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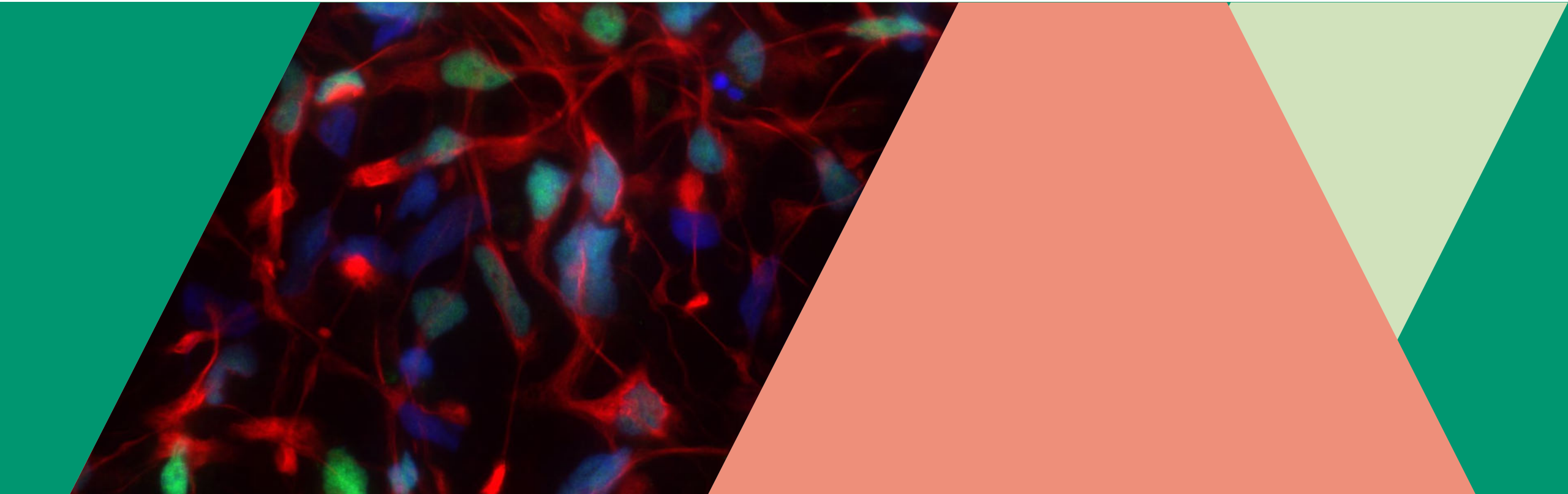
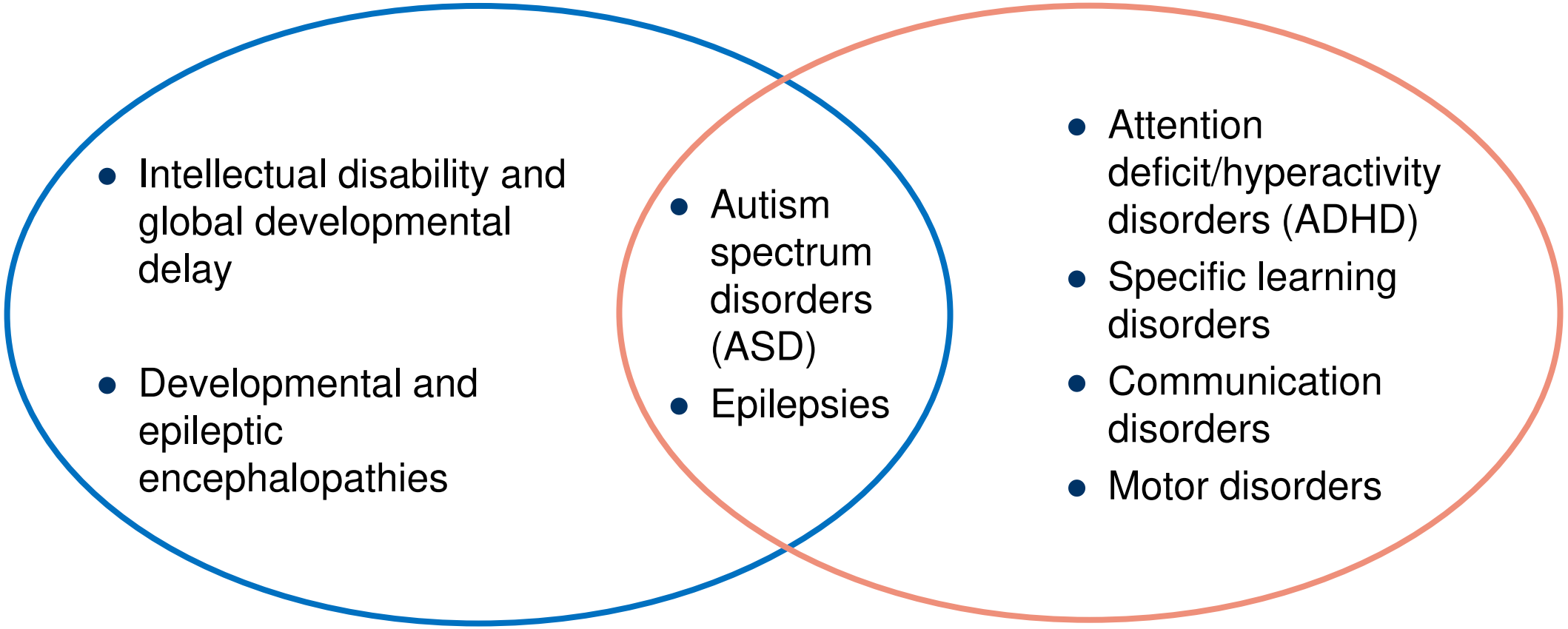
Genetics of neurodevelopmental disorders (NDDs)

Dr. rer. nat. Anne Gregor, Department of Human Genetics



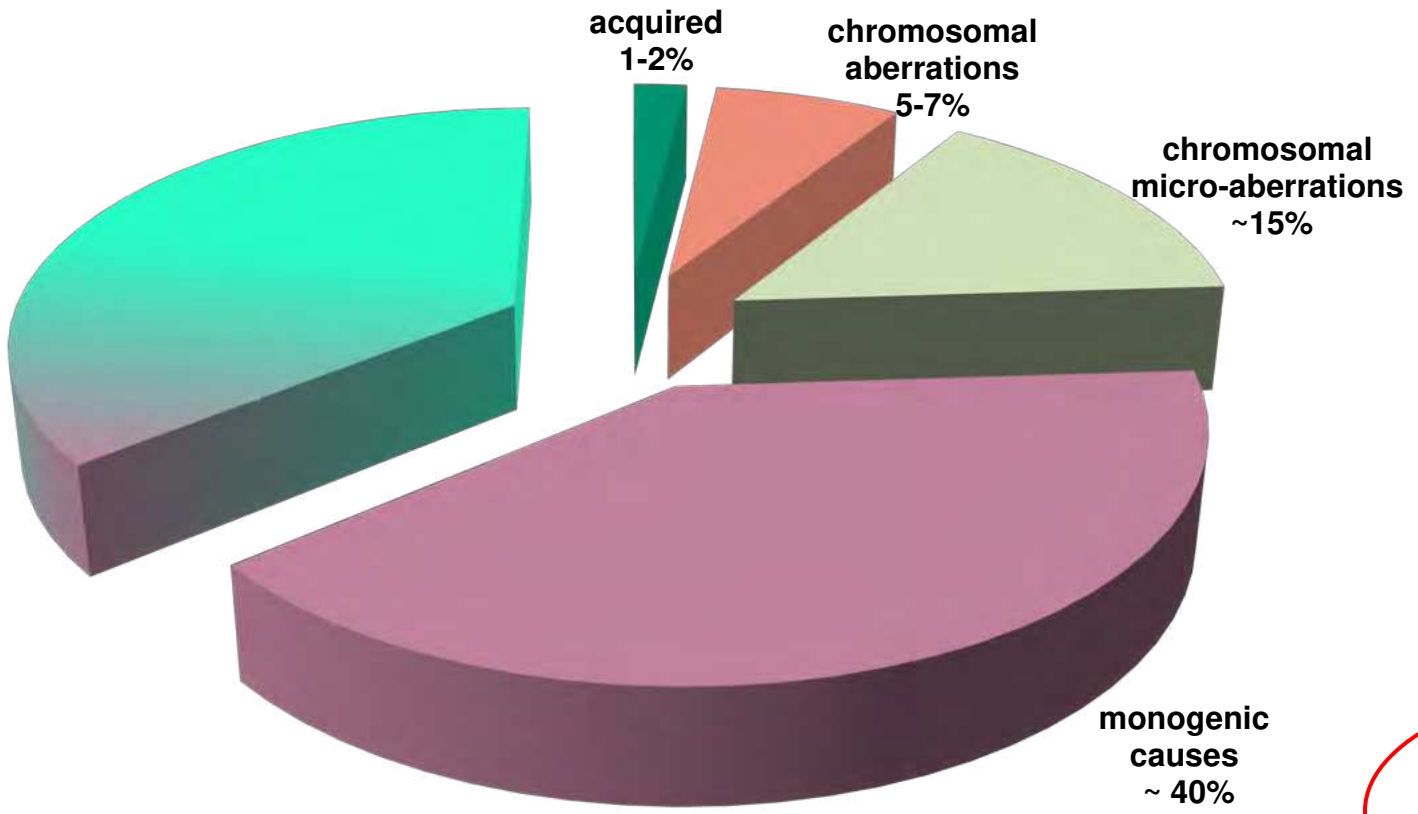
Which NDDs are relevant for genetic testing?



