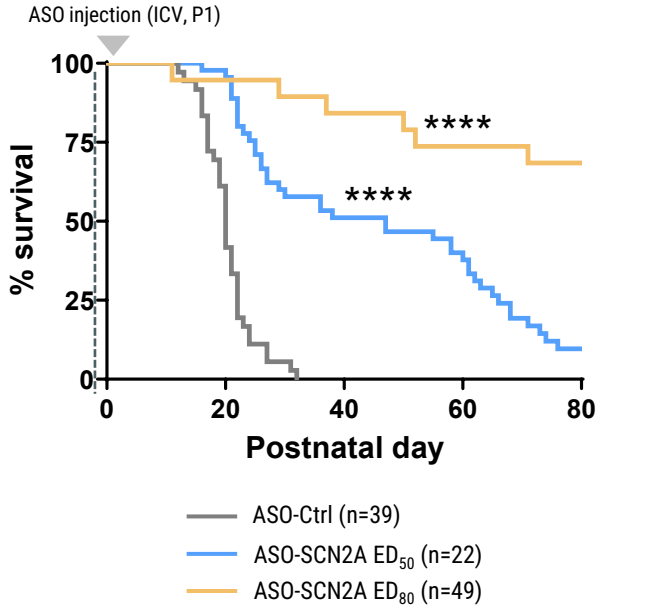
Scn2a ASO Increases Survival of *SCN2A*-DEE mice with a Single Dose

Potent Knockdown of Scn2a mRNA in Mouse Cortex Dosing at P1



ICV on P1

Protection from Premature Lethality

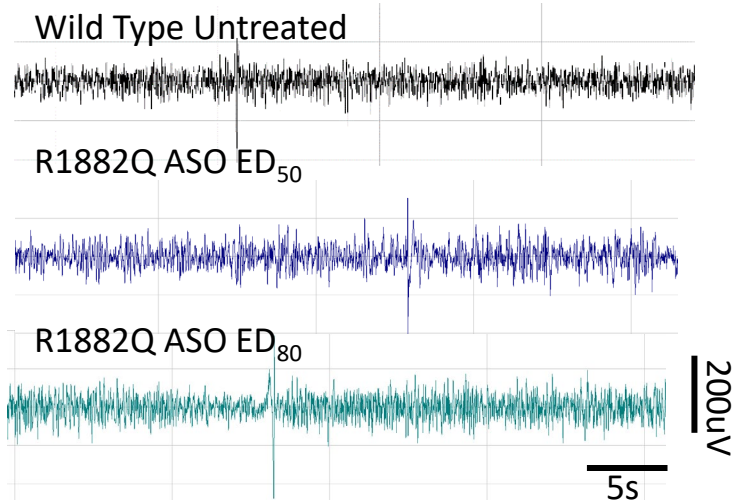


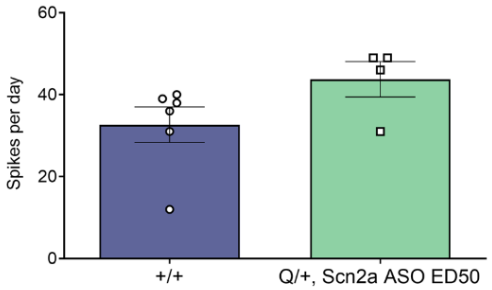
***p<0.001
****p<0.0001
All experiments conducted with SCN2A R1882Q mouse model

Li, M. *et al. J Clin Invest* **131**, e152079 (2021).

