

Genetic testing in epilepsy: why, who, how, interpretation and clinical utility

Ingrid E Scheffer AO MBBS PhD
FRACP FAES HonLLD FAHMS FAA
FRS

University of Melbourne, Austin and Royal
Children's Hospitals, Florey and Murdoch
Institutes, Melbourne, Australia

Why?

Who?

How?

Answer for
patient/family

Comorbidities

Prognosis

Guide
management

Avoid
unnecessary
investigations

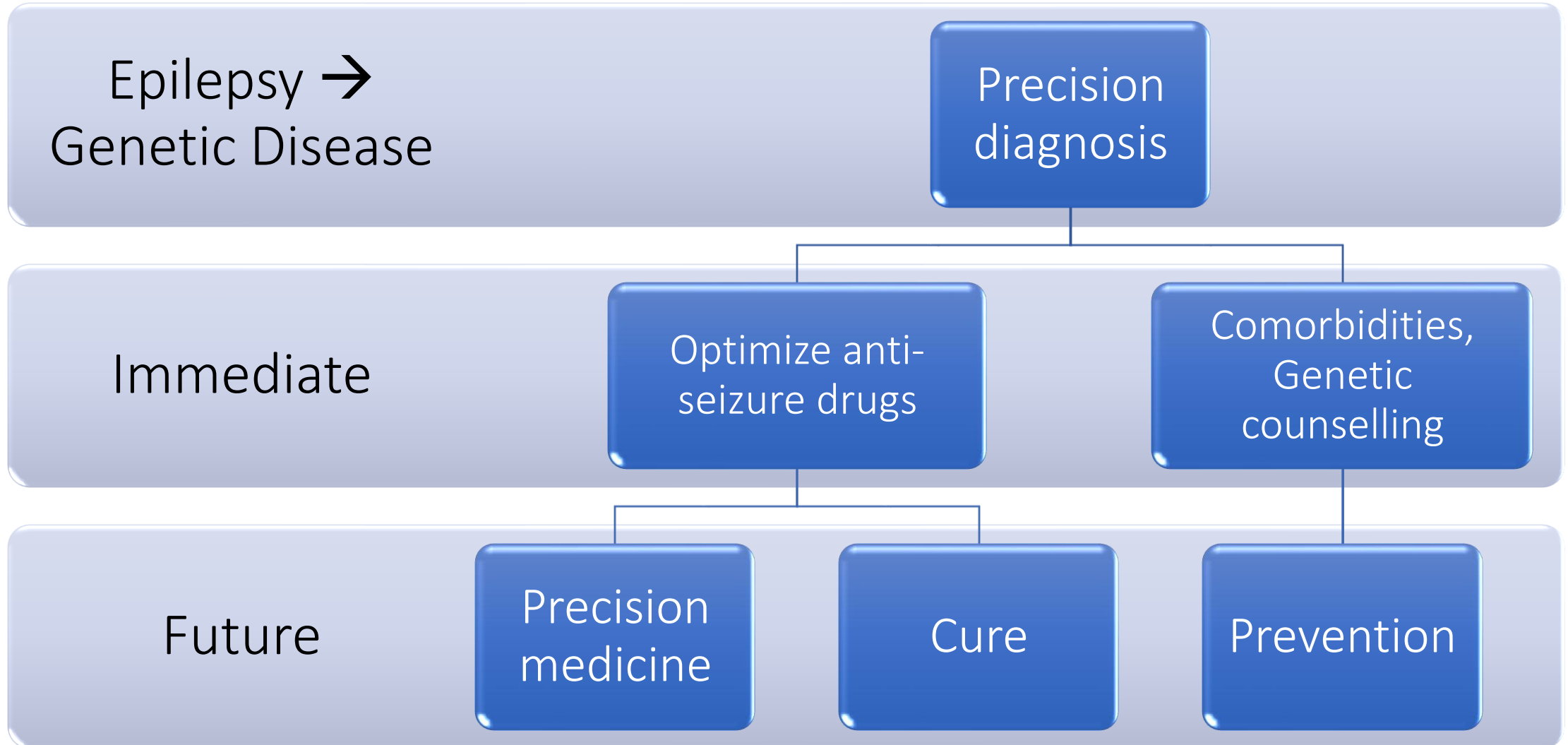
Recurrence
risk

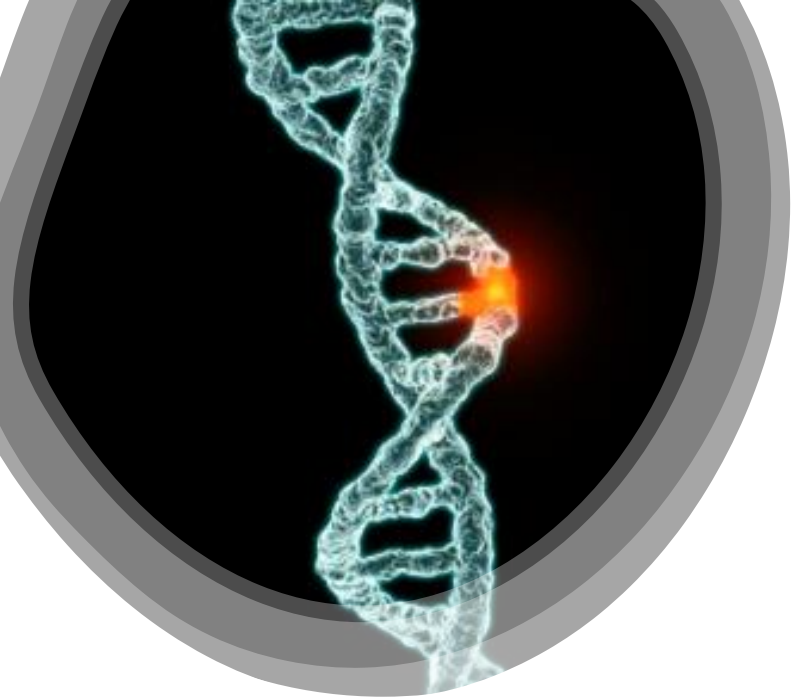
International
community

Prevention

Aetiology – why does it matter?

Genetic testing – How does it make a difference?





Genetic diagnosis
→ Anti-Seizure Medicine selection



Precision medicine

Wh

Who?

How?

Operational definition of developmental and epileptic encephalopathies to underpin the design of therapeutic trials

Ingrid E. Scheffer^{1,2,3}  | Jacqueline French⁴  | Kette D. Valente⁵  |
Stéphane Auvin^{6,7,8}  | J. Helen Cross⁹  | Nicola Specchio^{10,11} 

nature reviews disease primers

<https://doi.org/10.1038/s41572-024-00546-6>

Primer

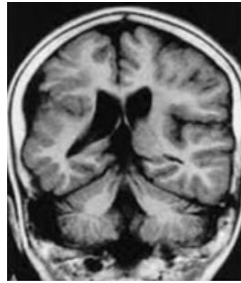
 Check for updates

Developmental and epileptic encephalopathies

Ingrid E. Scheffer^{1,2,3}  , Sameer Zuberi^{4,5} , Heather C. Mefford⁶ , Renzo Guerrini^{7,8}  & Amy McTague^{9,10} 

Developmental and Epileptic Encephalopathy

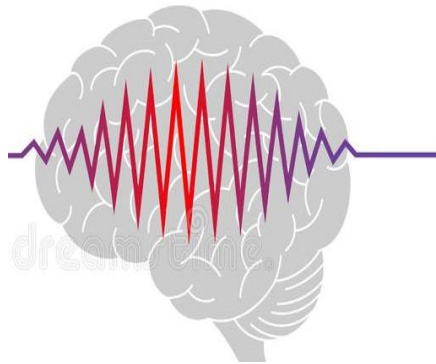
Operational definition



Aetiology



1. Frequent seizures and/or epileptiform activity on EEG
2. History of developmental slowing or regression
3. Underlying aetiology also contributes to developmental impairment