chromosomal / monogenic

multifactorial/genetically complex

Genetic heterogeneity of neurodevelopmental disorders



November 2023: > 1600 confirmed disease genes, > 1300 candidate genes

genetic causes of neurodevelopmental disorders

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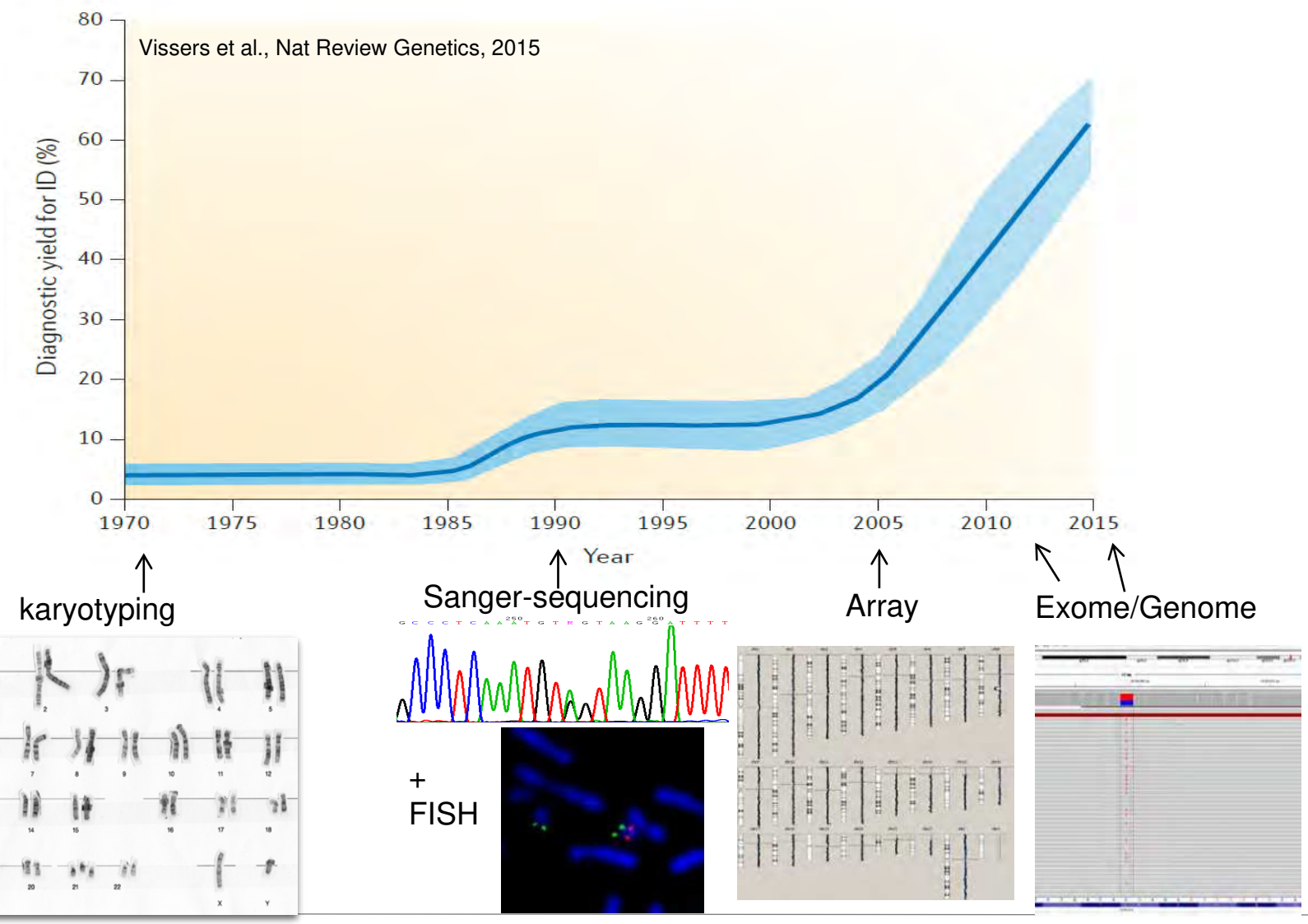
What is the benefit of having a genetic diagnosis?

- **Knowledge about cause of symptoms/disorder**
- better prognosis of clinical course
- **potential preventive (screening) tests and checkups**
- assessment of recurrent risks

  → de novo or inherited?

  → poss. pre-implantation diagnostics / prenatal diagnostics
- consequences for specific therapies (e.g. antiepileptic drugs)
- no further diagnostic odyssey
- communication with other affected families

Detection rate in diagnostics of rare disorders



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Recurrent microdeletions and -duplications

Microdeletion syndromes 1991-2006 (pre- array)	«new», recurrent microdeletions and -duplications
1p36.3 deletion syndrome	1q21.1 deletion/duplication
2q37 Albright-Osteodystrophy-like syndrome	2q11.2 deletion/duplication
4p16 Wolf-Hirschhorn syndrome	2q21.1 deletion
5p15 Cri-du-chat syndrome	3q29 deletion/duplication
7q11.23 Williams(-Beuren) syndrome	8p23.1 deletion/duplication
15q12 Prader-Willi / Angelman syndromes	15q11.2 deletion/duplication
17p13.3 Miller-Dieker syndrome	15q13.3 deletion/duplication
17p11.2 Smith-Magenis syndrome	16p11.2 deletion/duplication
22q11.2 deletion / DiGeorge / Shprintzen syndrome	16p12.1 deletion/duplication
	16p13.11 deletion/duplication
	17q12 deletion
	17q21.31 deletion

Next Generation Sequencing: a booster in NDD gene identification

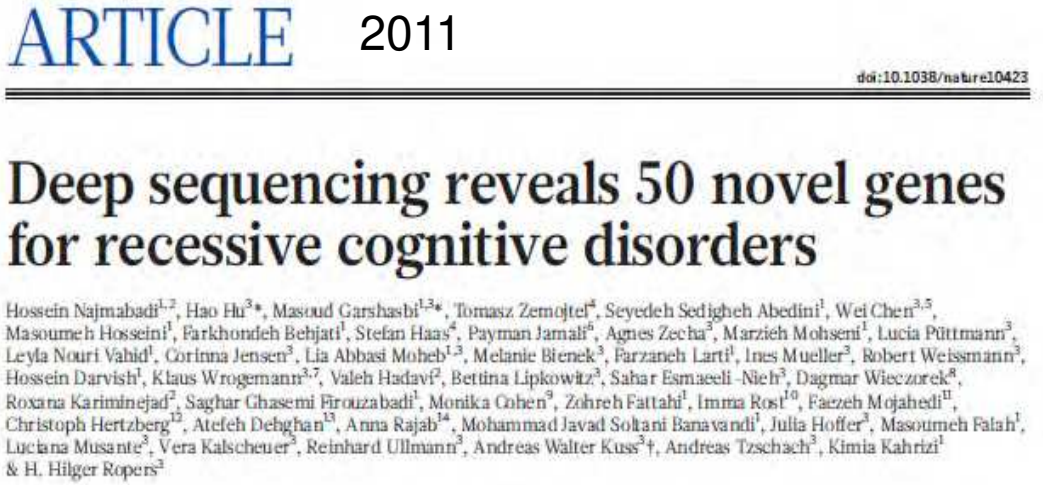

LETTERS

nature genetics 2010

A *de novo* paradigm for mental retardation

Lisenka E L M Vissers[1,2], Joep de Ligt[1,2], Christian Gilissen[1], Irene Janssen[1], Marloes Steehouwer[1], Petra de Vries[1], Bart van Lier[1], Peer Arts[1], Nienke Wieskamp[1], Marisol del Rosario[1], Bregje W M van Bon[1], Alexander Hoischen[1], Bert B A de Vries[1], Han G Brunner[1,3] & Joris A Veltman[1,3]

