2020 Epilepsy Genetics Update

Molecular studies of genes associated with epilepsies: *KCNQ2*

Alfred L. George, Jr., M.D.
Department of Pharmacology
Northwestern University

Northwestern Medicine®
Feinberg School of Medicine

Disclosures – Alfred L. George, Jr., M.D.

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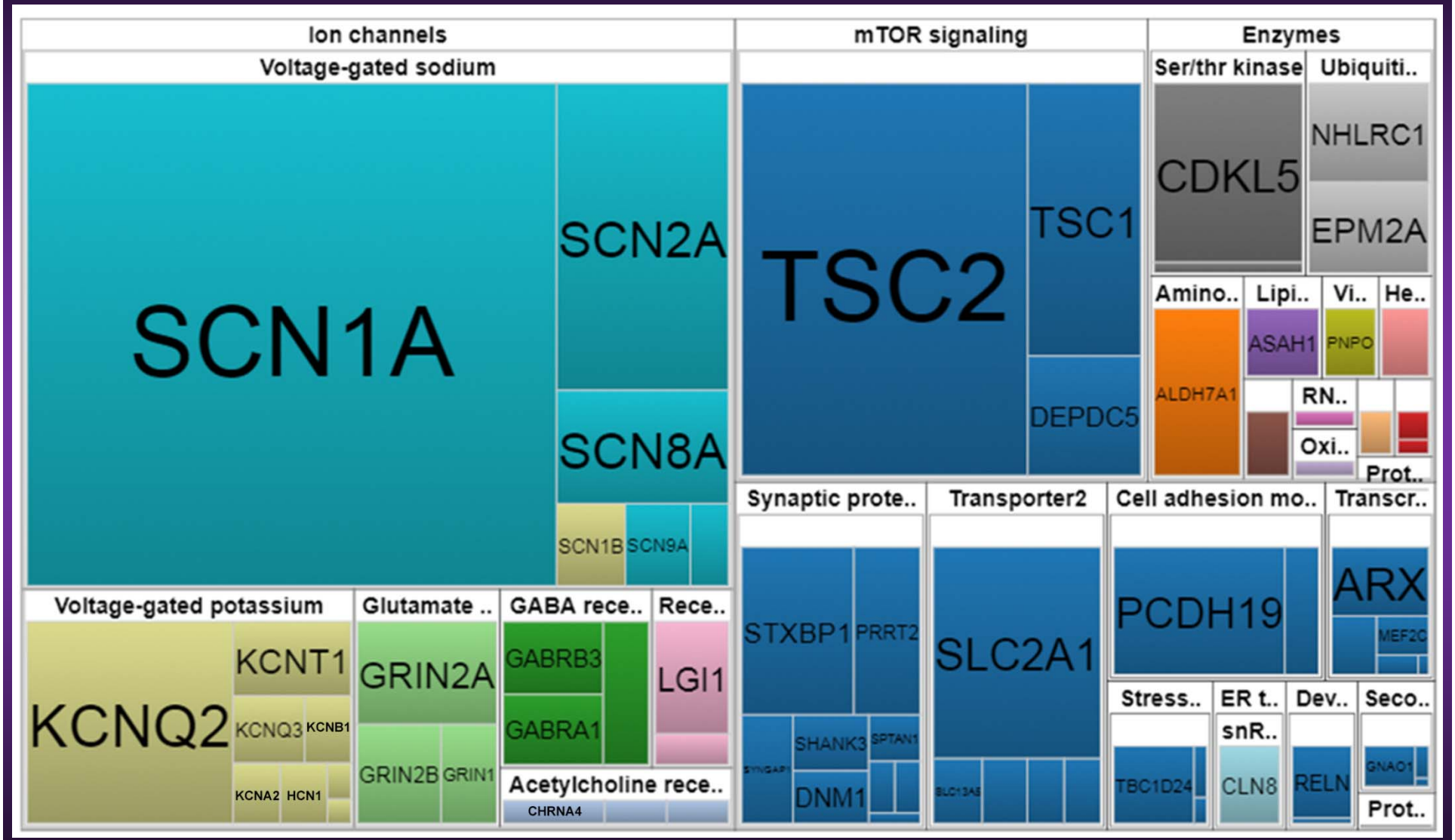
Allelic Heterogeneity in the Epilepsies



Source: HGMD® professional 2017.2 (6,125 variants)

Jennifer Kearney, Ph.D

Allelic Heterogeneity in the Epilepsies

Gene	Number of variants
SCN1A	1934
SCN2A	375
SCN3A	19
SCN8A	173
KCNQ2	**379**
KCNQ3	40
KCNT1	65
KCNB1	55
KCNA2	*24*
Glutamate receptors	*332*
GABA receptors	*228*
Acetylcholine receptors	*45*

Source: HGMD® professional 2020.1

Diagnostic outcomes for genetic testing of 70 genes in 8565 patients with epilepsy and neurodevelopmental disorders

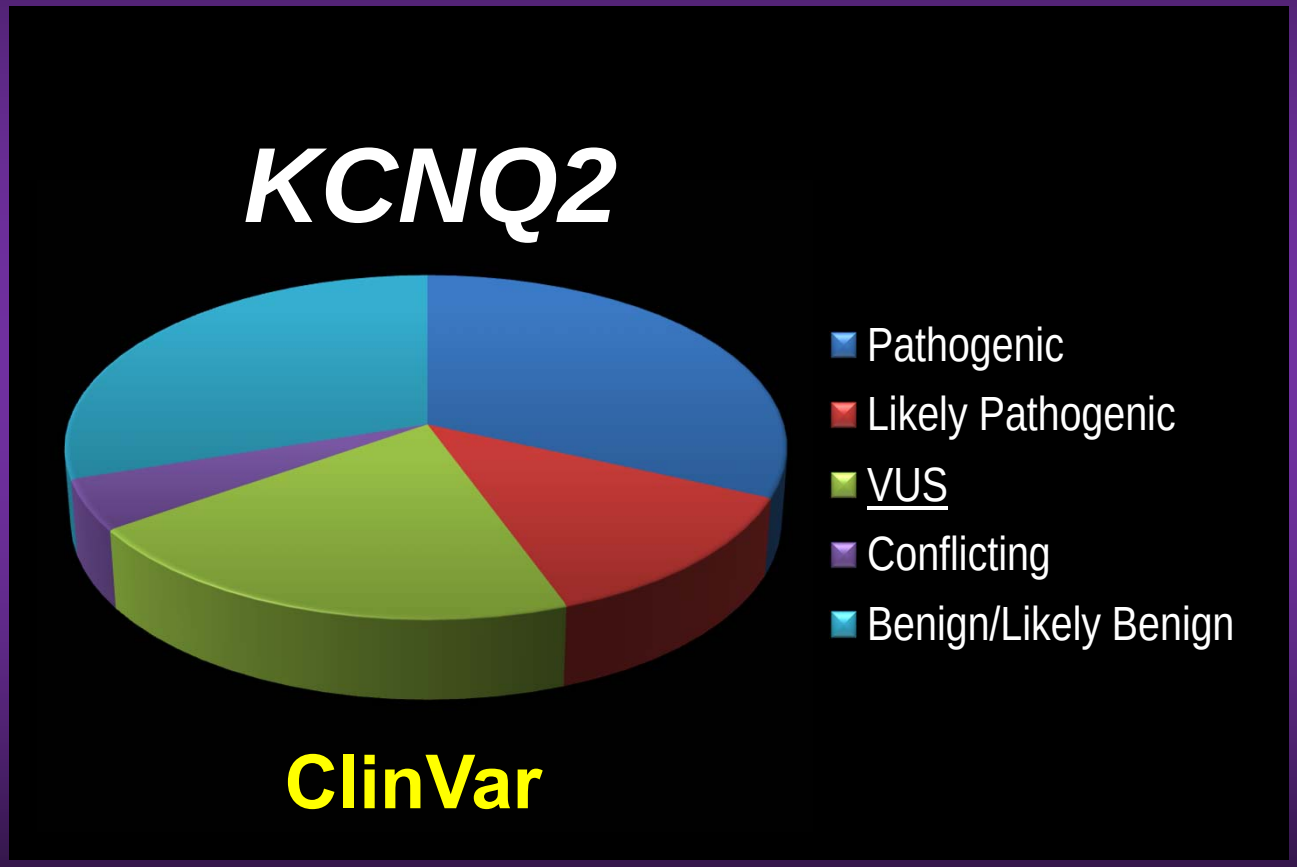
Amanda S. Lindy | Mary Beth Stosser | Elizabeth Butler | Courtney Downtain-Pickersgill | Anita Shanmugham | Kyle Retterer | Tracy Brandt Gabriele Richard | Dianalee A. McKnight

