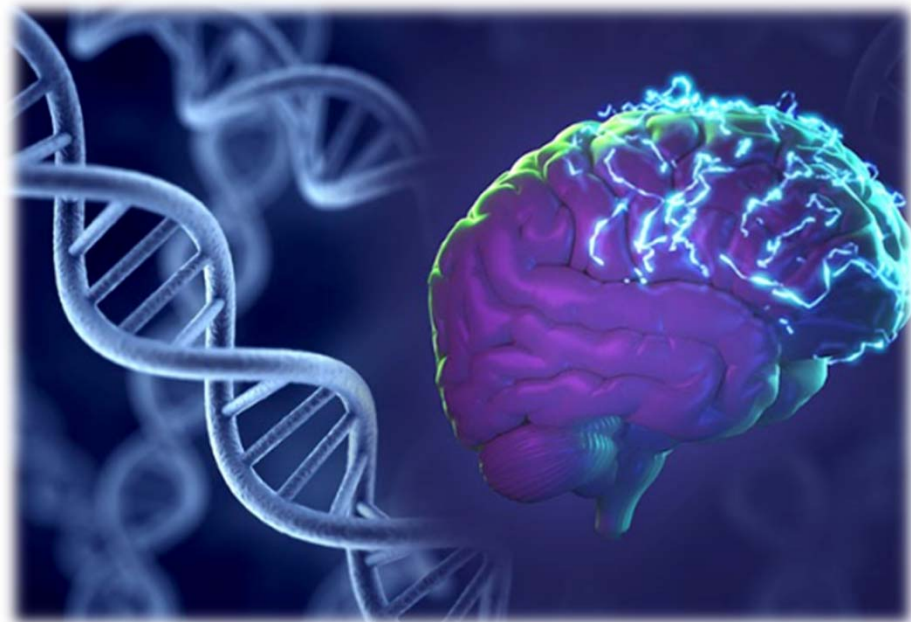


Selecting the Correct Genetic Test and Interpretation in Epilepsy



Mo, Sep 21st, 2020

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Why is genetic testing useful in caring for epilepsy patients?

- Understanding the genetic basis of the condition might help give information about the **progress** of the condition, possible **preventive actions** or **treatment**.
- Patients with a genetic condition may just find it helpful to know why their signs and symptoms occur.
 - Many Gene-Disorder Family Organizations have been formed **raising funds for research** and **provide support** for new patients and families.

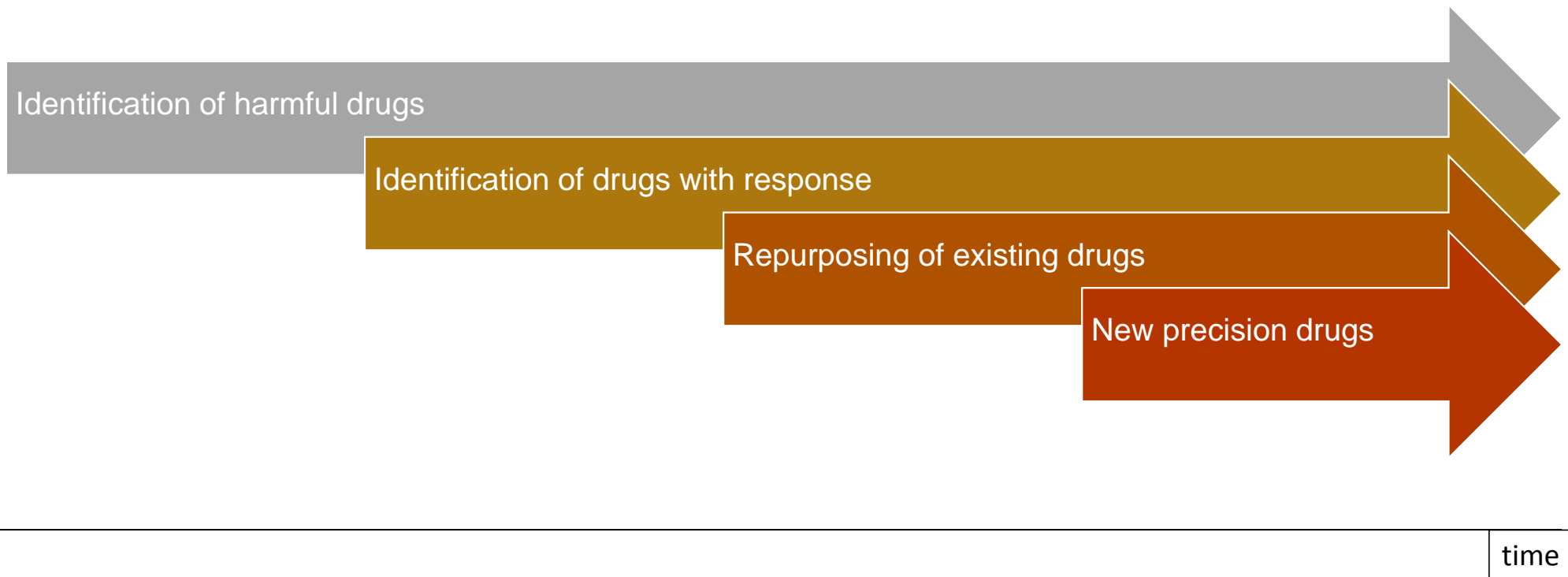
Examples of precision medicine in epilepsy

Table1: Precision medicine examples of frequently mutated epilepsy genes. AED = Anti-Epileptic Drug, GOF = Gain-of-function variant, LOF = Loss-of-function variant, data selected from recent reviews⁹⁻¹¹.

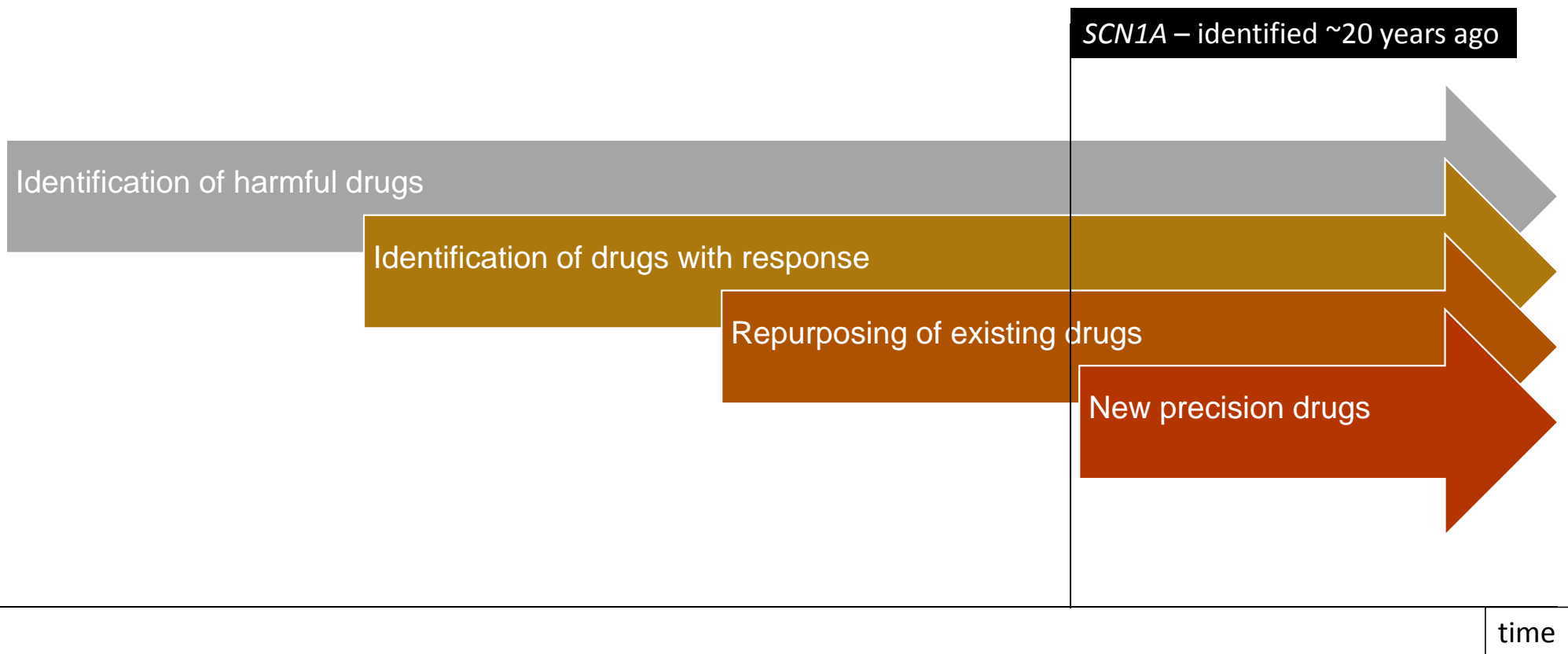
Gene	AEDs/treatments recommended	AED to avoid	Other management Implications	Rate of precision medicine responders
SLC2A1	Ketogenic Diet	-	-	Most respond
ALDH7A1 and PNPO	Pyridoxine	-	Lysine-restricted diet	A minority responds
SCN1A	Stiripentol, Valproate, Clobazam, Ketogenic Diet, Cannabidiol, Fenfluramine	Phenytoin/Carbamazepine /Lamotrigine/Oxcarbazepine		Minority responds to the current treatment regime, but clinical trials with Fenfluramine look promising
SCN2A	SCN2A-GOF: Carbamazepine, phenytoin, Oxcarbazepine	SCN2A-LOF: Carbamazepine, phenytoin, Oxcarbazepine	Consider high-dose IV phenytoin for status epilepticus	Variable response rates for SCN2A-GOF
SCN8A	Carbamazepine, phenytoin, Oxcarbazepine	-	-	Minority respond. It depends if mutations lead to gain- or loss-of-function
KCNQ2	Carbamazepine, phenytoin, Retigabine	-	-	Minority responds. It depends if mutations lead to gain- or loss-of-function
PCDH19	Clobazam, steroids	-	-	Minority responds
PRRT2	Carbamazepine	-	-	Majority responds
KCNT1	Trial of Quinidine in early-onset seizures, Potassium bromides, Ketogenic Diet, Vigabatrin	-	-	Minority responds