Trio sequencing


ARTICLE 2011

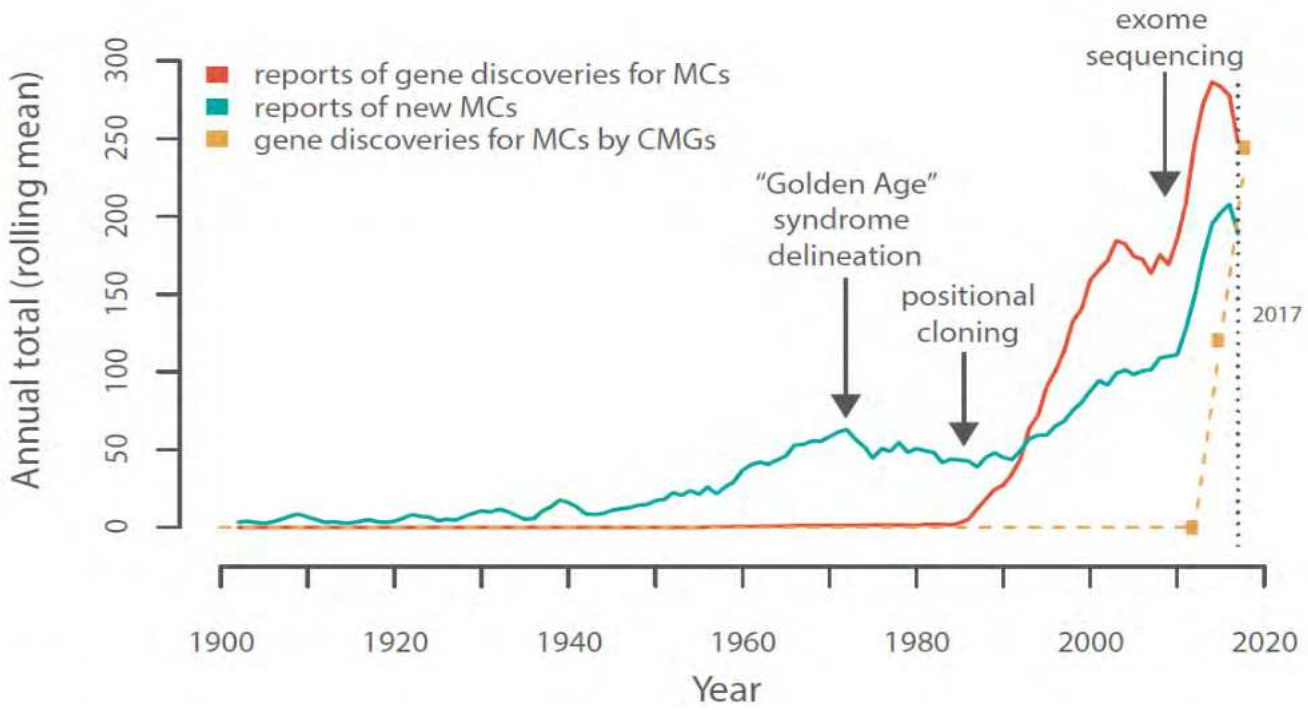
doi:10.1038/nature10423

Deep sequencing reveals 50 novel genes for recessive cognitive disorders

Hossein Najmabadi[1,2], Hao Hu[3]*, Masoud Garshasbi[1,3]*, Tomasz Zemojtel[4], Seyedeh Sedigheh Abedini[1], Wei Chen[3,5], Masoumeh Hosseini[1], Farkhondeh Behjati[1], Stefan Haas[4], Payman Jamali[6], Agnes Zecha[3], Marzieh Mohseni[1], Lucia Püttmann[3], Leyla Nouri Vahid[1], Corinna Jensen[3], Lia Abbasi Moheb[1,3], Melanie Bienek[3], Farzaneh Larti[1], Ines Mueller[3], Robert Weissmann[3], Hossein Darvish[1], Klaus Wrogemann[3,7], Valeh Hadavi[2], Bettina Lipkowitz[3], Sahar Esmaeeli-Nieh[3], Dagmar Wieczorek[8], Roxana Kariminejad[2], Saghar Ghasemi Firouzabadi[1], Monika Cohen[9], Zohreh Fattahi[1], Imma Rost[10], Faezeh Mojahedi[11], Christoph Hertzberg[12], Atefeh Dehghan[13], Anna Rajab[14], Mohammad Javad Soltani Banavandi[1], Julia Hoffer[3], Masoumeh Falah[1], Luciana Musante[3], Vera Kalscheuer[3], Reinhard Ullmann[3], Andreas Walter Kuss[3]†, Andreas Tzschach[3], Kimia Kahrizi[1] & H. Hilger Ropers[3]

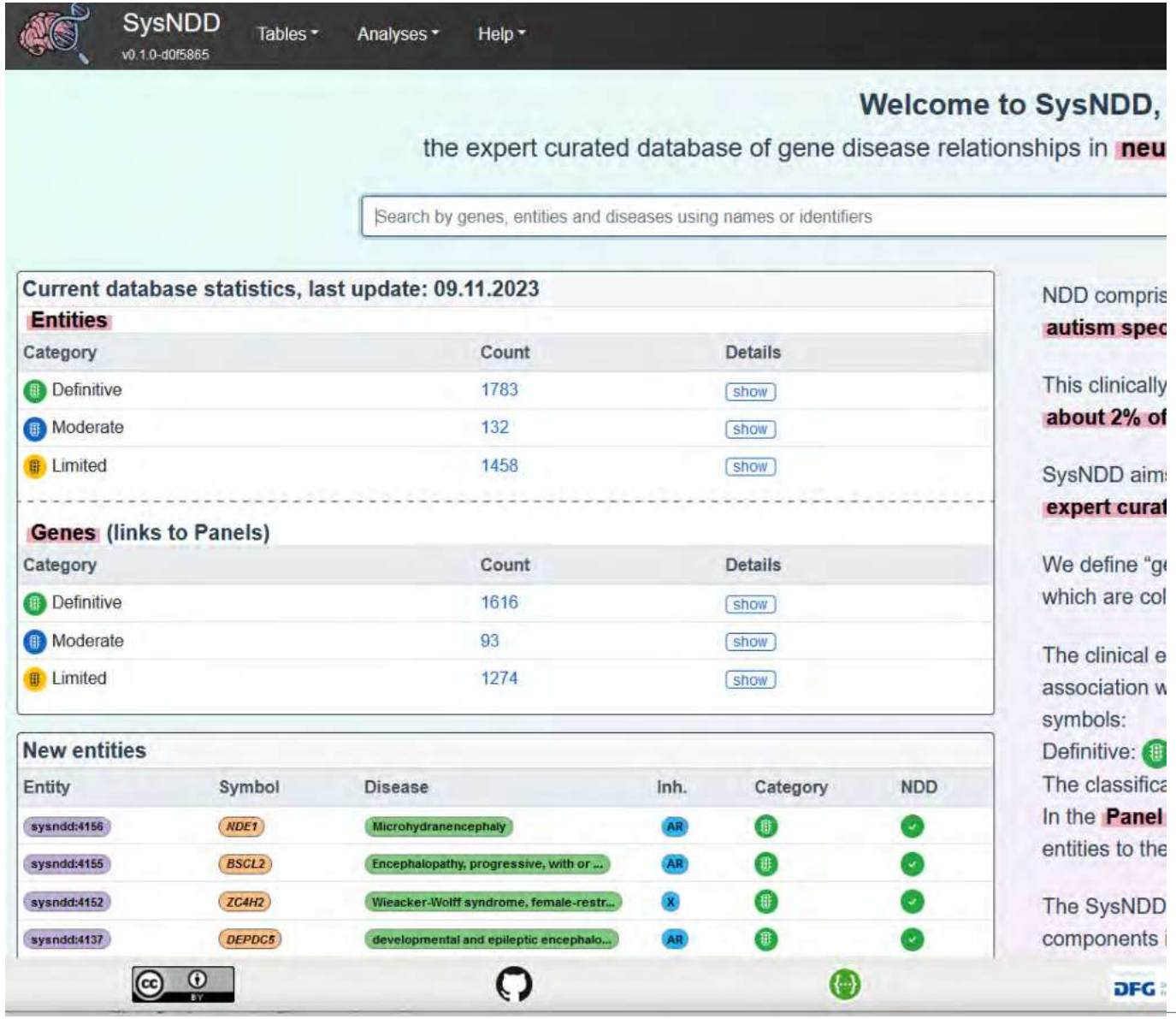
Homozygosity mapping

Gene and syndrome identification in the last decades



Bamshad et al., Mendelian Gene Discovery:
Fast and Furious with No End in Sight, AJHG, 2019

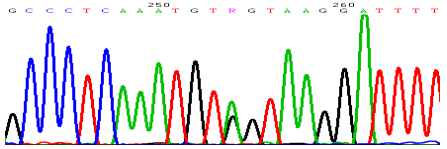
Monogenic NDDs (https://sysndd.dbmr.unibe.ch/)



- Entities: gene – inheritance – disease
- filtering possibilities: e.g. phenotypes, inheritance patterns
- Nov 2023: 1616 confirmed NDD genes

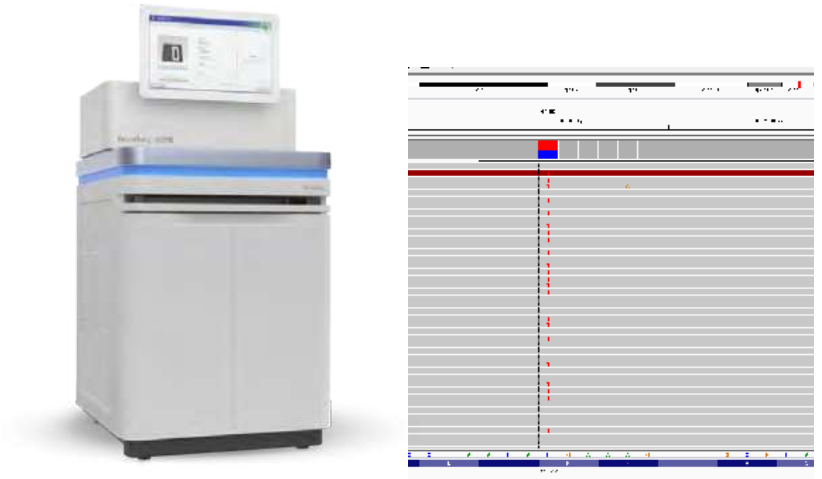
  (Aug 2016: 893 genes)
  - 60% autosomal-recessive
  - 33% autosomal-dominant
  - 7% X-linked
- plus >1300 published candidate genes

Testing for monogenic disease causes



Sanger-Sequencing

- single genes
- known (familial) variants



Next-Generation-Sequencing

- Panel-Sequencing (~ 5-100 genes)
- clinical exome/mendeliome: ~ 8000 known disease genes
- **(Trio) Exome-Sequencing: all coding genes (~ 20.500)**
- genome sequencing: coding and non-coding regions

Variant interpretation in known disease genes

Frequencies in patients and/or controls

- variant included as (likely) pathogenic in databases such as ClinVar, HGMD etc. or literature
- variant absent or very rarely in control databases such as gnomAD
- variant absent or very rarley in in-house data

Location and nature of variant

- e.g. truncating or missense
- located in functional domains or mutational hotspots

Segregation

- *de novo* or segregating with the phenotype

Functional effects

- mutational consequences by prediction programs (e.g. CADD score, PolyPhen2, Mutation Taster, REVEL)
- available information on gene/protein
- functional validation

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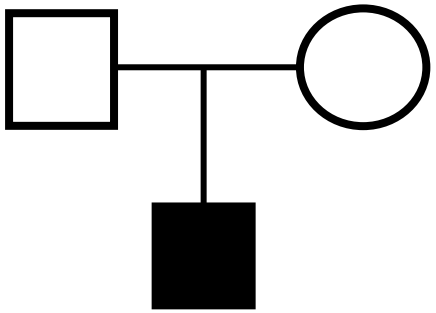
ACMG classification

