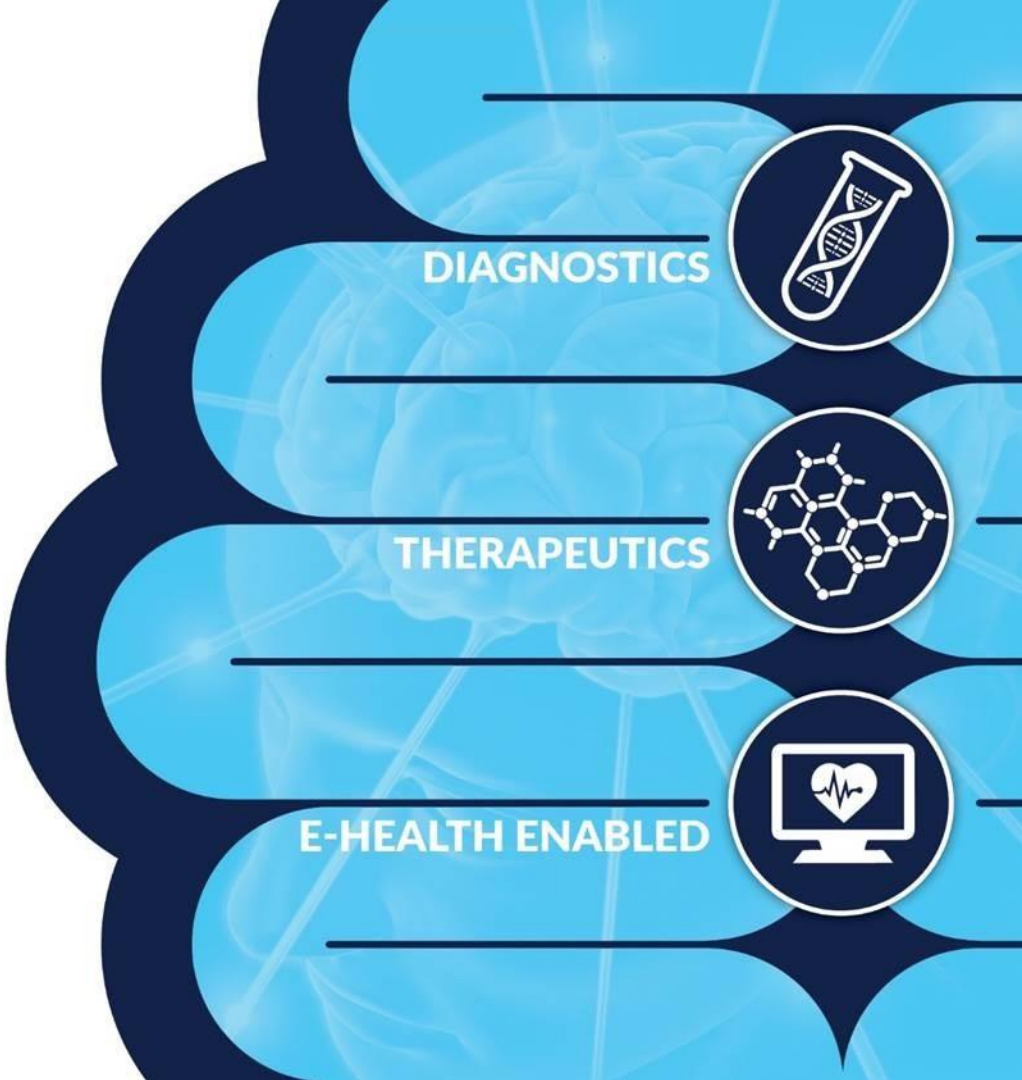
Genetics as a guide for therapeutics in the epilepsies

Gianpiero Cavalleri



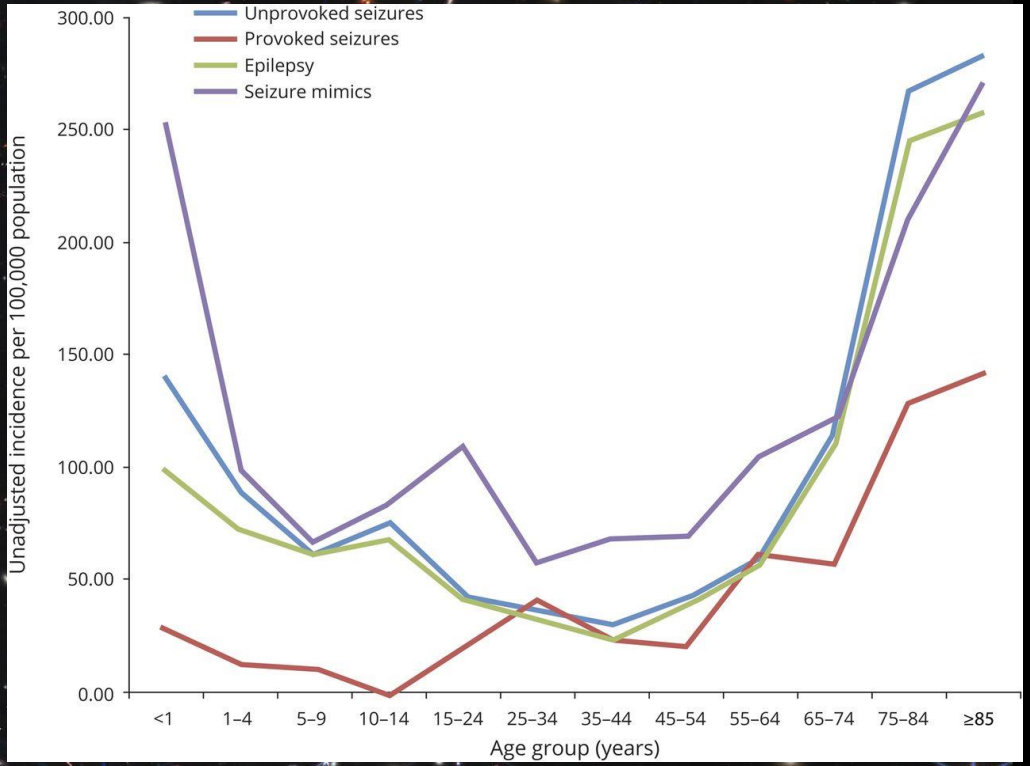
Conflict of interest

- I am in receipt of research funding from Congenica & Janssen Pharmaceuticals

Overview..

1. What are the epilepsies..? (causes, types, genetics of)
2. Clinical indications for genomic diagnostic testing…and associated yield
3. Some examples of precision medicine in the epilepsies
4. Latest (germline) gene discovery efforts
5. Application of PRS to the epilepsies

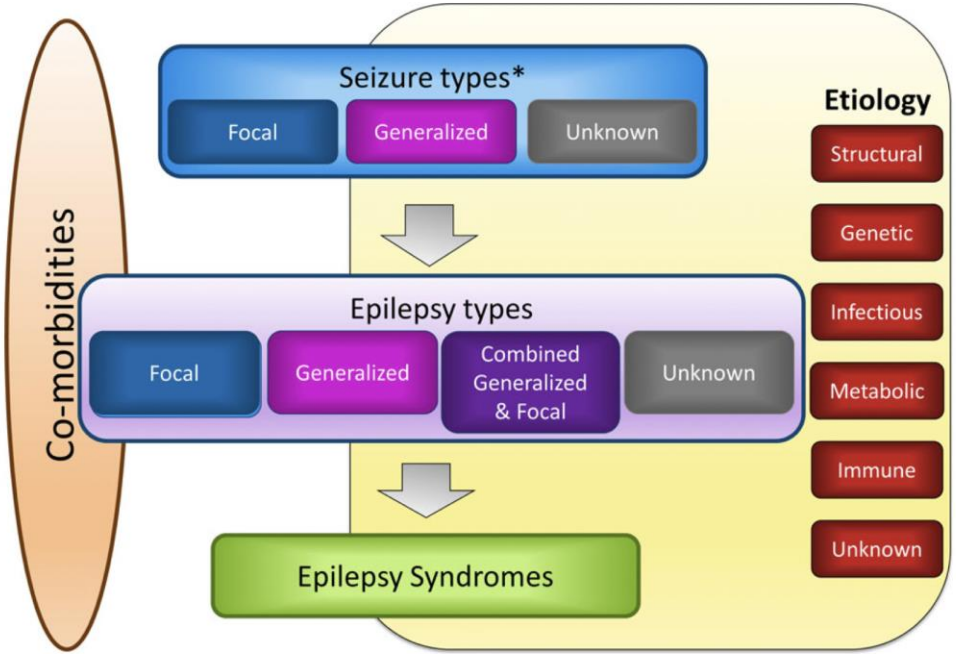
The epilepsies..