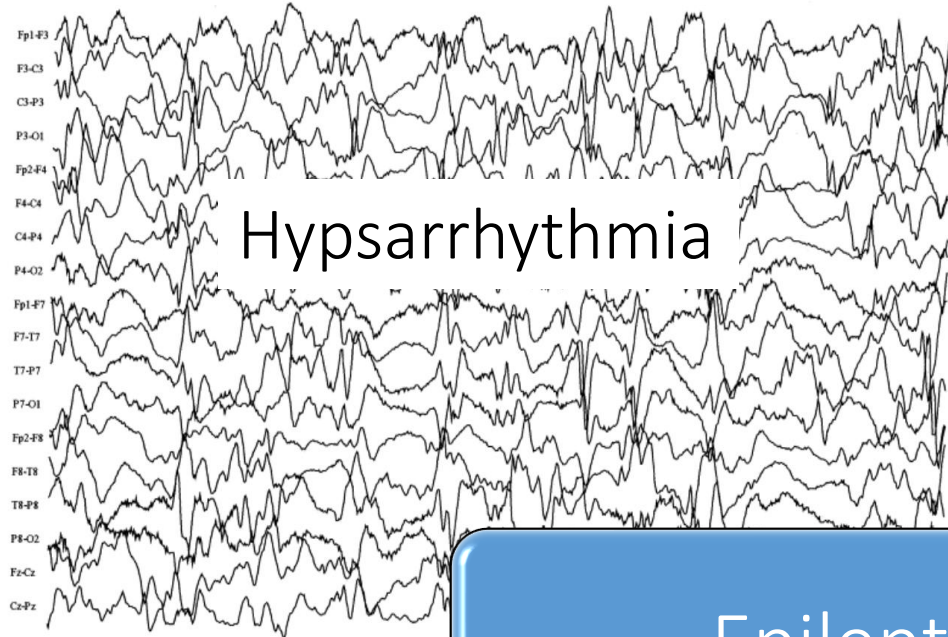


Epileptiform
activity

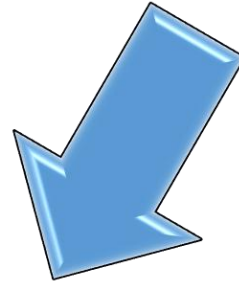
Developmental
Impairment



Epileptic Encephalopathy



Aetiology

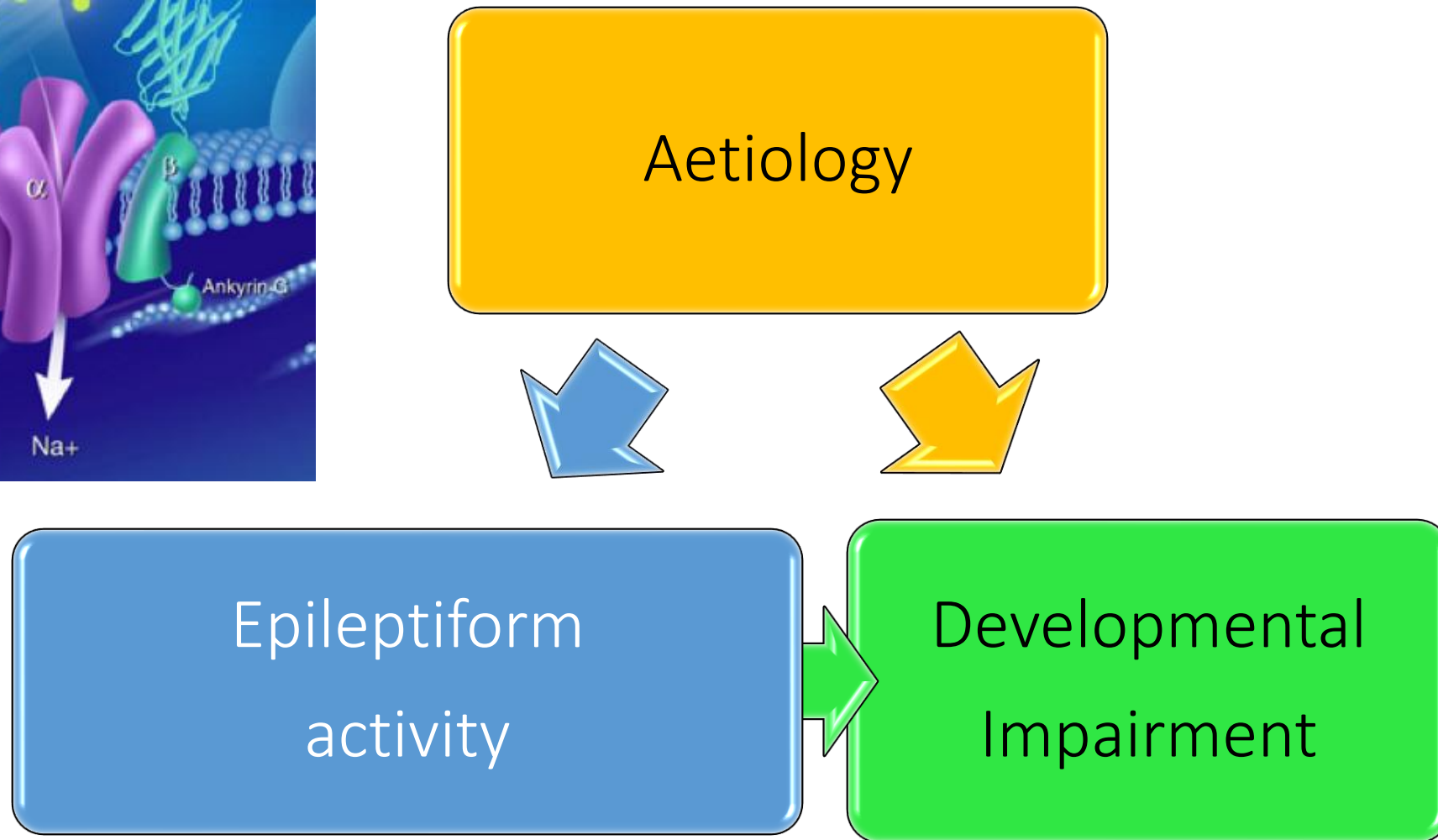
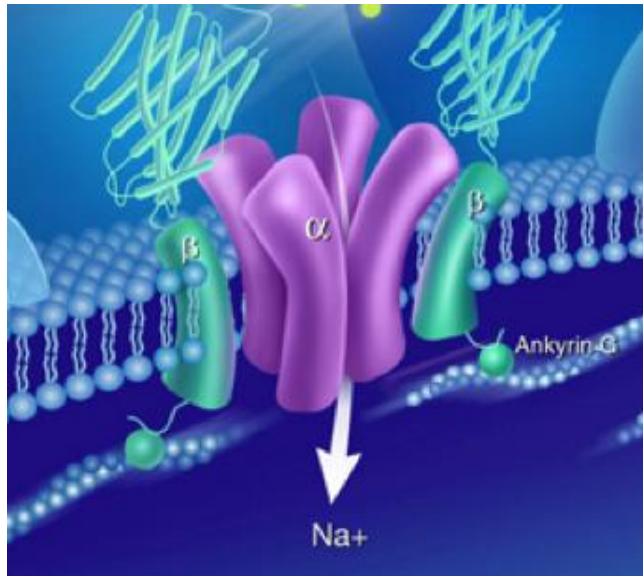


