3. EFFECT OF GENETIC VARIANTS

Functional annotation

G is the more pathogenic variant…….but is it really pathogenic?

3. EFFECT OF GENETIC VARIANTS

- ➢ Pathogenic
- ➢ Likely pathogenic
- ➢ Benign
- ➢ Likely benign
- ➢ Uncertain significance

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

Functional interpretation

ACMG STANDARDS AND GUIDELINES

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data – benign BP6	Reputable source – pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Figure 1 Evidence framework. This chart organizes each of the criteria by the type of evidence as well as the strength of the criteria for a benign (left side) or pathogenic (right side) assertion. Evidence code descriptions can be found in **Tables 3** and **4**. BS, benign strong; BP, benign supporting; FH, family history; LOF, loss of function; MAF, minor allele frequency; path., pathogenic; PM, pathogenic moderate; PP, pathogenic supporting; PS, pathogenic strong; PVS, pathogenic very strong.

3. EFFECT OF GENETIC VARIANTS

A
B
C
D
E
F
G
H

- Pathogenic
- Likely pathogenic
- Benign
- Likely benign
- Uncertain significance

ACMG STANDARDS AND GUIDELINES

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
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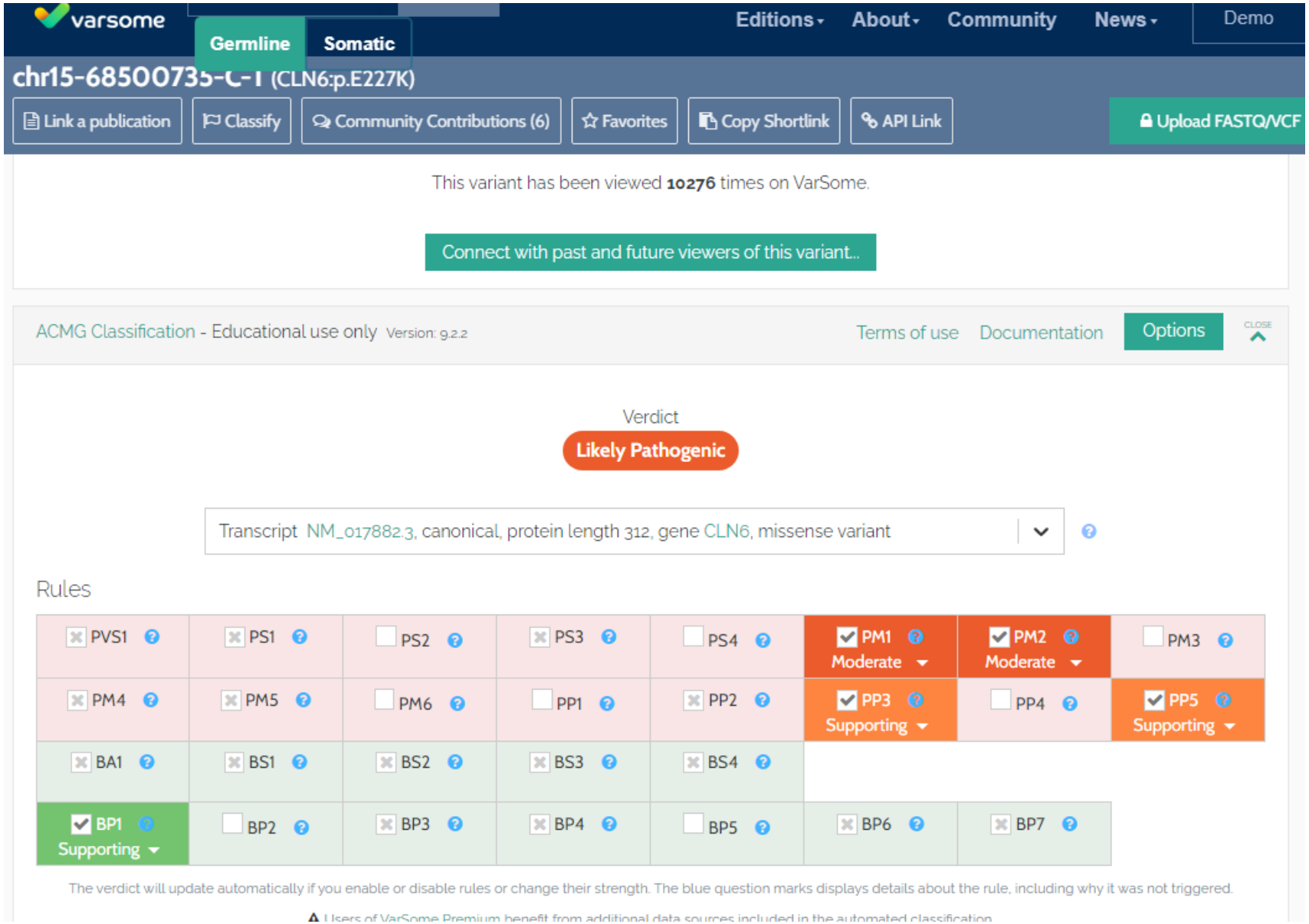
3. EFFECT OF GENETIC VARIANTS

Functional annotation



3. EFFECT OF GENETIC VARIANTS

Functional annotation

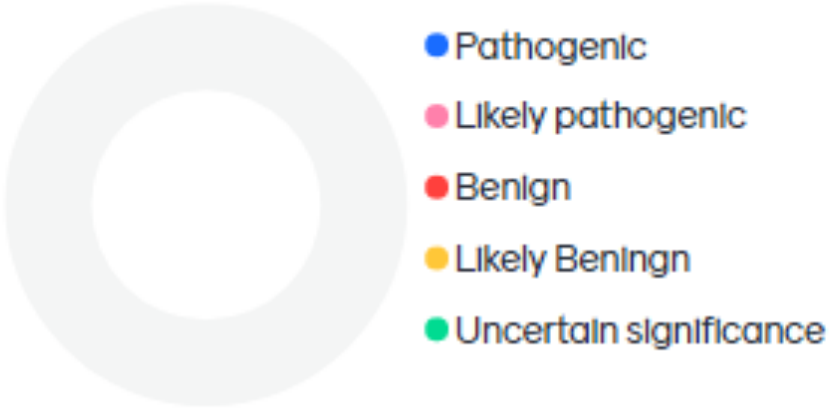
Genetic variant	Position	Change	Consequence	Gene	Freq	SIFT	Polyphen	Mutation Assessor	CADD	Condel	LoF tol	RVIS
A	3:1403401-1403401	A/C	missense	CNTN6	0.003	0.47	0	Neutral	9.52	Neutral	0.974	-0.92 (9.81%)
B	12:49022301-49022301	G/A	missense	KMT2D	-	0	1	Medium	32	0.945	-	-5.29 (0.06%)
C	3:25634002-25634002	G/A	missense	TOP2B	4×10^{-6}	0.01	0.152	Medium	29.3	0.778	0.82	-0.15 (42.28%)
D	15:72353105-72353105	C/T	missense	HEXA	5×10^{-5}	0	1	Damaging	32	0.945	0.17	-0.33 (30.7%)
E	4:38797314-38797314	C/A	synonymous	TLR1	0.38	-	-	-	0.29	-	0.963	1.32 (94.07%)
F	7:117639961-117639961	C/T	intron	CFTR	6×10^{-5}	-	-	-	1.08	-	0.0235	-0.51 (21.73%)
G	17:31230383-31230383	G/A	splice_donor	NF1	4×10^{-6}	-	-	-	34	-	0.116	-3.09 (0.47%)
H	11:63290453-63290453	G/A	stop_gained	SLC22A10	0.434	-	-	-	36	-	0.7	1.85 (97.12%)