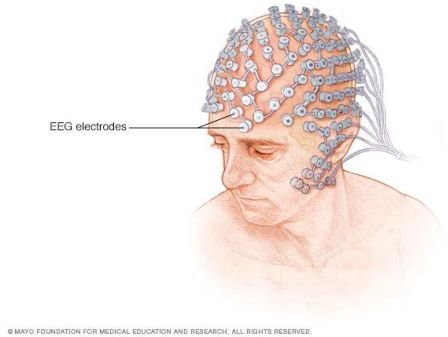
….. are characterised by recurrent unprovoked seizures……affect all age groups ……often of unknown cause…….

may have significant consequences in terms of adverse educational, … psychosocial functioning, and physical morbidity (and potential mortality), especially in the one third of patients with drug-resistant disease.

Classification and causes of epilepsy

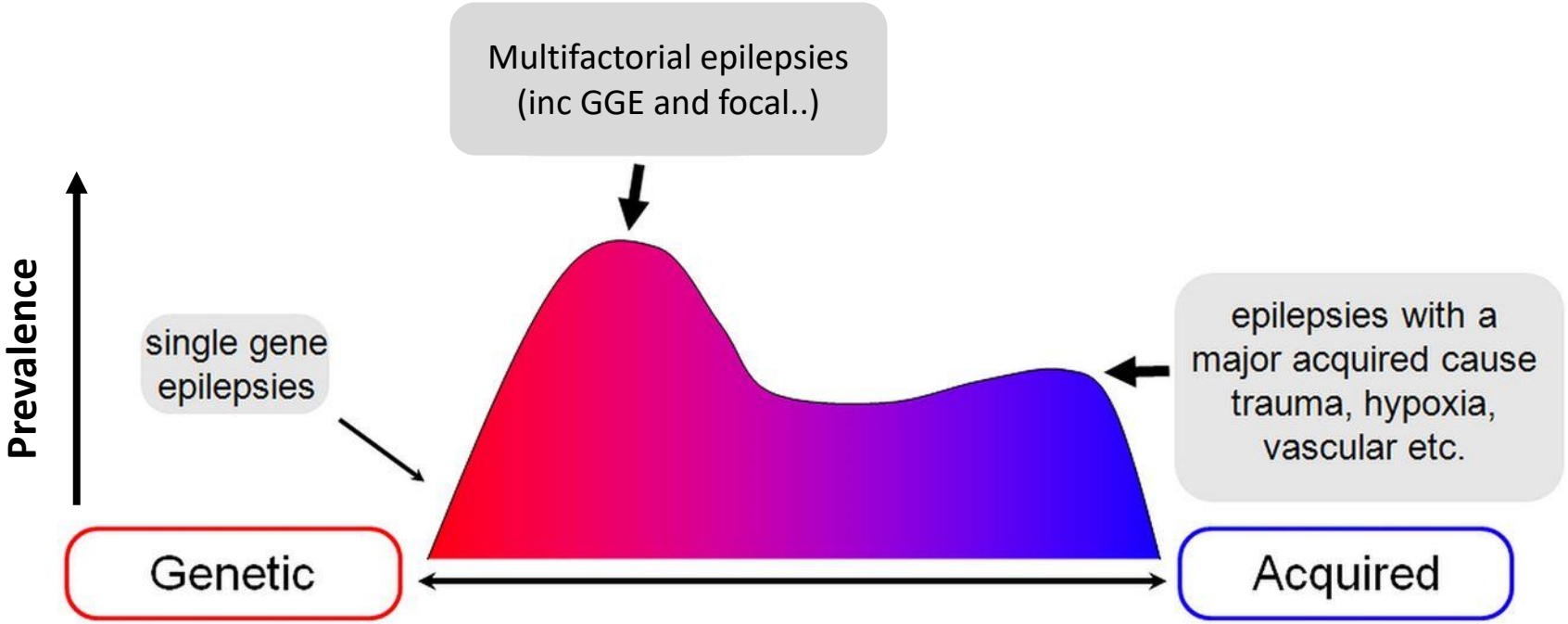


Scheffer et al *Epilepsia* 2017



- **Focal epilepsies (FE)**
- **Generalised epilepsies (GGE)**
- **Developmental and epileptic encephalopathies (DEE)**

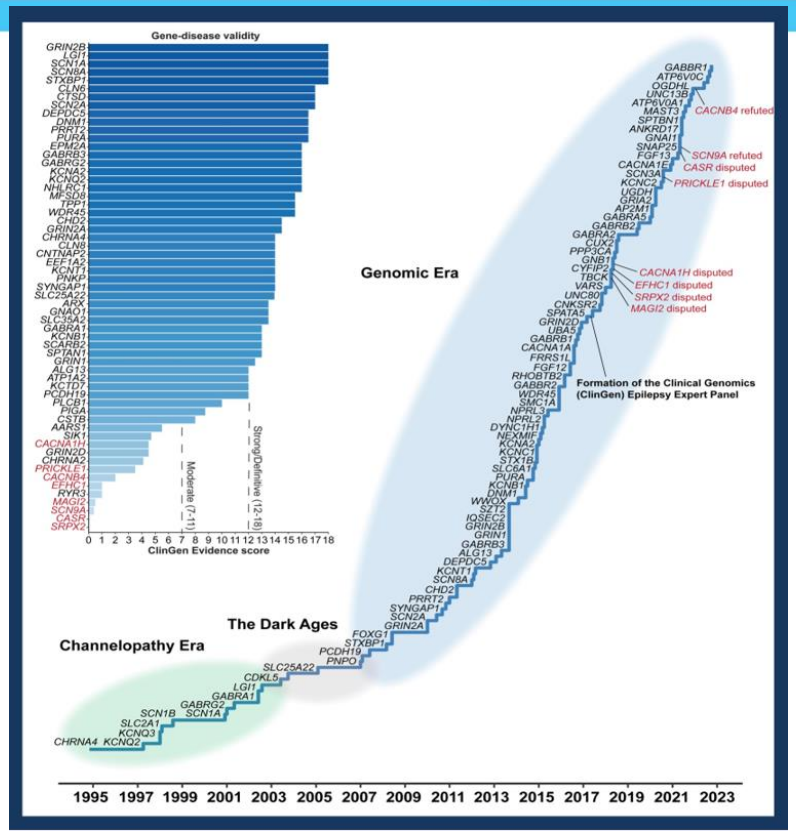
The epilepsies have a spectrum of genetic architectures..



Modified from Hildebrand et al J Med Genetics, 2013

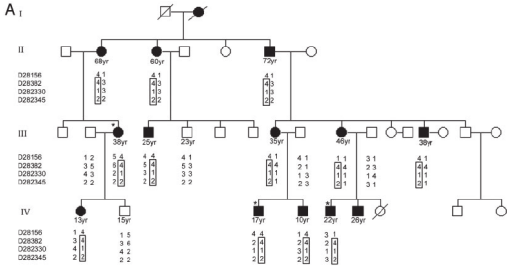
Clinical indications for genomic diagnostic testing..

A brief history of gene discovery in the epilepsies

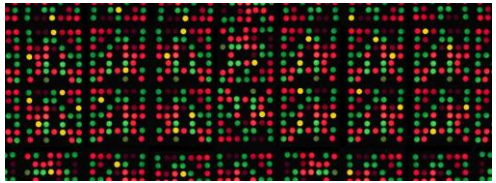


Ruggiero et al. *Curr Opin Neurol*, 2023

Linkage:



Array CGH:



NGS

Clinical indications for diagnostic genetic testing in the epilepsies..

