Using Dynamic Action Potential Clamp Modeling of $Na_V1.2$ Variants to Support the Prediction of Clinical Phenotype in DEE

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Drug Discovery for Ion Channels XXII, Feb 18, 2022

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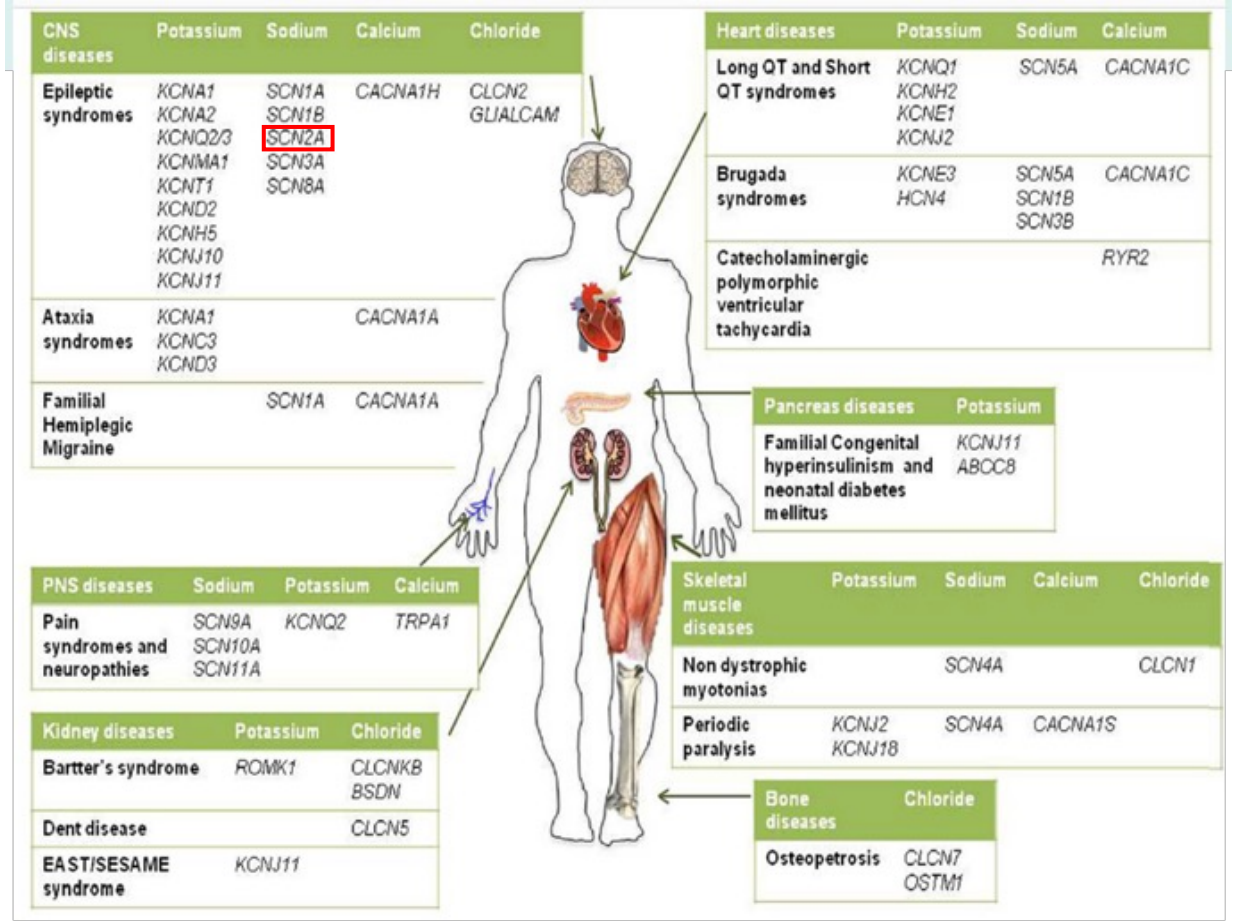
Genetic Variants Cause a Range of CNS Diseases Associated with Altered Neuronal Excitability

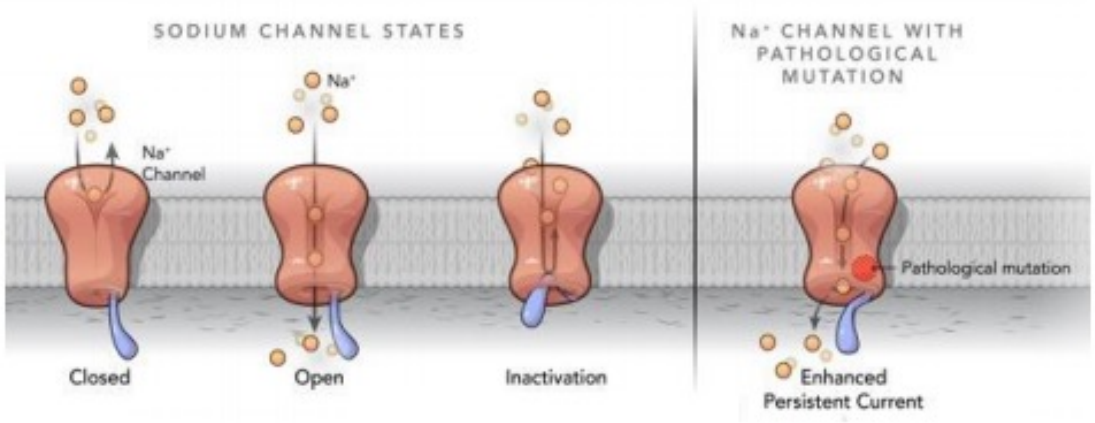


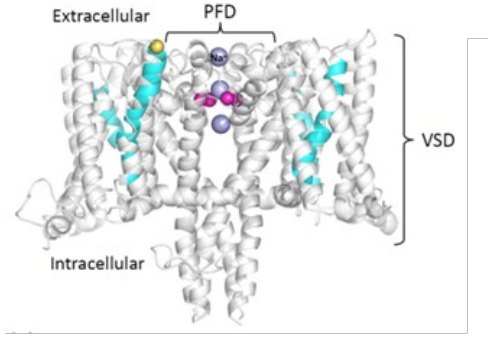
- Disorders of Hyper- and Hypo- Excitability
- Identification of variants is increasingly easy
- Understanding how variants alter activity of the encoded protein is challenging
- Improving variant characterization assays is critical to building a comprehensive understanding of disease and selection of treatment
- Particularly difficult when a single gene causes a spectrum of phenotypes