- class 1: benign
- class 2: likely benign
- class 3: variant of unknown significance (VUS)
- class 4: likely pathogenic
- class 5: pathogenic

Evidence of pathogenicity		Category
Very strong	PVS1	**Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease.** *Caveats:* • *Beware of genes where LOF is not a known disease mechanism (e.g., GFAP, MYH7)* • *Use caution interpreting LOF variants at the extreme 3' end of a gene* • *Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact* • *Use caution in the presence of multiple transcripts*
Strong	PS1	**Same amino acid change as a previously established pathogenic variant regardless of nucleotide change** • *Example: Val→Leu caused by either G>C or G>T in the same codon* • *Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level*
	PS2	**De novo (both maternity and paternity confirmed) in a patient with the disease and no family history** *Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to non maternity.*
	PS3	**Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product** *Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well established.*
	PS4	**The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls** *Note 1: Relative risk or OR, as obtained from case–control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0.* *See the article for detailed guidance.* *Note 2: In instances of very rare variants where case–control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.*
Moderate	PM1	**Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation.**
	PM2	**Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium** • *Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing.*
	PM3	**For recessive disorders, detected in trans with a pathogenic variant** *Note: This requires testing of parents (or offspring) to determine phase.*
	PM4	**Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants**
	PM5	**Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before** • *Example: Arg156His is pathogenic; now you observe Arg156Cys* • *Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.*
	PM6	**Assumed de novo, but without confirmation of paternity and maternity**

Richards et al., Genetics in Medicine 2015

Trio exome/genome sequencing: first-tier test in NDDs



→ Trio exome/genome sequencing: patient plus healthy parents

→ filtering for inheritance patterns

de novo

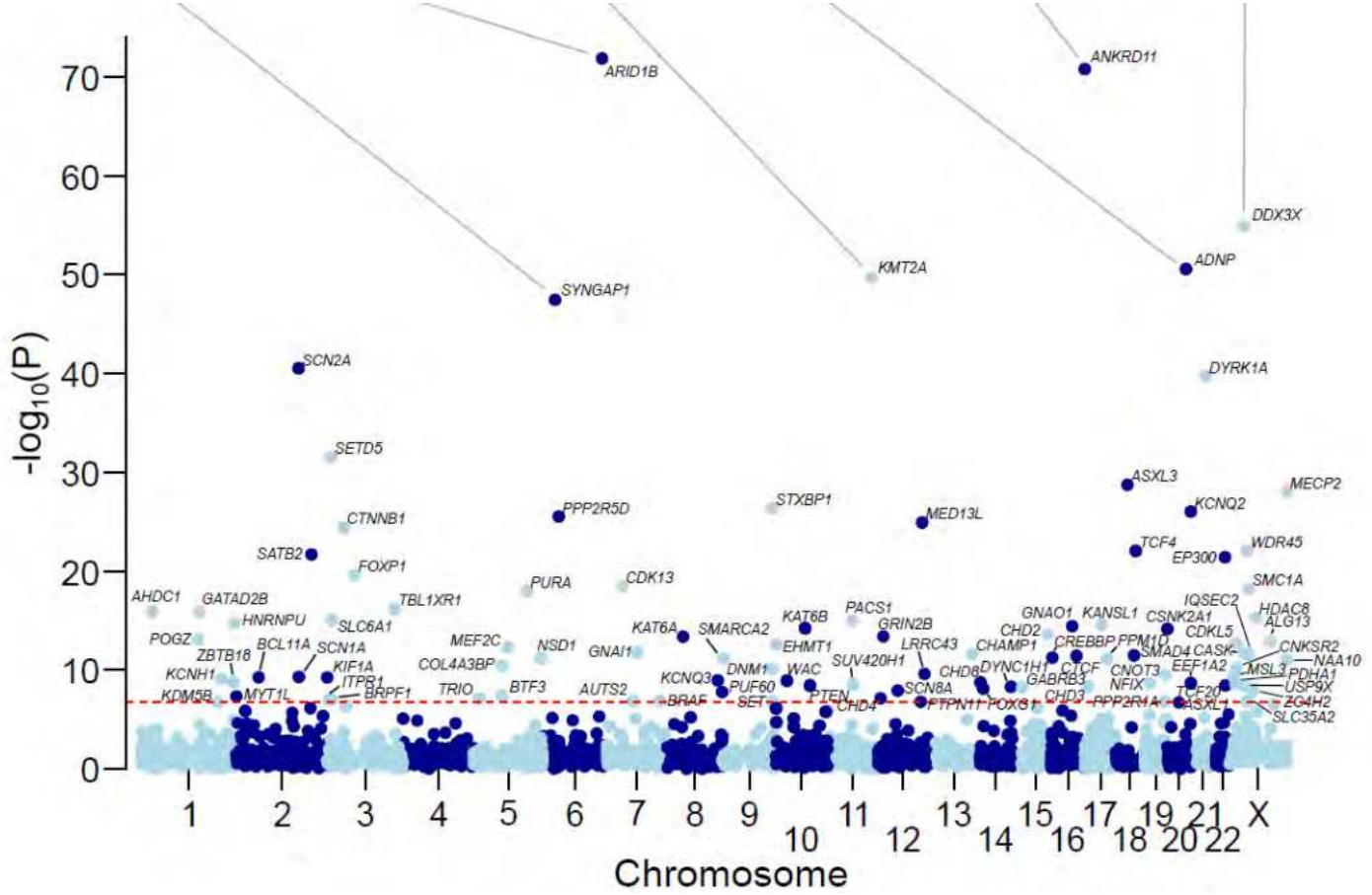
homozygous/compound heterozygous

X-chromosomal: maternally inherited

(autosomal-dominant inheritance)

pathogenic variant in known ID gene or candidate gene?

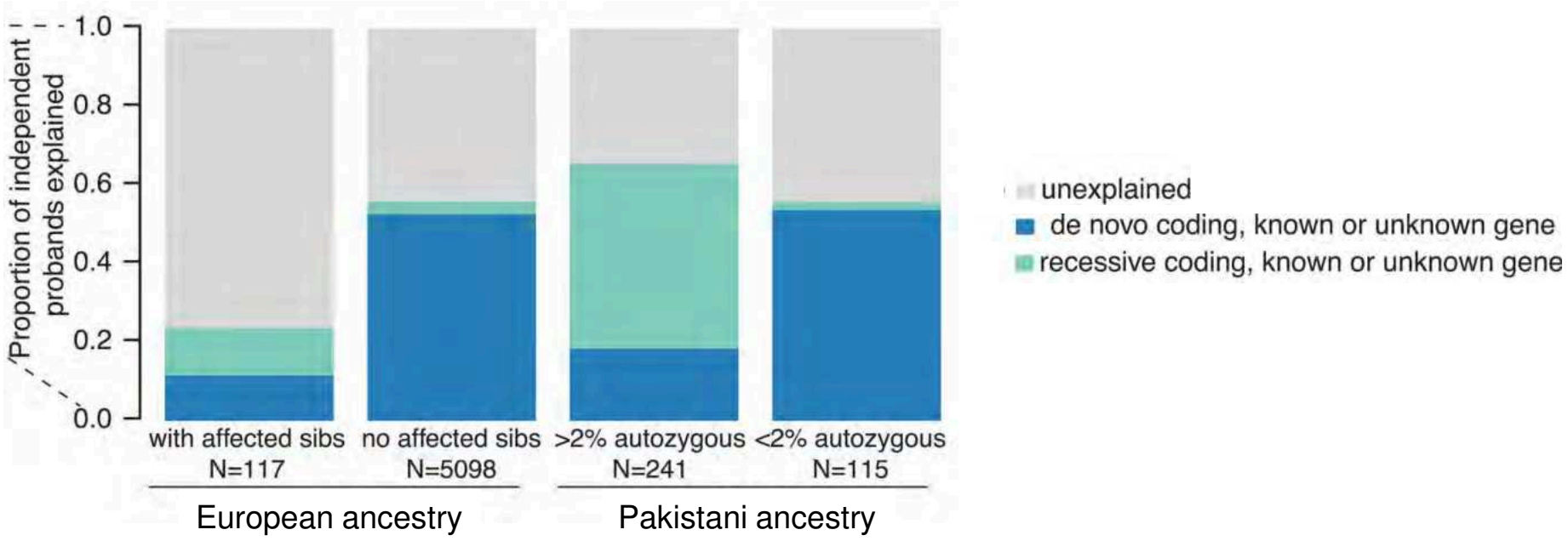
Deciphering Developmental Disorders Study in the UK



→pathogenic, *de novo* variants in the coding sequence in 42% of 7.000 patients

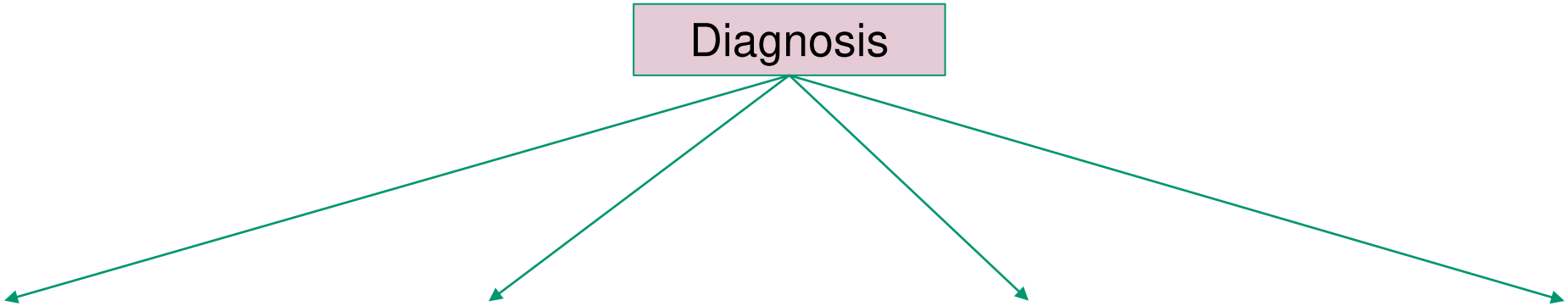
DDD Study: Mc Rae et al., Nature, 2017

Distribution *de novo* and recessive causes depends on ancestry and familial consanguinity



Martin et al., Nat Genet 2018

Different questions when making a genetic diagnosis



Correct gene?