3. EFFECT OF GENETIC VARIANTS

Go to **www.menti.com** and use the code **39 77 01 9**

Classification of variant G according to the ACMG guidelines

Mentimeter

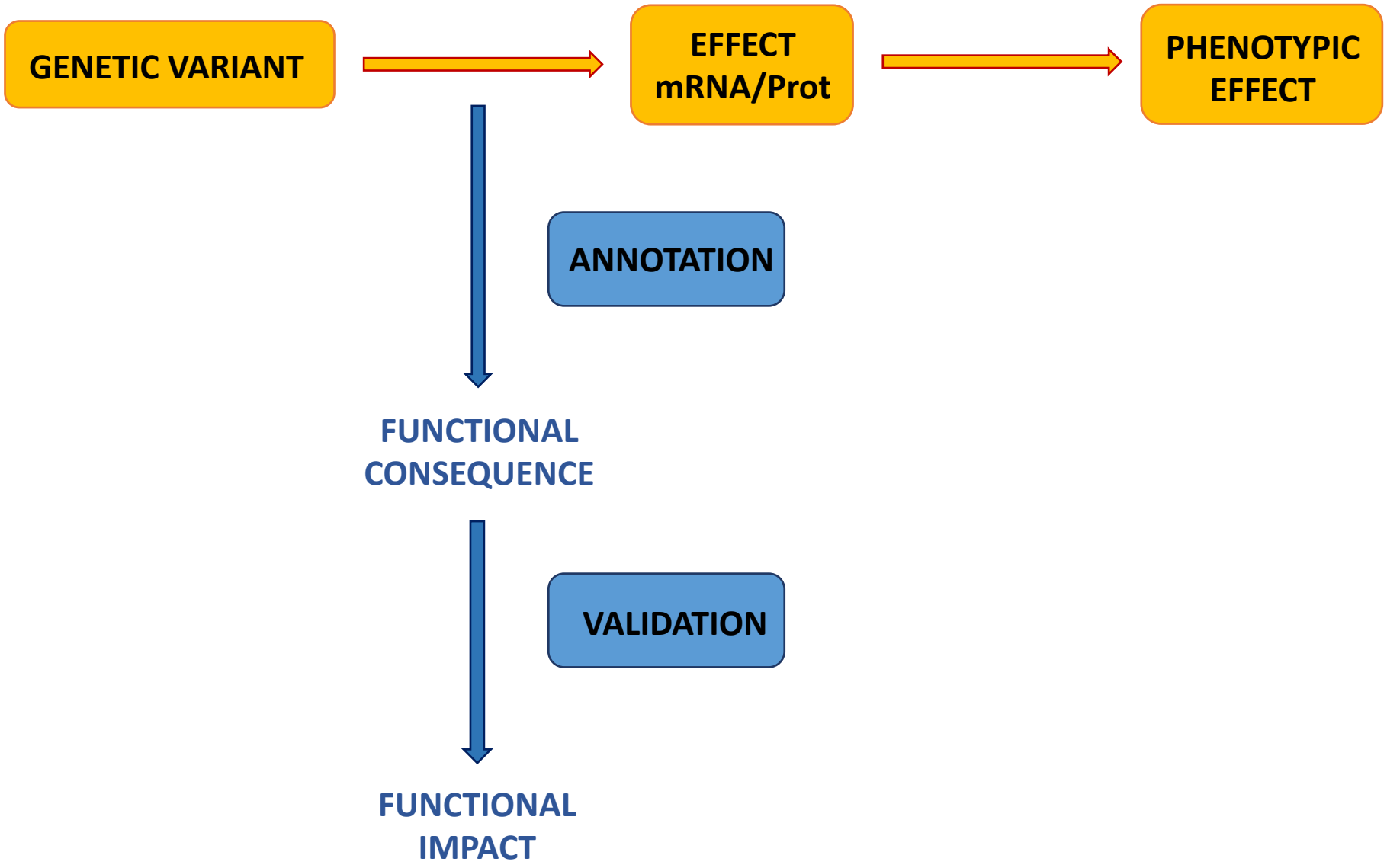


Using the ACMG standard guidelines, how would you classify a genetic variant that:

-Is a nonsense mutation never described in the population. The variant has been described in the patient but is absent in both parents, and the paternity has been confirmed.

