Targeted therapy in Drug Resistant Epilepsies

A magic remedy in the Post-Genomic Era

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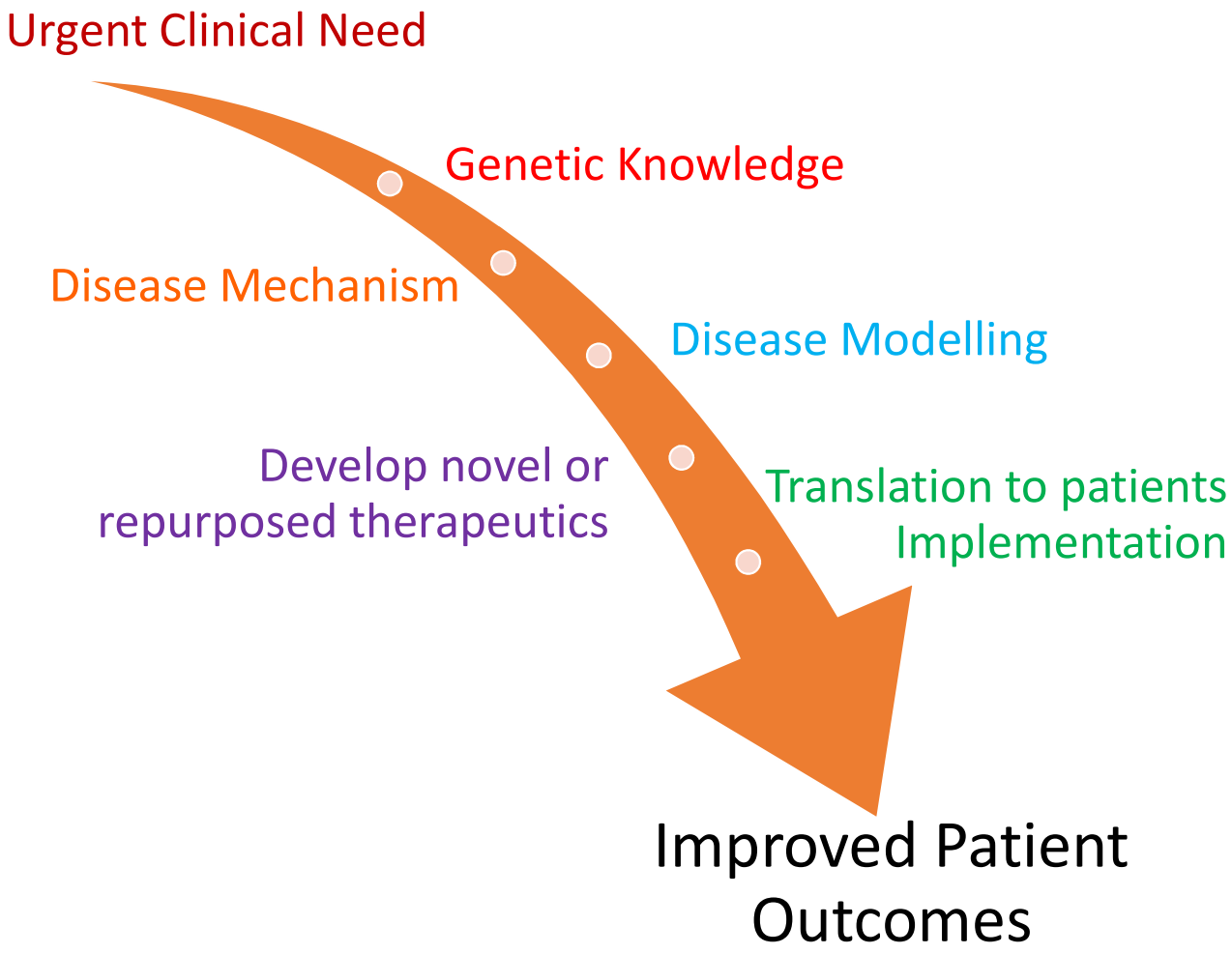
Pasiri Sithinamsuwan

Sasipa Thammongkol

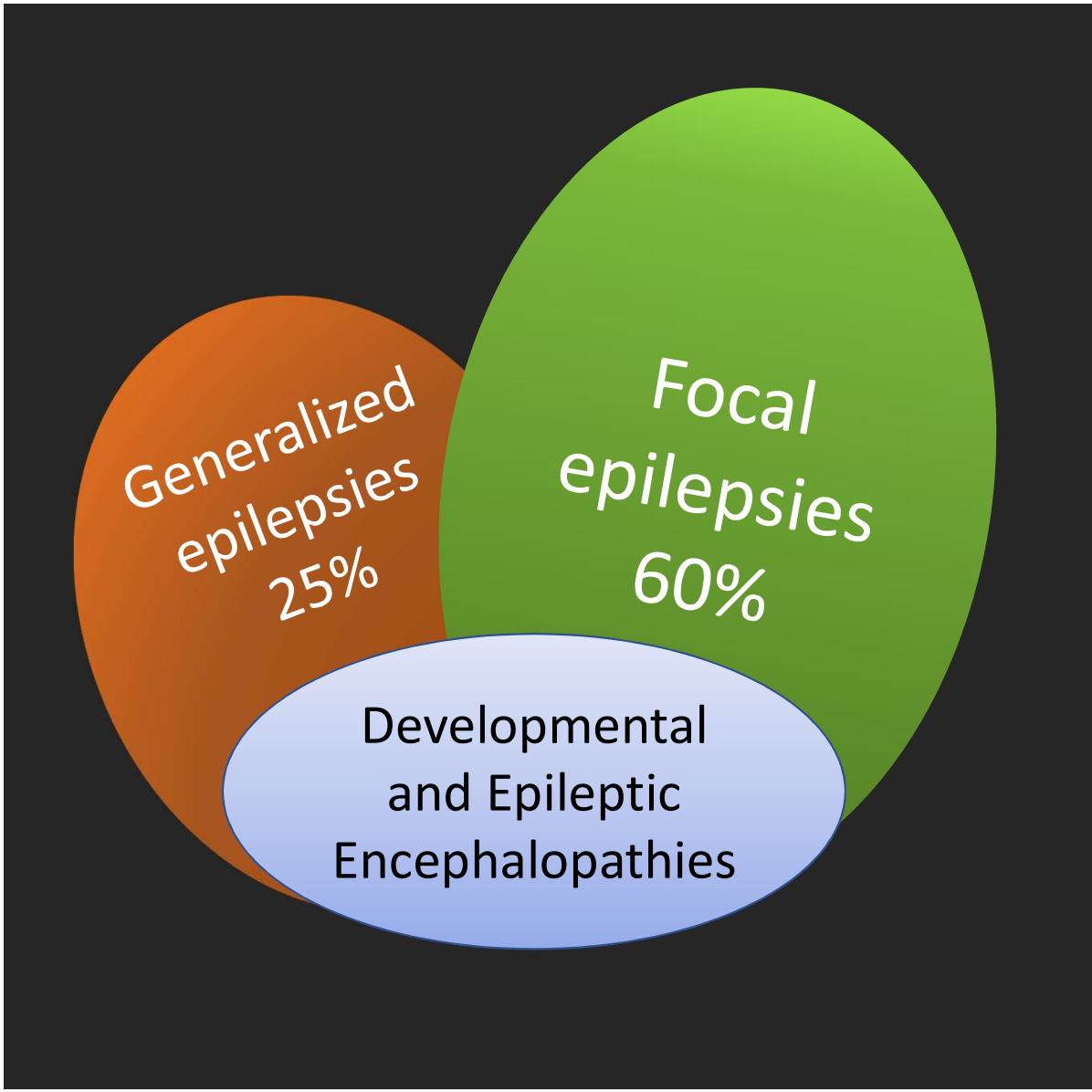
Suthida Yenjun

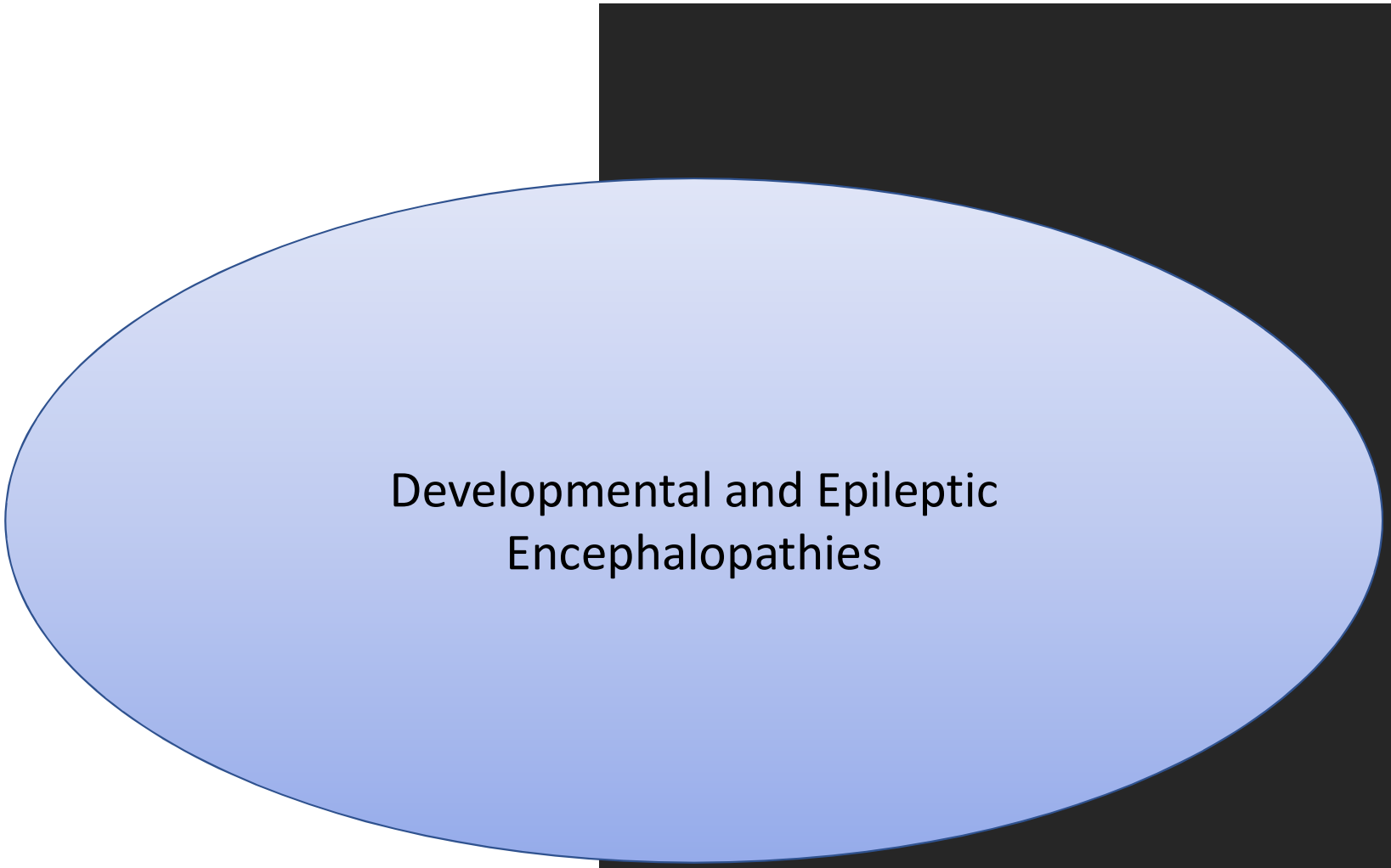
Somjit Sriudomkajorn



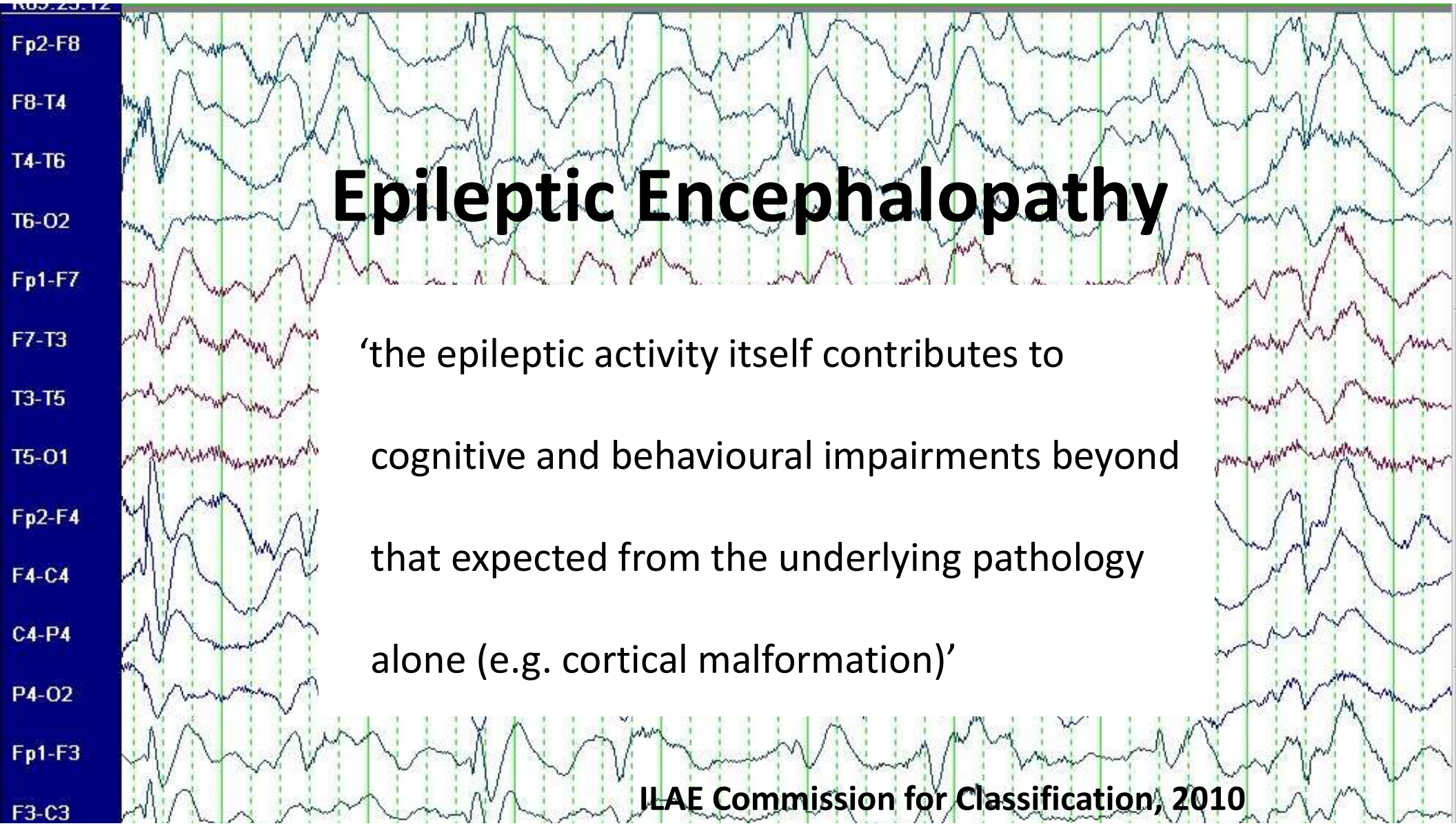
Gene discovery underpins Precision Medicine

Epilepsies


Generalized epilepsies 25%
Focal epilepsies 60%
Developmental and Epileptic Encephalopathies



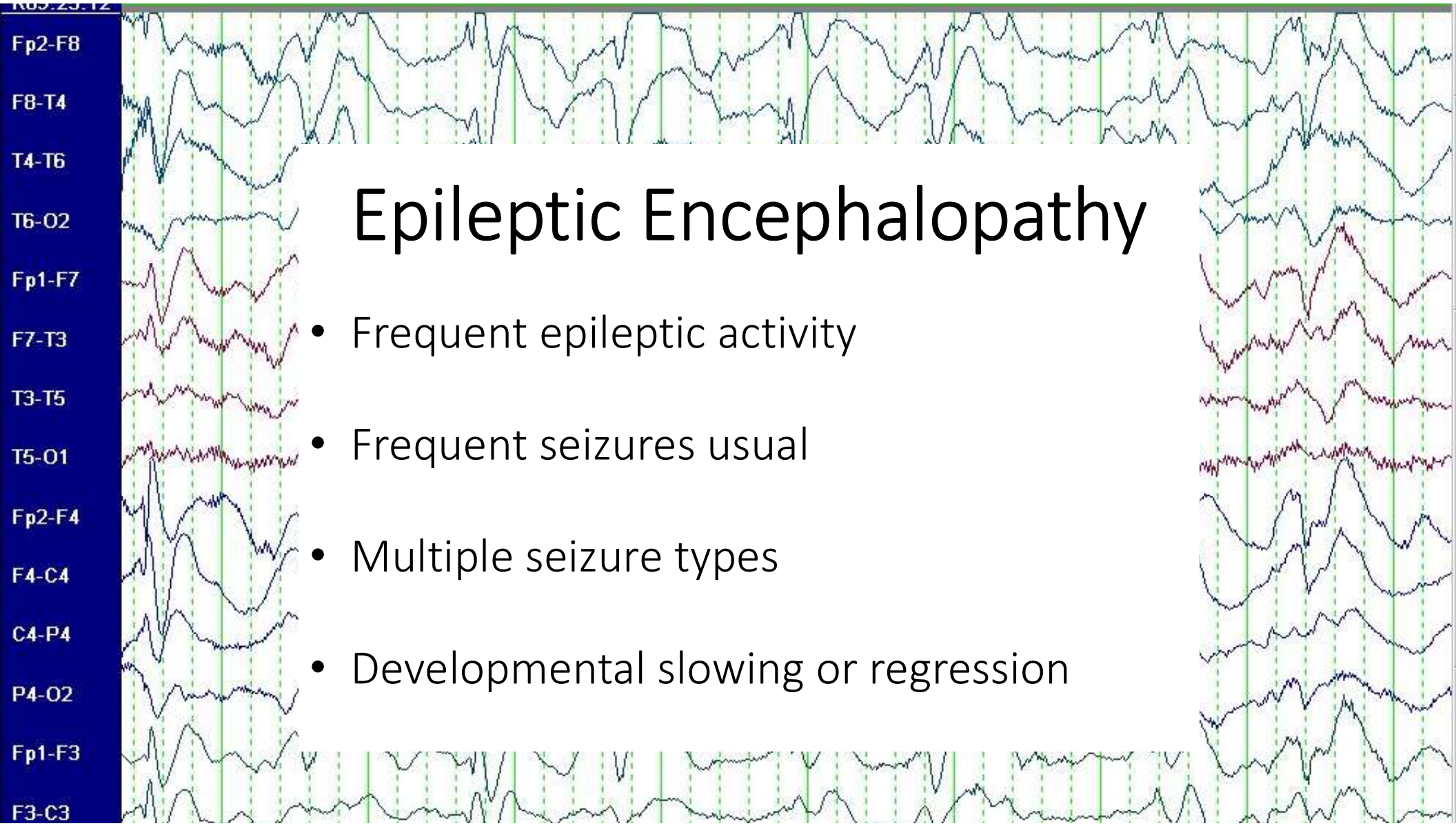
Scheffer et al ILAE Classification 2017



Epileptic Encephalopathy

'the epileptic activity itself contributes to cognitive and behavioural impairments beyond that expected from the underlying pathology alone (e.g. cortical malformation)'

