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High throughput screening of missense variants in *KCNH2*

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Dr Victor Chang AC 1936-1991, Pioneering Cardiothoracic Surgeon and Humanitarian



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New Years Eve



Feb 10, 2020



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Why?

Goal: To establish a causally cohesive link from genotype to phenotype for every variant in every gene (starting with *KCNH2*).

- (i) Potential consequences of a pathogenic *KCNH2* variant are severe
(1/59 genes currently listed on ACGM guidelines for reporting incidental findings)

LQTS: 1:2000, LQTS2: 1:5000

SCD risk (overall): 13% before age 40, ~0.3% p.a.

KCNH2 variants:

Pathogenic: ~0.02%

Total with MAF<0.1% (Gnomad): ~0.8%

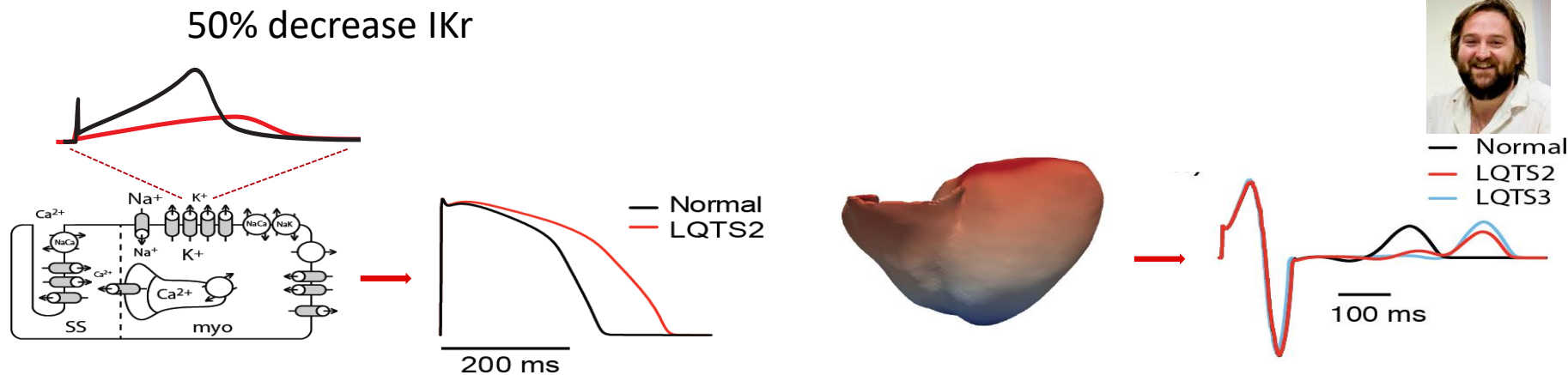
>95% are benign

>99% will not have a SCD before age 40

Why?

Goal: To establish a causally cohesive link from genotype to phenotype for every variant in every gene (starting with *KCNH2*).