- so far unknown gene
- genetic heterogeneity
- multiple diagnoses

Correct variant?

- VUS
- reduced penetrance
- mosaics

Matching inheritance pattern?

- 1 gene – different inheritance patterns

Does the phenotype fit?

- 1 gene – different phenotypes
- expansion of clinical spectrum
- variability and incomplete penetrance
- multiple diagnoses

Variants in unkown genes: a tiny step from research to diagnostics

- girl, 10 years
- epilepsy
- severe ID, no speech
- microcephaly
- movement disorder

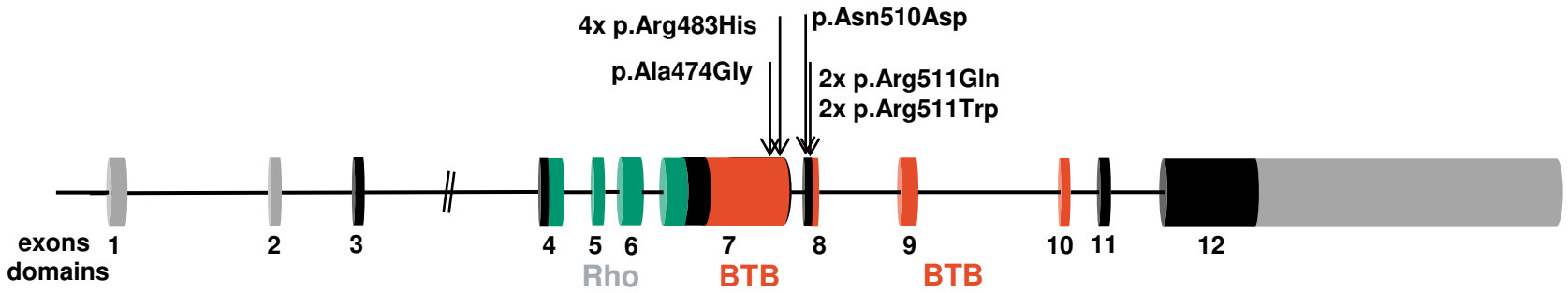
→ Trio-exome: ***de novo* missense variant in *RHOBTB2***

<u>at that time:</u>

- atypical RhoGTPase, implicated in cancer, not yet in NDDs
- highly expressed in the nervous system
- gnomAD constraint scores: z score 2.66, pLI score 0.01

«Genetic» confirmation of *RHOBTB2* as a new disease gene

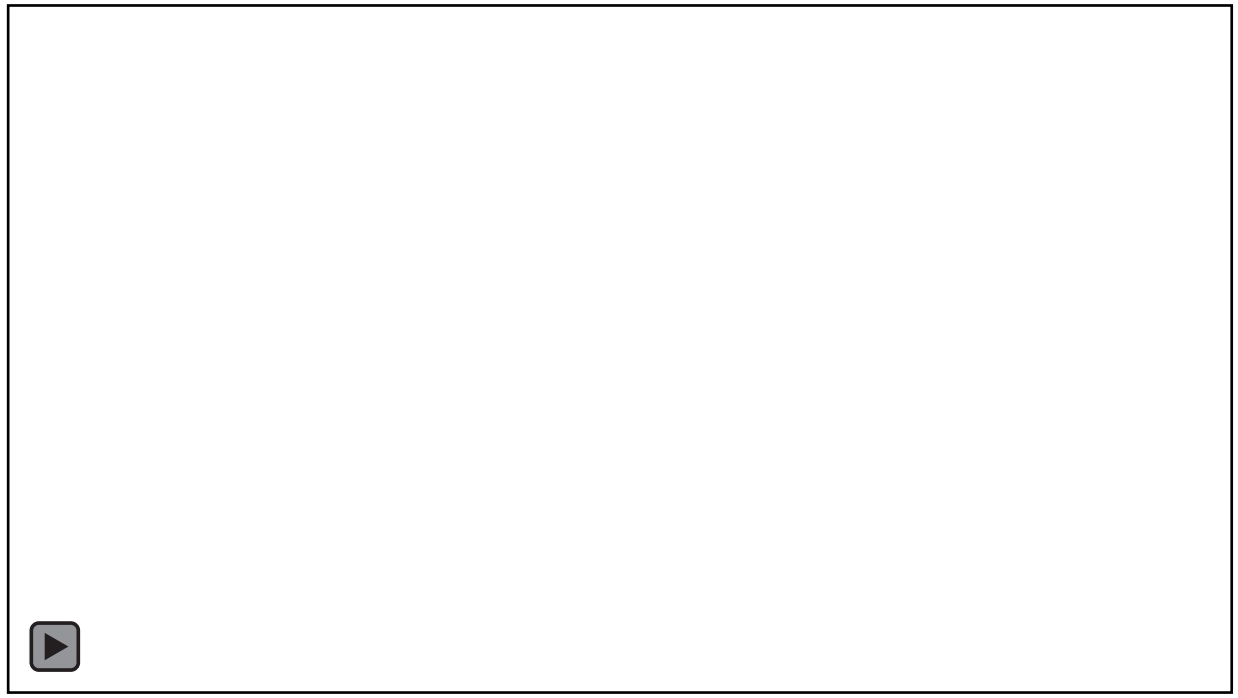
- matchmaking via **Genematcher**: 9 further cases with *de novo* variants in *RHOBTB2*
- similar phenotype: early-onset epilepsy, severe ID, neurologial anomalies, microcephaly
- similar mutational spectrum



Straub*,Konrad* et al., AJHG, 2018

Further support from model systems

- Bang sensitivity (seizure susceptibility) upon pan-neuronal RhoBTB-overexpression in *Drosophila*

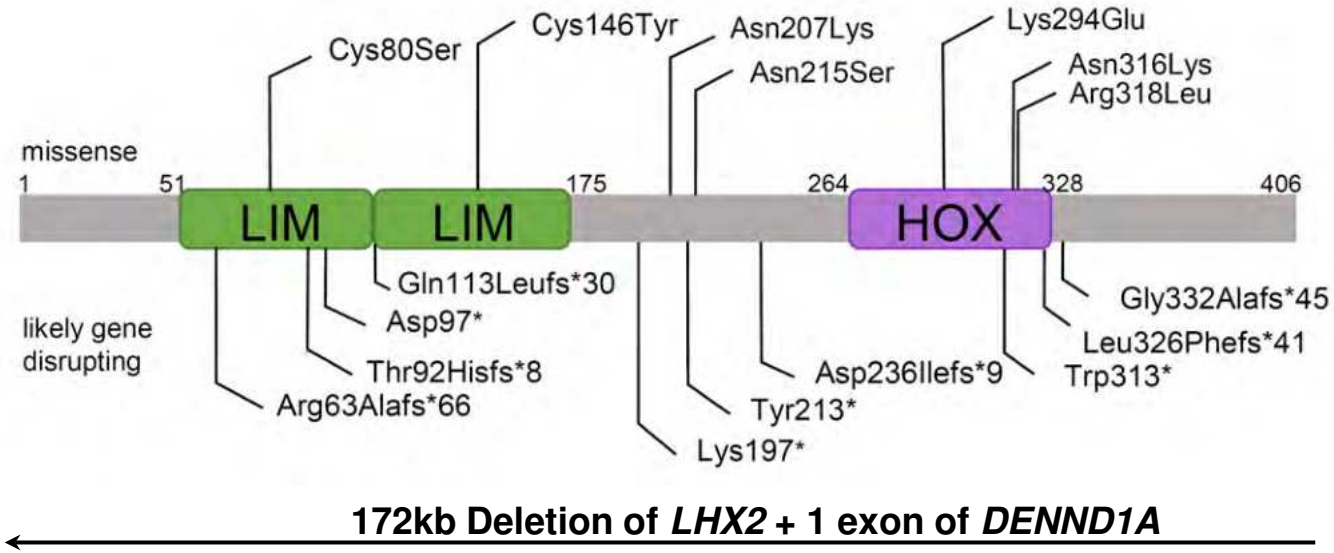


→ close link between diagnostics and research aids discovery of novel NDD-associated genes

Straub*,Konrad* et al., AJHG, 2018

Assessment of variant pathogenicity: Missense variants in *LHX2*

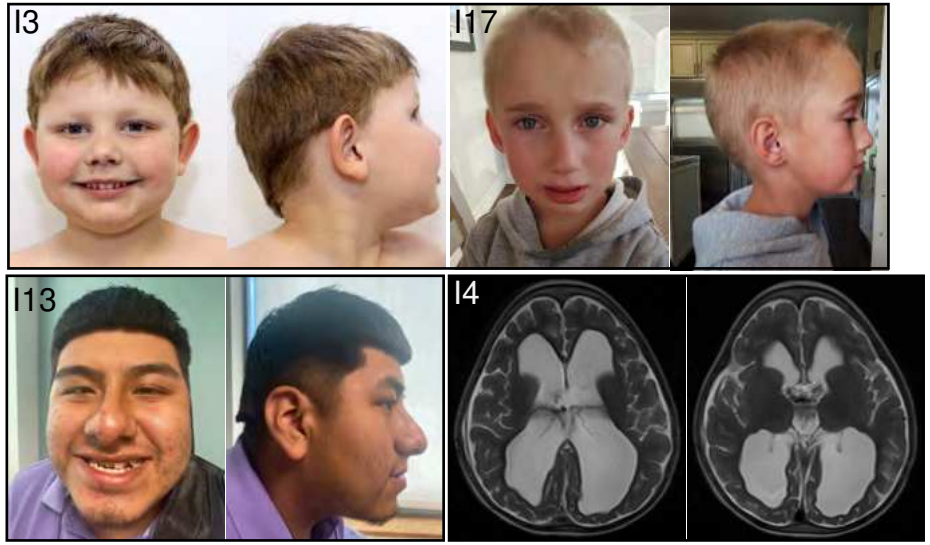
Correct variant?