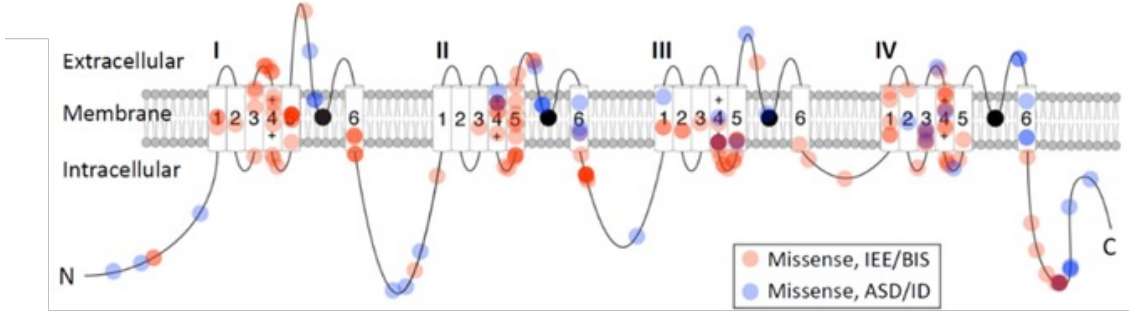
P. Imbrici *et al.*, *Front Pharmacol.* **7**, 121 (2016).

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





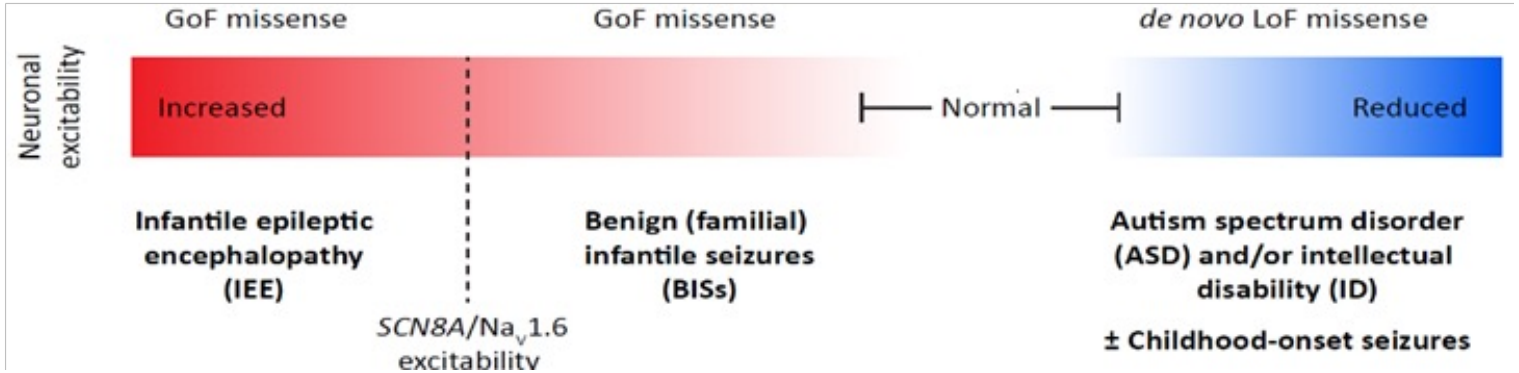


S. J. Sanders *et al.*, *Trends Neurosci.* **41**, 442–456 (2018).

Current Model of SCN2A Pathophysiology in Early Onset versus Later Onset Disease

Onset < 3 months of age -> predicts Gain of Function

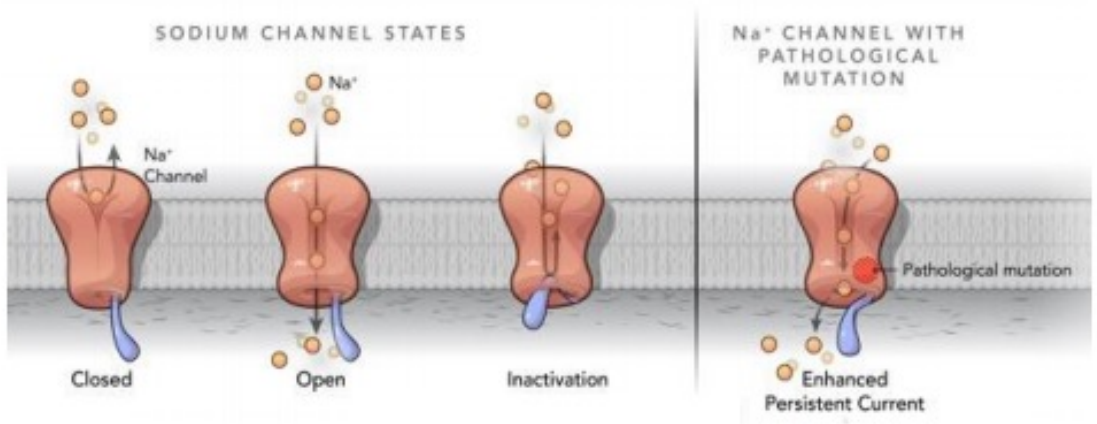
Onset > 3 months of age -> predicts Loss of Function