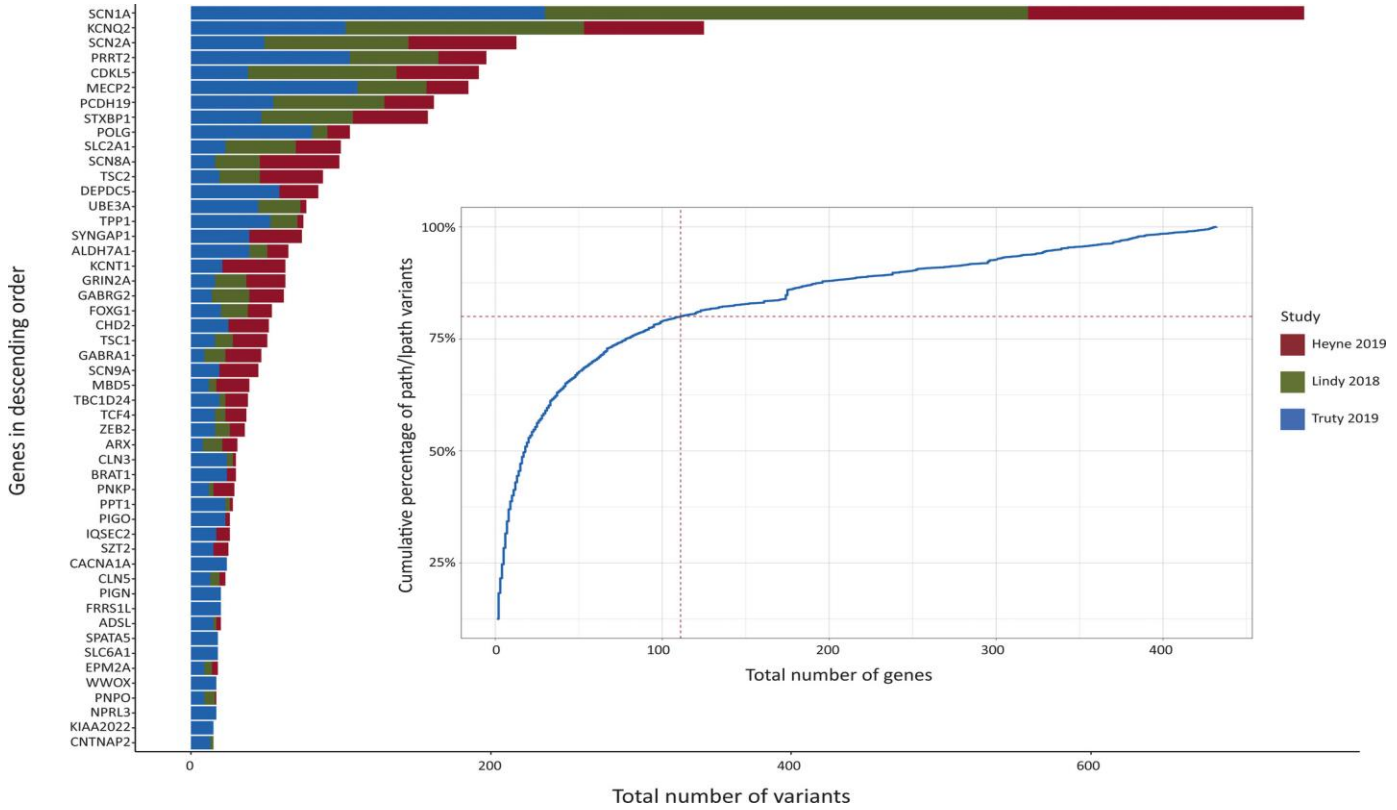
- **From clinical experience & gene discovery:**
  - Strong family history
  - Neonatal or infantile seizures
  - Developmental and epileptic encephalopathies
  - Epilepsy plus other neurodevelopmental comorbidities
- **Recent systematic review:**

"strongly recommend that individuals with unexplained epilepsy be offered genetic testing, without limitation of age".. Smith et al, 2022 *J Genetic Couns*

Grouping	Subgroup	No. of incl. cohorts	No. of incl. individuals	Diagnostic yield (95% CI)
Overall		103	32 310	23.7% (22%–26%)
By disorder	ASD	14	1530	17.1% (11%–25%)
	Epilepsy	72	27 923	24.0% (22%–27%)
	ID	21	2863	28.2% (22%–35%)
By seizure type	FE	15	1944	15.8% (10%–24%)
	GE	7	1258	24.3% (18%–32%)
	GE & FE	59	26 888	24.8% (22%–28%)
By disorder subtype	Epilepsy without ID	8	1224	9.3% (4%–23%)
	ASD with ID or DD	7	591	24.6% (18%–32%)
	Epilepsy with ID	15	1290	27.9% (24%–33%)
By other DEEs	WS	16	768	19.3% (14%–26%)
	Other DEEs	8	232	38.8% (23%–57%)
By age of onset	Any Age	5	1080	6.6% (2%–22%)
	Childhood	3	171	14.7% (4–42%)
	Neonatal/ Infantile	13	986	29.3% (23%–36%)
By sequencing technology	Panel	73	28 665	22.6% (20%–25%)
	ES	36	3720	27.3% (24%–31%)

Table from Stefanski et al 2021 *Epilepsia*:

Epilepsy genes.. by yield.. From >25,000 individuals



Knowles et al. *Epilepsia*, 2022

Some examples of precision medicine in the epilepsies..

Precision or Targeted Therapies in Genetic Epilepsies

- **TSC, DEPDC5, NPRL2/3**
  - ***Everolimus***, cann
- **SCN1A**
  - Loss-of-function: sodium channel b
  - Gain-of-function:
- **SCN2A**
  - Gain-of-function:
  - Loss-of-function:
- **SCN8A**
  - Gain-of-function: **riluzole**
- **SLC2A1 (GLUT1 def)**
  - ***Ketogenic diet***
- **KCNQ2**
  - Loss-of-function: retigabine, sodium channel blocker
  - Gain-of-function: amitriptyline
- **PRRT2**
  - Carbamazepine (drug of choice)

e, L-serine, dextromethorphan

Neurology trial)

), levetiracetam

yridoxine

FULL-LENGTH ORIGINAL RESEARCH

Epilepsia Open® Open Access

Possible precision medicine implications from genetic testing using combined detection of sequence and intragenic copy number variants in a large cohort with childhood epilepsy

Rebecca Truty[1] | Nila Patil[2] | Raman Sankar[2] | Joseph Sullivan[3] | John Millichap[4] |
Gemma Carvill[5] | Ali Entezam[1] | Edward D. Esplin[1] | Amy Fuller[1] | Michelle Hogue[1] |
Britt Johnson[1] | Amirah Khouzam[1] | Yuya Kobayashi[1] | Rachel Lewis[1] |
Keith Nykamp[1] | Darlene Riethmaier[1] | Jody Westbrook[1] | Michelle Zeman[1] |
Robert L. Nussbaum[1,6] | Swaroop Aradhya[1]

..” the testing had possible precision medicine implications in 33% of individuals who received definitive diagnostic results”

- **CHRNA2, CHRNA4, CHRNB2**
  - Transdermal nicotine
- **KCN2A**
  - 4-aminopyridine (fampridine)

Everolimus: a therapy for the GATOR1-related epilepsies? a case series/open label observational study

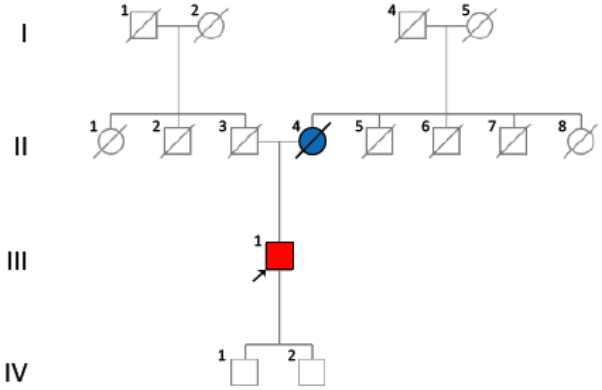
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Epilepsy type	Sleep-related hypermotor epilepsy	Sleep-related hypermotor epilepsy	Frontal lobe epilepsy	Sleep-related hypermotor epilepsy	Temporal lobe epilepsy
Duration of treatment	31 months	12 months	12 months	7 months	27 months
Everolimus dose at last review	15mg	10mg	10mg	12.5mg	15mg
Everolimus level at last review	3.2ng/mL	5.1ng/mL	8.9ng/mL	4.2ng/mL	6.2ng/mL
Baseline MMSF	86	11	7.66	49.66	18.33
MMSF at 3 months on treatment	36	4.33	2.66	51.33	16.66
MMSF at 6 months on treatment	21.66	3.66	1.5	61.5	11.83
MMSF at 12 months on treatment	14.58	2.83	1.08	-	11.91
MMSF at 18 months on treatment	11.94	-	-	-	9.94
MMSF at last review on treatment	12.03	2.83	1.08	62.57	10.29
Monthly seizure burden reduction	86.1%	74.3%	85.9%	No reduction (26% increase in seizure burden)	43.9%
Treatment- emergent adverse events	Stomatitis (mild)	Stomatitis (moderate), low mood and insomnia (moderate)	Stomatitis (mild), acneiform rash (mild)	Stomatitis (severe)	High cholesterol and triglycerides
Everolimus retention	Yes	No (stopped after 12 months due to adverse events)	Yes	No (stopped after 7 months due to lack of efficacy)	Yes
Variant type	Stopgain	Frameshift deletion	Deletion	Splicing	Missense