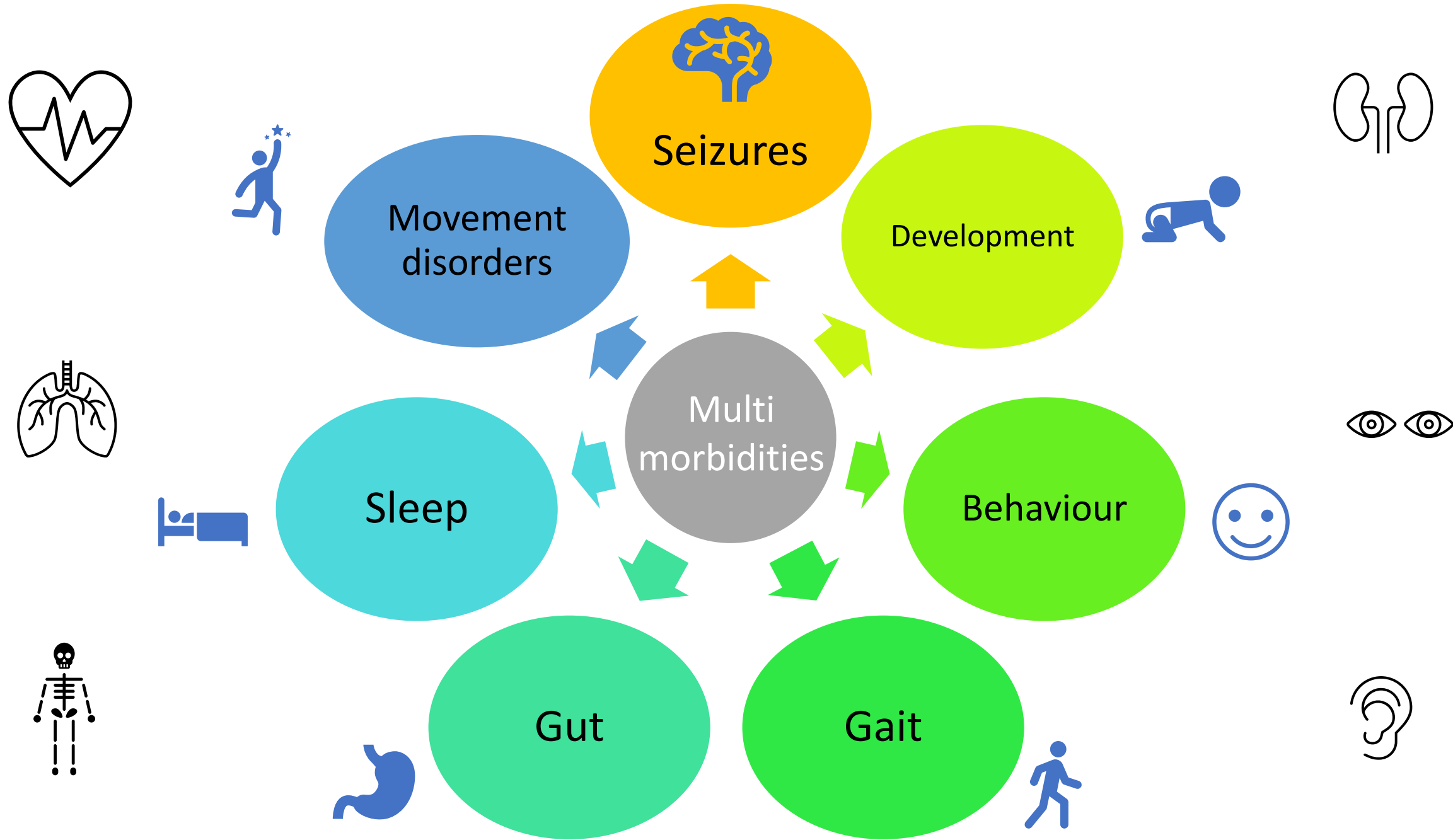
Epileptiform
activity



Developmental
Impairment

Developmental and Epileptic Encephalopathy





Who?

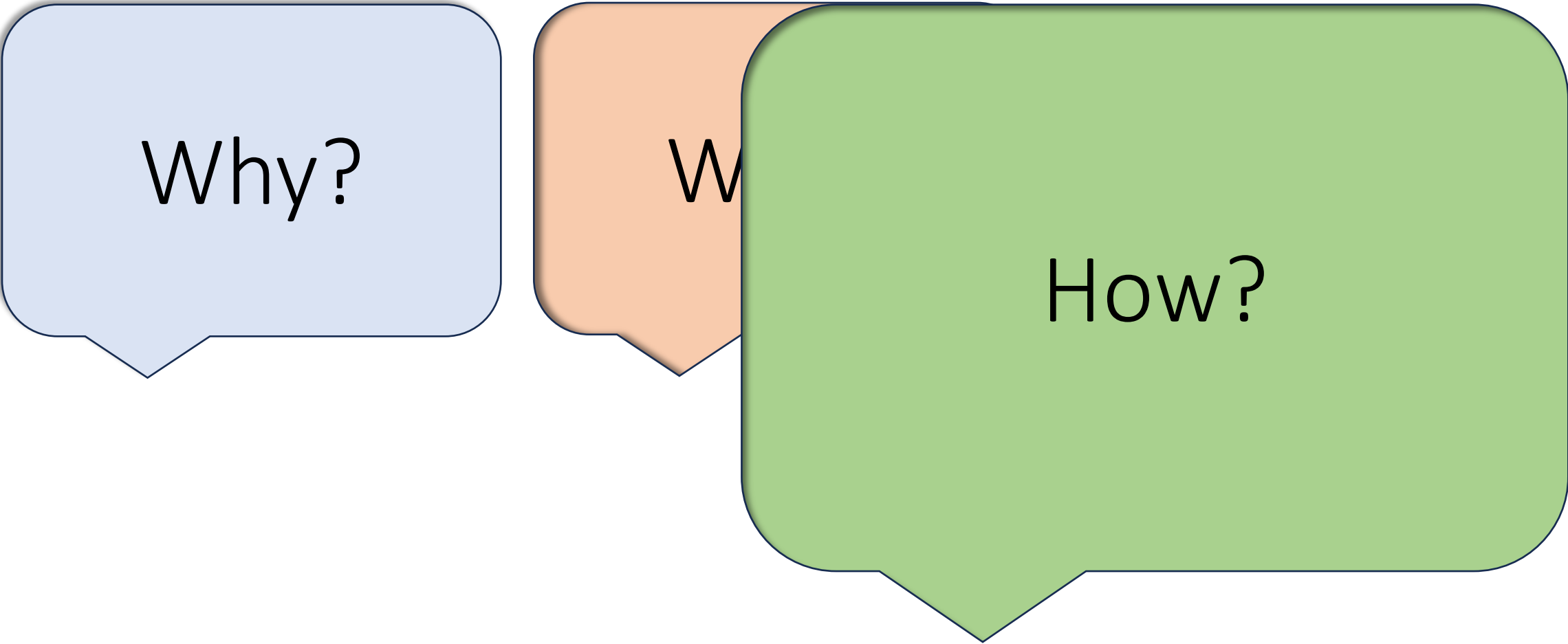
ALL patients with
Developmental &
Epileptic
Encephalopathies

- Adults
- Children

All patients with
Drug-Resistant
Epilepsies

Consider in
Idiopathic
Generalized
Epilepsies

Consider in Self-
Limited infantile
epilepsies

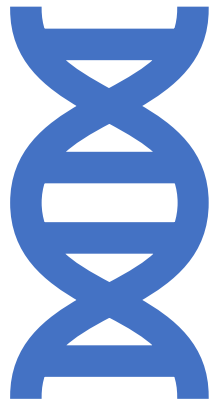


Why?

