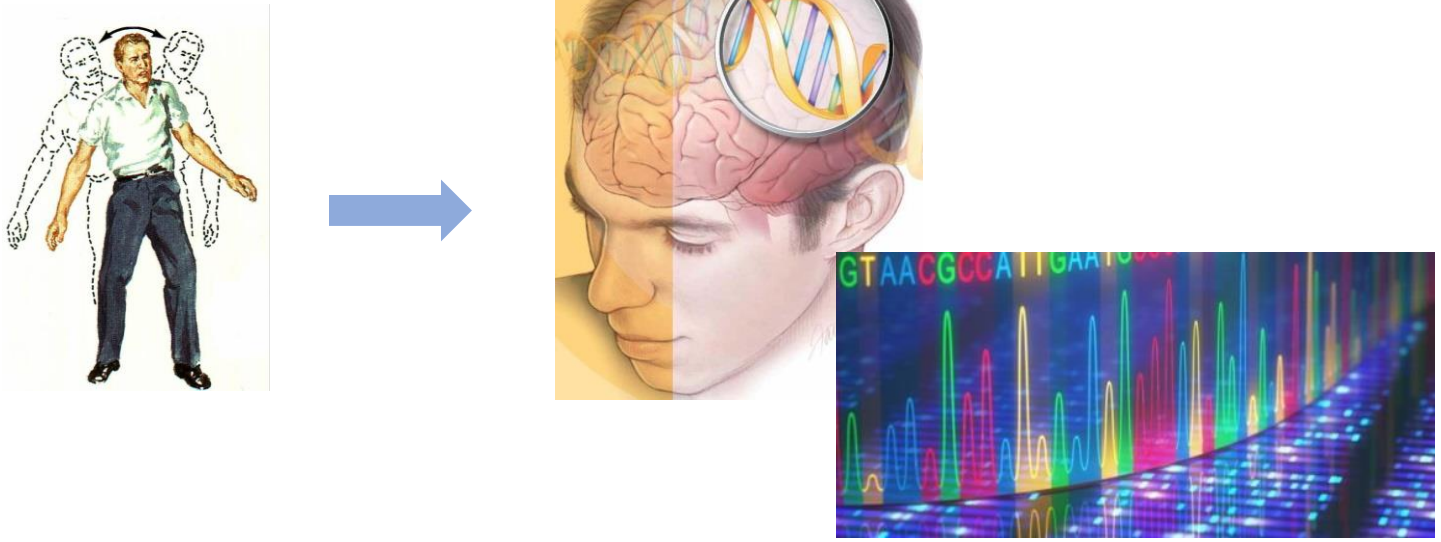
ATAXIA GLOBAL INITIATIVE
worldwide platform for clinical research in ataxias

NGS analysis in ataxias: what it is and how it is done?



for starters ☺

Matthis Synofzik, Andrea Nemeth, Stephan Zuchner
on behalf of the AGI WG Next-generation genomics

AGI webinar, January 18th, 2022

ATAXIA GLOBAL INITIATIVE
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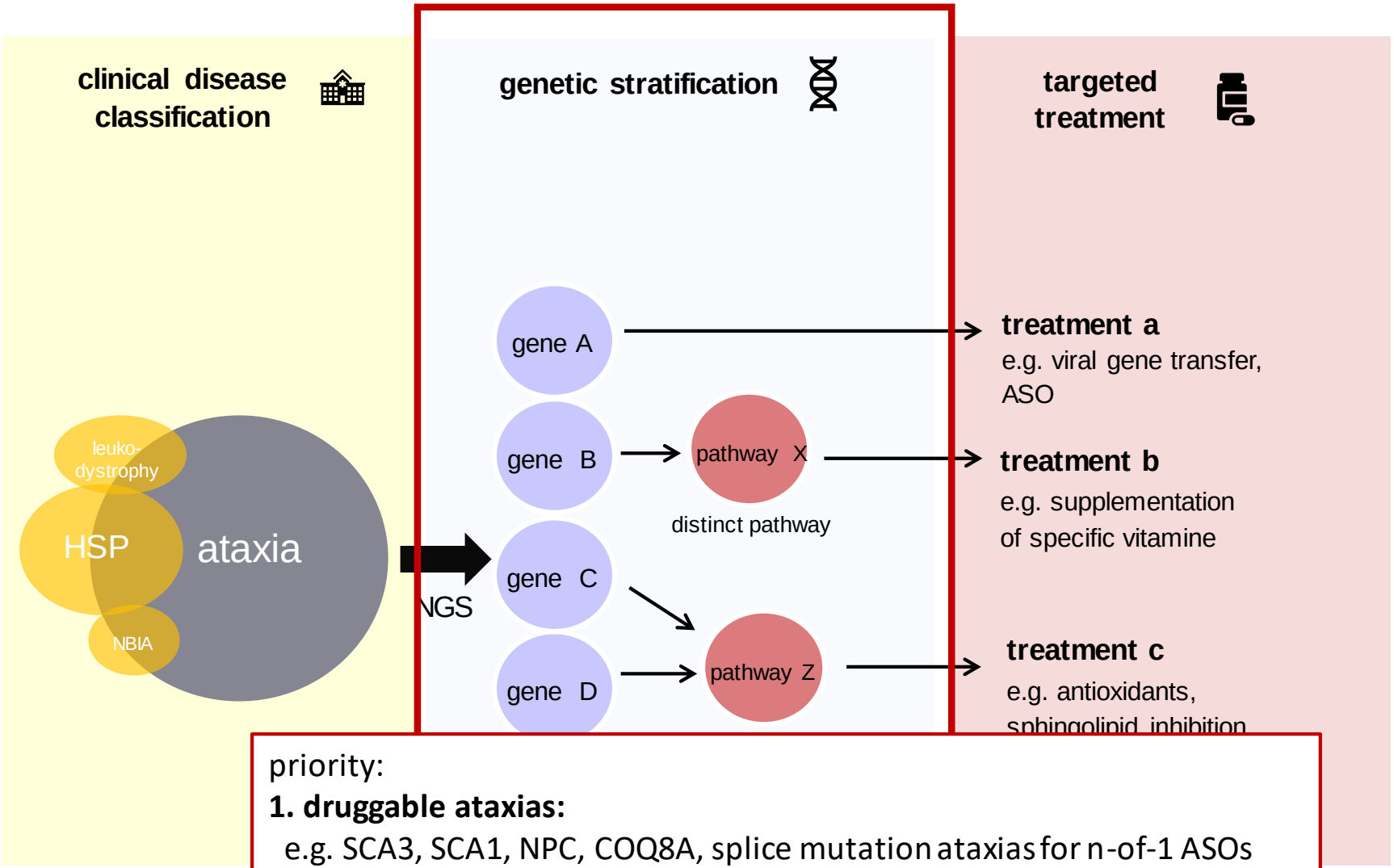
outline of the webinar

1. introduction
2. *Sanger, panel, exome, genome*: what are the differences, strengths and weaknesses?
3. *filter settings*: the crucial key in understanding NGS diagnostic and research reports
4. major international NGS ataxia pipelines - and ways to contribute
5. outlook: the next steps in ataxia genomics
6. discussion & questions

Genetic stratification is key to ataxia trial-readiness: from *DIAGNOSTICS* to *THERANOSTICS*



priority:

1. druggable ataxias:

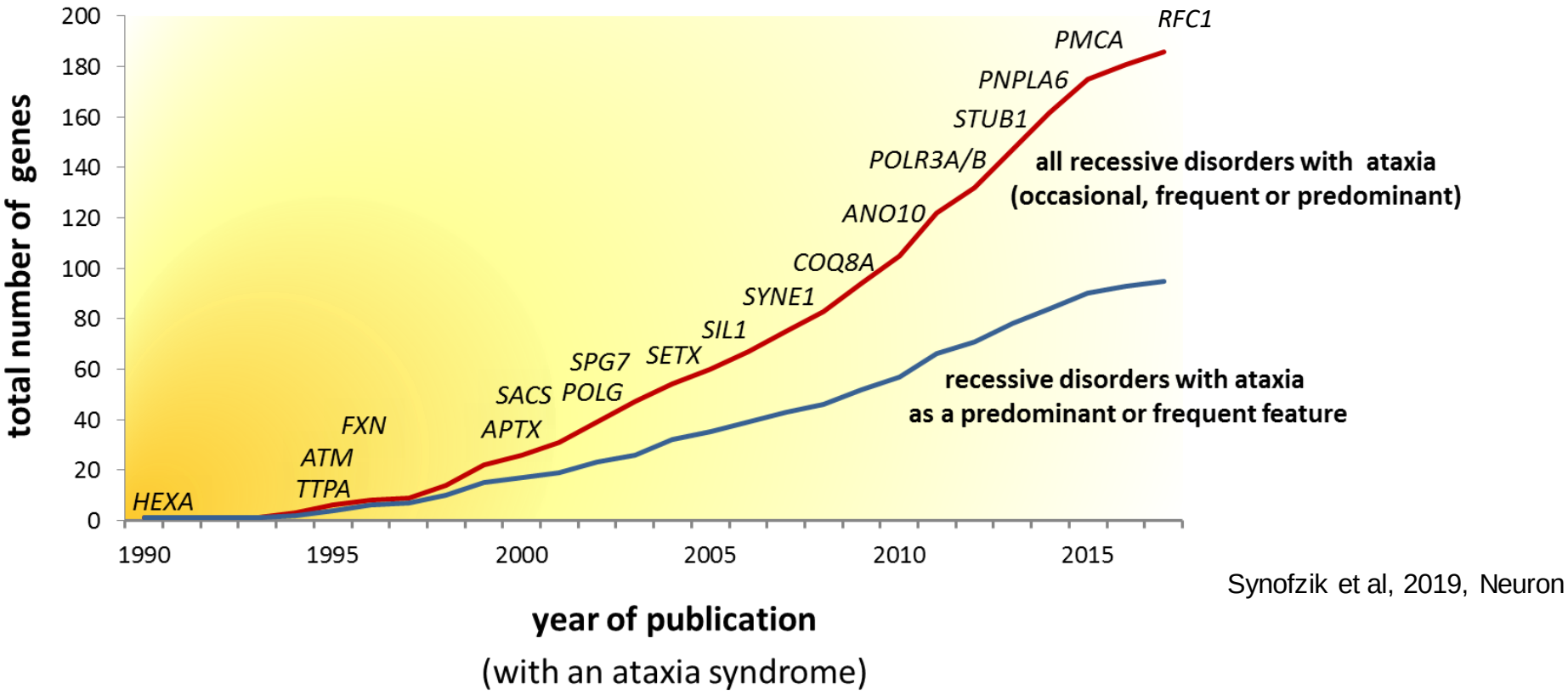
e.g. SCA3, SCA1, NPC, COQ8A, splice mutation ataxias for n-of-1 ASOs

2. trial-ready ataxias

e.g. ARSACS, SPG7, RFC1

Synofzik et al, 2019, Neuron

ataxia genetics: the current status



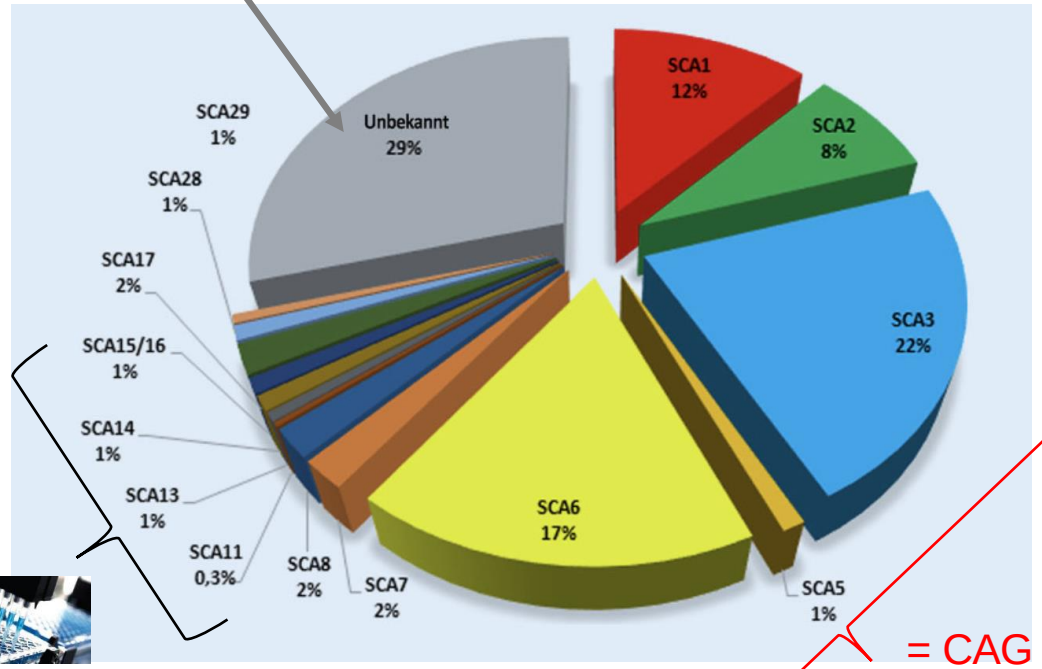
Synofzik et al, 2019, Neuron

- single gene sequencing (=Sanger sequencing) not really helpful ➔ NGS needed!
- any „hardwired“ NGS panel is out of date already at time of sequencing