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

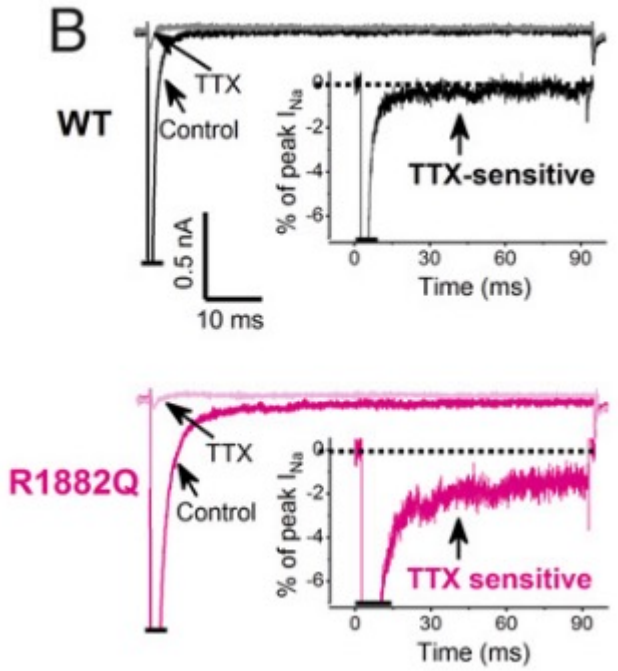
Limitation – Model created from a small subset of SCN2A variants and some variants present with mixed GoF/LoF features

Variants in SCN2A alter $Na_V1.2$ Activity



Persistent I_{Na} is a common feature of $Na_V1.2$ Gain of Function

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).

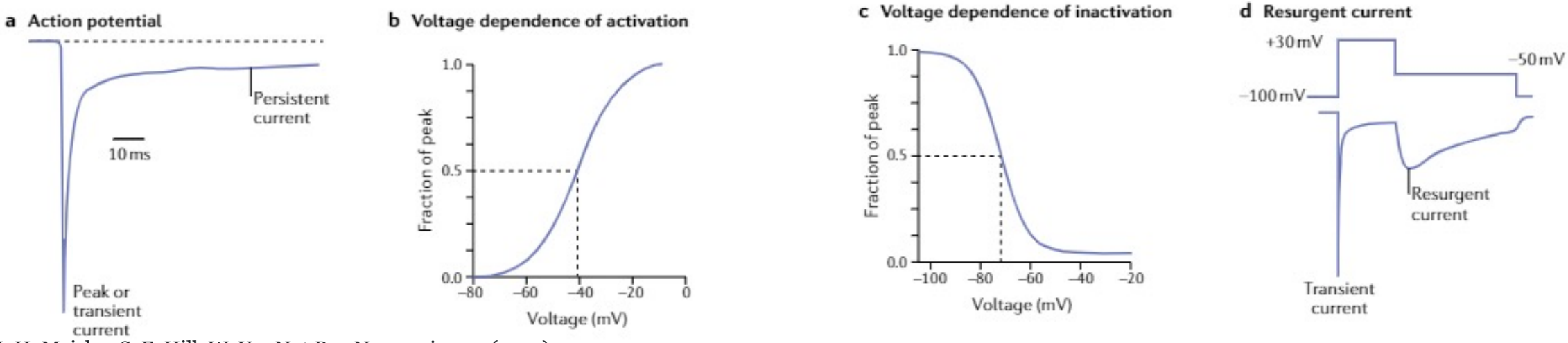
Full characterization of a variant requires measurement of >10 gating activities when using traditional Voltage Clamp

Current Density
I_{Na} Kinetics
Persistent I_{Na}

Activation

Fast Inactivation

Resurgent Current

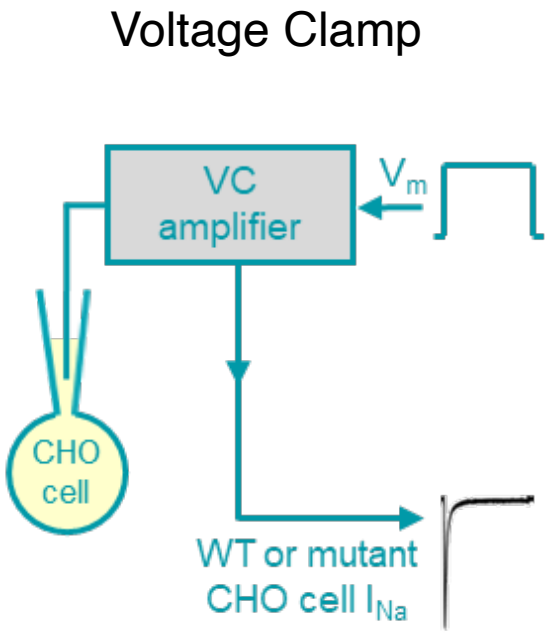


M. H. Meisler, S. F. Hill, W. Yu, *Nat Rev Neurosci*, 1–15 (2021).

Additional properties to measure: Recovery from Fast Inactivation, Use Dependent Activity, Slow Inactivation, Omega Current……. and more

Variant characterization is time intensive using traditional voltage clamp.

Traditional Assessment of Na_V Variants Employs Multiple Voltage Clamp Assays to Measurement Properties in Isolation



- Measure one property at a time
- Time intensive (per variant)

Manual Patch Clamp	1-1.5 months
Automated Patch Clamp	2-3 weeks

- Integrate findings using experience and intuition to make call of GoF of LoF
- Recent progress has been made adapting automated electrophysiology platforms to variant characterization in voltage clamp mode

Thompson et.al. #216, AES Meeting 2020.

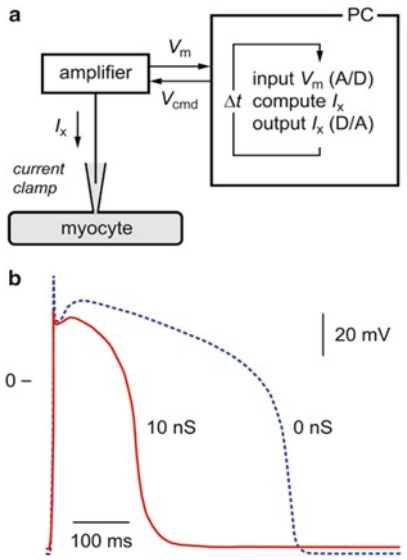
Rapid assay needed to quickly and confidently sort variants in to probable GoF and probable LoF categories at time of clinical presentation

Dynamic Action Potential Clamp Integrates a Real Conductance into a Simulated Action Potential

