EAC 2025

When and What to Order and How to Interpret Genetic Testing in Patients with IEIs

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Professor and Division Chief

Learning Objectives

- What are some of the benefits of genetic testing in IEIs?
- What kind of genetic variants does whole exome sequencing miss?
- How does one work up a “variant of unknown significance”?
- How can artificial intelligence help find patients with IEIs?

Genetics of Primary Immunodeficiency

- IEI is (largely) caused by monogenic variants that impair function of immune development, homeostasis or response
  - **everyone with IEI should have a genetic diagnosis**

- Rare disease requires **rare variants**

- We do not believe that one gene = one disease anymore
  - **multiple phenotypes are possible**
  - **consider the pathways involved**

- VUS “Variants of unknown significance”
  - Don’t ignore these
  - Look at the transcript, the protein
  - **Functional testing trumps everything**

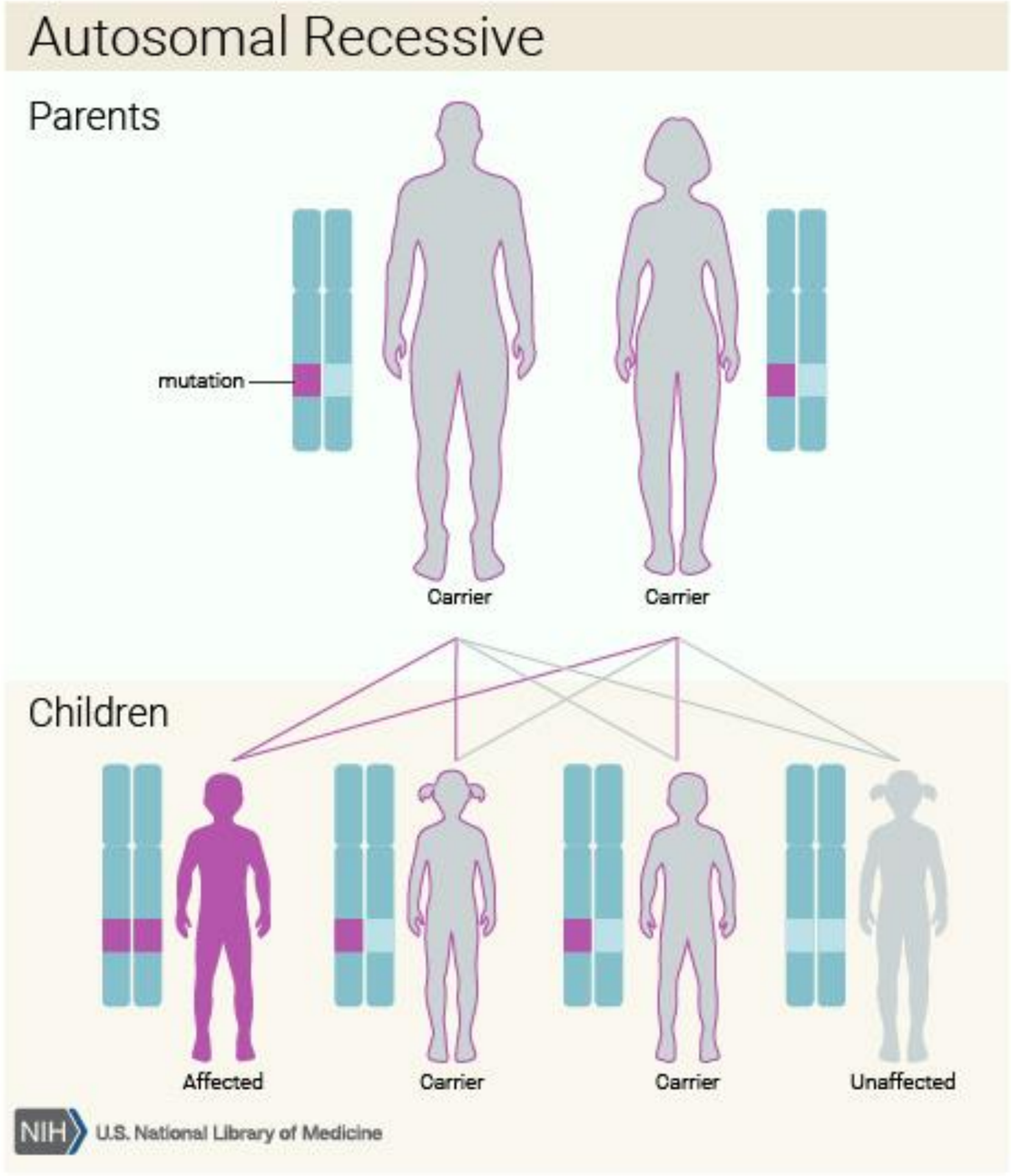
Overview of Genetic inheritance

- Autosomal dominant
  - Inherited
  - De novo (new variant)
- Autosomal recessive
- X-linked recessive
- Mitochondrial inheritance

- Advanced: Somatic mosaicism

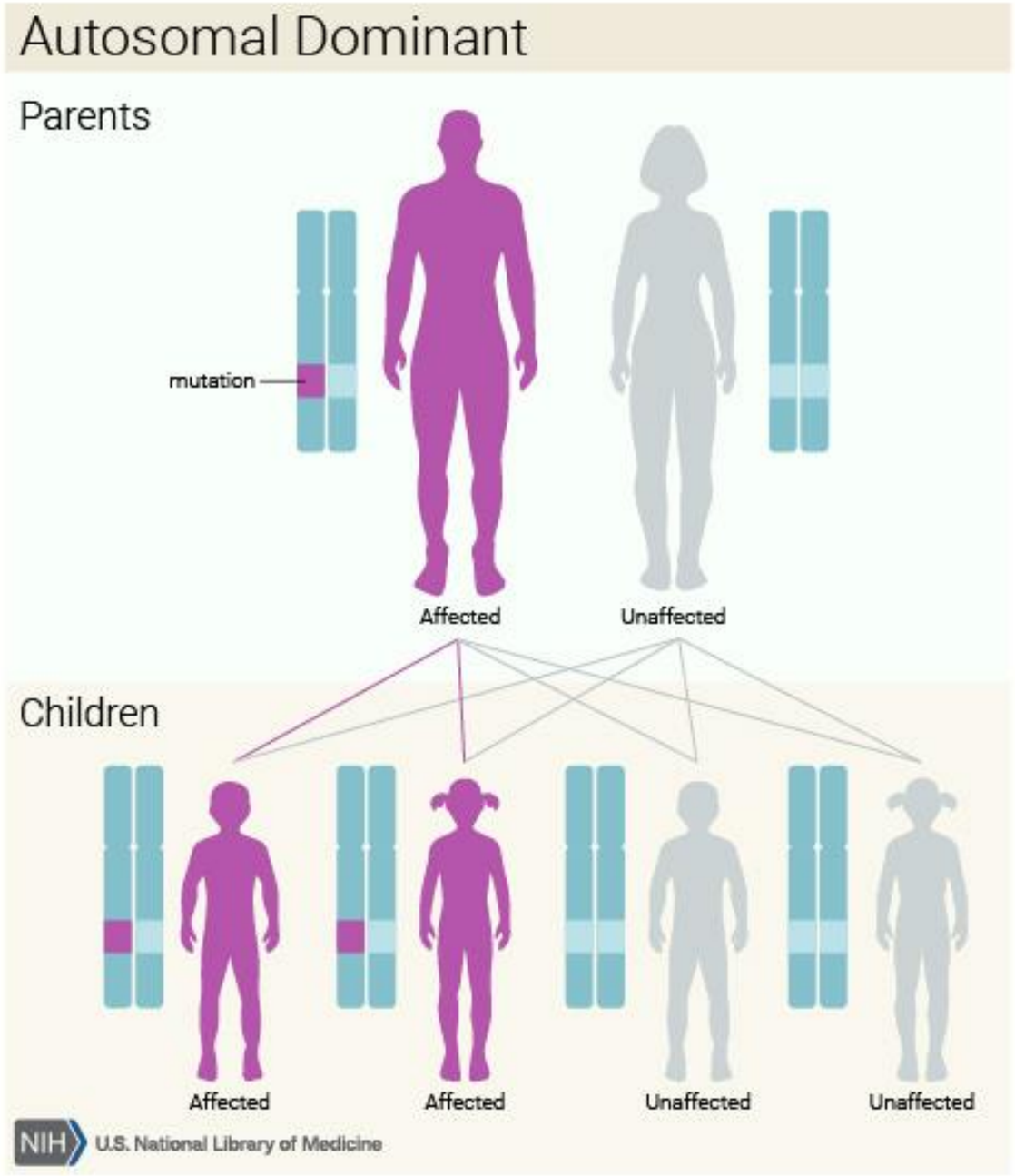
- not discussed:
  - Y dominant, X-dominant, mitochondrial
  - epigenetic inheritance