Scheffer et al., 2020 *Eur J Paediatr Neurol.*
 Musto E et al., 2019 *Eur J Paediatr Neurol.*
 Symonds JD, McTague A. 2020 *Eur J Paediatr Neurol.*

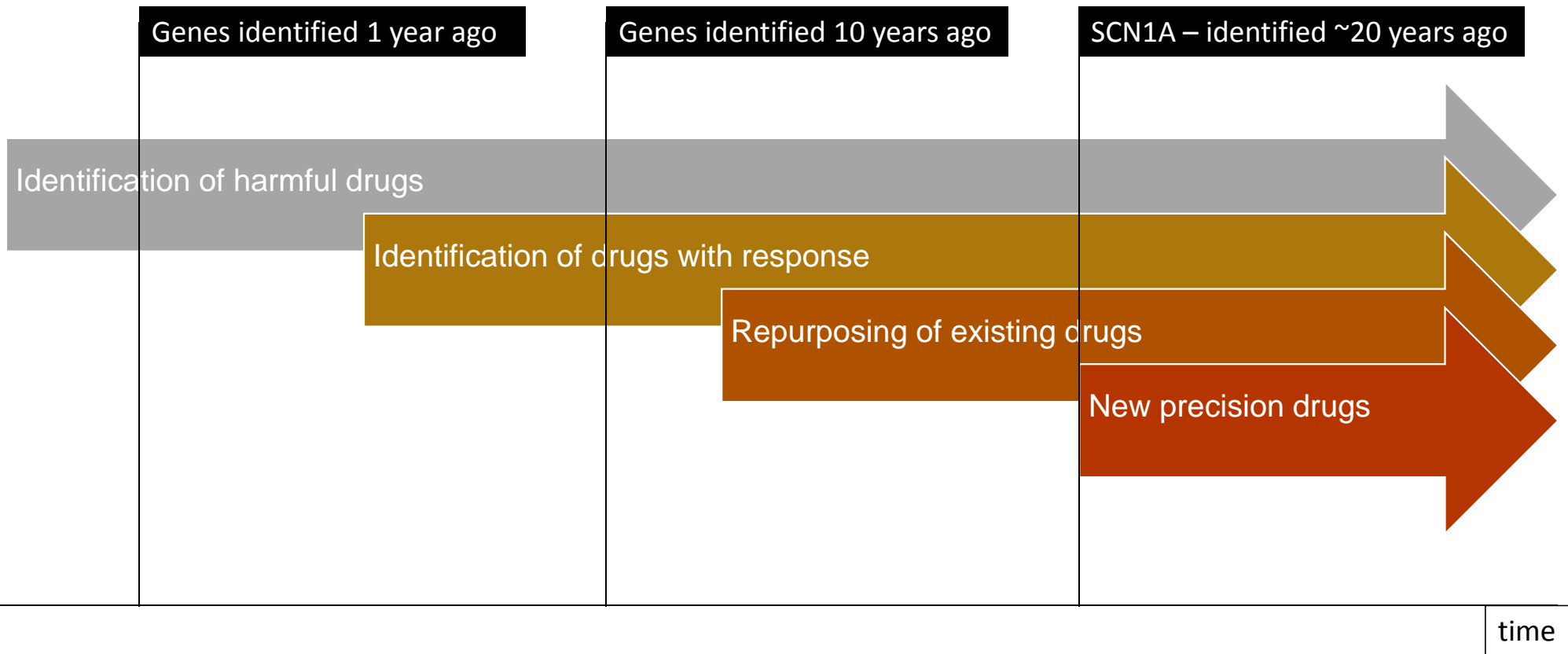
Precision medicine in epilepsy – where are we?



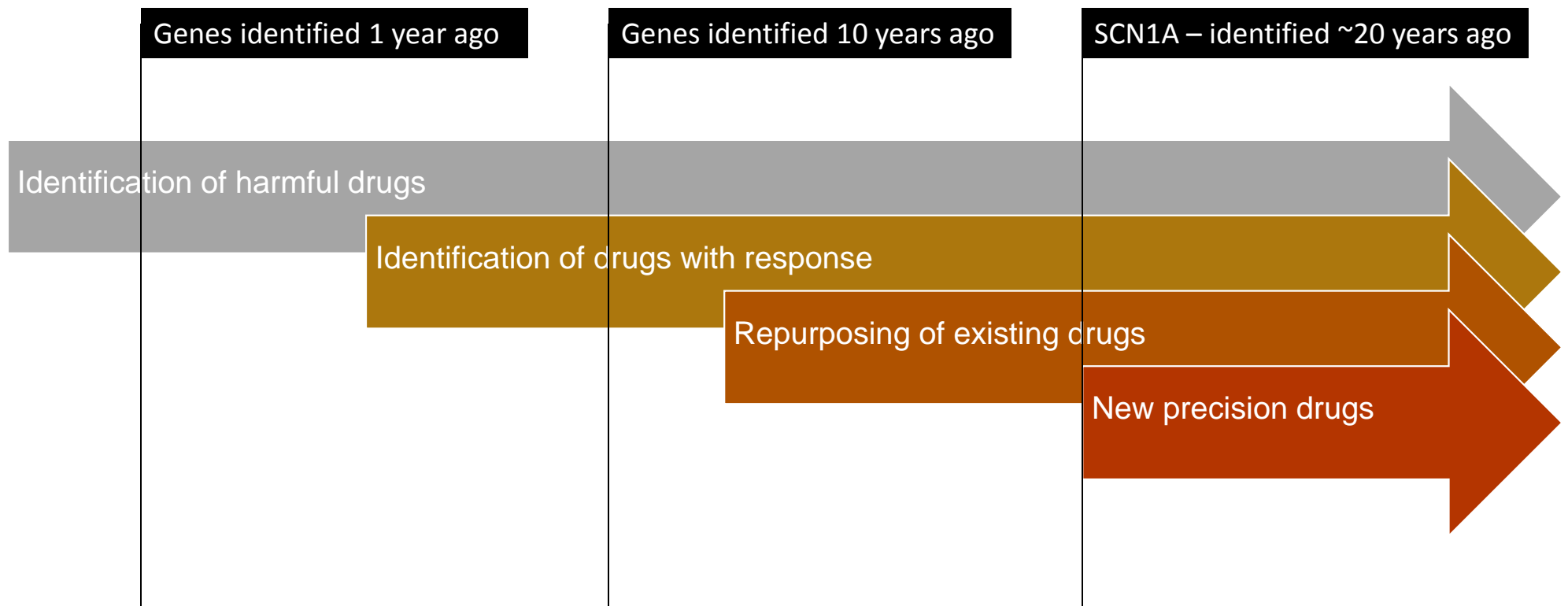
Precision medicine in epilepsy – where are we?



Precision medicine in epilepsy – where are we?



Precision medicine in epilepsy – where are we?



For complex genetic etiologies such as large copy number variants or somatic epilepsies progress is slower

time

What % of epilepsy patients are “genetic”?

We don't know.

A population based comprehensive genetic screen across all age ranges and tissue types has not been performed.

What % of epilepsy patients are “genetic”?

We don't know.

A population based comprehensive genetic screen across all age ranges and tissue types has not been performed.

Most epilepsy genetic cohort screening studies focus on epilepsy subtypes, ascertained through tertiary care centers & perform targeted testing of rare variants

What % of epilepsy patients are “genetic”?