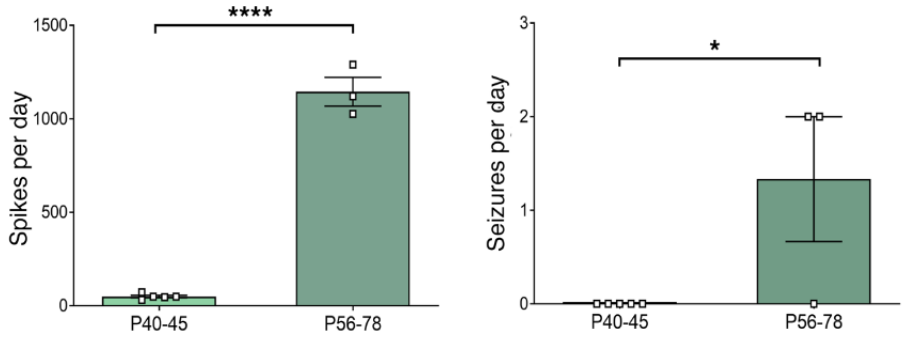
ASO Treatment Significantly Reduces Seizures and Rescues EEG Properties of *SCN2A*-DEE mice

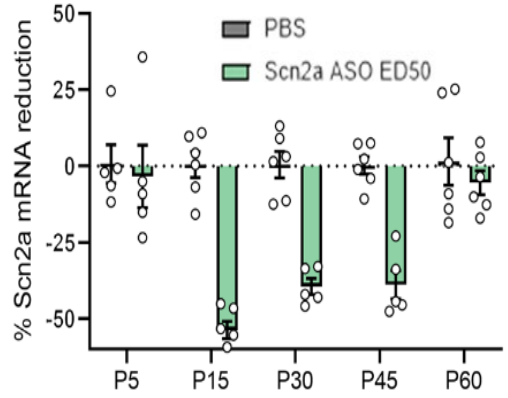
Normalization of Interictal Spikes





Return of Seizures/Spikes with loss of ASO Activity



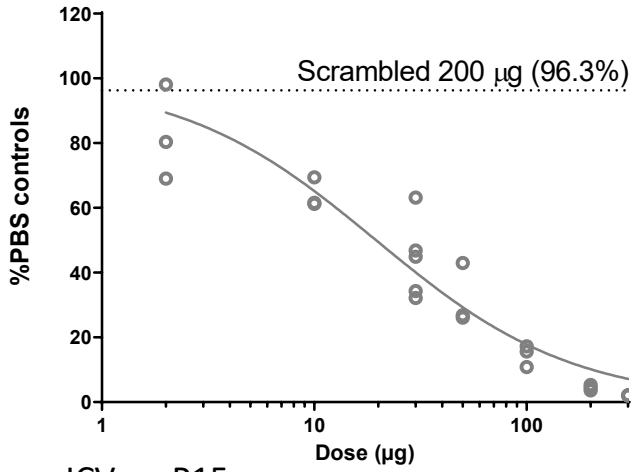


Improved survival associated with reduction in Scn2a mRNA, seizures and interictal spikes

Li, M. *et al. J Clin Invest* **131**, e152079 (2021).

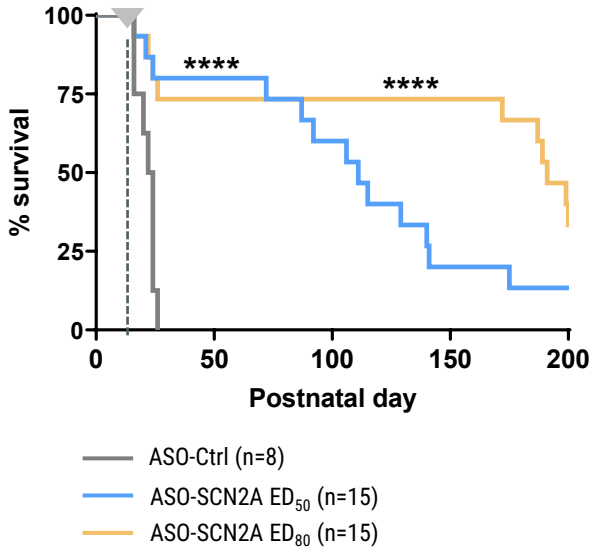
Administration of ASO After Disease Onset Extends Survival of *SCN2A*-DEE Mice