"Conventional" DC

- ~ 1 simulated conductance
- Measure effect on real action potential

Scott, 1979; Tan and Joyner, 1990 – cardiac e-phys.
Sharp et al. 1993a,b – neurobiology

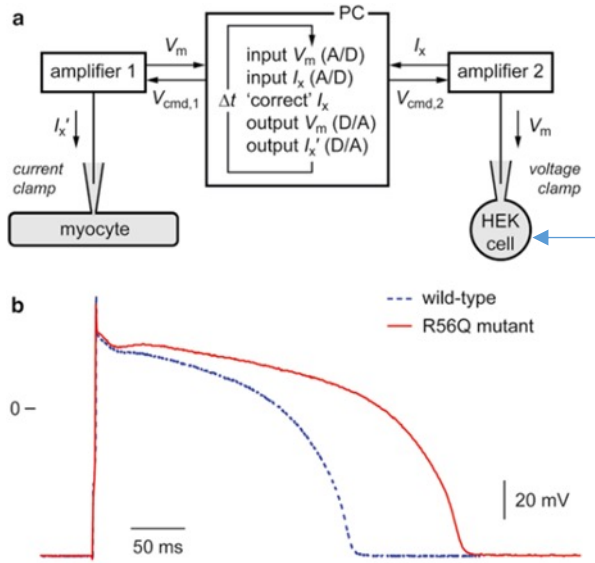


Verkerk et al. 2000

Dynamic action potential clamp

- ~ 1 real conductance
- Measure effect on simulated action potential

Berecki et al. 2005, 2006 – cardiac channelopathy
Berecki et al. 2018, 2019 – CNS channelopathy

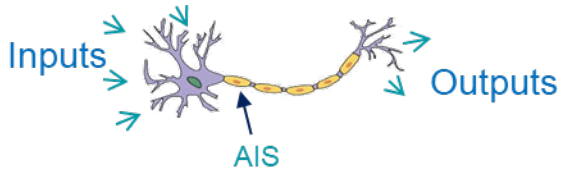


Na_V Variant

Berecki et al. 2005

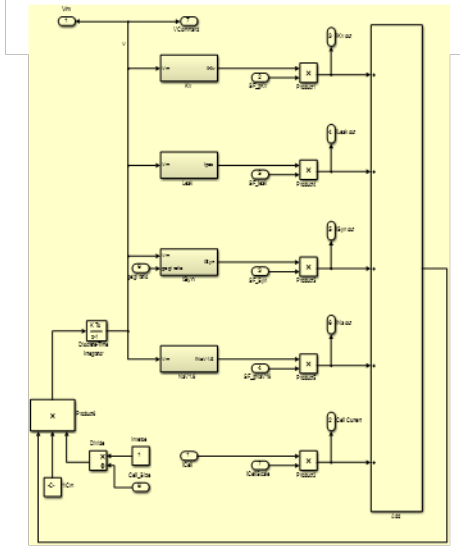
Figures from Berecki et al. 2014. Methods Mol Biol. 1183: 1183:309–326

Dynamic Action Potential Clamp uses AIS model to Predict Impact on Cellular Excitability

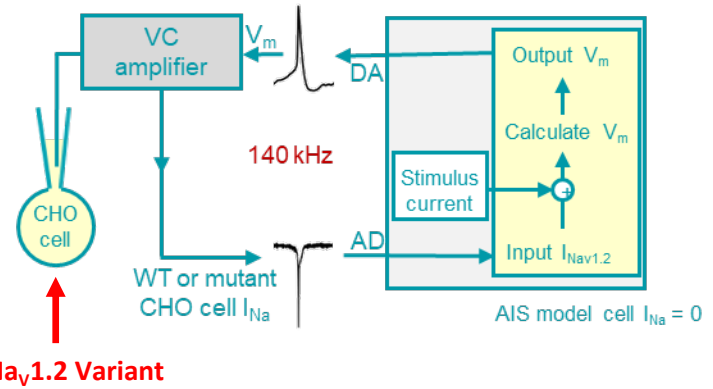


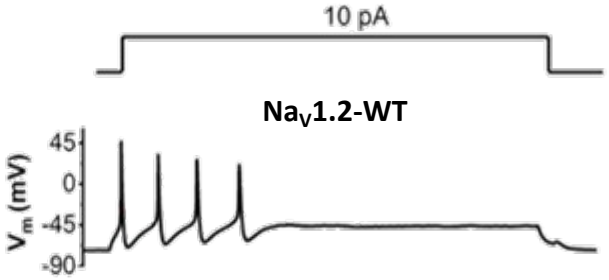
AIS model cell

Hu et al. 2009. Nat Neurosci 12:996–1002



Dynamic action potential clamp (DAPC)