We tried to answer this question based on (incomplete) data available



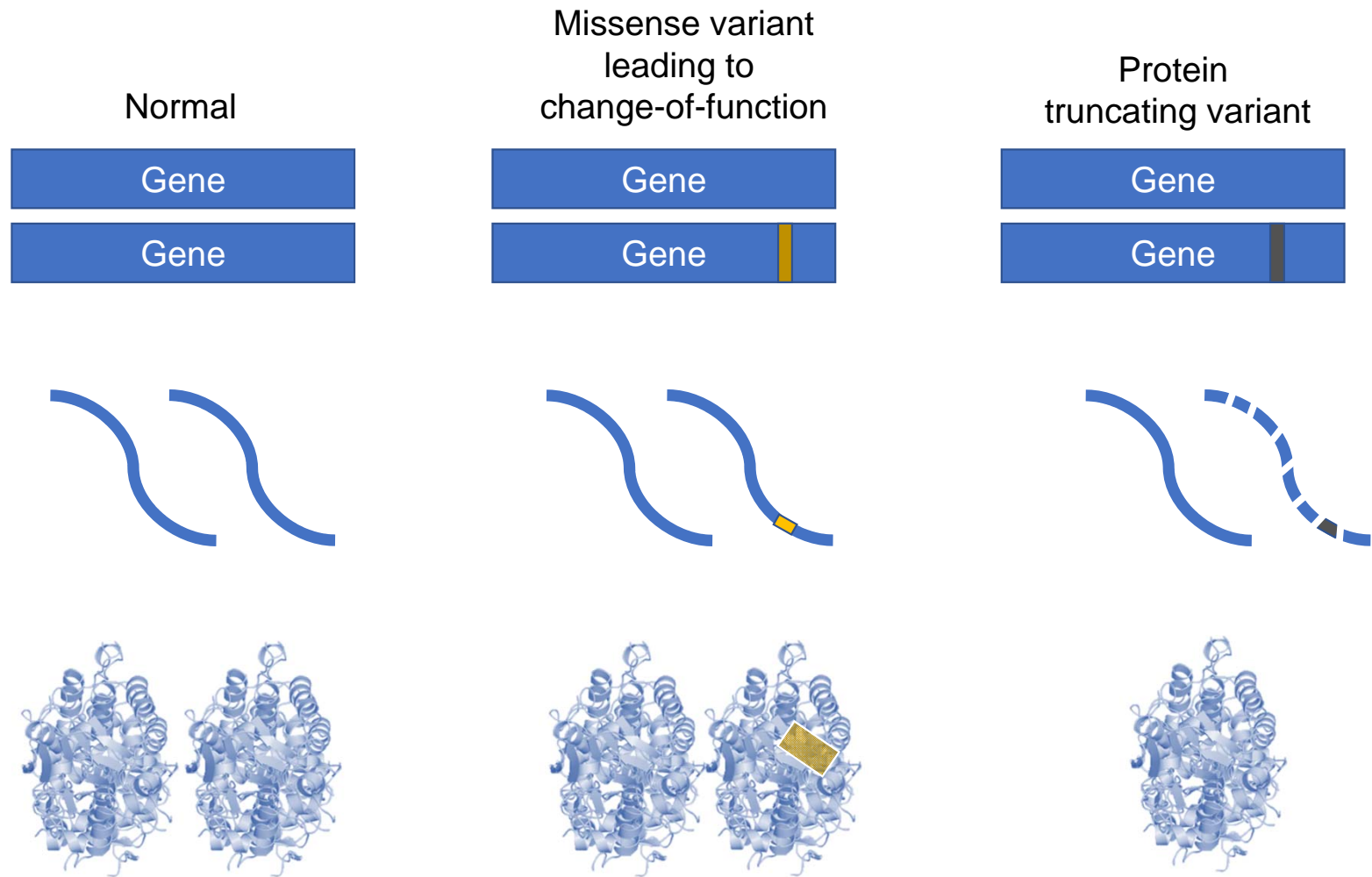
The screenshot shows the top portion of a medRxiv preprint page. On the left is the medRxiv logo with the tagline 'THE PREPRINT SERVER FOR HEALTH SCIENCES'. In the center are logos for CSH (Cold Spring Harbor Laboratory), BMJ, and Yale. On the right, there are navigation links for 'HOME' and 'ABC', and a search bar with the text 'Search'. Below the logos, there is a blue link that says 'Comment on this paper'. The main title of the preprint is 'Clinical sequencing yield in epilepsy, autism spectrum disorder, and intellectual disability: A systematic review and meta-analysis'. Below the title, the authors are listed as 'Arthur Stefanski, Yamile Calle Lopez, Costin Leu, Eduardo Perez Palma, Elia Pestana-Knight, Dennis Lal'. At the bottom of the preprint information, the DOI is provided as 'https://doi.org/10.1101/2020.05.04.20089896'.



Arthur Stefanski

Stefanski et al., 2020 in review

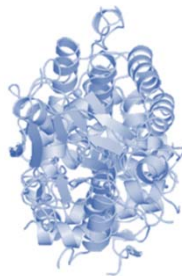
Most common clinically screened types of pathogenic genetic variants



Most common clinically screened types of pathogenic genetic variants

Full gene deletion

Gene

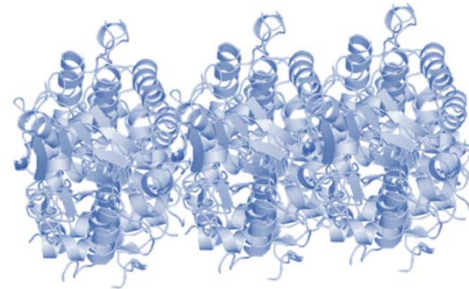
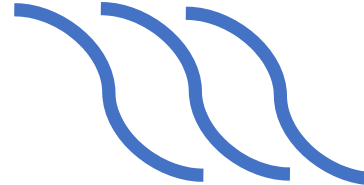