Just because it's genetic doesn't mean it's inherited

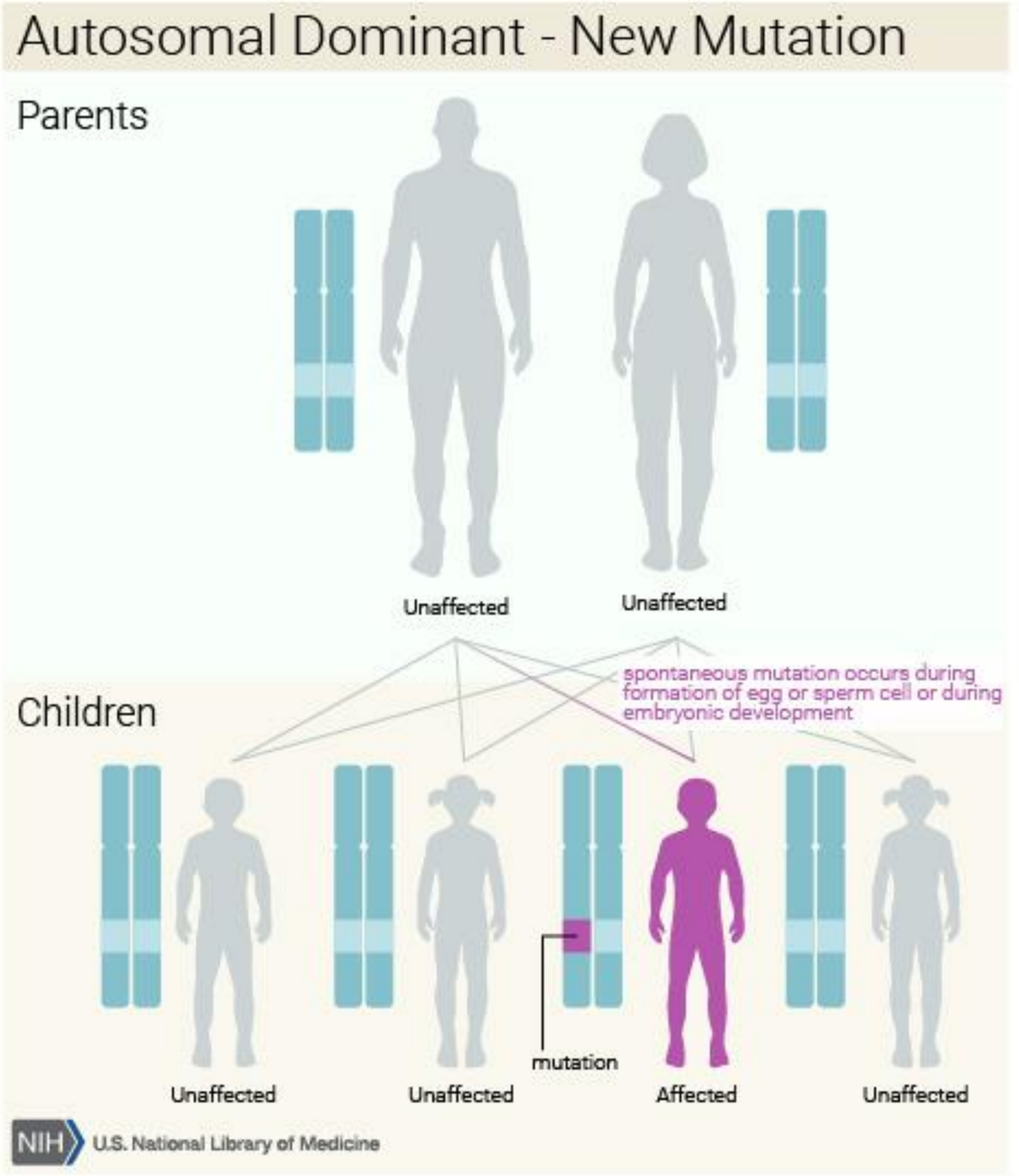


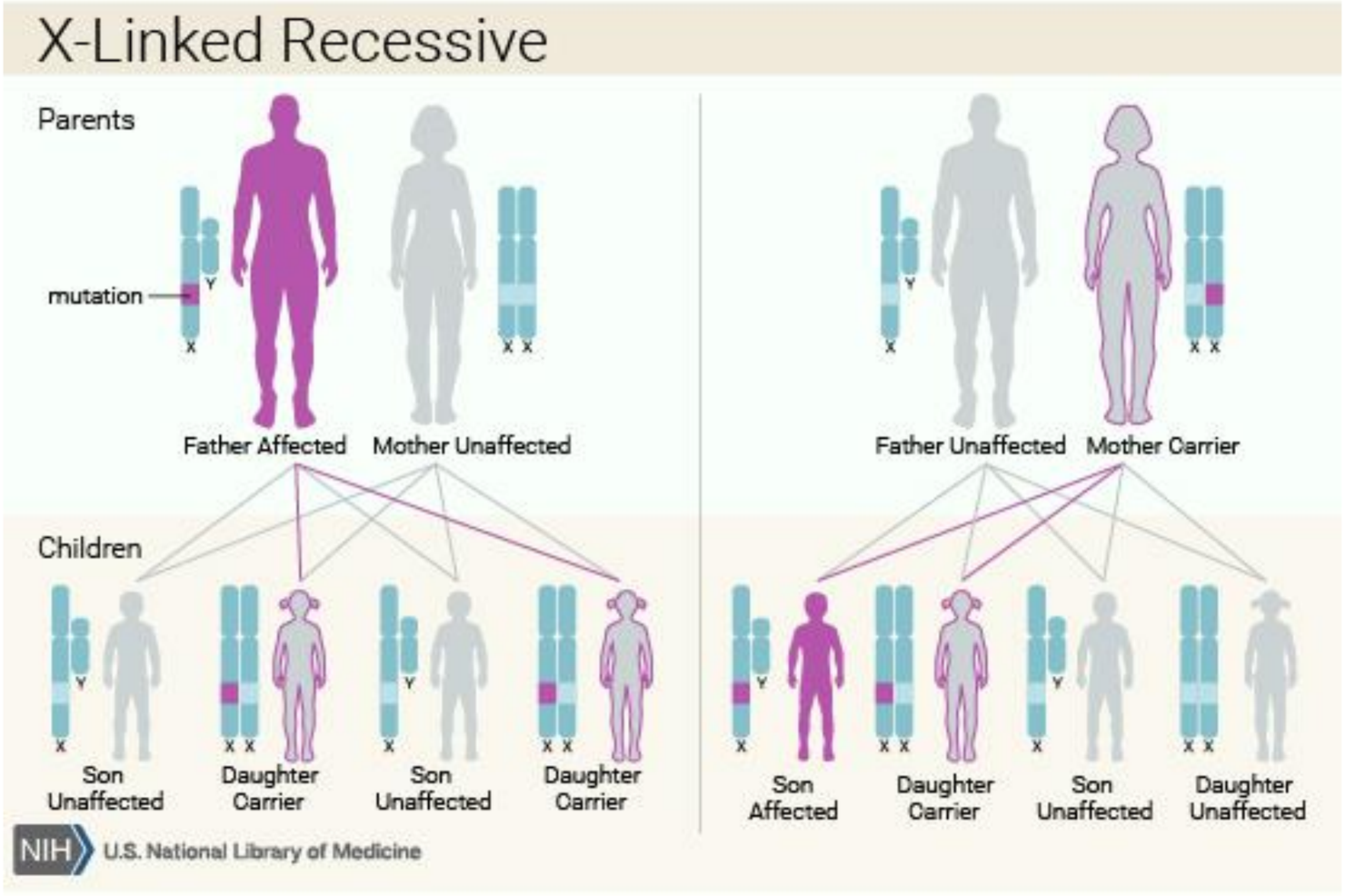
Example
“CVID” genes:
ARHGEF1
BAFF-R

Example
SCID genes:
RAG1
IL7R



Example
“CVID” genes:
PIK3CD
TWEAK
NKFB1
NKFB2
IZKF1
IRFBP2
SEC61A1


Autosomal Dominant - New Mutation
Parents
Unaffected
Unaffected
spontaneous mutation occurs during formation of egg or sperm cell or during embryonic development
Children
mutation
Unaffected
Unaffected
Affected
Unaffected
NIH
U.S. National Library of Medicine



Example
“CVID” genes:

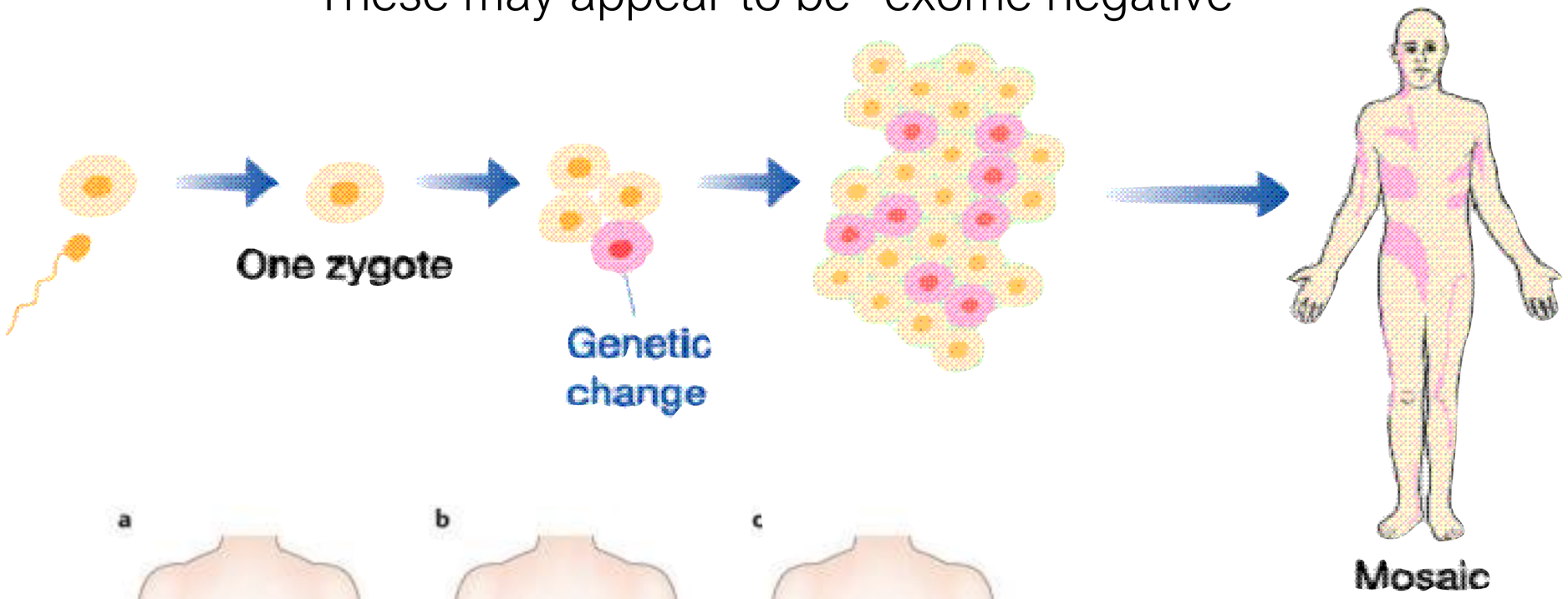
ATP6AP1
SH3KBP1

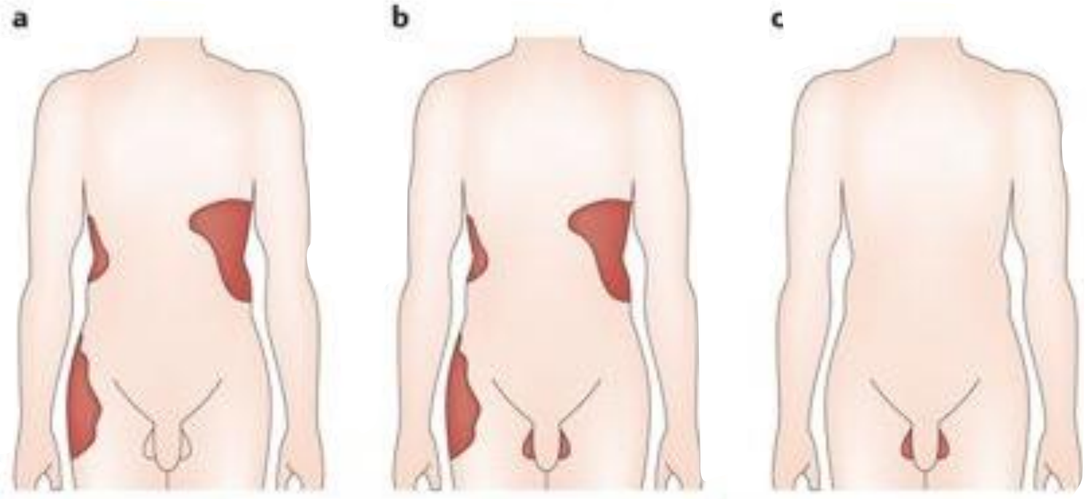
Well known
IEIs:

BTK
WAS
IL2RG

Don’t forget Somatic mosaicism

These may appear to be “exome negative”