W

How?

Genomic studies



Copy number variant studies

Panels

Exomes

Genomes

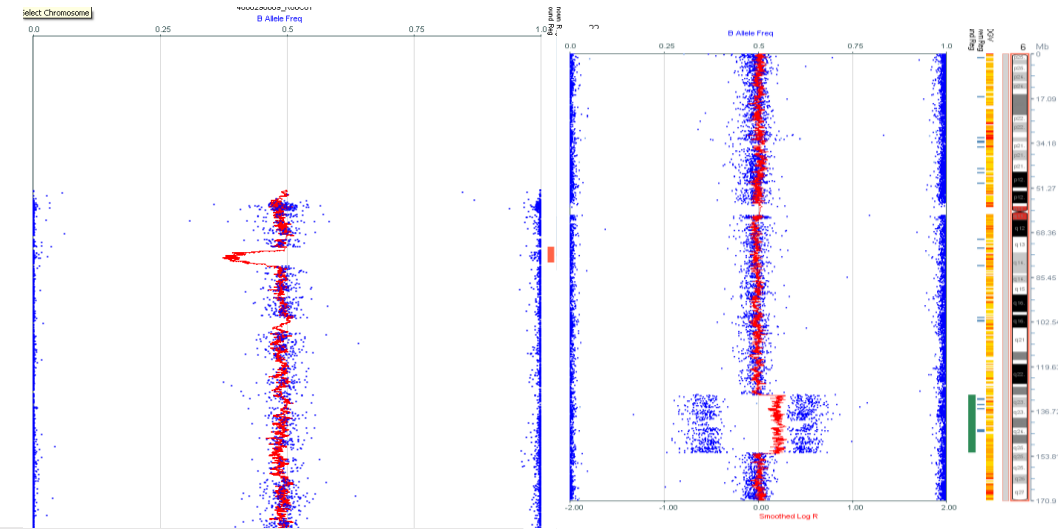
Trio genomes

Hidden pathogenic variants

Microarray

Copy number analysis
of whole genome

Microdeletion
Microduplication
Complex
rearrangements



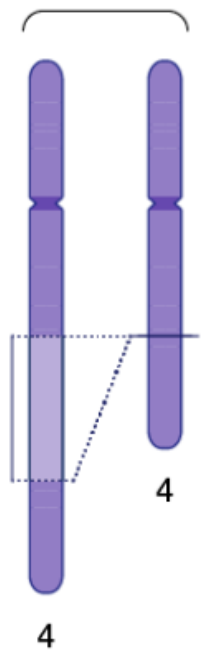
Deletion

Duplication

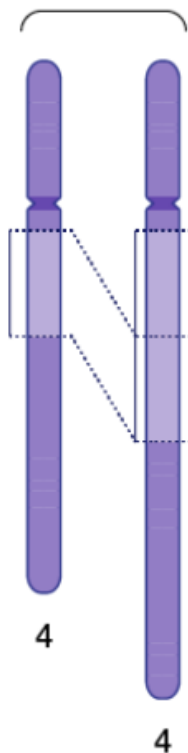
Molecular karyotype

Copy number variants

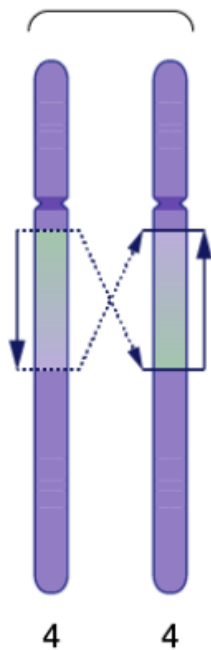
Deletion



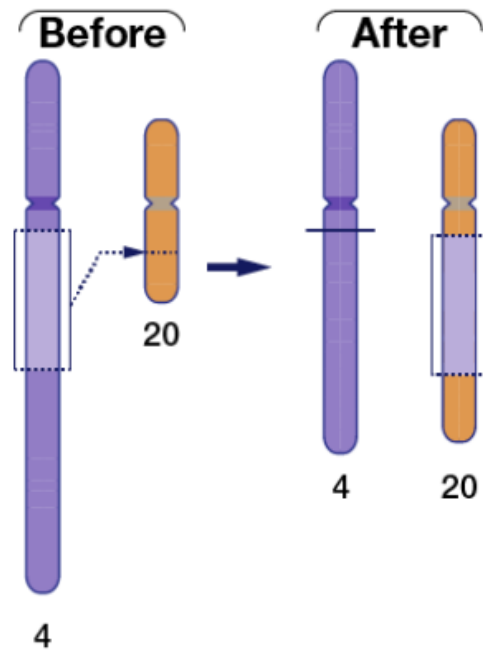
Duplication



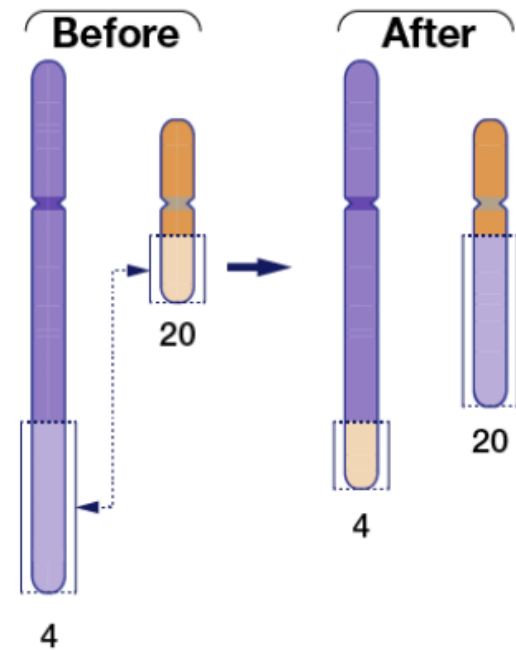
Inversion

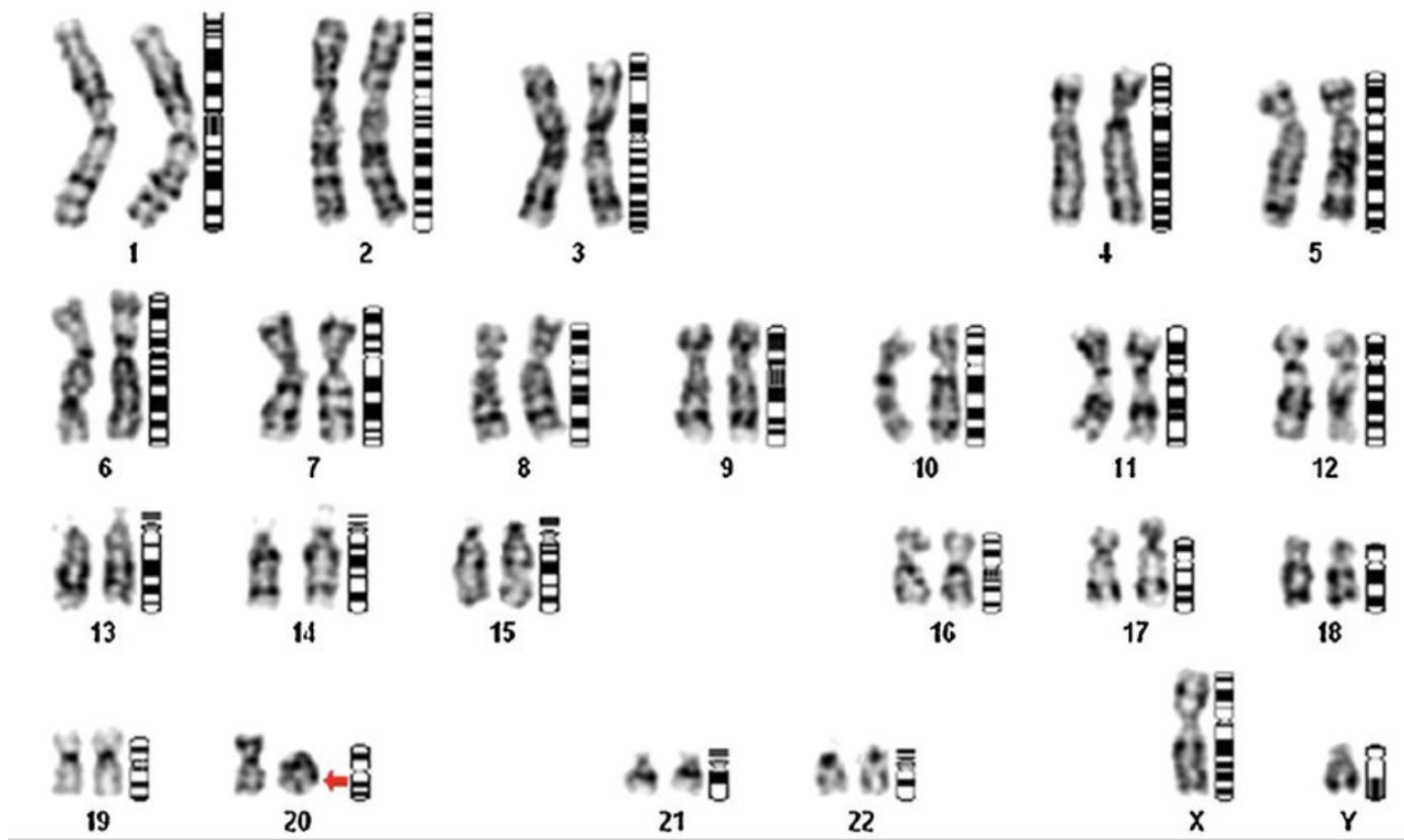


Insertion



Translocation





Classical karyotype

Ring 20

Epilepsy Gene Panels

- Compared 4 diagnostic providers: Invitae, GeneDx, Fulgent Genetics, Blueprint Genetics + 2 research panels: PanelApp Australia, ClinGen