Full gene duplication

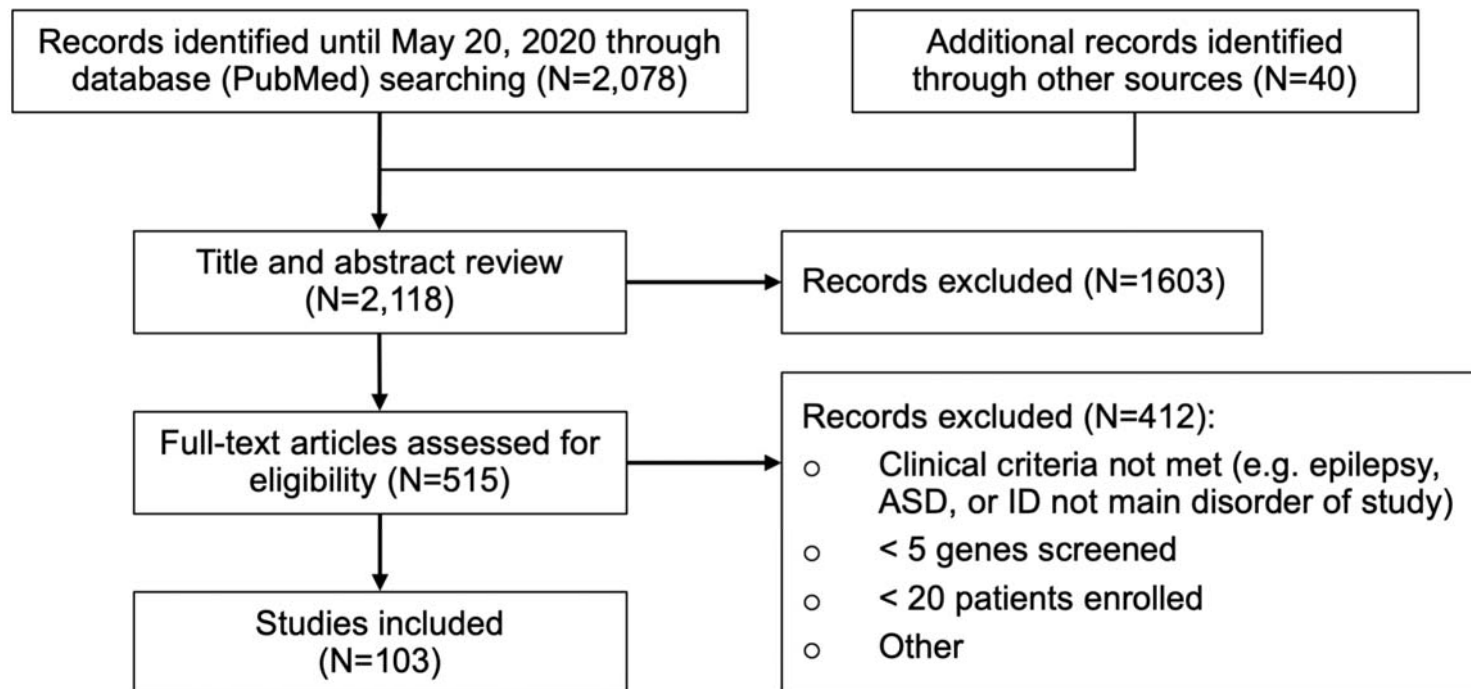
Gene

Gene

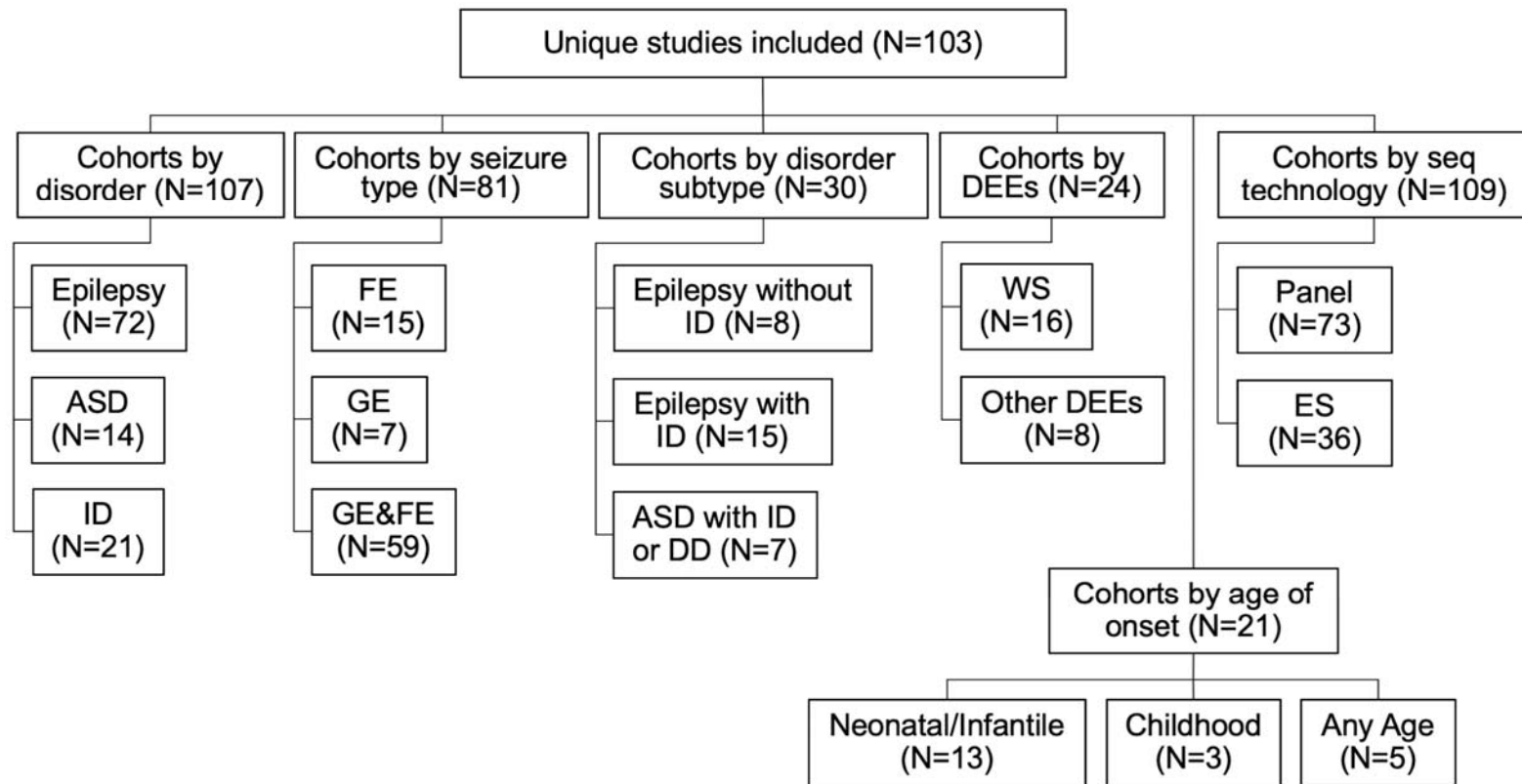
Gene



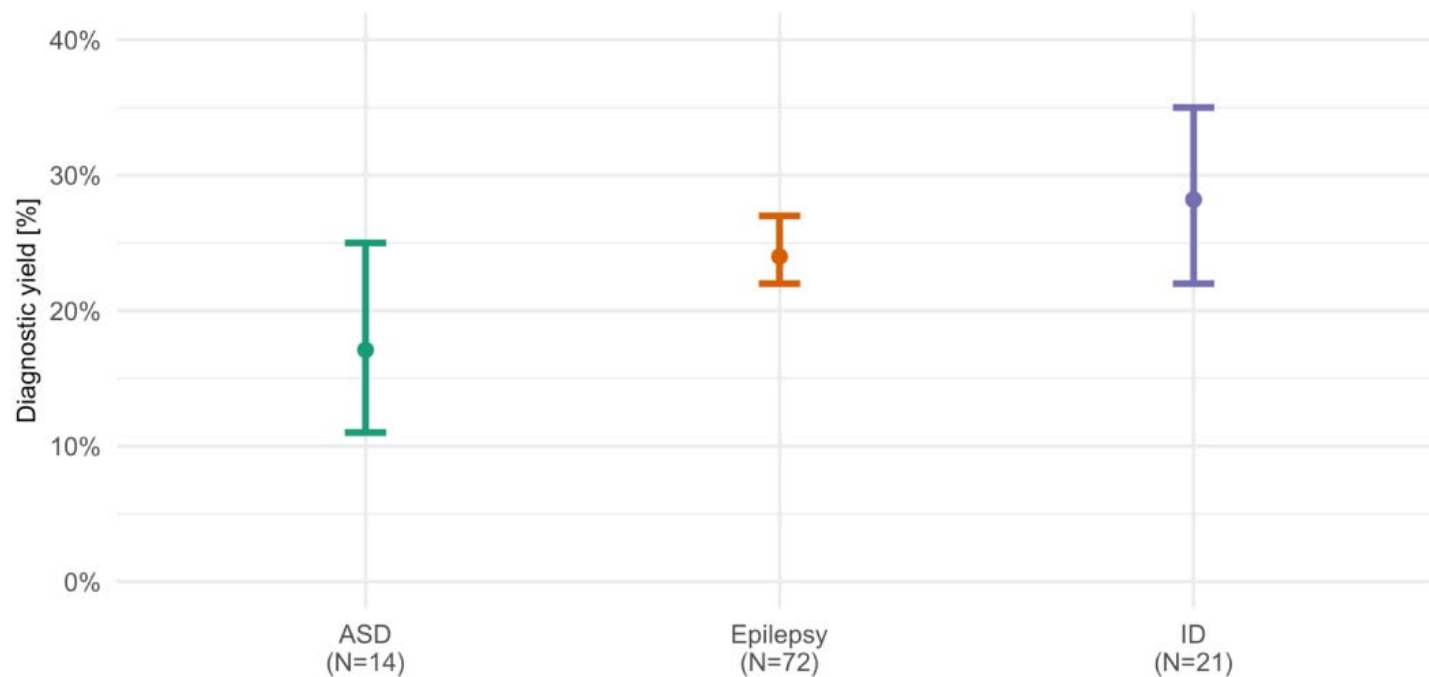
We identified 103 studies, including 72 epilepsy specific ones, to estimate the % of genetic test positive people with a neurodevelopmental disorder



The 103 studies were categorized for additional downstream analyses

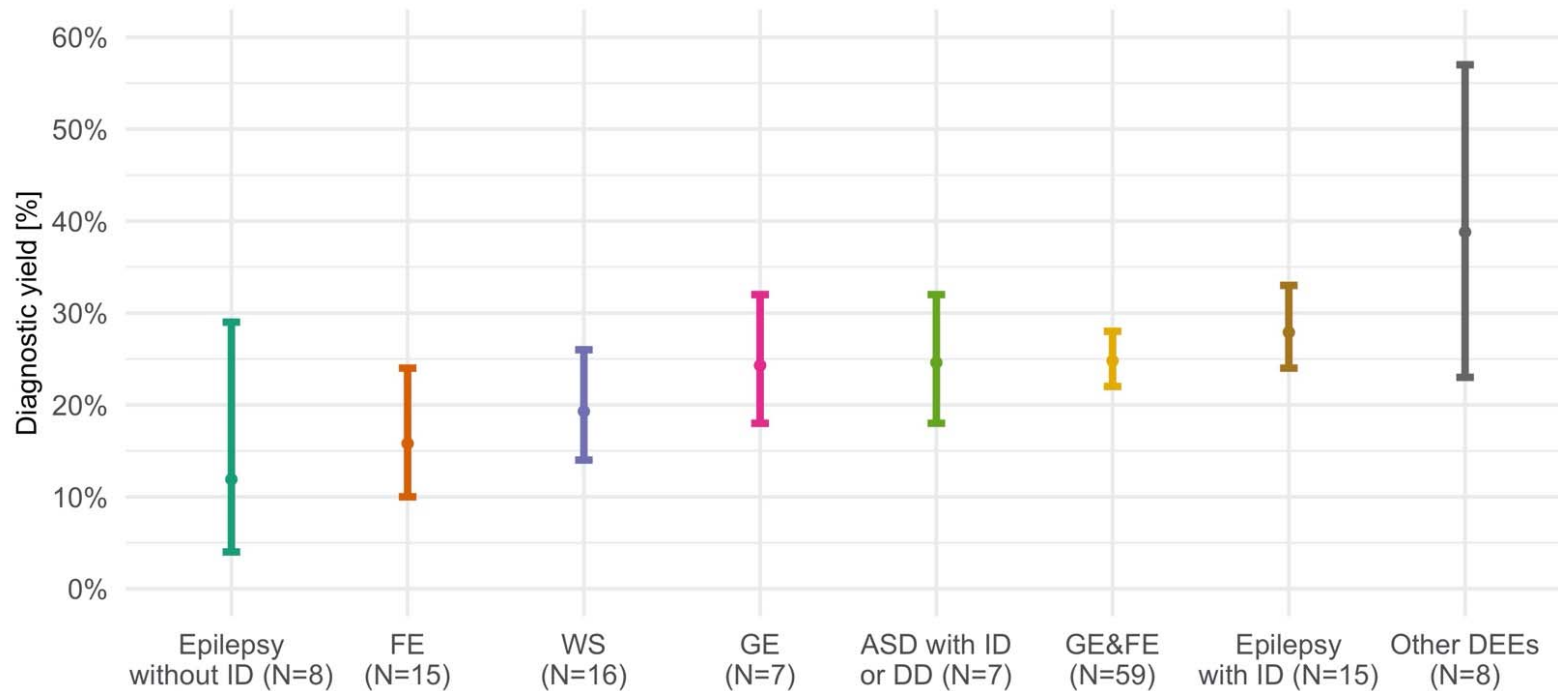


In the disorder analysis about 1 in 3-6 people with a neurodevelopmental disorder are genetic test positive