➔ **exome first strategy!**

ataxia genetics: the current status

autosomal-dominant ataxias (SCAs)



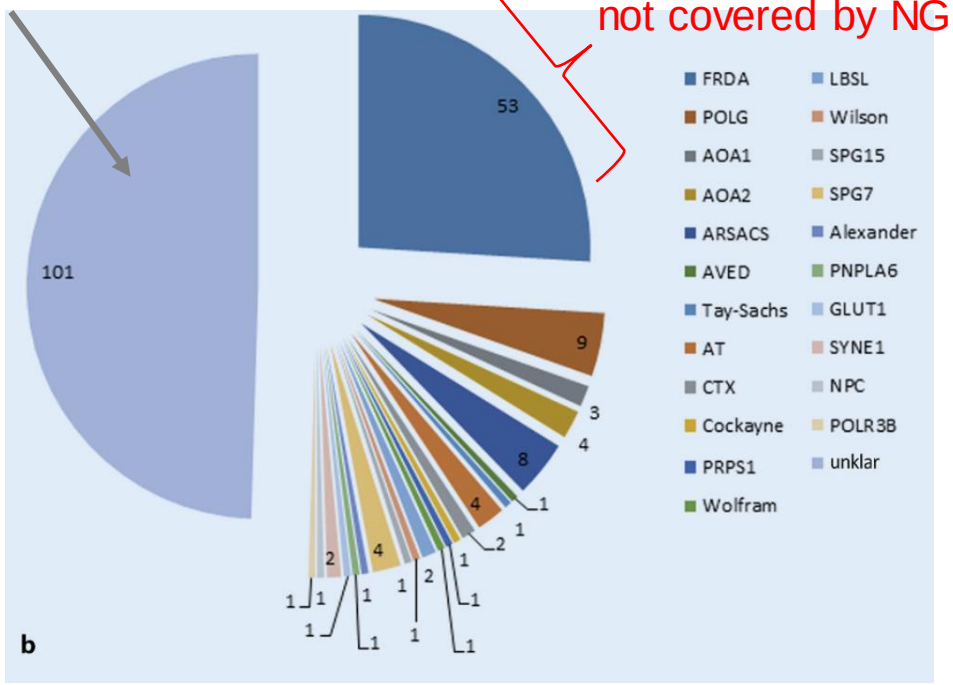
NGS

= CAG repeat SCAs:
not covered by NGS!

➔ **SCA 1,2,3 and 6: >70% of all dominant ataxias !**

autosomal-recessive ataxias (ARCAs)

+RFC1 repeat
= FA repeat
not covered by NGS!



➔ **FA and RFC1: >40% all recessive ataxias !**

The following statement about ataxia genetics is true:

Repeat expansions in ataxias

1. are much rarer than e.g. in ALS, HSP or FTD
2. account for the most frequent autosomal-dominant and autosomal-recessive ataxias
3. have now already all been identified, with no new repeat ataxias in the last 3 years

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Andrea Nemeth

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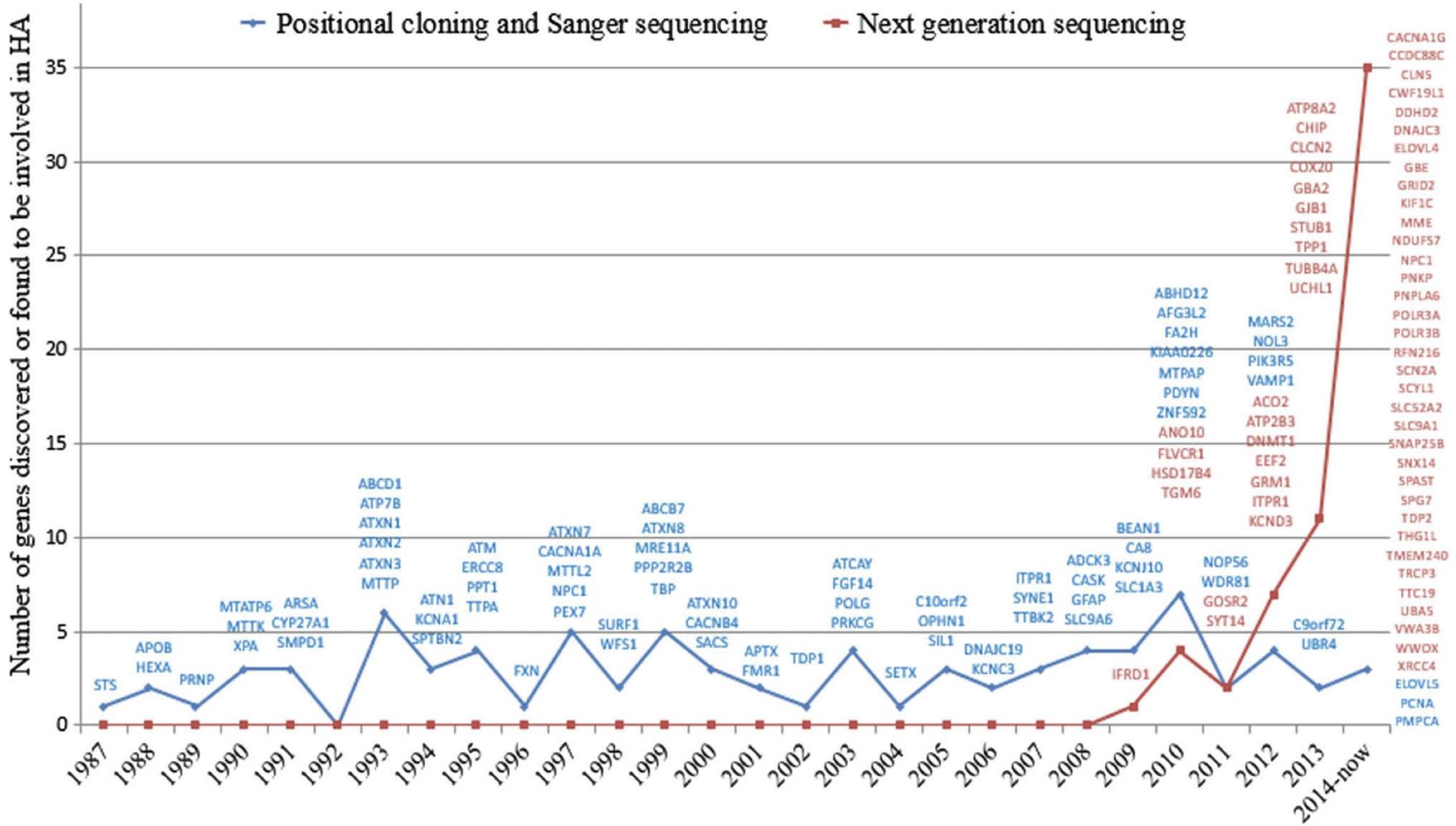
UNIVERSITY OF MIAMI

