KCNQ2 gene discovery; Epilepsy mechanism, and other ion channel mutations in epilepsy

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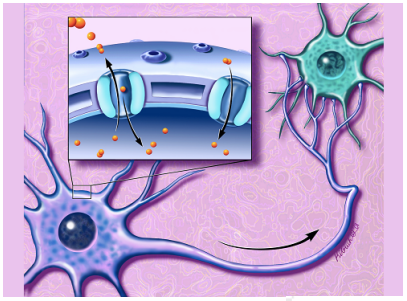
- No relevant financial relationships with any commercial interests.

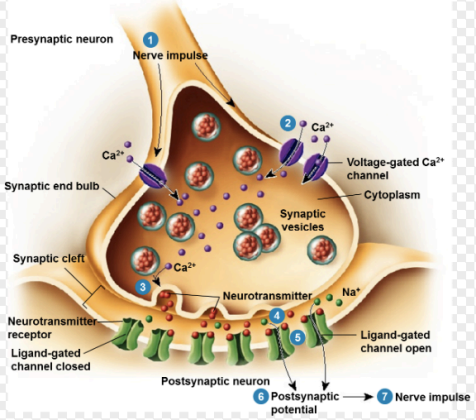
INTRODUCTION: WHAT IS EPILEPSY?

Epilepsy



Common

- Prevalence 4-8/1000
- Life time incidence 3%

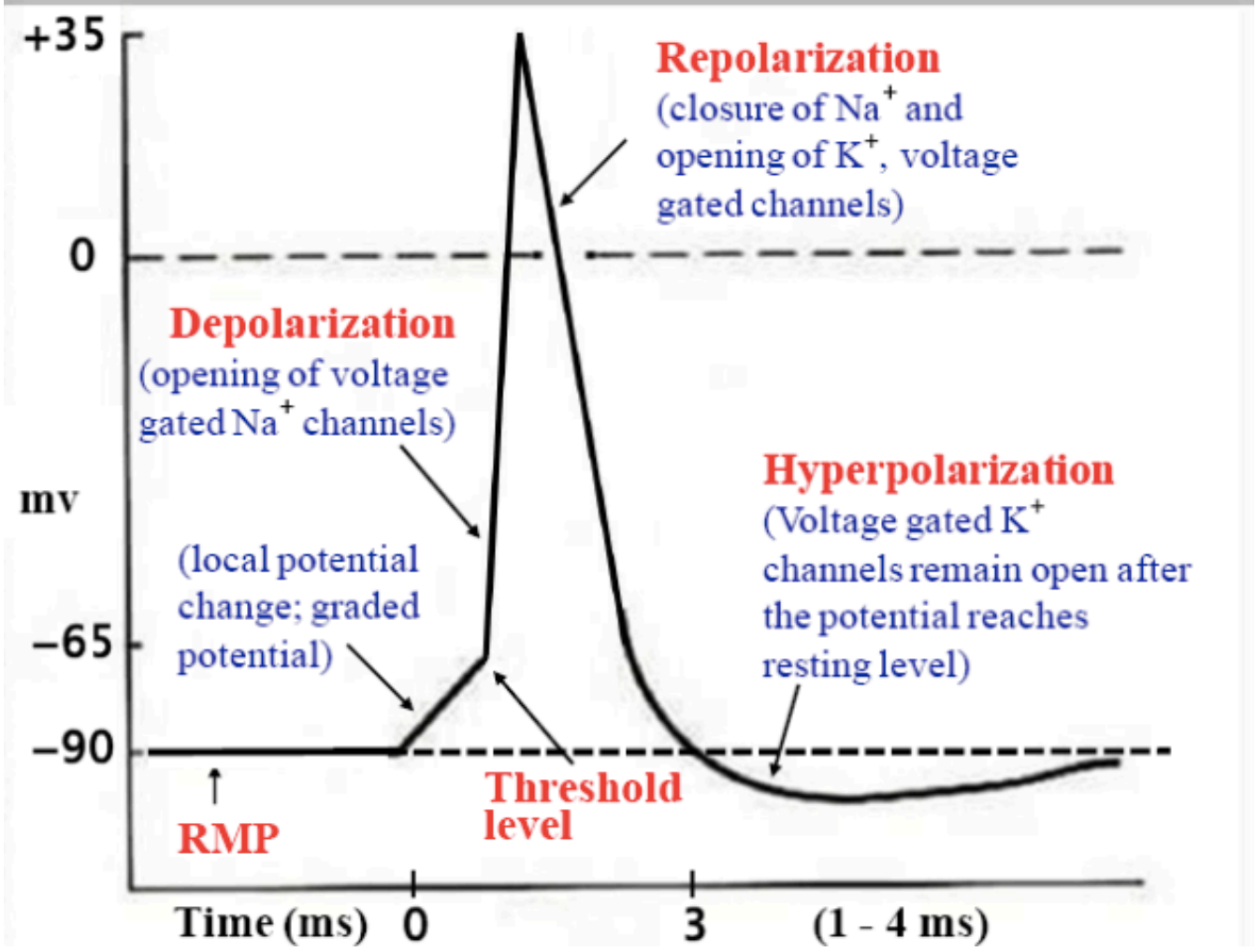


Key symptom = seizures

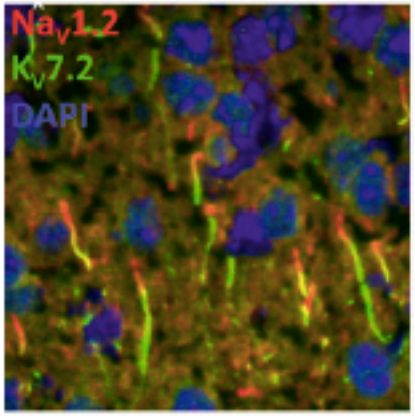
- Abnormal synchronization of cortical neurons
- Hyperexcitable neurons