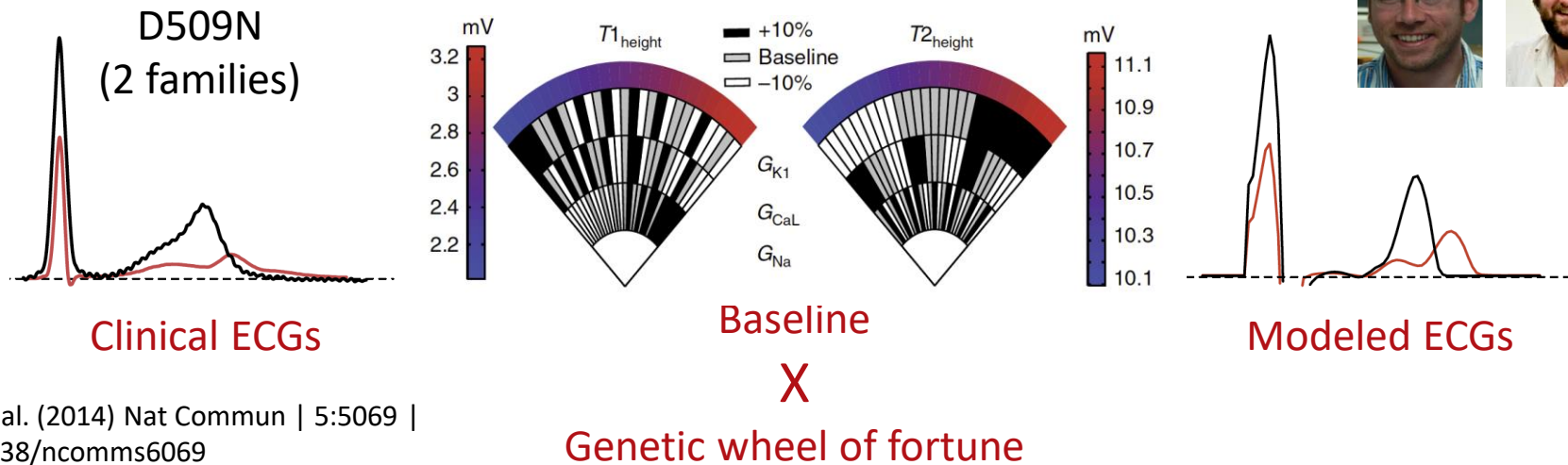
(ii) Disease mechanism for *KCNH2* LOF variants is very well understood



Why?

Goal: To establish a causally cohesive link from genotype to phenotype for every variant in every gene (starting with *KCNH2*).

(iii) Can model effects of genetic background (and environmental insults)

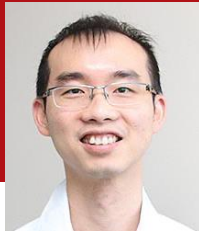


How ?

Functional characterisation of variants

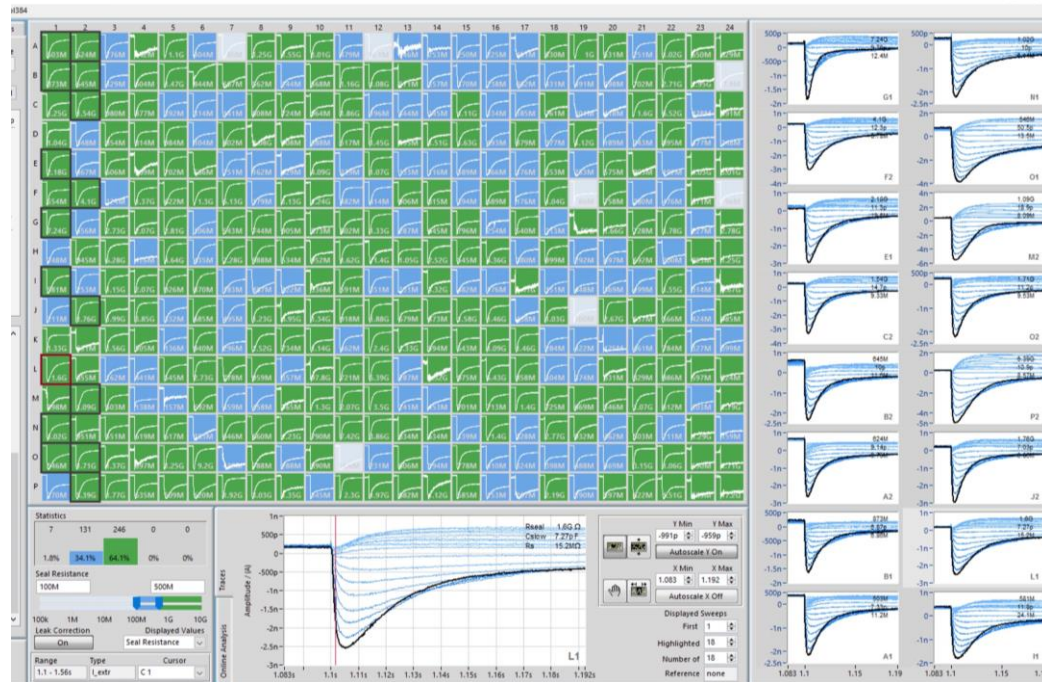
- a) Variants synthesised (gene blocks)
- b) Bi-cistronic vector to express WT + variant alleles
- c) Flp-In cell line system to establish stable cell lines with heterozygous variants
- d) Syncropatch 384 PE automated patch engine
 - single hole wells
 - 10 lines (32 wells each) + positive controls on each plate
 - all test lines repeated in duplicate
 - Overall, 5 mutants per plate

How ?