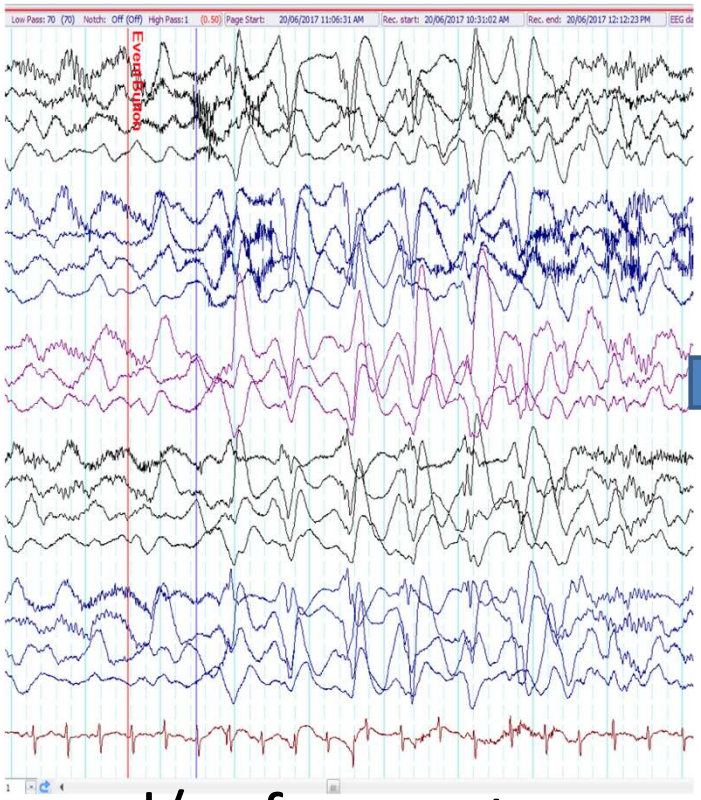
ILAE Commission for Classification, 2010



Epileptic Encephalopathy

- Frequent epileptic activity
- Frequent seizures usual
- Multiple seizure types
- Developmental slowing or regression

Epileptic Encephalopathy – any age

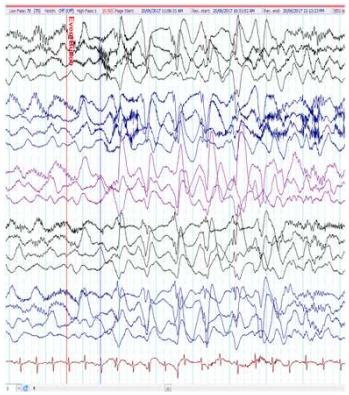


Developmental slowing or regression

Seizures

and/or frequent epileptiform activity

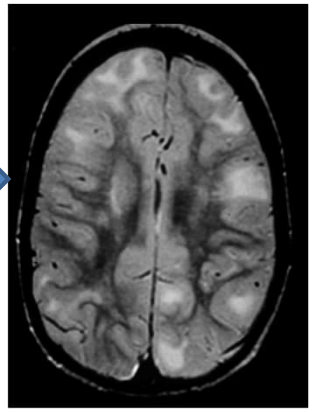
Epileptic Encephalopathy



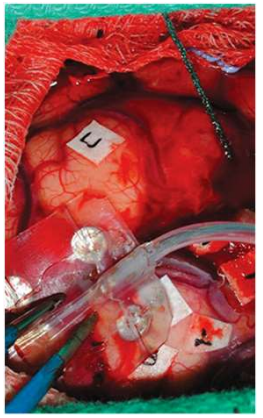
Seizures and/or frequent epileptiform activity

Etiology

Structural

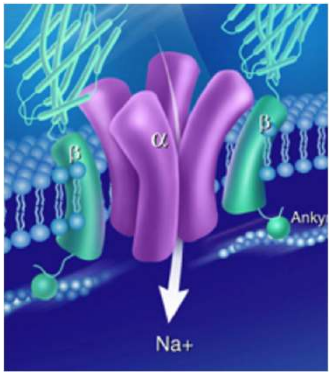


Surgery



Resolution

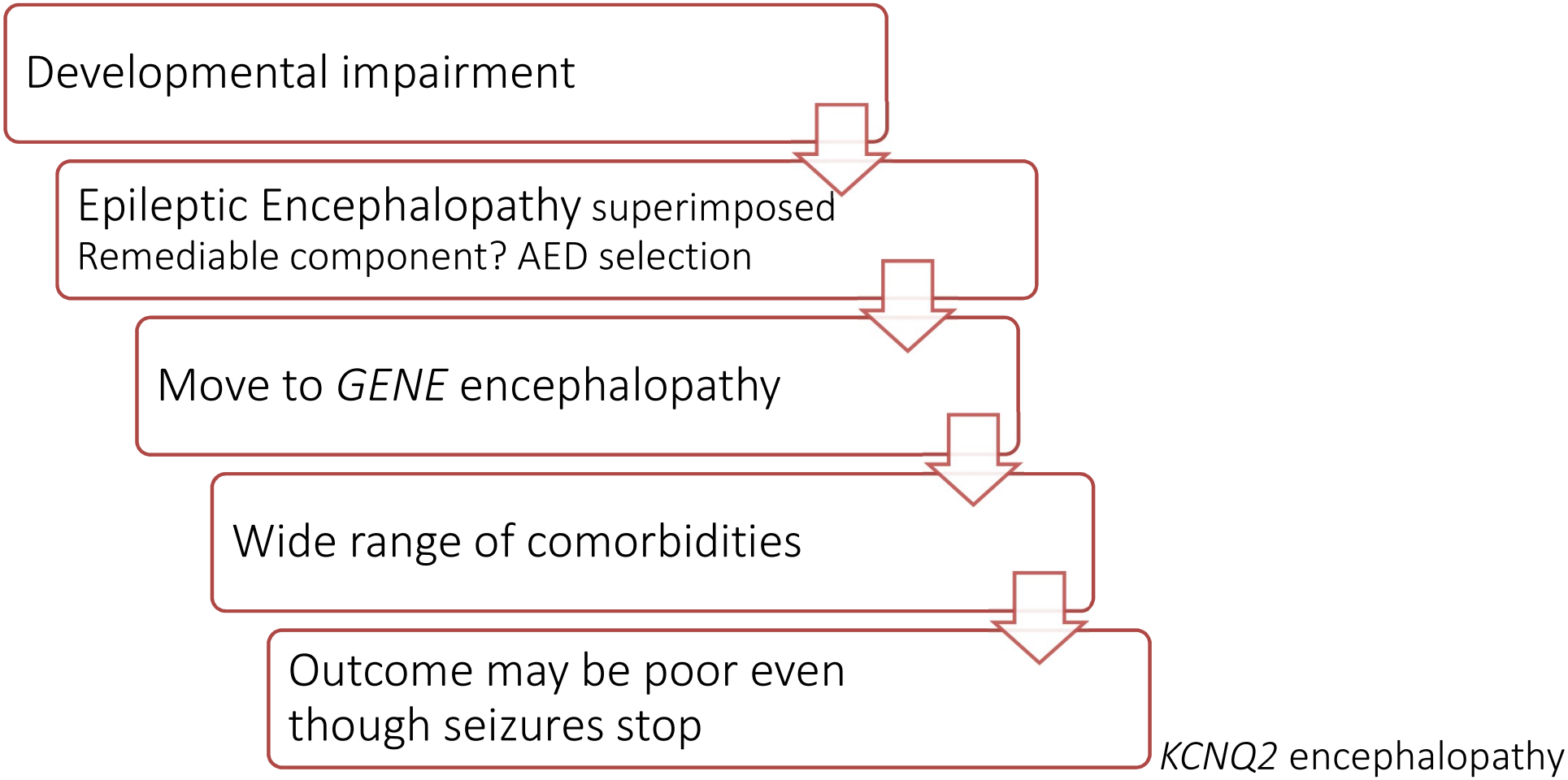
Genetic



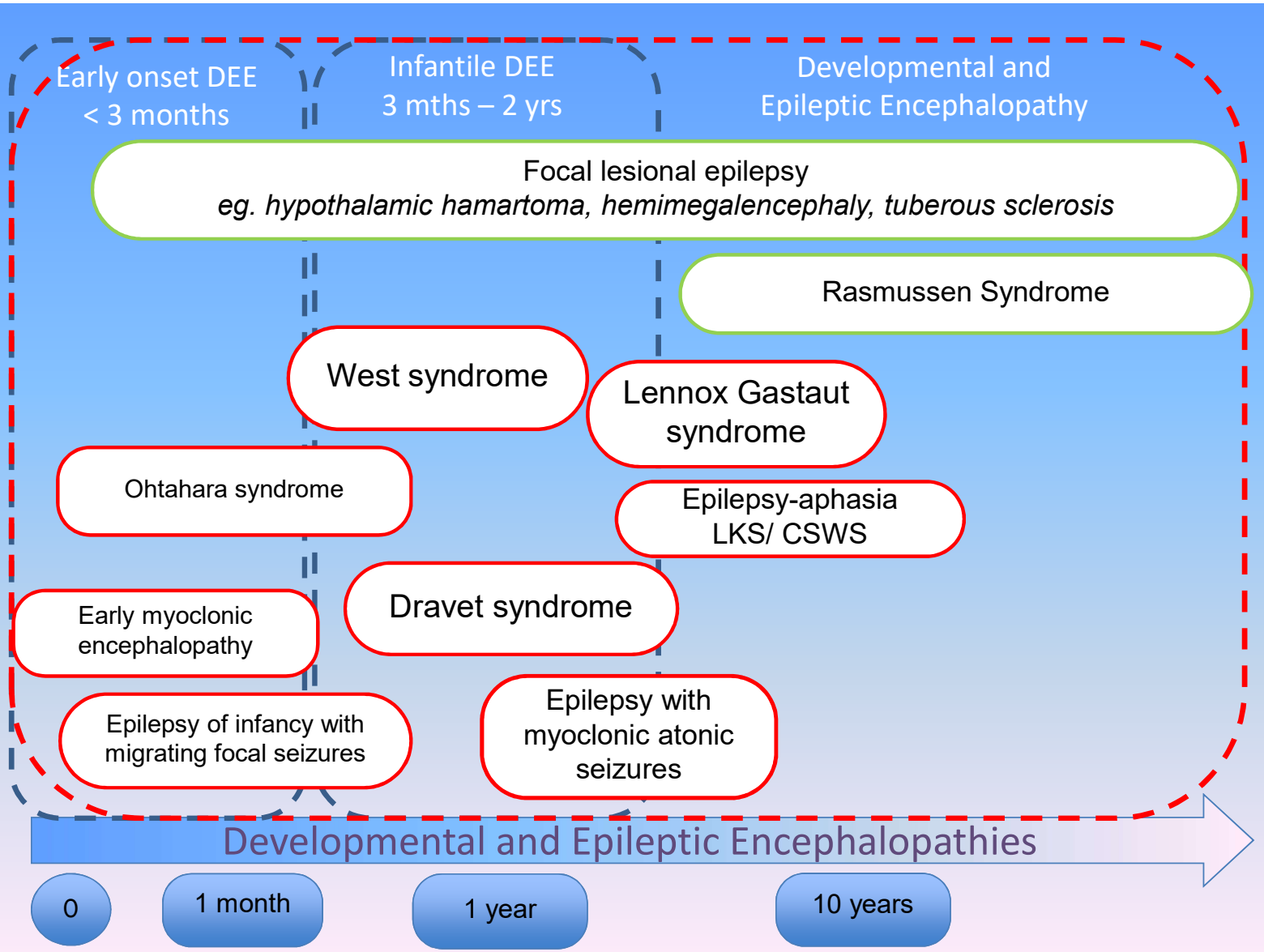
Dravet syndrome: Stop CBZ Start TPM, STP, FEN

Improve cognition

Developmental and Epileptic Encephalopathy

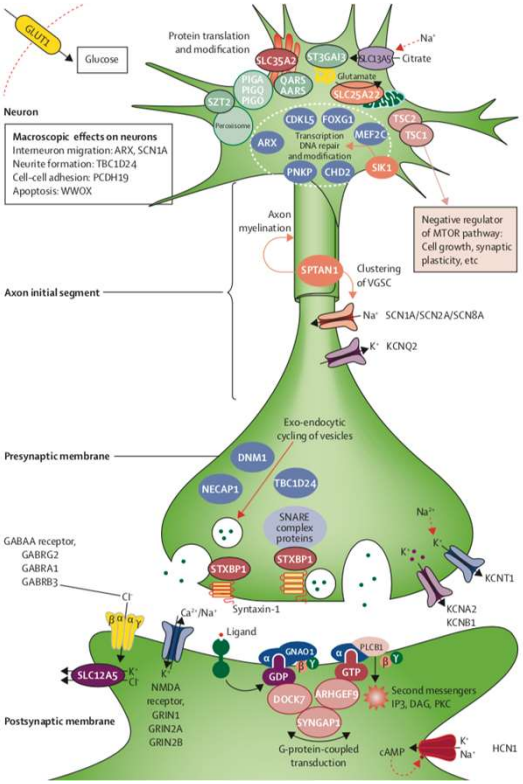



Developmental and Epileptic Encephalopathies
0
1 month
1 year
10 years


Early onset DEE
< 3 months
Infantile DEE
3 mths – 2 yrs
Developmental and
Epileptic Encephalopathy
Focal lesional epilepsy
eg. hypothalamic hamartoma, hemimegalencephaly, tuberous sclerosis
Rasmussen Syndrome
West syndrome
Lennox Gastaut syndrome
Ohtahara syndrome
Epilepsy-aphasia
LKS/ CSWS
Dravet syndrome
Early myoclonic encephalopathy
Epilepsy of infancy with migrating focal seizures
Epilepsy with myoclonic atonic seizures
Developmental and Epileptic Encephalopathies
0
1 month
1 year
10 years

Developmental and Epileptic Encephalopathies

> 100 genes



DNA repair
Transcriptional regulation
Axon myelination
Protein translation/modification
Peroxisomal function
Channelopathies
Synaptic dysfunction

The genetic landscape of the epileptic encephalopathies of infancy and childhood

Amy McTague, Katherine B Howell*, J Helen Cross, Manju A Kurian, Ingrid E Scheffer*

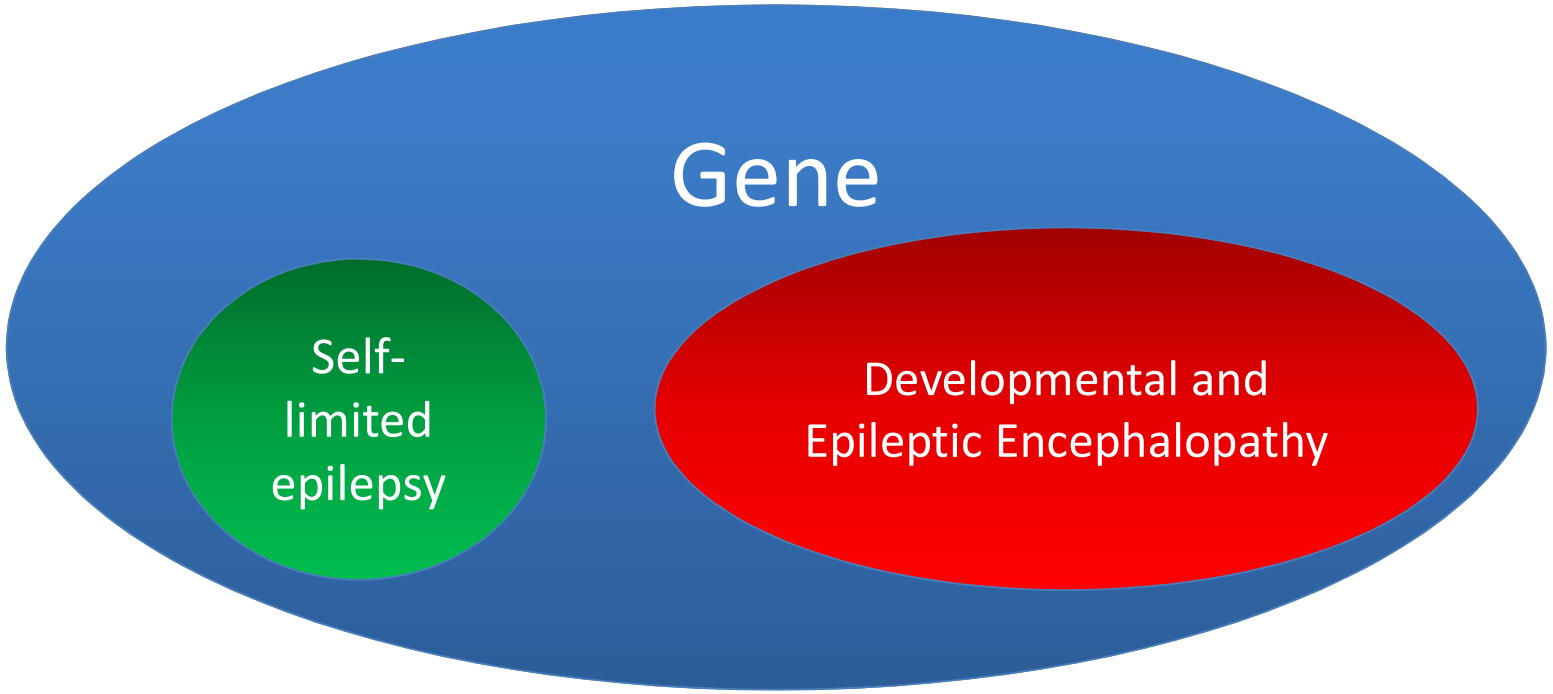
www.thelancet.com/neurology **Vol 15 March 2016**