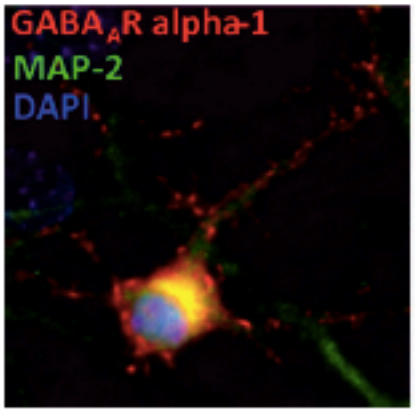
Epilepsy = channelopathy

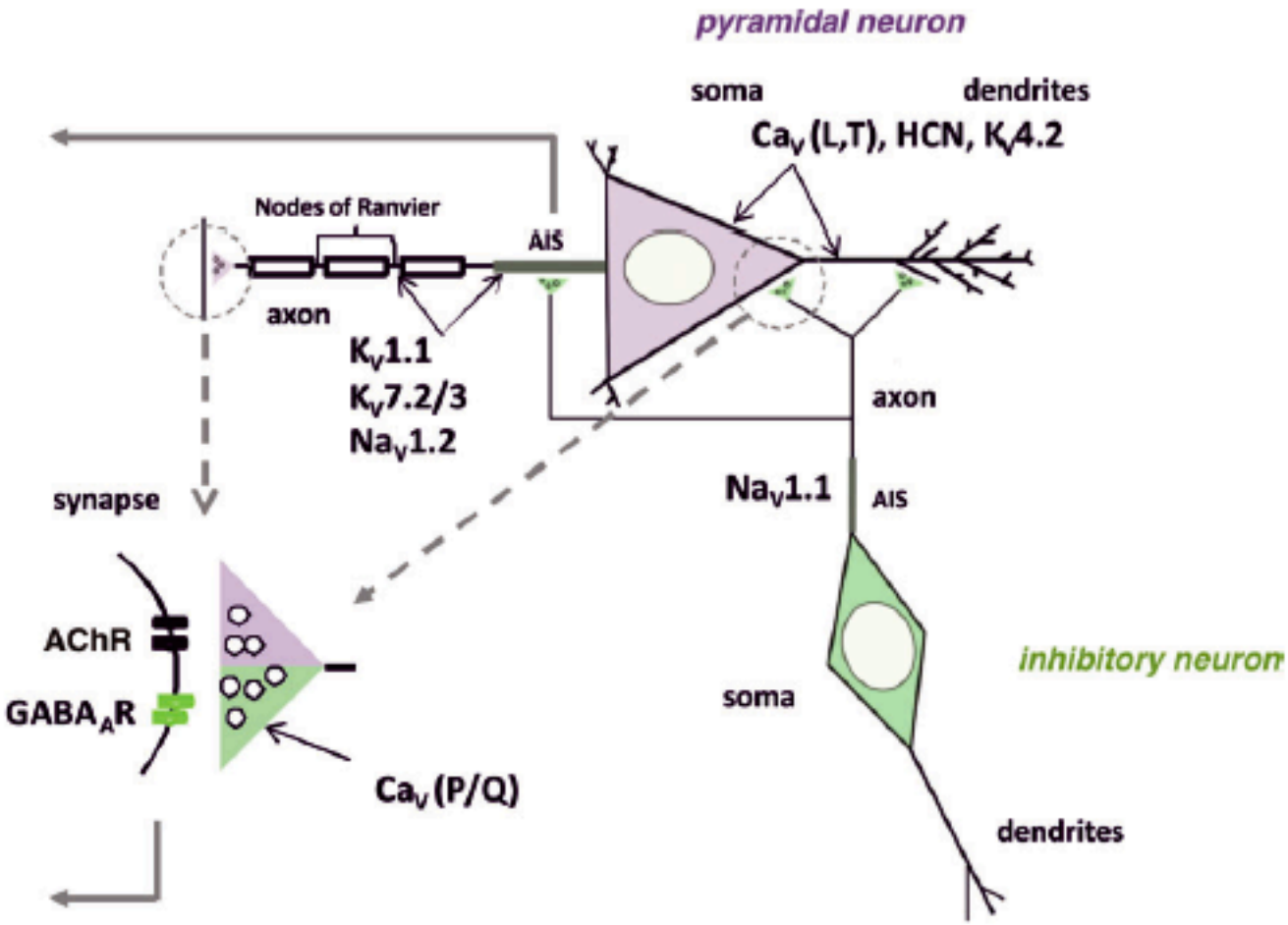
Action potential



Ligand and voltage gated ion channels







H. Lerche et al., J. Physiol. 2013

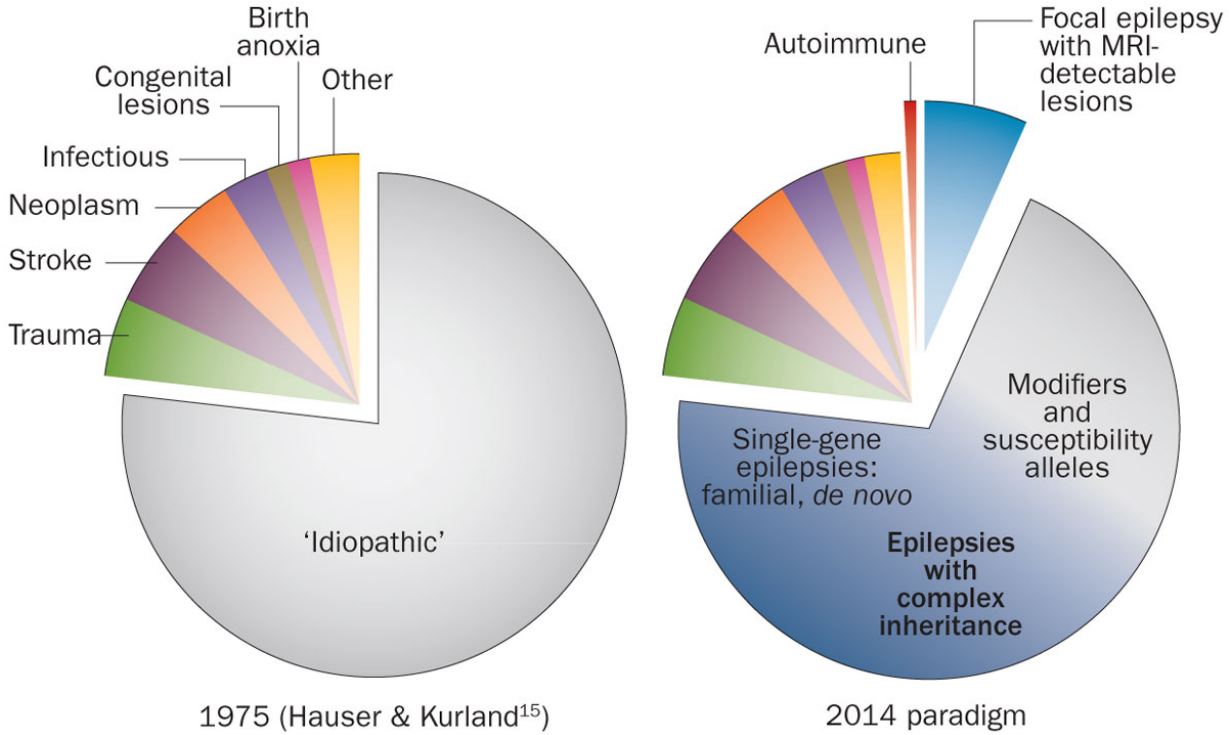
Epilepsy

↓ Inhibition

↑ **Excitation**

Causes of epilepsy

Figure 1 Advances in understanding the causes of epilepsy



Thomas, R. H. & Berkovic, S. F. (2014) The hidden genetics of epilepsy—a clinically important new paradigm
Nat. Rev. Neurol. doi:10.1038/nrneurol.2014.62

Monogenetic epilepsies (non-exhaustive)

ALG13	GABRG2	PLCB1
ARHGEF9	GRIN2A	PRRT2
ARX	GRIN2B	PNKP
ATP1A2	HCN1	SCN1A
CDKL5	KCNJ11	SCN1B
CHD2	KCNQ2	SCN2A
CHRNA4	KCNQ3	SCN8A
CHRNB2	KCNMA1	SLC25A22
CHRNA2	KCNT1	SLC2A1
DEPDC5	LGI1	SPTAN1
FOXG1	MEF2C	STXBP1
GABRA1	NRX1	SYNGAP1
GABRB3	PCDH19	TBC1D24

Ion channel genes

THE GENE *KCNQ2* IN EPILEPSY

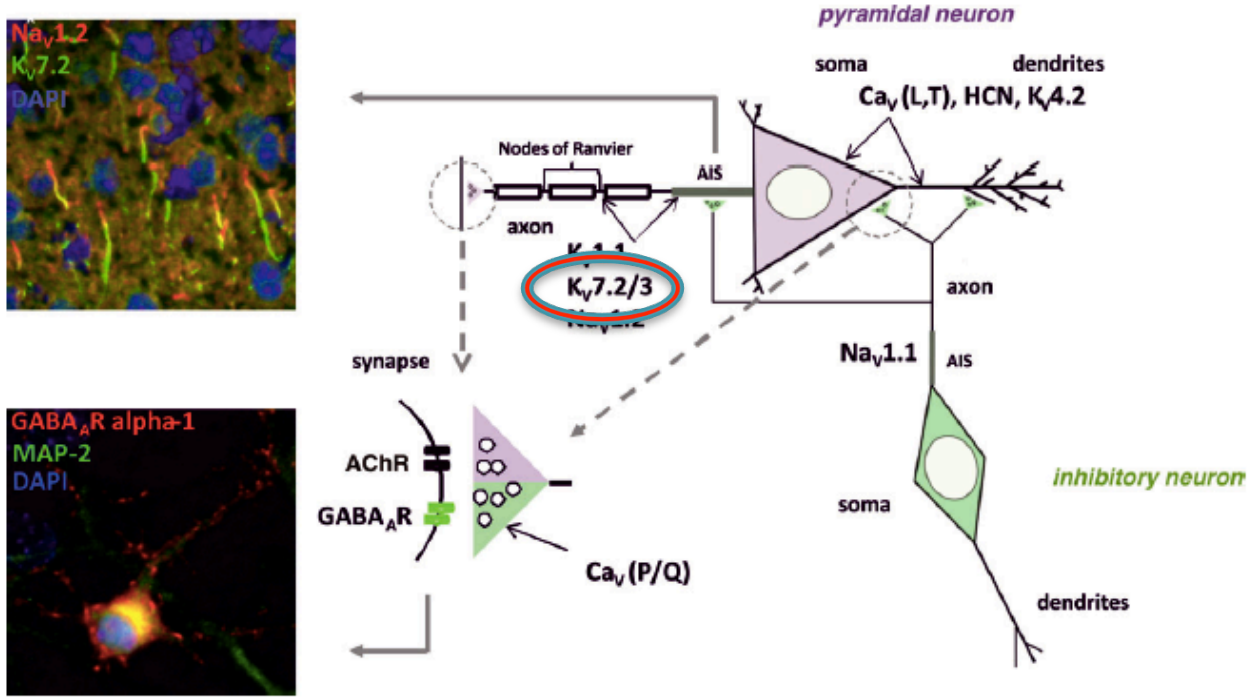
Voltage gated potassium channels

- Humans: > 70 potassium channel genes

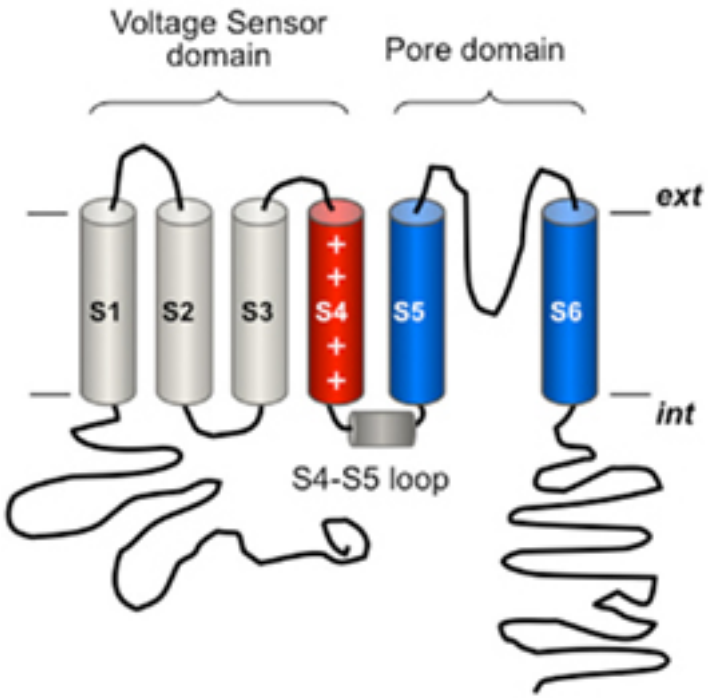
- Small family of voltage gated KCNQ genes: KCNQ1-5
  - KCNQ1: cardiac arrythmia (LQTS)
  - KCNQ2/3: epilepsy
  - KCNQ4: deafness