Understanding NGS diagnostics

Stephan Zuchner, MD, PhD

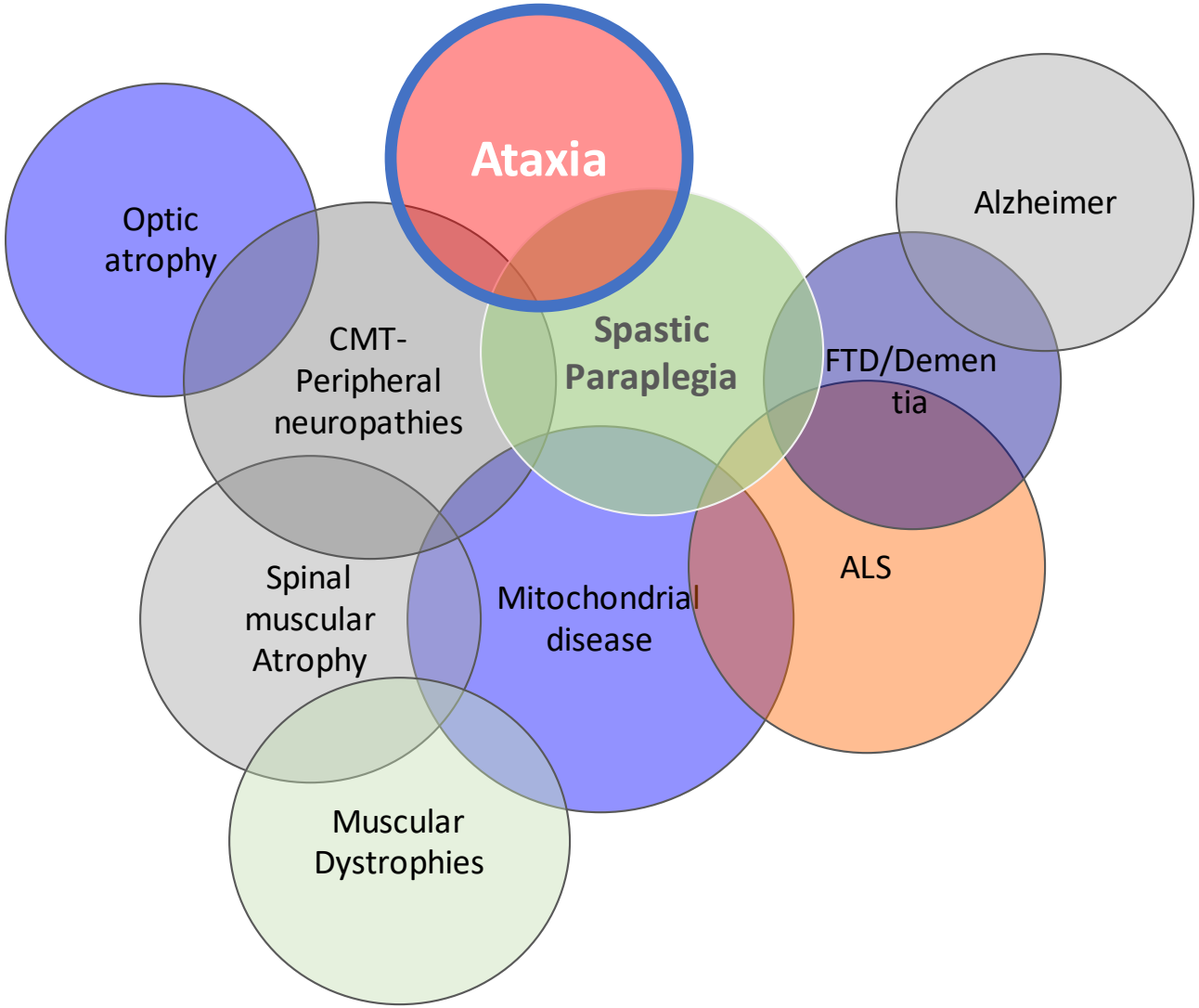
MANY genes can cause ataxia

Filippo M. Santorelli and colleagues

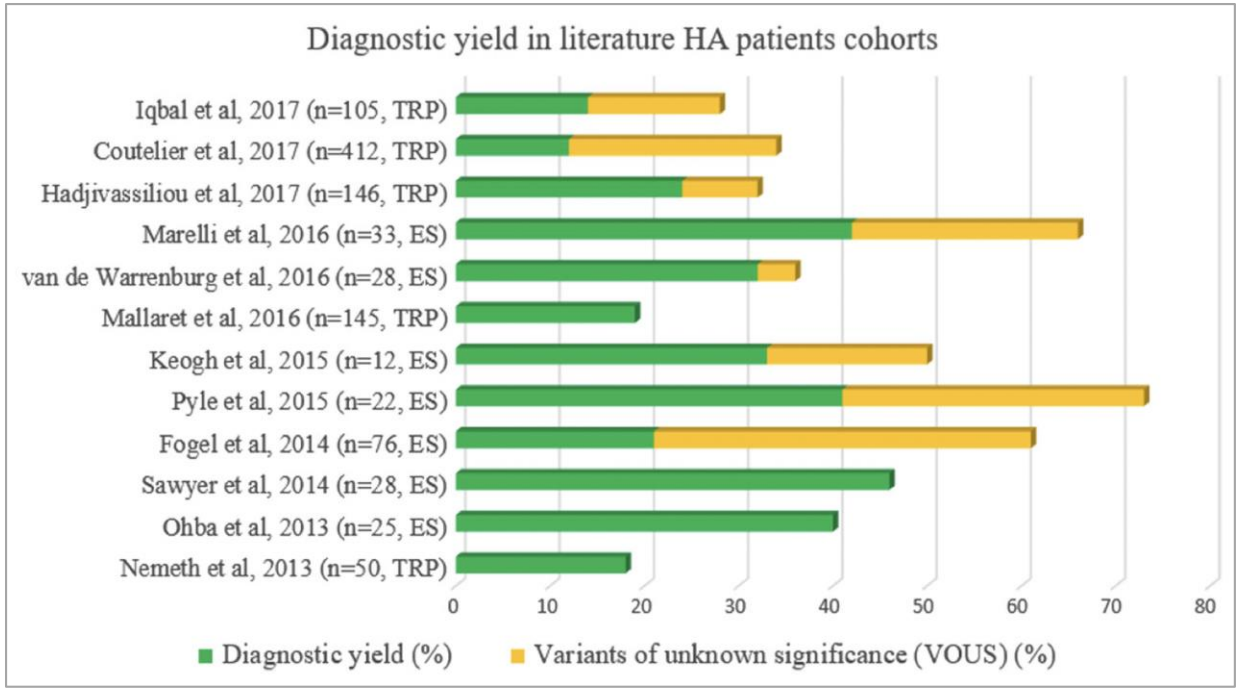


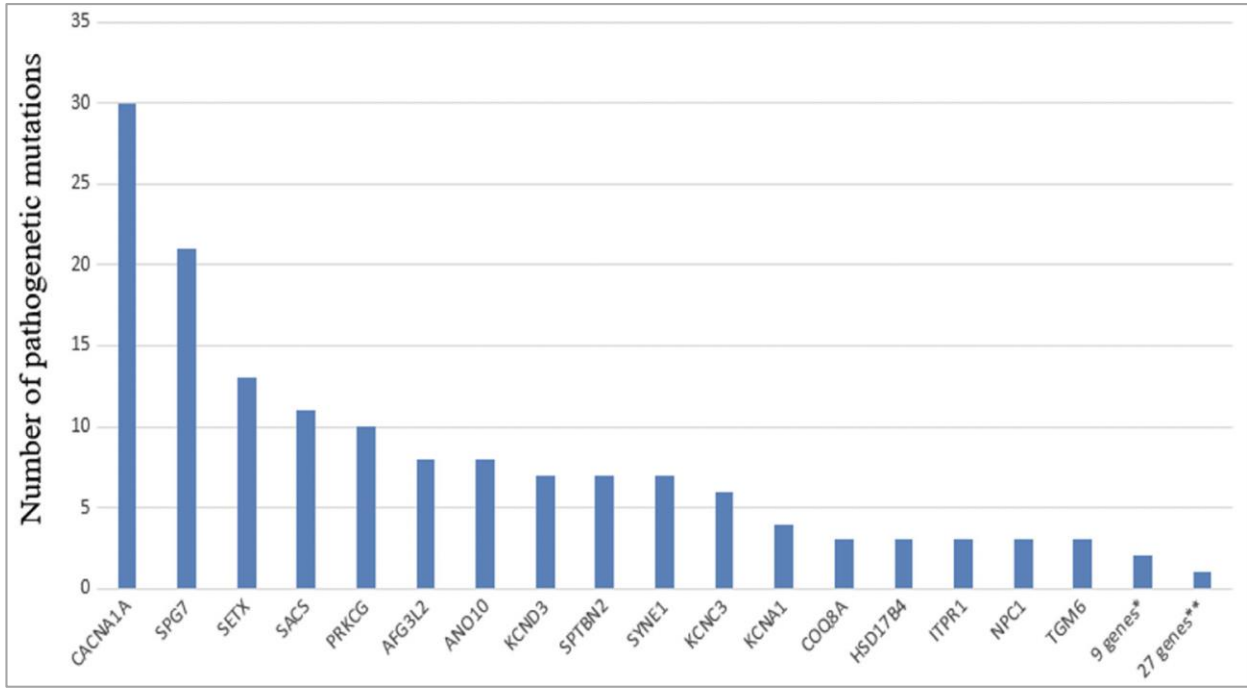
Overlap between neurological diseases

- Overlapping phenotypes and genotypes
- An ataxia gene list might contain “HSP” genes



The diagnostic gap in ataxias is still substantial





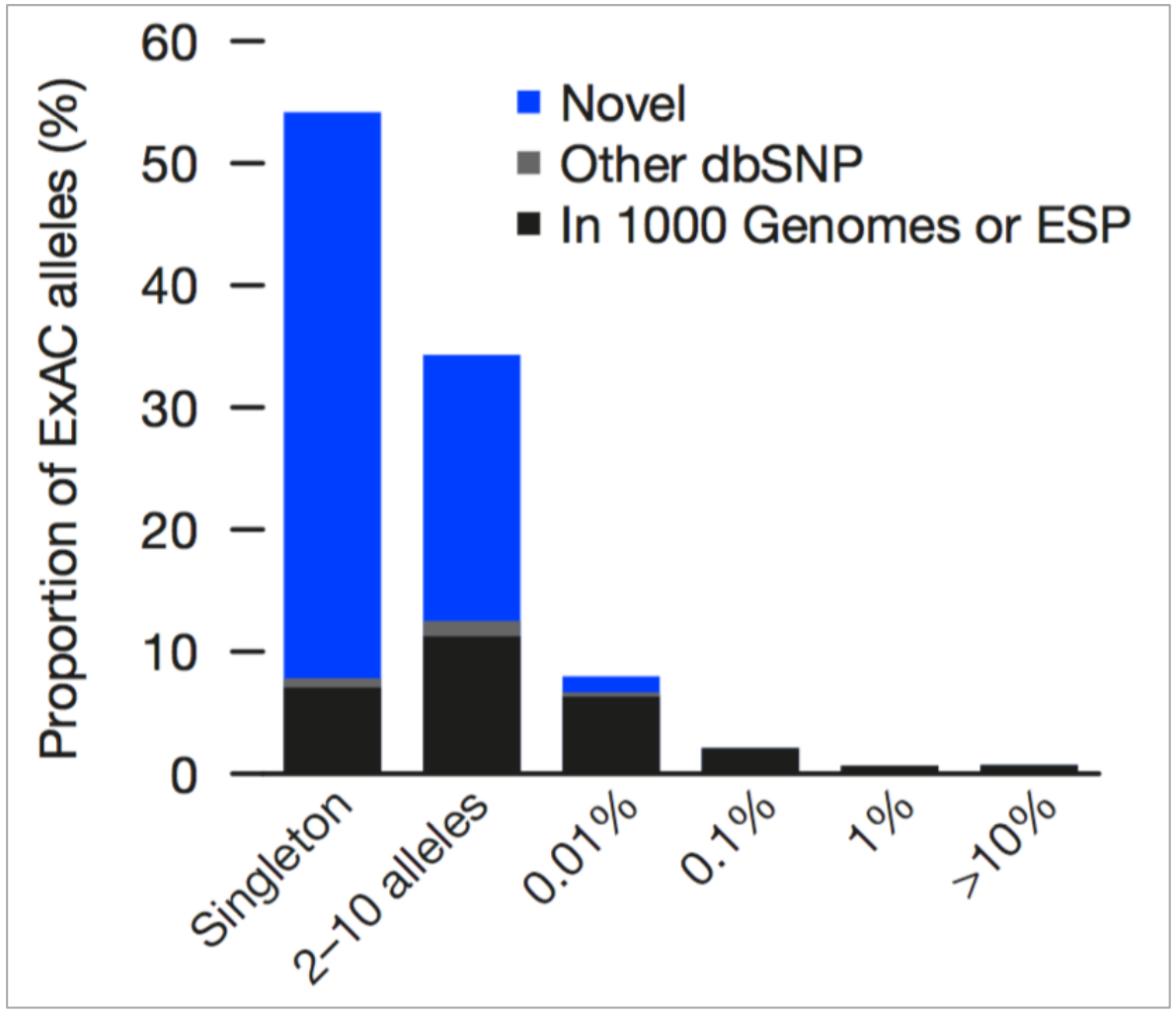
Clinical application of next generation sequencing in hereditary spinocerebellar ataxia: increasing the diagnostic yield and broadening the ataxia-spasticity spectrum. A retrospective analysis

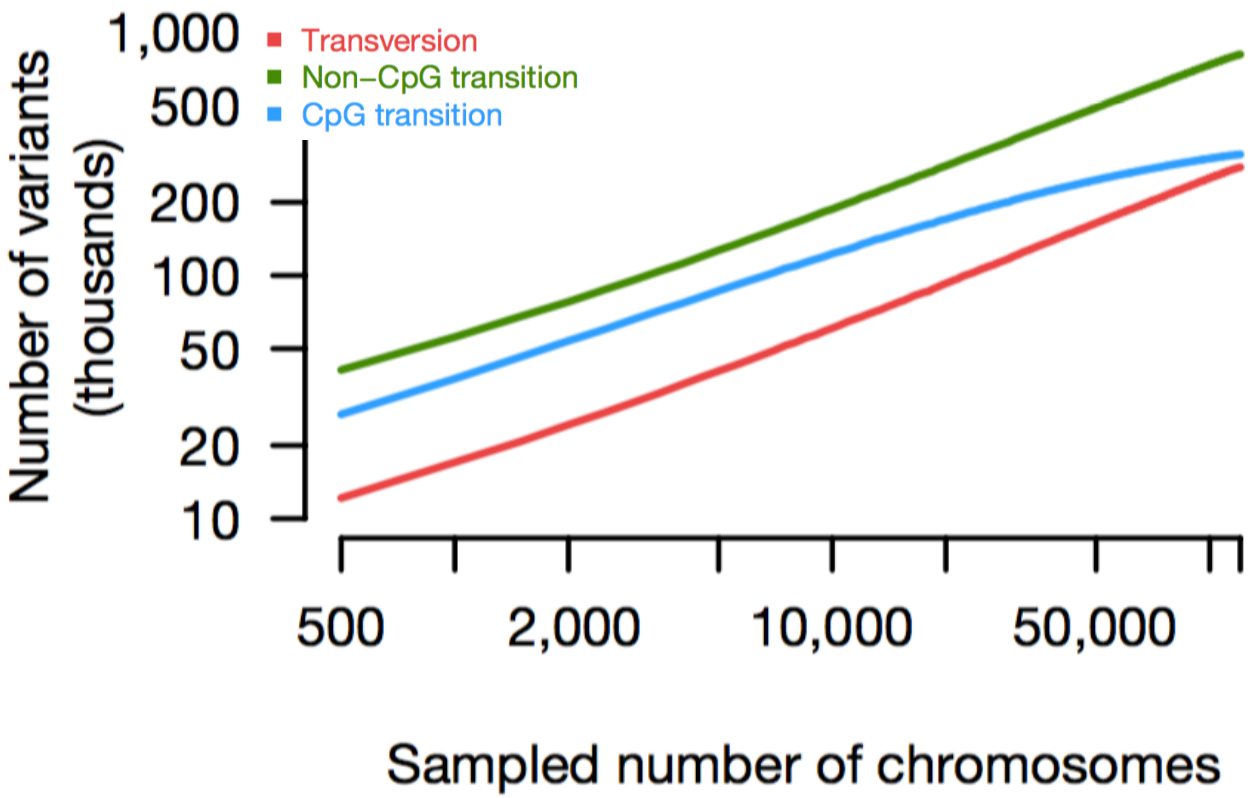
Daniele Galatolo [1] · Alessandra Tessa [1] · Alessandro Filla [2] · Filippo M. Santorelli [1]