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Phase 1: Validation with known mutants



Quality Control

$R_{\text{seal}} > 300 \text{ M}\Omega$

$R_s < 20 \text{ M}\Omega$

Capacitance 5-50 pF

$\text{Leak}_{(-80/-120 \text{ mV})} < 40 \text{ pA}$

Success rate (20 plates): 65%



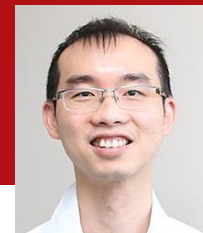
Heart Rhythm
Available online 23 September 2019
In Press, Uncorrected Proof



High-throughput phenotyping of heteromeric human ether-à-go-go-related gene potassium channel variants can discriminate pathogenic from rare benign variants

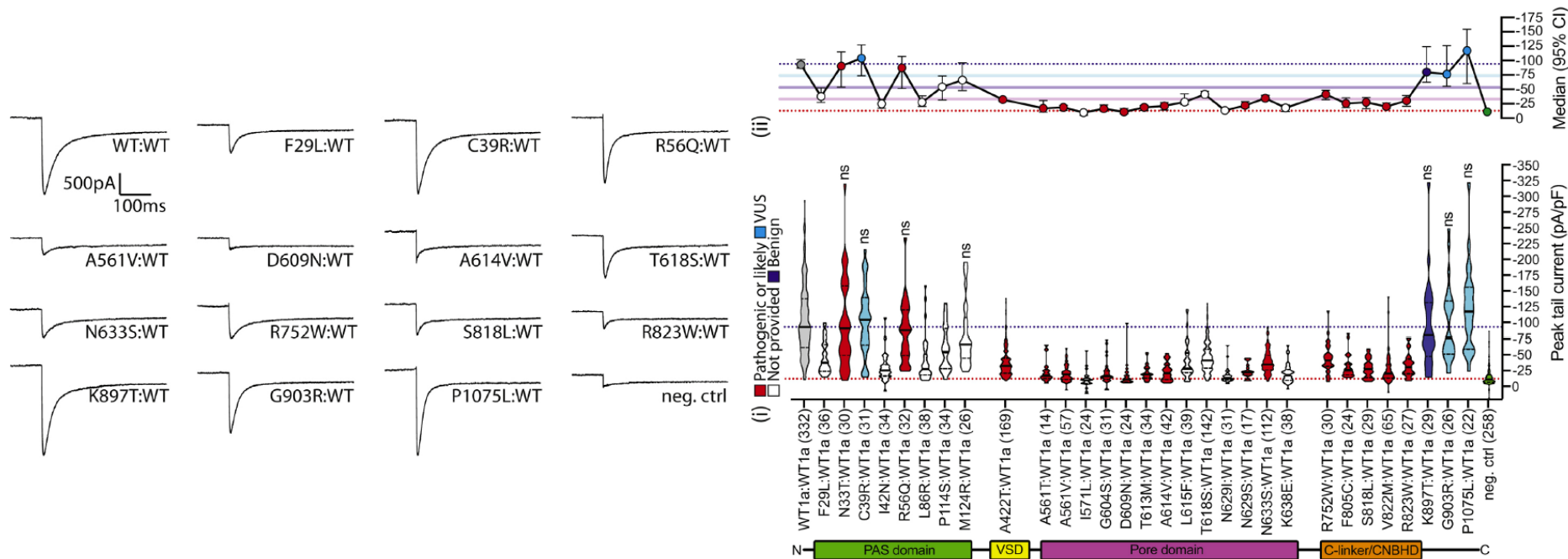
Chai-Ann Ng PhD ^{*,†,1}, Matthew D. Perry PhD ^{*,†,1}, Whitney Liang ^{*,†}, Nicola J. Smith PhD ^{*,†}, Brian Foo PhD [†], Alvin Shrier PhD [†], Gergely L. Lukacs MD, PhD ^{†,§}, Adam P. Hill PhD ^{*,†}, Jamie L. Vandenberg MBBS, PhD, FHRS ^{*,†}

How ?