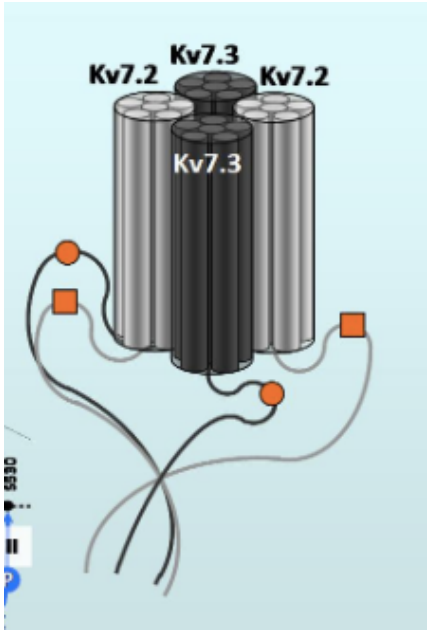
KCNQ2

- Encoding voltage gated potassium channel subunit $K_V7.2$
  - Hyperpolarizing M current
  - Stabilizes neuronal excitability



Heteromeric *KCNQ2/KCNQ3* channels



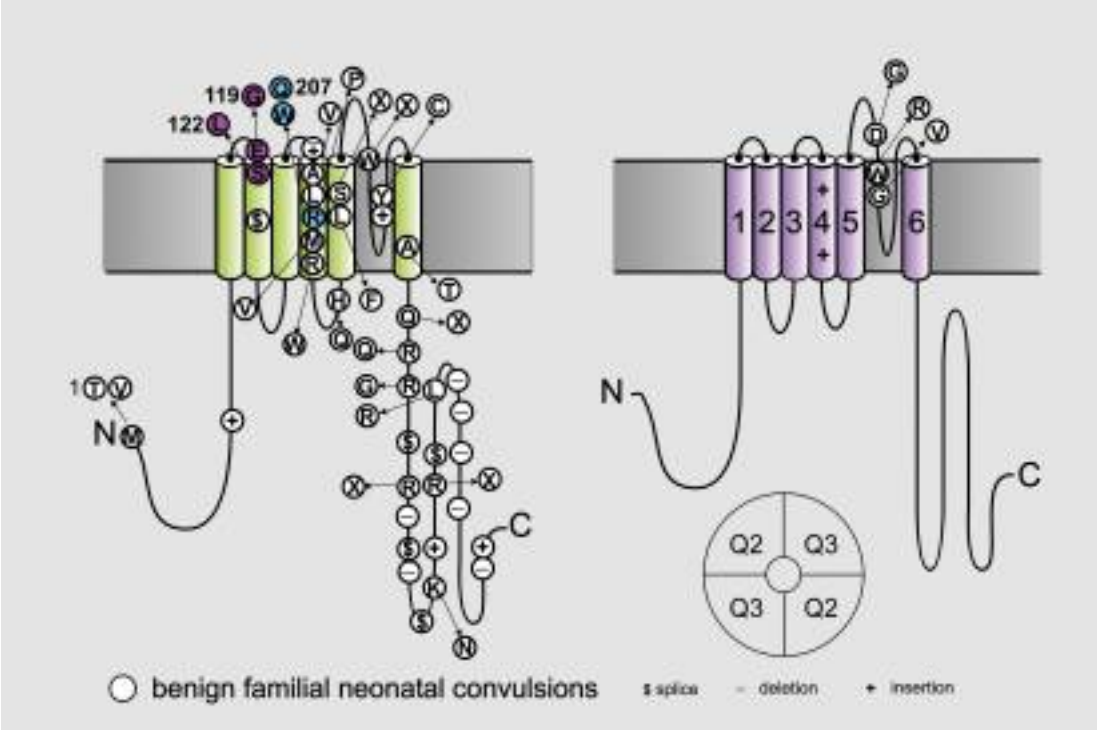


Front. Pharmacol., 23 March 2012

Benign Familial Neonatal Seizures (BFNS)

- Autosomal dominant
- Seizures onset between 2 - 8 days, remission within first months of life
- Seizures: tonic -> autonomic and motor changes, uni- or bilateral. Often with episodes of apnea
- Investigations normal
- Psychomotor development normal
- Small increased risk of recurring seizures later in life

1998: *KCNQ2* and *KCNQ3* in BFNS



Missense, nonsense, splicing, frameshift mutations, intragenic insertions/deletions, whole gene deletions described

Where the story starts

- *KCNQ2* screening offered for neonatal seizures in diagnostic unit
  - 2010: 1 patient: refractory seizures and psychomotor regression

- Literature
  - 4 case reports of patients with neonatal seizures and intellectual disability

(Dedek et al 2003, Borgatti et al 2004, Schmitt et al 2005, Steinlein et al. 2007)

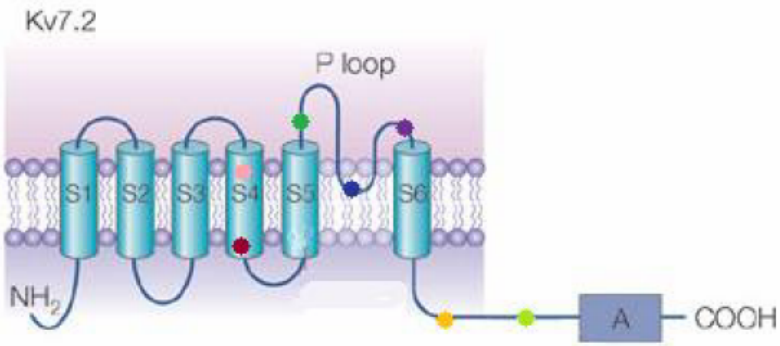
Methods

- *KCNQ2* and *KCNQ3* screening in 80 patients with unexplained neonatal or early onset epileptic encephalopathy
  - Onset < 3 months
  - Slowing of psychomotor development
  - Metabolic screening normal
  - Imaging: no explanation
  - Genetic screening for relevant genes normal

Weckhuysen et al, Ann. Neurol. 2012

Results

- No *KCNQ3* mutations
- 7 novel *KCNQ2* missense mutations in 8/80 patients (10%)
- Not present in 276 ethnically matched controls
- Inheritance
  - 6 mutations *de novo*
  - 1 paternal DNA unavailable
  - 1 mosaic father



Weckhuysen et al, Ann. Neurol. 2012

Ann Neurol. 2012 Jan;71(1):15-25. doi: 10.1002/ana.22644.

KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy.

Weckhuysen S, Mandelstam S, Suls A, Audenaert D, Deconinck T, Claes LR, Deprez L, Smets K, Hristova D, Yordanova I, Jordanova A, Ceulemans B, Jansen A, Hasaerts D, Roelens F, Lagae L, Yendle S, Stanley T, Heron SE, Mulley JC, Berkovic SF, Scheffer IE, de Jonghe P.

Neurogenetics Group, VIB-Department of Molecular Genetics, University of Antwerp, Antwerp, Belgium.

Whole Exome Sequencing Identifies *KCNQ2* Mutations in Ohtahara Syndrome

Hirotomo Saitsu, MD, PhD,[1] Mitsuhiro Kato, MD, PhD,[2]
Ayaka Koide, MD, PhD,[3] Tomohide Goto, MD, PhD,[3]
Takako Fujita, MD,[4] Kiyomi Nishiyama, PhD,[1]

Epilepsia. 2013 Jul;54(7):1282-7. doi: 10.1111/epi.12200. Epub 2013 Apr 26.

Clinical spectrum of early onset epileptic encephalopathies caused by KCNQ2 mutation.

Kato M, Yamagata T, Kubota M, Arai H, Yamashita S, Nakagawa T, Fujii T, Sugai K, Imai K, Uster T, Chitayat D, Weiss S, Kashii H, Kusano R, Matsumoto A, Nakamura K, Oyazato Y, Maeno M, Nishiyama K, Kodera H, Nakashima M, Tsurusaki Y, Miyake N, Saito K, Hayasaka K, Matsumoto N, Saitsu H.

Orphanet J Rare Dis. 2013 May 22;8:80. doi: 10.1186/1750-1172-8-80.

Similar early characteristics but variable neurological outcome of patients with a de novo mutation of KCNQ2.

Milh M, Boutry-Kryza N, Sutera-Sardo J, Mignot C, Auvin S, Lacoste C, Villeneuve N, Roubertie A, Heron B, Carneiro M, Kaminska A, Altuzarra C, Blanchard G, Ville D, Barthez MA, Heron D, Gras D, Afenjar A, Dorison N, Doummar D, Billette de Villemeur T, An I, Jacquette A, Charles P, Perrier J, Isidor B, Vercueil L, Chabrol B, Badens C, Lesca G, Villard L.

Neurology. 2013 Nov 5;81(19):1697-703. doi: 10.1212/01.wnl.0000435296.72400.a1. Epub 2013 Oct 9.

Extending the KCNQ2 encephalopathy spectrum: Clinical and neuroimaging findings in 17 patients.

Weckhuysen S, Ivanovic V, Hendrickx R, Van Coster R, Hjalgrim H, Møller RS, Grønborg S, Schoonjans AS, Ceulemans B, Heavin SB, Eltze C, Horvath R, Casara G, Pisano T, Giordano L, Rostasy K, Haberlandt E, Albrecht B, Bevot A, Benkel I, Syrbe S, Sheidley B, Guerrini R, Poduri A, Lemke JR, Mandelstam S, Scheffer I, Angriman M, Striano P, Marini C, Suls A, De Jonghe P; KCNQ2 Study Group.