Phenotypic spectrum of genetic epilepsies

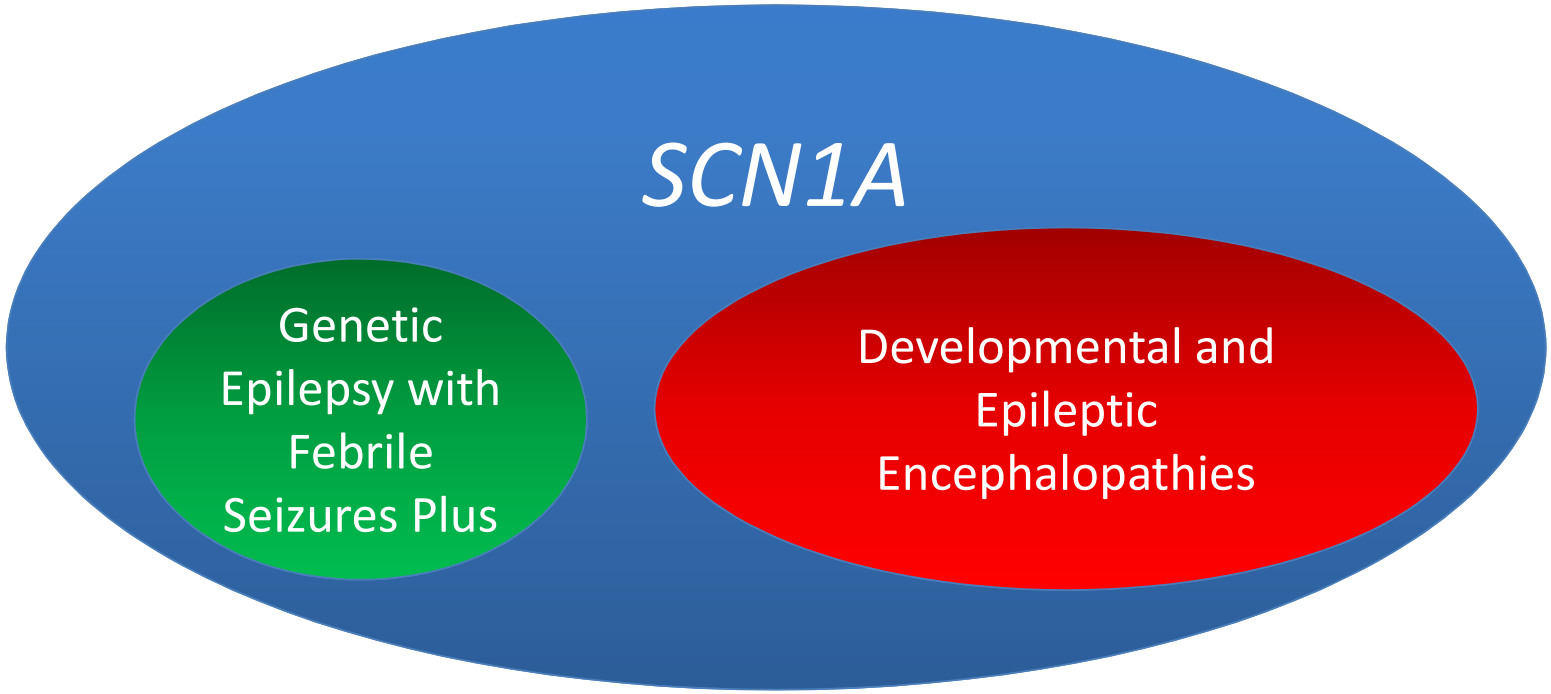
Self-limited epilepsies

Developmental and
Epileptic Encephalopathies

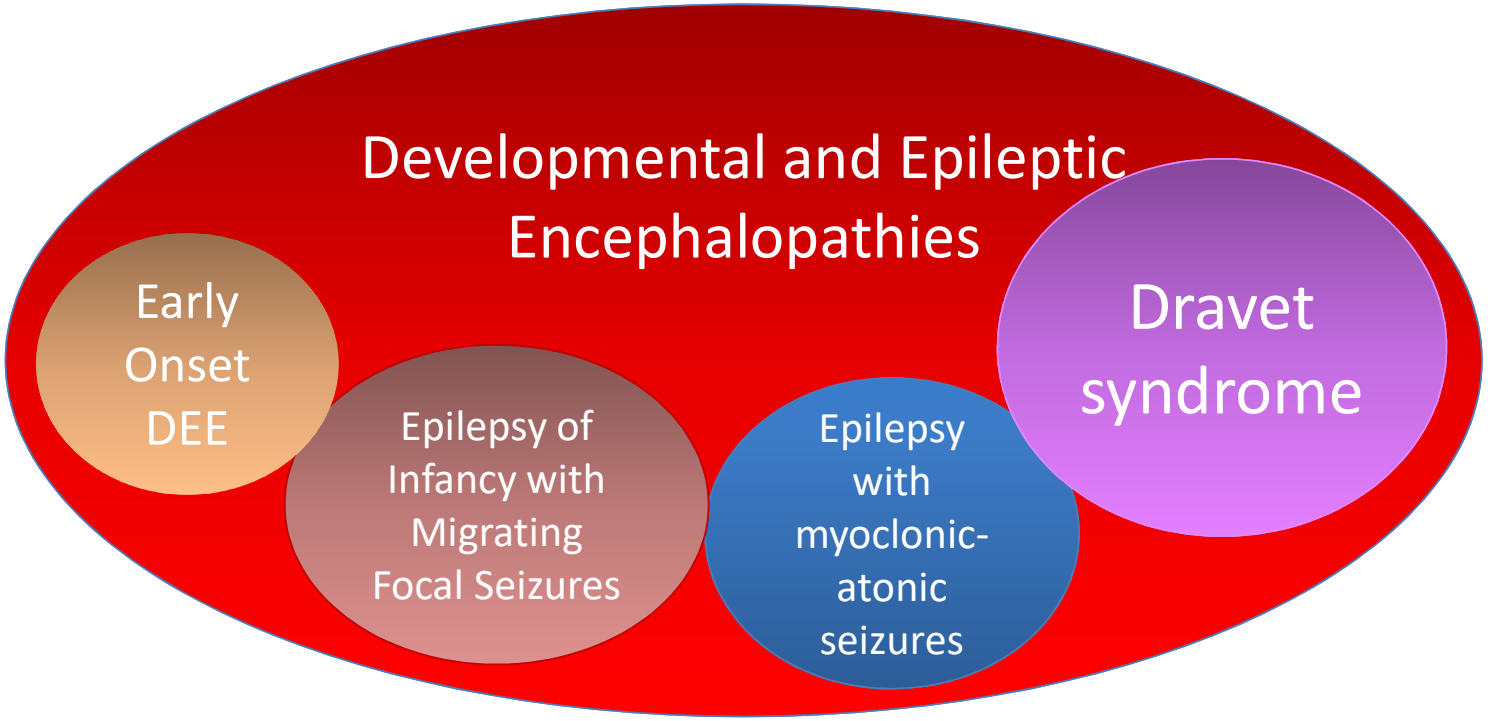
Epilepsy syndrome ≠ Genetic disease

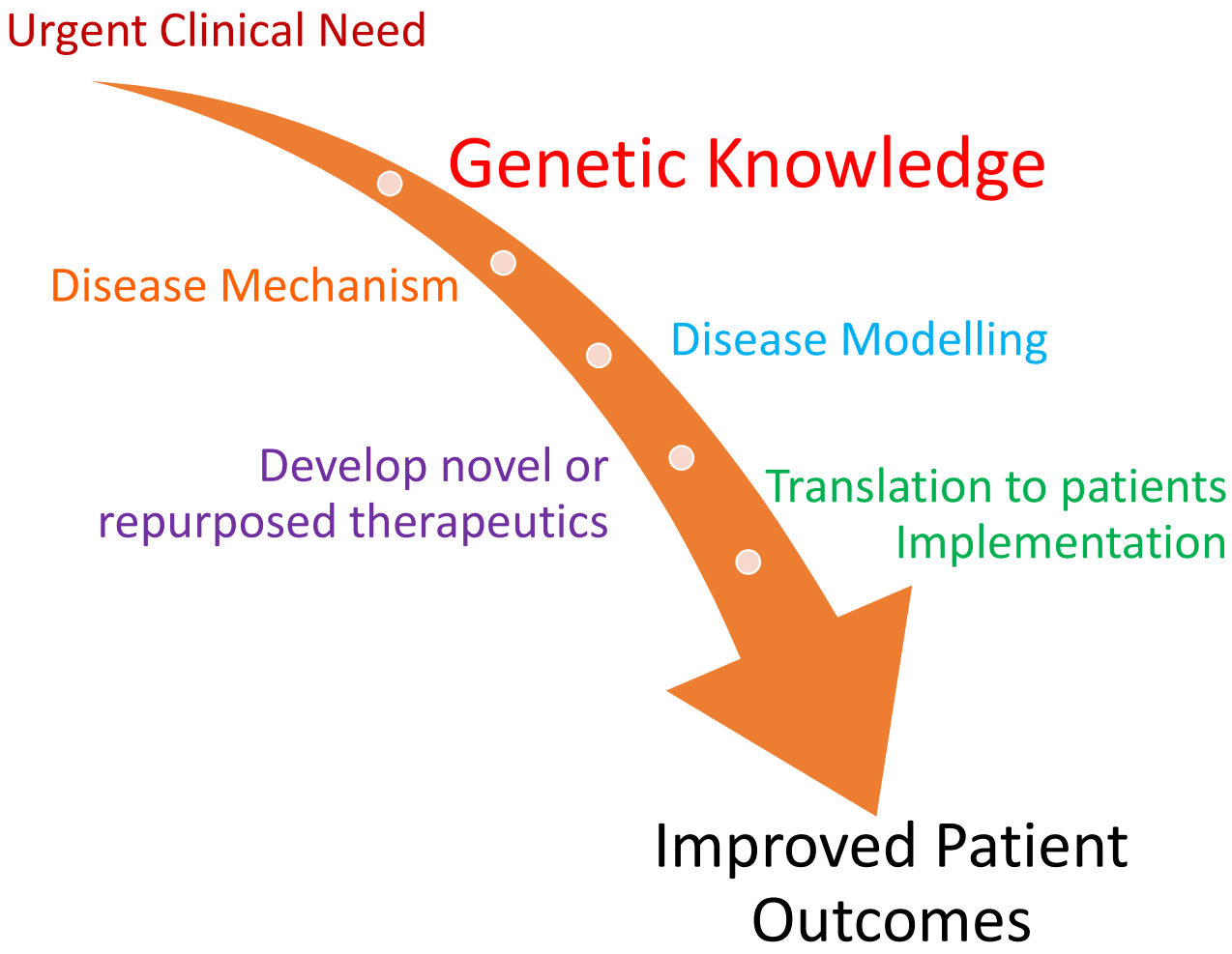


One gene → many syndromes



SCN1A


Developmental and Epileptic Encephalopathies
Early Onset DEE
Epilepsy of Infancy with Migrating Focal Seizures
Epilepsy with myoclonic-atonic seizures
Dravet syndrome

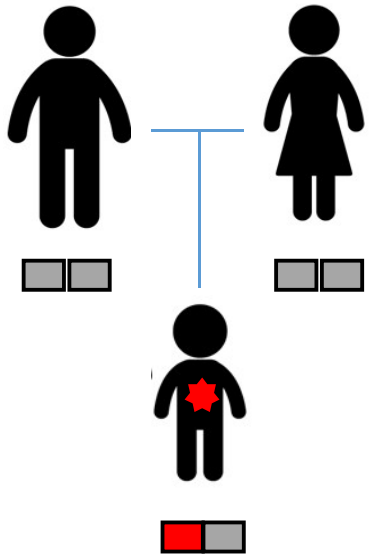


Gene discovery underpins Precision Medicine

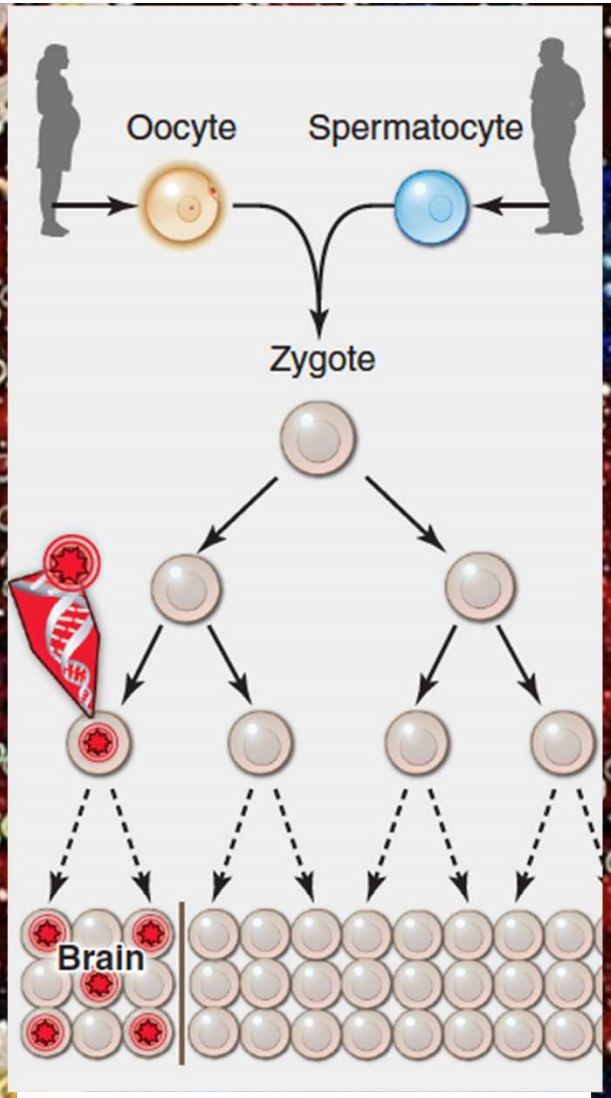

Mosaicism

Developmental and Epileptic Encephalopathies

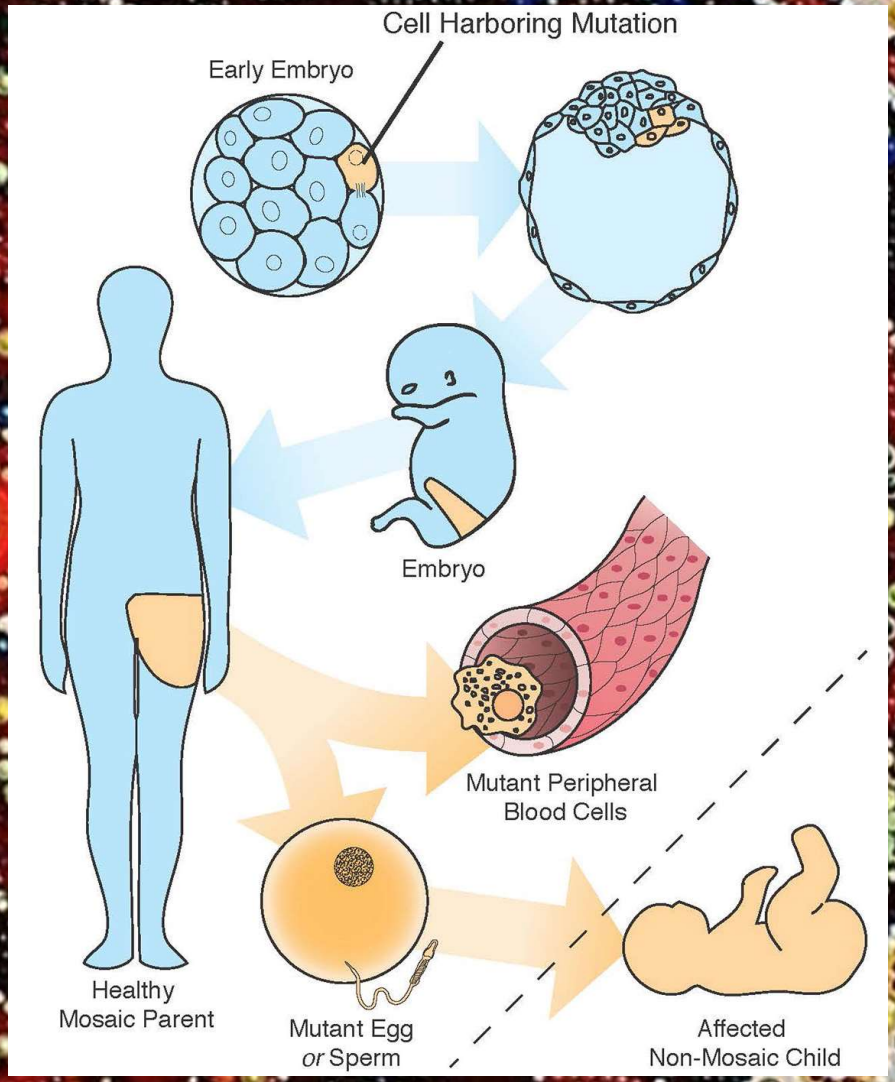
- Most patients have a dominant *de novo* mutation
- Recessive, X-linked and mitochondrial inheritance can also occur