- Number of genes 144 – 511
- 713 unique genes in 2022
- **Only 111 (15.5%) genes in all 4 clinical panels!**
- 2026: >1000 monogenic genes
→ 976 cause DEEs

List publicly available:

github.com/bahlolab/epilepsygenes

Exome sequencing has replaced gene panels

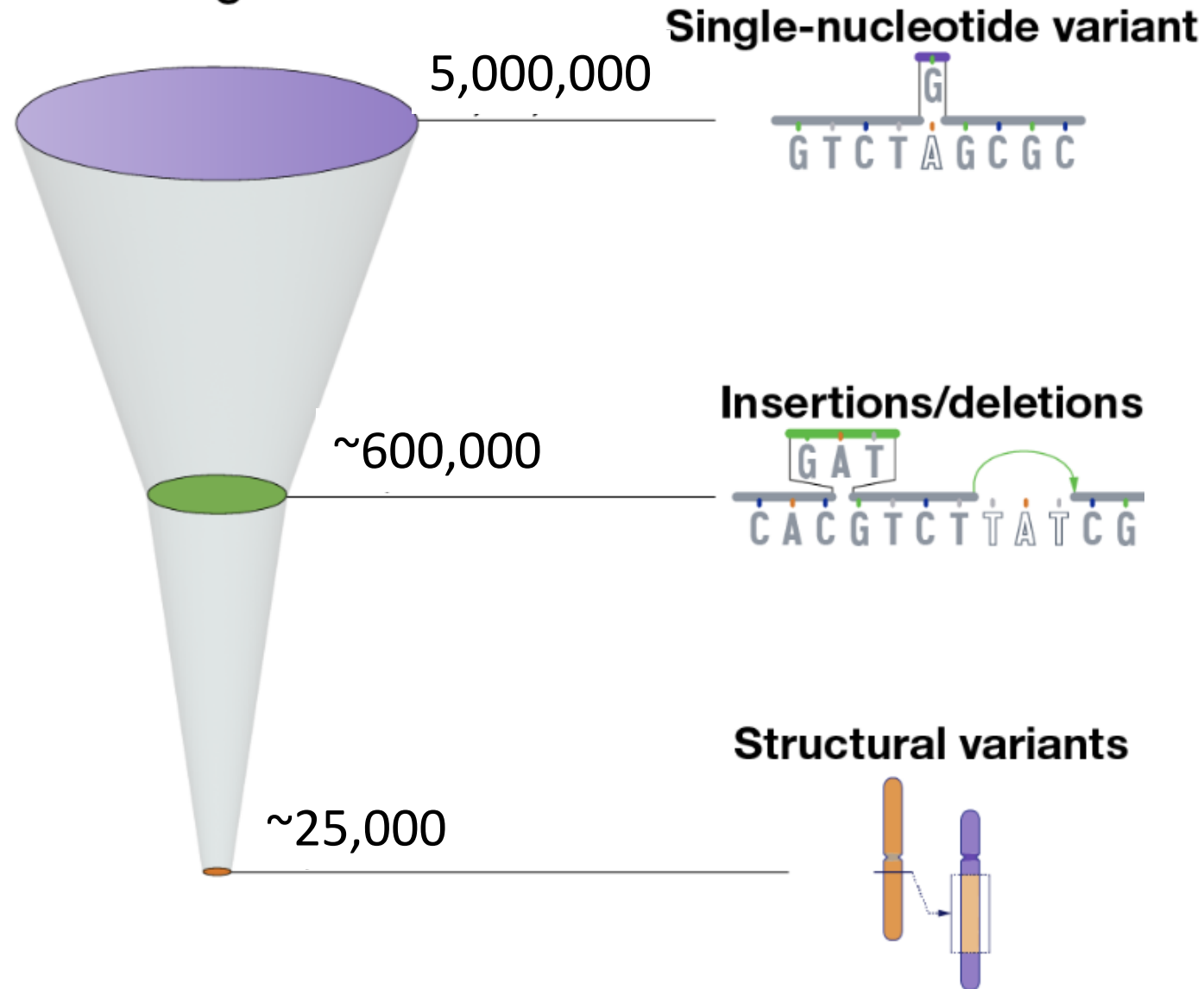
- 1-2% of genome that encodes proteins
- Most pathogenic variants in exome
- Trio Exome Sequencing
 - Identify pathogenic variants
 - Need to understand Inheritance pattern eg. dominant, recessive
- Reinterrogate data as new genes discovered

Genome Sequencing

- 6 billion nucleotide sequence
- 5 million single nucleotide variants



Number of each variant type in a human genome



Trio Genome Sequencing

Huge datasets

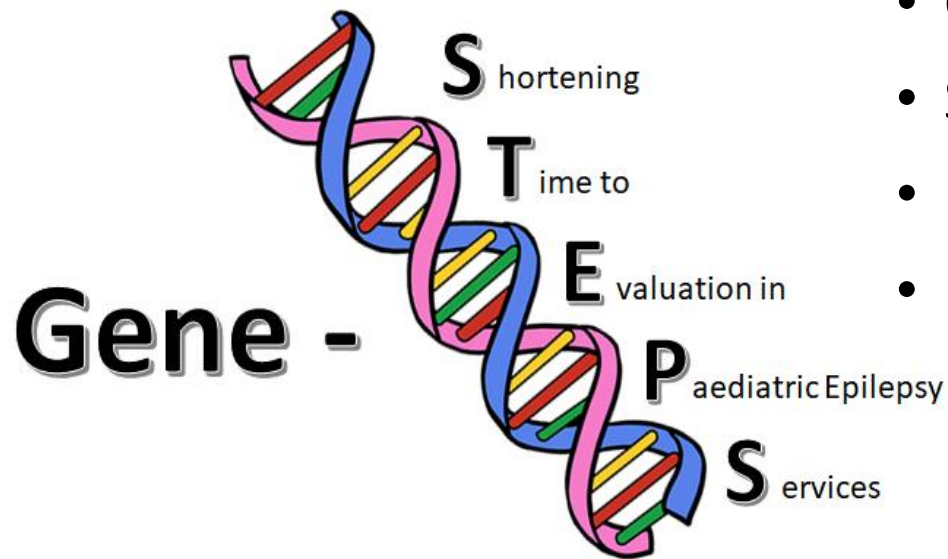
Trios → identify novel variants in patient

Is a variant pathogenic?