Neurology. 2014 Jan 28;82(4):368-70. doi: 10.1212/WNL.0000000000000060. Epub 2013 Dec 26.

KCNQ2 encephalopathy: delineation of the electroclinical phenotype and treatment response.

Numis AL[1], Angriman M, Sullivan JE, Lewis AJ, Striano P, Nabbout R, Cilio MR.

- September 2014:
  - 62 patients with KCNQ2 encephalopathy described in literature
    - 44 different mutations
  - 23 additional non-reported European patients
    - 8 novel mutations

KCNQ2 encephalopathy

- 10% of patients with neonatal EE of unknown etiology
- *KCNQ2* encephalopathy mutations
  - All missense mutations
    - Several mutations recurrent in multiple patients
    - Several mutations in same codon
  - Novel: not reported in BFNS
- Inheritance
  - *de novo*
  - 1 mosaic father with BNS phenotype

KCNQ2 encephalopathy

- Neonatal onset

  1 patient onset at 5 months
- Seizure type at onset

  Prominent tonic component

  Often autonomic features: apnea, desaturation, bradycardia
- Dramatic onset, multiple sz daily
- EEG at onset burst-suppression pattern or multifocal epileptic activity
- Range of cognitive outcome, mostly severe to profound intellectual disability

MECHANISM

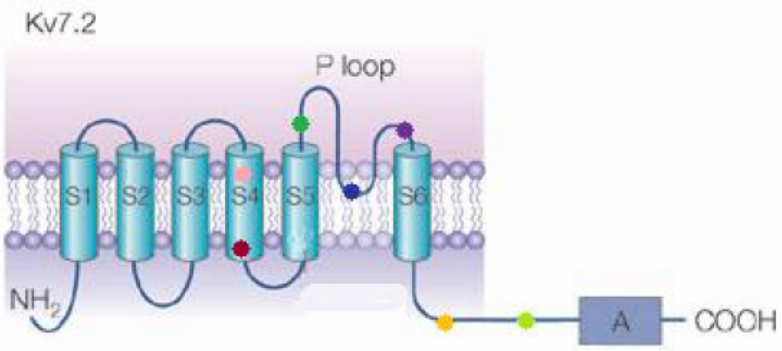
?

BFNS ← *KCNQ2* mutation → *KCNQ2* encephalopathy

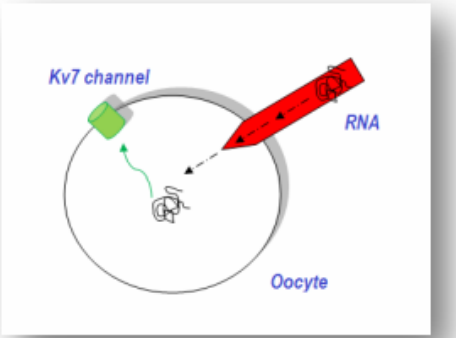
20-50% reduction of M current.
(Few cases with dominant negative effect: BFNS+myokymia)
=> Haploinsufficiency

Mechanism

I205V (S4-segment)
R213Q (S4-segment)
A 256P (pore-loop)
T 273M (pore-loop)
G290D (S6-segment)
M518V (C-terminus)
R532W (C-terminus)

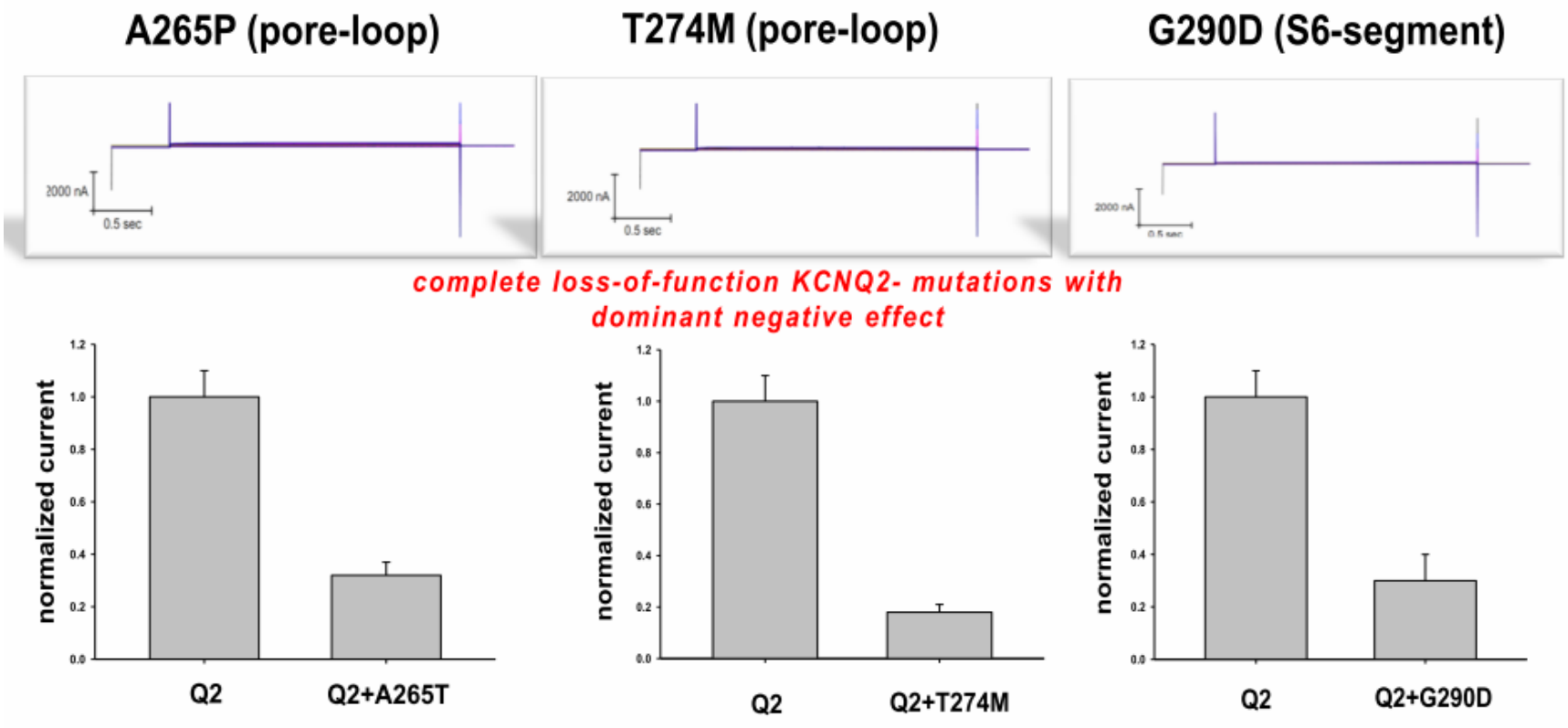


Heterologous expression of KCNQ-mutations in Oocytes



Orhan et al., Annals of Neurology, 2014

Mechanism



5/7 mutations: dominant negative effect on channel function

Orhan et al., Annals of Neurology, 2014

Mouse model of dominant negative mutation

Nat Neurosci. 2005 Jan;8(1):51-60. Epub 2004 Dec 19.

Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior.

Peters HC[1], Hu H, Pongs O, Storm JF, Isbrandt D.

- Transgenic adult mice carrying dominant negative *KCNQ2* mutation
  - recurrent spontaneous seizures
  - impaired spatial learning
  - marked hyperactivity
- Pyramidal neurons
  - diminished M-type K+ current, hyperexcitable
- Studies of hippocampal sections
  - laminar disorganization of area CA1