3. EFFECT OF GENETIC VARIANTS

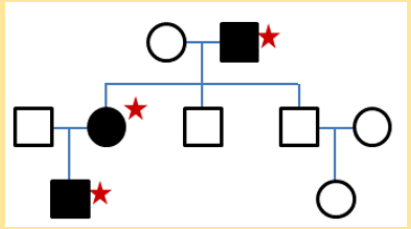
Functional annotation

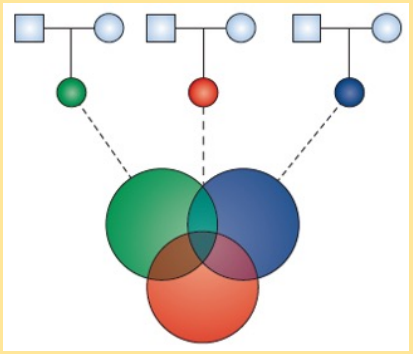


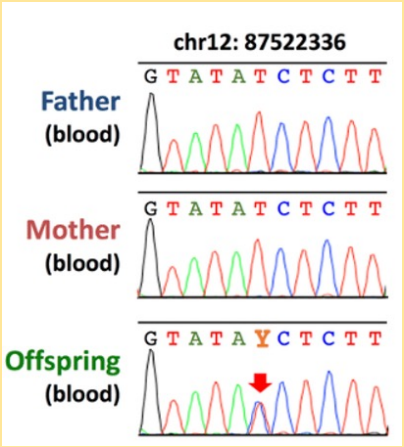
3. EFFECT OF GENETIC VARIANTS

Functional annotation

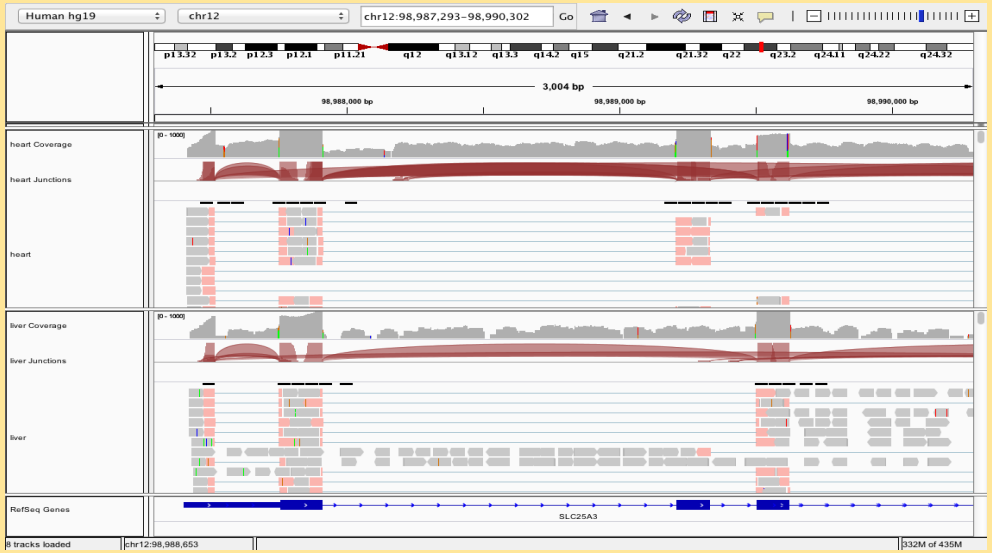
Segregation pattern

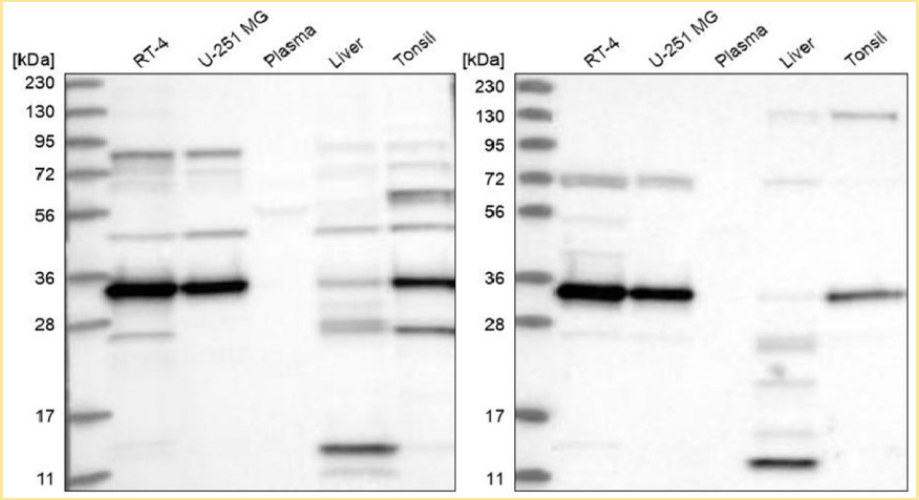