Analysis of protein-coding genetic variation in 60,706 humans

Monkol Lek, Daniel McArthur

Excess of rare variation in the human genome at population level



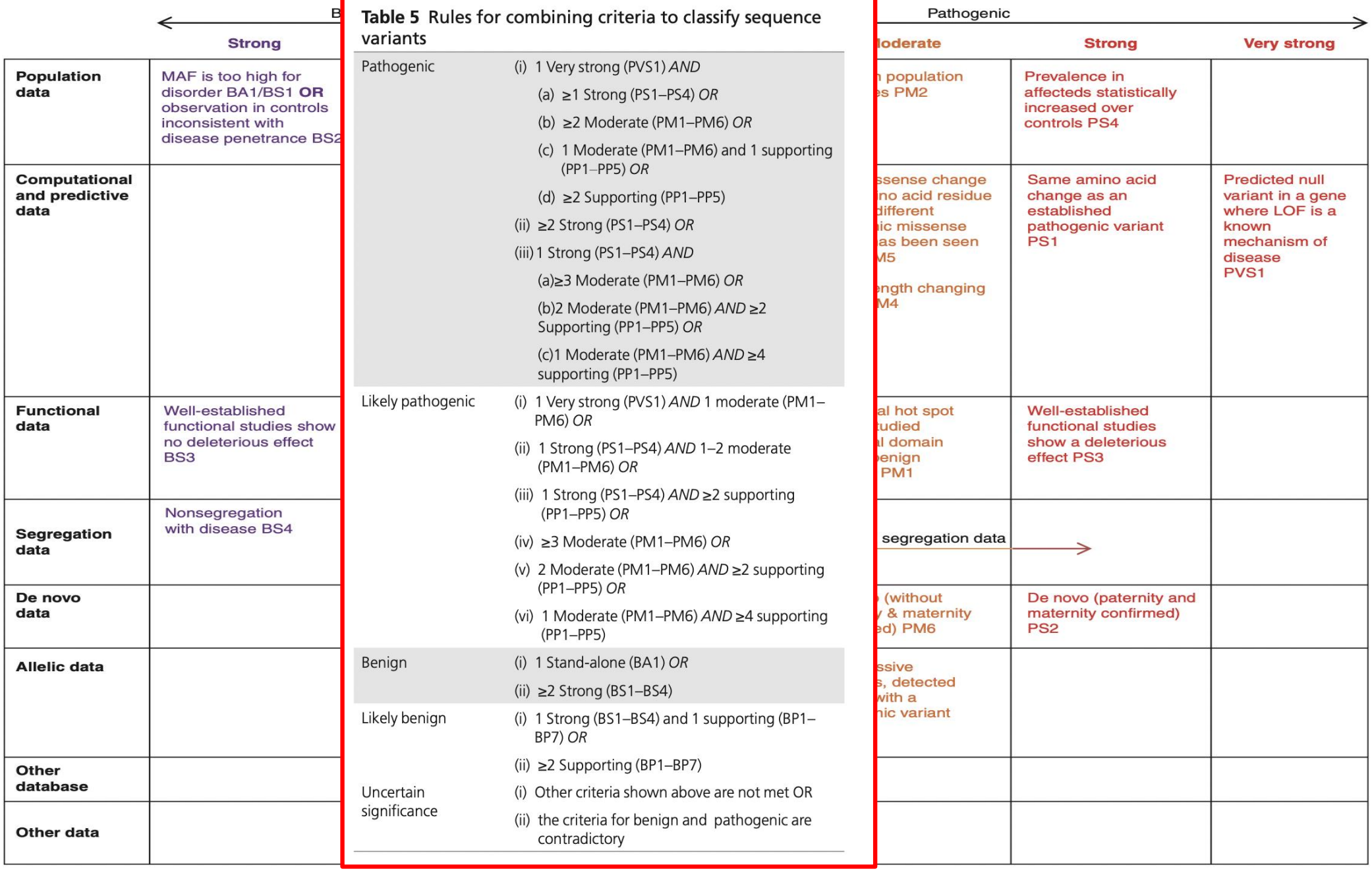


ACMG criteria

https://www.acmg.net/docs/Standards_Guidelines_for_the_Interpretation_of_Sequence_Variants.pdf

Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory



The challenge

Every exome contains:

- ~20,000 coding variants
- several hundred variants never seen before
- False positive variants calls
- False negative variants calls

Goal is to find the ONE disease causing change

Filtering of coding variation

What are reasonable filters to consider (without eliminating the disease variant)?

1. Inheritance pattern

- Family history may give essential clues to dominant or recessive inheritance, consanguinity *(always take careful family history)*

2. Quality of variations

- Low quality variants may be false positive ‘calls’; exclude low coverage and low genotype quality calls

Filtering of coding variation

What are reasonable filters to consider (without eliminating the disease variant)?

3. Minor allele frequency (MAF) in the population

- A rare disease (prevalence <<1:1,000) especially caused by multiple different genes (locus heterogeneity) and many different mutations per gene (allele heterogeneity) cannot be caused by a variant that is present in 20% of all people in a population -> otherwise the disease prevalence would be higher

- For recessive cases: Minor allele frequency typically less than 1% in controls

- For dominant cases: Minor allele frequency typically 0% in controls

Example: recessive ataxia family (AAD-CAE-JUL-252-003)

- ✗ Inheritance pattern
- ✗ Allele frequency
- ✓ Quality
- ✗ Known ataxia genes only

Review genotypes from 10000 records returned.

Download Results | View parameters

Genomic Position	Reference Allele	Alternate Allele	Family Number	Clinical Diagnosis	Variant Type	Gene Name	Protein Notation	Maverick Score (NS)	Functional Rating	Conservation Rating	Allele Frequency
Chr12:9248233	T/T	C/C	AAD-CA...	183518	Missense	A2M	Asn639Asp	0.00			Other
Chr12:9004892	C/C	A/A	AAD-CA...	183518	Missense	A2ML1	Asp850Glu	0.00		★★	Other
Chr12:9009820	G/G	G/A	AAD-CA...	183518	Missense	A2ML1	Cys970Tyr	0.00	★★★	★★★★	Other
Chr12:9016573	A/A	G/G	AAD-CA...	183518	Missense	A2ML1	His1229Arg	0.00		★★	Other
Chr12:9020489	A/A	G/G	AAD-CA...	183518	Missense	A2ML1	Met1257Val	0.00		★	Other
Chr3:137843106	T/T	C/C	AAD-CA...	183518	Stop retained	A4GNT		N/A		★	Other
Chr3:137843476	G/G	T/T	AAD-CA...	183518	Missense	A4GNT	Ala218Asp	0.00		★	Other
Chr5:178195673	T/T	T/C	AAD-CA...	183518	Splice region	AACSP1		N/A		★	Other
Chr3:151545601	G/G	A/A	AAD-CA...	183518	Missense	AADAC	Val281Ile	0.00	★	★★★★	Other
Chr3:151463421	G/G	G/T	AAD-CA...	183518	Missense	AADACL2	Ala186Ser	0.00		★	Other
Chr1:12776344	A/A	T/T	AAD-CA...	183518	Start lost	AADACL3	Met1?	N/A		★	Other
Chr1:12779618	T/T	C/C	AAD-CA...	183518	Missense	AADACL3	Ser47Pro	N/A		★★★★	Other
Chr1:12785494	G/G	T/T	AAD-CA...	183518	Missense	AADACL3	Cys195Phe	N/A		★	Other
Chr4:170987636	A/A	A/G	AAD-CA...	183518	Splice region	AADAT		N/A		★★	Other
Chr15:67528374	T/T	T/G	AAD-CA...	183518	Missense	AAGAB	Ile132Leu	0.00		★★★	Other

Example: recessive ataxia family (AAD-CAE-JUL-252-003)

- ✗ Inheritance pattern
- ✓ Allele frequency
- ✓ Quality
- ✗ Known ataxia genes only

Review genotypes from 496 records returned.

Download Results | View parameters

Genomic Position	Reference Allele	Alternate Allele	Family Number	Clinical Diagnosis	Variant Type	Gene Name	Protein Notation	Maverick Score (NS)	Functional Rating	Conservation Rating	Allele Frequency
Chr1:155026813	A/A	A/G	AAD-CA...	183518	Missense	ADAM15	Glu148Gly	0.75	★	★★★★	Private
Chr1:161161016	G/G	G/A	AAD-CA...	183518	Missense	ADAMTS4	Thr809Met	0.00	★	★★	Rare
Chr11:130288992	G/G	G/A	AAD-CA...	183518	Missense	ADAMTS8	Arg306Cys	0.90	★★★★★	★★★★	Rare
Chr4:100263945	A/A	A/T	AAD-CA...	183518	Splice region	ADH1C		N/A		★	Rare
Chr14:105404756	G/G	G/A	AAD-CA...	183518	Stop gained	AHNAK2	Gln5678Ter	0.75		★★★	Rare
Chr6:151669838	G/G	G/T	AAD-CA...	183518	Splice region	AKAP12		N/A		★	Rare
Chr14:33015531	A/A	A/G	AAD-CA...	183518	Missense	AKAP6	Asn558Asp	0.00		★	Rare
Chr6:13470102	C/C	C/T	AAD-CA...	183518	Stop retained	AL583828.1		N/A		★	Rare
Chr4:113359634	T/T	T/C	AAD-CA...	183518	Splice region	ALPK1		N/A			Rare
Chr2:131521383	G/G	G/A	AAD-CA...	183518	Missense	AMER3	Gly580Arg	0.01		★	Rare
Chr19:17394492	C/C	C/T	AAD-CA...	183518	Missense	ANKLE1	Pro307Ser	0.02		★	Rare
Chr2:97499281	TA/TA	TA/T	AAD-CA...	183518	Splice region	ANKRD23		N/A			Rare
Chr10:37478440	G/G	G/T	AAD-CA...	183518	Missense	ANKRD30A	Ala767Ser	0.00		★	Other
Chr2:96514607	T/T	T/TA	AAD-CA...	183518	Splice region	ANKRD36C		N/A		★	Rare

Example: recessive ataxia family (AAD-CAE-JUL-252-003)

- ✓ Inheritance pattern
- ✓ Allele frequency
- ✓ Quality
- ✗ Known ataxia genes only

Review genotypes from 68 records returned.