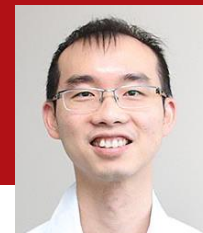


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Phase 1: Validation with known mutants – current density summary



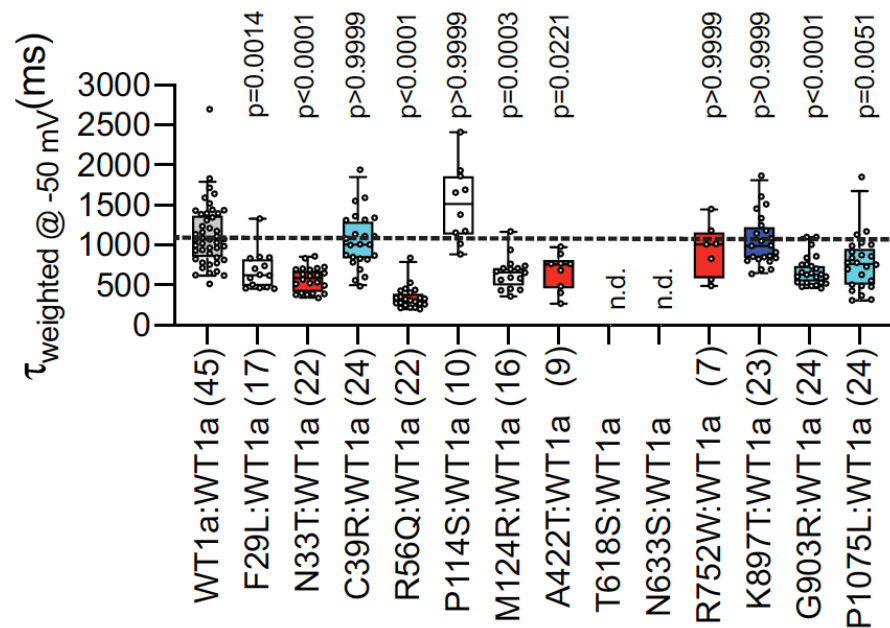
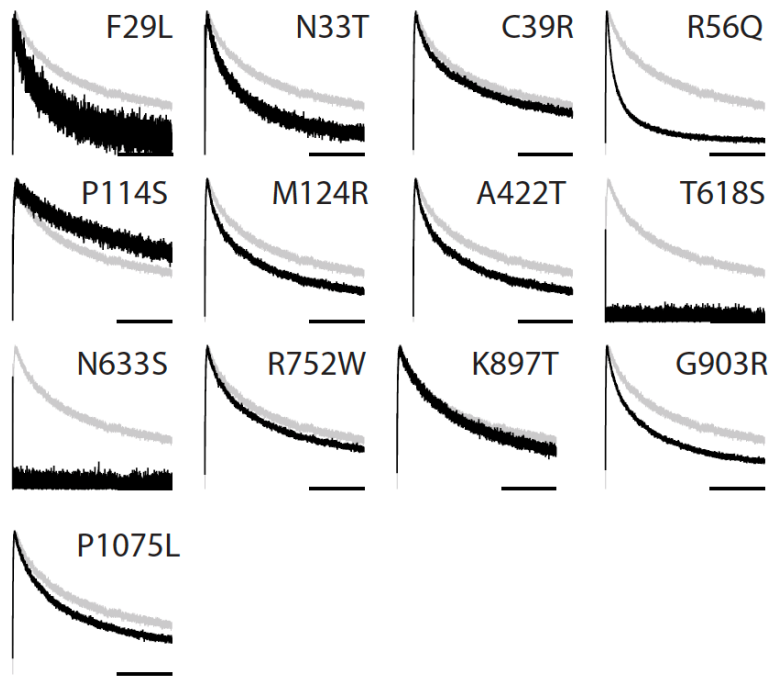
How ?