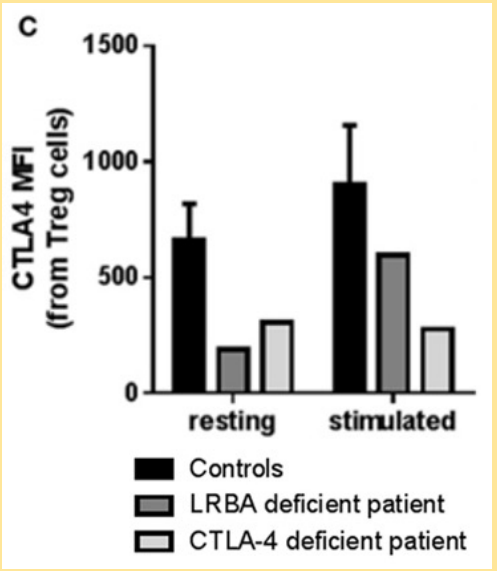


RNA and protein analysis

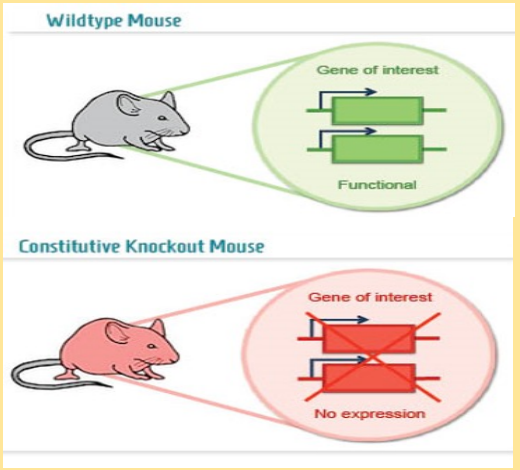




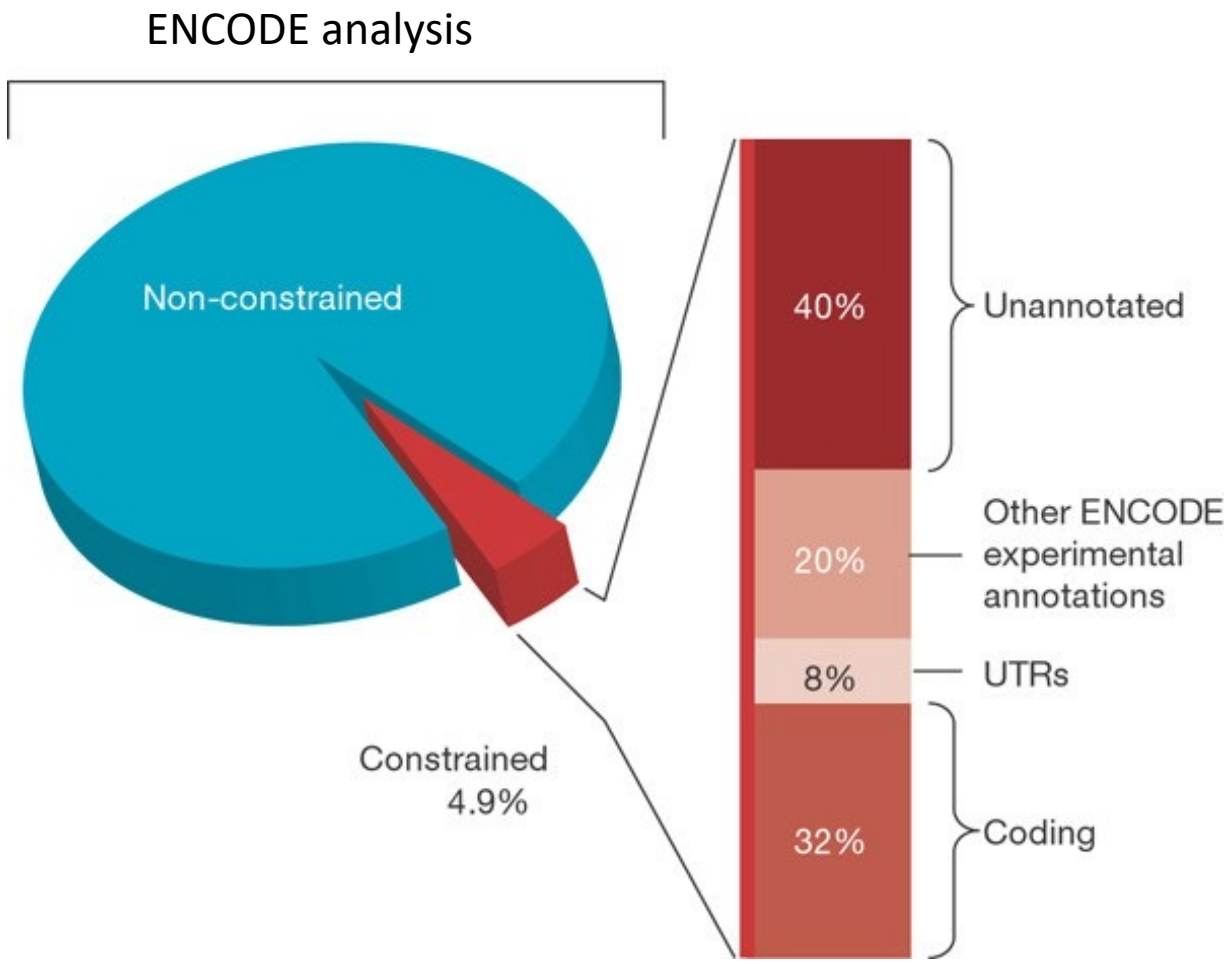
Cellular studies



Model organism



4. BIOLOGICAL MEANING OF GENETIC VARIANTS



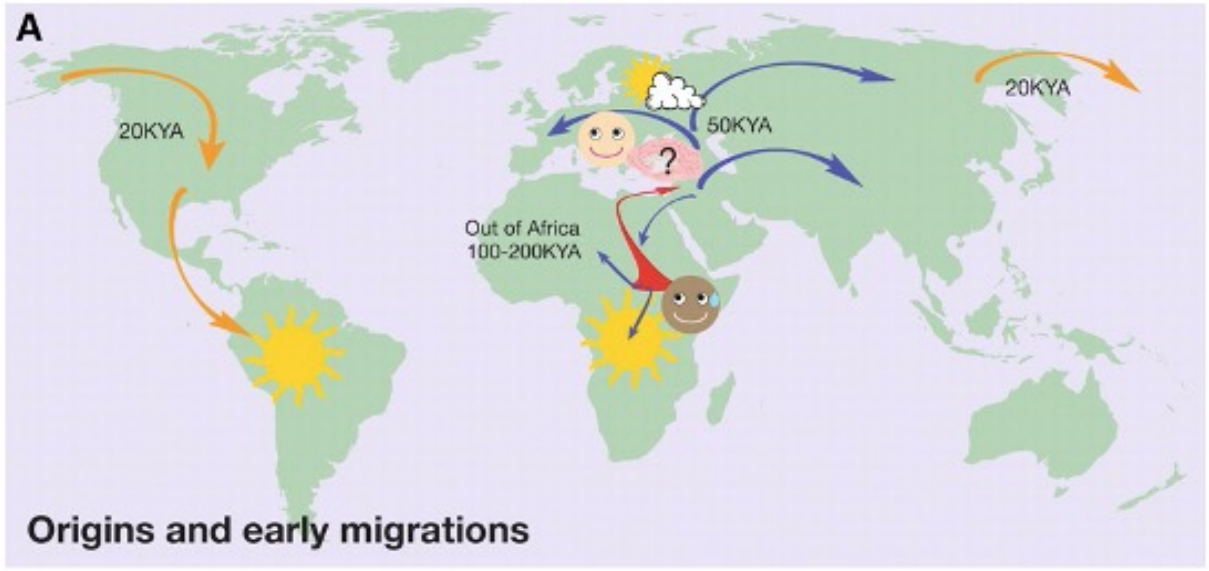
GENETIC DISEASE
- **Mendelian**
- **Complex**