Download Results | View parameters

Genomic Position	Reference Allele	Alternate Allele	Family Number	Clinical Diagnosis	Variant Type	Gene Name	Protein Notation	Maverick Score (REC)	Functional Rating	Conservation Rating	Allele Frequency
Chr11:108115638	G/G	G/T	AAD-...	183518	Missense	ATM	Leu262Phe	0.53	★	★★	Private
Chr11:108175463	A/A	A/T	AAD-CA...	183518	Missense	ATM	Asp1853Val	0.00	★★★	★★★★	Rare
Chr17:65907298	A/A	A/G	AAD-CA...	183518	Missense	BPTF	Lys1100Glu	0.00	★	★★★★	Rare
Chr17:65907644	T/T	T/C	AAD-CA...	183518	Missense	BPTF	Ile1215Thr	0.00		★	Rare
Chr11:64604201	GAA/GAA	GAA/G	AAD-CA...	183518	Splice region	CDC42BPG		N/A			Private
Chr11:64607024	C/C	C/T	AAD-CA...	183518	Missense	CDC42BPG	Val201Ile	0.00		★	Rare
Chr4:104030085	C/C	C/T	AAD-CA...	183518	Missense	CENPE	Arg2629Gln	0.00		★	Rare
Chr4:104070041	G/G	G/A	AAD-CA...	183518	Missense	CENPE	Thr1268Ile	0.00		★	Other
Chr4:104080217	A/A	A/C	AAD-CA...	183518	Missense	CENPE	Ser851Ala	0.00	★	★★★	Other
ChrX:47486217	C/C	T/T	AAD-CA...	183518	Missense	CFP	Asp299Asn	0.00	★	★★	Rare
Chr20:61452878	C/C	C/A	AAD-CA...	183518	Missense	COL9A3	Pro122His	0.32	★★★★	★★★	Rare
Chr20:61453970	G/G	G/T	AAD-CA...	183518	Missense	COL9A3	Ala169Ser	0.06		★	Rare
Chr8:144940649	G/G	G/A	AAD-CA...	183518	Missense	EPPK1	Thr2258Met	0.59	★★	★★★★	Rare
Chr8:144941879	G/G	G/A	AAD-CA...	183518	Missense	EPPK1	Ala1848Val	0.00		★	Rare

Example: recessive ataxia family (AAD-CAE-JUL-252-003)

- Inheritance pattern
- Allele frequency
- Quality
- Known ataxia genes only

Review genotypes from 4 records returned.

Download Results | View parameters

Genomic Position	Reference Allele	Alternate Allele	Family Number	Clinical Diagnosis	Variant Type	Gene Name	Protein Notation	Maverick Score (REC)	Functional Rating	Conservation Rating	Allele Frequency
Chr11:108115638	G/G	G/T	AAD-CA...	183518	Missense	ATM	Leu262Phe	0.53	★	★★	Private
Chr11:108175463	A/A	A/T	AAD-CA...	183518	Missense	ATM	Asp1853Val	0.00	★★★	★★★★	Rare
Chr16:732223	C/C	C/T	AAD-CA...	183518	Missense	STUB1	Pro243Leu	0.72	★★★★	★★★★	Rare
Chr16:731512	A/A	A/C	AAD-CA...	183518	Missense	STUB1	Lys145Gln	0.01	★★	★★★★	Rare

GENESIS demo of additional details

Q&A

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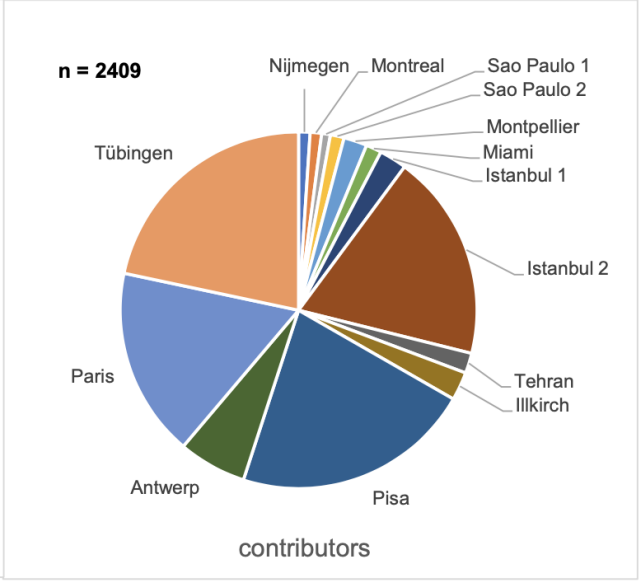
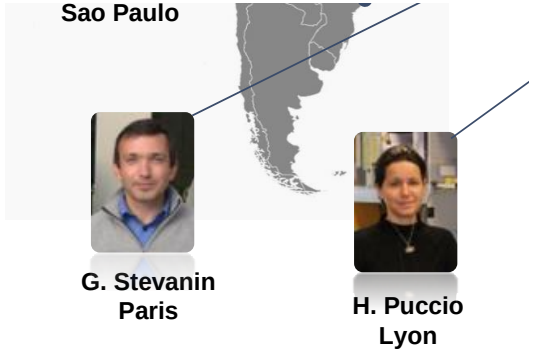
PREPARE GENESIS. The world's largest collaborative ataxia pipeline

use of a shared web-based NGS pipelin

GENESIS



GENESIS 2.0



The power of global NGS ataxia data aggregation: example PNPLA6

A FORM OF FAMILIAL DEGENERATION OF THE CEREBELLUM.

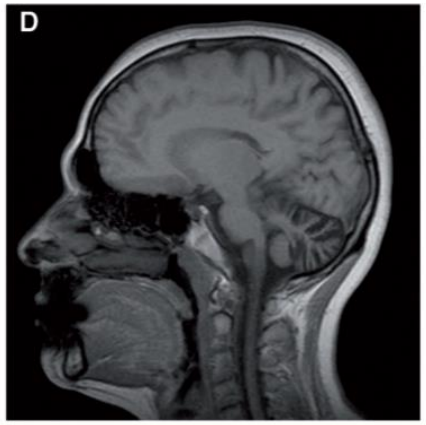
BY GORDON HOLMES, M.D.

(From the Neurological Research Department, National Hospital, Queen Square, London.)

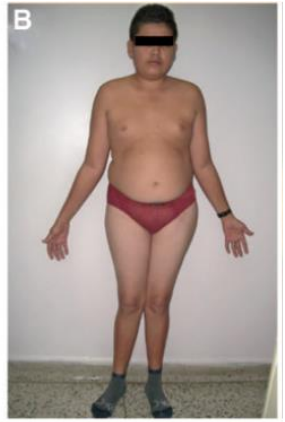
November 1907, *BRAIN*

Boucher Neuhäuser Syndrome

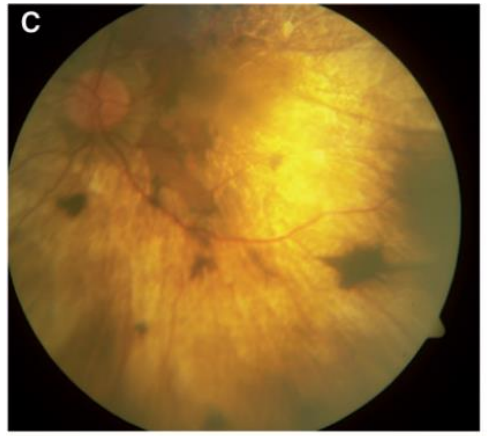
Gordon Holmes Syndrome



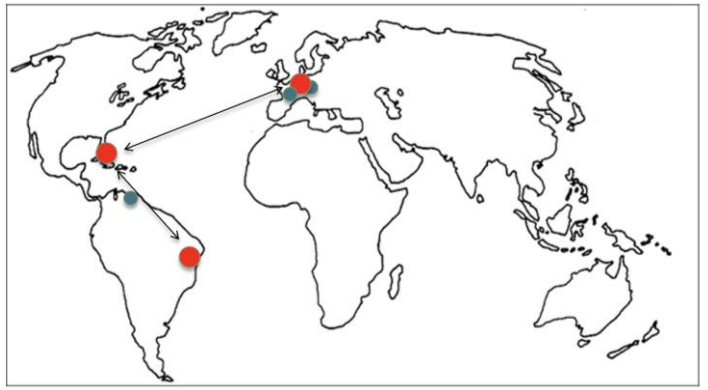
cerebellar atrophy



hypogonadism

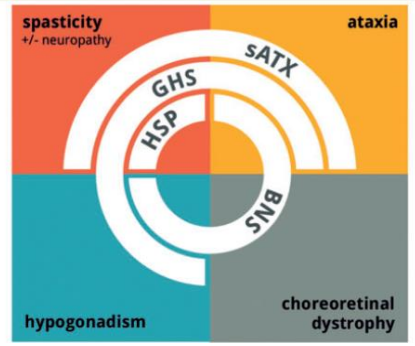


chorioretinal dystrophy



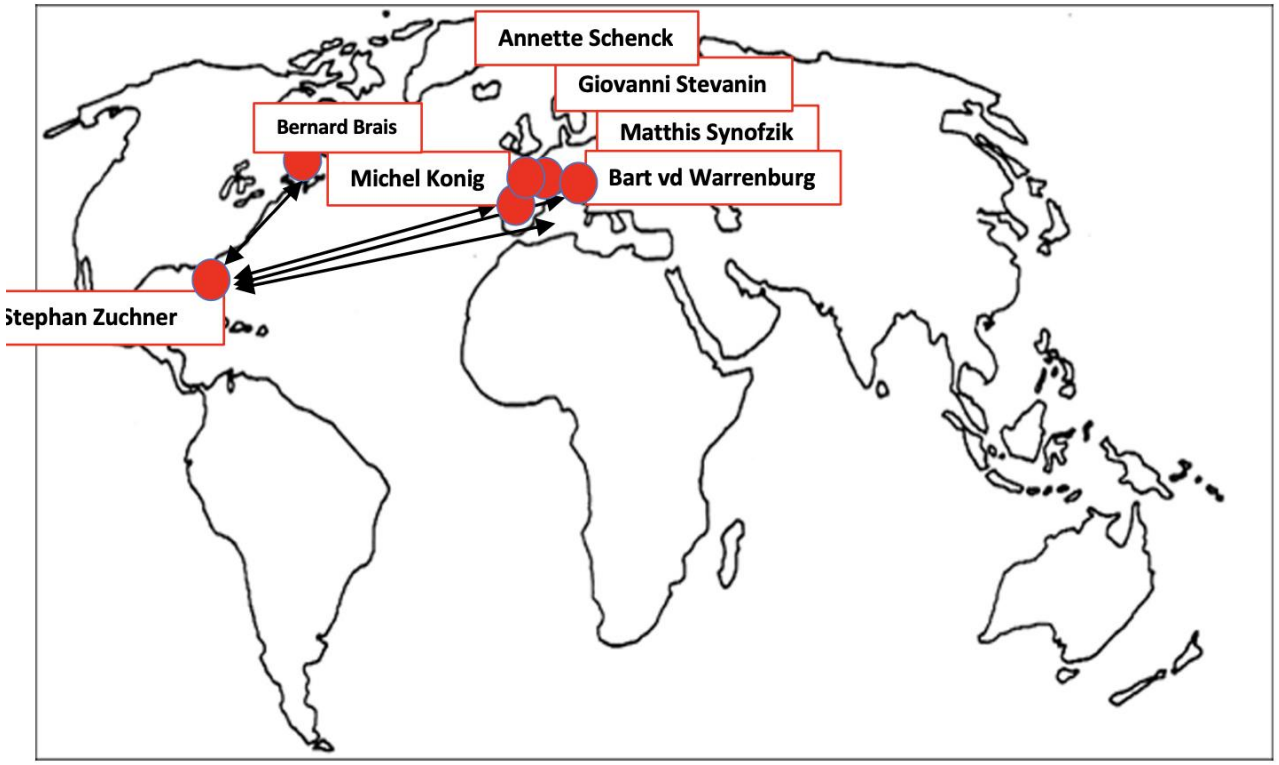
GENESIS 2.0

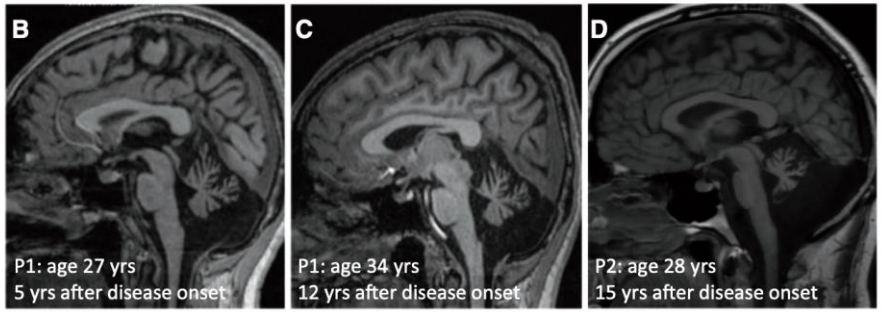
PNPLA6 (phospholipid remodelling enyzme)