Potent Knockdown of Scn2a mRNA in Mouse Cortex Dosing at P15



ICV on P15

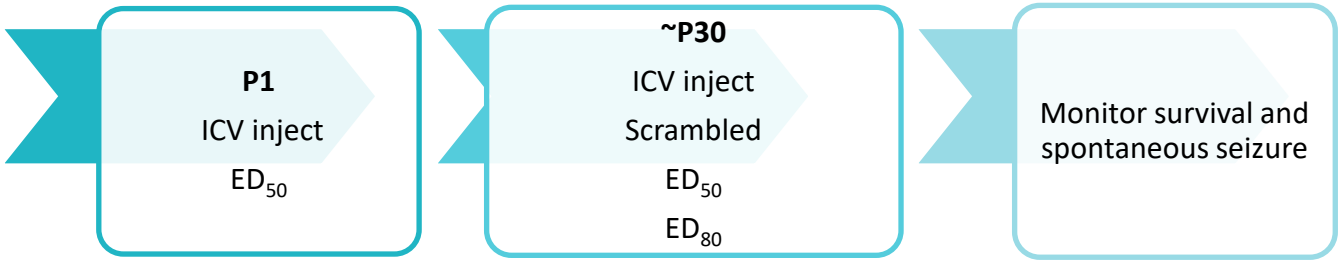
Protection from Premature Lethality



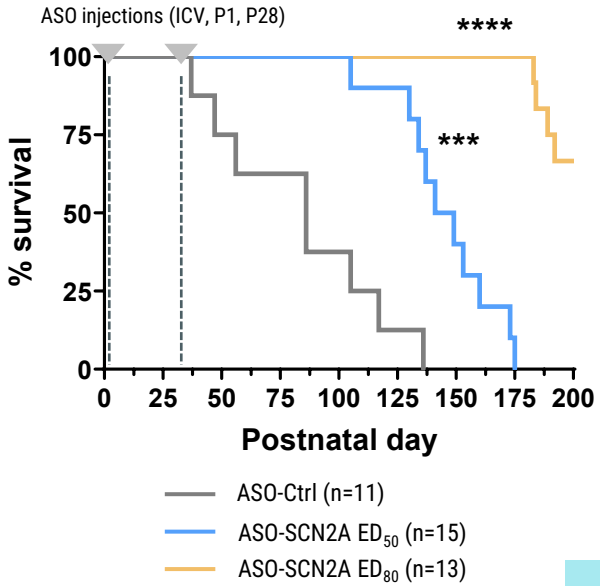
***p<0.001
****p<0.0001
All experiments conducted with SCN2A R1882Q mouse model

Li, M. *et al. J Clin Invest* **131**, e152079 (2021).

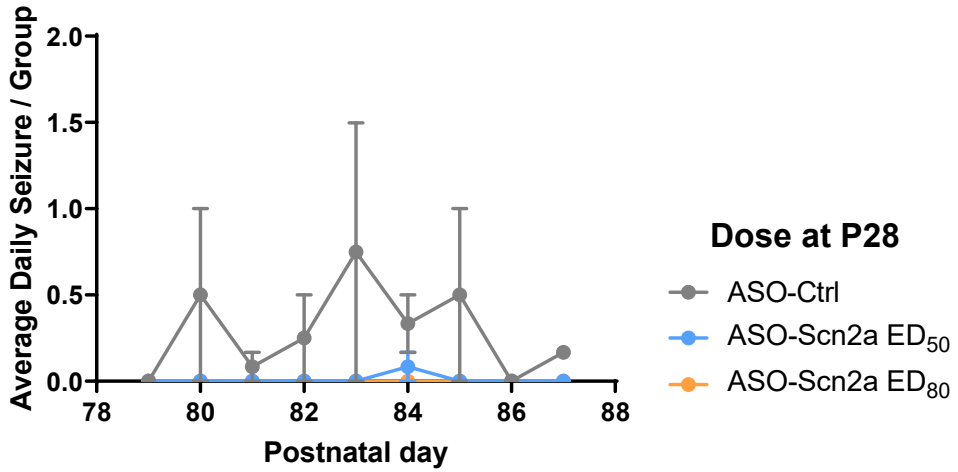
Re-Dosing Significantly Extends Survival of *SCN2A*-DEE mice



Re-Dosing Extends Survival



Seizure Reappearance 50 Days Post-Dose



Survival benefit extended with repeat dosing
Lethality observed as seizures return

Li, M. *et al. J Clin Invest* **131**, e152079 (2021).