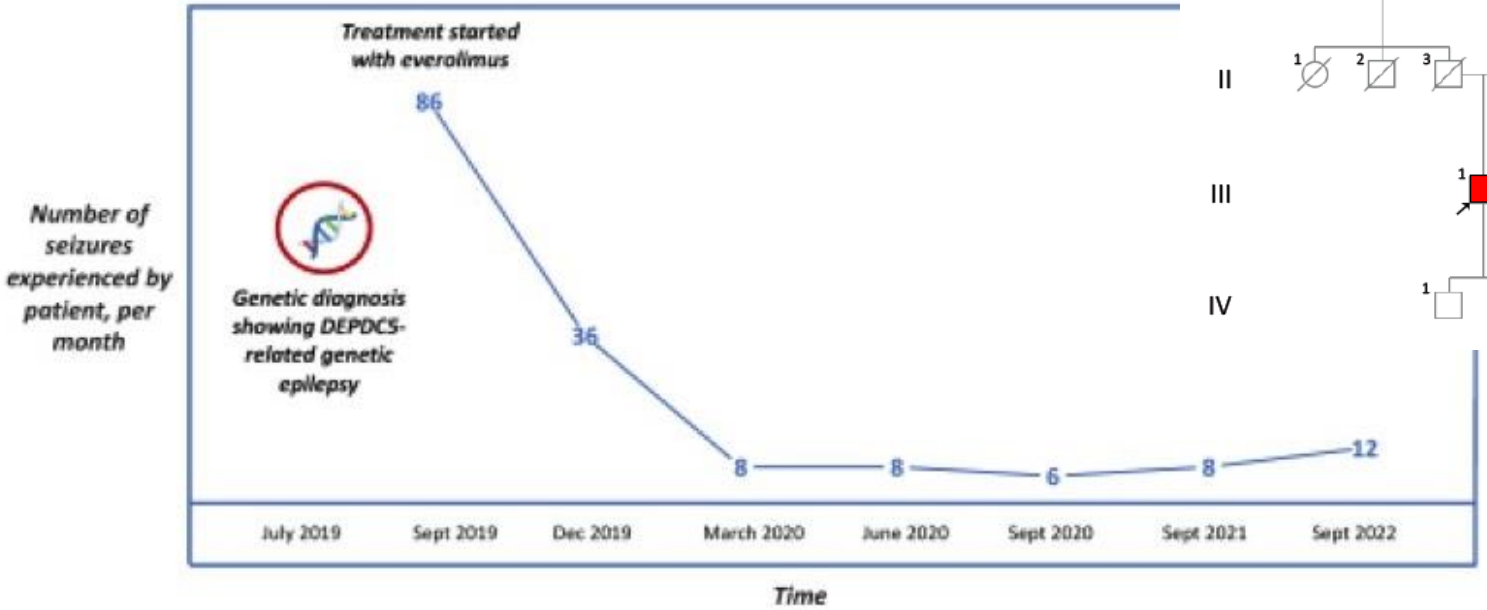
Maloney et al, under revision, *EJN*

Example of treatment response..

Patient 1 (50 yrs old, seizures since teenager)

DEPDC5 c.3436C>T (p.Gln1146*)



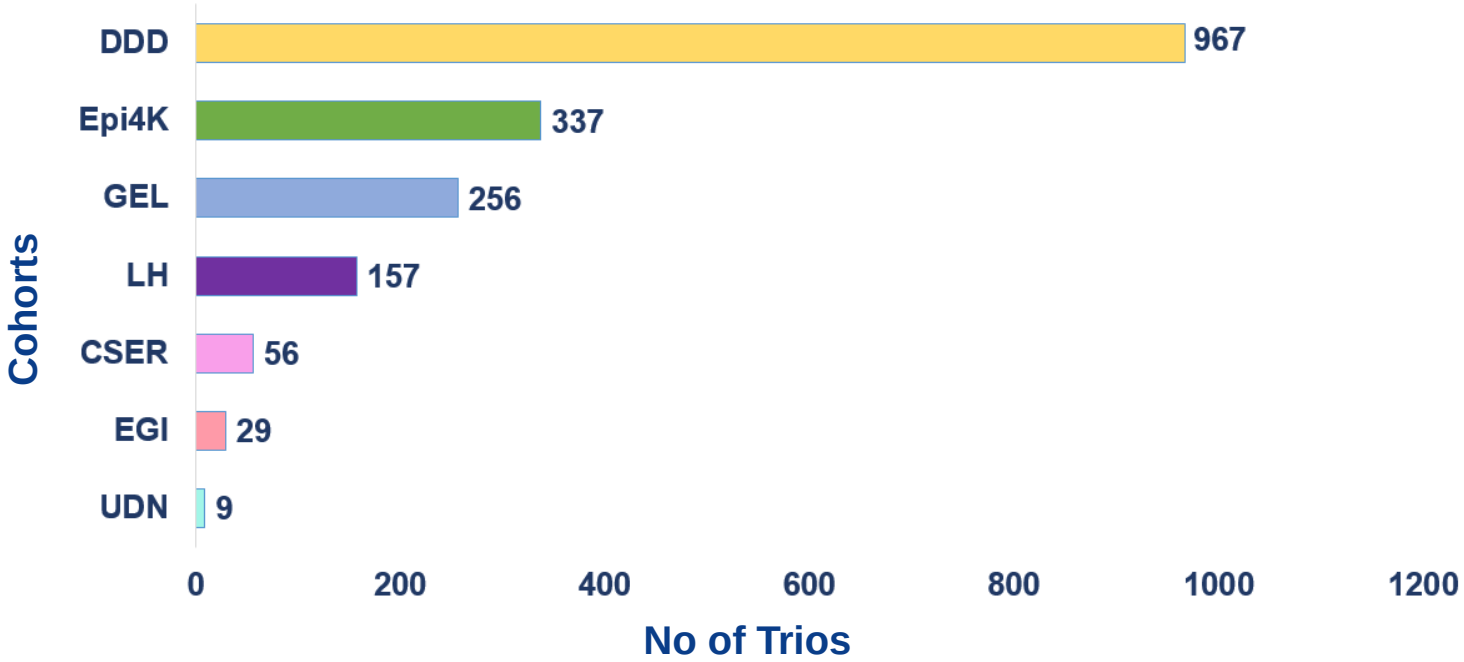


Pharmacogenomics in epilepsy..

Drug	Gene	Allele	Effect	ancestry	Clinical status?
Carbamazepine	HLA-B	*15:02	SJS/TEN	Han/SE Asian	FDA recommended
Phenytoin	HLA-B	*15:02	SJS/TEN	Han/SE Asian	FDA warning
Oxcarbazepine	HLA-B	*15:02	SJS/TEN	Han/SE Asian	FDA warning
Carbamazepine	HLA-A	*3101	SJS/TEN, MPE, and DRESS	European, Japanese, Korean	FDA warning
Carbamazepine	HLA-B	*57:01	SJS/TEN	EU	More data required
Phenytoin	CYP2C9	*3	SJS/TEN/DRESS	Han, Thai, European	(FDA warning)
Aromatic AEDs	IKZF1	rs4917014	SCAR	European	More data required
Carbamazepine	ALK	rs187926838	SCAR	European	More data required
Phenytoin	CFHR	rs78238784	MPE	European	More data required
Levetiracetam	PRS	various	psychosis	European	More data required

Latest (germline) gene discovery efforts

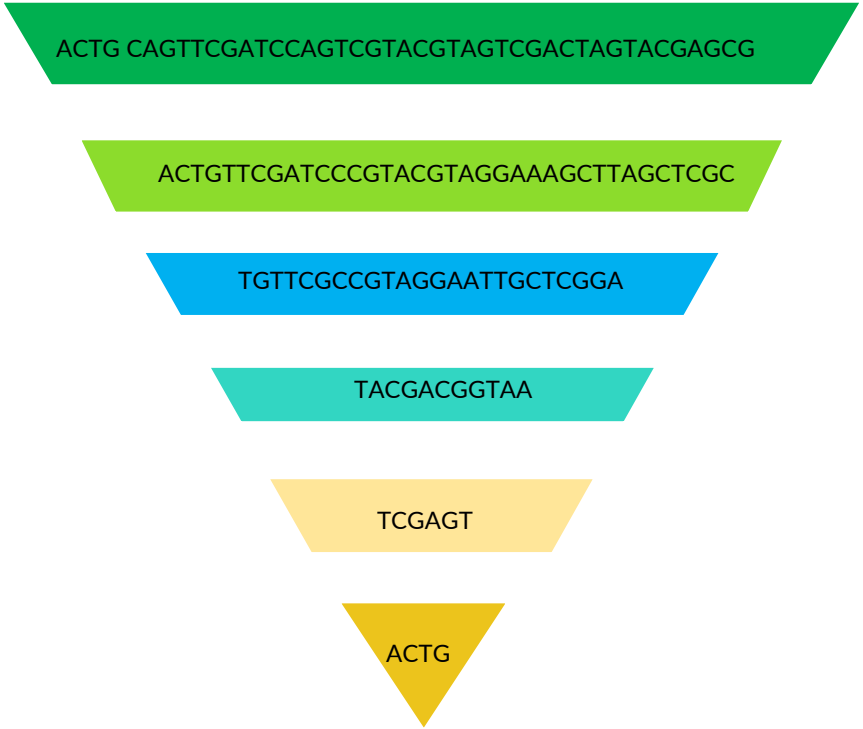
New gene discovery effort for DEE/epilepsy + ID