KCNQ2 spectrum

Functional aspects mutation + genetic background

BFNS — ***KCNQ2*** — *KCNQ2* encephalopathy

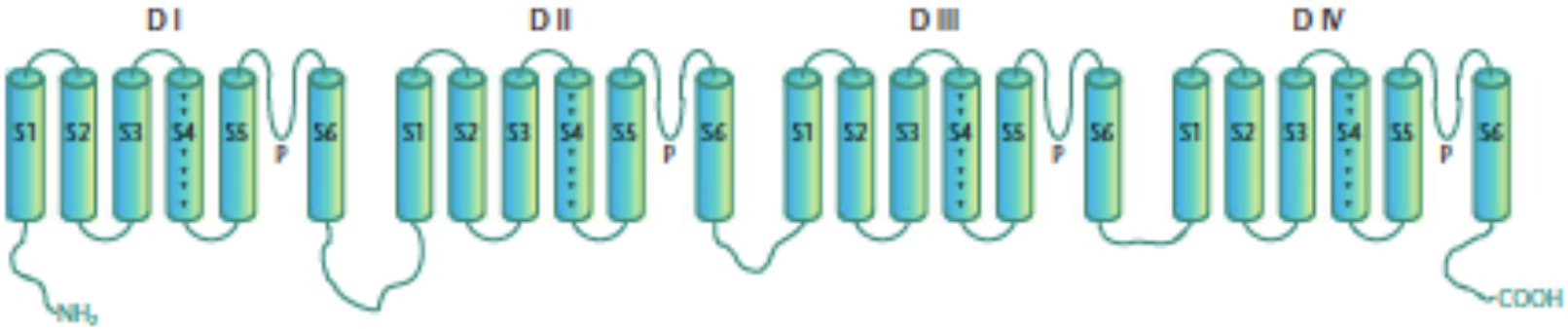
- Mostly Inherited

- Mostly *de novo*
- Mozaicism described in inherited cases

SCNIA

SCN1A

- Encoding $Na_v1.1$



SCN1A

FEVER SENSITIVITY

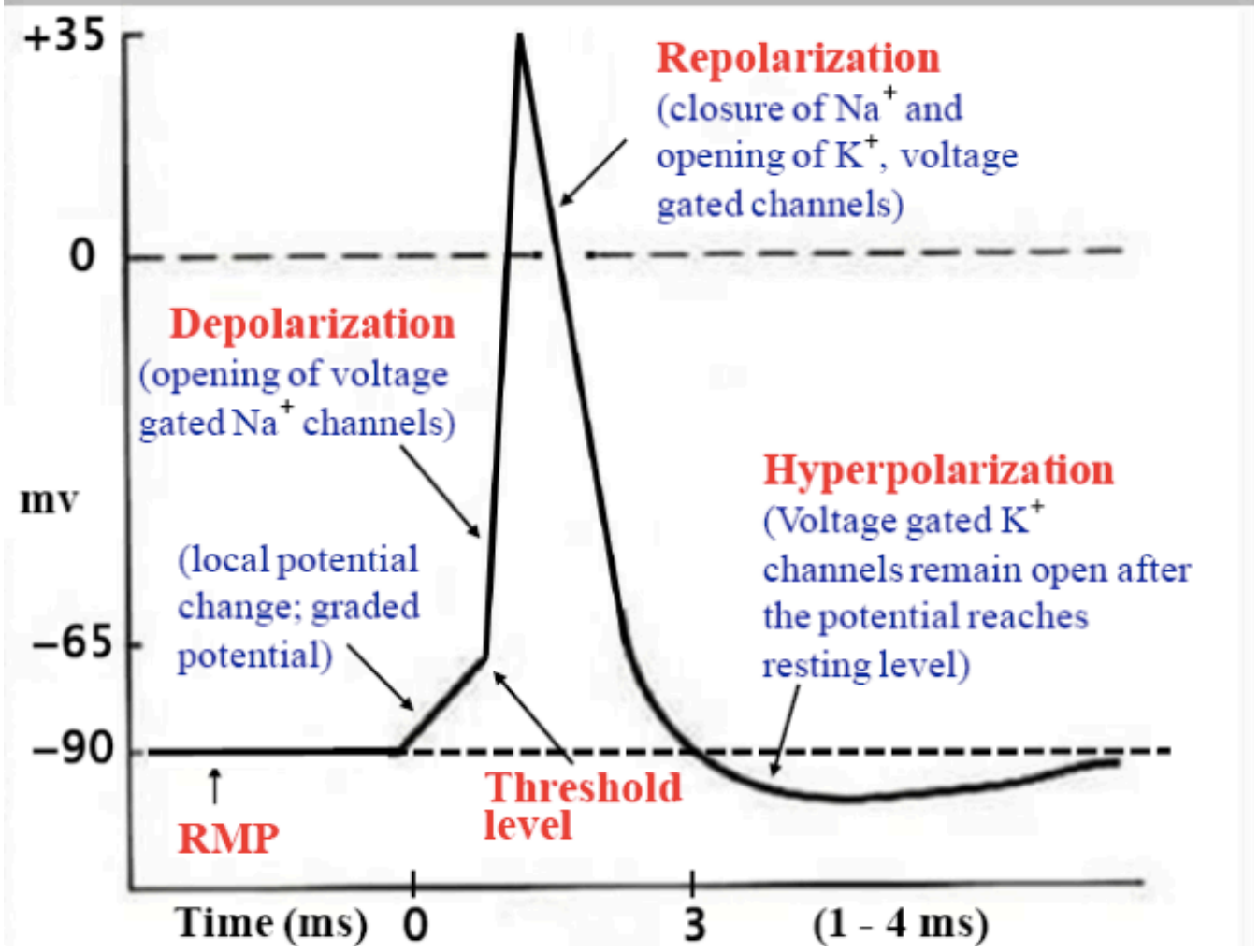
- 1997

  Clinical description GEFS+ syndrome (Scheffer et al.)
- 2000

  SCN1A mutation in large GEFS+ family (Escayg et al.)
- 2001

  SCN1A mutation in 7/7 patients with Dravet syndrome (Claes et al.)
- Follow up studies: *SCN1A* mutations/deletions in 70-80% of Dravet patients

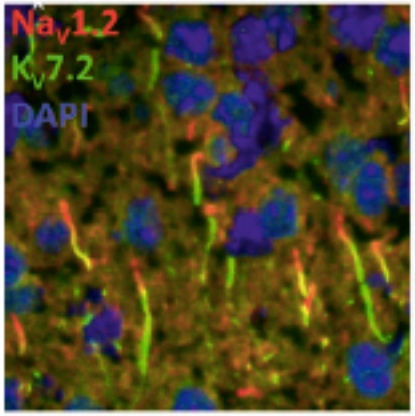
Action potential

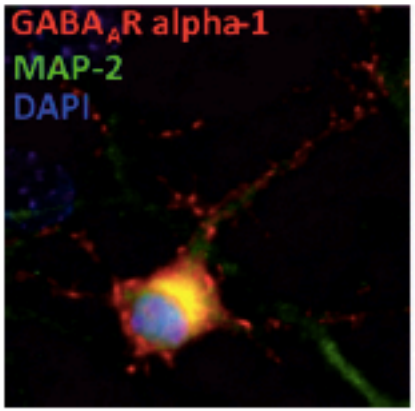


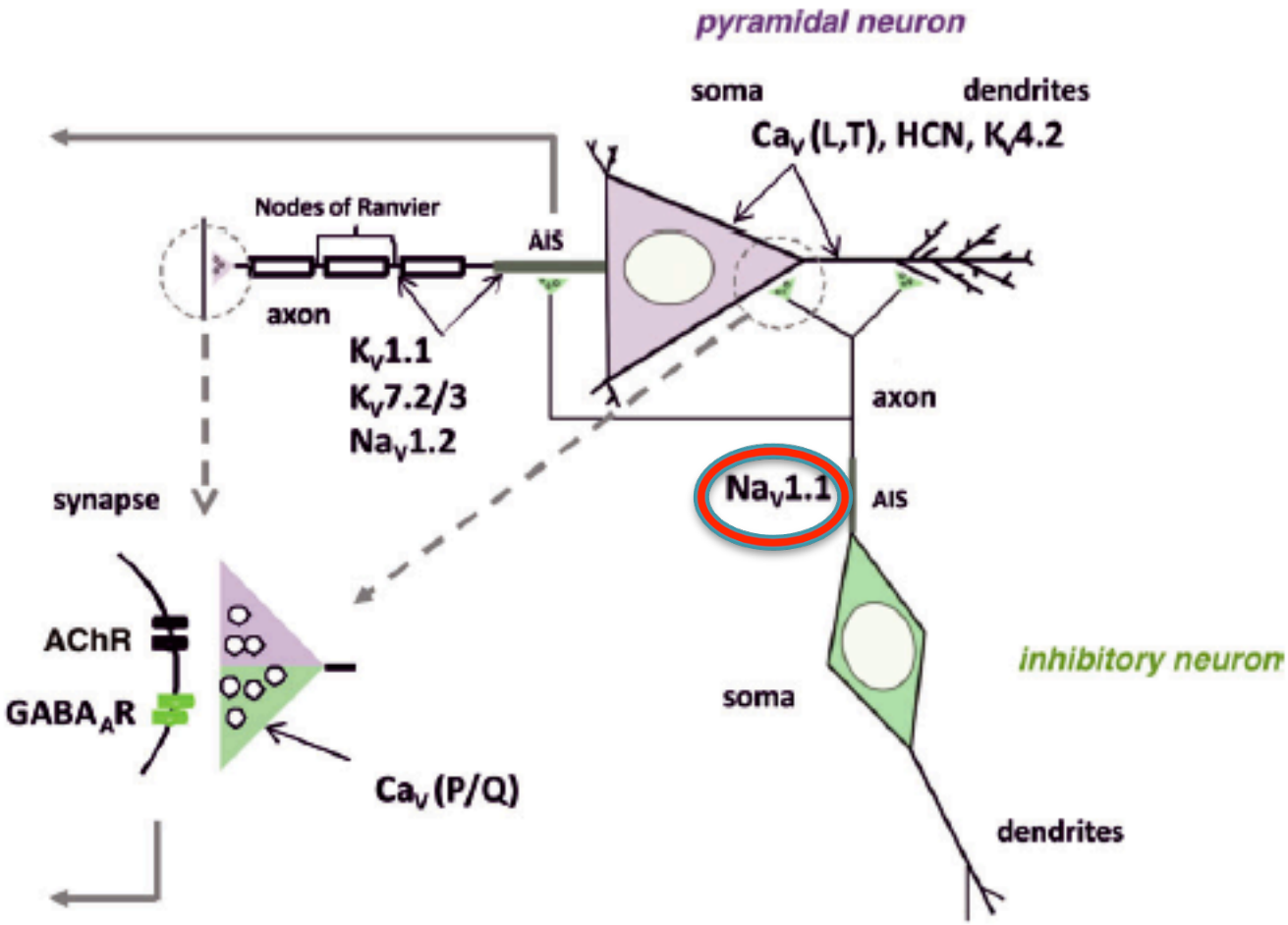
LOF mutation in excitatory ion channel subunit

??