Genetics of Epilepsy

- Selection of an appropriate target for ASO modulation

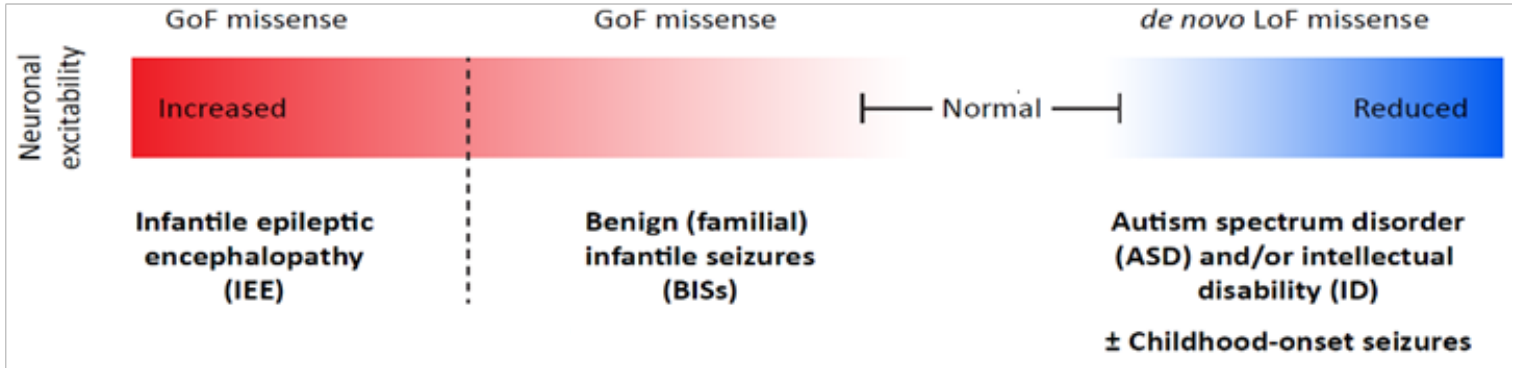
SCN2A-DEE

- Model for evaluating therapeutic intervention
- Model for variant characterization