Epilepsia May 2018

70-gene panel and array CGH

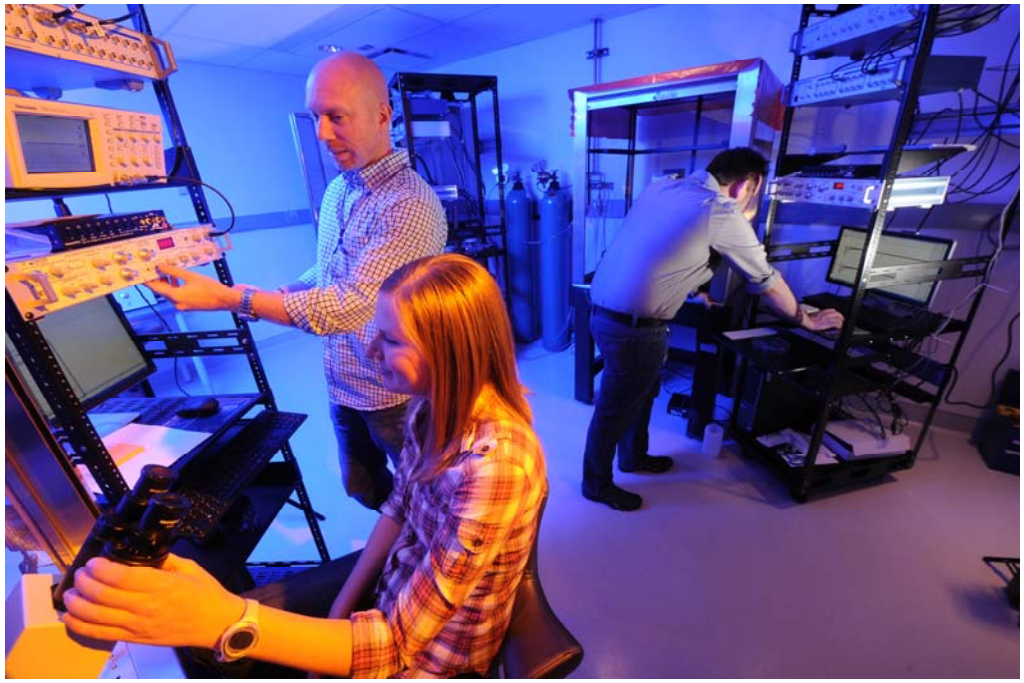
Gene	Positive Cases	% of Positive Cases
SCN1A	322	24.8%
KCNQ2	**159**	**13.2%**
SCN2A	96	7.4%
SCN8A	30	3.6%
Totals	**607**	**49%**

Variants of Unknown Significance in Channelopathy-associated Epilepsy



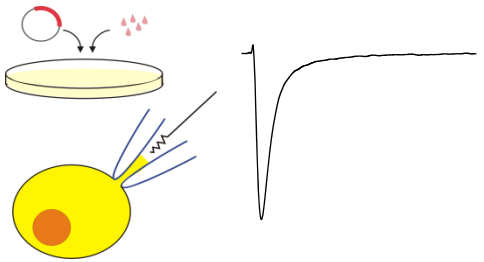
Functional Evaluation of Ion Channels Variants

Patch clamp electrophysiology is the gold standard



Conventional patch clamp:

- 16 measurements/person/day
- days-weeks per mutation



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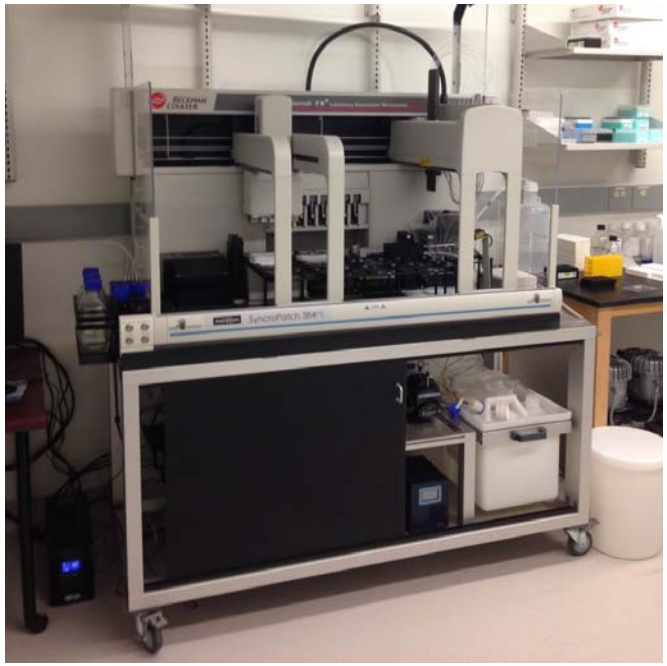
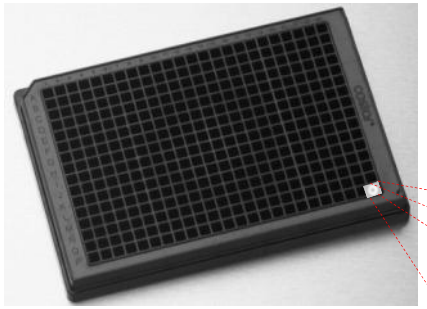
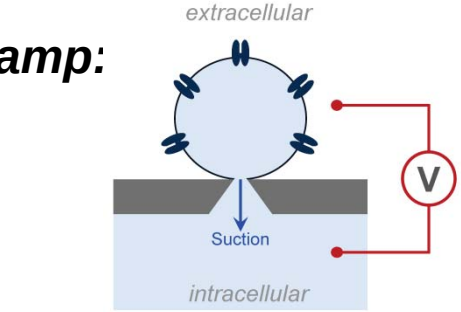
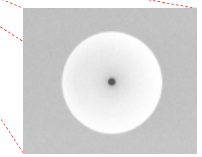


SyncroPatch 768PE

Automated patch clamp:





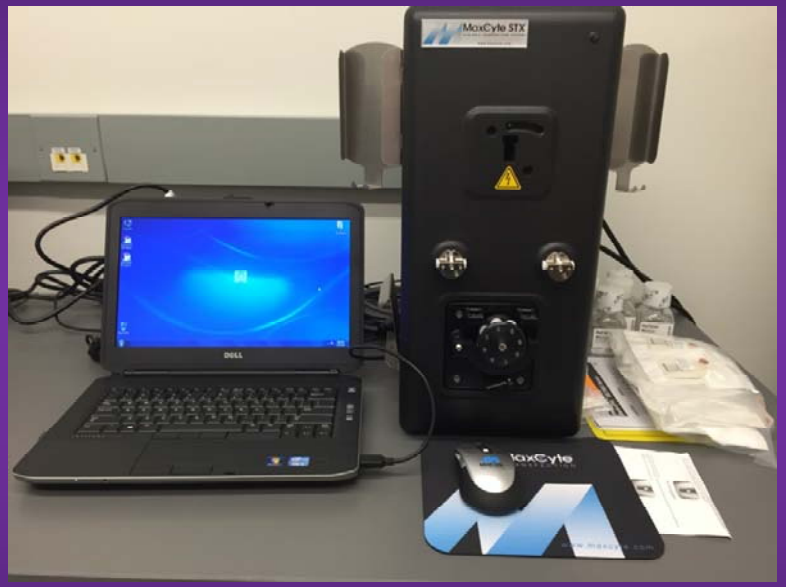


- ***768 measurements per hour***
- ***multiple variants per day***

#DasMachine-NU

Functional Evaluation of KCNQ2 Variants

Electroporation



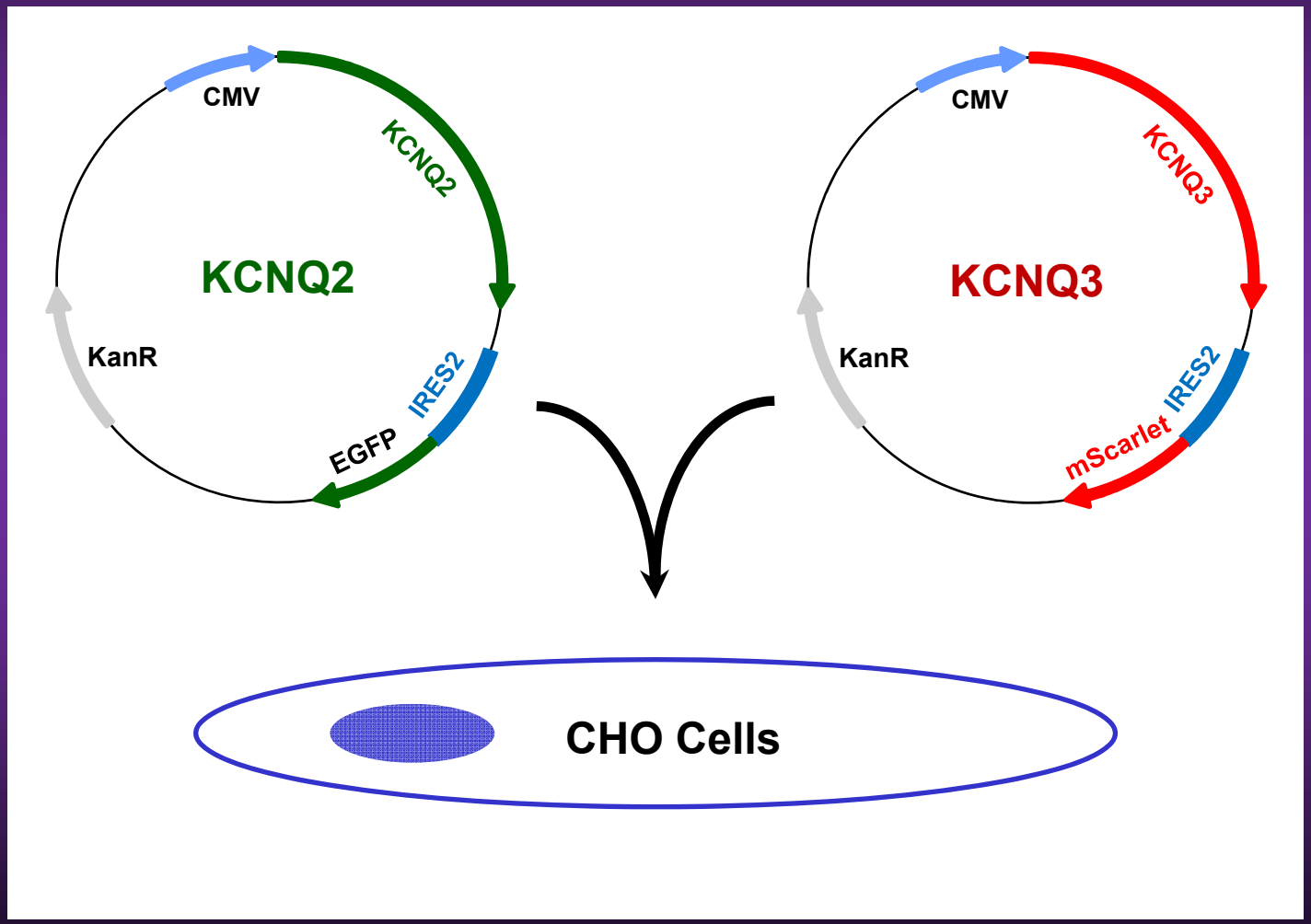
MaxCyte-STX

Automated patch-clamp

Syncropatch 768PE

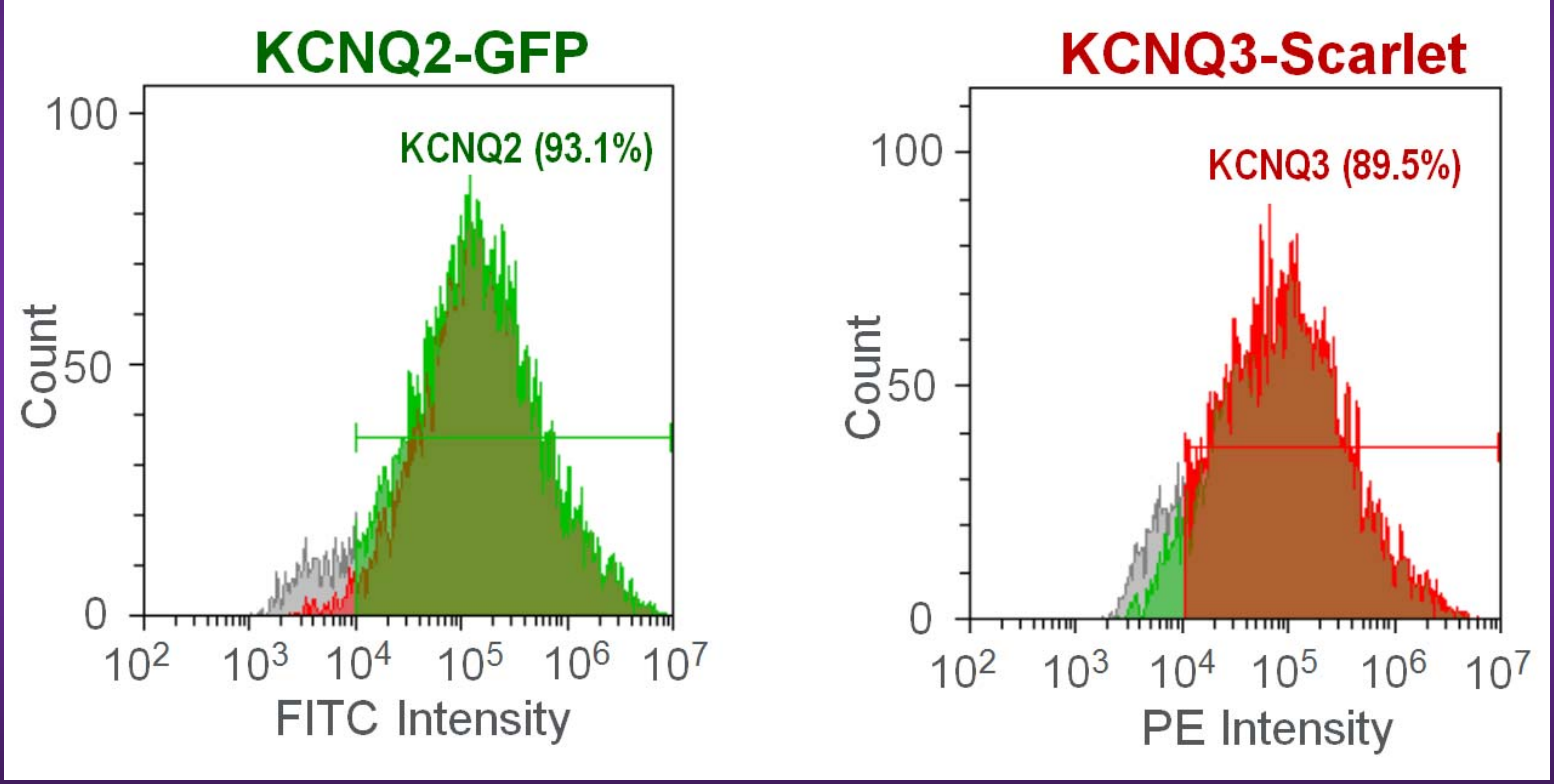
Functional Evaluation of KCNQ2 Variants

KCNQ2 + KCNQ3 co-expression



Functional Evaluation of KCNQ2 Variants

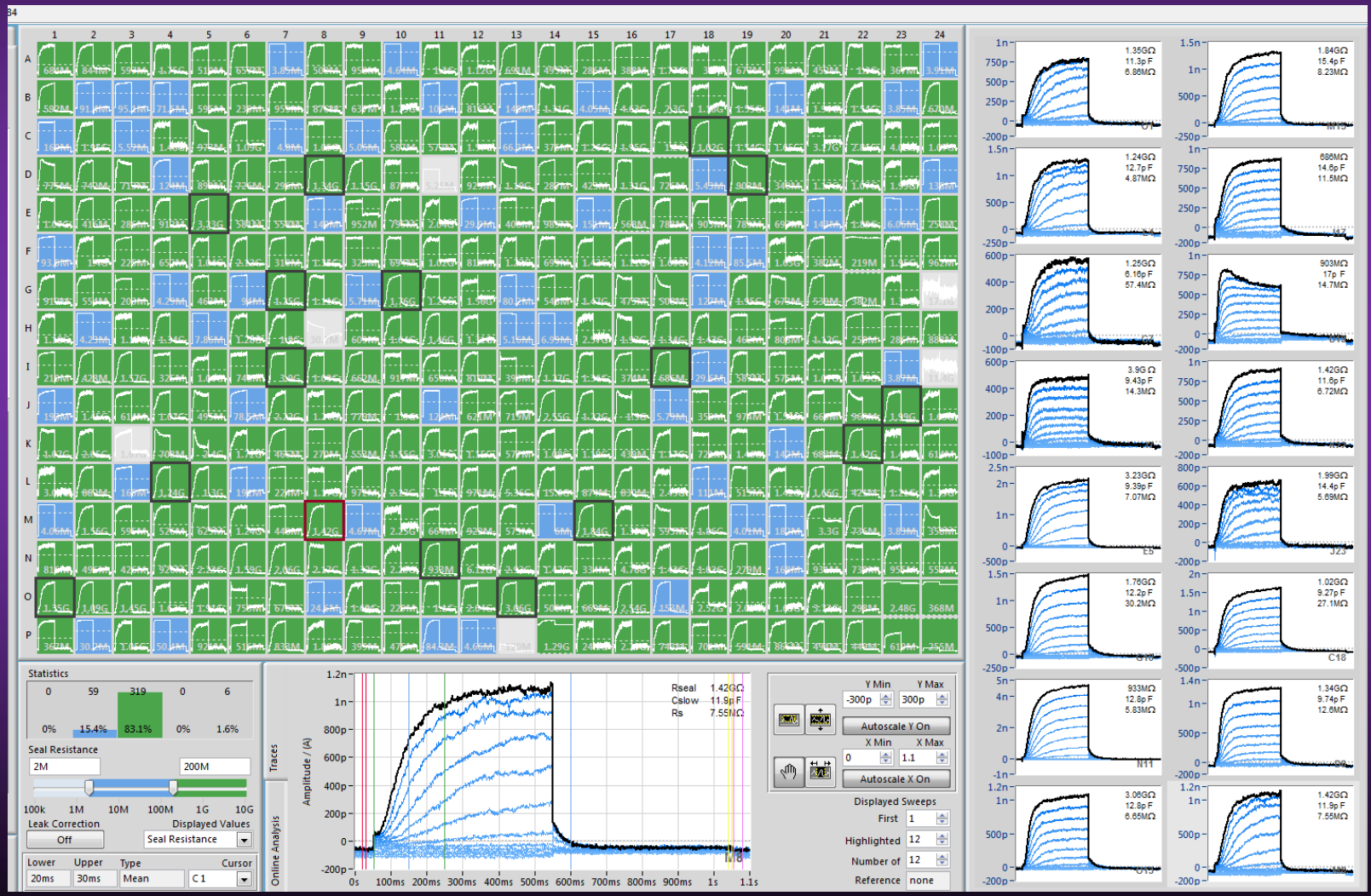
Electroporation of KCNQ2 and KCNQ3 in CHO cells evaluated by flow cytometry