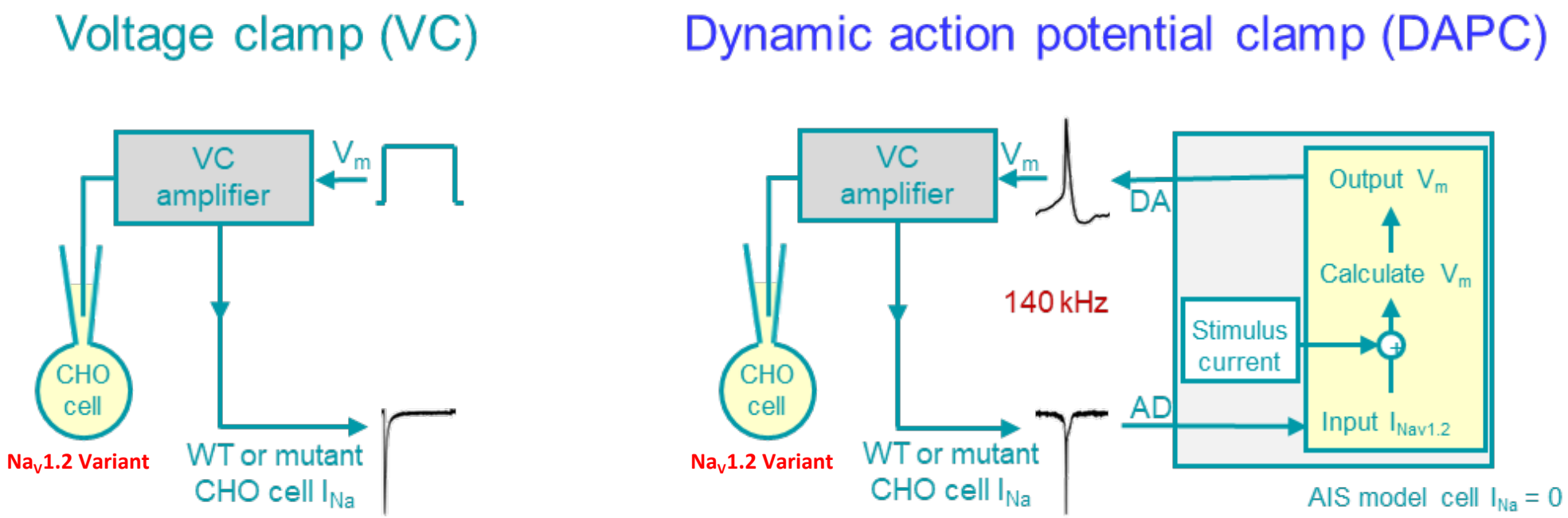


$$(C * CellSize)\frac{dVm}{dt} = SF\_gKv * I_{Kv} + SF\_leak * I_{pas} + SF\_Syn * I_{Syn}$$
$$+SF\_gNaV1.6 * I_{NaV1.6} + ICellScale * I_{Cell}$$

- Cell capacitance (Cm),
- Passive leak current (I_{pas}),
- Potassium current (I_{Kv}),
- Sodium $Na_v1.6$ current ($I_{Nav1.6}$),
- Excitatory & inhibitory synaptic currents (I_{Syn}),
- External input $Na_v1.2$ current (I_{Cell}).

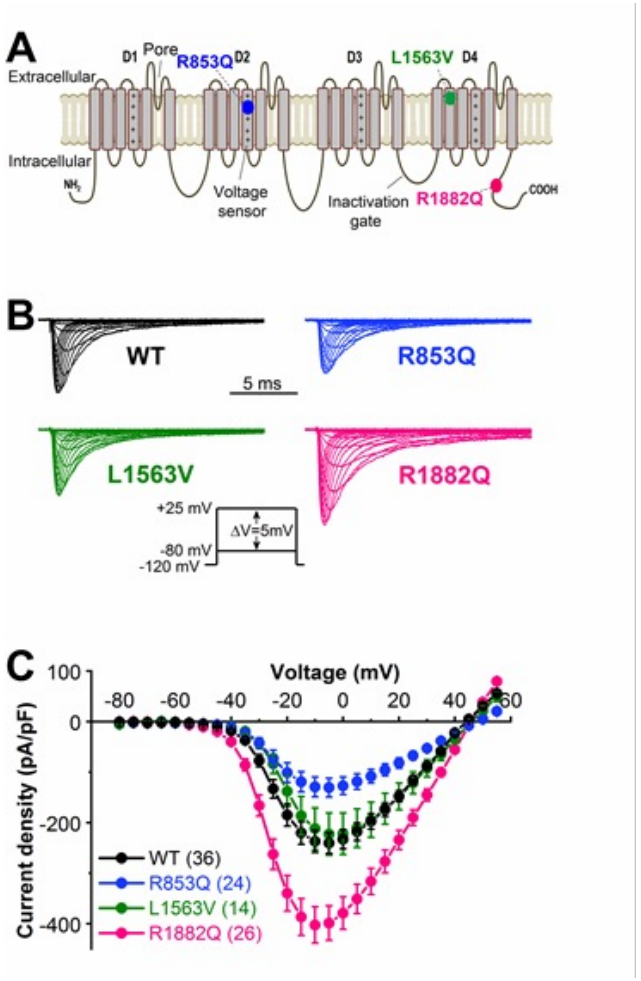
Dynamic Action Potential Clamp Accelerates Characterization of Ion Channel Variants

Measure both VC (selected parameters) and DAPC from each cell



Rapid prediction of the impact of a variant on excitability

Previous Work Using Dynamic Clamp to Predict Affect of SCN2A Variation on Neuronal Excitability



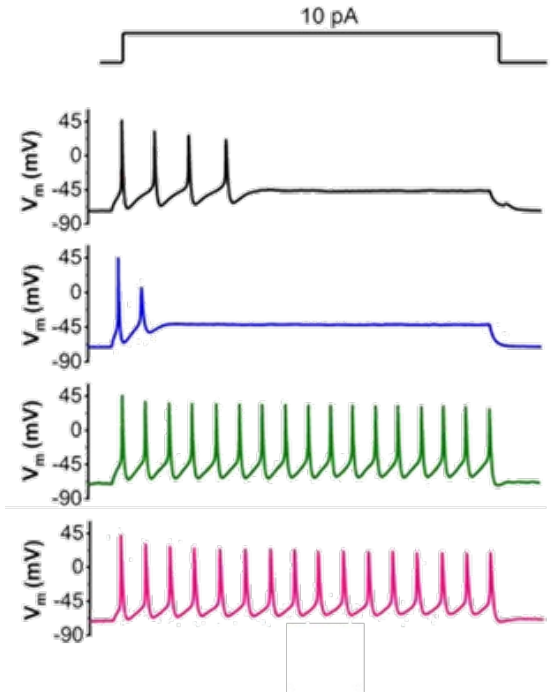
Voltage Clamp Assessment of Various Gating Parameters Cleanly Predicts Gain or Loss of Function for this Set