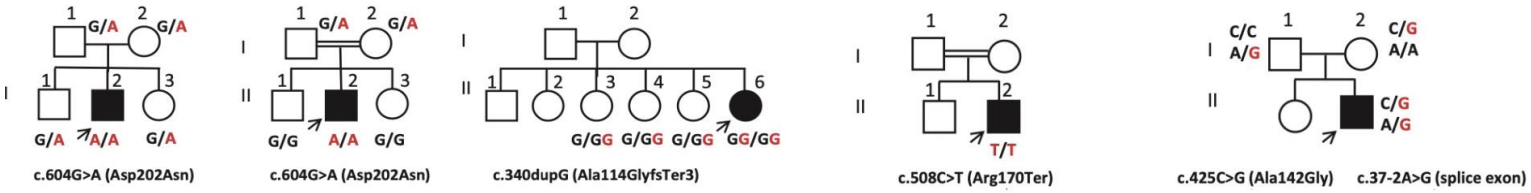


Synofzik et al, 2014, *BRAIN*

The power of global NGS ataxia data aggregation: PRDX3







BRAIN
ORIGINAL ARTICLE

Biallelic loss-of-function variations in PRDX3 cause cerebellar ataxia

Adriana P. Rebelo,[1] Ilse Eidhof,[2] Vivian P. Cintra,[1] Léna Guillot-Noel,[3,4] Claudia V. Pereira,[5] Dagmar Timmann,[6] Andreas Traschütz,[7,8] Ludger Schöls,[7,8] Giulia Coarelli,[3] Alexandra Durr,[3,9] Mathieu Anheim,[10,11] Christine Tranchant,[10,11] Bart van de Warrenburg,[12] Claire Guissart,[13] Michel Koenig,[13] Jack Howell,[1] Carlos T. Moraes,[5] Annette Schenck,[2] Giovanni Stevanin,[3,4] Stephan Züchner[1,2] and Matthis Synofzik[7,8,2] on behalf of the PREPARE network

Rebelo et al, 2021, *BRAIN*

3 key collaborative NGS platforms used by the AGI

	GENESIS	GPAP	CAGC
go-live date	year 2011	year 2015	year 2020
ATX NGS datasets	2409	1746	548
new ATX genes	17	number not actively tracked	1

➔ full systematic overview table ready for circulation for all AGI members

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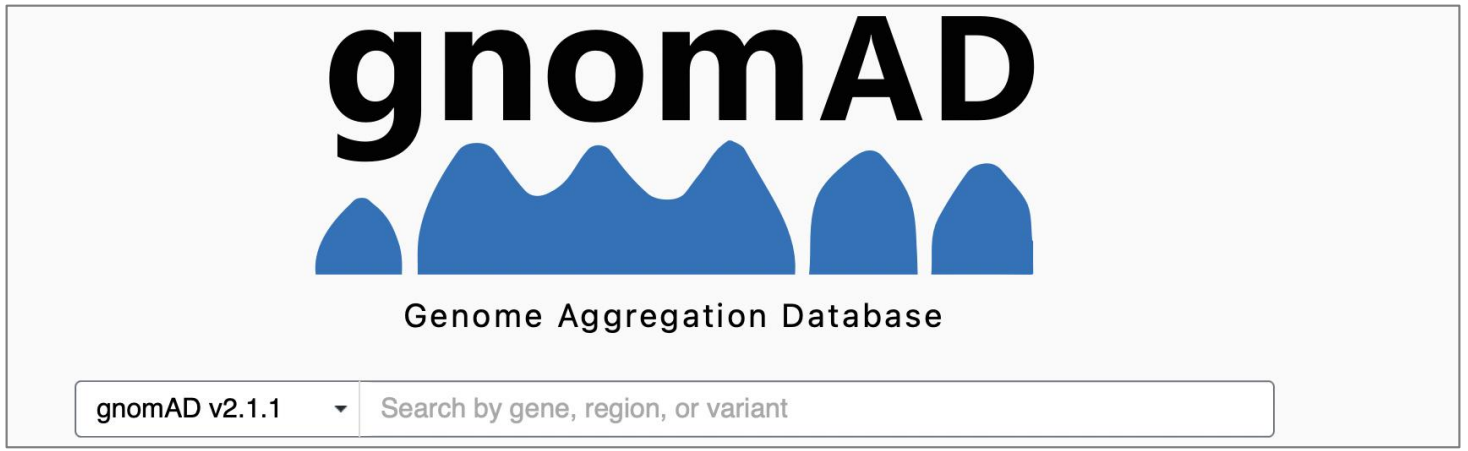
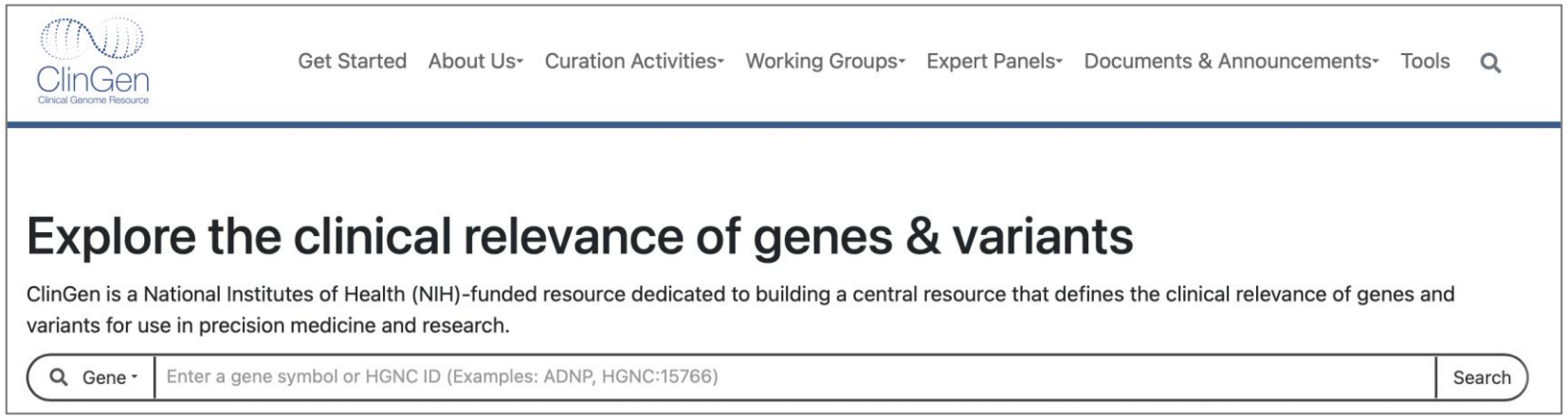
outline of the webinar

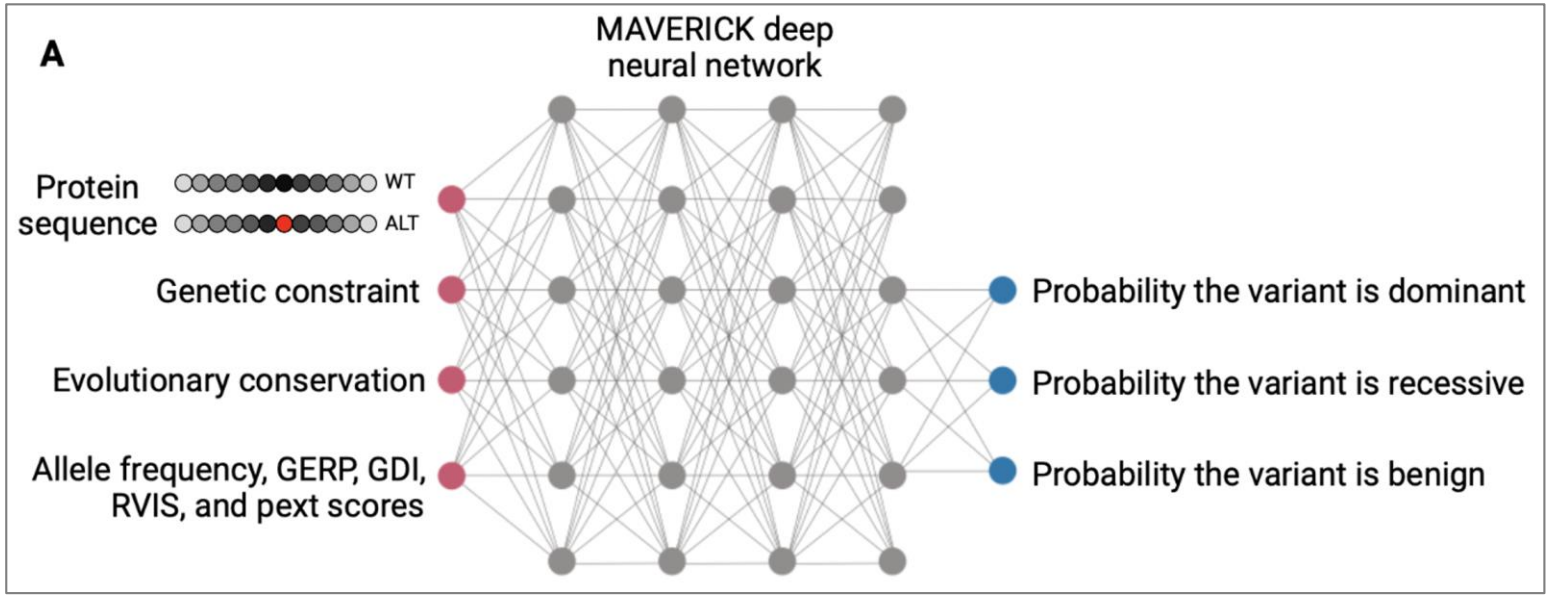
1. introduction
2. *Sanger, panel, exome, genome*: what are the differences, strengths and weaknesses?
3. *filter settings*: the crucial key in understanding NGS diagnostic and research reports
4. major international NGS ataxia pipelines - and ways to contribute
5. outlook: the next steps in ataxia genomics
6. discussion & questions