Percent Co-transfected: 88%

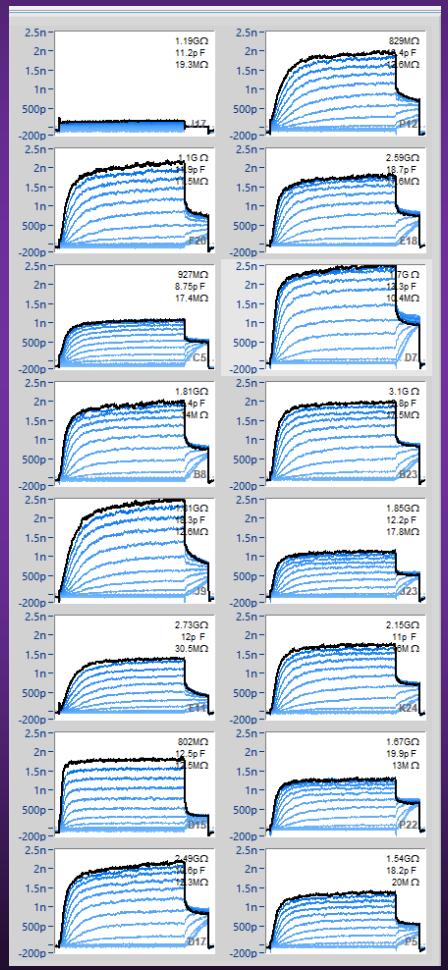
Functional Evaluation of KCNQ2 Variants

Syncropatch recording of KCNQ2 and KCNQ3



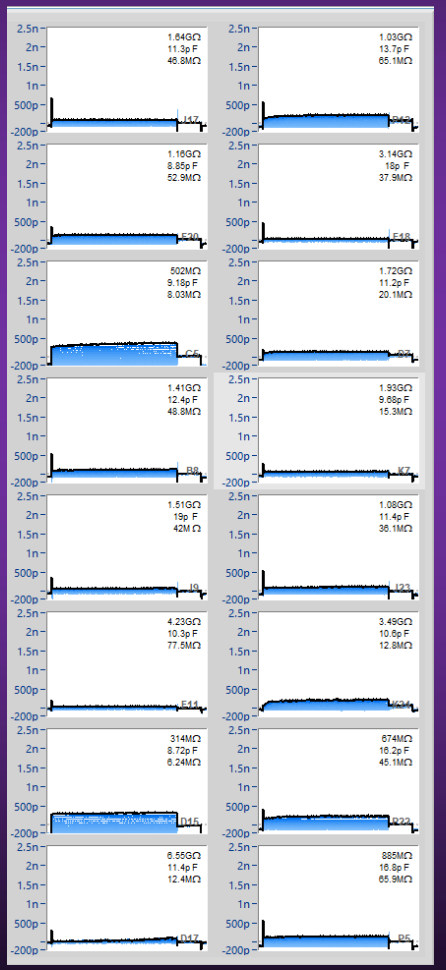
Functional Evaluation of KCNQ2 Variants

Syncropatch recording of KCNQ2 and KCNQ3

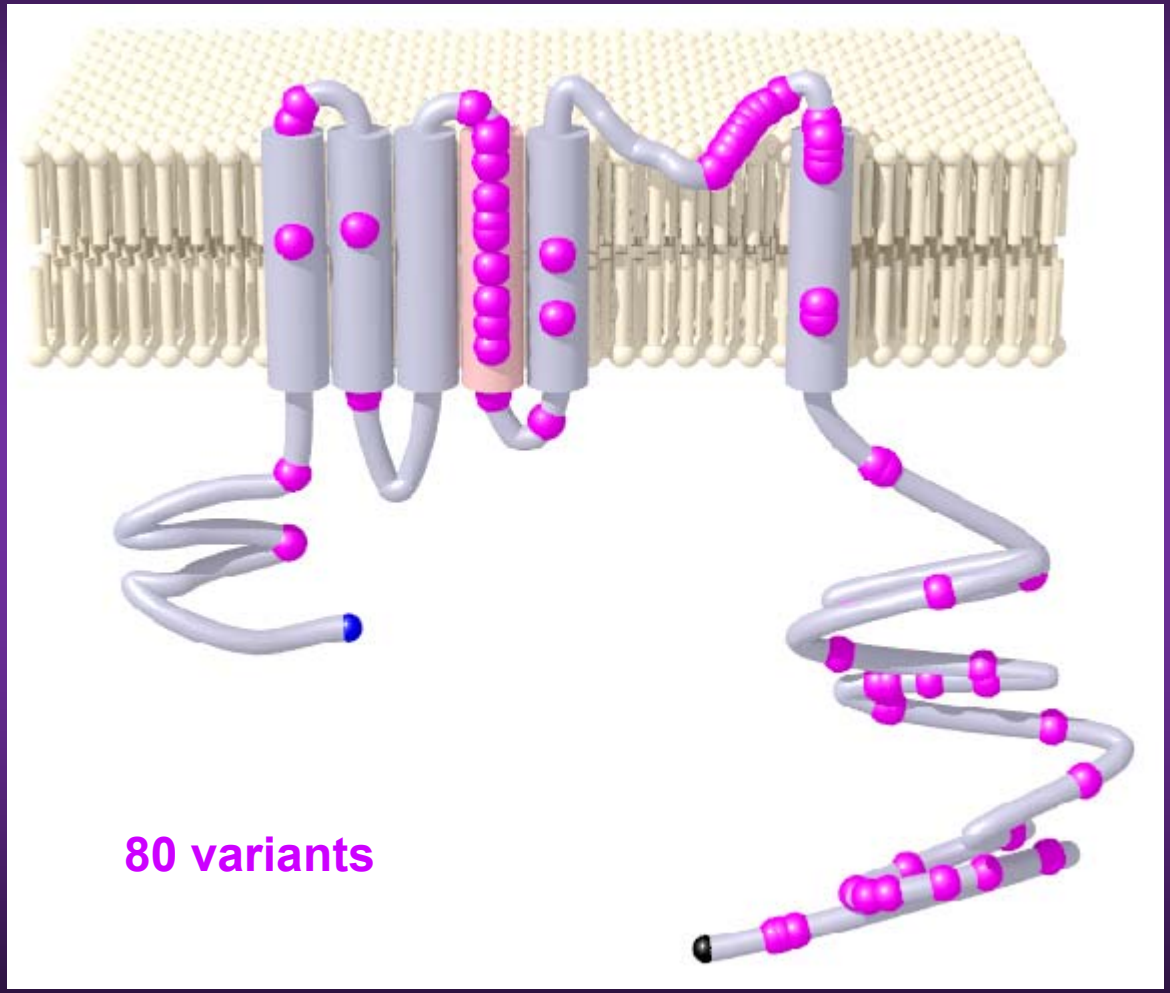


Block currents

20 µM XE991

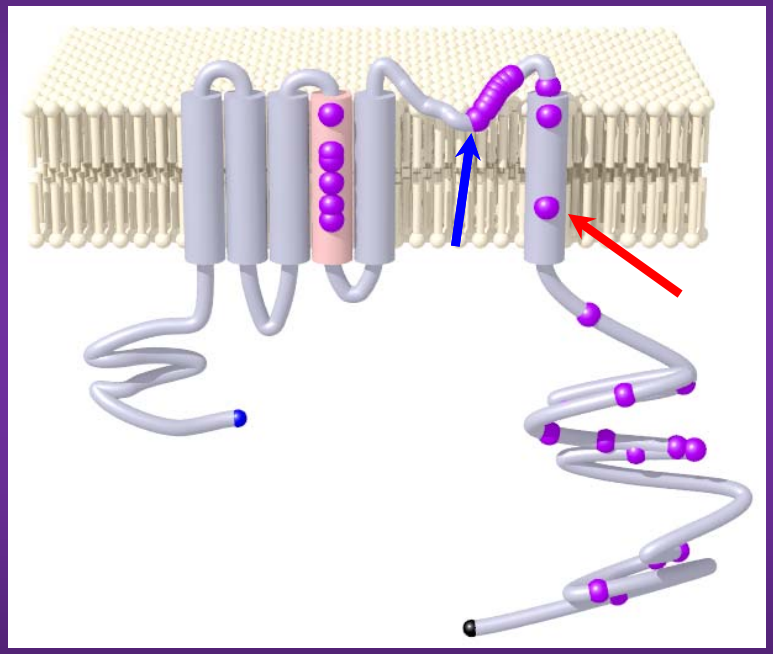


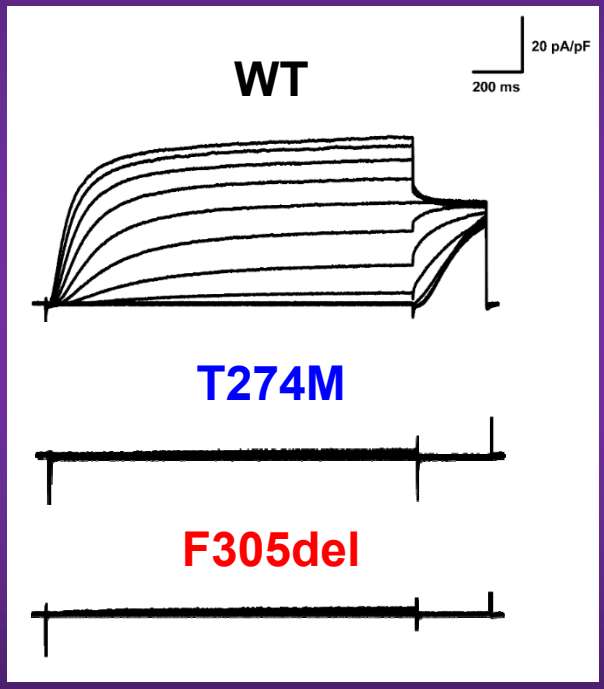
Functional Consequences of KCNQ2 Variants