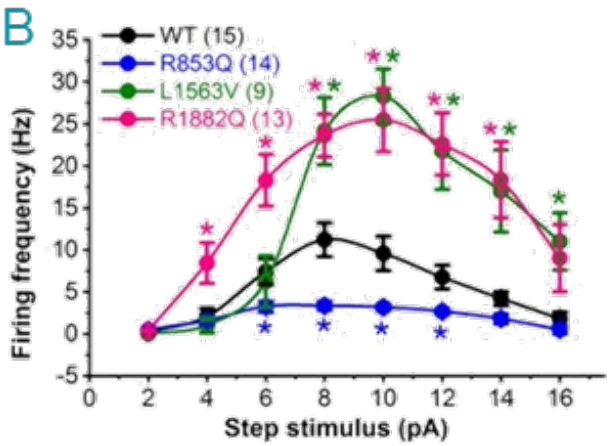
Variant / Phenotype	Change in Voltage Clamp Compared to WT	Predicted Effect on $Na_V1.2$ Activity
R853Q / Infantile Spasms Presents >3 months of age	↓ Current Density ↓ Window Current Left Shift Activation ↑ Slow Inactivation	LoF Decreased Activity
L1563V / BFNIS Presents 0-13 months of age	↑ Window 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

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

Number of Dynamic Clamp Evoked Action Potentials 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



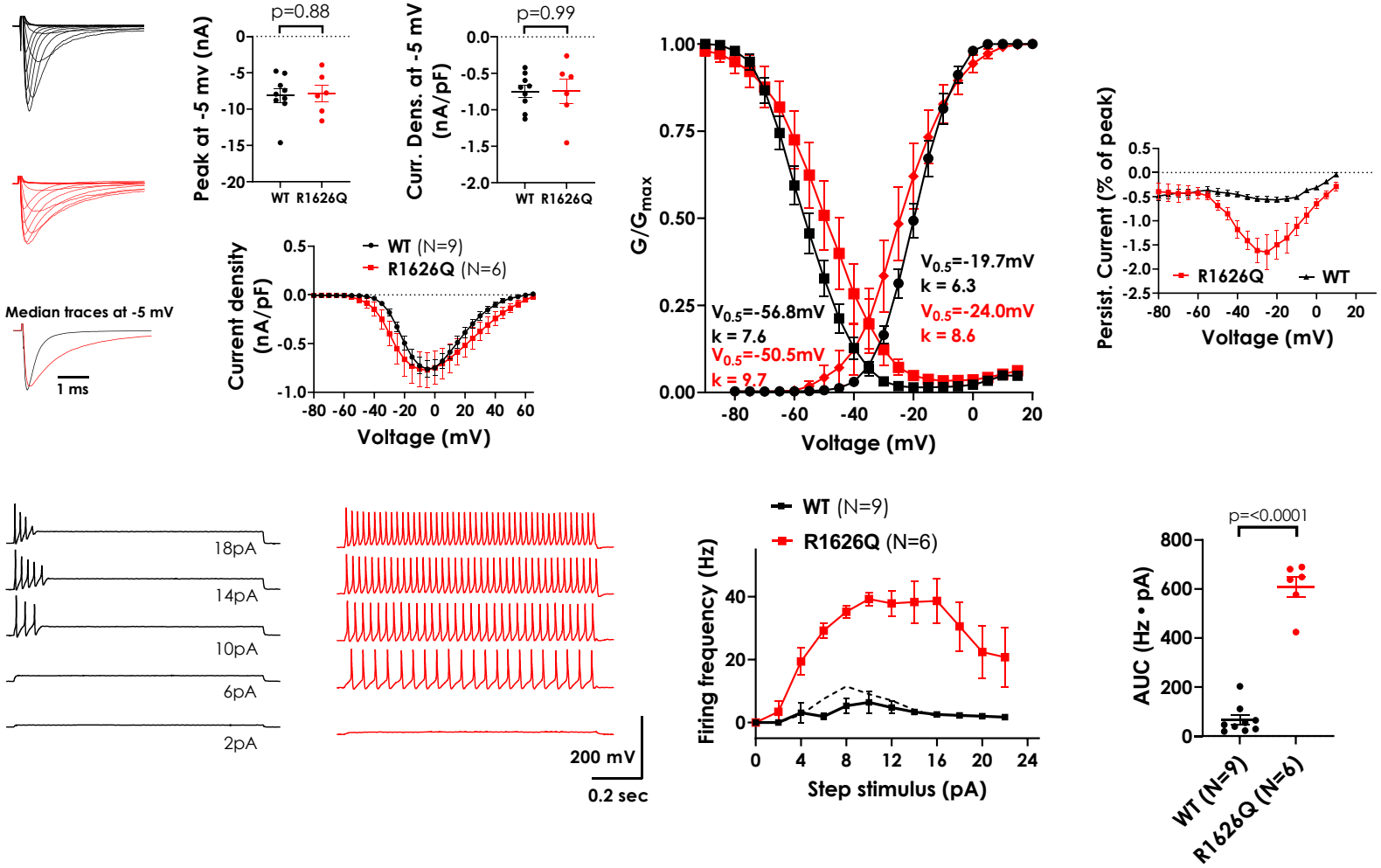


Encouraging results, need to expand analysis to larger set of variants

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

Dynamic Action Potential Clamp Predicts Early Onset DEE Variant R1626Q Causes Neuronal Excitability