- Absent from population databases eg. gnomAD
- *In silico* tools, recurrent
- Phenotypic match

Novel mechanisms eg intronic variants

Periodic reanalysis of exome or genome sequencing data is important!



- Gene-STEPS study: 312 infants
 - Seizure onset <1 year: self-limited + drug-resistant
 - Rapid genome sequencing – 46% solved
 - 176 unsolved → genome reanalysis → + 5% solved
 - VUS with new evidence for pathogenicity
 - Variants missed
 - Mosaic variants
 - Structural variants
 - Repeat expansion
- All findings had clinical utility

Interpreting genetic results

- Variants of uncertain significance (VUS) are NOT the answer
- Not clear they are pathogenic
- Until further evidence emerges
 - eg. recurrent in patient with same phenotype
- Should review every 1-2 years
- Keep looking....



Explaining results

- Be clear – explain what a genetic cause is
- VUS – *no* need to explain because we don't know what it means!
- We need to ensure families understand findings – if negative, it can still have a genetic basis!
- Genetic counseling – ask your friendly geneticist
- Follow up and review

Hidden Genetic causes

- Structural variants
- Intronic variants
- Mosaicism
- Alternate transcripts
- Repeat expansions
- Epigenetics

50% DEEs unsolved

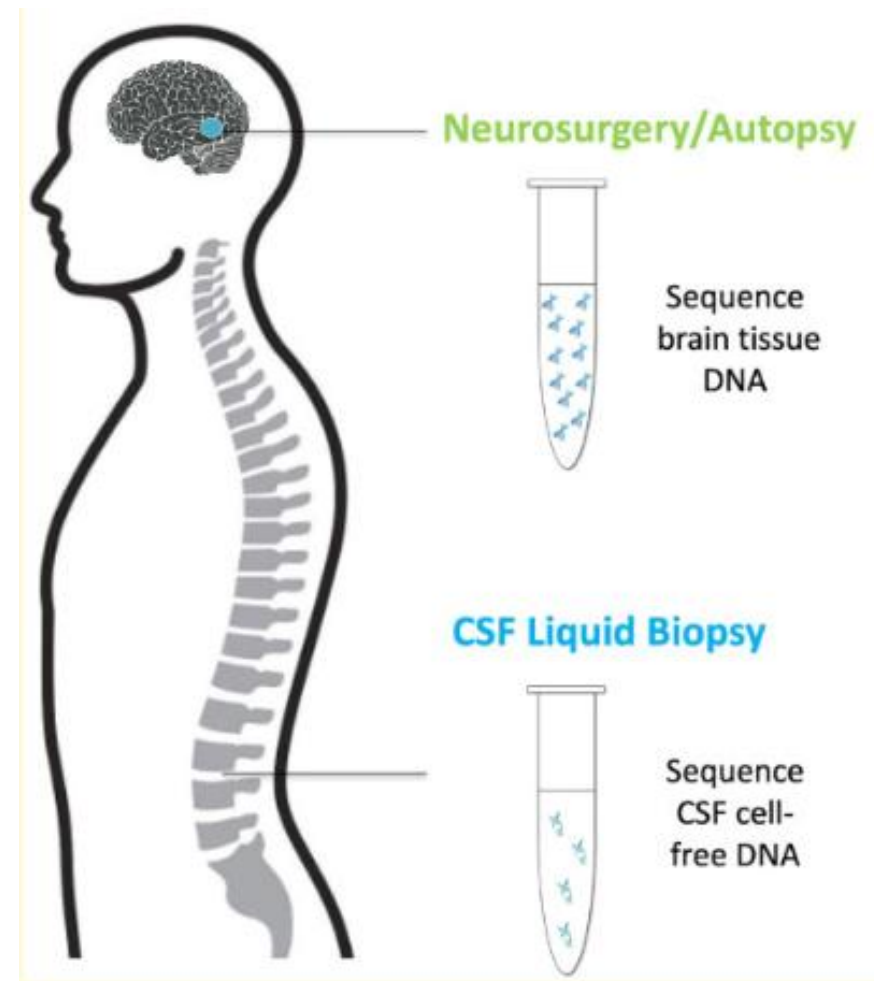
Malformations



BRAIN COMMUNICATIONS

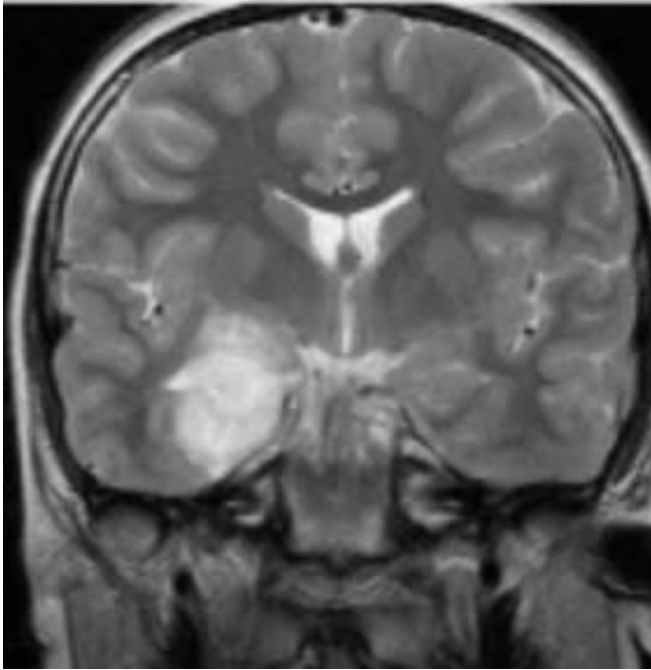
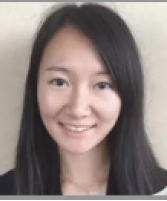
Cerebrospinal fluid liquid biopsy for detecting somatic mosaicism in brain

Zimeng Ye,¹ Zac Chatterton,² Jahnvi Pflueger,^{3,4} John A. Damiano,¹ Lara McQuillan,¹ Anthony Simon Harvey,^{5,6,7} Stephen Malone,⁸ Hongdo Do,^{9,10,11} Wirginia Maixner,¹² Amy Schneider,¹ Bernadette Nolan,⁸ Martin Wood,¹³ Wei Shern Lee,^{5,6} Greta Gillies,^{5,6} Kate Pope,⁵ Michael Wilson,^{5,6} Paul J. Lockhart,^{5,6} Alexander Dobrovic,^{10,11,14,*} Ingrid E. Scheffer,^{1,5,6,7}  Melanie Bahlo,^{15,16} Richard J. Leventer,^{5,6,7} Ryan Lister,^{3,4} Samuel F. Berkovic,^{1,†} and Michael S. Hildebrand^{1,5,†}