Functional Consequences of KCNQ2 Variants

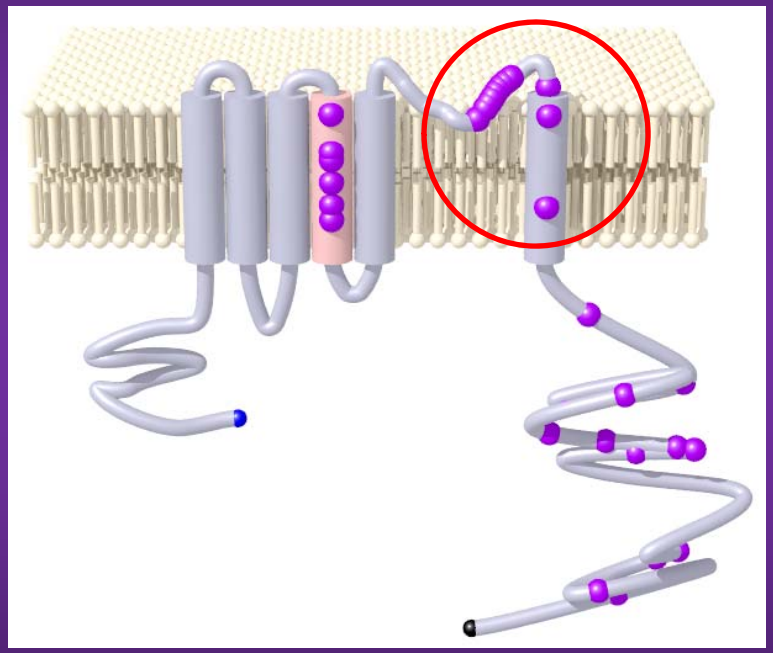
Variants with prominent loss-of-function

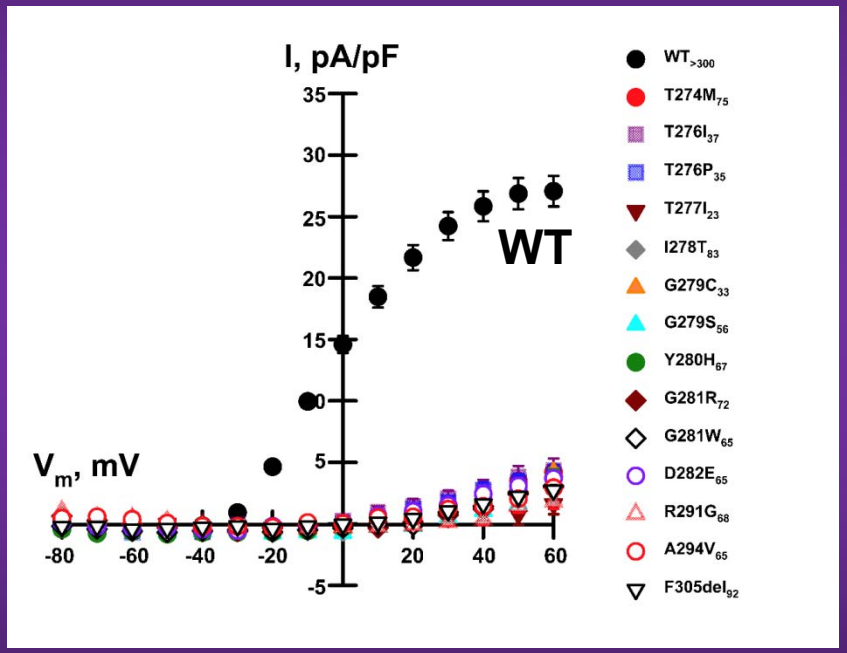




Functional Consequences of KCNQ2 Variants

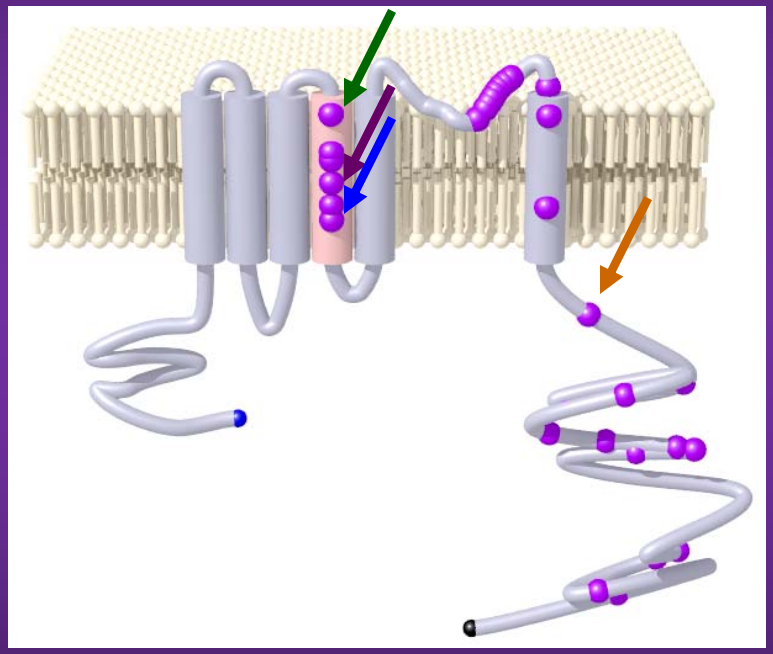
Variants with prominent loss-of-function

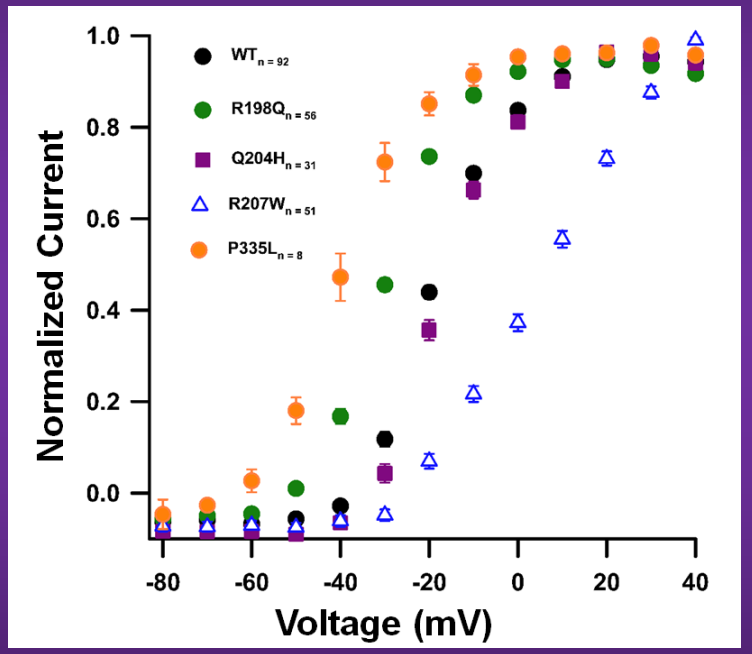




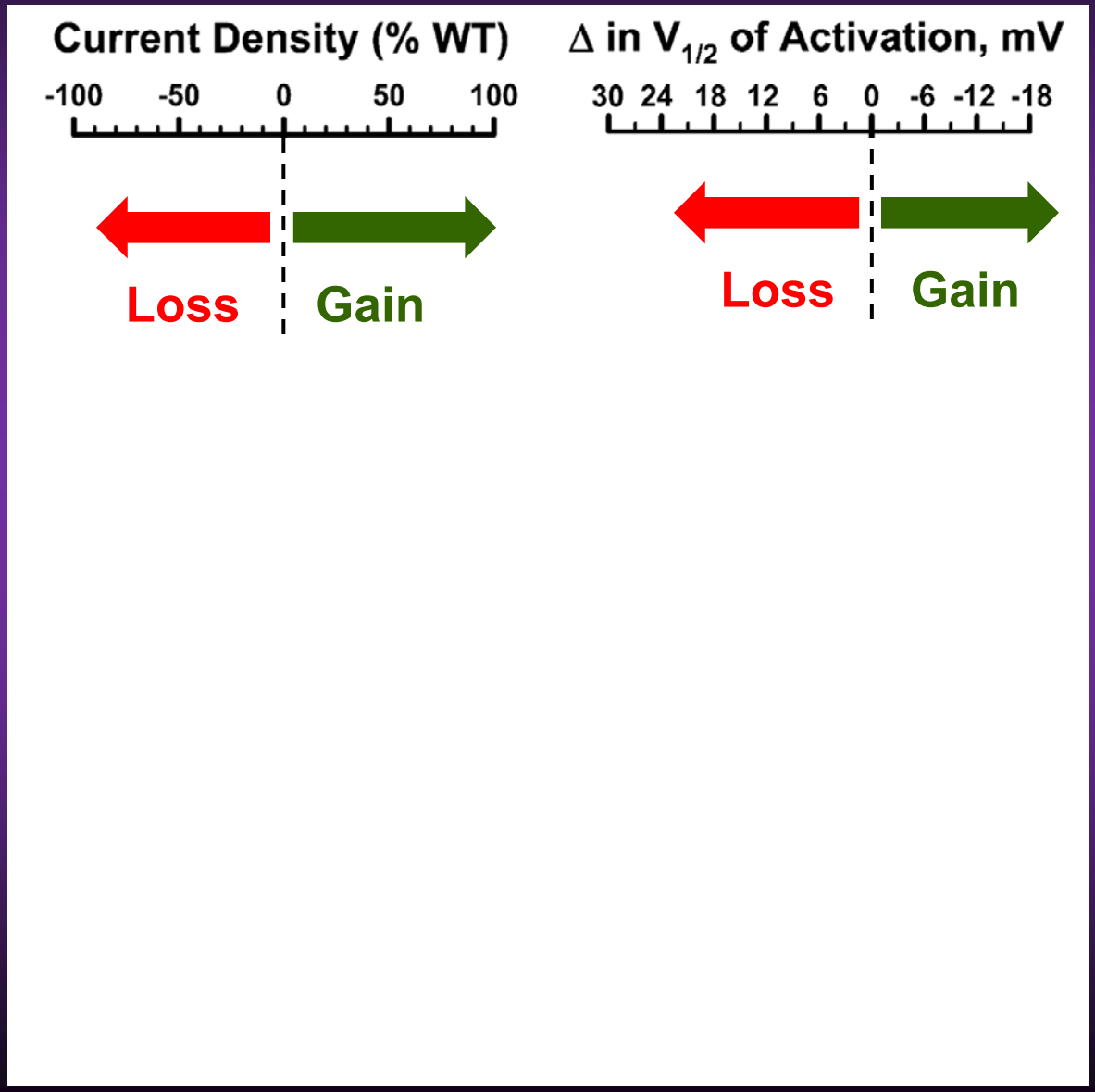
Functional Consequences of KCNQ2 Variants