Congenica
Enabling genomic medicine

DDD=Deciphering Developmental Disorders
Epi4K=Epi4K data
GEL=Genomics England
LH=Lighthouse
CSER=HudsonAlpha Institute for Biotechnology Clinical Sequencing Exploratory Research (CSER)
EGI=Epilepsy Genetics Initiative
UDN=Undiagnosed Diseases Network

De novo variants were processed and filtered



Input variants (bioinformatics pipeline)

Filter 1: Include those that are seen in the child for the first time

Filter 2: Include those that are not seen/ultra rare in "normal" control groups (GnomAD Non-Neuro / Epi25 Browser)

Filter 3: Include predicted to be damaging (LOF + missense) variants by in-silico tool (CADD ≥ 15)

Filter 4: Inspect for true variants using IGV

Final De-novo variants Output

Gene-based burden test of LoF and damaging missense DNVs by DeNovoWEST

1)Initial run using DNVs input:
- Synonymous
- Missense
- Nonsynonymous

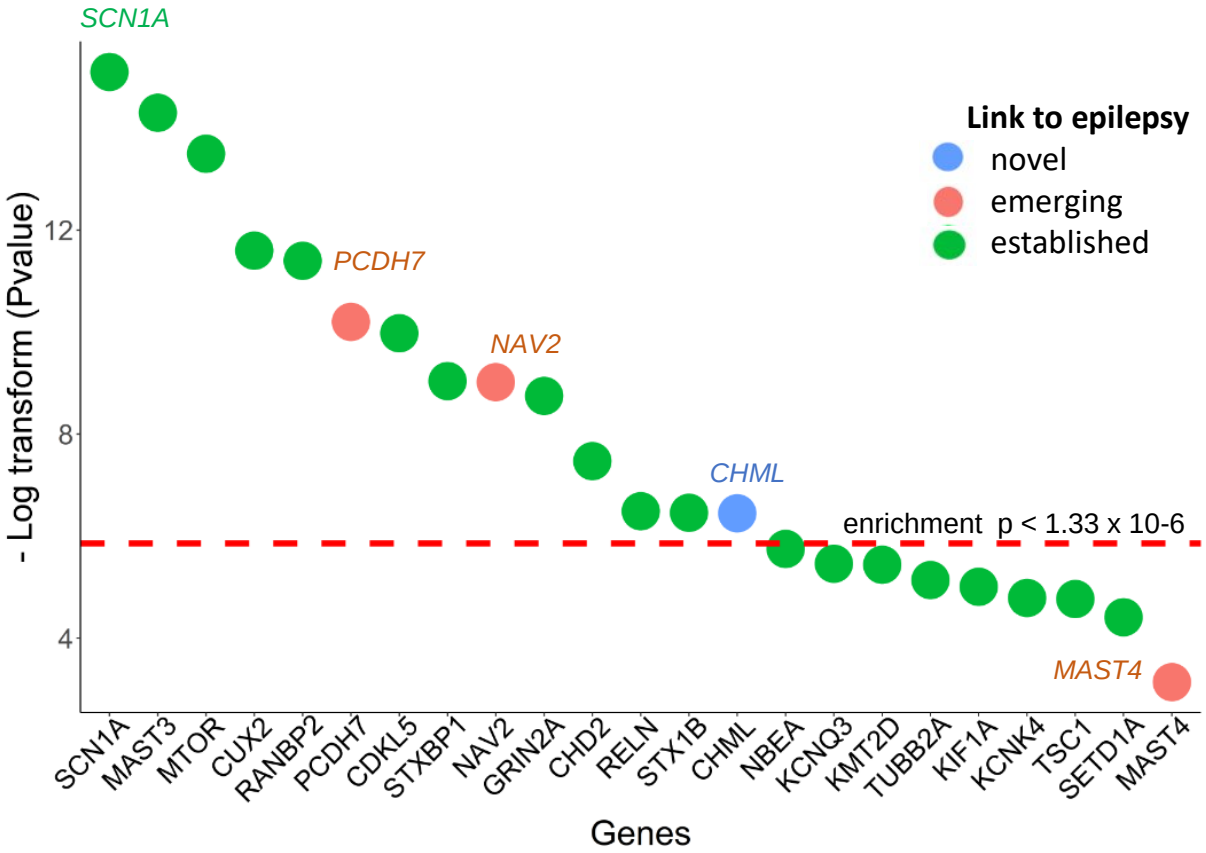
2) Genes nominally enriched by DNVs ($P < 0.001$)

3) Bonferroni-corrected for no of genes (n=18,762*2 tests per gene)
New threshold :
$p < 1.33 \times 10\text{-}6$

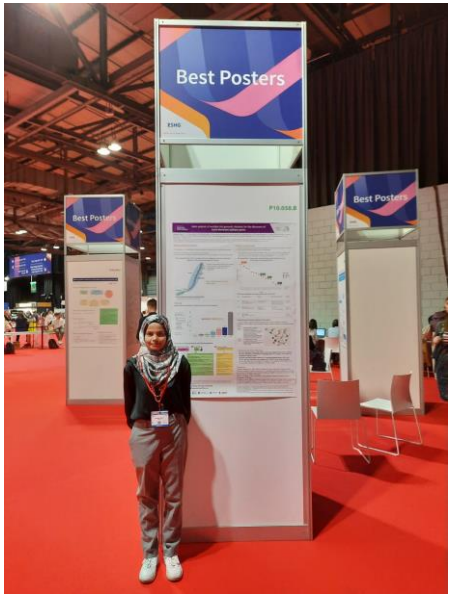
4) Check for the brain expression (GTEx) and supporting evidence

Novel Genes

We identified 14 genes that reached exome-wide significance for enrichment of damaging DNVs



ESHG 2023/Glasgow



GWAS in the epilepsies – 'ILAE3' Study Design

Main Analyses	
Focal	16,384
Generalized	7,407
All Epilepsies	29,944
Controls	52,538

Sub-analyses	
Childhood Absence Epilepsy	1,072
Juvenile Absence Epilepsy	671
Juvenile Myoclonic Epilepsy	1,813
Generalised Tonic-Clonic Seizures	499
Focal Epilepsy, Lesion Negative	6,367
Focal Epilepsy, Hippocampal Sclerosis	1,375
Focal Epilepsy, Other Lesion	4,661

ILAE Consortium on Complex Epilepsies

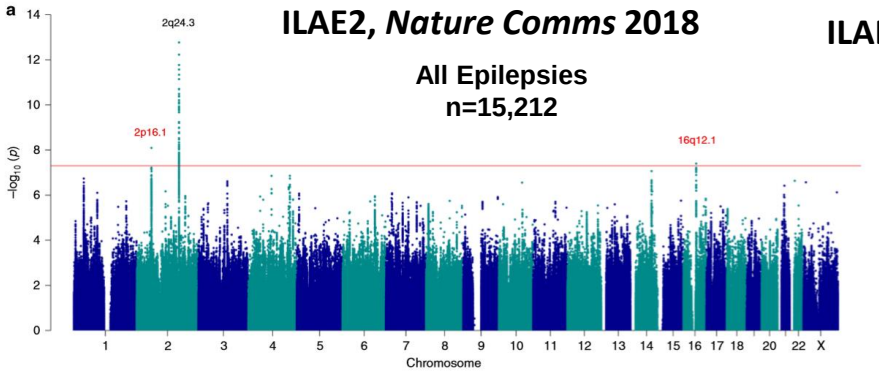
HPC @ Uni.lu
High Performance Computing in Luxembourg