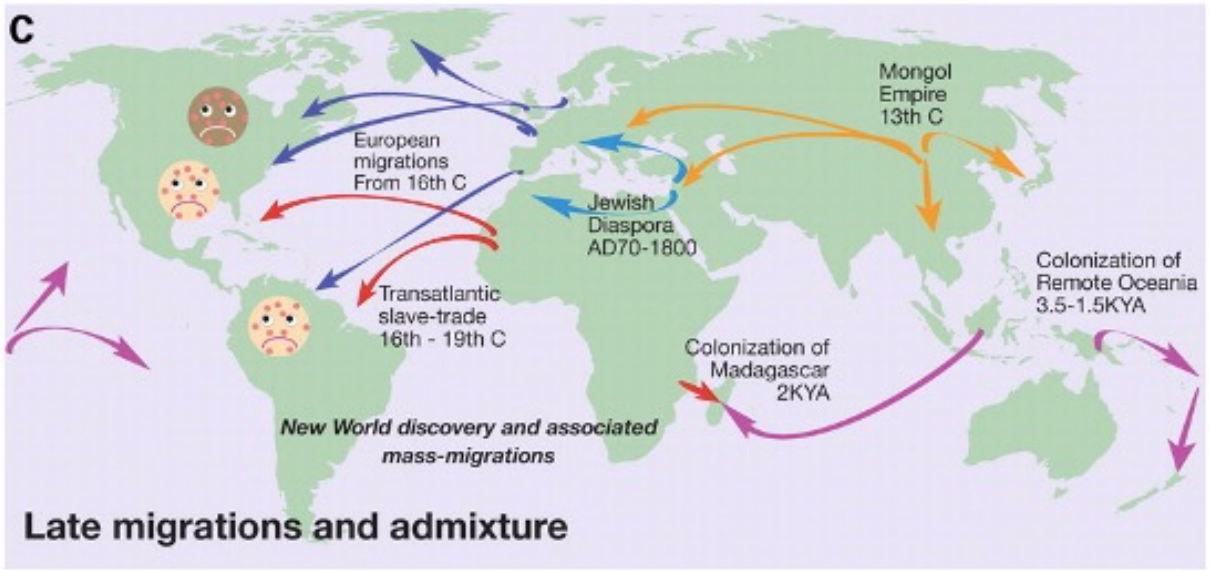
ADAPTATION

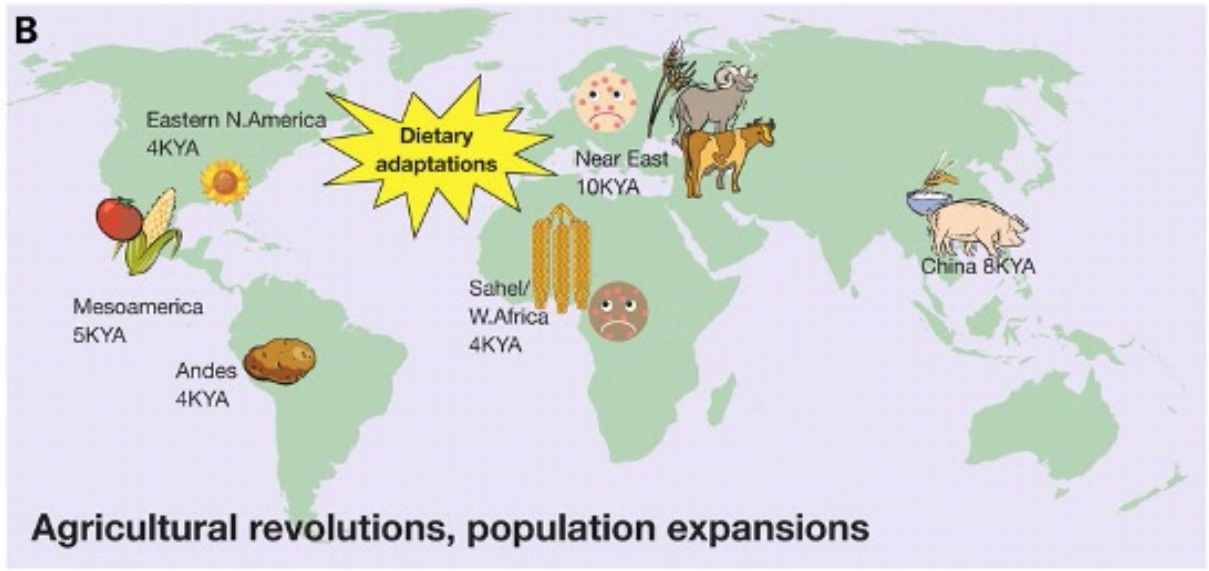
NEUTRAL

4. BIOLOGICAL MEANING OF GENETIC VARIANTS

Adaptative variants





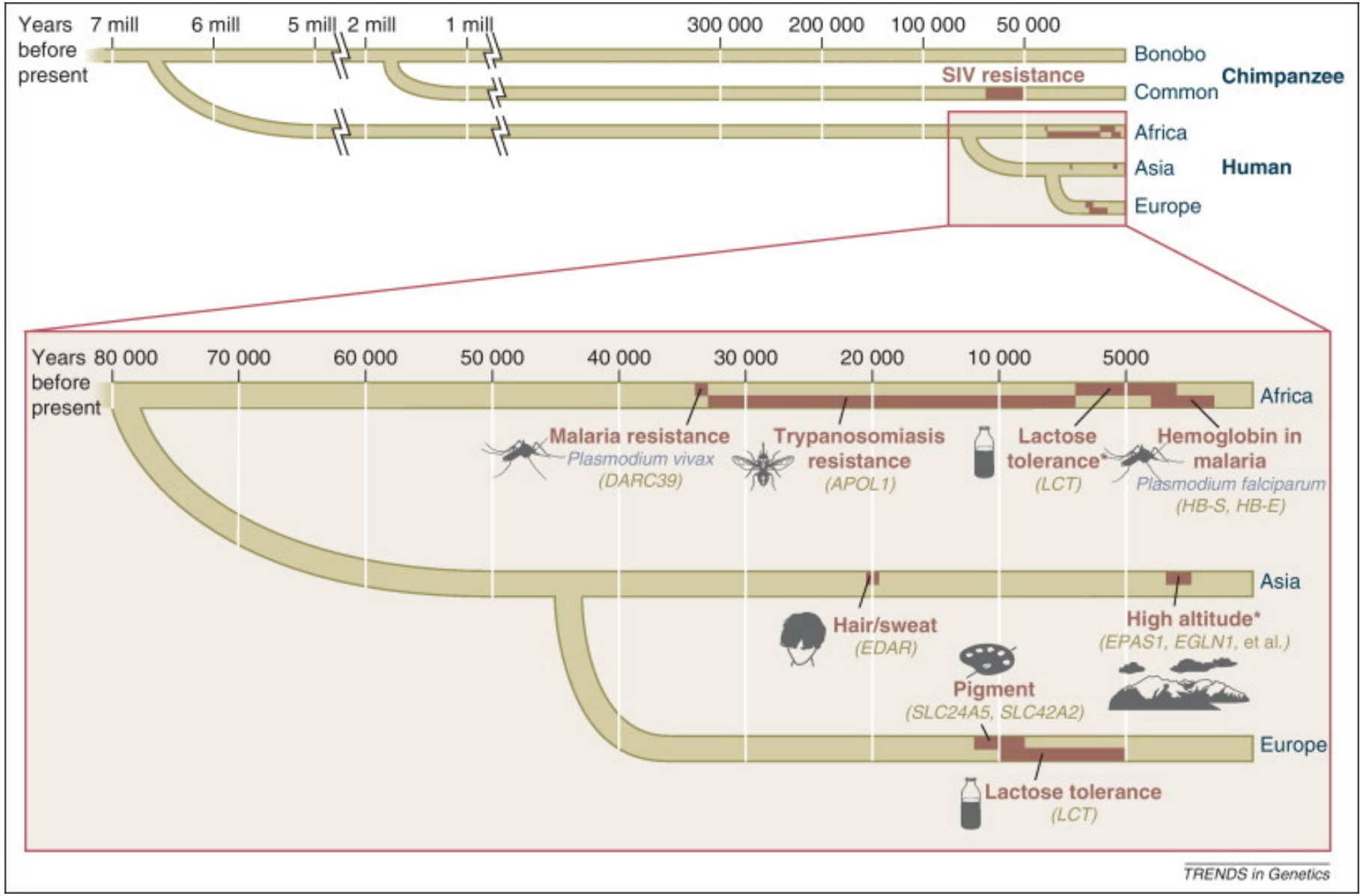


- New environments
- Food production – new diet
- Population growth– infectious diseases

"Challenges in human genetic diversity: demographic history and adaptation", Balaresque et al. (2007)

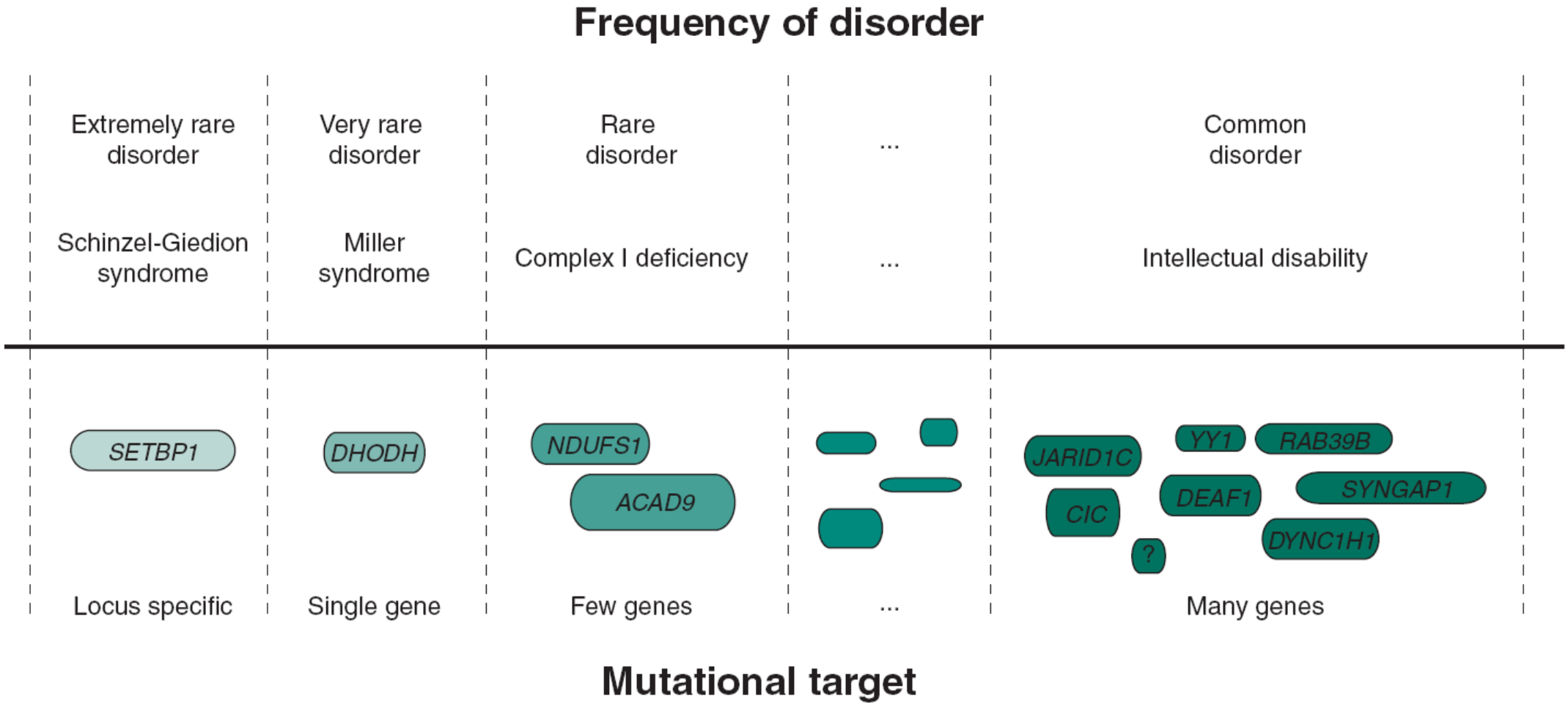
4. BIOLOGICAL MEANING OF GENETIC VARIANTS