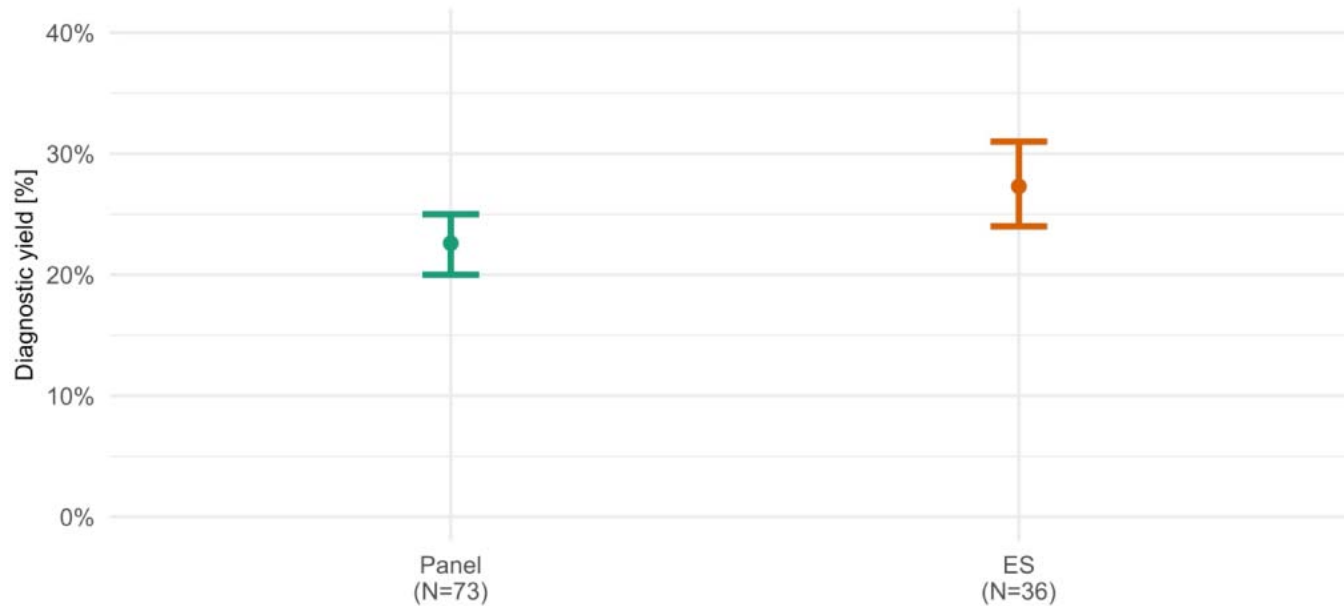
Abbreviations: ASD = autism spectrum disorder, ID = intellectual disability.

Epilepsy patients with cognitive problems have greater genetic burden



Abbreviations: GE&FE = combined generalized and focal epilepsy, FE = focal epilepsy, GE = generalized epilepsy, WS = West syndrome, ASD with ID or DD = autism spectrum disorder with intellectual disability or developmental delay.

Diagnostic yield is higher in Whole Exome Sequencing vs. gene panel sequencing studies



The diagnostic yield across Panel and ES.

Abbreviations: Panel = targeted gene panel sequencing, ES = exome sequencing.

Diagnostic yield in early onset epilepsies is greater compared to later onset epilepsies

Epilepsy Studies by Age of Onset (N=21) Cases Total Proportion 95% CI

Neonatal/Infantile (N=13)

Study	Cases	Total	Proportion	95% CI
Shellhaas et al., 2017	17	26	0.65	[0.44; 0.83]
Gokben et al., 2016	12	30	0.40	[0.23; 0.59]
Rochtus et al., 2020	50	125	0.40	[0.31; 0.49]
Rim et al., 2018	28	74	0.38	[0.27; 0.50]
Zhou et al., 2018	24	70	0.34	[0.23; 0.47]
Zhang et al., 2017	55	174	0.32	[0.25; 0.39]
Wirrell et al., 2015	11	38	0.29	[0.15; 0.46]
Krey et al., 2019	13	45	0.29	[0.16; 0.44]
Berg et al., 2017	42	147	0.29	[0.21; 0.37]
Kodera et al., 2013	11	53	0.21	[0.11; 0.34]
Arafat et al., 2017	13	68	0.19	[0.11; 0.30]
Michaud et al., 2014	7	44	0.16	[0.07; 0.30]
Muir et al., 2019	7	92	0.08	[0.03; 0.15]
Random effects model		986	0.29	[0.23; 0.36]

Heterogeneity: $I^2 = 76%$ [59%; 86%]

Childhood (N=3)

Routier et al., 2019	11	27	0.41	[0.22; 0.61]
Angione et al., 2019	5	57	0.09	[0.03; 0.19]
Licchetta et al., 2019	6	87	0.07	[0.03; 0.14]
Random effects model		171	0.15	[0.04; 0.42]

Heterogeneity: $I^2 = 89%$ [69%; 96%]

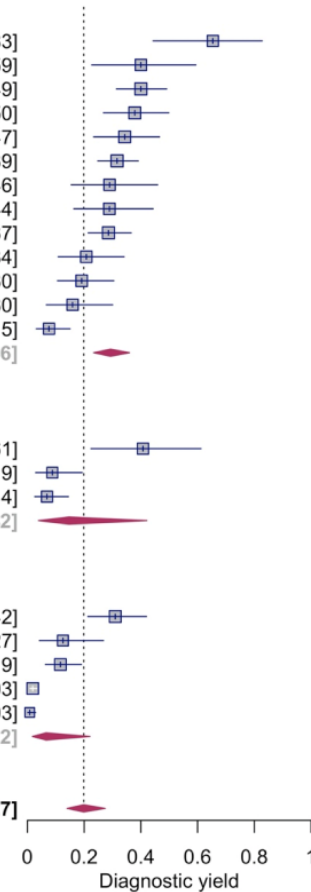
Any Age (N=5)

Muona et al., 2014	26	84	0.31	[0.21; 0.42]
Perucca et al., 2017	5	40	0.12	[0.04; 0.27]
Krenn et al., 2020	13	112	0.12	[0.06; 0.19]
Tsai et al., 2018	11	593	0.02	[0.01; 0.03]
Hildebrand et al., 2016	2	251	0.01	[0.00; 0.03]
Random effects model		1080	0.07	[0.02; 0.22]

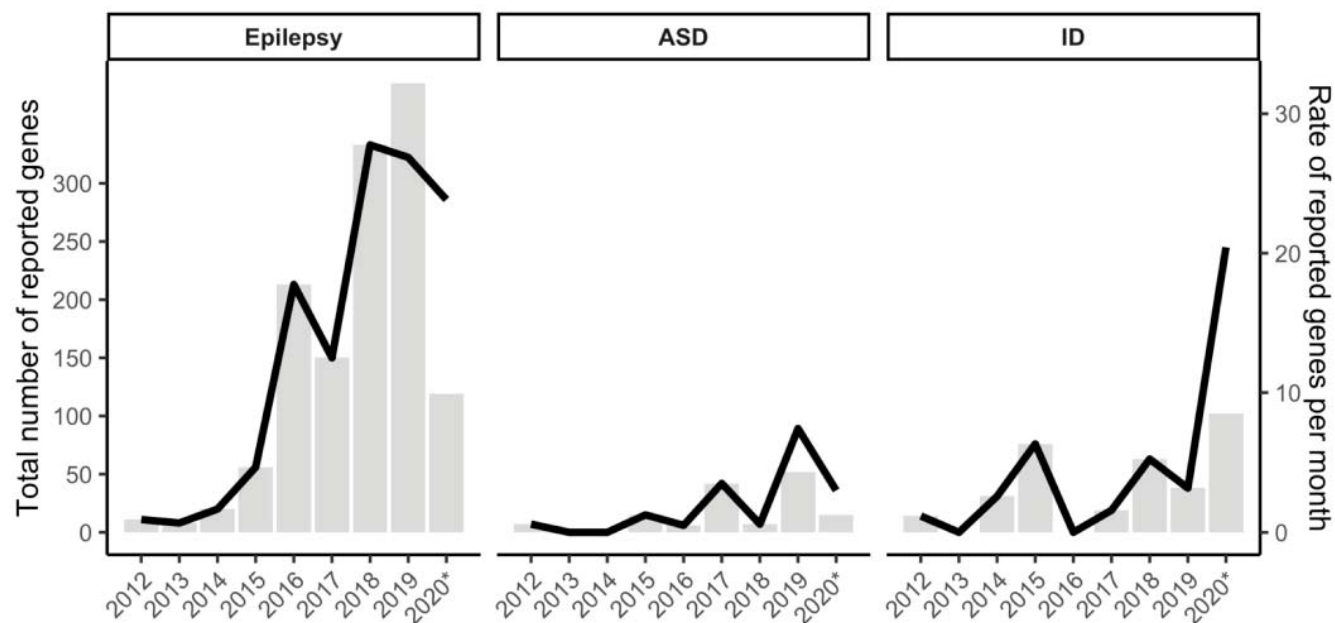
Heterogeneity: $I^2 = 95%$ [91%; 97%]

Random effects model

Heterogeneity: $I^2 = 91%$ [88%; 93%]