Uni Lux

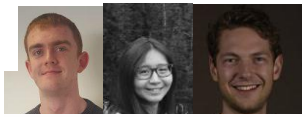
Epi25

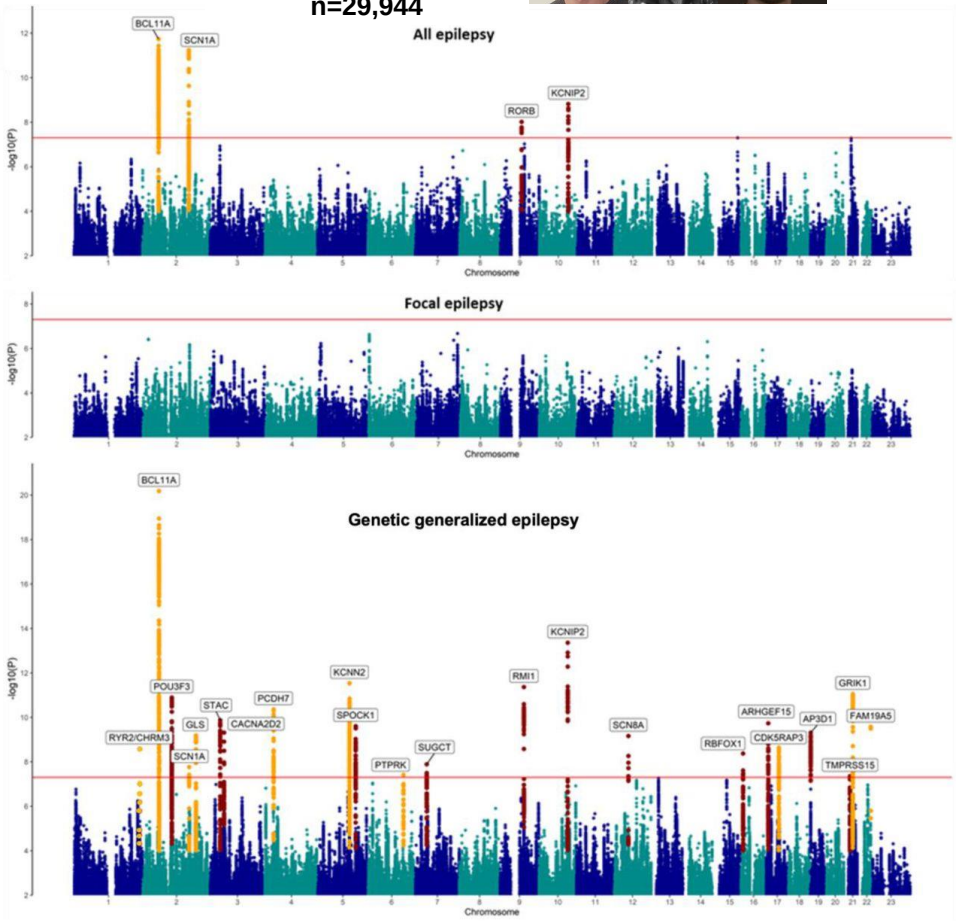
Imputation: Sanger Imputation Server, Haplotype Reference Consortium (release 1.1), 4.9m SNPs
Association: GLM (SAIGE)

ILAE2, *Nature Comms* 2018

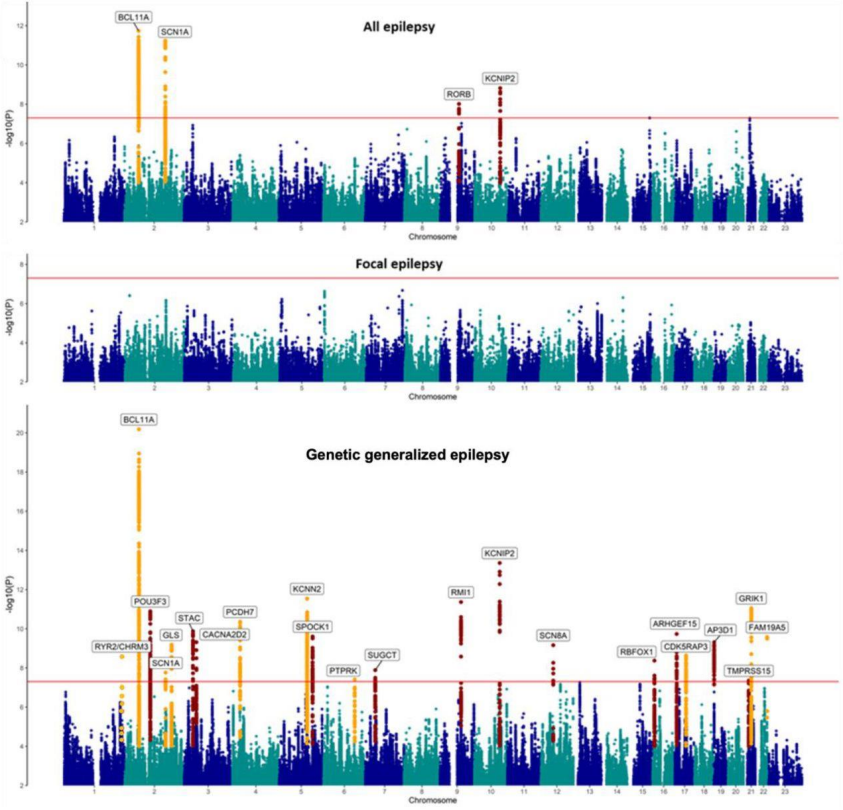


ILAE3, *Nat Gen.* 2023 (under revision)