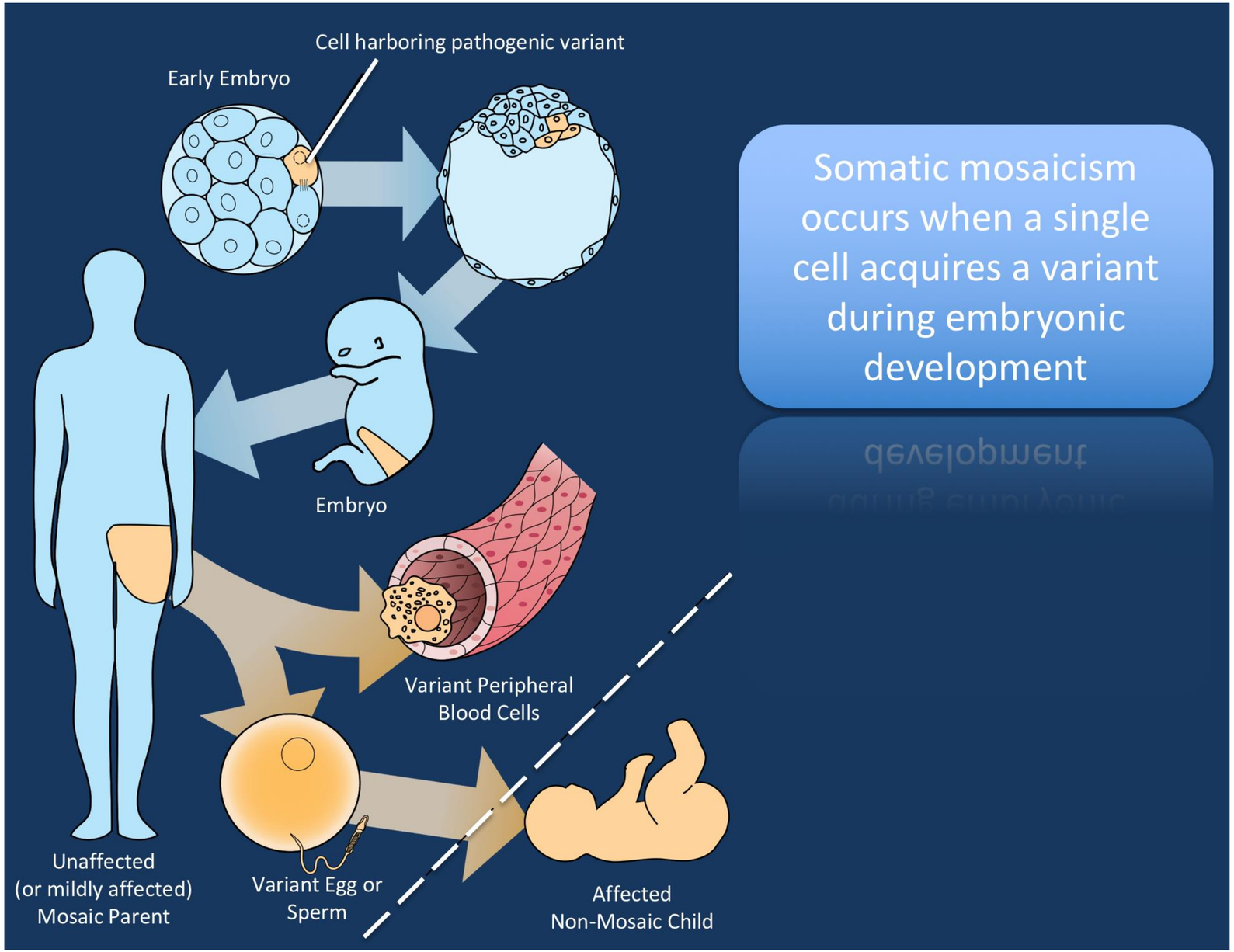




wiringthebrain.org

Biesecker Nat Rev Gen 2013

Inherited somatic mosaicism



Helbig (CHOP)

Unexpected relevant role of gene mosaicism in patients with primary immunodeficiency diseases

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Barcelona, Madrid, Las Palmas de Gran Canaria, Murcia, Esplugues, Oviedo, Granada, Santiago de Compostela, Santander, Alcorcon, Badalona, Leon, Zaragoza, and Palma de Mallorca, Spain; Mexico City, Mexico; San Francisco, Calif; Tunis, Tunisia; Sao Paulo, Brazil; Bogota and Cartagena, Colombia; Leuven, Belgium; Kuala Lumpur, Malaysia; and Prague, Czech Republic