Epilepsy


$Na_V1.2$
$K_V7.2$
DAPI


$GABA_AR$ alpha-1
MAP-2
DAPI


pyramidal neuron
soma
dendrites
Ca_V (L,T), HCN, $K_V4.2$
Nodes of Ranvier
AIS
axon
$K_V1.1$
$K_V7.2/3$
$Na_V1.2$
axon
$Na_V1.1$
AIS
synapse
AChR
$GABA_AR$
Ca_V (P/Q)
inhibitory neuron
soma
dendrites

??

GEFS+ ← *SCN1A* mutation → Dravet

General Rule

- GEFS+
  - Missense mutations
    - Outside pore region
- SMEI
  - Truncating mutations, splice site mutations, deletions
  - Missense mutations
    - In pore region
    - More often changes in AA polarity

BUT exceptions!!

Ion channel spectrum

Functional aspects mutation + genetic background

BFNS — ***KCNQ2*** — *KCNQ2* encephalopathy

- Mostly Inherited

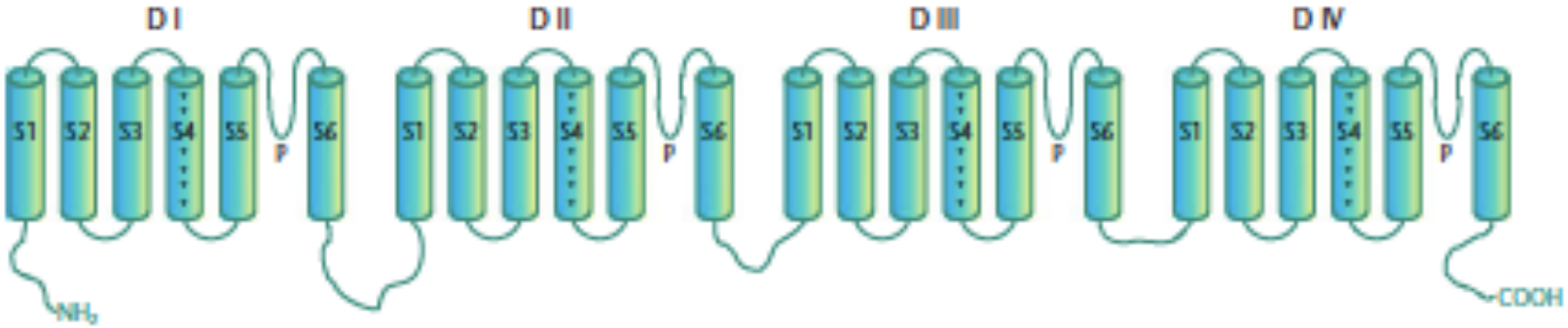
- Mostly *de novo*
- Mozaicism described in inherited cases

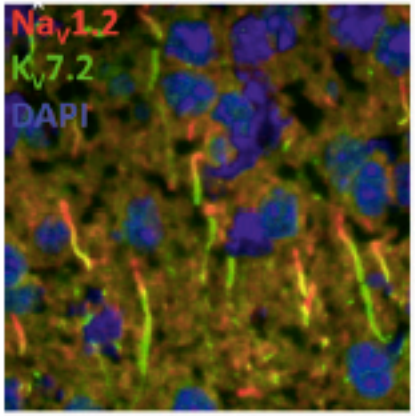
GEFS+ — ***SCN1A*** — Dravet Syndrome

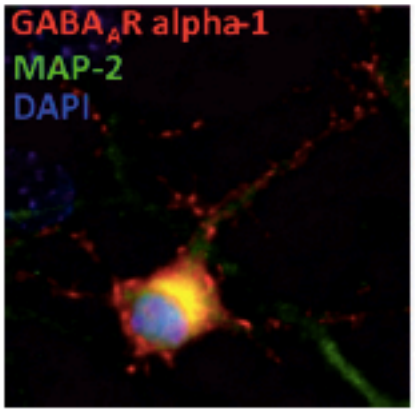
Functional aspects mutation + genetic background

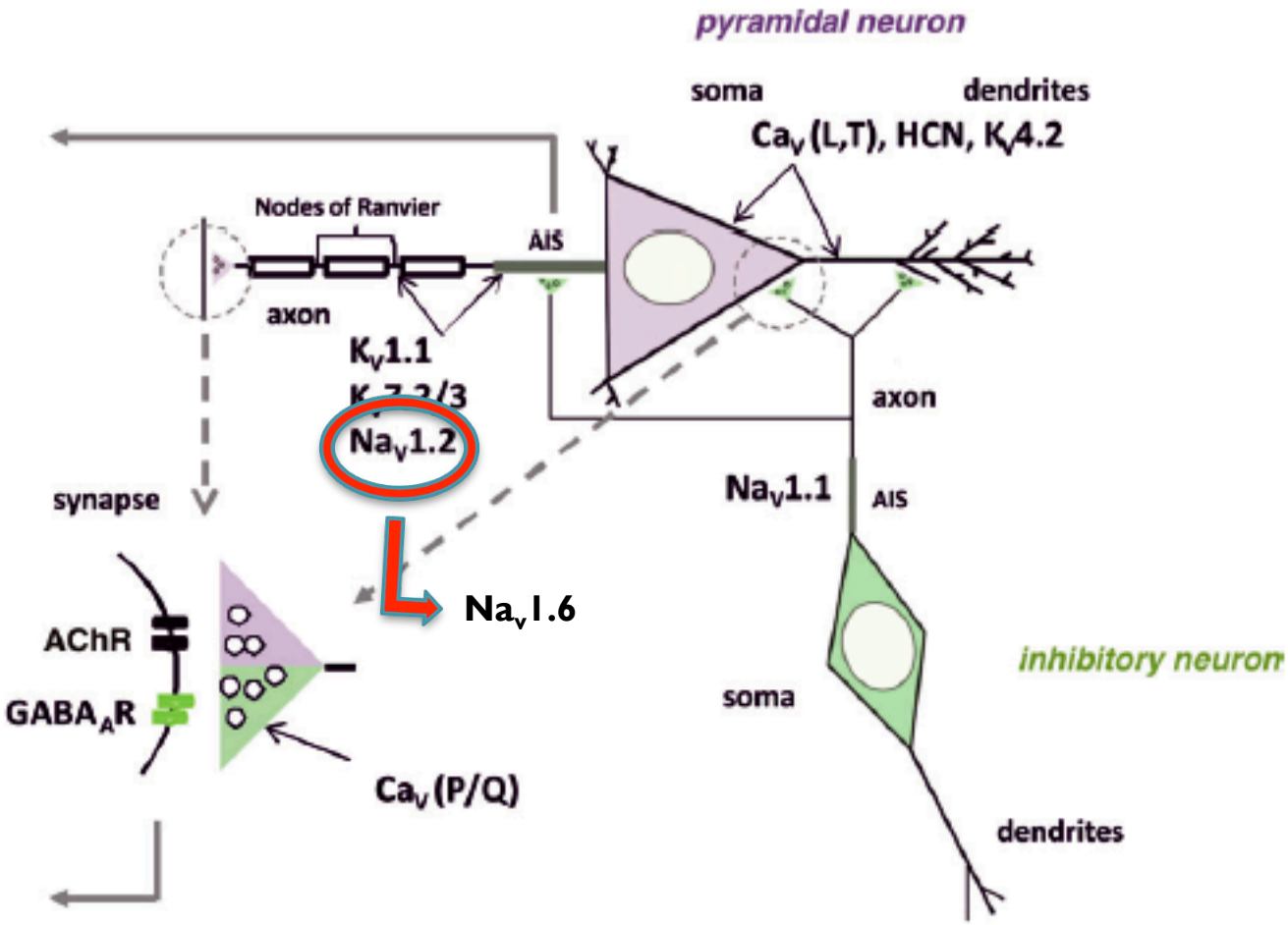
SCN2A

- Encoding $Na_v1.2$




$Na_V1.2$
$K_V7.2$
DAPI


$GABA_AR$ alpha-1
MAP-2
DAPI


pyramidal neuron
soma
dendrites
Ca_V (L,T), HCN, $K_V4.2$
Nodes of Ranvier
AIS
axon
$K_V1.1$
$Na_V1.2$
axon
$Na_V1.1$
AIS
synapse
$Na_V1.6$
AChR
$GABA_AR$
Ca_V (P/Q)
inhibitory neuron
soma
dendrites

SCN2A in BFNIS

- Benign familial neonatal-infantile seizures
  - Autosomal dominant
  - Seizures onset between 2 days and 7 months, remission by 12 months of life
  - Investigations normal; Psychomotor development normal

2002: *SCN2A* mutations in BFNIS. All missense mutations

SCN2A encephalopathy

J N ar 17;24(11):2690-8.

ation of the sodium channel gene SCN2A in a patient with intractable epilepsy and mental

Ka azaki E, Okamura N, Montal M, Makita N, Tanaka M, Fukushima K, Fujiwara T, Inoue Y, Yamakawa K.
Laborator nce Institute, Saitama 351-0198, Japan.

Brain Dev. 20 016/j.braindev.2009.08.009. Epub 2009 Sep 23.

Missense mut channel gene SCN2A causes Dravet syndrome.

Shi X, Yasumoto S, Nakaga S, Hirose S.

Neurology. 2009 Sep 29;73(13):1046-53. doi: 10.1

De novo mutations of voltage-gated s gene SCN2A in intractable epilepsies.

Ogiwara I, Ito K, Sawaishi Y, Osaka H, Mazaki E, Inoue I, Mo ujiwara T, Inoue Y, Kaneda M, Yamakawa K.