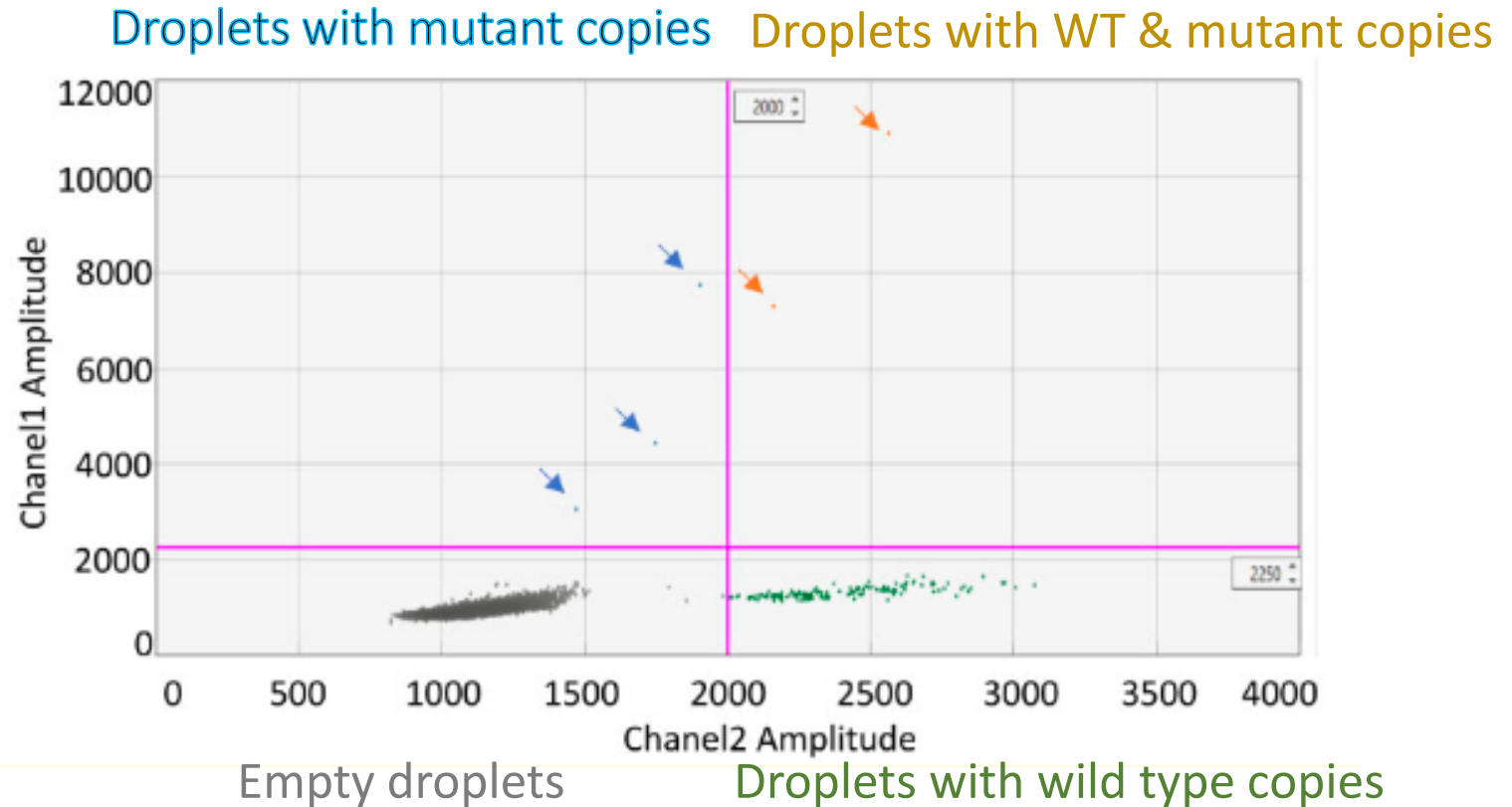


CSF Liquid biopsy can detect somatic mosaicism in brain

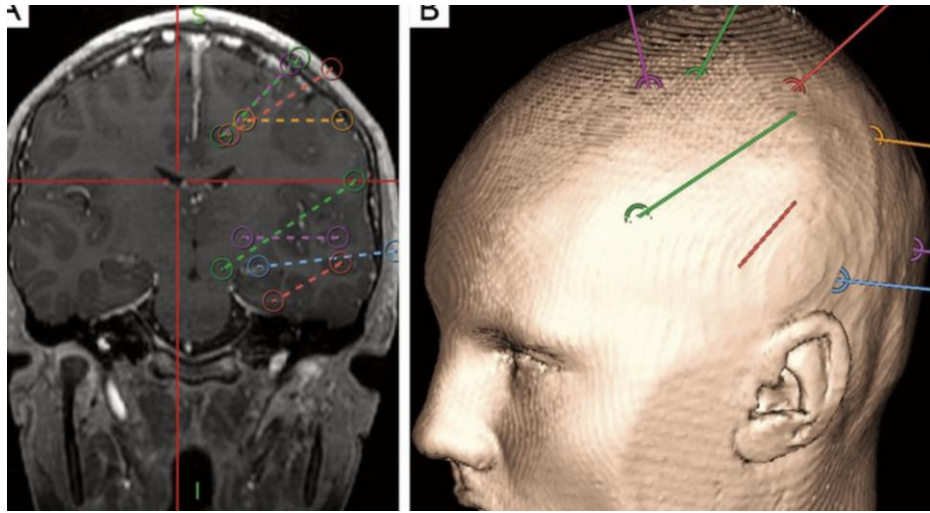
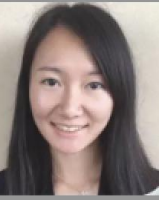