Genome-wide meta-analysis of over 29,000 people with epilepsy …

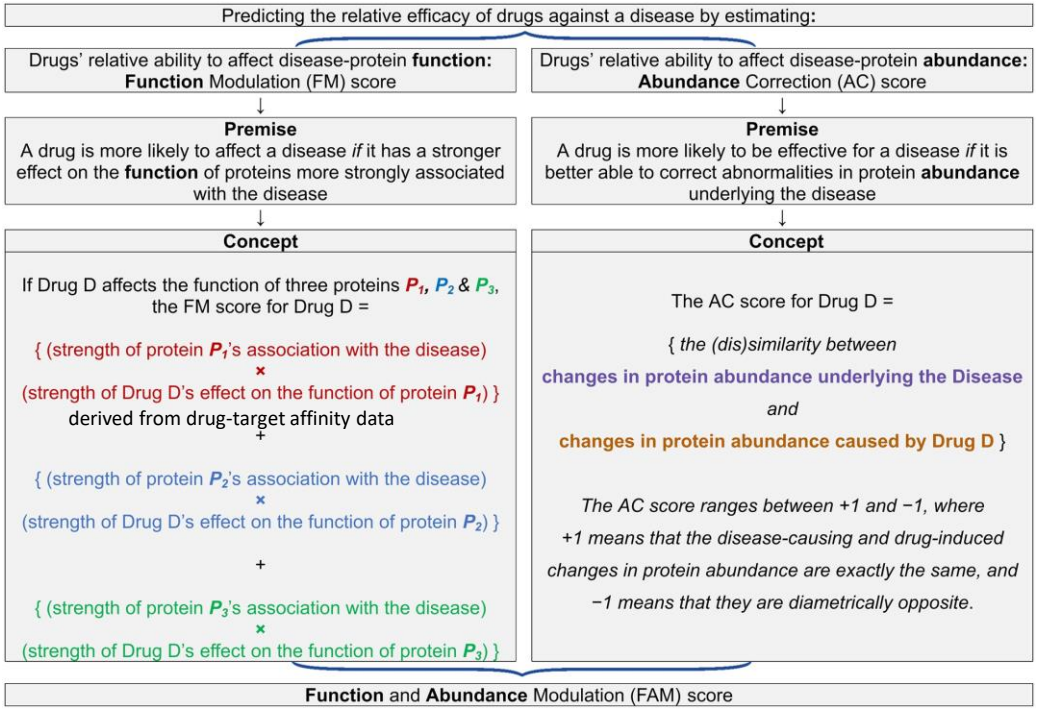


Phenotype	Locus	Novel / Replication	Lead SNP (A1:A2)	Freq1	Z-score	P-value	Gene	Total	Missense	TWAS	SMR	MAGMA	PoPS	Brain exp	brain-coX	KO mouse	AED target	Monogenic
All epilepsy	2p16.1	Replication	rs13032423 (A:G)	0.53	-7.04	1.85E-12	*BCL11A*	5										
	2q24.3	Replication	rs59237858 (T:C)	0.23	-6.89	5.746E-12	*SCN1A*	8										
	9q21.13	Novel	rs4744696 (A:G)	0.82	-5.74	9.694E-09	*RORB*	4										
	10q24.32	Novel	rs3740422 (C:G)	0.33	6.04	1.517E-09	*KCNIP2*	3										
GGE	1q43	Replication	rs876793 (T:C)	0.67	-5.95	2.644E-09	*RYR2*	4										
							CHRM3	4										
	2p16.1	Replication	rs11688767 (A:T)	0.53	9.38	6.58E-21	*BCL11A*	5										
	2q12.1	Novel	rs62151809 (T:C)	0.43	6.77	1.277E-11	*POU3F3*	3										
	2q24.3	Replication	rs11890028 (T:G)	0.72	5.63	1.728E-08	*SCN1A*	8										
	2q32.2	Replication	rs6721964 (A:G)	0.66	-6.18	6.542E-10	*GLS*	4										
	3p22.3	Novel	rs9861238 (A:G)	0.41	-6.42	1.333E-10	*STAC*	2										
	3p21.31	Novel	rs739431 (A:G)	0.84	6.23	4.822E-10	*CACNA2D2*	6										
	4p15.1	Replication	rs1463849 (A:G)	0.59	-6.59	4.377E-11	*PCDH7*	3										
	5q22.3	Replication	rs4596374 (T:C)	0.55	-6.98	2.906E-12	*KCNN2*	6										
	5q31.2	Novel	rs2905552 (C:G)	0.48	-6.33	2.492E-10	*SPOCK1*	5										
	6q22.33	Replication	rs13219424 (T:C)	0.29	-5.49	3.872E-08	*PTPRK*	3										
	7p14.1	Novel	rs37276 (T:G)	0.26	-5.69	1.288E-08	*SUGCT*	2										
	9q21.32	Novel	rs2780103 (T:C)	0.26	-6.93	4.342E-12	*RMI1*	5										
	10q24.32	Novel	rs11191156 (A:G)	0.67	-7.55	4.409E-14	*KCNIP2*	4										
	12q13.13	Novel	rs4762030 (T:G)	0.02	6.17	6.90E-10	*SCN8A*	6										
	16p13.3	Novel	rs62014006 (T:G)	0.47	5.88	4.223E-09	*RBFOX1*	5										
	17p13.1	Novel	rs2585398 (A:C)	0.53	-6.37	1.842E-10	*ARHGEF15*	6										
	17q21.32	Replication	rs16955463 (T:G)	0.25	-5.97	2.3E-09	*CDK5RAP3*	4										
	19p13.3	Novel	rs75483641 (T:C)	0.14	-6.22	4.852E-10	*AP3D1*	5										
	21q21.1	Novel	rs1487946 (A:G)	0.59	5.47	4.409E-08	*TMPRSS15*	1										
	21q22.1	Replication	rs7277479 (A:G)	0.36	-6.82	8.935E-12	*GRIK1*	4										
	22q13.32	Novel	rs469999 (A:G)	0.31	-6.32	2.647E-10	*FAM19A5*	2										
CAE	2p16.1	Replication	rs12185644 (A:C)	0.70	-7.12	1.04E-12	*BCL11A*	5										
JME	4p12	Replication	rs17537141 (T:C)	0.85	-5.47	4.62E-08	*GABRA2*	6										
	8q23.1	Novel	rs3019359 (T:C)	0.41	-5.55	2.89E-08	*RSPO2*	3										
							TMEM74	3										
	16p11.2	Replication	rs1046276 (T:C)	0.35	6.19	6.05E-10	*STX1B*	5										
							CACNA1I	5										

Nature Genetics, in revision – manuscript on MedBioArchives

future neuro

Drug repurposing..



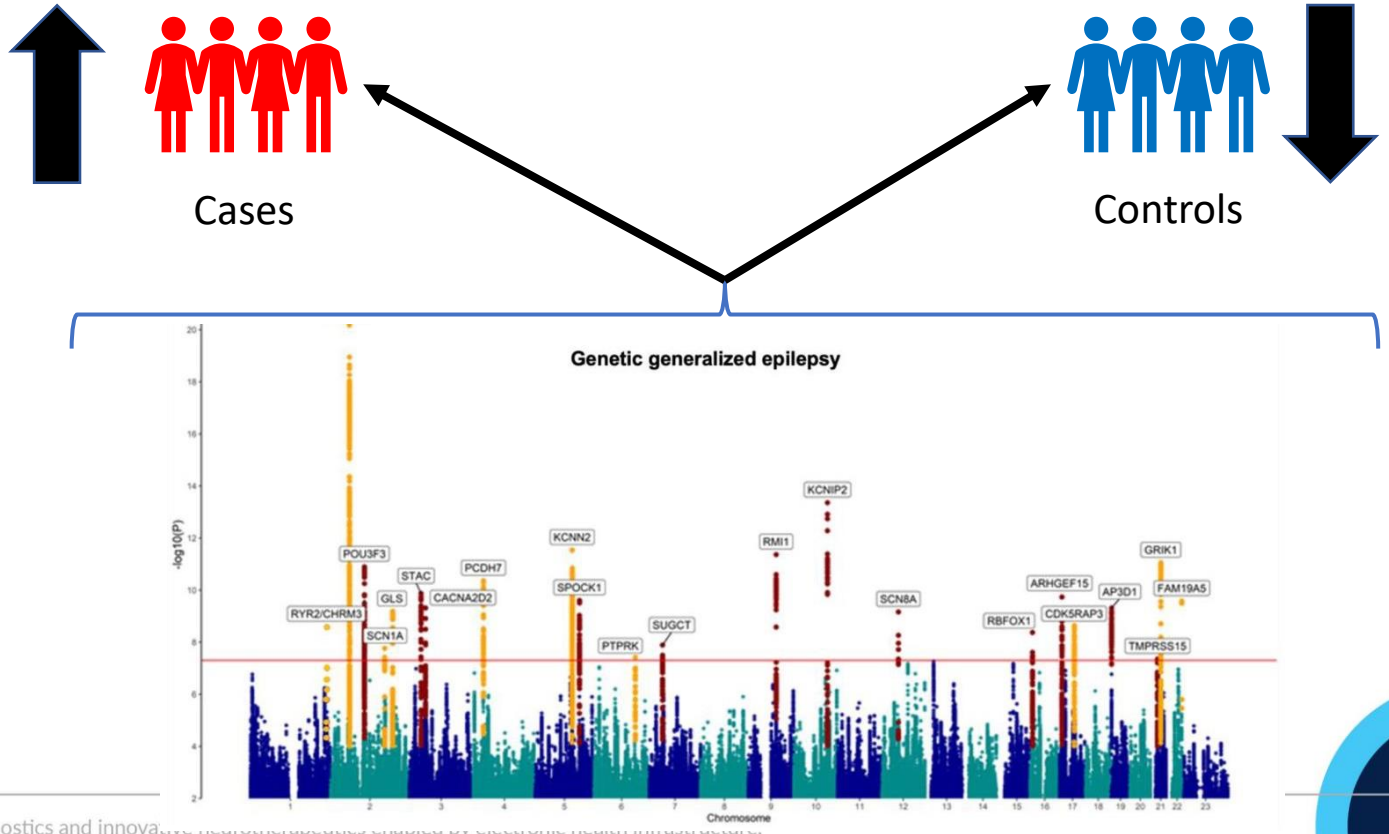
Method: Mirza et al, *Brain Communications* 2021

current ASMs ranked higher than expected by chance ($p < 1\times10^{-6}$)

For GGE, broad-spectrum ASMs more effective than narrow-spectrum antiseizure drugs ($p < 1\times10^{-6}$),

Application of PRS to the epilepsies

Polygenic risk scores (PRS) and the epilepsies



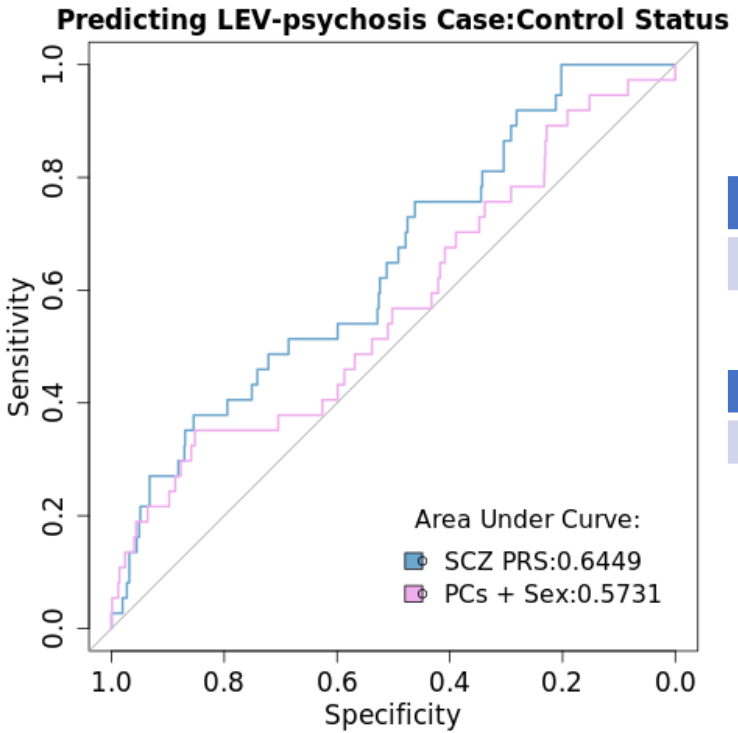
PRS as a predictor of LEV-induced psychosis