Variants with abnormal activation voltage dependence





Functional Consequences of KCNQ2 Variants



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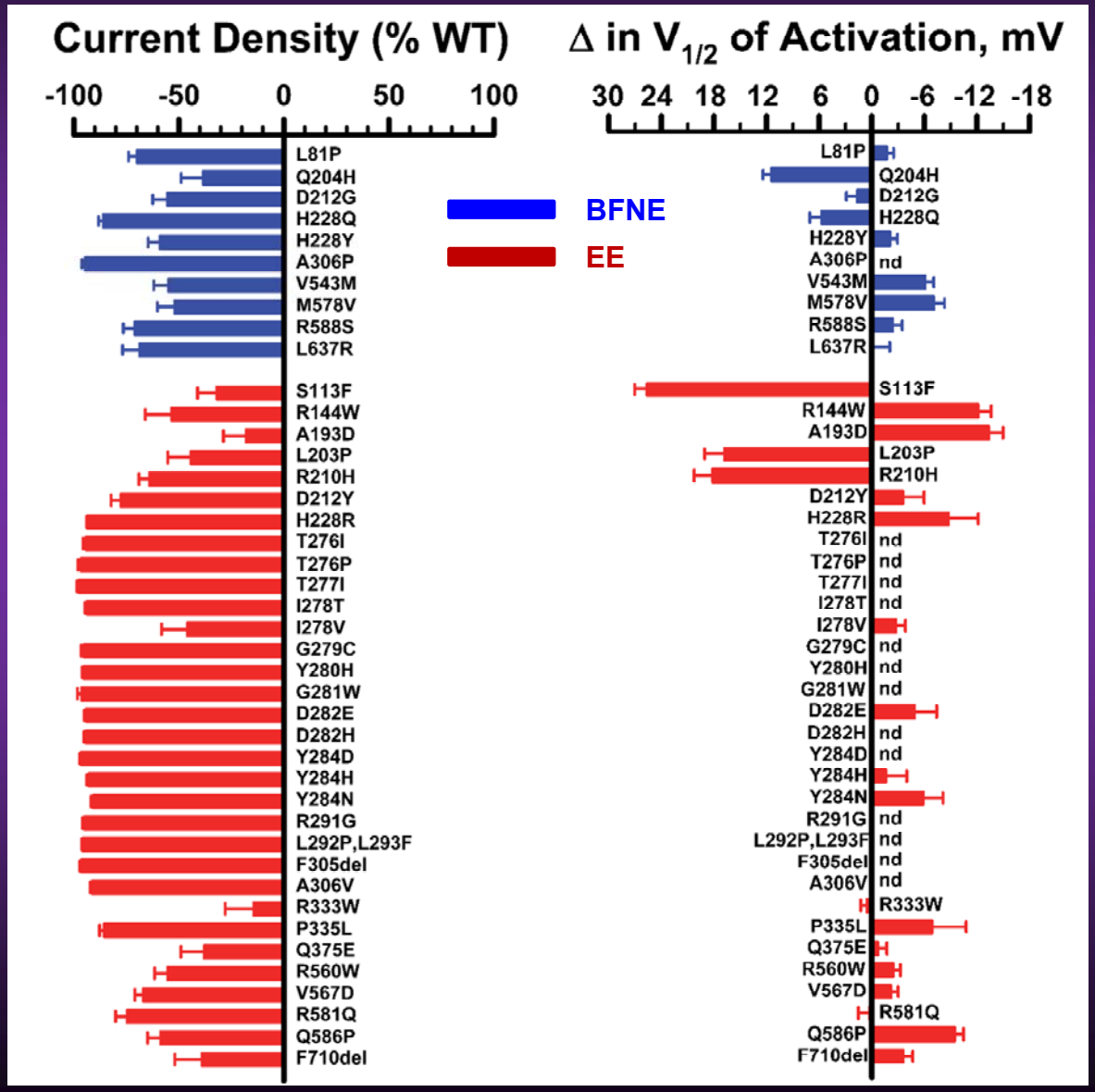
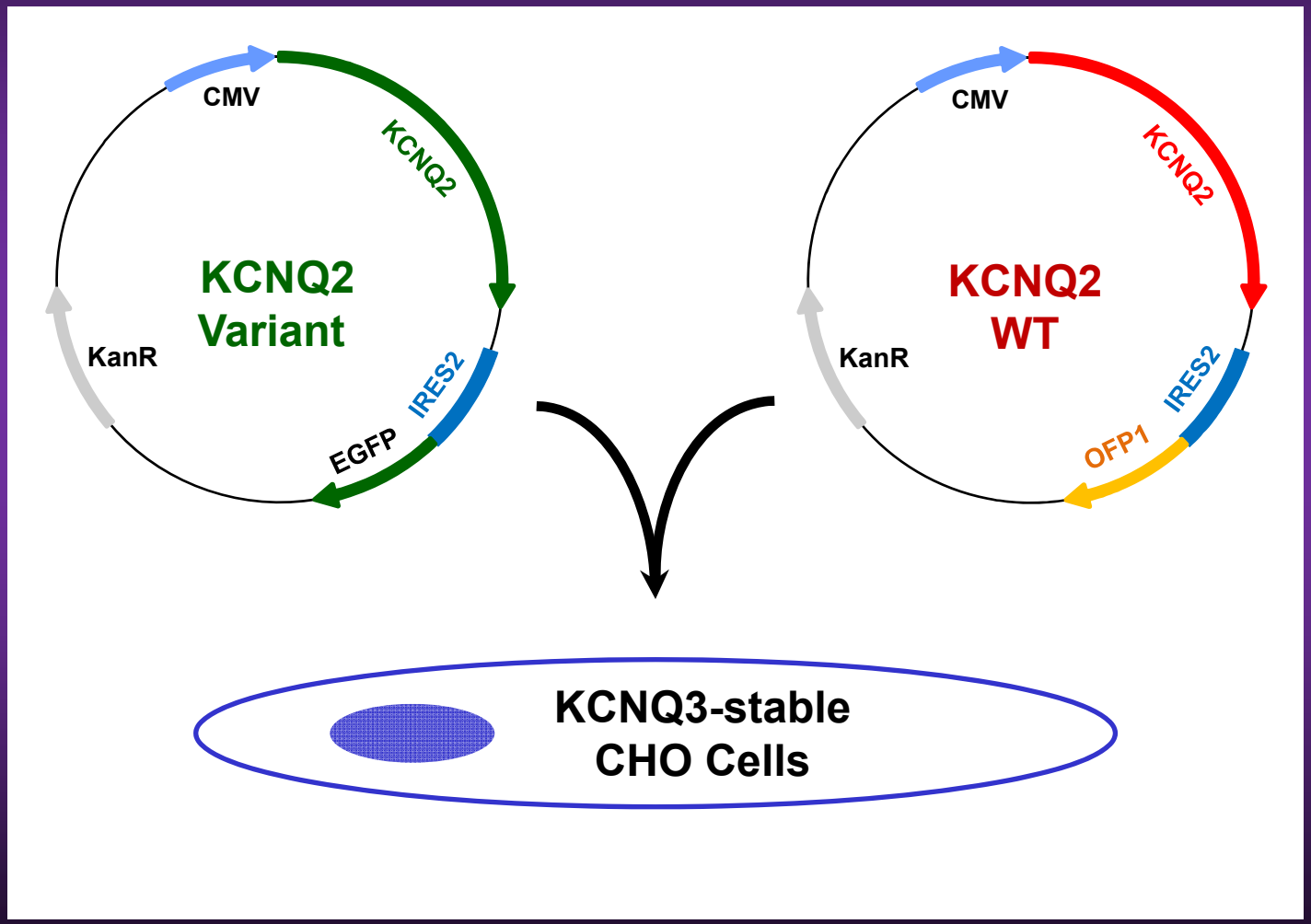