SCN2A WT vs R1626Q



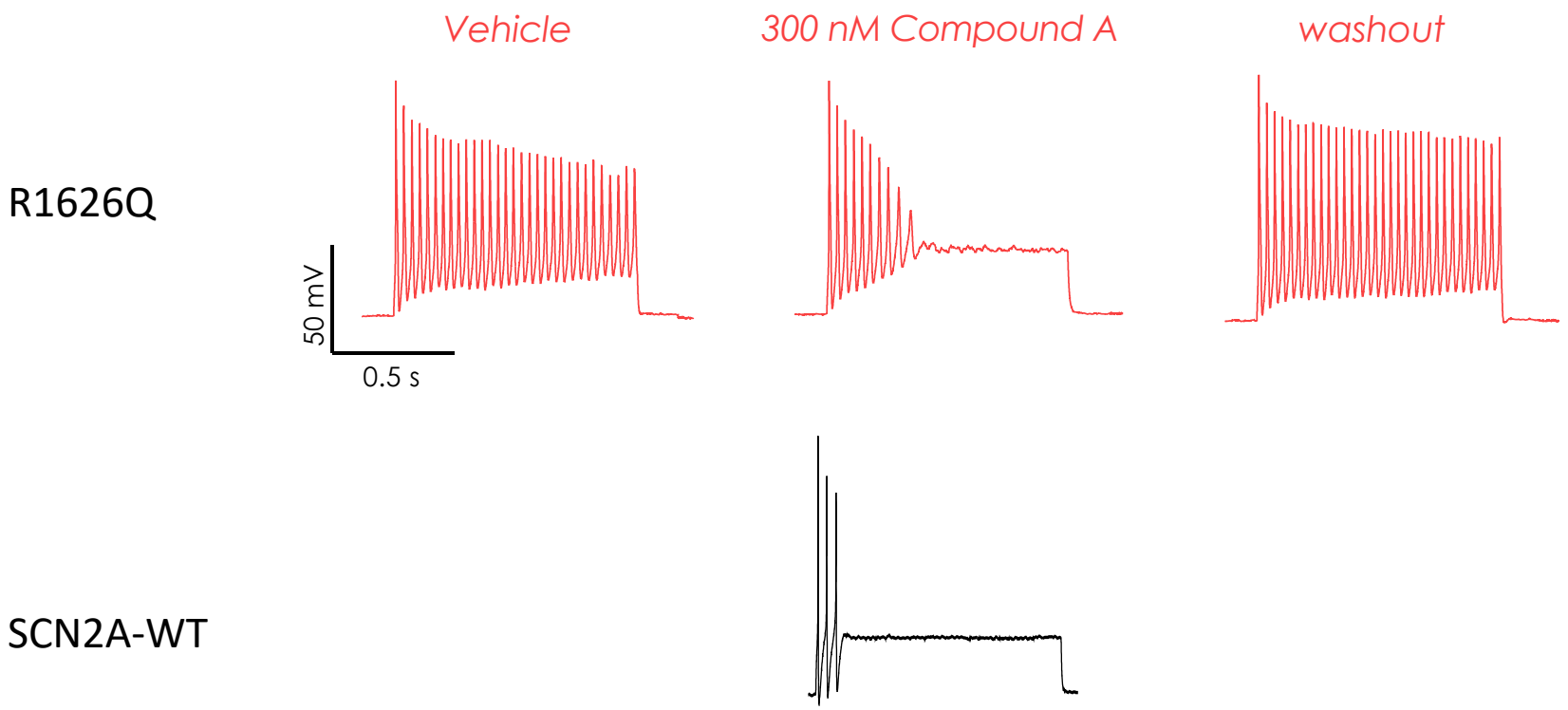
Summary of R1626Q

Parameter	R1626Q	Prediction
Current Density	No Change	No Change
Whole Cell Current Decay	Slower	GoF
Persistent INa	Increase	GoF
Activation	Left Shift	GoF
Fast Inactivation	Right Shift	GoF
Dynamic Clamp		Predicts GoF

GoF, Gain of Function

Pharmacological Screening Using Dynamic Action Potential Clamp

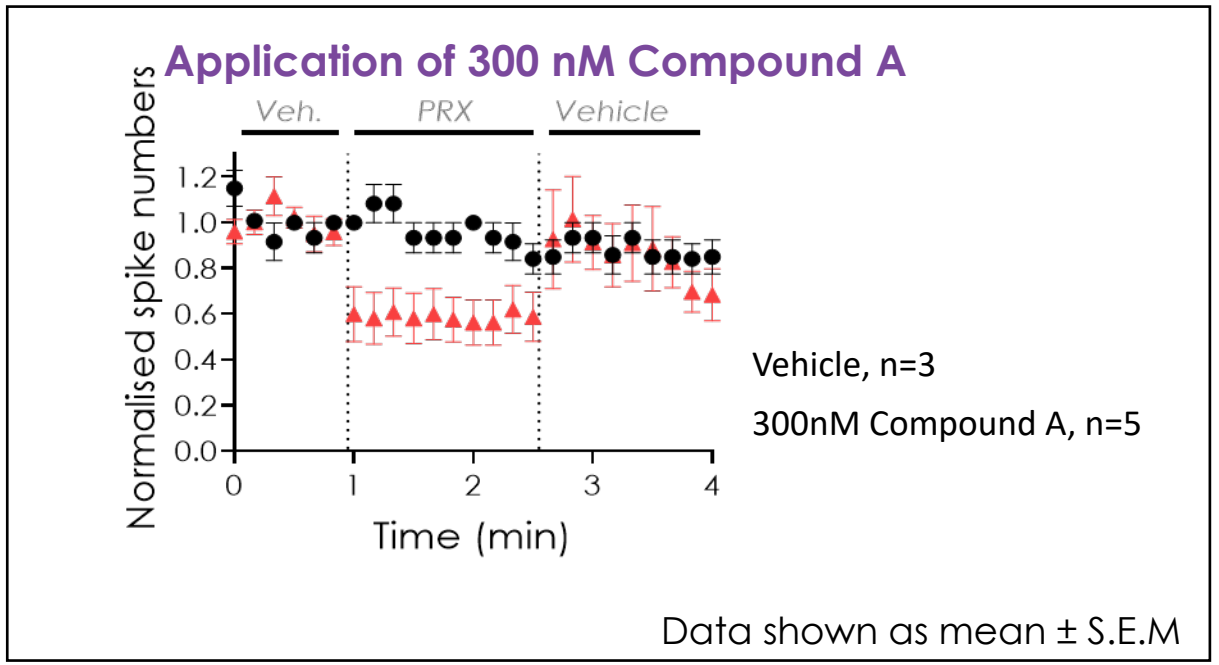
Reversal of R1626Q Gain of Function With Acute Application of Compound A



- Protocol: 1s current step at maximal spiking frequency (10 pA for R1626Q)
  - 10 s sweep – sweep interval