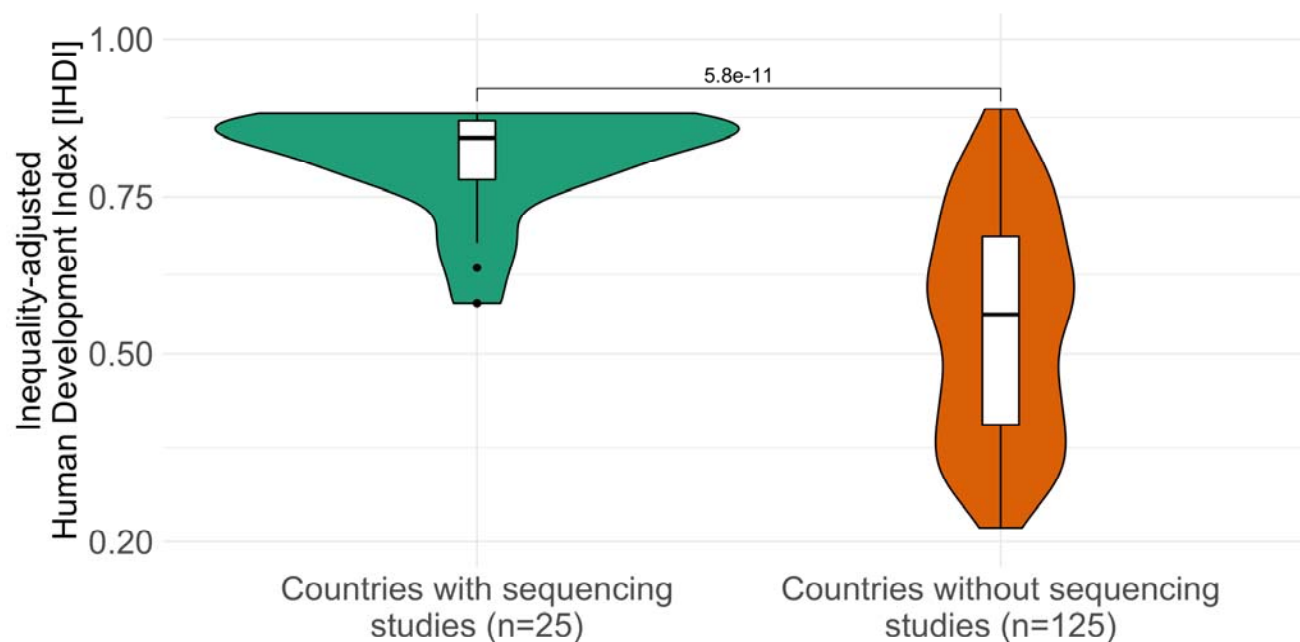
The number of genes with pathogenic variant is still increasing



The bars represent the number of genes with pathogenic variants. The line represents the rate of genes per month and year. The number of identified genes increased rapidly in recent years for epilepsy. For ASD and ID the number of reported genes with pathogenic variants is low.

*Abbreviations: ASD = autism spectrum disorder, ID = intellectual disability, * = Data were collected until May 20, 2020.*

Likely health disparities: No access to genetic testing?



Countries with sequencing studies have a significantly higher mean Inequality-adjusted Human Development Index (IHDI), compared to countries without sequencing studies ($P = 5.8 \times 10^{-11}$)

Summary of our meta-analysis

- 1 in 2-12 people with epilepsy with receive a diagnosis through currently available genetic tests
- Earlier epilepsy onset and cognitive challenges increase the chance of a positive genetic test
- More comprehensive genetic tests have a greater diagnostic yield
- The number of epilepsy-associated genes is increasing
- Not everyone has access to genetic testing



Arthur Stefanski

Summary of our meta-analysis

- 1 in 2-12 people with epilepsy with receive a diagnosis through currently available genetic tests
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- The number of epilepsy-associated genes is increasing
- Not everyone has access to genetic testing

What did the studies miss?

-> Most studies analyzed only known epilepsy genes



Arthur Stefanski

There are several classes of epilepsy associated genetic variants, which are currently not routinely screened or interpreted

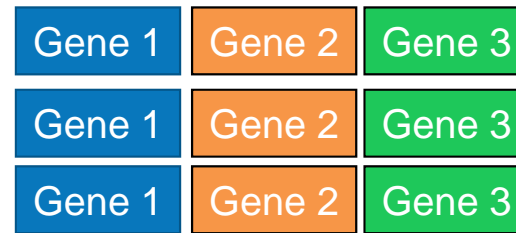
Large pathogenic copy number variants not affecting an established epilepsy gene

DNA level

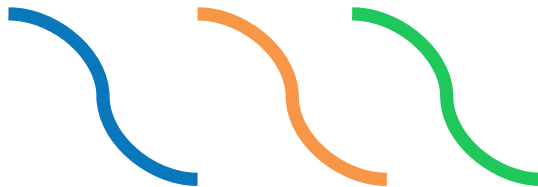
Large multi-gene deletion



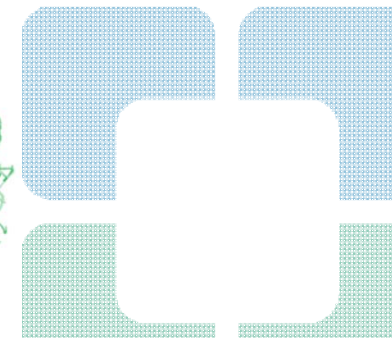
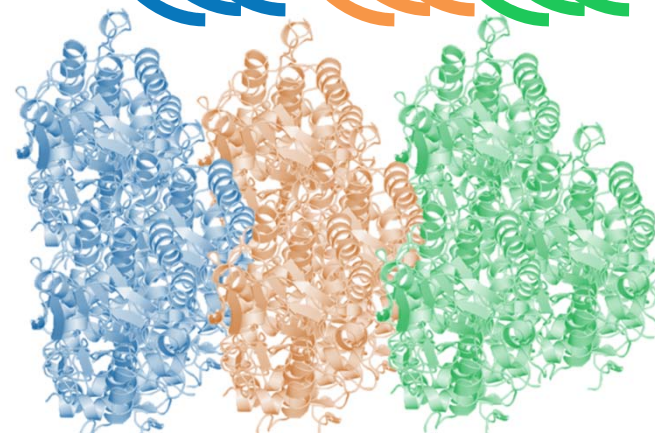
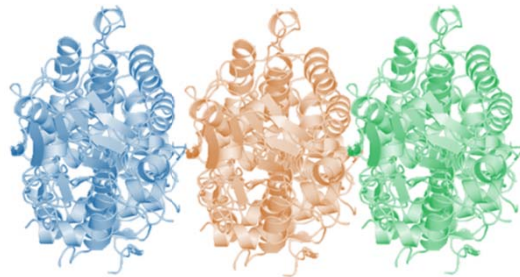
Large multi-gene duplication



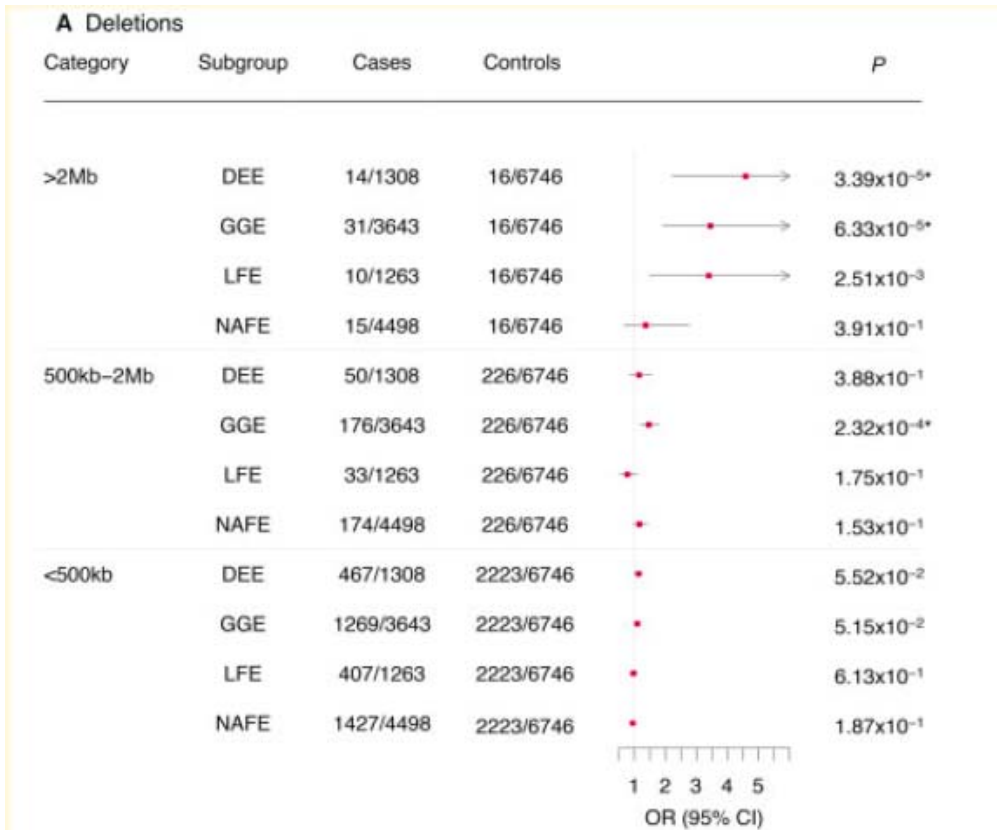
mRNA level



Protein level



Large pathogenic copy number variants not affecting an established epilepsy gene





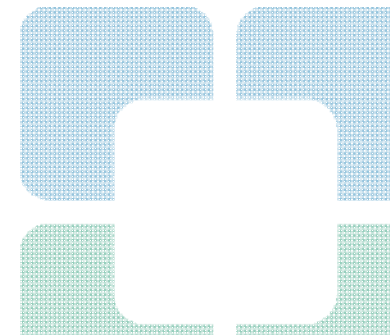
doi:10.1093/brain/awaa171

BRAIN : Page 1 of

BRAIN
A JOURNAL OF NEUROLOGY

Epilepsy subtype-specific copy number burden observed in a genome-wide study of 17 458 subjects

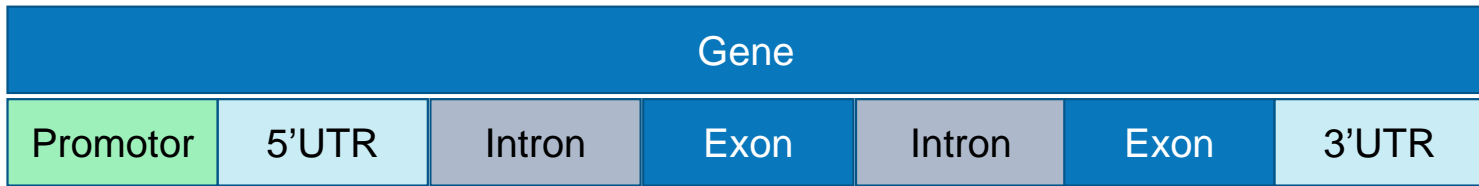
 Lisa-Marie Niestroj,¹ Eduardo Perez-Palma,² Daniel P. Howrigan,³ Yadi Zhou,² Feixiong Cheng,^{2,4,5}  Elmo Saarentaus,⁶ Peter Nürnberg,^{1,7,8} Remi Stevelink,^{9,10} Mark J. Daly,^{3,6,11} Aarno Palotie^{3,6,11} and Dennis Lal^{1,2,3,12} on behalf of the Epi25 Collaborative*