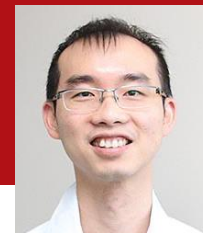
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Phase 1: Validation with known mutants – rate of deactivation

Manual fitting of bi-exponential decay



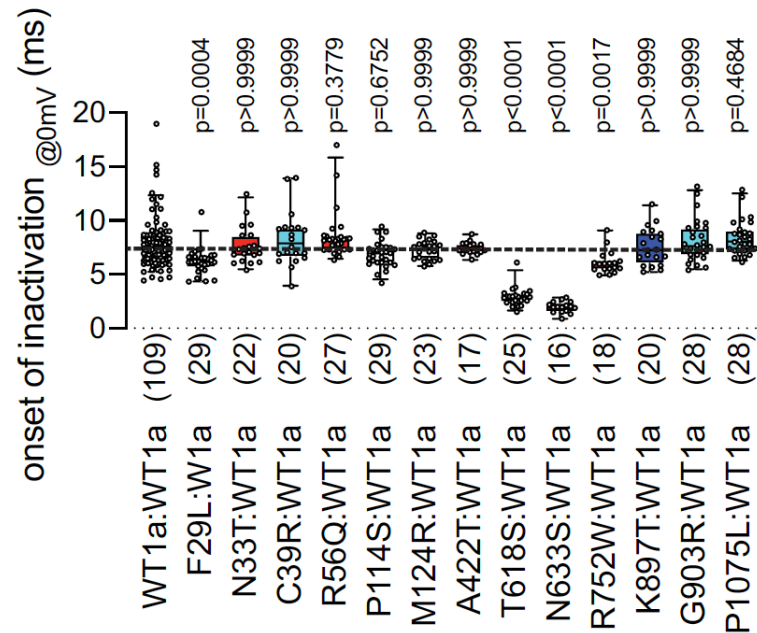
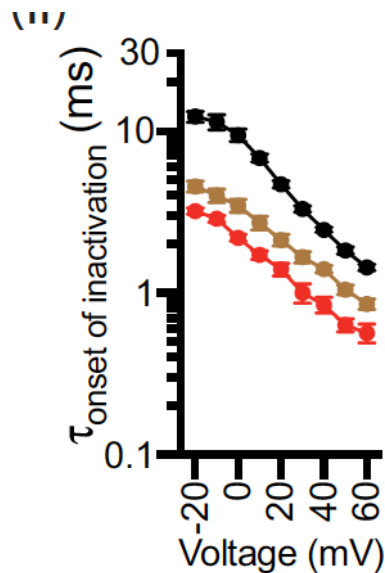
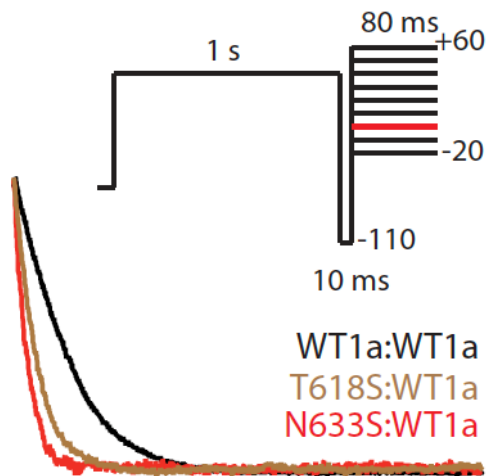
How ?