Functional Consequences of KCNQ2 Variants

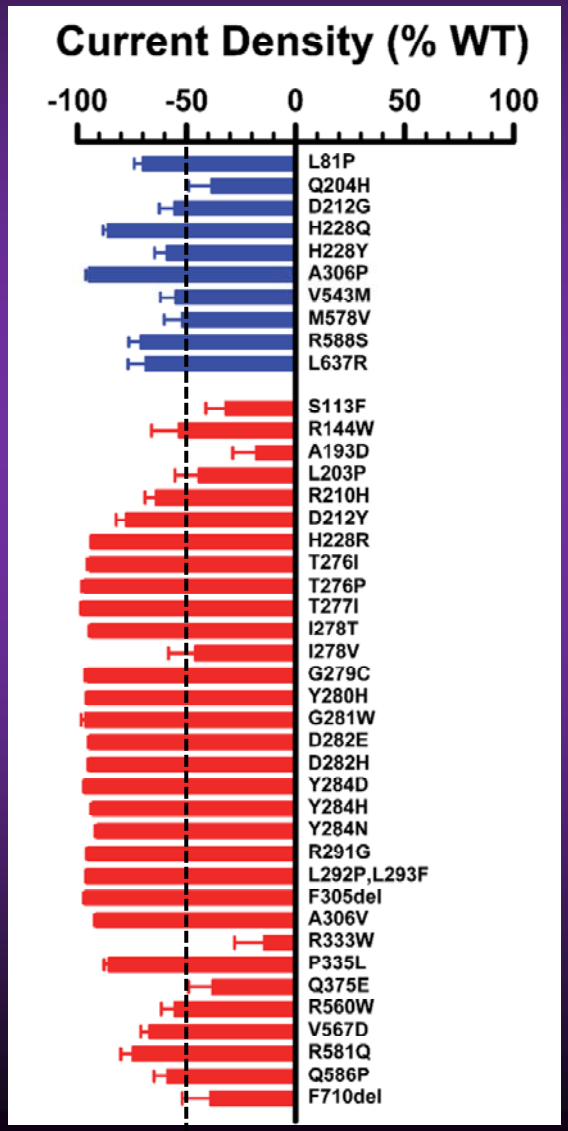
Dominant-negative paradigm

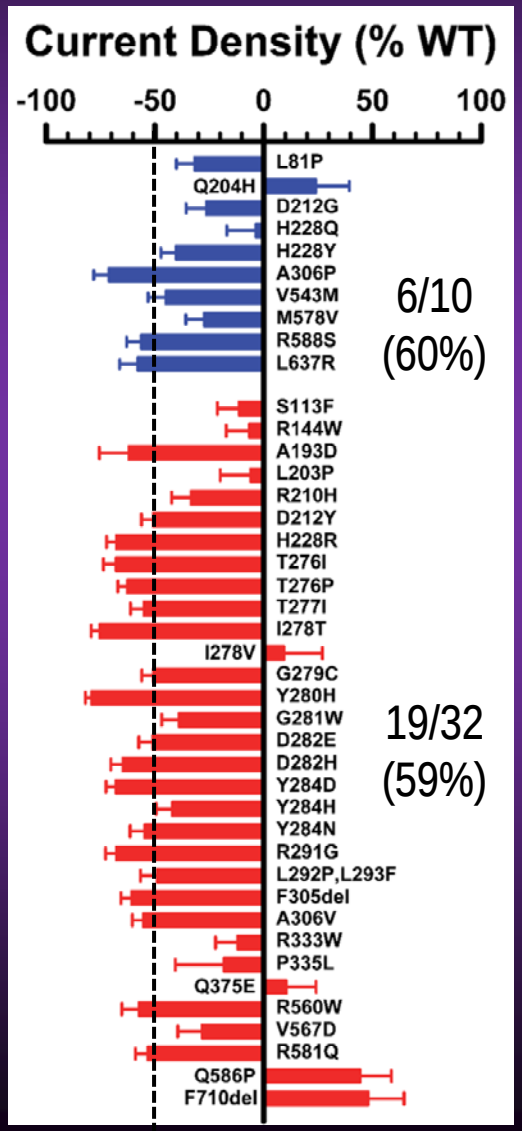


Functional Consequences of KCNQ2 Variants

Dominant-negative paradigm

Homozygous KCNQ2 variant



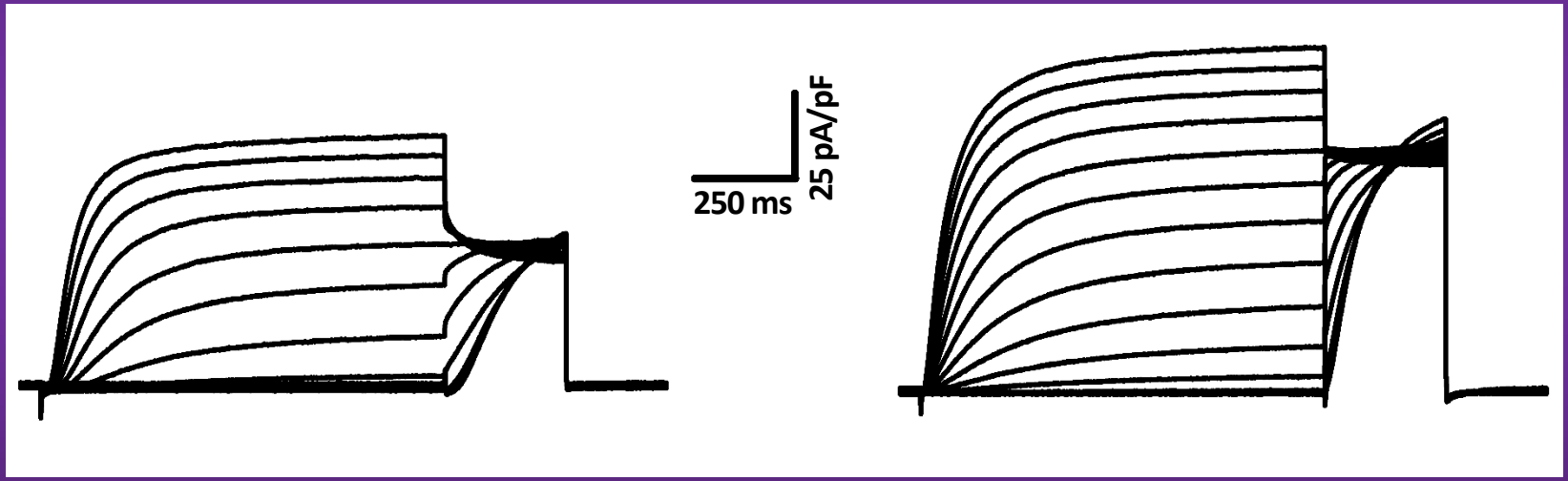


Heterozygous KCNQ2 variant

Pharmacological Effects of Retigabine

Expression of WT KCNQ2 in KCNQ3 stable CHO cells

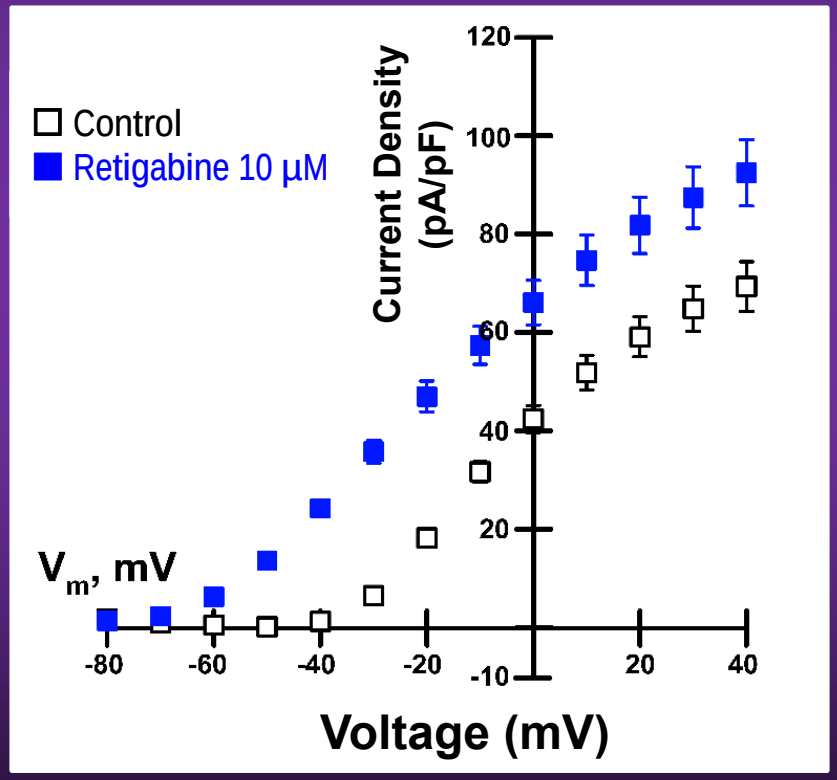
Control **Retigabine 10 μM**



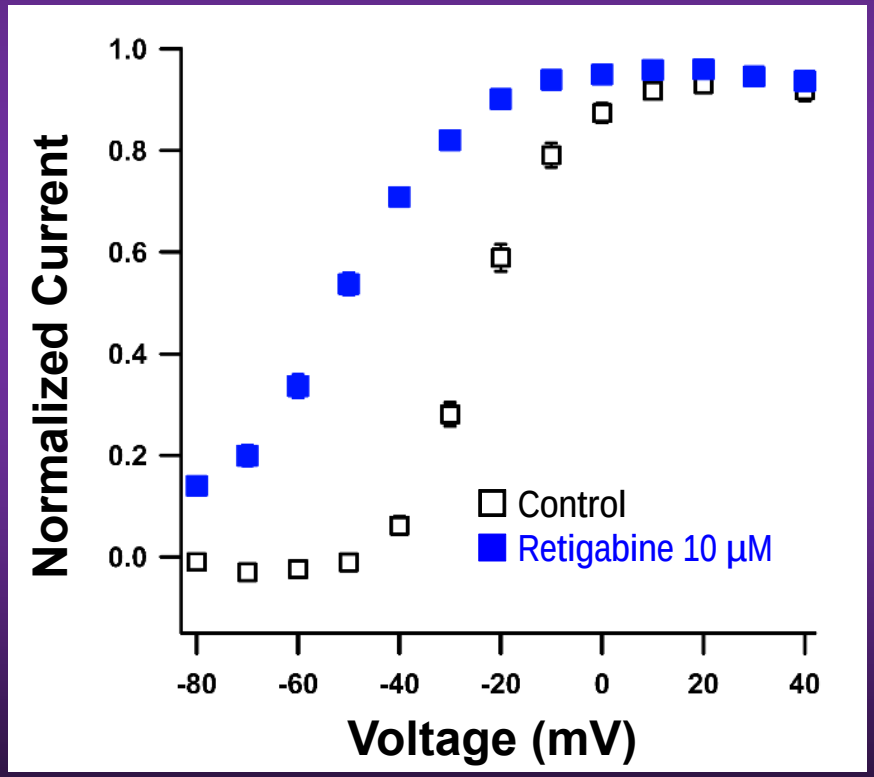
Pharmacological Effects of Retigabine

Expression of WT KCNQ2 in KCNQ3 stable CHO cells

Current-voltage



Activation

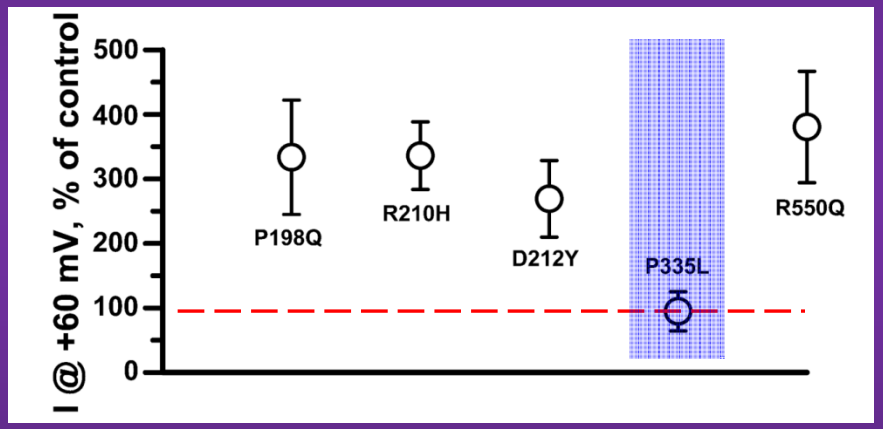


KCNQ2 Variant Responses to Retigabine

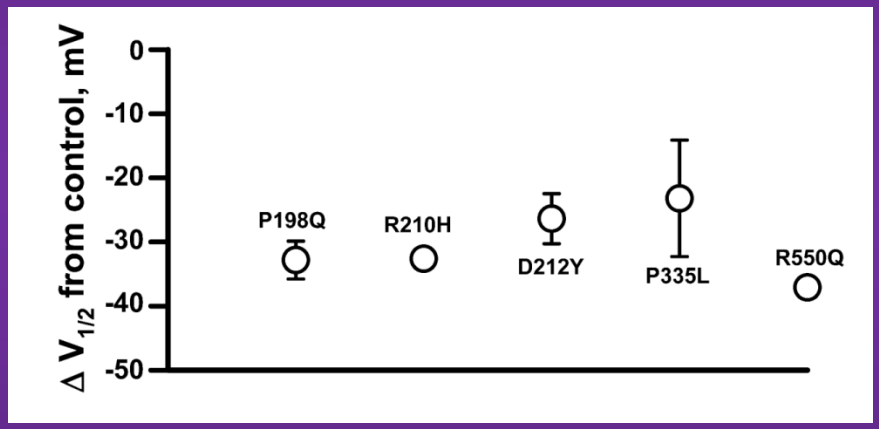
Expression of KCNQ2 variants in KCNQ3 stable CHO cells

Response to 10 µM retigabine

Peak current (% control)



Activation $\Delta V_{1/2}$



Not all KCNQ2 variants respond to retigabine

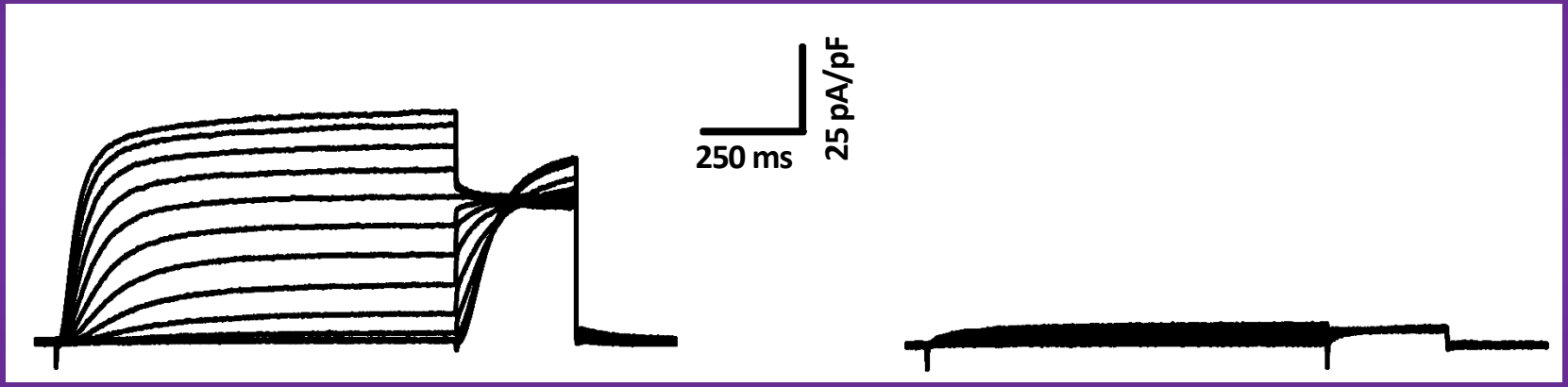
KCNQ2 Variant Responses to Retigabine

Expression of KCNQ2 variants in KCNQ3 stable CHO cells

KCNQ2-R144Q

Control

Retigabine 10 µM



Not all KCNQ2 variants respond to retigabine

KCNQ2 Variant Responses to Retigabine

Expression of KCNQ2 variants in KCNQ3 stable CHO cells

Retigabine 10 µM

	%WT	ΔV½
A43V	155.2%	-29.23
L81P	51.2%	-26.38
F104L	179.9%	-23.90
S113F	130.8%	-2.54
I115L	156.7%	-34.62
E130K	12.3%	-1.04
R144Q	46.8%	-39.91
R144W	82.9%	-45.57
A193D	116.5%	-41.51
A196V	134.8%	-19.14
R198Q	177.9%	-38.89
R201C	292.2%	-31.74
R201H	268.2%	-38.74
L203P	83.3%	-7.06
Q204H	132.6%	-16.48
R207Q	208.1%	-14.72
R207W	198.6%	-1.02
R210H	88.6%	-14.15
D212G	62.4%	-20.08
D212Y	37.9%	-26.45