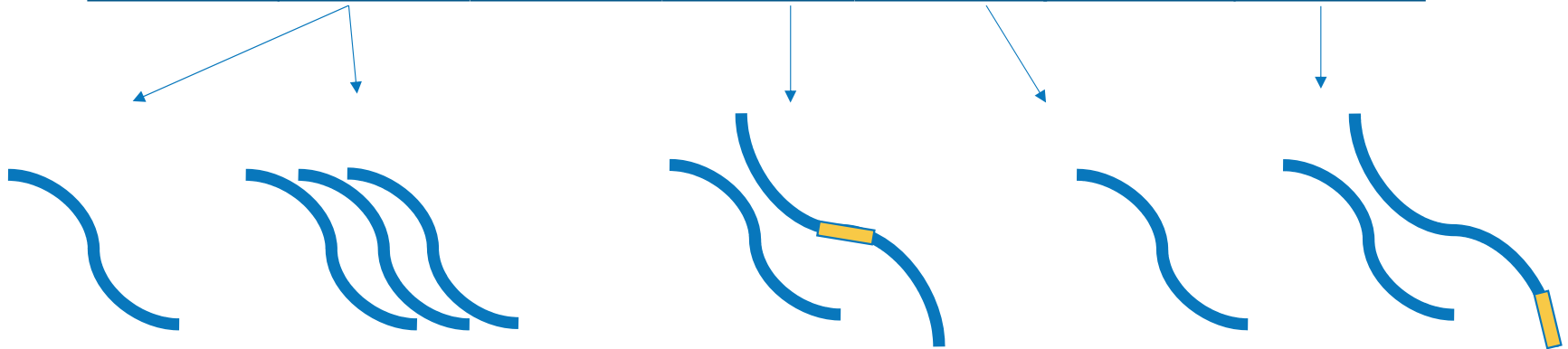
Nucleotide expansion pathogenic genetic variants

DNA level

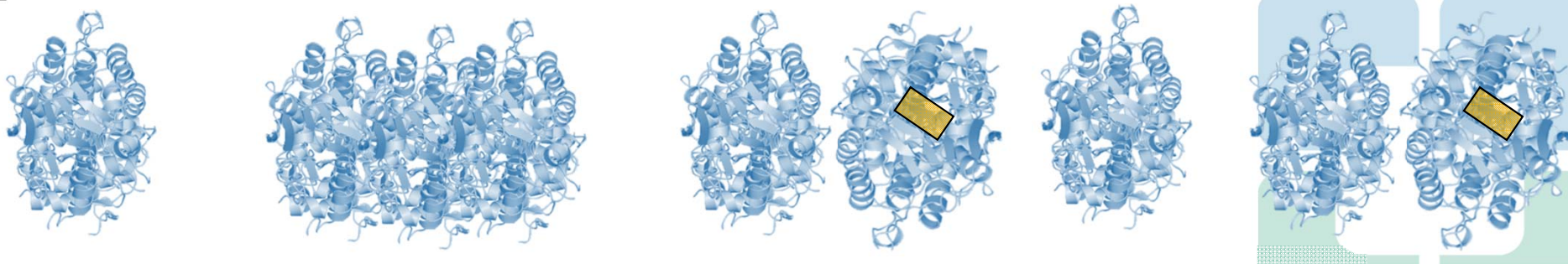
Nucleotide expansion disorders affecting different parts of a gene



mRNA level



Protein level




Types of nucleotide repeat pathogenic genetic variants





REPORT

TTTCA repeat in YEATS2 in benign epilepsy type 4

 Patra Yeetong,¹ Monnat Pongr
Adjima Assawapitaksakul,^{4,5} Var
Chaipat Chunharas,⁶ Kanya Sup



Expansions of intronic TTTCA and TTTTA repeats in benign adult familial myoclonic epilepsy

Hiroyuki Ishiura , Koichiro Doi², Jun Mitsui , Jun Yoshimur
Asao Fujiyama³, Yasuko Toyoshima⁴, Akiyoshi Kakita⁴, Hitoshi
Sumio Sugano⁶, Wei Qu², Kazuki Ichikawa², Hideaki Yurino⁷, K
Aki Mitsue¹, Masaki Tanaka¹, Yaeko Ichikawa⁹, Yuji Takahashi¹⁰
Junko Kanda¹, Fumiko Kusunoki Nakamoto¹, Mana Higashihara
Mutsuo Sasagawa¹⁴, Yasuko Kuroha¹³, Naoya Hasegawa¹⁵, Nor



ARTICLE

<https://doi.org/10.1038/s41467-019-12763-9>

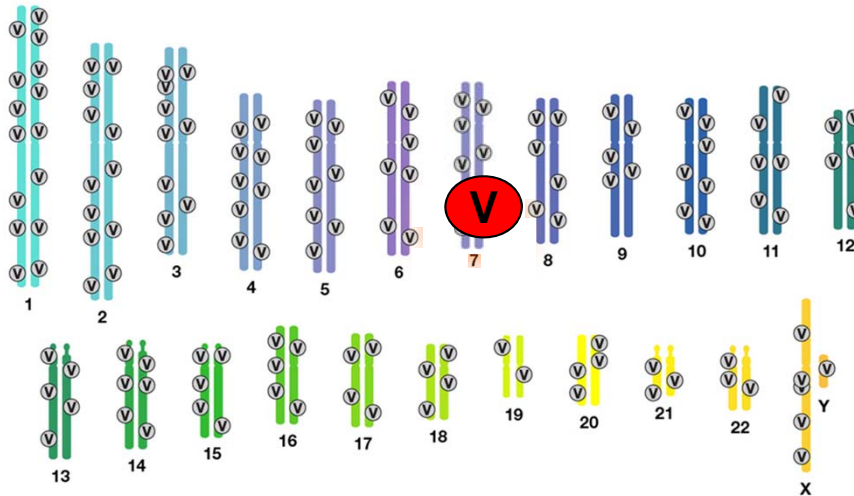
OPEN

Unstable TTTTA/TTTCA expansions in *MARCH6* are associated with Familial Adult Myoclonic Epilepsy type 3

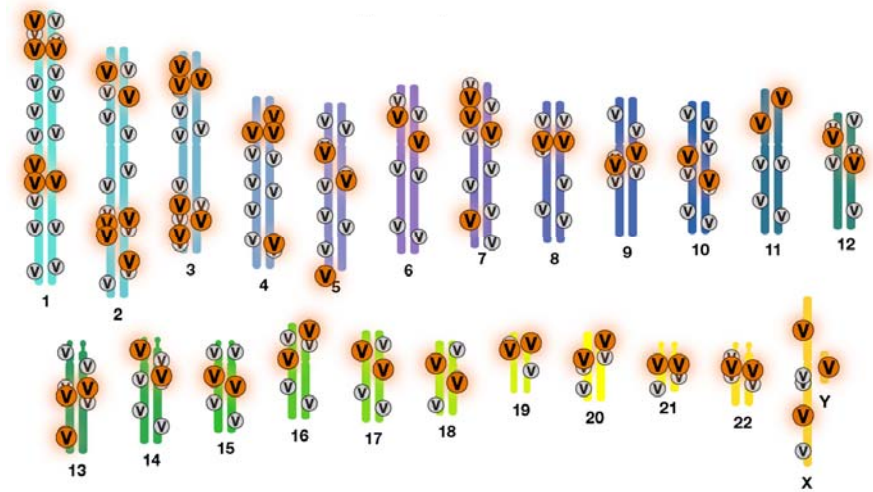
Rahel T. Florian et al.[#]