Epilepsia. 2013 May;54(5):e81-5. doi: 10.1111/epi.12137. Epub 2013 Mar 28.

Whole genome sequencing identifies SCN2A mutation in monozygo ndrome and unique neuropathologic findings.

Touma M, Joshi M, Connolly MC, Grant PE, Hansen AR, Khwaja O, Berry GT, Kinney HC, Poduri A, Agrawa

Neurology. 2013 Sep 10;81(11):992-8. doi: 10.1212/WNL.0b013e3182a43e57. Epub 2013 Aug 9.

Clinical spectrum of SCN2A mutations expanding to Ohtahara syndrome.

Nakamura K, Kato M, Osaka H, Yamashita S, Nakagawa E, Haginoya K, Tohyama J, Okuda M, Wada T, Shimakawa S, Imai K, Ta ev D, Lerman-Sagie T, Cervantes-Barragán DE, Villarroel CE, Ohfu M, Writzl K, Gnidovec Strazisar B, Hirabayashi S, Chitayat D, Myles Rei s, Kodera H, Nakashima M, Tsurusaki Y, Miyake N, Hayasaka K, Matsumoto N, Saitsu H.

BMC Med Genet. 2014 Mar 20;15:35. doi: 10.1186/1471-2350-15-35.

De novo SCN2A splice site mutation in a boy with Autism spectrum disorder.

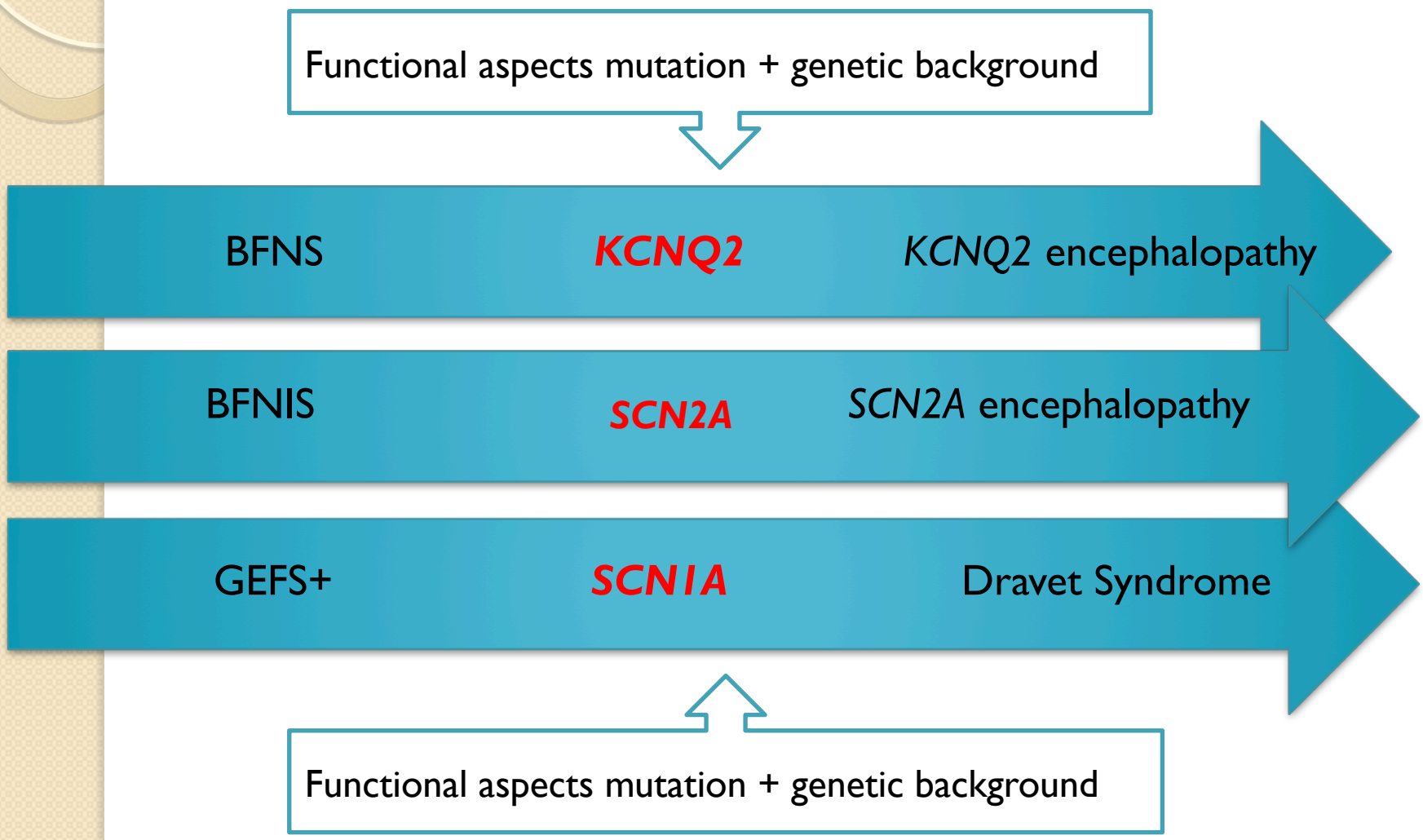
Tavassoli T, Kolevzon A, Wang AT, Curchack-Lichtin J, Halpern D, Schwartz L, Soffes S, Bush L, Grodberg D, Cai G, Buxbaum JD.

Genotype-phenotype correlation

- Across all phenotypes
  - Most mutations = missense mutations
    - BFNIS predominantly in transmembrane domains (TMD), "severe" mutations more outside TMD
    - Both net gain vs. loss of function described
  - All truncating mutations => EE/ID/autism

- No conclusive results (yet)

SCN2A spectrum



KCNT1

KCNT1

- Sodium-gated potassium channel
- Slow hyperpolarization that follows repetitive firing
- C-terminal cytoplasmic domain interacts with protein network including FMRP

KCNT1

Nat Genet. 2012 Nov;44(11):1188-90. doi: 10.1038/ng.2440. Epub 2012 Oct 21.

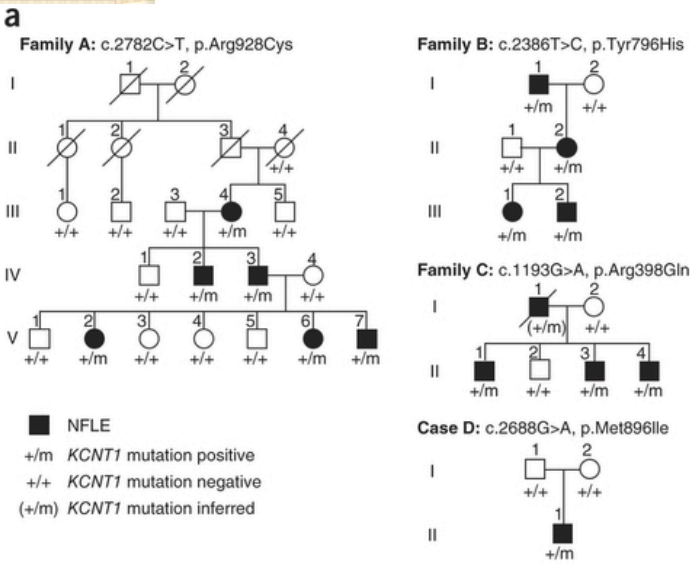
Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy.

Heron SE[1], Smith KR, Bahlo M, Nobili L, Kahana E, Licchetta L, Oliver KL, Mazarib A, Afawi Z, Korczyn A, Plazzi G, Petrou S, Berkovic SF, Scheffer IE, Dibbens LM.

Nat Genet. 2012 Nov;44(11):1255-9. doi: 10.1038/ng.2441. Epub 2012 Oct 21.

De novo gain-of-function KCNT1 channel mutations cause malignant migrating partial seizures of infancy.

Barcia G[1], Fleming MR, Deligniere A, Gazula VR, Brown MR, Langouet M, Chen H, Kronengold J, Abhyankar A, Cilio R, Nitschke P, Kaminska A, Boddaert N, Casanova JL, Desguerre I, Munnich A, Dulac O, Kaczmarek LK, Colleaux L, Nabbout R.



ADNFLE

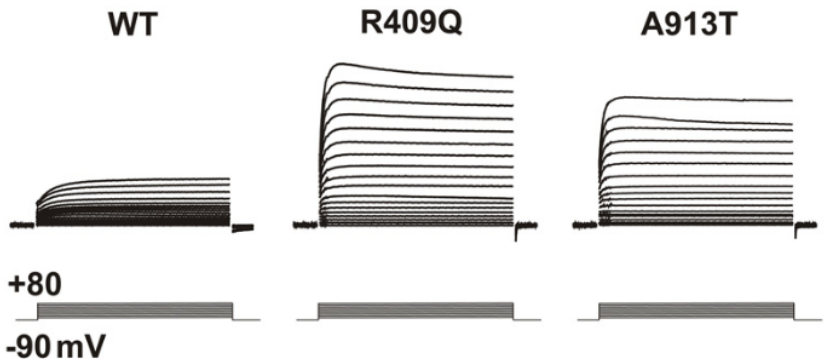
			Age of onset		Other clinical features				
Family	Number of affected individuals	Origin	Mean ± s.d.	Median (range) in years	Intellectual disability	Behavioral or psychiatric problem	Refractory epilepsy[a]	*KCNT1* mutation	KCNT1 amino-acid change
A	6	Australian[b] (British descent)	4.6 ± 5.9	2 (1–15)[d]	3/6	5/6	5/6	c.2782C>T	p.Arg928Cys
B	4	Italian[c]	5.5 ± 2.1	5.5 (3–8)	3/4	2/4	2/4	c.2386T>C	p.Tyr796His
C	4	Israeli (Sephardic Jewish)	8.5 ± 6.4	5.5 (5–18)	0/4	2/4	0/4	c.1193G>A	p.Arg398Gln
D	1	Australian (British descent)	9.0	N/A	0/1	1/1	1/1	c.2688G>A	p.Met896Ile