- **Hypothesis**: do people experiencing LEV-psychosis have higher PRS for schizophrenia than LEV tolerant individuals?
- **Cases**: LEV-treated, psychosis as a side-effect (n=37)
  - ADR within 6 months of commencing drug treatment
  - Led to a dose reduction or withdrawal of drug treatment
  - Symptoms stop after dose reduction / withdrawal
  - Not attributable to another cause (e.g. underlying psychiatric illness)
- **Controls**: LEV-treated, no side-effects (n=902)

PRS alleles for SCZ selected from: Ripke et al *Nature* 2014

Campbell et al, *Epilepsia*, 2022

LEV PRS



Cases	Controls
37	920

PRS	pT	Estimate	SE	OR	95%CI	P	R2
SCZ	0.5	-0.4866	0.1881	13.2	9.2000<->19.159	0.0059	0.04

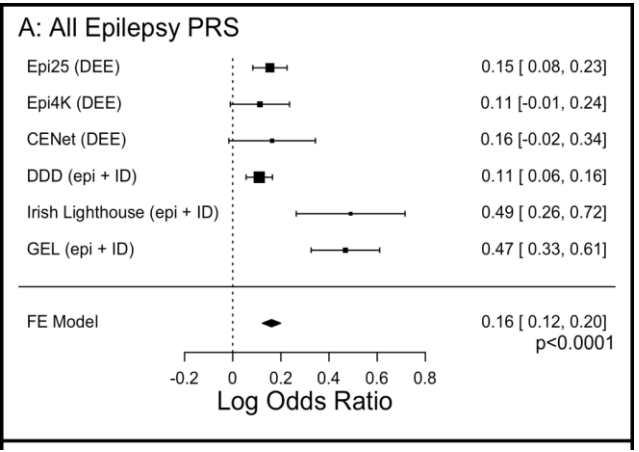
Campbell et al, *Epilepsia*, 2022

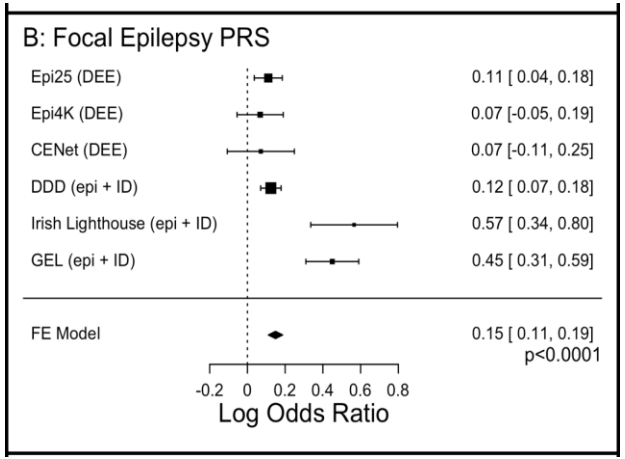
Combining common & rare: Do cases of epileptic encephalopathy carry a genomic burden for epilepsy?

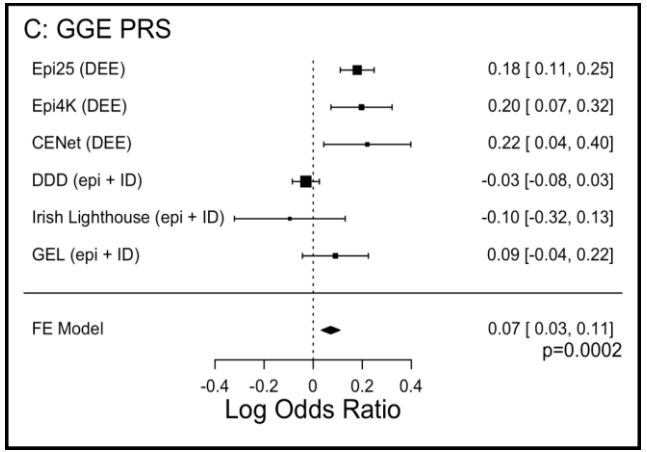
Cohort	Epilepsy	Screen-positive	Screen-negative	Controls	Phenotype	Data types
Epi25	1,094	163	931	210	DEE	Microarray + exome
Partner's Biobank	0	0	0	19,762	Controls only	Microarray
Epi4K	266	44	77	0	DEEs	Microarray + exome
QSkin	0	0	0	15,717	Controls only	Microarray
CENet	171	40	86	0	DEE	WGS + microarray
Canadian Controls	0	0	0	6,901	Controls only	Microarray
DDD	897	152	745	0	Seizures + ID	Microarray + exome
UK Biobank	0	0	0	400,835	Controls only	Microarray
Irish Lighthouse	82	29	53	0	Epilepsy + ID	Exome (trios) + microarray (probands)
Irish Controls	0	0	0	2,404	Controls only	Microarray
Genomics England	249	32	217	1,931	Epilepsy + ID and controls	WGS
Total	2,759	460	2,109	447,760		

Cambell et al, *ebiomedicine*, 2022

Epilepsy-related PRS in the epileptic encephalopathies

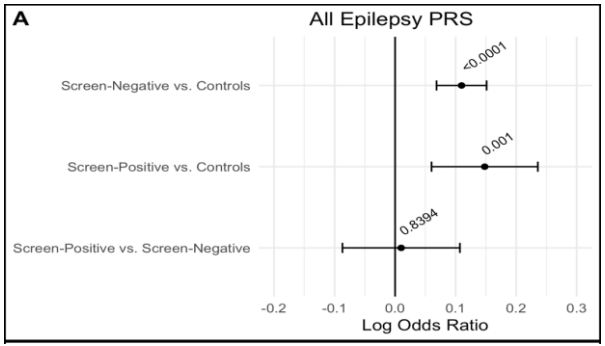


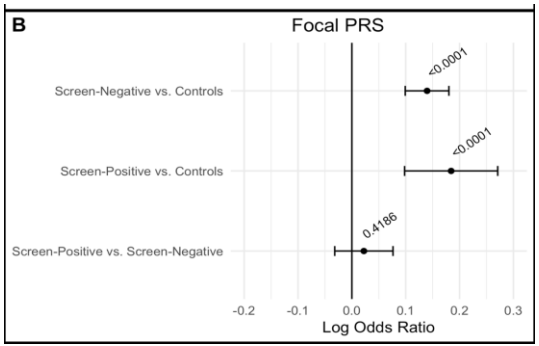


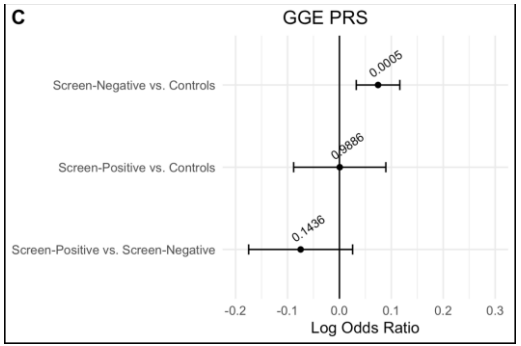


Cambell et al, *ebiomedicine*, 2022

If we stratify by screen +ive vs –ive..







Cambell et al, *ebiomedicine*, 2022

Conclusions

- **Pathogenic variants in hundreds of genes can cause monogenic epilepsies**
  - **Yield depends on epilepsy type (DEE = 30-40%)**
  - **Some diagnosis will have treatment implications..**
- **Rare variant and GWAS studies are delineating genetic architecture & pointing to drug targets**
  - **There is genetic continuity between common and rare forms of epilepsy.**
  - **“Monogenic” forms appear to have a polygenic component..**

Acknowledgements…

















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Marie Sklodowska-Curie COFUND: Pre-notification of call

NeuroInsight - Advanced Skills for Data Analytics in Neurological Diseases

Call 3 for proposals opens 1st August 2023 and will close on 31 October 2023

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