Polygenic disease 'risk' variants

A person with a disease causing variant on chromosome 7



A person with thousands of disease risk conferring variants across the genome





Polygenic disease 'risk' variants

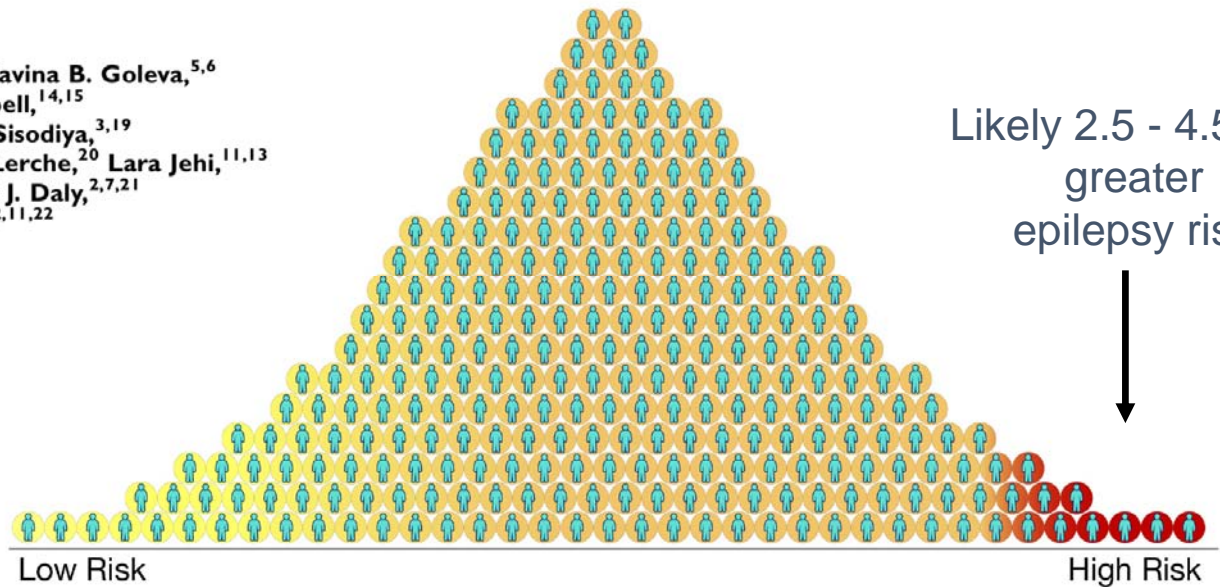
doi:10.1093/brain/awz292

BRAIN 2019; 142: 3473–3481

BRAIN
A JOURNAL OF NEUROLOGY

Polygenic burden in focal and generalized epilepsies

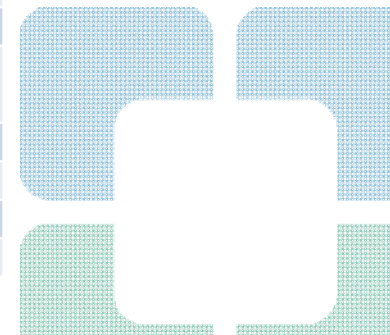
 Costin Leu,^{1,2,3} Remi Stevelink,⁴ Alexander W. Smith,² Slavina B. Goleva,^{5,6}
Masahiro Kanai,^{2,7,8,9,10} Lisa Ferguson,^{11,12,13} Ciaran Campbell,^{14,15}
 Yoichiro Kamatani,^{10,16} Yukinori Okada,^{10,17,18} Sanjay M. Sisodiya,^{3,19}
Gianpiero L. Cavalleri,^{14,15} Bobby P.C. Koeleman,⁴ Holger Lerche,²⁰ Lara Jehi,^{11,13}
Lea K. Davis,^{5,6} Imad M. Najm,^{11,13} Aarno Palotie,^{2,21} Mark J. Daly,^{2,7,21}
Robyn M. Busch,^{11,12,13} Epi25 Consortium and Dennis Lal^{1,2,11,22}



Somatic variants

- An underlying disease causing genetic variant can be identified in 1 in 2-9 patients with a lesional epilepsy (e.g., low grad tumors or cortical malformation)
- Examples of focal cortical dysplasia brain tissue sequencing studies

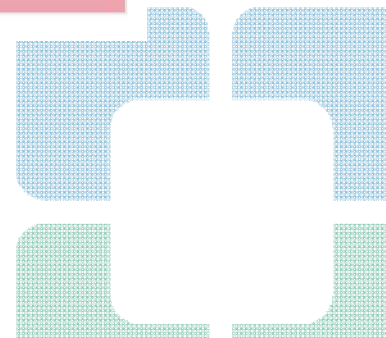
Study	Total sample size	FCD I	FCD II	Carrier of somatic variants	Success rate (% yield)
Zhang et al. (2020)	17	0	17	7	41.18
Baldassari et al. (2019)	80	18	62	37	46.25
Sim et al. (2019)*	75	4	71	18	24.00
Niestroj et al. (2019)	15	0	15	2	13.33
Zhao et al. (2019)	8	0	8	1	12.50
D'Gama et al. (2017)	66	0	66	14	21.21
Lim et al. (2017)	40	0	40	5	12.50
Møller et al. (2016)	19	3	16	6	31.58
Mirzaa et al. (2016)	8	0	8	4	50.00
Nakashima et al. (2015)	24	6	18	6	25.00
Lim et al. (2015)	77	0	77	12	15.58
Jansen et al. (2015)	33	0	33	4	12.12
D'Gama et al. (2015)	53	0	53	6	11.32
Total (no duplicates)	491	31	460	122	24.85



Variant interpretation is in its infancy

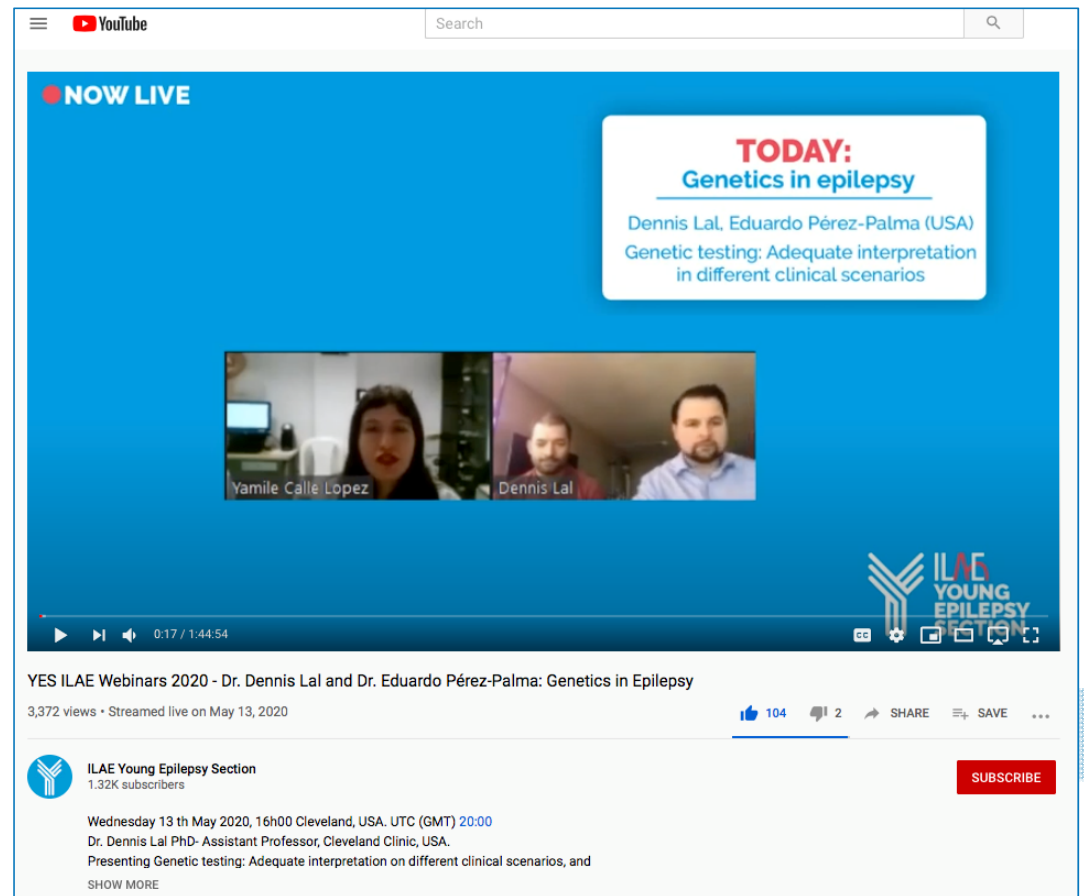
Important to acknowledge!

- Rare missense variants are not rare as an aggregate
- The chance to find in a genetic test a benign variant in a epilepsy gene that 'fits the broader phenotype' is high
- A large amount of previous pathogenic classified variants found in disease databases are becoming now reclassified as benign



Resources relevant for variant interpretation

- We have a ~2h overview presentation online in the young epilepsy ILAE youtube channel



The screenshot shows a YouTube live stream interface. At the top, the YouTube logo and a search bar are visible. Below the search bar, a blue banner features the text "NOW LIVE" and a white box with the following information:

TODAY:
Genetics in epilepsy
Dennis Lal, Eduardo Pérez-Palma (USA)
Genetic testing: Adequate interpretation in different clinical scenarios

The video player shows a split-screen view of two speakers: Yamile Calle Lopez on the left and Dennis Lal on the right. The video progress bar indicates 0:17 / 1:44:54. Below the video player, the video title is "YES ILAE Webinars 2020 - Dr. Dennis Lal and Dr. Eduardo Pérez-Palma: Genetics in Epilepsy", with 3,372 views and a note that it was streamed live on May 13, 2020. The channel name is "ILAE Young Epilepsy Section" with 1.32K subscribers. A red "SUBSCRIBE" button is present. The video description includes the date and time: "Wednesday 13 th May 2020, 16h00 Cleveland, USA, UTC (GMT) 20:00", the speaker information: "Dr. Dennis Lal PhD- Assistant Professor, Cleveland Clinic, USA.", and the topic: "Presenting Genetic testing: Adequate interpretation on different clinical scenarios, and". A "SHOW MORE" link is also visible.

Summary

- 1 in 2-12 people with epilepsy with receive a diagnosis through currently available genetic tests
- Earlier epilepsy onset and cognitive challenges increase the chance of a positive genetic test
- More comprehensive genetic tests have a greater diagnostic yield
- The number of epilepsy-associated genes is increasing
- Not everyone has access to genetic testing
- **Several in 'research well-established' different types of pathogenic or risk variants are currently not screened in the clinic. This will change in the near future. Subsequently, the role of genetics in clinical management of patients will further increase.**
- **Not all varriants identified in clinical genetic testing are pathogenic**

Acknowledgement

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Patrick May

University of Cologne, Germany

Juliana Du
Eduardo Perez
Lisa-Marie Niestroj
Peter Nuernberg

DAAD

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 **Cleveland Clinic**

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University of Cologne