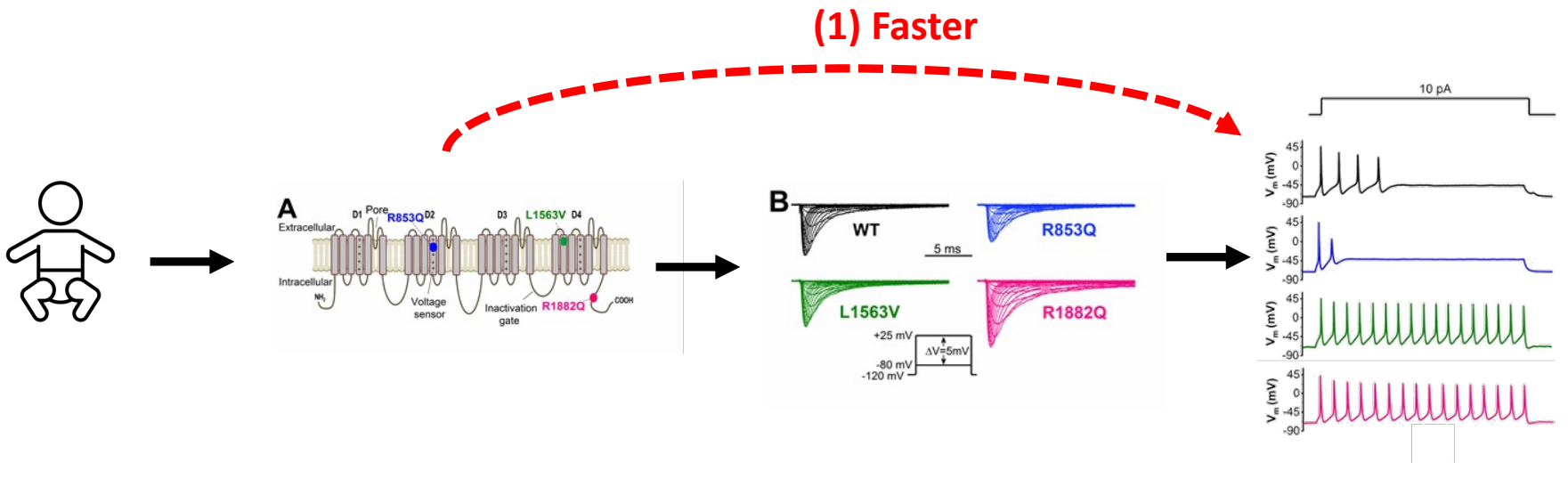
Pharmacological Screening Using Dynamic Action Potential Clamp

Reversal of R1626Q Gain of Function With Acute Application of Compound A



Platform to investigate the acute reversal of a GoF $Na_V1.2$ phenotype

Why the Focus on Dynamic Action Potential Clamp?



(2) Selection of Treatment

Standard of Care
Small Molecule Nav Inhibitor
Carbamazepine, Oxcarbazepine, etc.

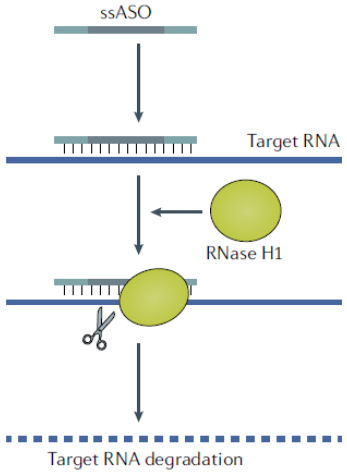
Precision Therapy

Future Precision Options
ASO targeted to *SCN2A*

Selective reduction of $Na_V1.2$ expression could be ideal for SCN2A-DEE patients

Antisense Oligonucleotides Are a Novel Method of Modulating Na_Vs

Mechanism of ASO Mediated RNA Knockdown



S. T. Crooke, B. F. Baker, R. M. Crooke, X. Liang, *Nat Rev Drug Discov*, 1–27 (2021).

Advantages of ASOs over Small Molecules

- Absolute selectivity for isoform of interest
- Consistent modulation of target due to long half-life (minimal C_{max}/C_{min})
- Restricted CNS activity with intrathecal dosing

Long duration of ASO activity requires thoughtful patient selection

DAPC should rapidly identify patients who will not tolerate $Na_V1.2$ knockdown

Proof of Concept: Antisense Oligonucleotide Mediated Efficacy in Mouse Model of Scn2a-DEE (R1882Q)

Therapeutic Strategy

$Na_V1.2$ Gain of Function

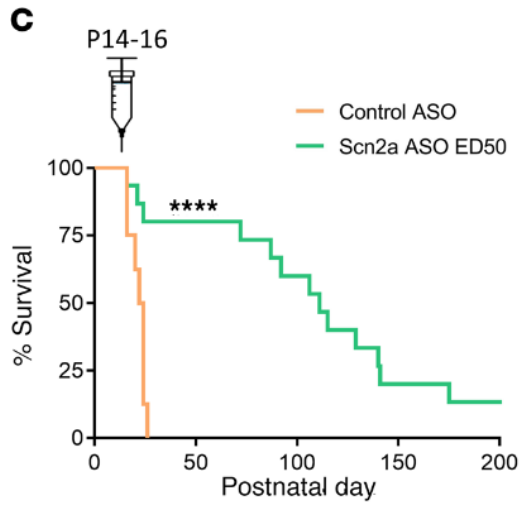
↓

Treat with ASO

↓

Reduced $Na_V1.2$ Activity

Reducing Scn2a RNA protects against premature lethality



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

DAPC may fill the critical need to quickly identify patients appropriate for a small molecule or ASO based therapy

Limitations of Dynamic Action Potential Clamp

- Currently only available as module on manual patch clamp stations
  - Slow and labor intensive
  - Engineering and programming challenges
  - Dedicated hardware for real-time signal processing

- Adaptation to automated instruments plausible
  - Absolute requirement for high quality / low noise recordings
  - Scaling hardware/software represent primary challenge

- Beyond AIS model - need to establish and validate additional neuron models

Conclusions and Future Plans

- Dynamic Action Potential Clamp directly predicts the impact an ion channel variant has on neuronal excitability
  - Contribution of variant to AP firing is determined without making assumptions to the voltage dependence or kinetic properties of the channels
  - Approach is well positioned to advance functional studies and drug discovery
- Future studies will expand SCN2A variants tested to further evaluate correlation between GoF/LoF prediction from DAPC and clinical phenotype

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