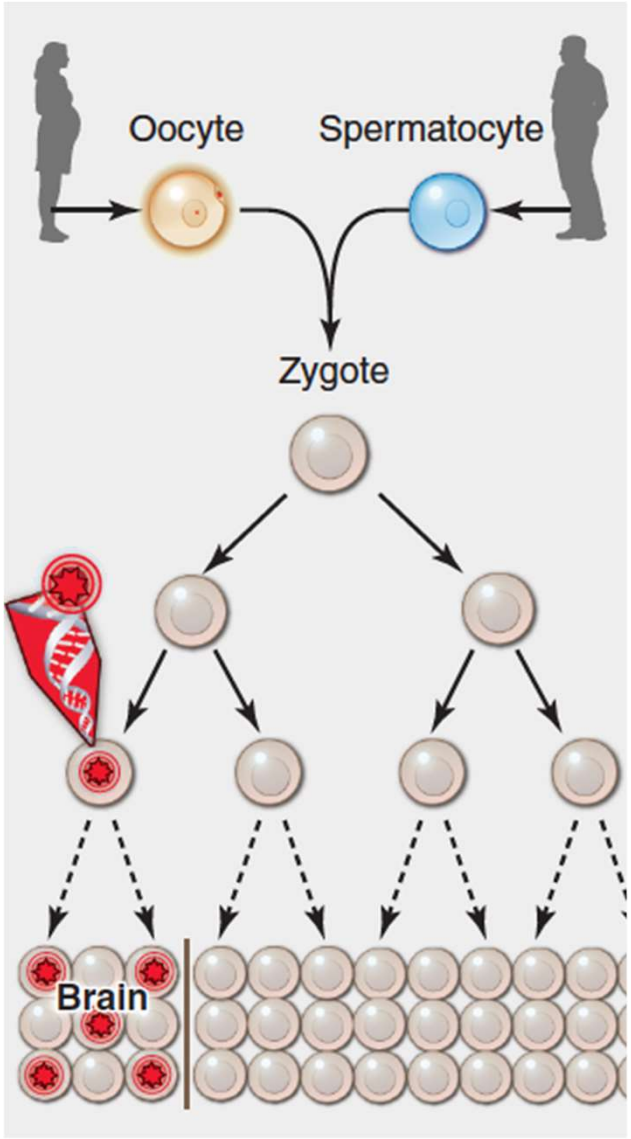


- Recurrence risk given: very low



Poduri et al Science 2013





Mosaicism in the patient

Accepted: 16 January 2018
DOI: 10.1111/epi.14021

FULL-LENGTH ORIGINAL RESEARCH

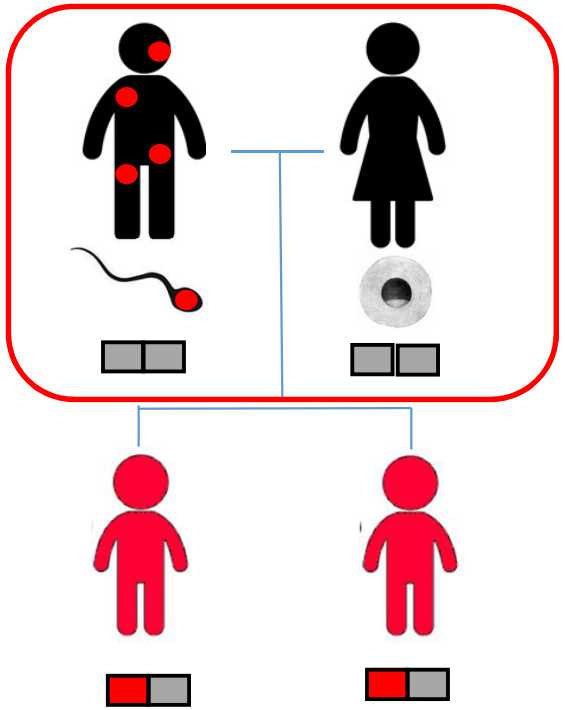
Epilepsia

Mosaicism of de novo pathogenic *SCN1A* variants in epilepsy is a frequent phenomenon that correlates with variable phenotypes

Iris M. de Lange[1] | Marco J. Koudijs[1] | Ruben van 't Slot[1] | Boudewijn Gunning[2] |

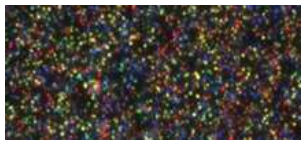
- 8/113 (7.5%) Dravet probands mosaic
- Milder phenotype if truncating mutation
- Deep sequencing of mutation
  - median coverage 1281 x, 20-7320x)
- Genetic counseling implications

Two affected children and unaffected parents



Targeted sequencing to detect post-zygotic mutation in unaffected parents

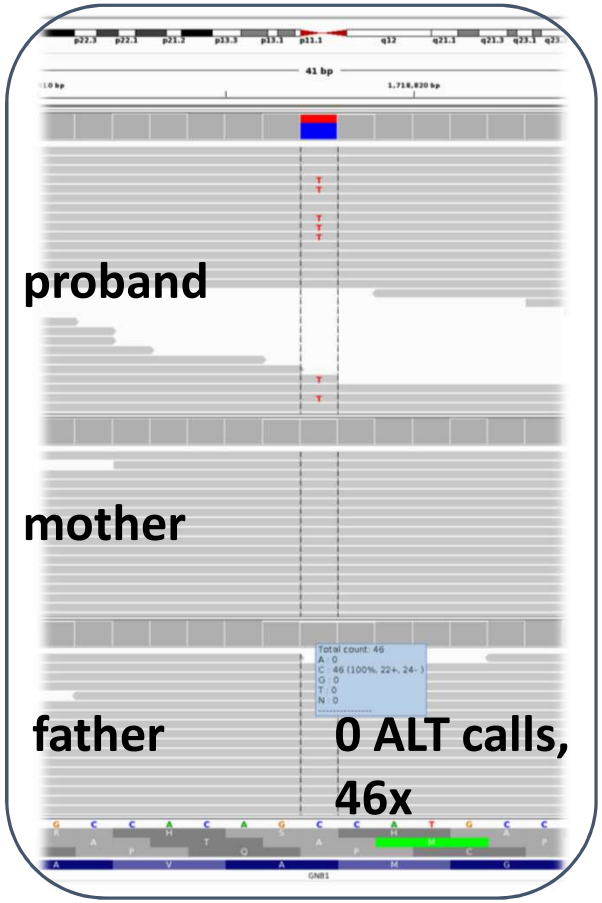
"capture" pathogenic variant, multiplex with universal primers



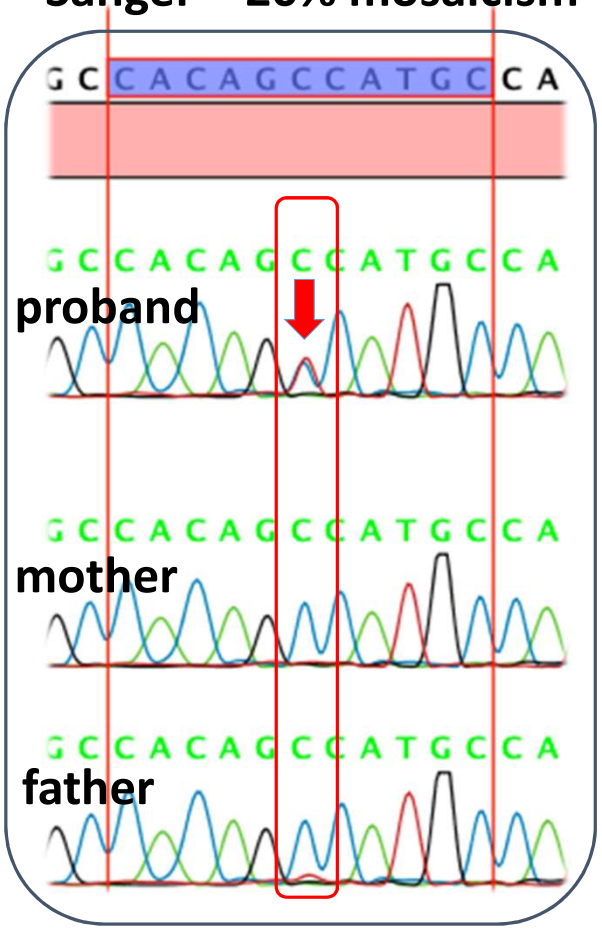
ACTTCGGATGTCCG
ACTTCGGATGACCG

Sensitivity to detect low level mosaicism

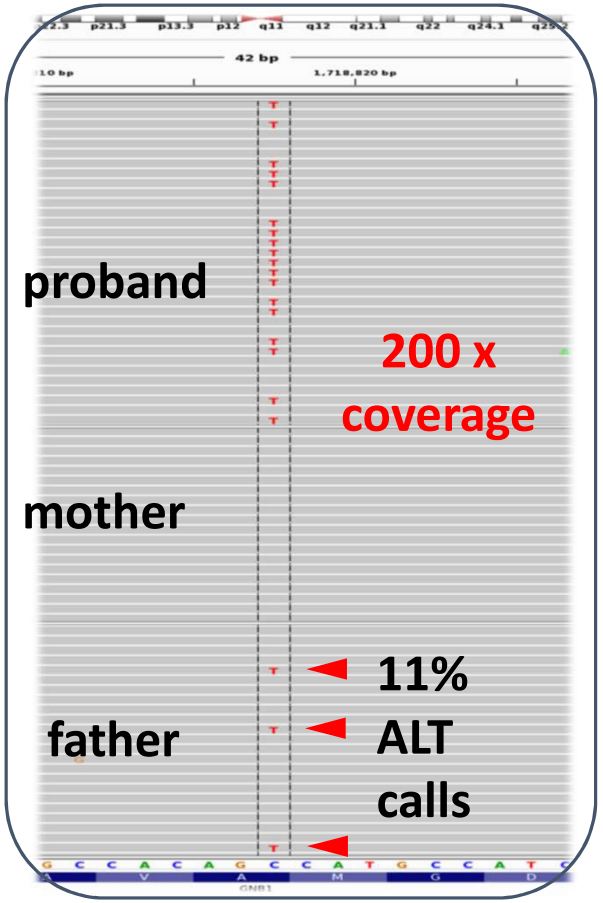
Whole Exome Sequencing



Sanger – 20% mosaicism



Single molecule MIPs

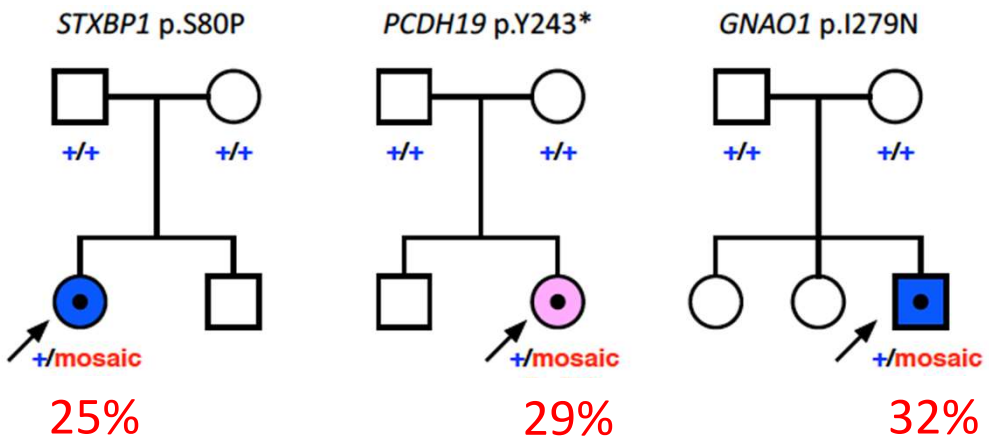


Mosaicism rates in patients with apparently '*de novo*' mutations

154 trios with mutations in 33 genes

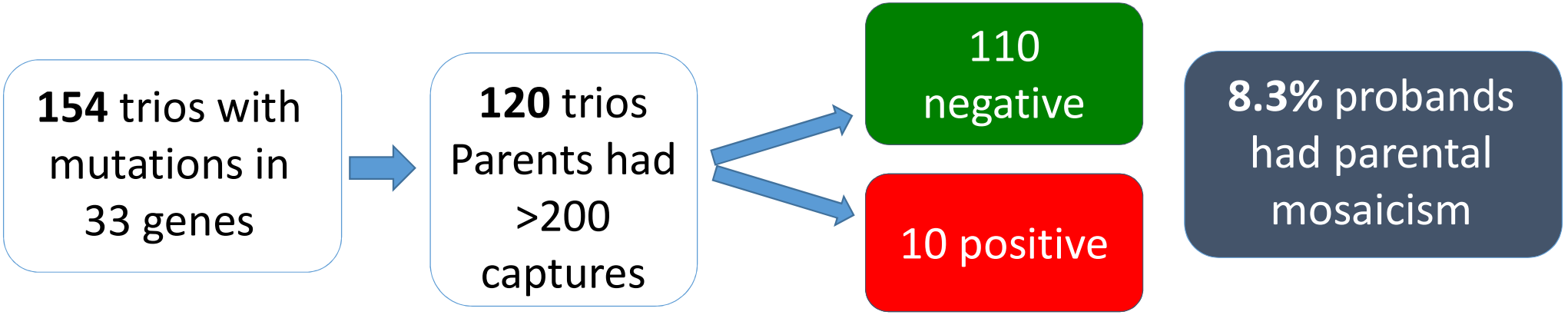
123 trios Parents had >200 captures

3 probands were mosaic
STXBP1, PCDH19, GNAO1



Myers, Scheffer, Mefford et al, NEJM, *2018*

Parental mosaicism rates in patients with apparently '*de novo*' mutations



Myers, Scheffer, Mefford et al, NEJM, *2018*

Parental mosaicism

Mutation
SCN1A p.Ile483Metfs*18
SCN1A p.S1516*
SCN1A p.R101W
SCN8A p.L1331V
GNB1 p.A326T
KCNT1 p.R950Q
SLC6A1 p.A334P
DNM1 p.R237Y
CACNA1A p.A713T
KCNQ2 p.V567D

Parental mosaicism

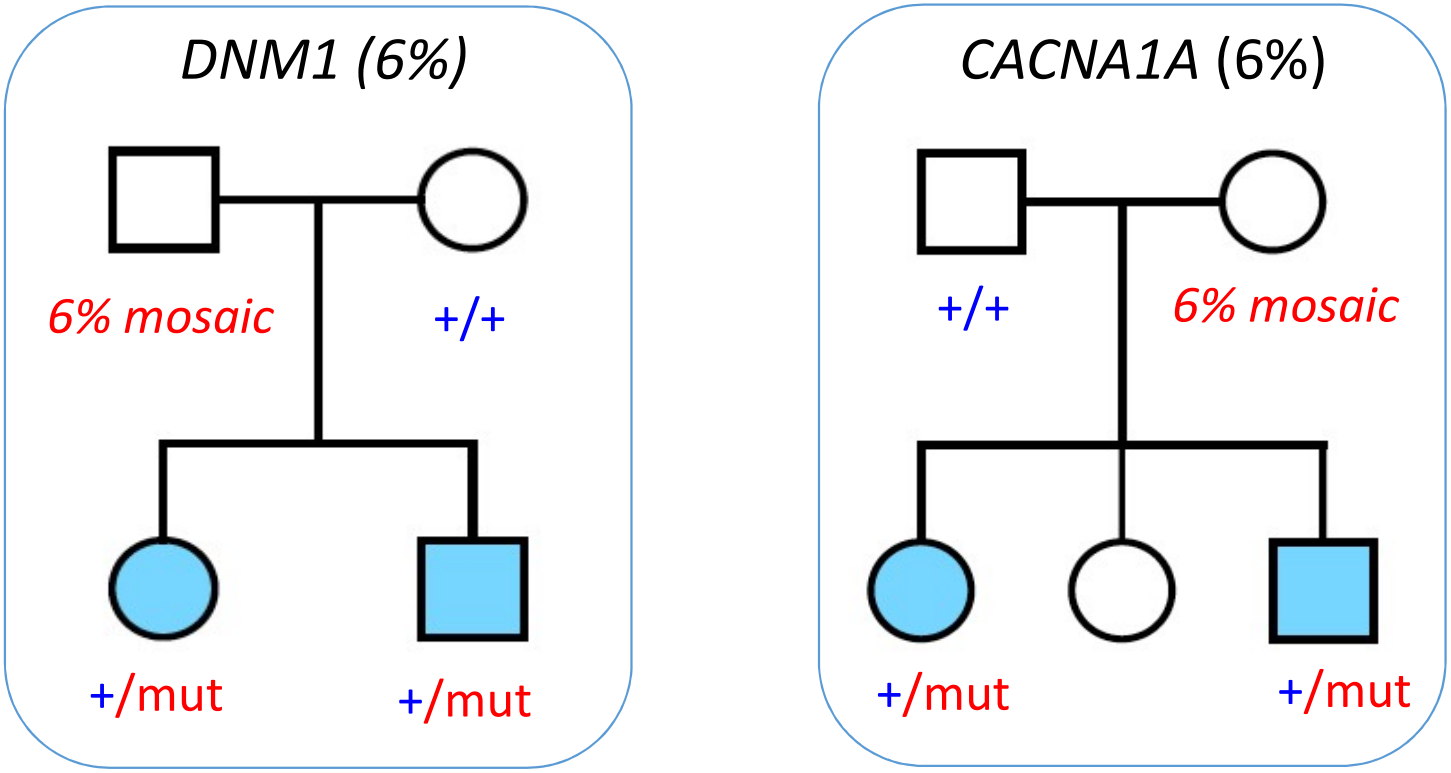
Mutation	Origin
SCN1A p.Ile483Metfs*18	Father
SCN1A p.S1516*	Mother
SCN1A p.R101W	Father
SCN8A p.L1331V	Father
GNB1 p.A326T	Father
KCNT1 p.R950Q	Father
SLC6A1 p.A334P	Mother
DNM1 p.R237Y	Father
CACNA1A p.A713T	Mother
KCNQ2 p.V567D	Mother

Parental mosaicism

Mutation	Origin		% Mosaicism
SCN1A p.Ile483Metfs*18	Father	*	28% (Affected)
SCN1A p.S1516*	Mother		17% (Affected)
SCN1A p.R101W	Father		16%
SCN8A p.L1331V	Father		13% (Affected)
GNB1 p.A326T	Father		11%
KCNT1 p.R950Q	Father		9%
SLC6A1 p.A334P	Mother		9%
DNM1 p.R237Y	Father		6%
CACNA1A p.A713T	Mother		6%
KCNQ2 p.V567D	Mother		3%