172kb Deletion of *LHX2* + 1 exon of *DENND1A*

key clinical features

- intellectual disability
- behavioral anomalies
- microcephaly



gnomAD constraint scores:

pLI = 1

Z = 2.03

Schmid*, Gregor* et al., Genet Med 2023

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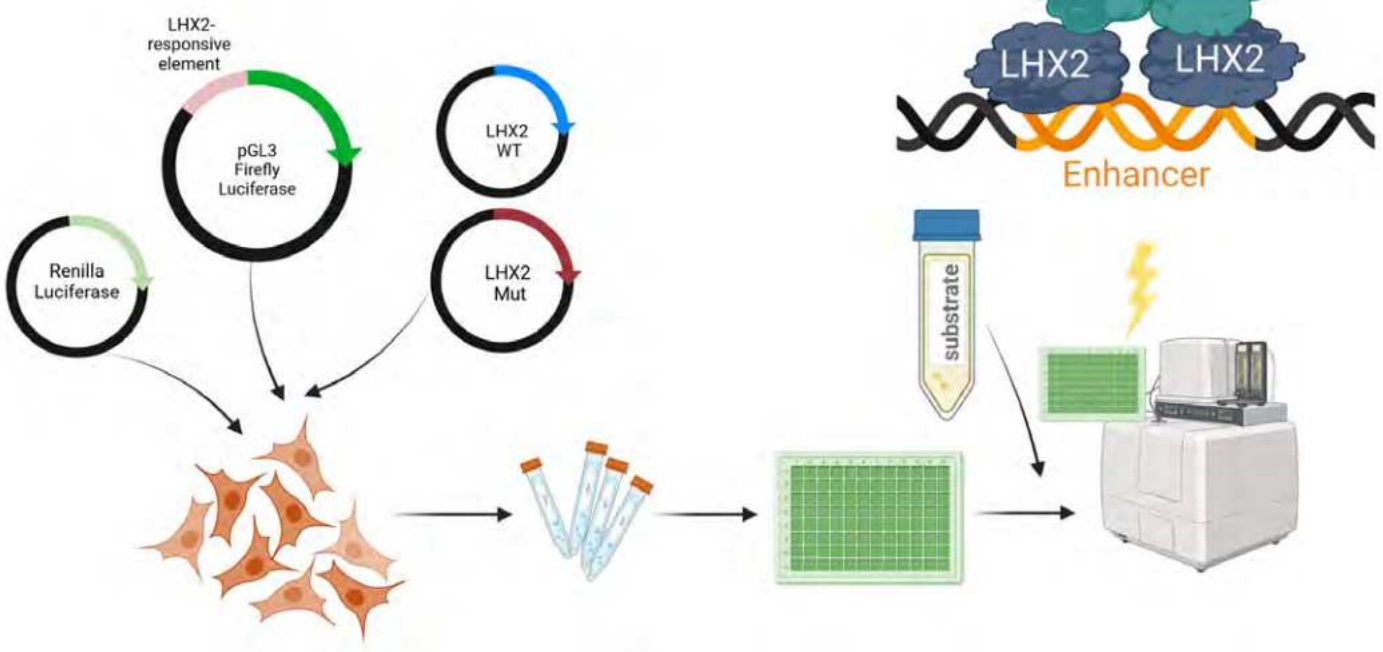
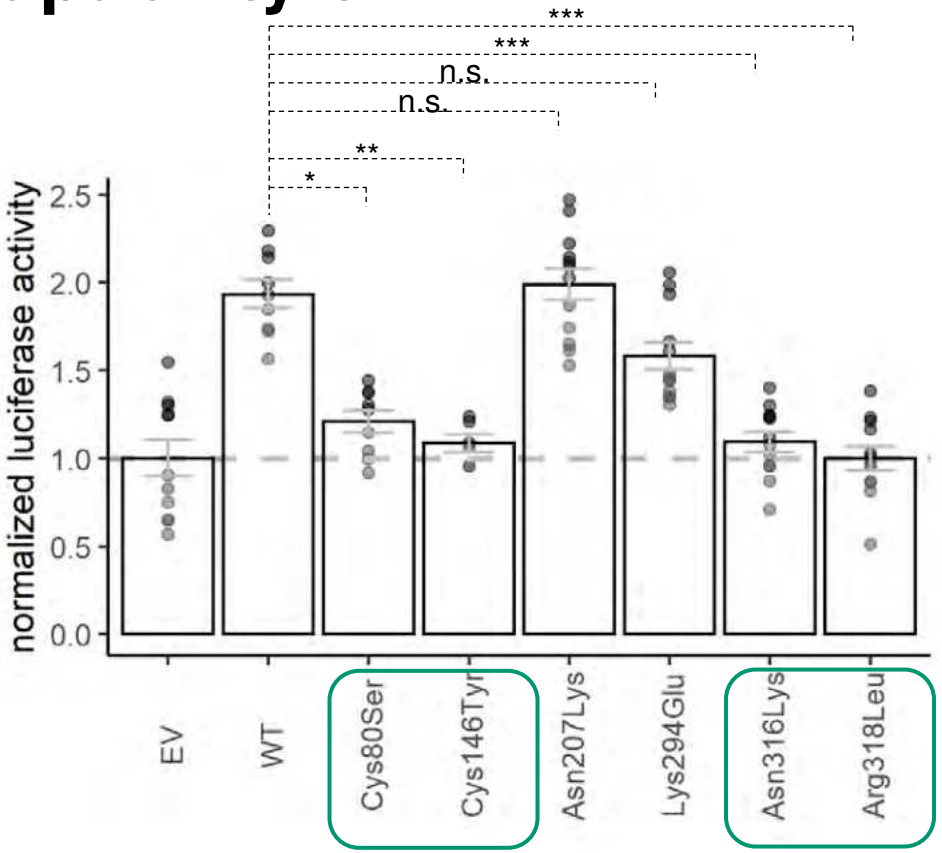
Assessment of variant pathogenicity: Impaired transactivation capability of LHX2





→4 missense variants classified as likely pathogenic
→loss-of-function mechanism likely also for missense variants
→haploinsufficiency most likely disease mechanism

Schmid*, Gregor* et al., Genet Med 2023

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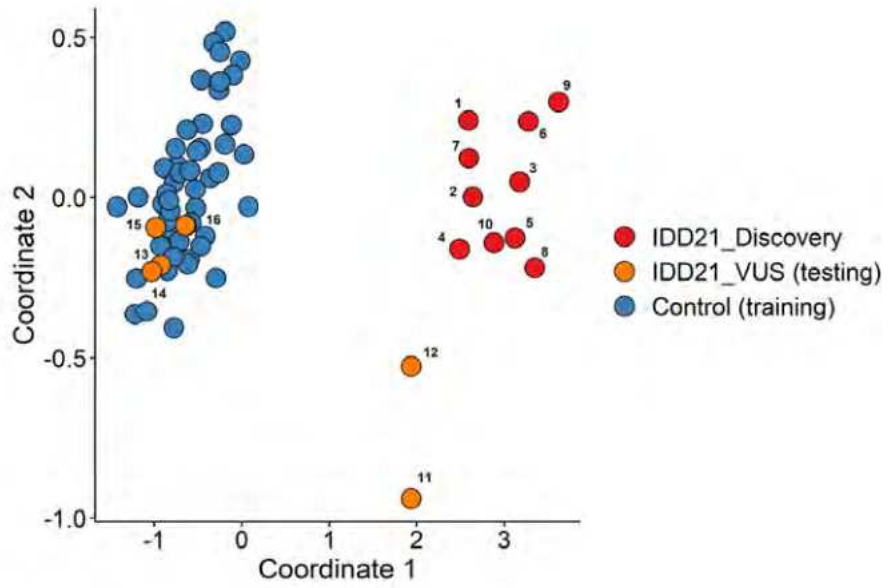
Assessment of variant pathogenicity: Episignatures

- variants in chromatin-regulating genes (and transcriptional regulators) affect DNA-methylation
- DNA methylation can be assessed genome-wide using microarrays
- specific methylation signatures for disease genes/gene groups (n > 70)
- can be used to aid classification of VUS

VUS classification CTCF



→ **functional assays can help improve variant classification and decrease number of VUS**

Karimi et al., Genet Med 2024

Assessing variant pathogenicity: Incomplete penetrance

- first year: normal development
- regression, severe ID, spasticity
- episodic joint swellings, jaundice sclerae, red skin patches
- family: two early deceased siblings and maternal aunts
- exome: variant in IFIH1 p.(Arg779His), inherited from healthy mother but reported as pathogenic in literature

Aicardi-Goutieres syndrome 7

- IFIH1: autosomal-dominant AGS
- progressive encephalopathy (often after initially normal development)
- infect-like anomalies
- neurological symptoms, spasticity, dystonia
- enlarged liver, elevated liver enzymes

→ **Incomplete penetrance**
(13,5% of variant carriers are asymptomatic (Rice et al., Hum Mutation, 2020))

Reduced penetrance in a family with Aicardi-Goutieres syndrome

→ *de novo* occurence important factor for pathogenicity

→ BUT: (mild) autosomal dominant NDDs can be inherited

→ incomplete penetrance (also possible for severe disorders)

→ challenge for variant interpretation, genetic counselling and prenatal diagnostics