2 y boy – No genetic testing

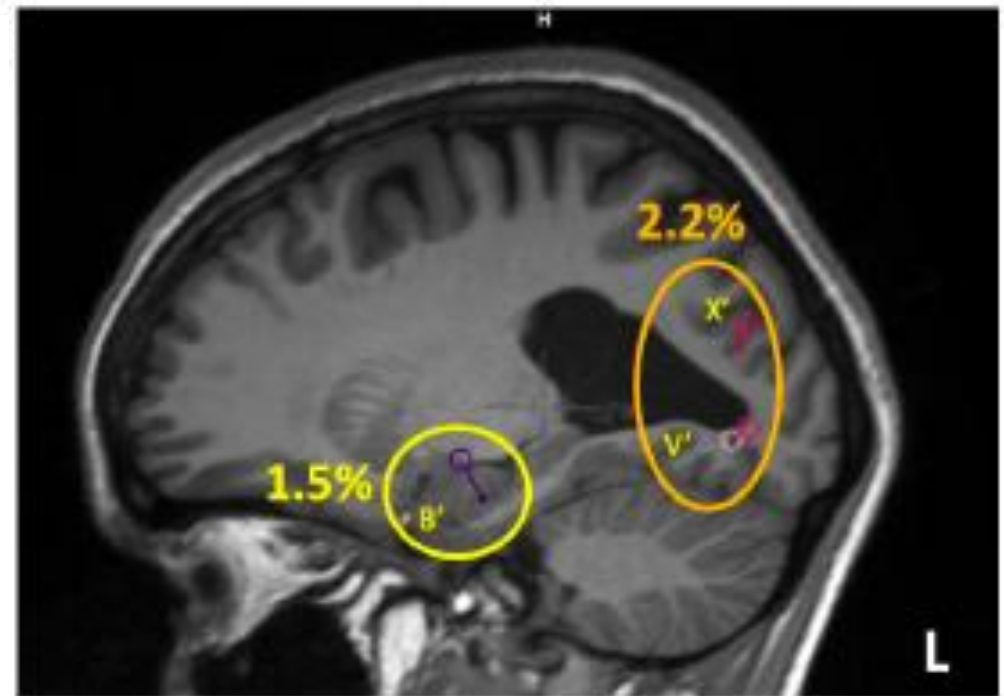
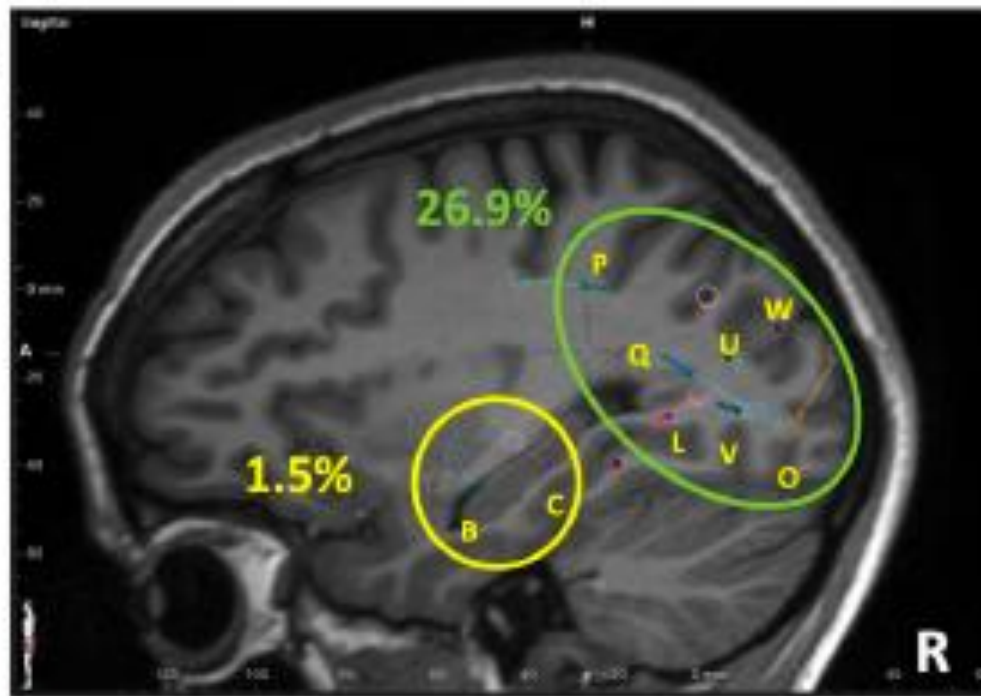
- Medial temporal ganglioglioma
- Recurrent *BRAF* mutation p.V600E in >50% patients with ganglioglioma



- Droplet Digital PCR on CSF to test cell free DNA
found 3.2% Variant Allele Frequency p.V600E
- Then validated in brain 20.8%
 - Not present in blood



Mosaic pathogenic *KCNT1* variant and anatomical gradient in brain DNA from trace tissue on stereo-EEG electrodes



New technologies



Long read technologies

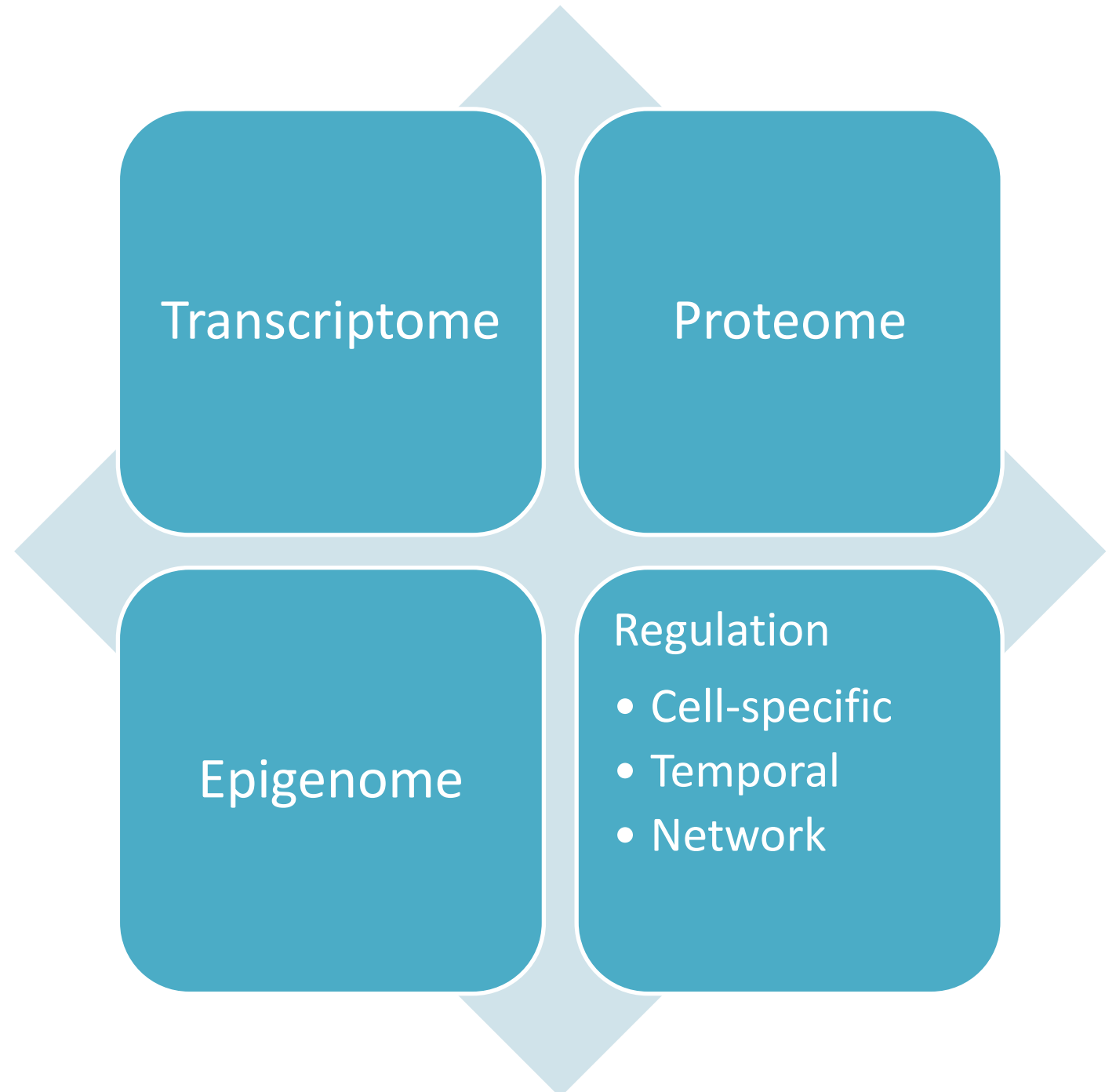


Single cell sequencing

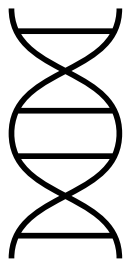


Big data
Artificial Intelligence

Multi-omics
to discover
hidden genetic
causes



Genetic
testing in
individuals
with
epilepsy



Make a syndromic and etiological diagnosis in **all** individuals with epilepsy

Chromosomal microarray – less expensive

Exome sequencing

Genome sequencing

If negative → continue searching for the aetiology!