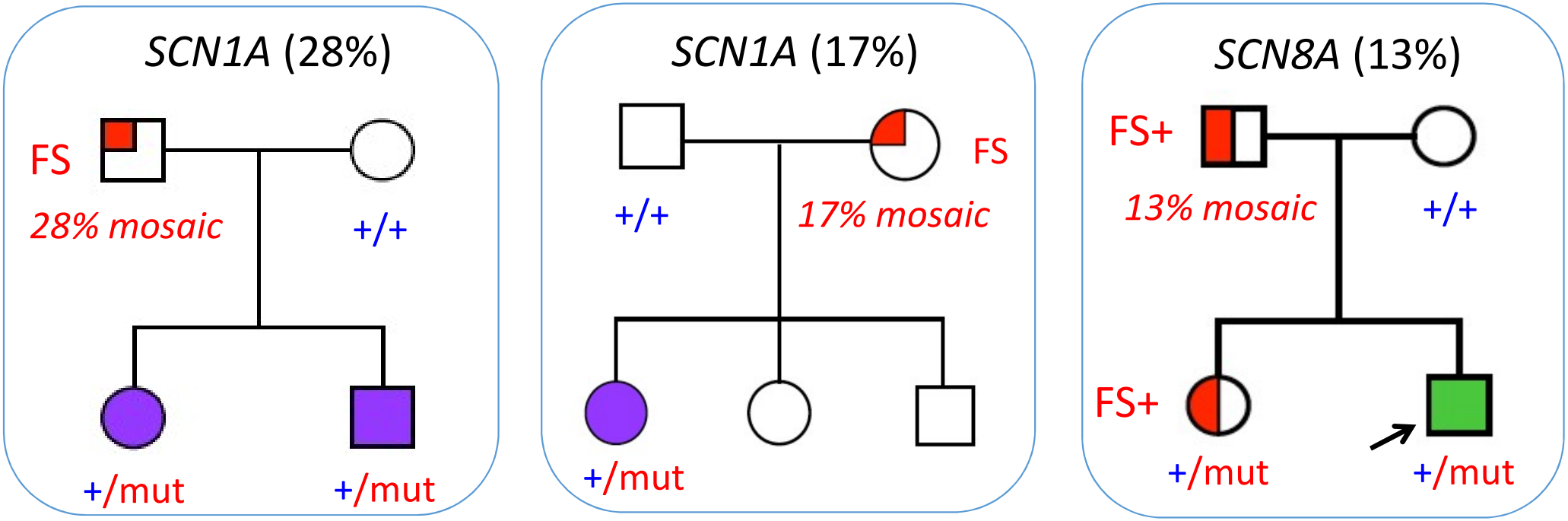
Lower
% mosaicism
Unaffected

Families with two affected children
Unaffected parents



Developmental and Epileptic Encephalopathy

3/10 families: mosaic parent mildly affected



Dravet syndrome

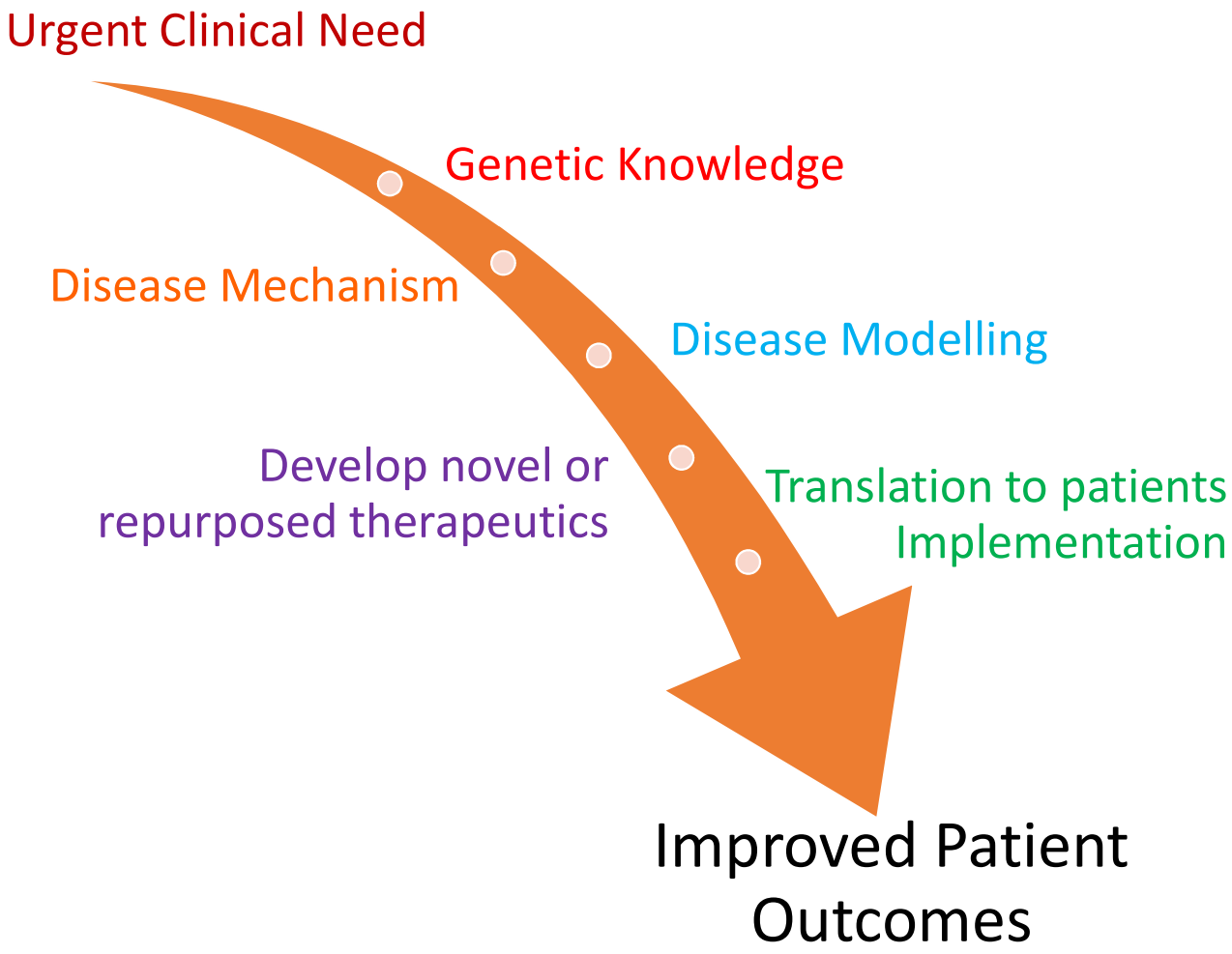
MAE – Epilepsy with Myoclonic-Atonic seizures

Myers, Scheffer, Mefford et al, NEJM, *2018*

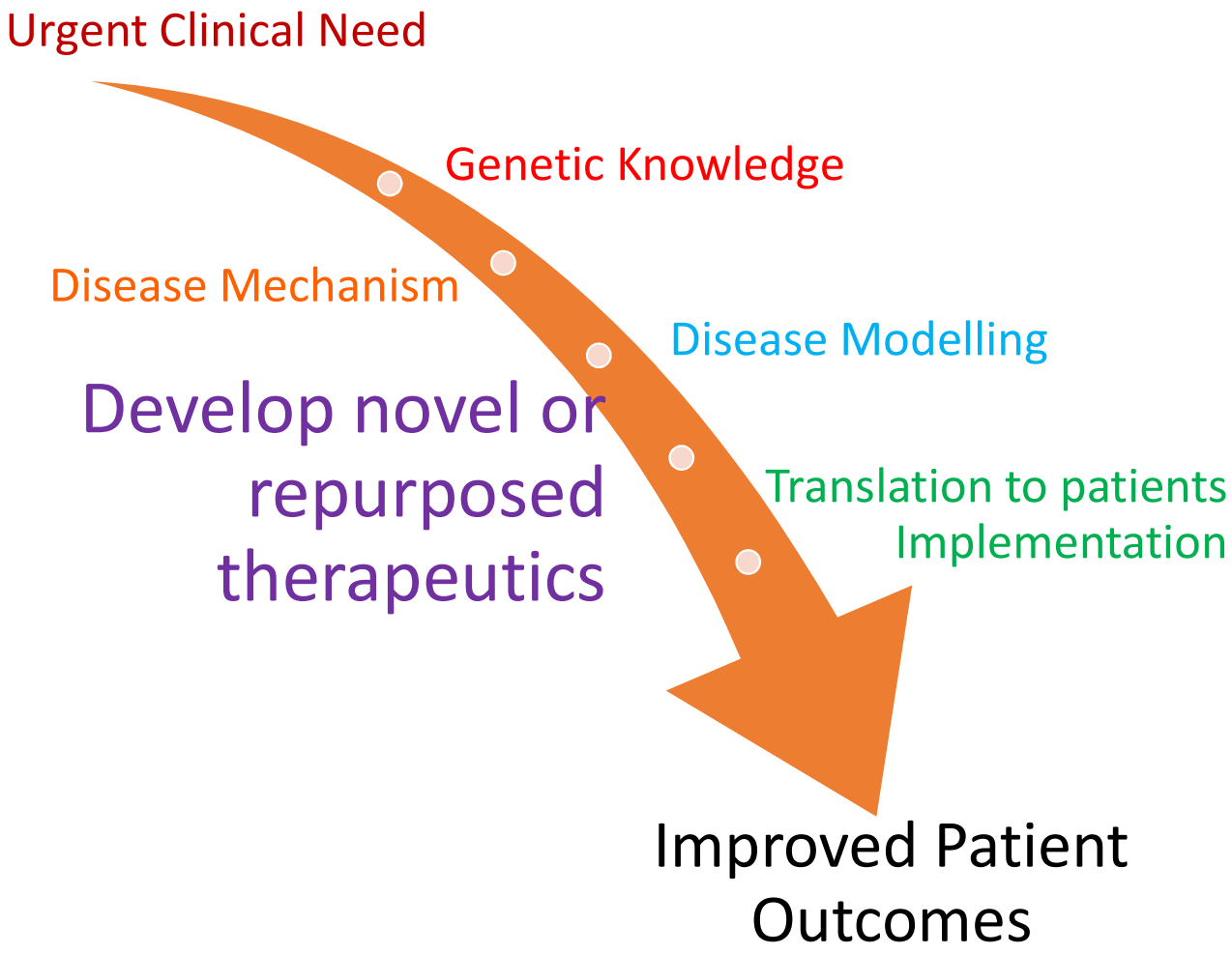
Parental Mosaicism underestimated

- 8% probands with DEE had parental mosaicism - also *SCN1A* (Xu et al 2015)
- More sensitive tools detect low levels of mosaicism
- Higher % mosaicism correlate with affected parents
- Detecting parental mosaicism crucial
  - *Before* second affected child
  - For reproductive counseling and family planning
  - Change testing practices in clinic

Myers, Scheffer, Mefford et al, NEJM, 2018

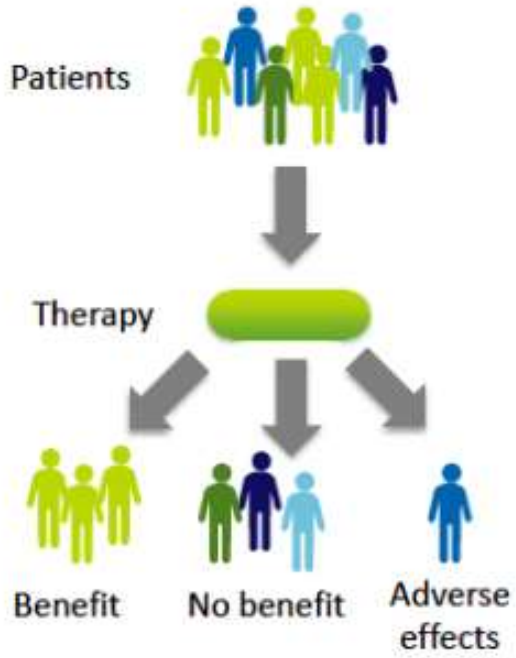


Gene discovery underpins Precision Medicine

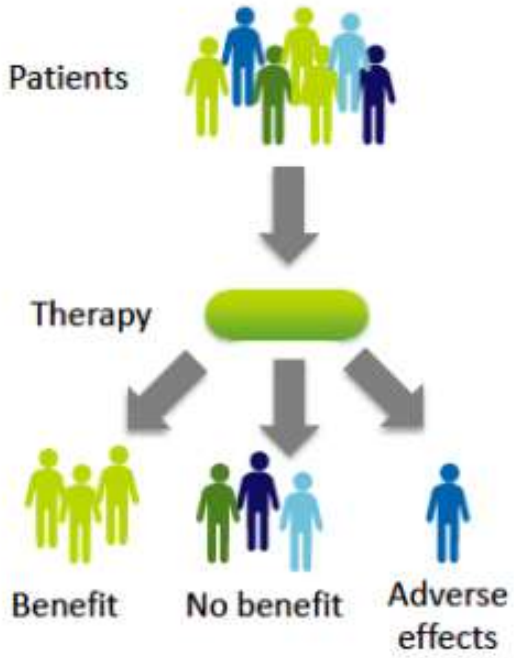


Gene discovery underpins Precision Medicine

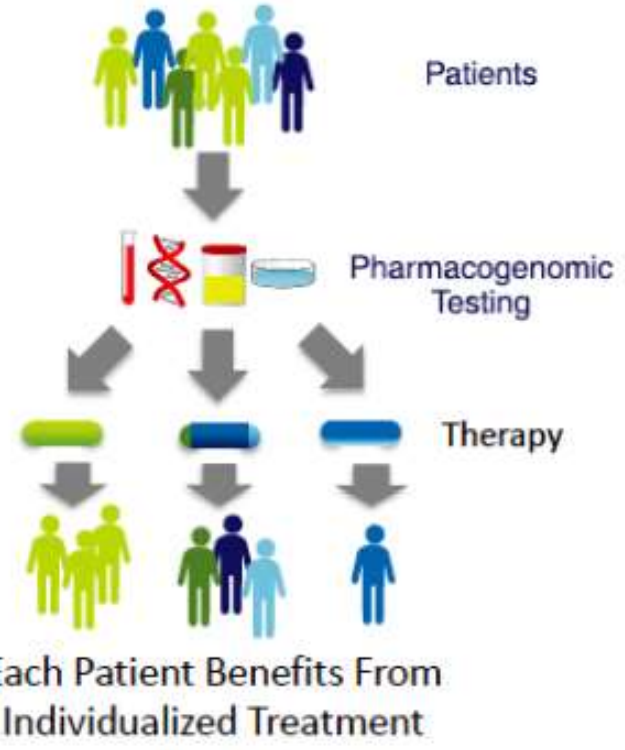
One-size-fits-all medicine



One-size-fits-all medicine



Precision Medicine



Precision Medicine

- Gene
- Function
  - Gain
  - Loss
- Pathway

Precision Medicine

- Gene
- Function
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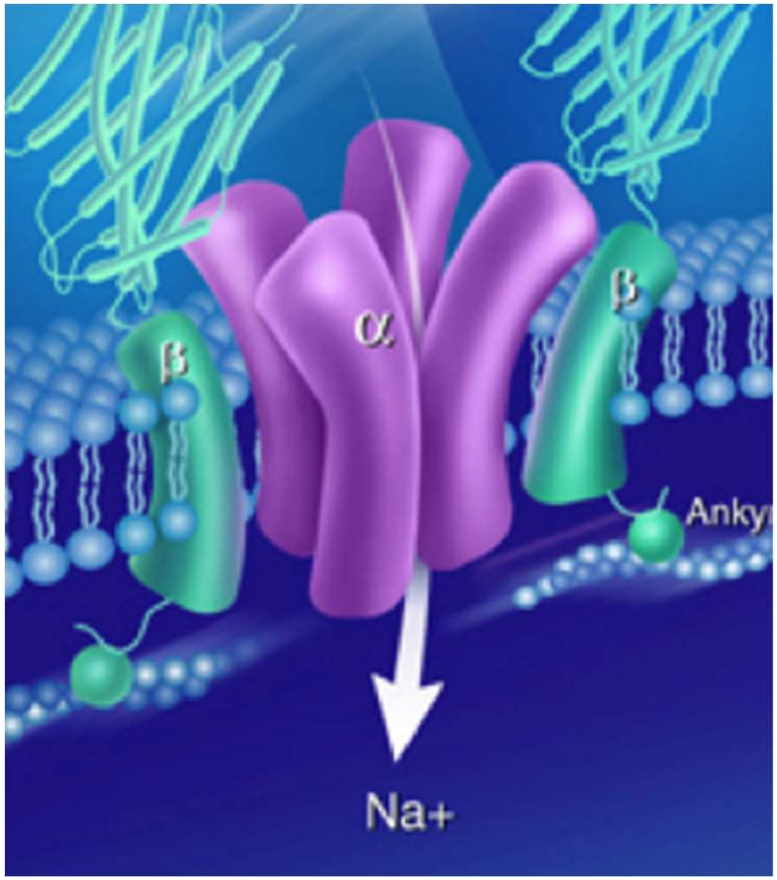
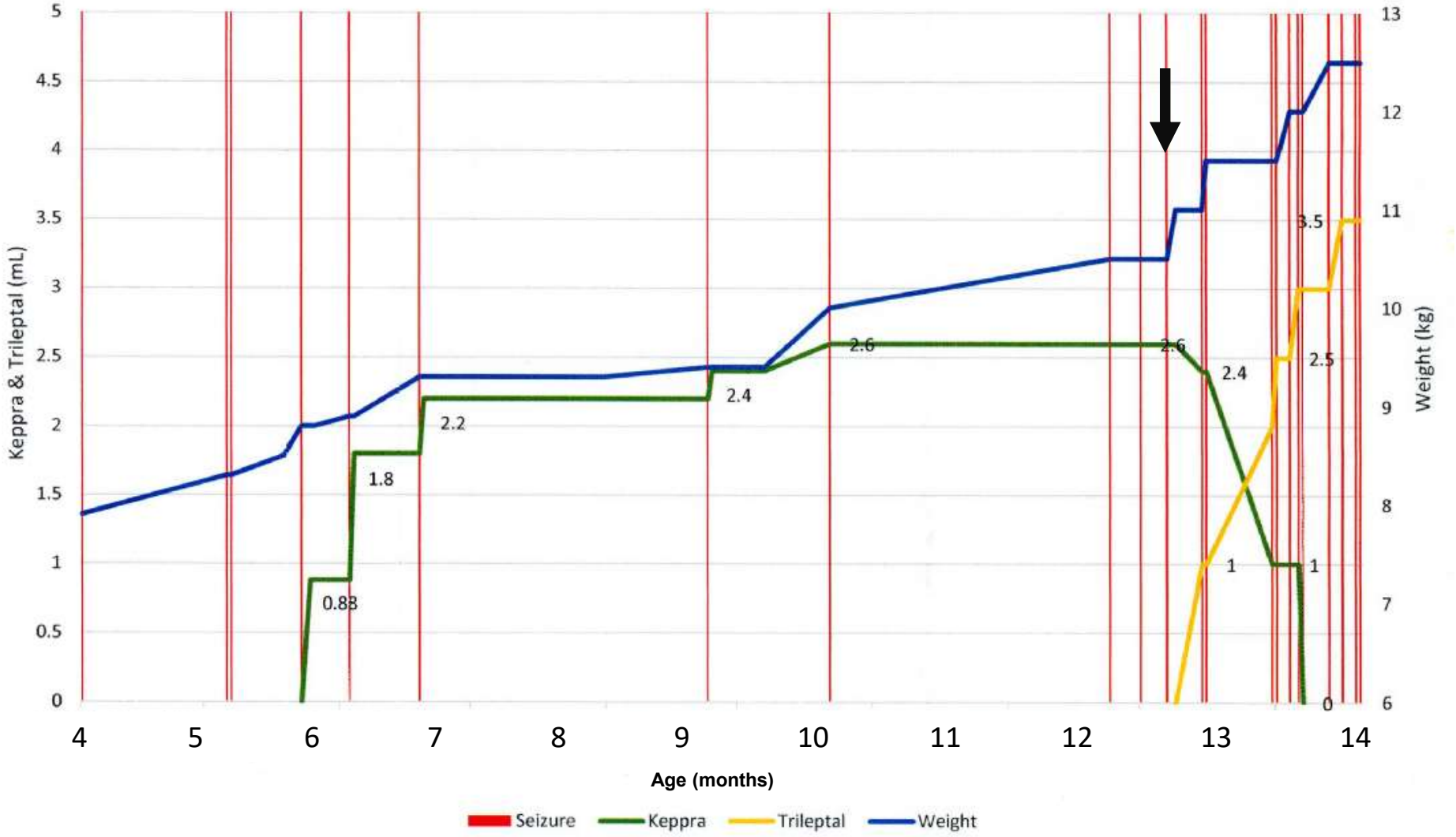
Does the specific sodium channelopathy matter?