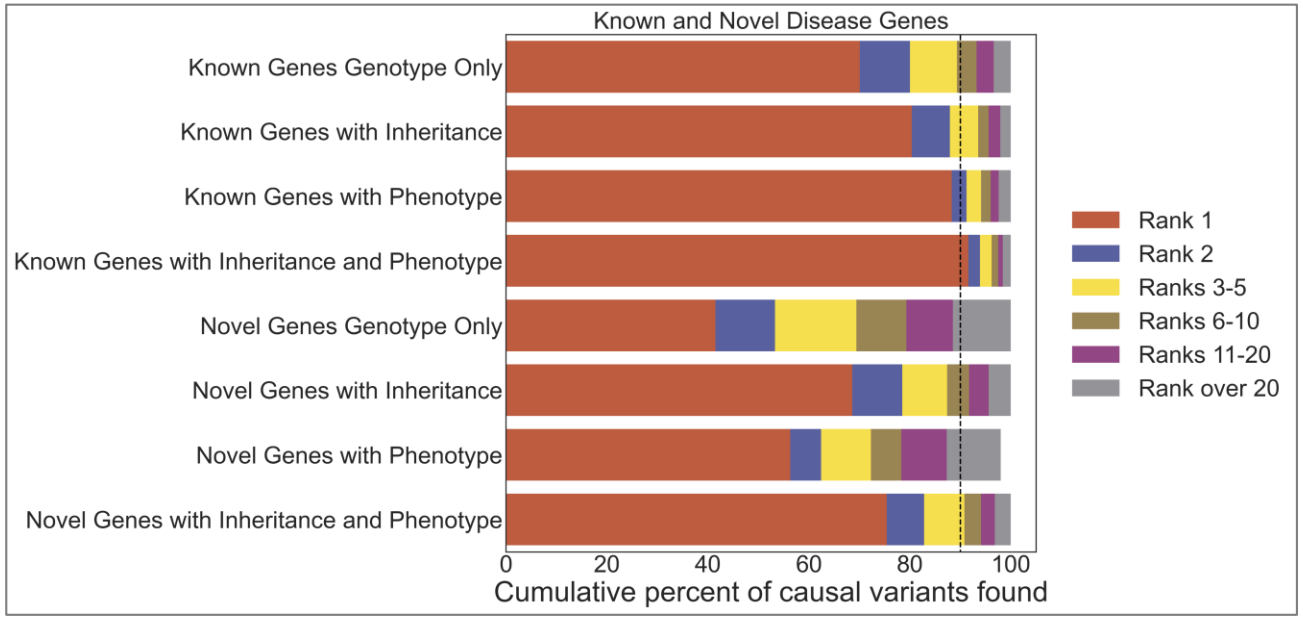
Better public resources





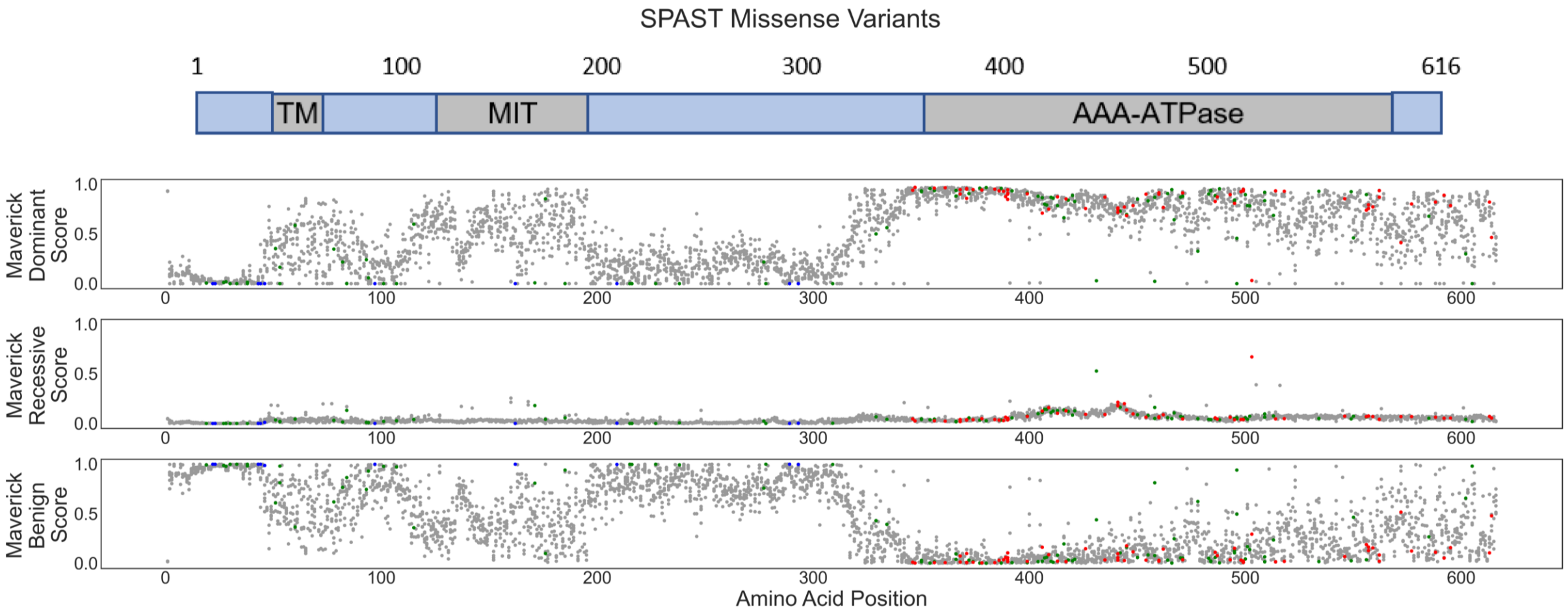
Machine learning in neural networks (MAVERICK)





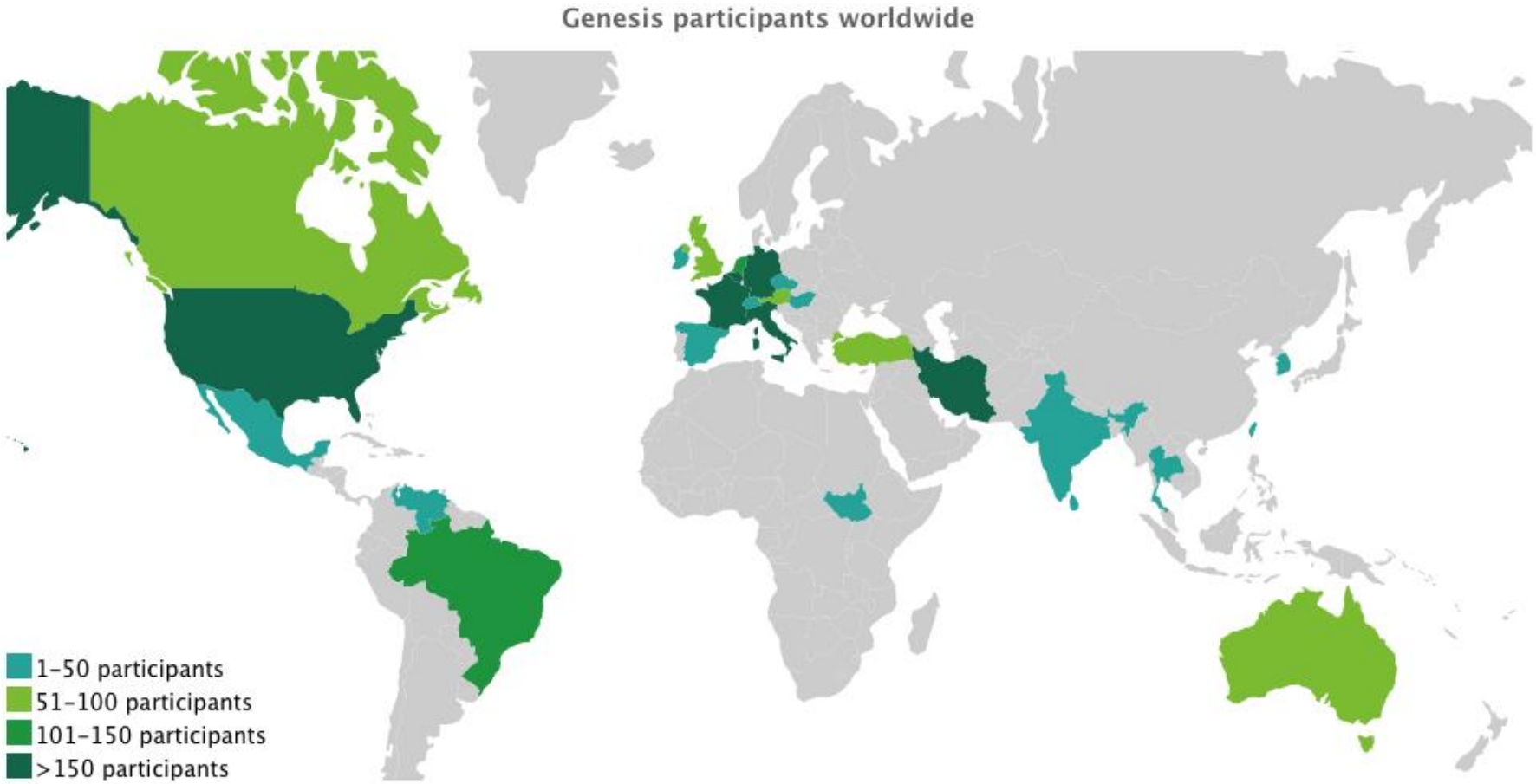
- We have created a transformer based neural network to predict pathogenicity
- Currently tested within GENESIS

Pathogenicity simulation of all possible changes in diseases genes



Known pathogenic or benign variants in SPAST

Global rare disease database GENESIS with >16,000 datasets



>80 Mendelian gene discoveries. **~200 citations** **70 phenotypes (mostly neuromuscular/ neurodegenerative)**

GENESIS: a success story of a collaborative ataxia NGS pipeline

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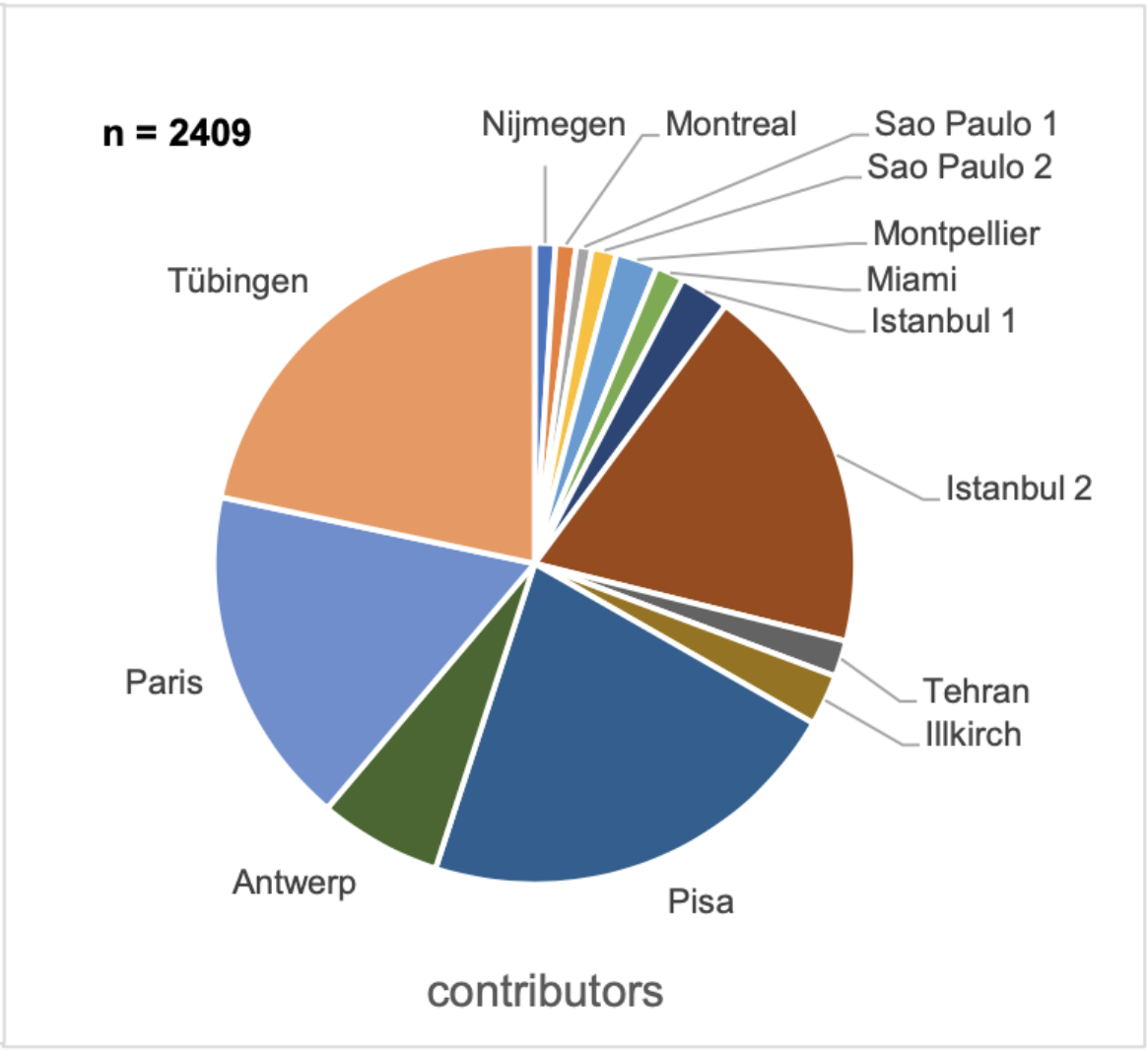
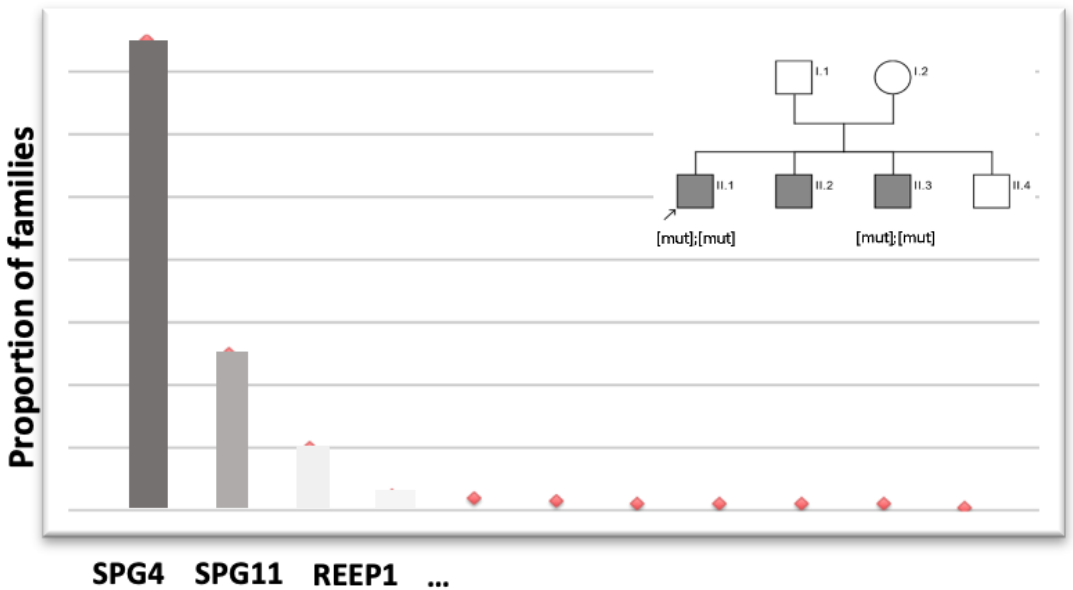
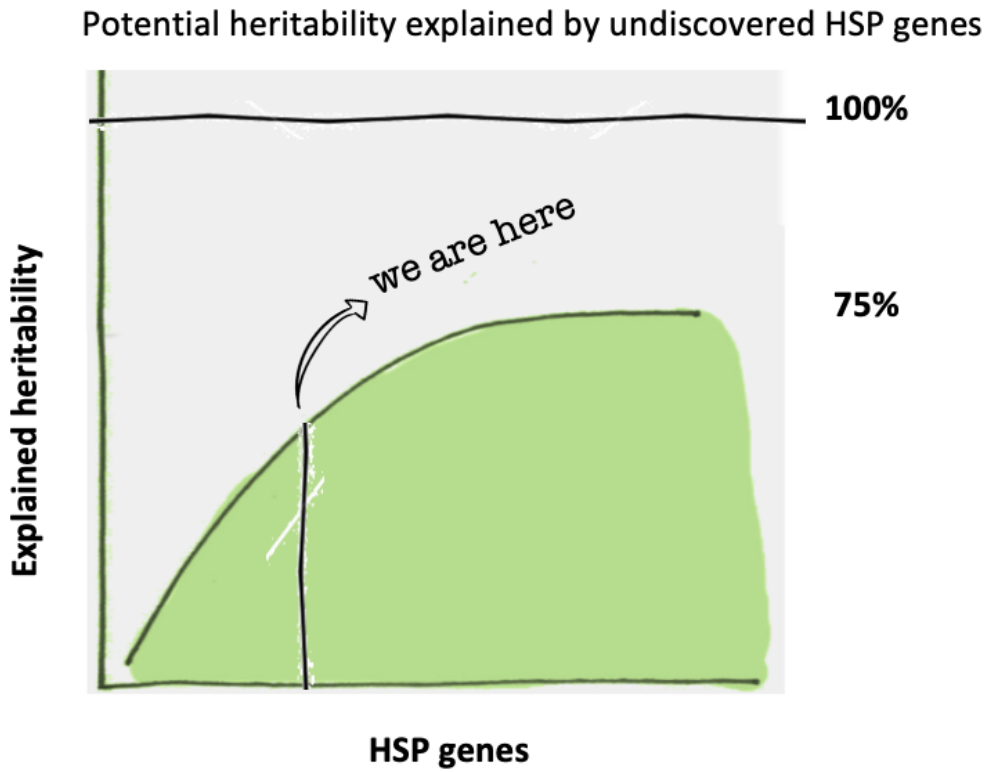