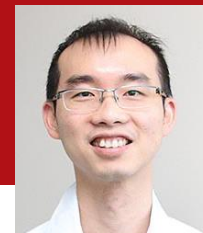
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Phase 1: Validation with known mutants – rate of inactivation

Manual fitting of exponential decay

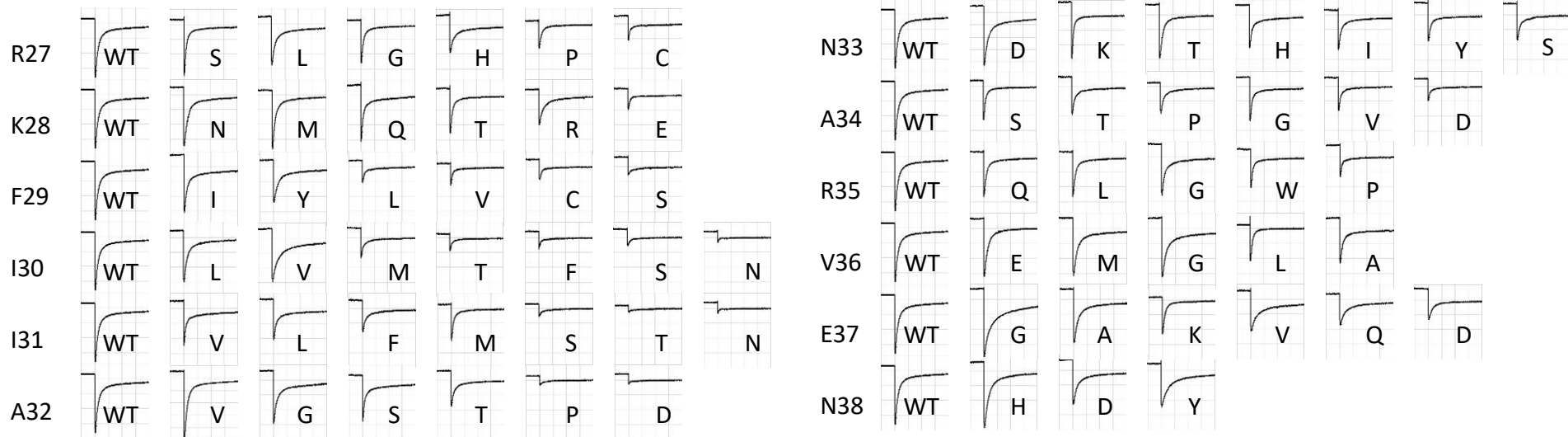


How ?

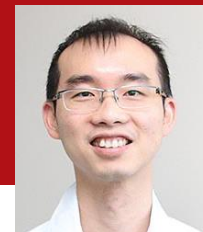


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Phase 2: All possible 458 missense SNVs in Exon 2 (Proof of concept)



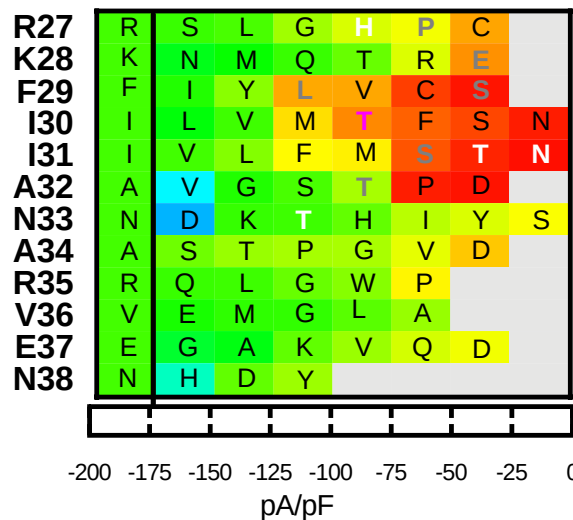
How ?