Keppra & Trileptal (mL)
Weight (kg)
Age (months)
0.88
1.8
2.2
2.4
2.6
2.6
2.4
1
1
0
3.5
2.5
Seizure
Keppra
Trileptal
Weight

Precision Medicine

- Gene
- Function
  - Gain
  - Loss
- Pathway

Does the specific sodium channelopathy matter?

SCN1A DEE - Dravet

- Seizure exacerbation with sodium channel blockers
  - ✗ carbamazepine
  - ✗ - ✓ lamotrigine
- Seizure control
  - ✓ topiramate
  - ✓ stiripentol
  - ✓ clobazam
  - ✓ valproate

SCN8A EE

SCN2A EE

- Seizure control with sodium channel blockers
  - ✓ carbamazepine
  - ✓ phenytoin
  - ✓ oxcarbazepine

Dalic et al DMCN 2014
Larsen et al Neurology 2015
Howell et al Neurology 2015

Precision Medicine

- Gene
- Function
  - Gain
  - Loss
- Pathway

Replace the protein deficit

Precision Medicine

- Gene
- Function
  - Gain
  - Loss
- Pathway

Replace the protein deficit

Cerliponase alfa for Neuronal Ceroid Lipofuscinosis CLN2

- 3 yr old - language delay, seizures, regression
- Lysosomal storage disease
- Recessive mutations TPP1
- Deficient Tripeptidyl peptidase 1
- Cerliponase alfa – recombinant TPP-1 enzyme

The NEW ENGLAND JOURNAL of MEDICINE

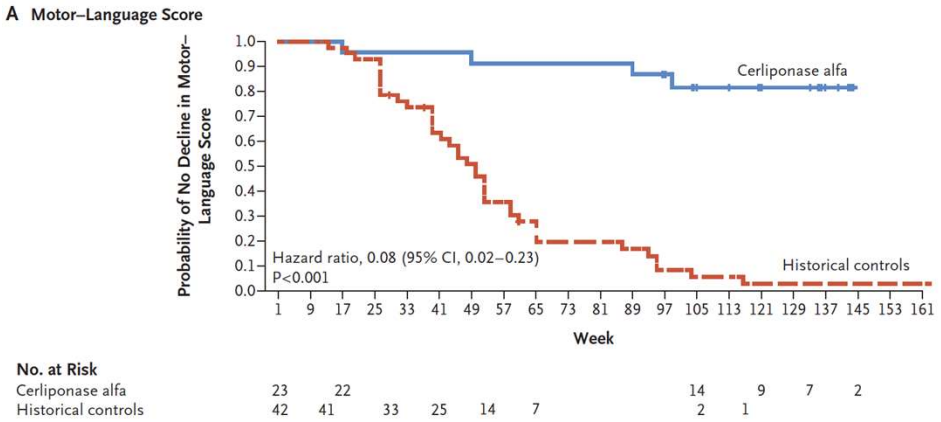
ORIGINAL ARTICLE

Study of Intraventricular Cerliponase Alfa for CLN2 Disease

Angela Schulz, M.D., Temitayo Ajayi, M.D., Nicola Specchio, M.D., Ph.D., Emily de Los Reyes, M.D., Paul Gissen, M.B., Ch.B., Ph.D., Douglas Ballon, Ph.D., Jonathan P. Dyke, Ph.D., Heather Cahan, M.D., Peter Slasor, Sc.D., David Jacoby, M.D., Ph.D., and Alfried Kohlschütter, M.D., for the CLN2 Study Group*

- Intra-cerebroventricular infusion in 24 children, 3-16 yrs, for 48 weeks

➢ Stop disease progression



Neuronal ceroid lipofuscinosis:
Enzyme replacement therapy

Precision Medicine

- Gene
- Function
  - Gain
  - Loss
- Pathway

Loss of function mutation

Increase function

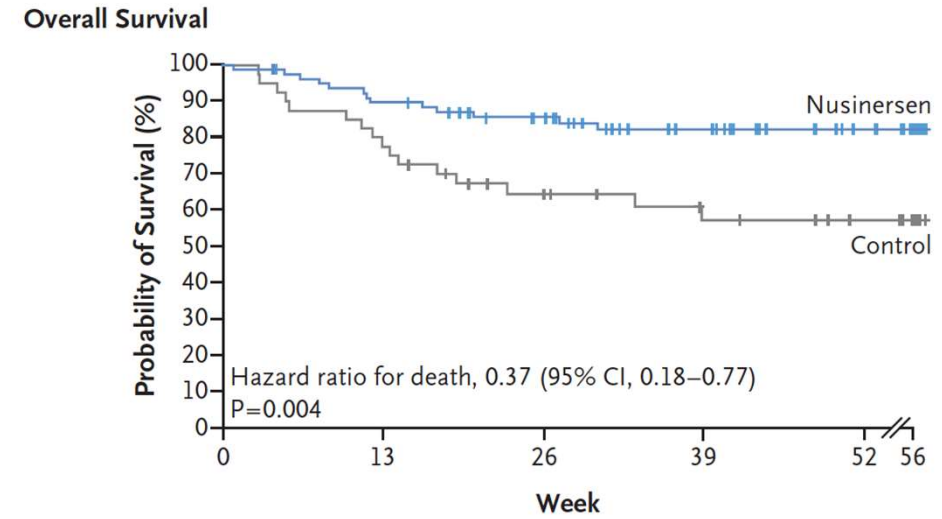
- Wild type allele
  - Increase expression
  - Block dominant negative effect
- Mutant allele
  - Skip truncation
- AntiSense Oligonucleotide (ASO)
  - Modifies pre-mRNA splicing to promote increased production of protein

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy

R.S. Finkel, E. Mercuri, B.T. Darras, A.M. Connolly, N.L. Kuntz, J. Kirschner, C.A. Chiriboga, K. Saito, L. Servais, E. Tizzano, H. Topaloglu, M. Tulinius, J. Montes, A.M. Glanzman, K. Bishop, Z.J. Zhong, S. Gheuens, C.F. Bennett, E. Schneider, W. Farwell, and D.C. De Vivo, for the ENDEAR Study Group*



Antisense Oligonucleotide (ASO) modifies pre-mRNA splicing of SMN2 gene to produce full length SMN protein

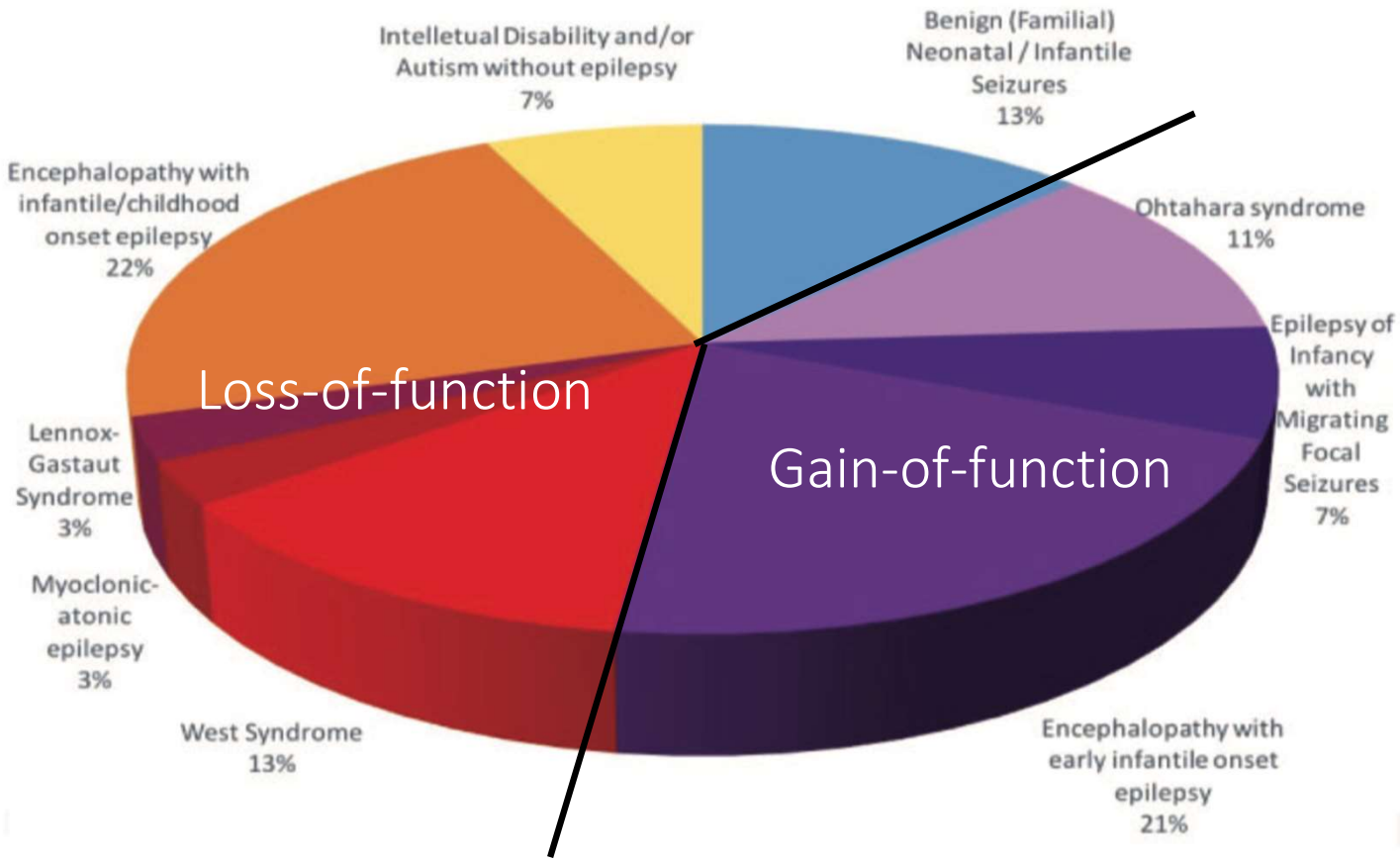
Precision Medicine

- Gene
- Function
  - Gain
  - Loss
- Pathway

Gain of function mutation

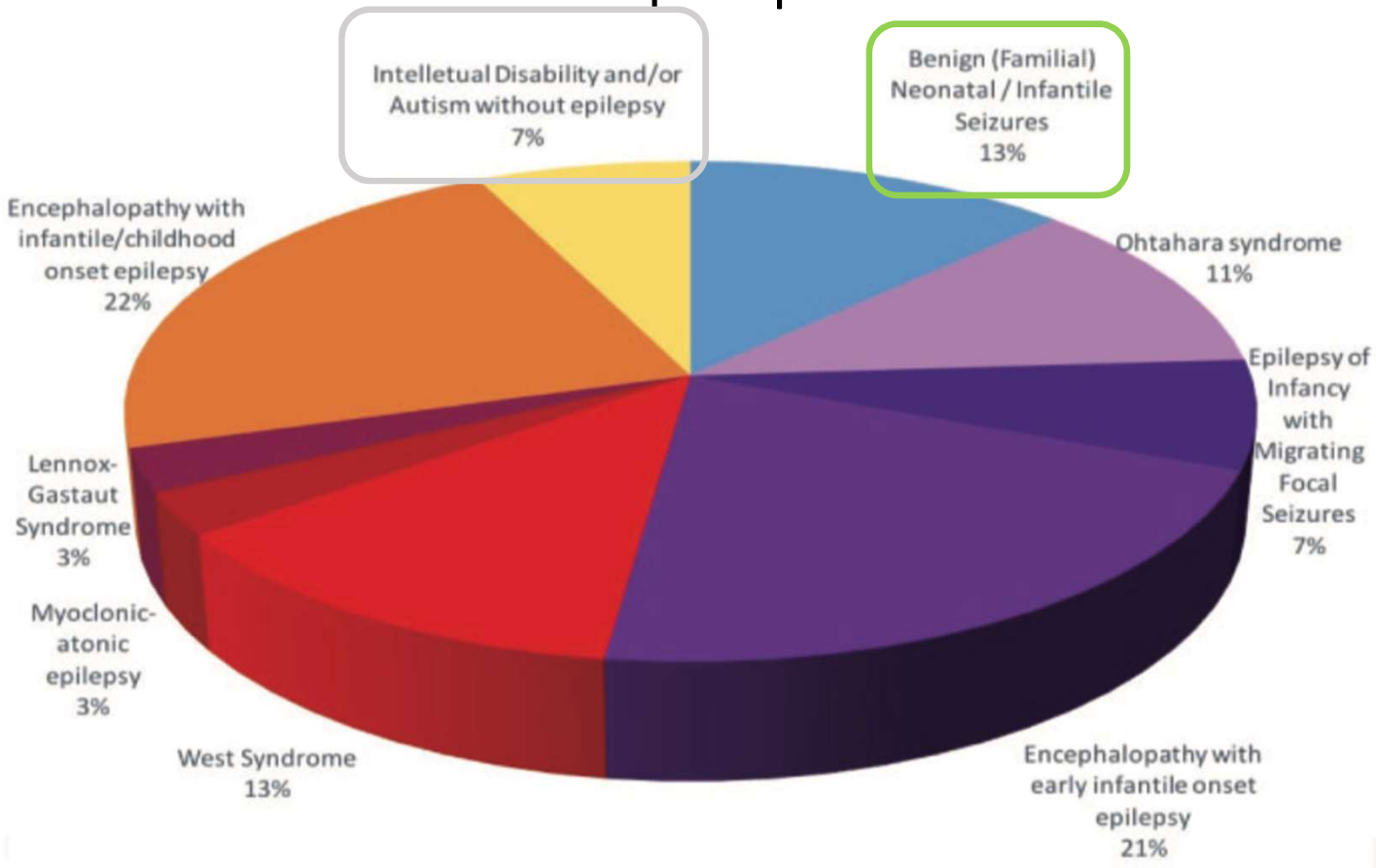
- Block gain of function
- Restore normal levels of function
- Challenges
  - Block increased function
    - In right cell
    - In right network
    - At right developmental time
  - *Not* to reduce to loss of function phenotype

SCN2A epilepsies

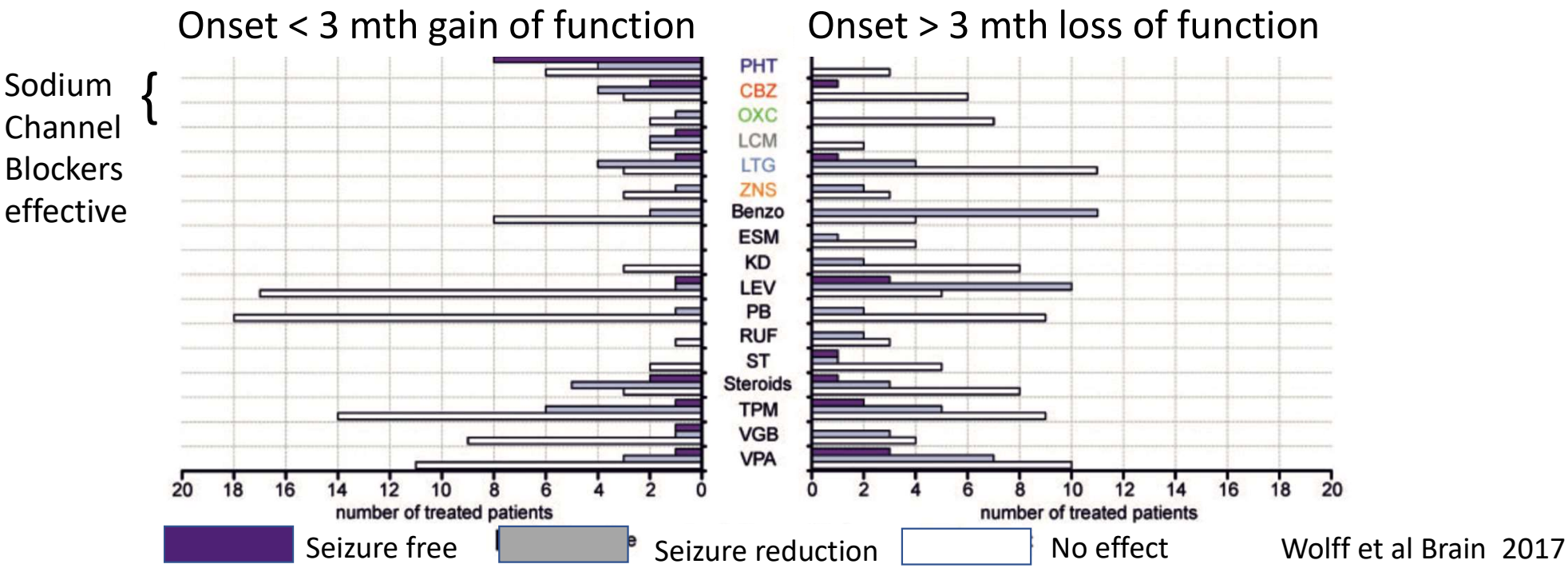


Heron et al Lancet 2002; Howell et al Neurol 2015; Wolff et al Brain 2017

SCN2A epilepsies



Heron et al Lancet 2002; Howell et al Neurol 2015; Wolff et al Brain 2017



Wolff et al Brain 2017

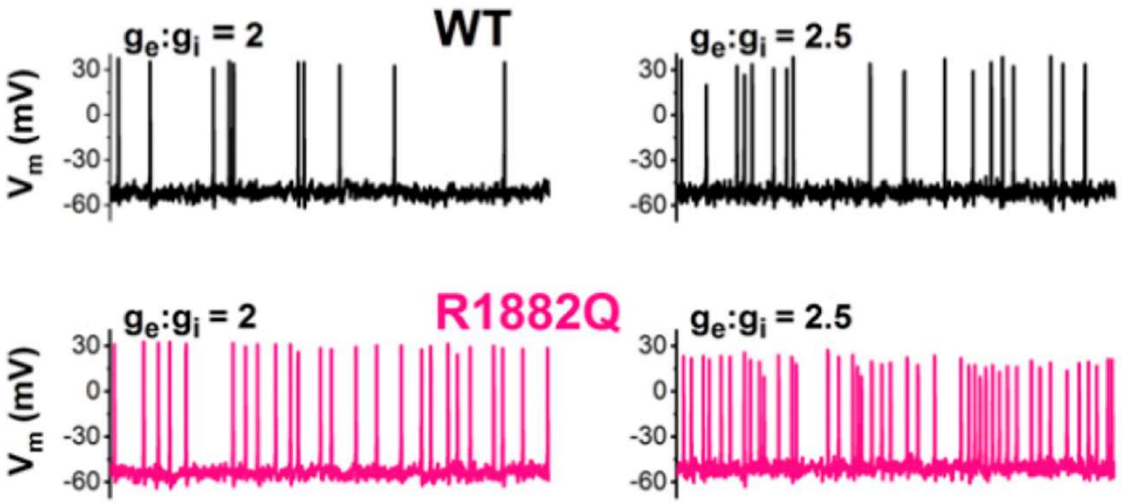
Anti-epileptic drug response in *SCN2A* encephalopathy