Adaptative variants



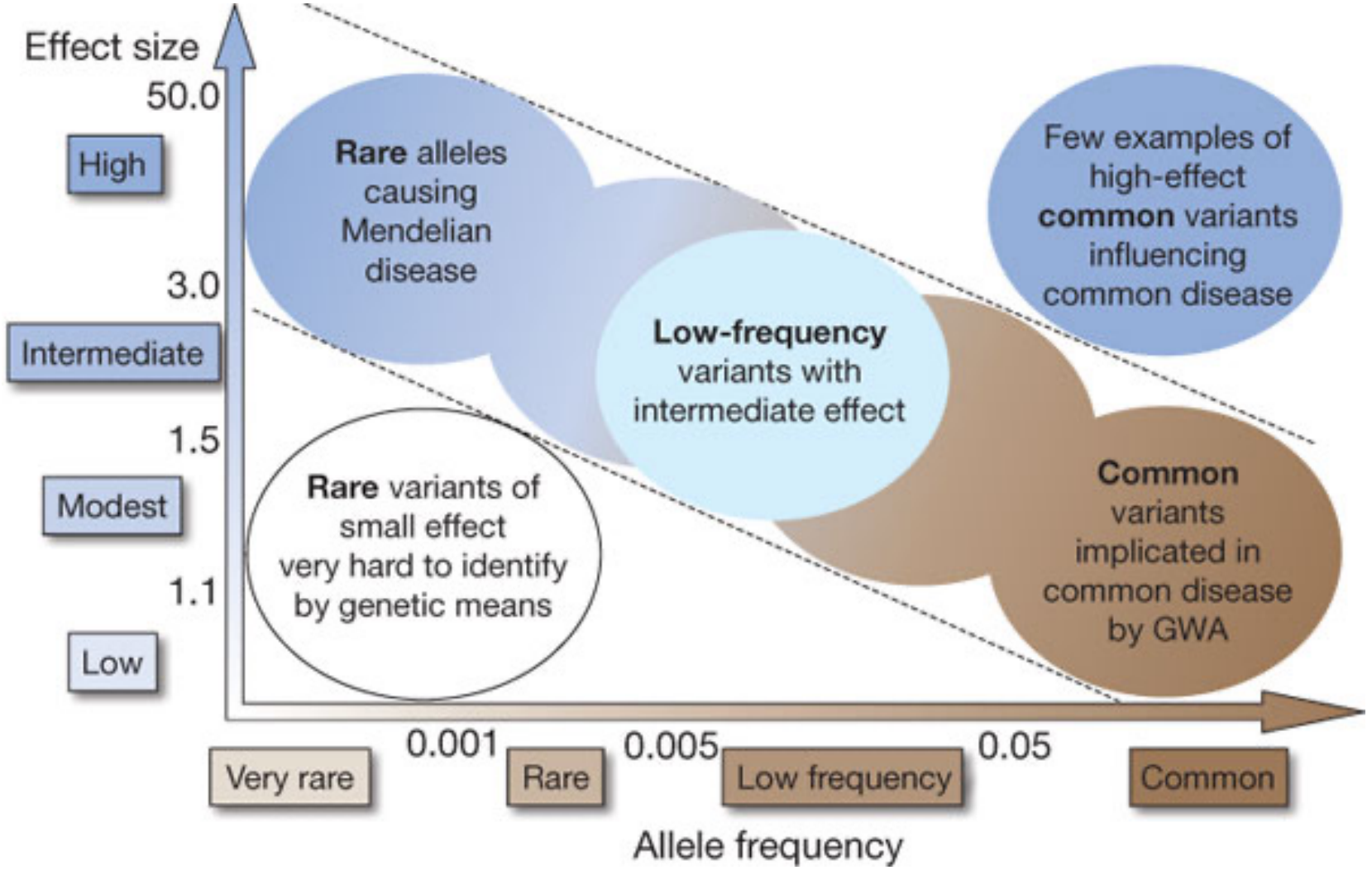
Pathogenic variants

Disease prevalence and genetic model

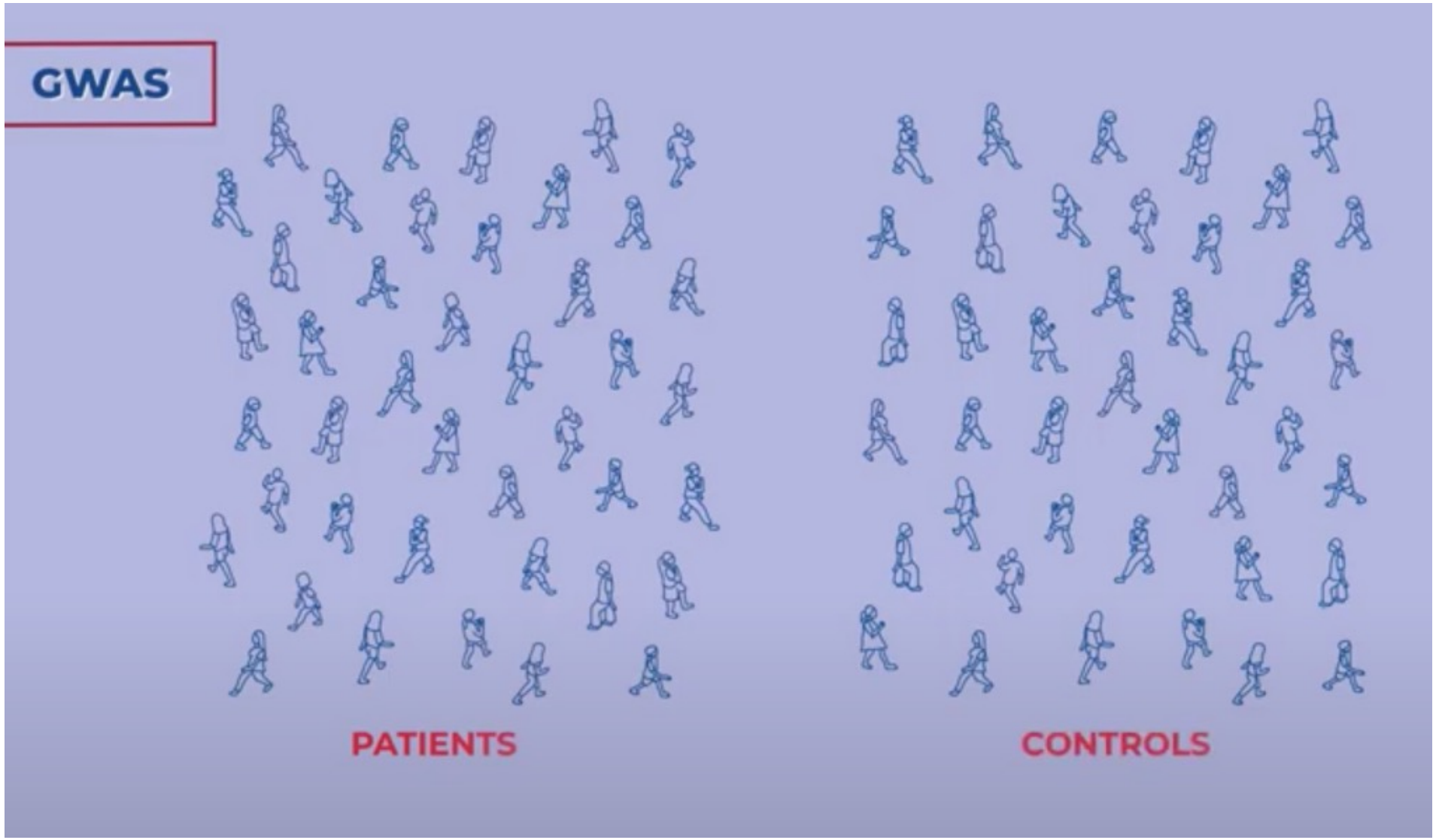


De: Gilissen *et al. Genome Biology 2011,* ***12:228***

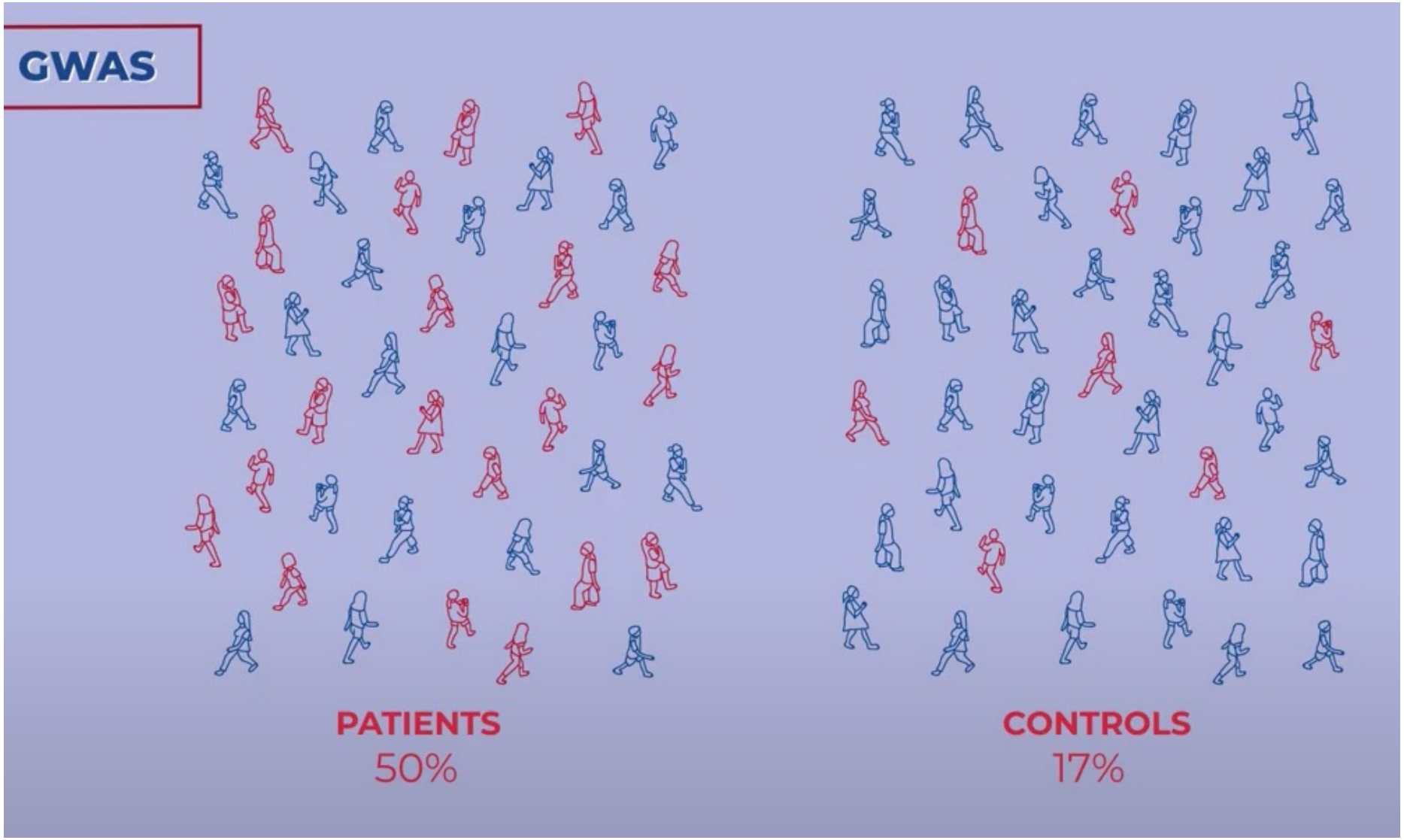
Pathogenic variants



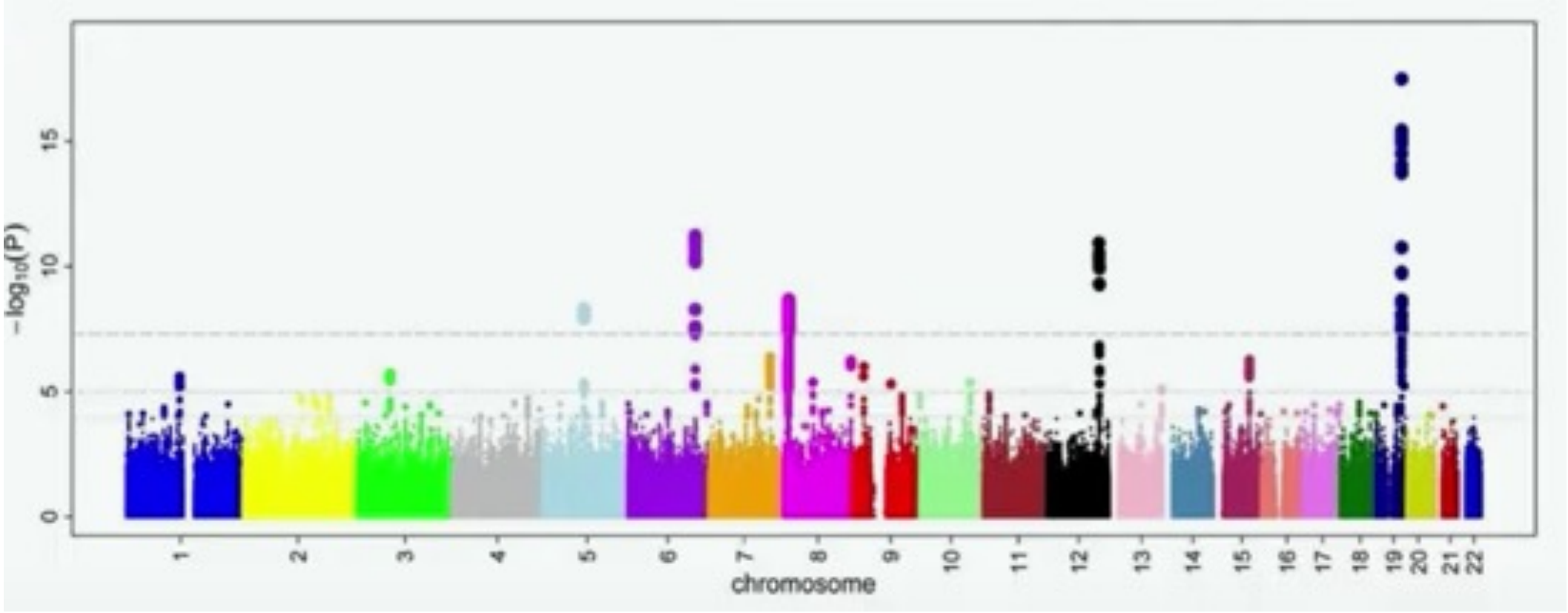
GENOME WIDE ASSOCIATION STUDIES



GENOME WIDE ASSOCIATION STUDIES