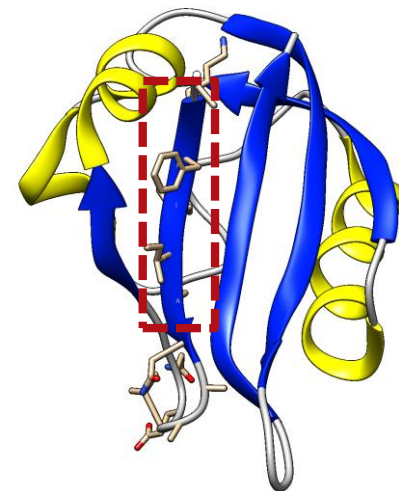
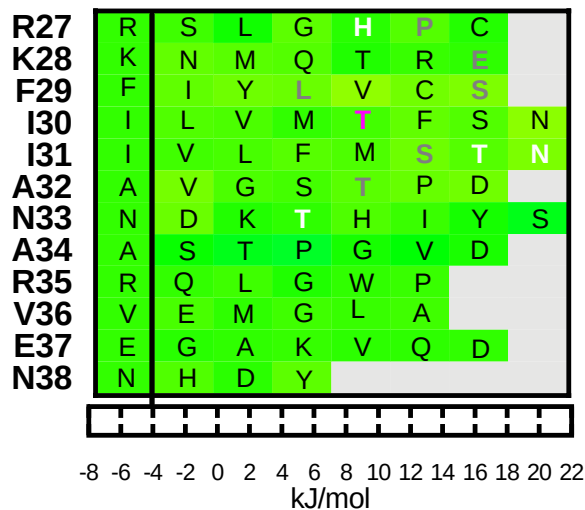
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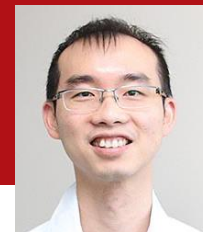
Current density



Steady-state activation



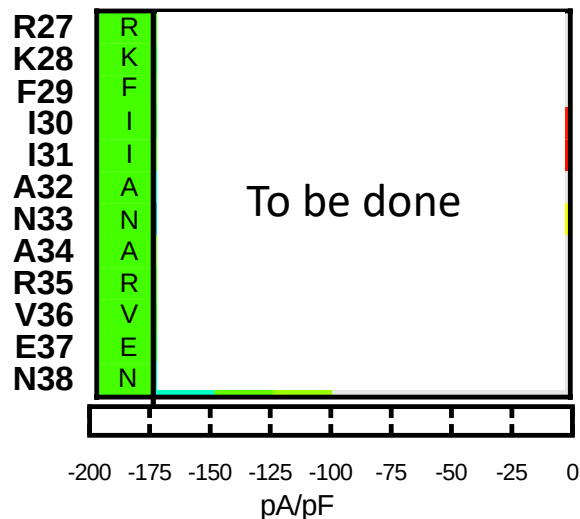
How ?



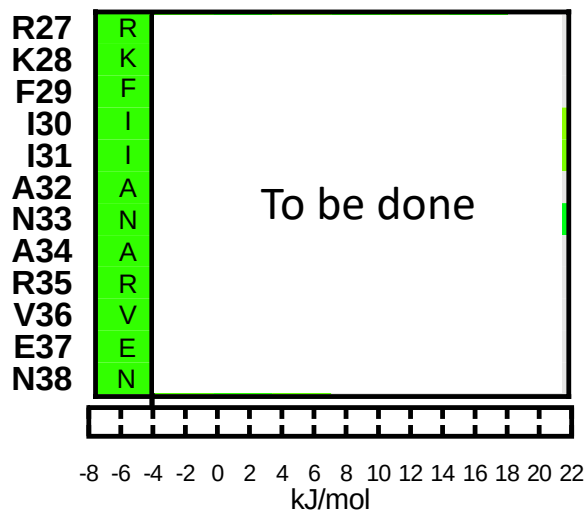
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Phase 2: All possible 458 missense SNVs in Exon 2 (Proof of concept)

Rate deactivation



Rate inactivation



Need to optimize an automated analysis routine for fitting biexponential functions at multiple voltages

Challenges ?

1. Define robust clinical phenotypes for verification (? Penetrance)
2. Incorporation of stringent QC to ensure clinical applicability
3. Optimise high throughput automated analysis routines
4. Application of FAIR data principles to ensure robustness of results
5. Development of secure data analysis and visualisation pipeline
6. Cost and who pays?

Acknowledgements



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