	%WT	ΔV½
R214W	146.6%	-33.99
H228R	5.2%	-32.41
H228Q	12.9%	-19.54
H228Y	48.4%	-30.73
I238V	118.0%	-25.71
L243F	12.4%	-25.78
T274M	7.9%	-14.96
T276I	8.6%	-28.06
T276P	3.5%	-26.90
T277I	3.2%	
I278T	5.0%	-27.84
I278V	77.7%	-40.30
G279C	2.9%	
G279S	4.4%	
Y280H	3.6%	-8.14
G281R	2.7%	
G281W	3.7%	
D282E	4.5%	-21.85
D282H	2.5%	-16.85
Y284C	66.7%	-30.81

	%WT	ΔV½
Y284D	4.6%	-23.44
Y284H	44.5%	-28.37
Y284N	13.4%	-33.20
R291G	2.5%	-30.29
L292P,L293F	5.2%	-7.71
A294G	40.5%	-32.04
A294V	4.5%	
F305del	1.3%	
A306P	9.9%	-27.11
A306T	56.0%	-25.86
A306V	6.8%	-24.55
R333Q	218.1%	-14.81
R333W	147.2%	-29.59
Q375E	105.5%	-25.88
P410L	70.1%	-39.25
A472V	76.6%	-40.98
E484D	142.3%	-28.25
V512M	82.7%	-33.47
R529W	66.3%	-42.92
V536D	53.2%	-29.75

	%WT	ΔV½
M547V	126.2%	-35.19
R550Q	52.0%	-38.39
Q555P	58.2%	-39.46
R557S	117.0%	-27.74
R573C	73.4%	-34.19
T574S	216.4%	-25.81
L606R	79.4%	-29.24
E632G	193.2%	-26.56
F670del	103.8%	-37.69
G706S	99.6%	-26.08
S720L	122.9%	-36.39
Y724C	199.5%	-30.35
G725S	211.3%	-23.87
R279H	163.5%	-37.35
T740I	79.2%	-31.23
N749T	100.4%	-40.84
V762L	104.4%	-34.83
R823C	131.2%	-41.35
T826I	160.5%	-29.96
G827S	100.8%	-32.11

Response — Non-response

Summary

- KCNQ2 channelopathy is an important cause of monogenic epilepsy
- Many KCNQ2 variants have unknown clinical significance
- Automated patch clamp can decrypt variants of unknown significance
- Loss of function KCNQ2 variants are not all retigabine responsive
- Functional studies may help classify KCNQ2 VUS

Acknowledgments

CHANNELOPATHY-ASSOCIATED EPILEPSY RESEARCH CENTER

CENTER WITHOUT WALLS
U54 NS108874

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NIH National Institute of Neurological Disorders and Stroke