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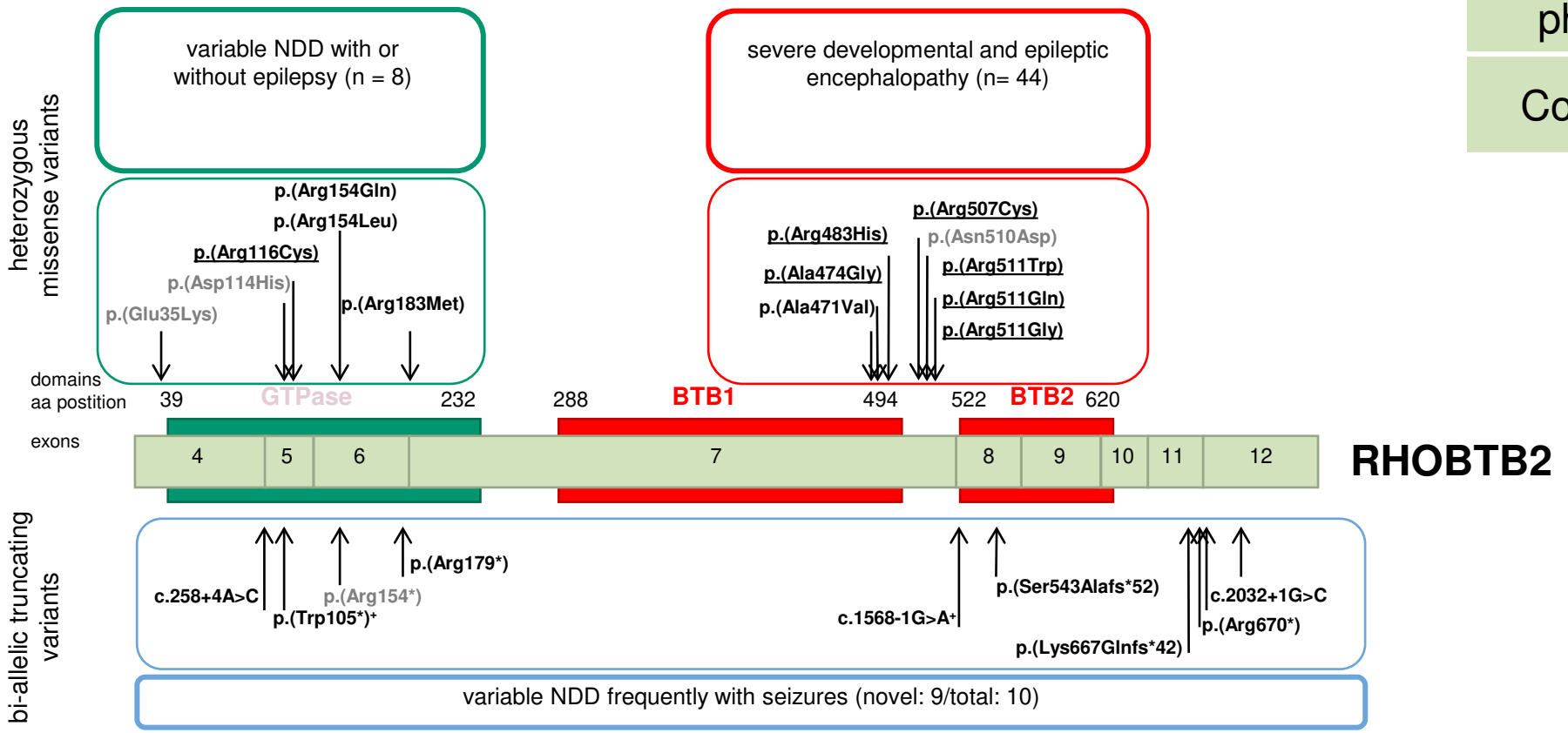
One gene – different phenotypes and inheritance patterns

Matching inheritance pattern?

Does the phenotype fit?

Correct variant?



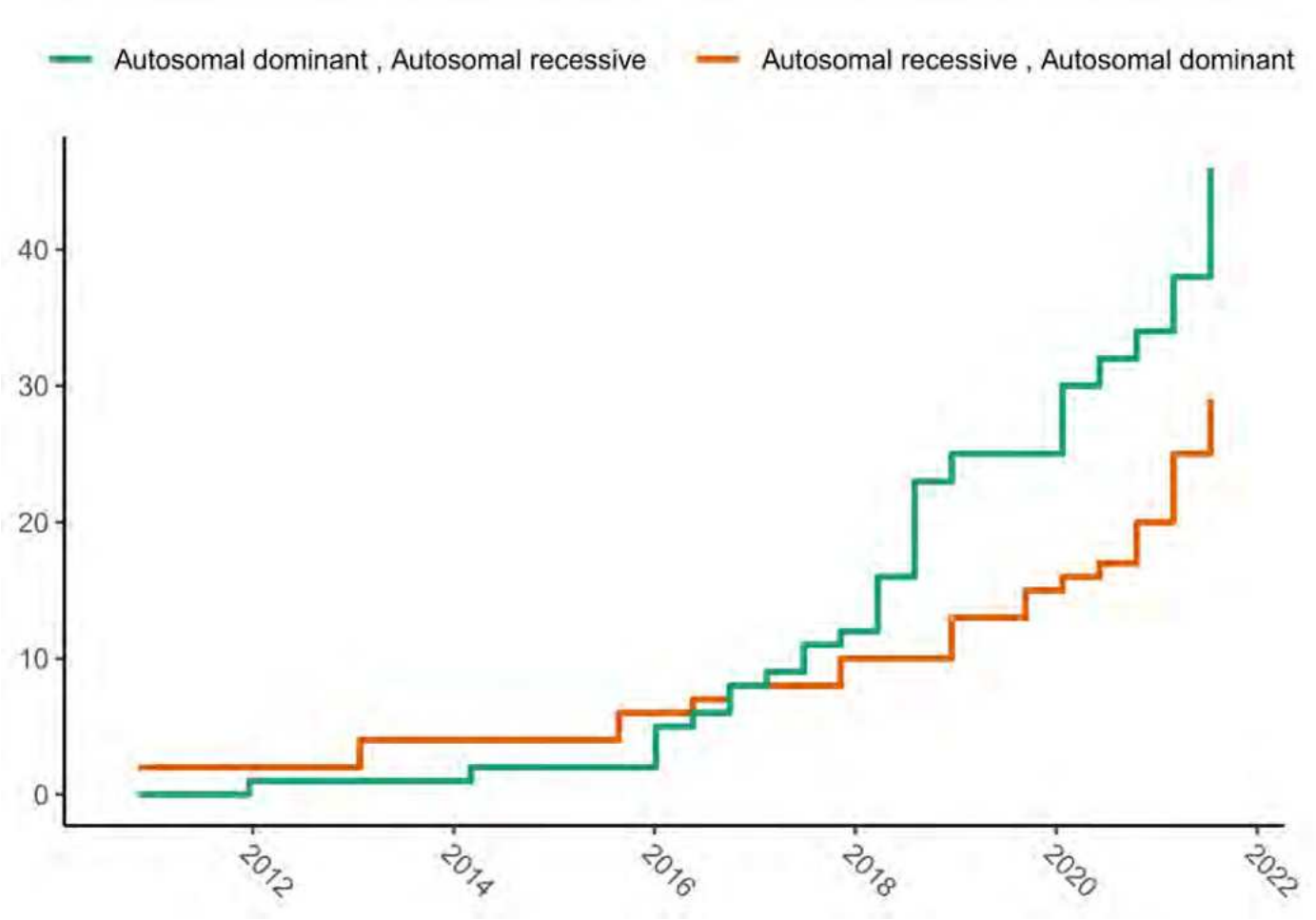
→ genotype-phenotype correlations

→ both autosomal-dominant (de novo) and autosomal-recessive inheritance

Langhammer et al., Genet Med, 2023

Genes with both autosomal-recessive and autosomal-dominant inheritance patterns

Matching inheritance pattern?



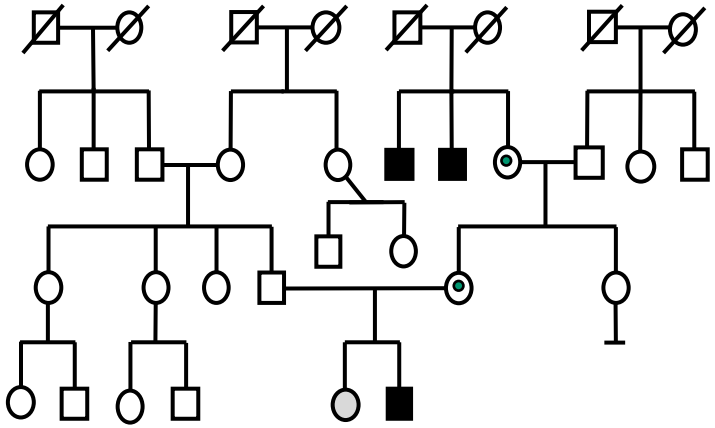
- some same/similar phenotypes
- some different phenotypes

based on data from SysNDD database

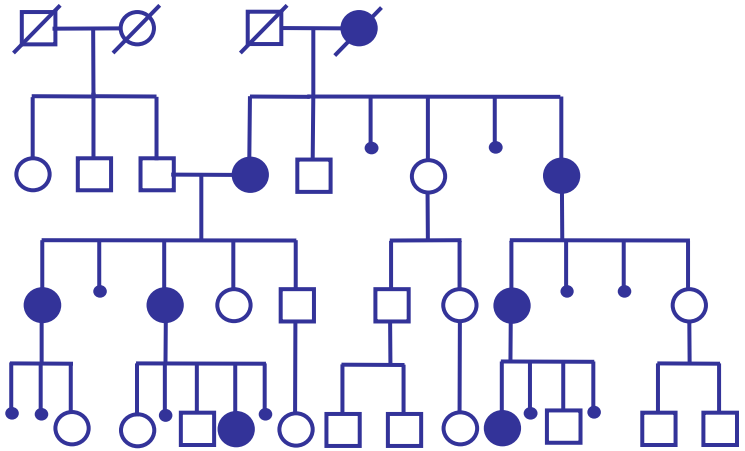
X-linked NDDs

Matching inheritance pattern?