SCN2A Loss of Function Variants can also Cause Seizures

Onset < 3 months of age -> predicts GoF

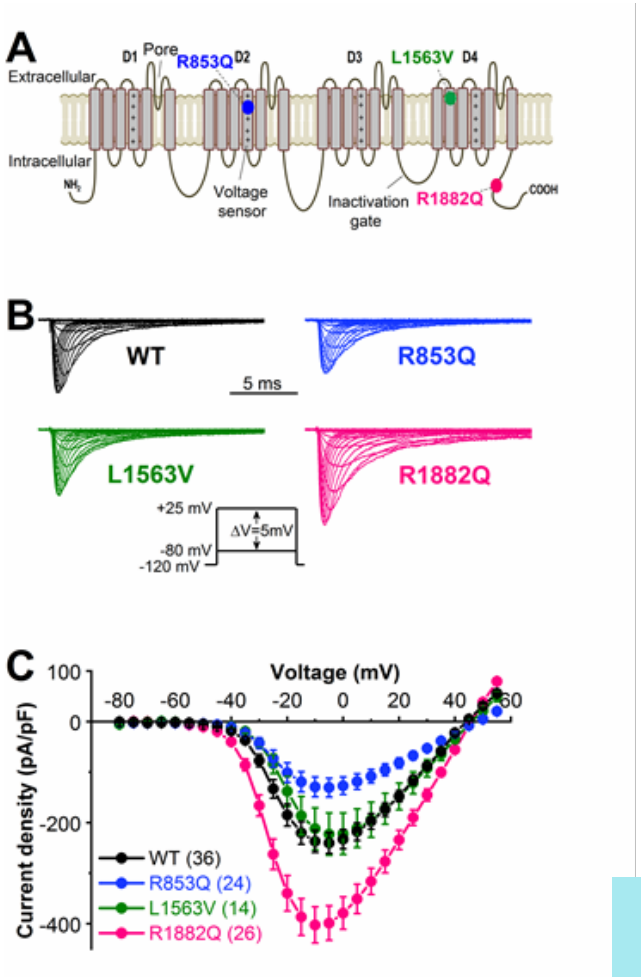
Onset > 3 months of age -> predicts LoF



S. J. Sanders *et al.*, *Trends Neurosci.* **41**, 442–456 (2018).

Assay needed to rapidly identify variants with predicted GoF features

Voltage Clamp Electrophysiology Can Predict Impact of Variant



Voltage Clamp Assessment of Various Gating Parameters

Variant / Phenotype	Change in Voltage Clamp Compared to WT	Predicted Effect on $Na_V1.2$ Activity
R853Q / Infantile Spasms Presents >3 months of age	↓ Current Density ↓ Window Current Left Shift Activation ↑ Slow Inactivation	LoF Decreased Activity
L1563V / BFNIS Presents 0-13 months of age	↑ Window Current ↑ Recovery Fast Inactivation ↓ Slow Inactivation	GoF Increase Activity
R1882Q / DEE Presents 0-3 months of age	↑ Current Density ↑Persistent I_{Na} ↑ Window Current Left Shift Activation Right Shift Fast Inactivation	GoF Increase Activity

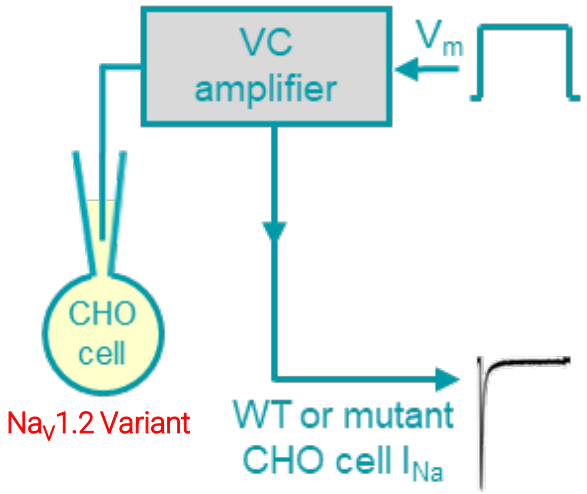
Traditional Assay is Time-Consuming (months) and Incomplete

G. Berecki *et al.*, *Proceedings of the National Academy of Sciences*. 115, E5516–E5525 (2018).

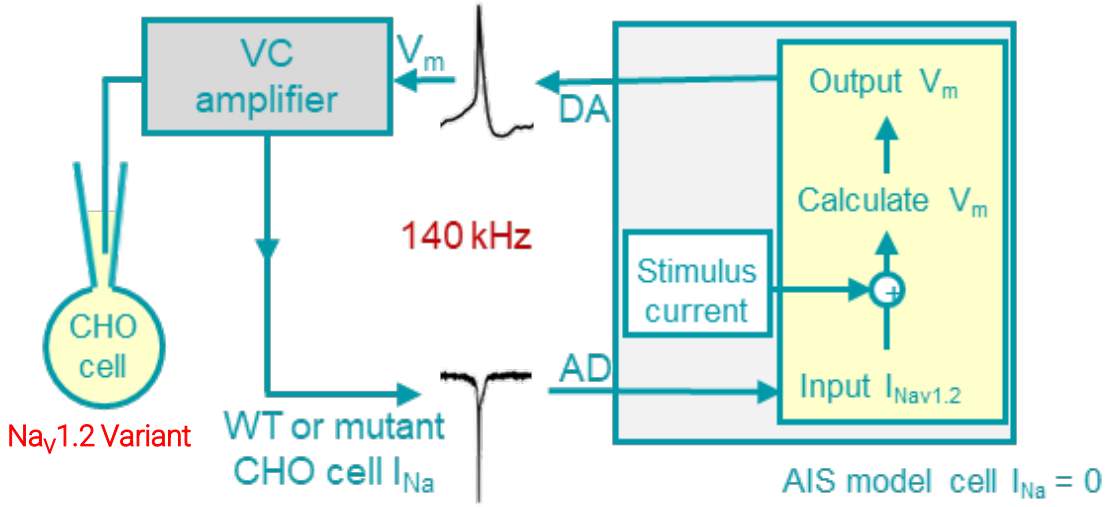
Dynamic Action Potential Clamp (DAPC) Accelerates Characterization of Ion Channel Variants