Heron et al., 2012

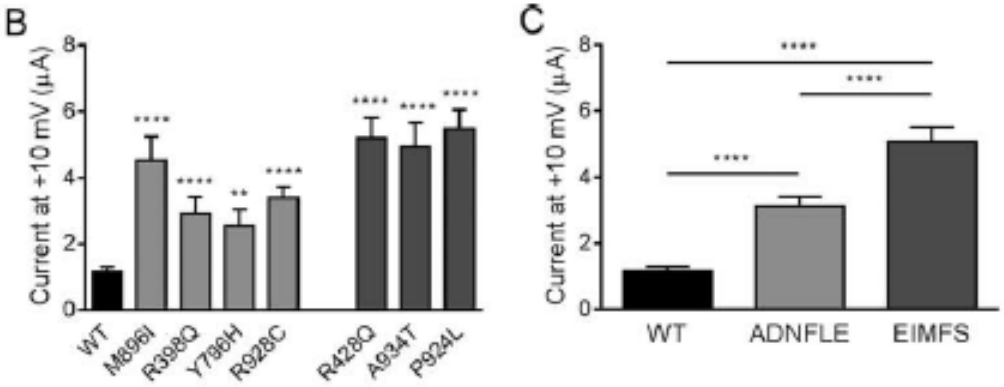
MMPSI (EIMFS)

- Onset < 6 months
- Polymorphic focal seizures
- Migrating ictal EEG pattern
- Arrest of psychomotor development

cDNA position	Protein change
c.2800G>A	p.Ala934Thr
c.1283G>A	p.Arg428Gln
c.1283G>A	p.Arg428Gln
c.1283G>A	p.Arg428Gln
c.1421G>A	p.Arg474His
c.2280C>G	p.Ile760Met



Barcia et al., 2012



Milligan et al., Annals of Neurology 2014

Ion channel genes in monogenetic epilepsies

Gene	Benign	Severe/Epileptic encephalopathies
CACNA1A	Absence epilepsy + EA	
CHRNA4	ADNFLE	
CHRNB2	ADNFLE	
CHRNA2	ADNFLE	
GABRA1	GEFS+, JME	Dravet
GABRB3		EE
GABRG2	GEFS+ (with CAE)	
GRIN2A	BRE	CSWS/LKS
GRIN2B		EE
HCN1		Dravet
KCNA1	Focal epilepsy + EA	
KCNJ11		Neonatal diabetes + Epi + DD
KCNQ2	BFNS	Ohtahara, neonatal EE
KCNQ3	BFNS	Neonatal epi +MR
KCNMA1	IGE + PD	
KCNT1	ADNFLE	MMPSI
SCN1A	GEFS+	Dravet, MMPSI
SCN1B	GEFS+	Dravet
SCN2A	BFNIS	Ohtahara, EE
SCN8A		EE

TREATMENT CONSEQUENCES?

Dravet syndrome with *SCN1A* mutation

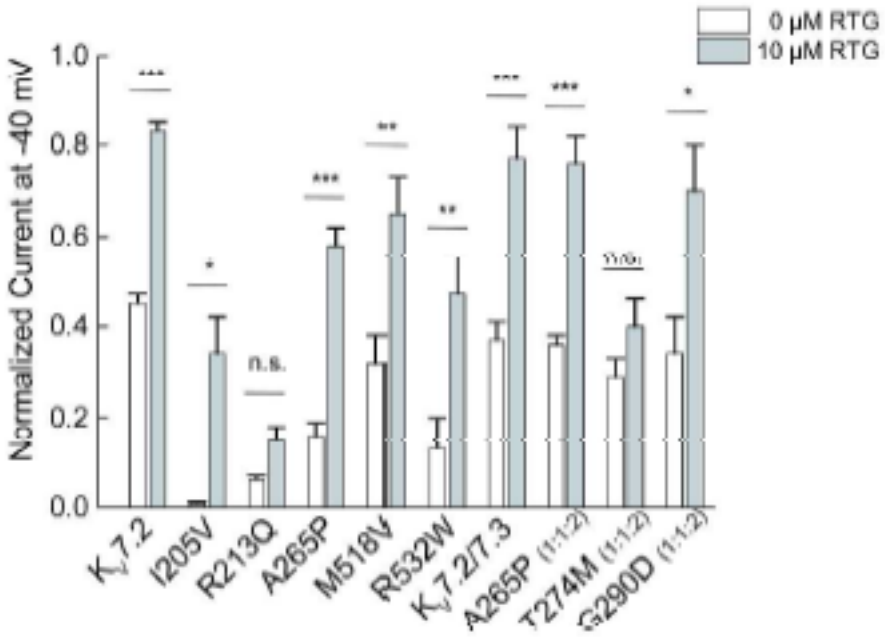
SCN1A loss of function

⇒ Aggravation of sz by sodium channel blockers

(carbamazepine, oxcarbazepine, lamotrigine,…)

KCNQ2 encephalopathy

- Dominant negative effect
  - Potassium channel opener retigabine



SCN2A encephalopathy

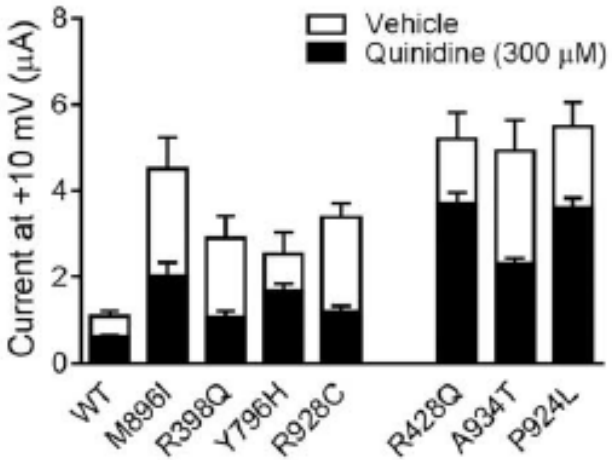
- Some patients respond well to sodium channel blockers (study ongoing)

MMPSI with *KCNT1* mutation

Ann Neurol. 2014 Apr;75(4):581-90. doi: 10.1002/ana.24128. Epub 2014 Apr 14.

KCNT1 gain of function in 2 epilepsy phenotypes is reversed by quinidine.

Milligan CJ[1], Li M, Gazina EV, Heron SE, Nair U, Trager C, Reid CA, Venkat A, Younkin DP, Dlugos DJ, Petrovski S, Goldstein DB, Dibbens LM, Scheffer IE, Berkovic SF, Petrou S.



Targeted Treatment of Migrating Partial Seizures of Infancy with Quinidine

David Bearden, MD,[1]
Alanna Strong, PhD,[2]
Jessica Ehnot, PharmD,[3]
Marissa DiGiovine, MD,[1]
Dennis Dlugos, MD, MSCE,[1] and
Ethan M. Goldberg, MD, PhD[1]

Migrating partial seizures of infancy is an early onset epileptic encephalopathy syndrome that is typically resistant to treatment. The most common cause is a gain of function mutation in the potassium channel *KCNT1*. The antiarrhythmic drug quinidine is a partial antagonist of *KCNT1* and hence may be a candidate drug for treatment of this condition. We report the case of a child with migrating partial seizures of infancy secondary to an activating mutation in *KCNT1* treated with quinidine. Treatment with quinidine was correlated with a marked reduction in seizure frequency and improved psychomotor development.

ANN NEUROL 2014;00:000–000

ORIGIN OF ID IN EPILEPSIES DUE TO ION CHANNEL MUTATIONS

Dravet syndrome

- Clinical
  - No strict correlation seizure severity - outcome
  - Treatment change later in life => improvement cognition

- Functional

Neurobiol Dis. 2013 Jun;54:297-307. doi: 10.1016/j.nbd.2012.12.021. Epub 2013 Jan 11.

Focal Scn1a knockdown induces cognitive impairment without seizures.

Bender AC[1], Natola H, Ndong C, Holmes GL, Scott RC, Lenck-Santini PP.

  - siRNA in basal forebrain adult rats for 4 days

KCNQ2 encephalopathy

- Clinical
  - No strict correlation seizure severity – outcome

- Functional

Nat Neurosci. 2005 Jan;8(1):51-60. Epub 2004 Dec 19.

Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior.

Peters HC[1], Hu H, Pongs O, Storm JF, Isbrandt D.

  - Normal hippocampal morphology, not hyperactive, no overt behavioral seizures
  - Impaired spatial learning
  - Reduced M-type K+ current and neuronal hyperexcitability

SCN2A

- De novo mutations in patients with ID/ autism without epilepsy

Epilepsy and ID

Seizures do not explain everything

=> Target cause not only symptom

=> New strategies for treatment development

Neurogenetics group - epilepsy

- Rik Hendrickx
- Tine Deconinck
- Jolien Roovers
- Tania Djémié
- Katia Hardies
- Arvid Suls
- Peter De Jonghe

SPECIAL THANKS TO:

Parents and patients with *KCNQ2* mutations

All treating physicians of patients with *KCNQ2* mutations

Contact: sarahweck@hotmail.com