Antisense oligonucleotide therapy for *SCN2A* gain-of-function epilepsies

Steven Petrou, Melody Li, Nikola Jancovski, Paymaan Jafar-najad, Lisseth Burbano, Alex Nemiroff, Kelley Dalby, Snezana Maljevic, Christopher Reid, Frank Rigo

Early infantile *SCN2A* encephalopathy

R1882Q Gain of function mutation



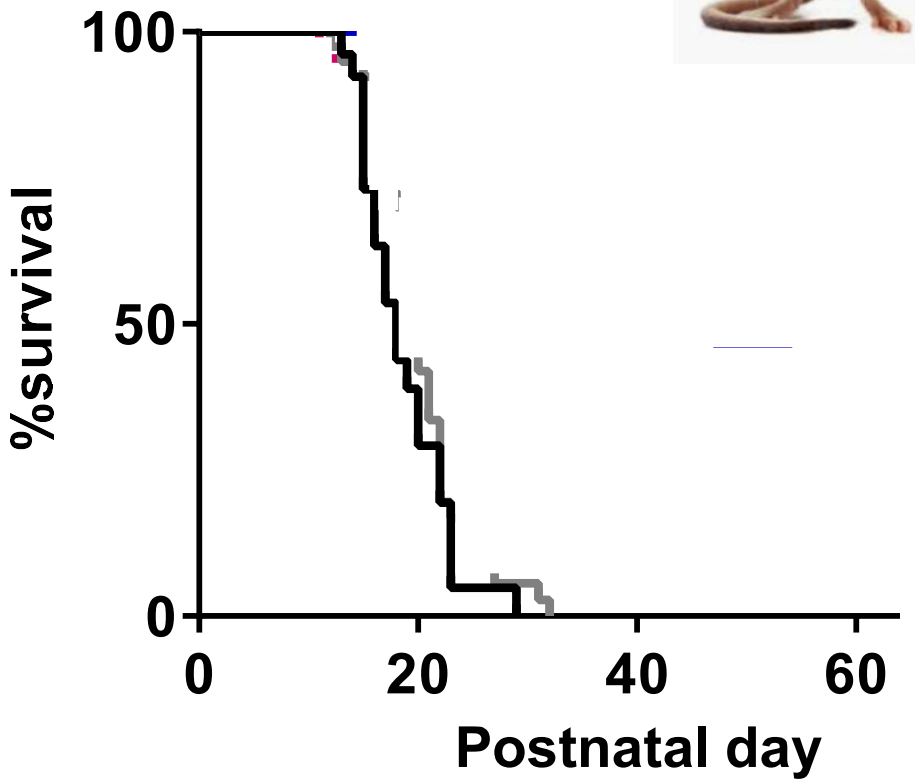
Action potential firing from dynamic clamp model

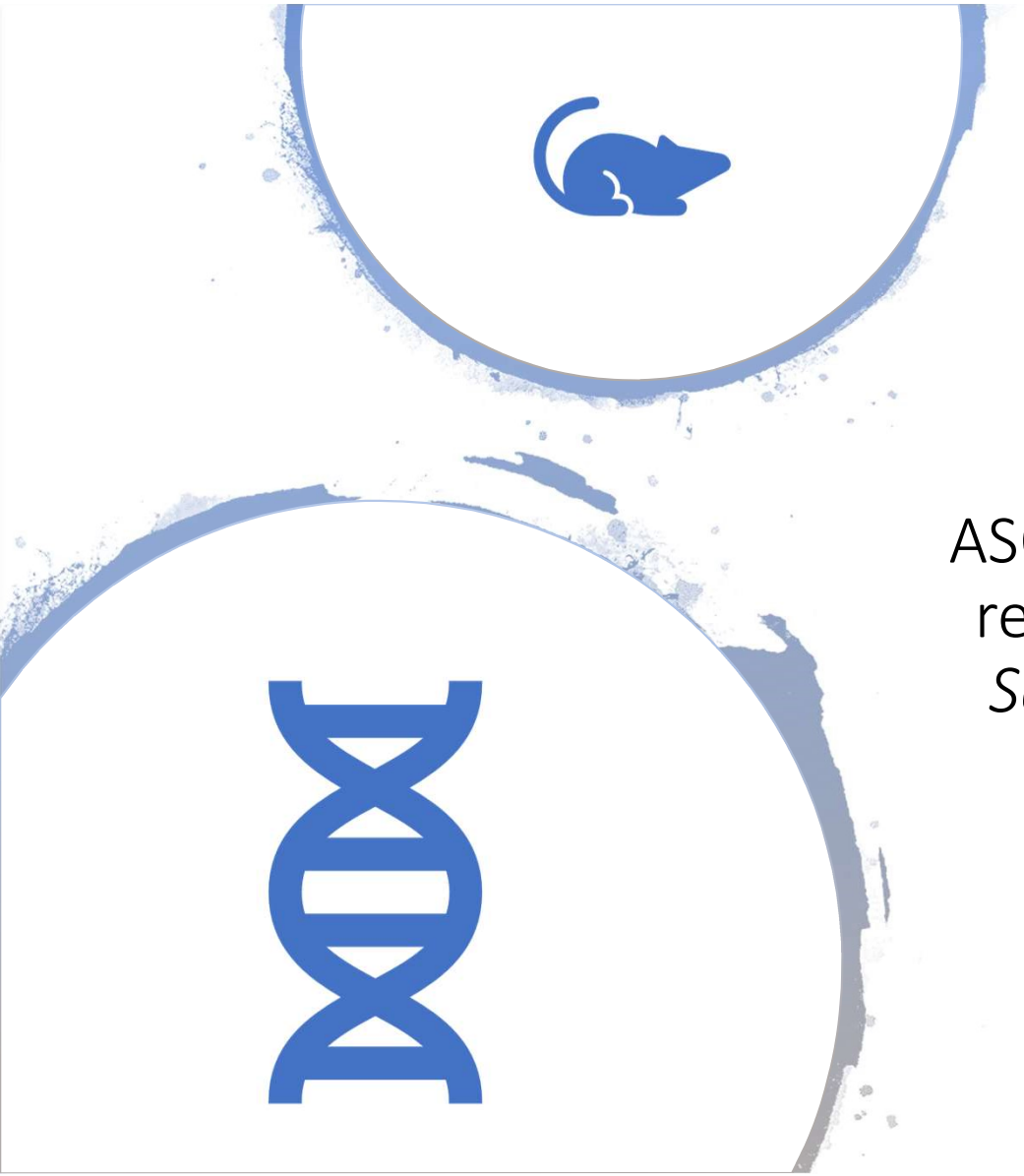
Berecki, Petrou et al PNAS 2018

Scn2a R1882Q mouse
Recurrent Early infantile DEE variant

- Strong seizure phenotype - onset P1
- Severe mortality – most die by P22

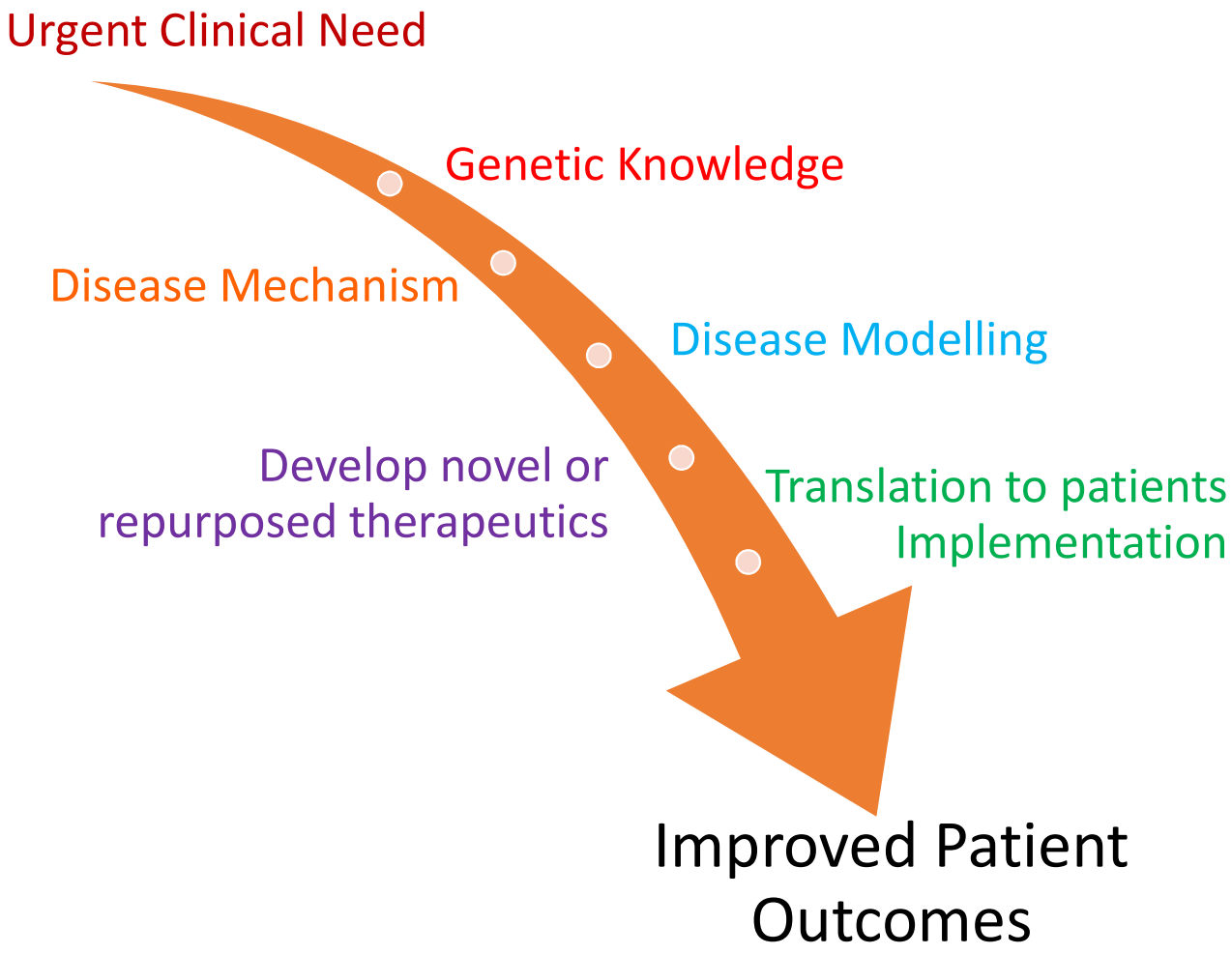
- ASO targeting mouse *Scn2a*
  → reduce gain of function and restore to normal levels of protein



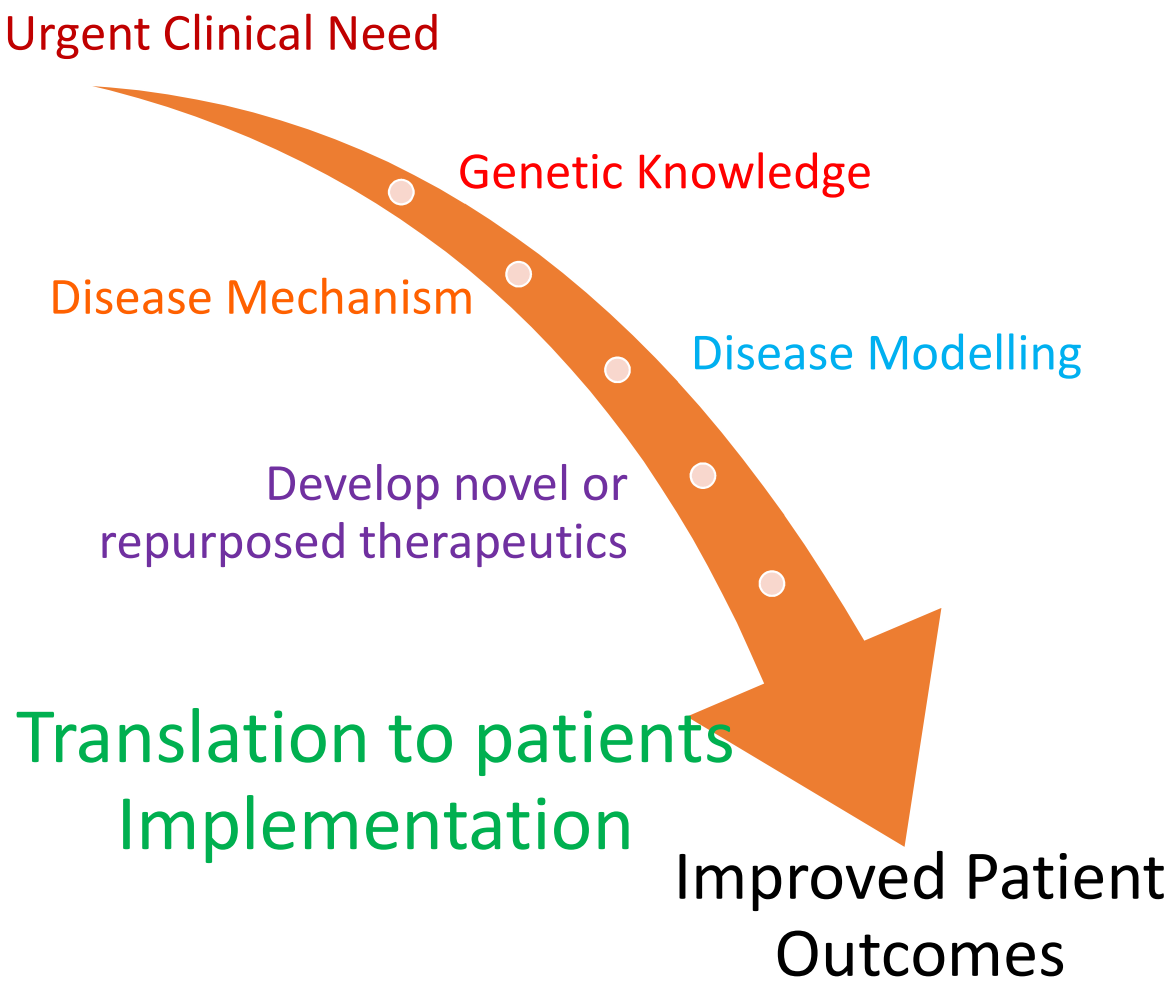


ASO mediated knockdown of *Scn2a* rescues the disease phenotype of *Scn2a* gain-of-function DEE mice

Petrou et al AES 2018



Gene discovery underpins Precision Medicine

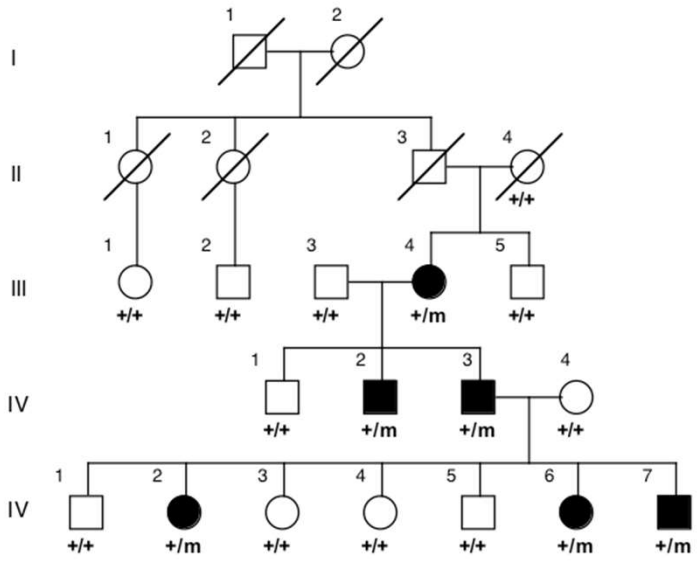


Gene discovery underpins Precision Medicine

Severe autosomal dominant nocturnal frontal lobe epilepsy associated with psychiatric disorders and intellectual disability