Assay Measures both VC (selected parameters) and DAPC from each cell

Voltage clamp (VC)

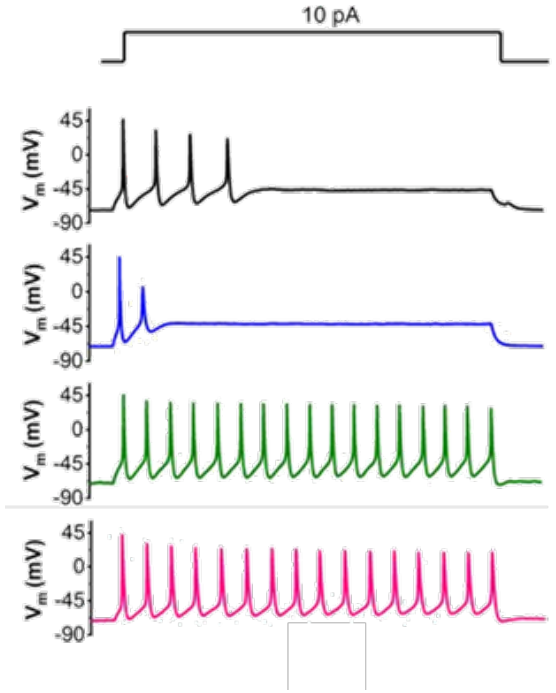


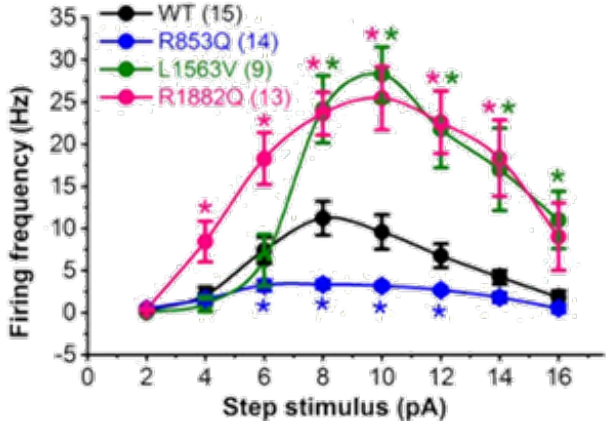
Dynamic action potential clamp (DAPC)



Dynamic Action Potential Clamp Correlates with Predictions Made from Voltage Clamp Analysis

Variant	VC Predicted Effect on $Na_V1.2$ Activity	DAPC Predicted Effect on $Na_V1.2$ Activity
R853Q	LoF Decreased Activity	Decrease in Excitability
L1563V	GoF Increase Activity	Increase in Excitability
R1882Q	GoF Increase Activity	Increase in Excitability





DAPC rapidly identifies variants with GoF features

G. Berecki *et al.*, *Proceedings of the National Academy of Sciences*. 115, E5516–E5525 (2018).; Berecki, G. et al. Commun Biology 5, 515 (2022).

Preclinical Models Inform Early Clinical Development of Oligonucleotides for *SCN2A*-DEE

PRAX-222

INTRATHECALLY-ADMINISTERED ASO WITH POTENTIAL TO BE DISEASE-MODIFYING FOR EARLY ONSET *SCN2A* GoF DEE

INITIATING PHASE 2 TRIAL (EMBRAVE)

ASO modality addresses underlying genetic cause of disease

Estimating degree of target knockdown for therapeutic response

Informing time course of intervention and possibility of disease reversal

Functional testing to inform on variant activity and identify appropriate patients

Acknowledgements

- Melody Li
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- Alex Nemiroff
- Steven Petrou

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Questions?

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