GENOME WIDE ASSOCIATION STUDIES, Manhattan Plot



CASES

CC	CT	TT
751	234	15

CONTROLS

CC	CT	TT
810	180	10

ODDS

TT is **1,5** times more frequent in cases

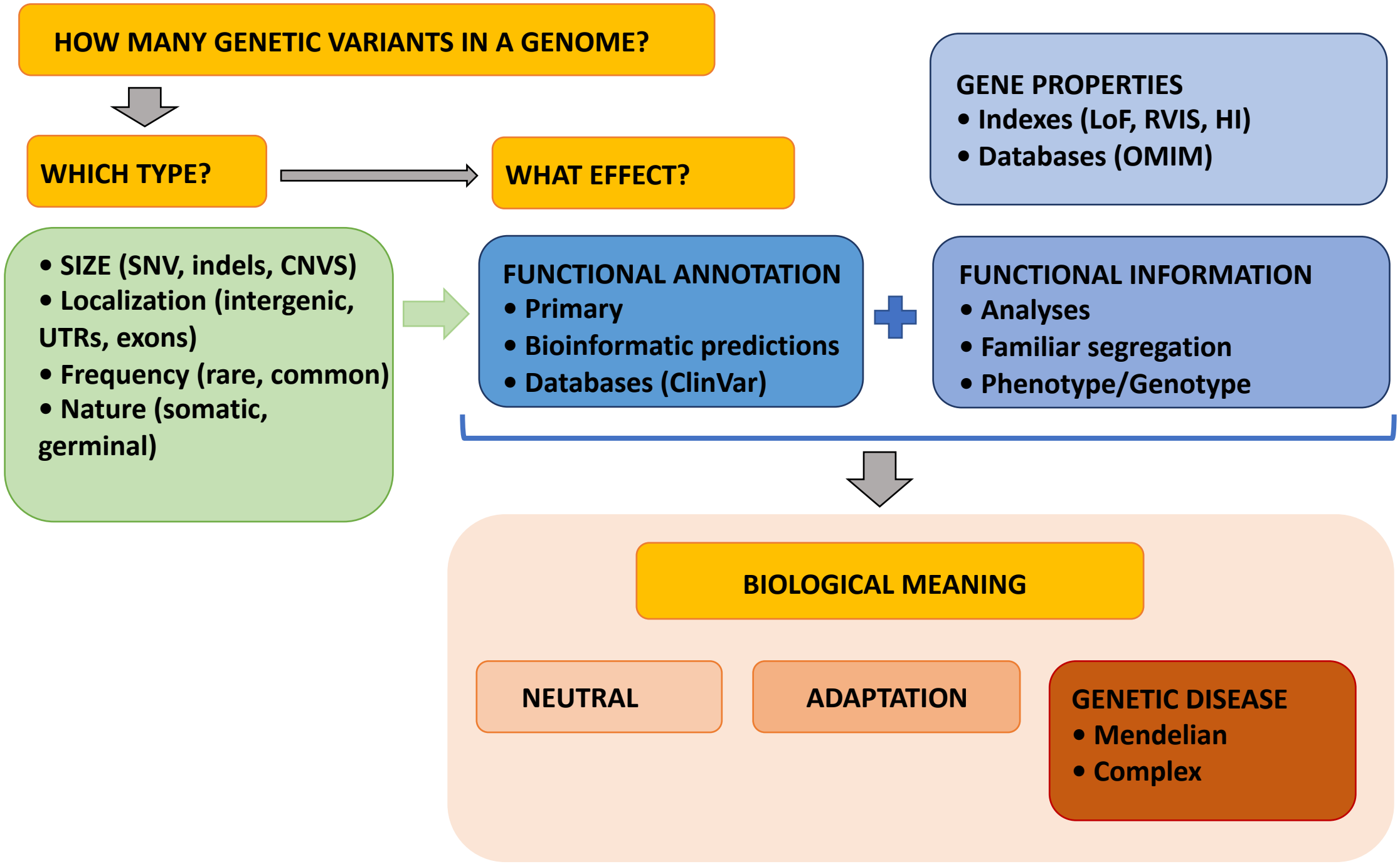
CT is **1,3** times more frequent in cases

CC is **0,9** times less frequent in cases

ODDS RATIO

The odds ratio for **TT** is **1.7**

The odds ratio for **CT** is **1.4**


HOW MANY GENETIC VARIANTS IN A GENOME?
WHICH TYPE?
WHAT EFFECT?
GENE PROPERTIES
• Indexes (LoF, RVIS, HI)
• Databases (OMIM)
• SIZE (SNV, indels, CNVS)
• Localization (intergenic, UTRs, exons)
• Frequency (rare, common)
• Nature (somatic, germinal)
FUNCTIONAL ANNOTATION
• Primary
• Bioinformatic predictions
• Databases (ClinVar)
FUNCTIONAL INFORMATION
• Analyses
• Familiar segregation
• Phenotype/Genotype
BIOLOGICAL MEANING
NEUTRAL
ADAPTATION
GENETIC DISEASE
• Mendelian
• Complex