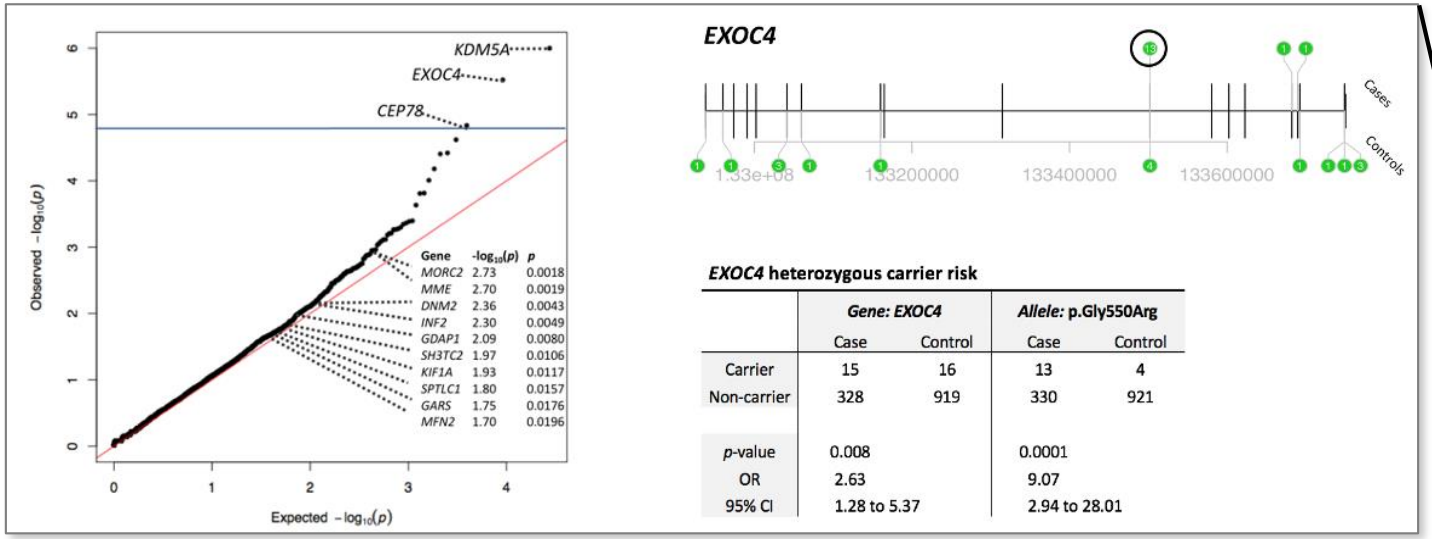
Remaining Heritability

- Some 30% of cases with HSP and related disorders are genetically undiagnosed
- New genes explain low proportion of heritability
- Many remaining genes not reaching 100%



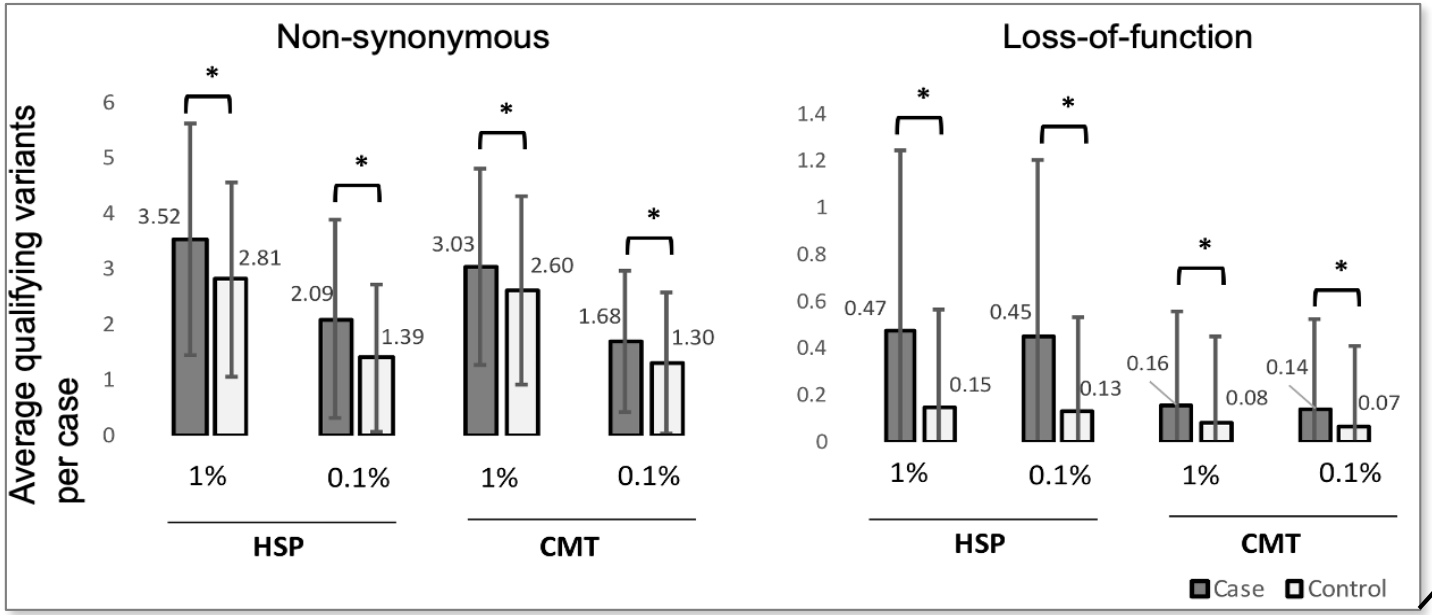


Rare Variant Burden on HSP and CMT genes



***EXOC4* heterozygous carrier risk**

	Gene: EXOC4		*Allele:* p.Gly550Arg	
	Case	Control	Case	Control
Carrier	15	16	13	4
Non-carrier	328	919	330	921
p-value	0.008		0.0001	
OR	2.63		9.07	
95% CI	1.28 to 5.37		2.94 to 28.01	



Exome Sequence Analysis Suggests That Genetic Burden Contributes to Phenotypic Variability and Complex Neuropathy

Claudia Gonzaga-Jauregui [1], Tamar Harel [2], Tomasz Gambin [2], Maria Kousi [3], Laurie B Griffin [4], Ludmila Francescatto [3], Burcak Ozes [5], Ender Karaca [2], Shalini N Jhangiani [6], Matthew N Bainbridge [6], Kim S Lawson [7], Davut Pehlivan [2], Yuji Okamoto [2], Marjorie Withers [2], Pedro Mancias [8], Anne Slavotinek [9], Pamela J Reitnauer [10], Meryem T Goksungur [11], Michael Shy [12], Thomas O Crawford [13], Michel Koenig [14], Jason Willer [3], Brittany N Flores [15], Igor Pediaditrakis [3], Onder Us [16], Wojciech Wiszniewski [2], Yesim Parman [11], Anthony Antonellis [17], Donna M Muzny [6], Baylor-Hopkins Center for Mendelian Genomics; Nicholas Katsanis [3], Esra Battaloglu [5], Eric Boerwinkle [18], Richard A Gibbs [19], James R Lupski [20]

Genetics inMedicine 2020

Assessing non-Mendelian Inheritance in Inherited Axonopathies

Dana M. Bis-Brewer[1], Ziv Gan-Or[3,4,12], Patrick Sleiman[2], Inherited Neuropathy Consortium, Hakon Hakonarson[2], Sarah Fazal[1], Steve Courel[1], Vivian Cintra[1], Feifei Tao[1], Mehrdad A. Estiar[3,4], Mark Tarnopolsky[5], Kym M. Boycott[6], Grace Yoon[7,8], Oksana Suchowersky[9], Nicolas Dupré[10,11], Andrew Cheng[13], Thomas E. Lloyd[13], Guy Rouleau[3,4,12], Rebecca Schüle[14], Stephan Züchner[1]

Dana Bis, PhD

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THANK YOU! ☺