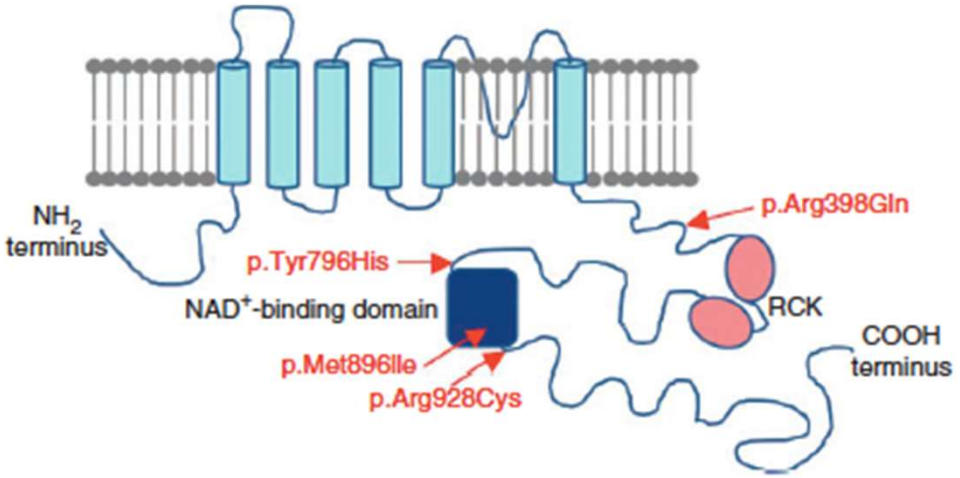
*†Christopher P. Derry, ‡Sarah E. Heron, *Fiona Phillips, §Stephen Howell, *Jacinta MacMahon, ‡Hilary A. Phillips, †John S. Duncan, ‡¶John C. Mulley, *Samuel F. Berkovic, and *#Ingrid E. Scheffer

Family A: c.2782C>T, p.Arg928Cys



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

Sarah E Heron[1,2], Katherine R Smith[3,4], Melanie Bahlo[3,5], Lino Nobili[6], Esther Kahana[7], Laura Licchetta[8], Karen L Oliver[8], Aziz Mazarib[9], Zaid Afawi[10], Amos Korczyn[11], Giuseppe Plazzi[12], Steven Petrou[13–15], Samuel F Berkovic[8], Ingrid E Scheffer[8,13,16,17] & Leanne M Dibbens[1,2,17]

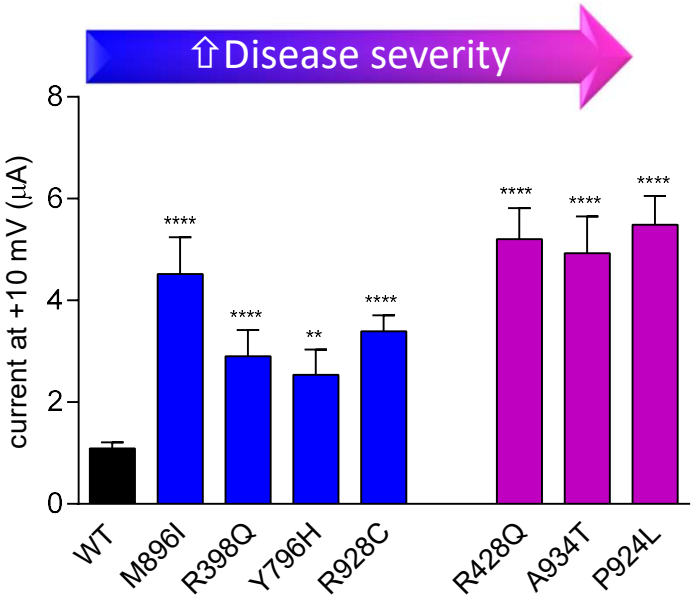


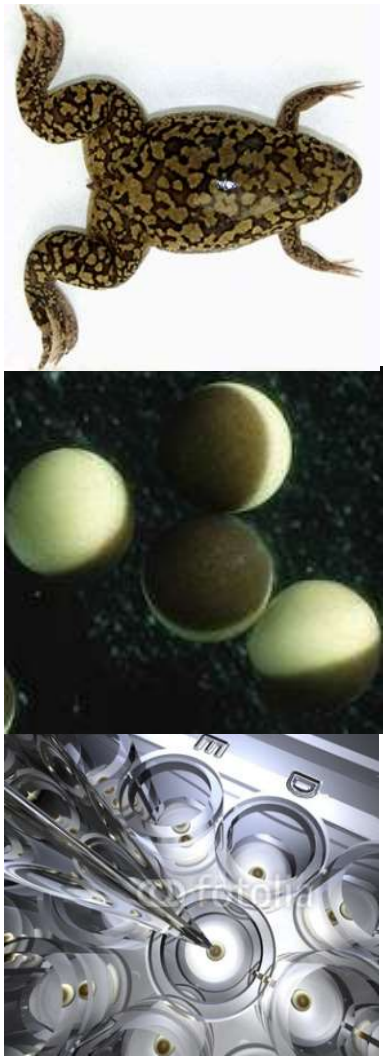
NOVEMBER 2012 NATURE GENETICS

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

Giulia Barcia[1,2,12], Matthew R Fleming[3,4,12], Aline Deligniere[1], Valeswara-Rao Gazula[3], Maile R Brown[3], Maeva Langouet[5], Haijun Chen[6], Jack Kronengold[3], Avinash Abhyankar[7], Roberta Cilio[8], Patrick Nitschke[9], Anna Kaminska[10], Nathalie Boddaert[11], Jean-Laurent Casanova[7], Isabelle Desguerre[1], Arnold Munnich[5], Olivier Dulac[1,2], Leonard K Kaczmarek[3,4], Laurence Colleaux[5] & Rima Nabbout[1,2]

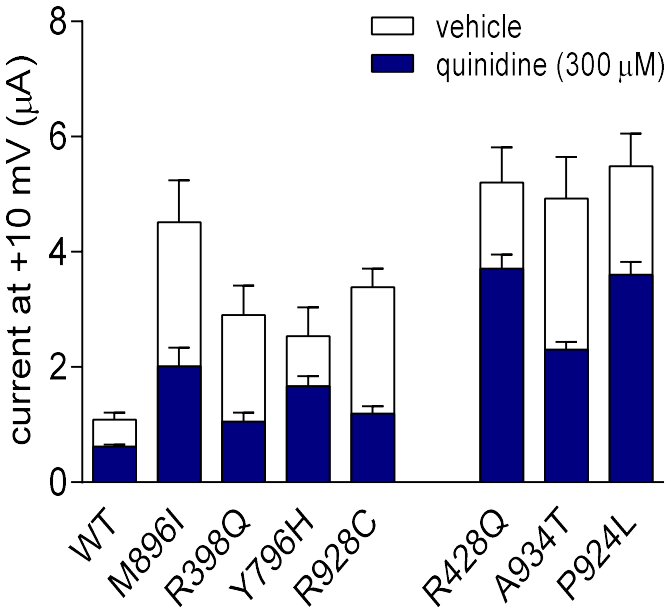
Gain of function correlates with epilepsy severity





Milligan et al Ann Neurol 2014

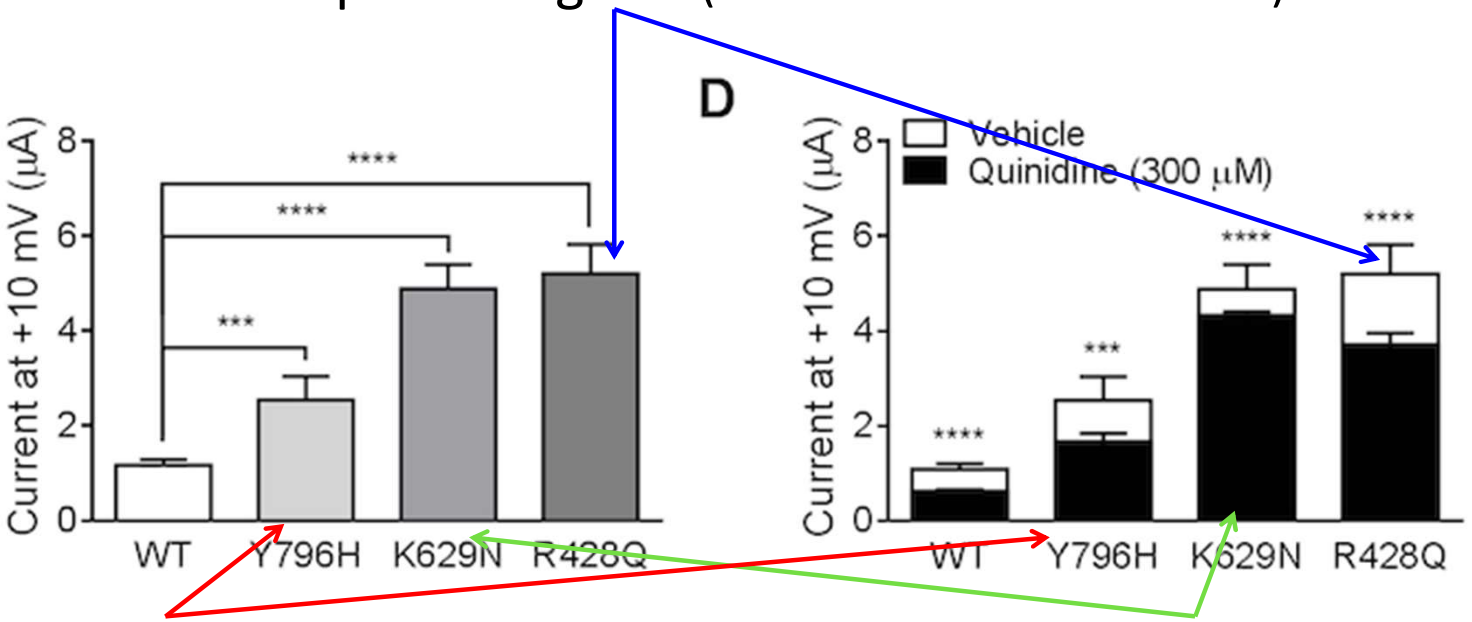
Cinchona Tree Bark



Quinidine - dose dependent reversible inhibition

Anecdotal reports of Precision Medicine in human *KCNT1* diseases

- 25 mth girl Migrating Focal Seizures – seizures improved, small developmental gains (Bearden Ann Neurol 2014)



Girl with focal epilepsy & regression
Quinidine: no benefit

Boy with Migrating Focal Seizures
80% seizure reduction

Mikati et al Ann Neurol 2015

Precision therapy for epilepsy due to *KCNT1* mutations

A randomized trial of oral quinidine

Saul A. Mullen, MBBS, PhD, Patrick W. Carney, MBBS, PhD, Annie Roten, BAppSc, Michael Ching, MPharm, PhD, Paul A. Lightfoot, BSc, Leonid Churilov, PhD, Umesh Nair, BSc, Melody Li, PhD, Samuel F. Berkovic, MBBS, MD, Steven Petrou, PhD, and Ingrid E. Scheffer, MBBS, PhD

Neurology® 2018;90:e67-72. doi:10.1212/WNL.0000000000004769

Quinidine in *KCNT1* severe NFLE

Randomised

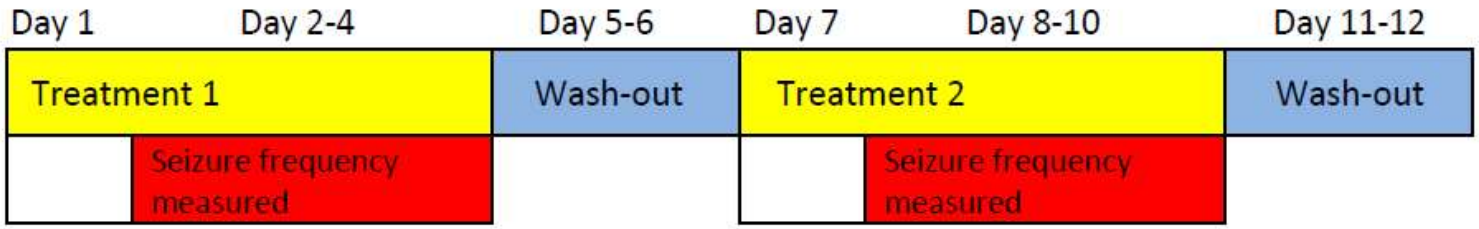
Double-blind

Cross-over

Placebo-controlled

Inpatient

Trial



- 3 days of quinidine 900 mg/day versus placebo
  - Decrease to 600 mg, then 300 mg if toxicity
  - Cardiac monitoring
- Video-EEG monitoring → precise seizure counts
- Mean 10 seizures per night (2-42)

Patient	Age	Dose / day	Focal seizures in 72 hours		
			Placebo	Active	Change
1 M	54y	900mg	-	-	-
2 M	43y	600mg	-	-	-

Quinidine in *KCNT1* severe NFLE

- Very large number of seizures (14/night)
- Exacerbation in 3 of 4 patients
- No statistical effect - adequately powered
- Doses too low?
- Study too short?
- Different ages and different diseases?

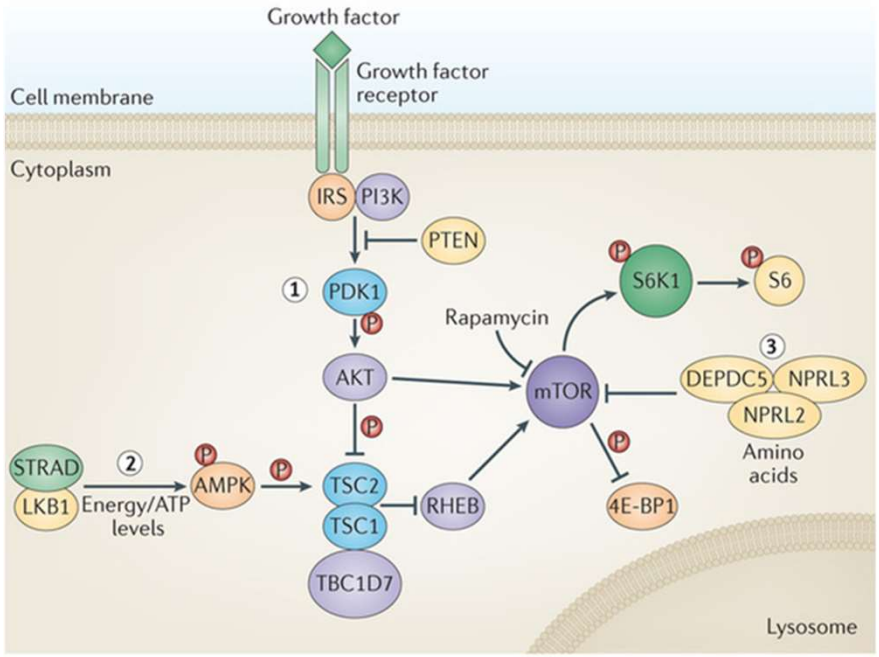
Mullen et al 2018

Precision Medicine

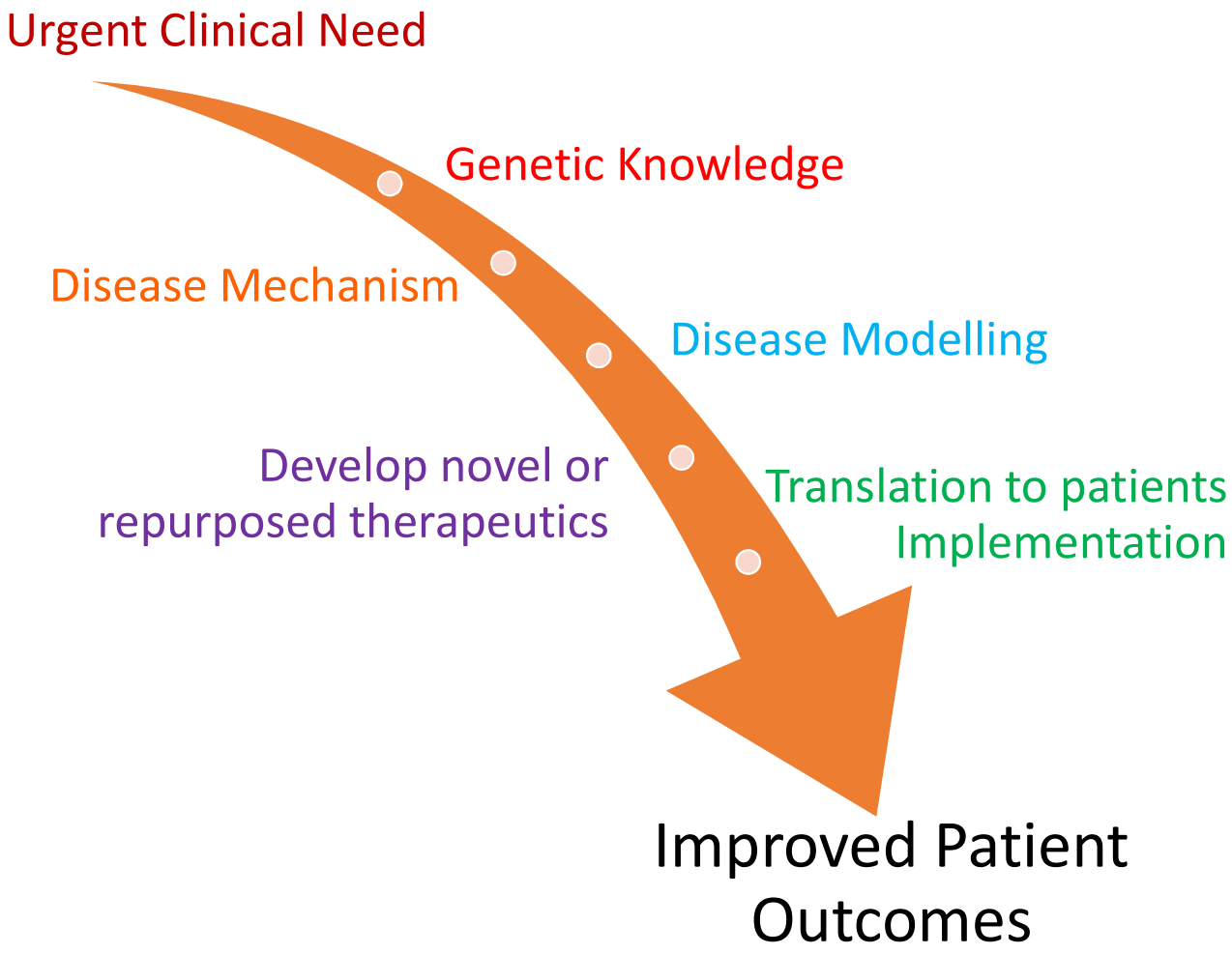
- Gene
- Function
  - Gain
  - Loss
- Pathway

Pathway precision therapies

- Too expensive for targeted therapy for each gene?
- Pathway: one treatment → many genetic diseases



- mTOR pathway
- Focal cortical dysplasia
- ? Surgery
- mTOR inhibitors



Epilepsy genetics *will* transform treatment of Drug-Resistant Epilepsies

- Reproductive counseling
  - Parental mosaicism
  - Patient mosaicism
- Gene therapy
  - ASO promising in mice
- Rigorous controlled trials of new therapeutics