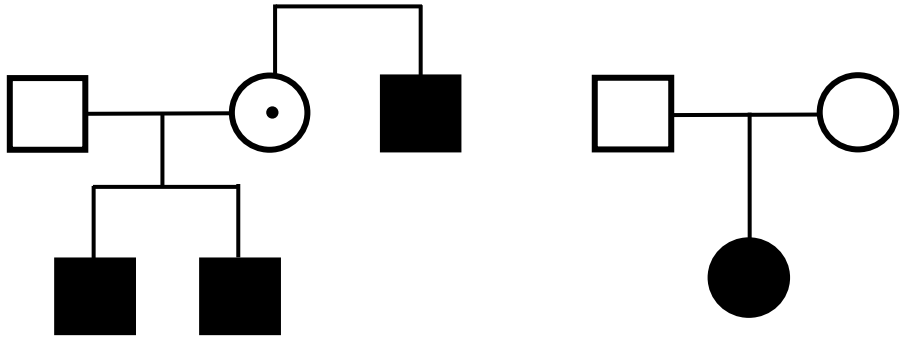
X-linked recessive



X-linked dominant



X-linked ID in both sexes



X-recessive

de novo

Example: Borjeson-Forssman-Lehmann syndrome

Matching inheritance pattern?

in males

- **X-chromosomal-recessive variants in *PHF6***
- variable ID
- typical facies
- obesity, gynecomastia
- hypogonadism, small genitals
- tapering fingers, short toes
- female carriers asymptomatic or only midly symptomatic
- XI skewed in half of the female carriers

in females

- ***de novo* variants in *PHF6***
- moderate to severe ID
- typical facies
- dental anomalies
- finger and toe deformities
- oligo- or amenorrhoe
- linear skin hyperpigmentation
- XI skewed in blood, random in fibroblasts

Matching inheritance pattern?

→variants in one gene can cause different phenotypes or diseases
→genotype-phenotype correlations

→don't stop searching for other similar cases when the phenotype does not seem to fit to the gene

→ one gene can be associated with multiple inheritance patterns

→ SysNDD database: 1616 genes associated with 1783 diseases

Challenge: Mulitple diagnosis

Correct gene?

Does the phenotype fit?

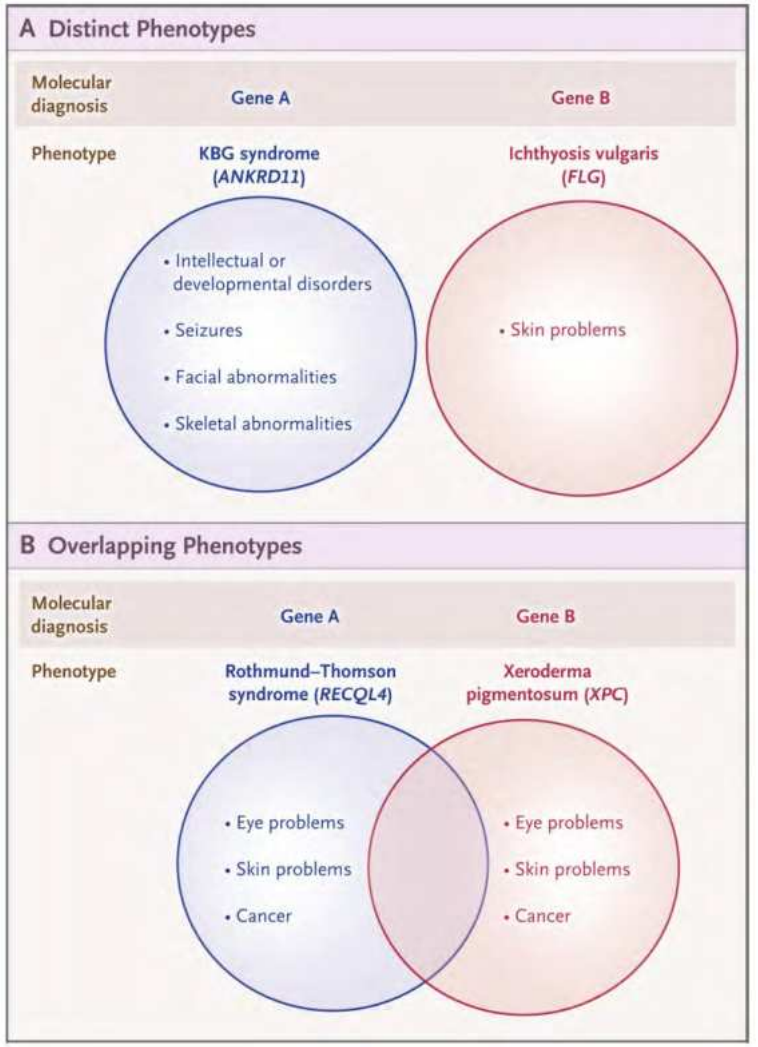
N Engl J Med. 2017 Jan 5;376(1):21-31.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation

Jennifer E. Posey, M.D., Ph.D., Tamar Harel, M.D., Ph.D., Pengfei Liu, Ph.D., Jill A. Rosenfeld, M.S., Regis A. James, Ph.D., Zeynep H. Coban Akdemir, Ph.D., Magdalena Walkiewicz, Ph.D., Weimin Bi, Ph.D., Rui Xiao, Ph.D., Yan Ding, M.D., Fan Xia, Ph.D., Arthur L. Beaudet, M.D., Donna M. Muzny, M.S., Richard A. Gibbs, Ph.D., Eric Boerwinkle, Ph.D., Christine M. Eng, M.D., V. Reid Sutton, M.D., Chad A. Shaw, Ph.D., Sharon E. Plon, M.D., Ph.D., Yaping Yang, Ph.D., and James R. Lupski, M.D., Ph.D., D.Sc.



Retrospective analysis of exome data of 7374 patients: → 4,9% molecular diagnosis at 2 or more disease loci

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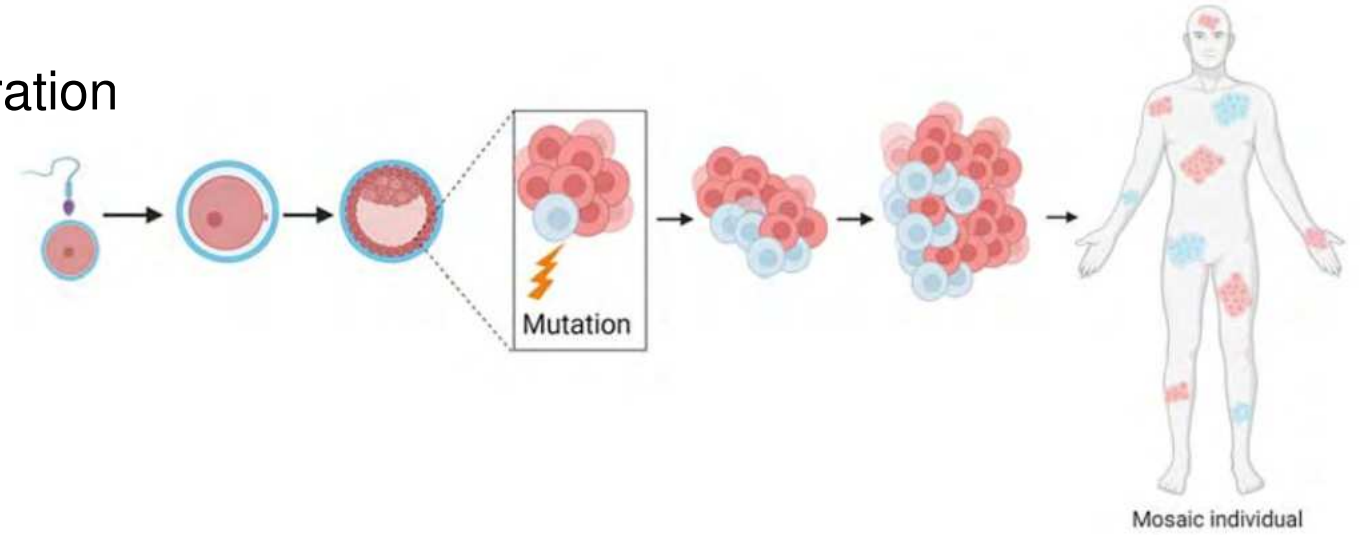
Challenge: Mosaics

- post-zygotic occurence of the genetic alteration
- only part of the body cells is affected
- tissue-specificity
- mosaics often not detectable in blood



→ Don’t stop at blood when you think it might be a mosaic

→ Don’t stop searching for more variants if the phenotype is «atypical»

→ Contribute to characterizing phenotypic spectra and natural histories

What do we miss with NGS?

- Boy, 5 years
- walking with 16 months
- speech delay, 5-10 single words
- Hyperactivity
- growth normal
- MRI normal
- array and trio-exome normal

example picture
https://en.wikipedia.org/wiki/Fragile_X_syndrome

Fragile X syndrome

- **repeat expansion in the FMR1-promoter (premutation 50-180 repeats, full mutation >200)**
- X-linked
- BUT: also ~50% of females with full mutations are symptomatic
- intellectual disability, speech > motor delay
- behavior: aggressivity, autism
- after puberty: macroorchidism

Take home messages

- diagnostics of NDDs is more than just diagnostics
- a genetic diagnosis requires close interaction between clinics and diagnostics
- genetics of NDDs is work in progress and needs continuous learning