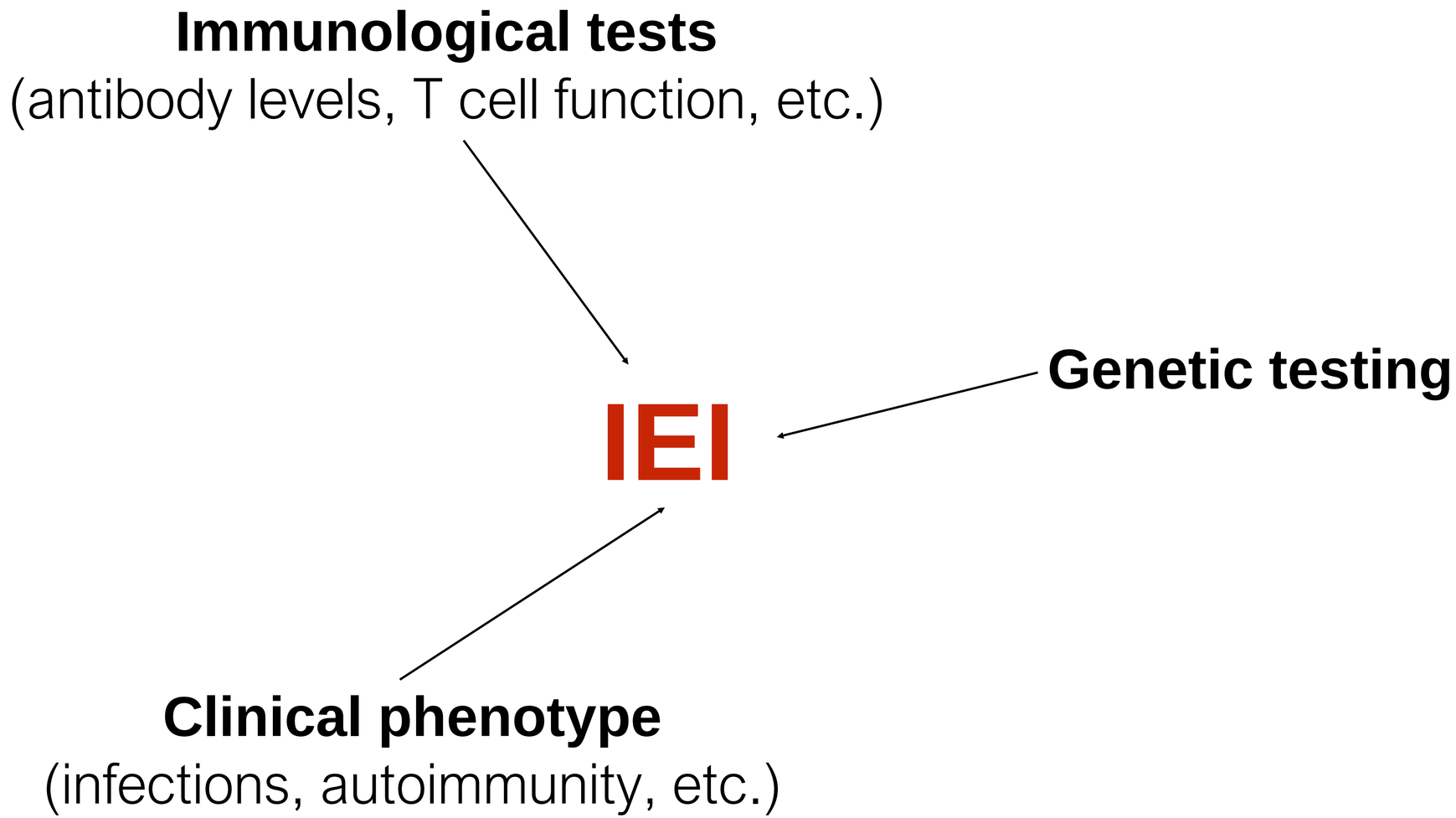
JACI Jan 2019

Somatic mosaicism was detected in 23% of PID patients

Somatic mosaicism causing disease with corresponding germline IEI.

Disease phenotype		Gene	Chr	Types of mosaicism	Cell types/ tissues affected	VAF	Mechanism
Autoimmune lymphoproliferative syndrome (ALPS)		*FAS*	Chr10	Somatic	PBMCs, DNTs	1–50%	LOF
RAS-associated autoimmune leukoproliferative disease (RALD)		*KRAS*	Chr12	Somatic	T,B, NK cells	NA	GOF
		NRAS	Chr1	Somatic	PBMCs	50%	GOF
Auto inflammatory disorders	CAPS	*NLRP3*	Chr1	Somatic	Multiple tissues	2–45%	GOF
	NLRC4 GOF	*NLRC4*	Chr2	Somatic	Multiple tissues	30%	GOF
	TRAPS	*TNFRSF1A*	Chr12	Somatic GS	B, NK cells; Multiple tissues, sperm cells (GS)	18–30%; 4–21%	GOF
	Blau syndrome	*NOD2*	Chr16	Somatic GS	Multiple tissues	4.9–11%; 0.9–12.9%	GOF
	SAVI	*TMEM173*	Chr5	Somatic	Multiple tissues	NA	GOF
	JAK1 GOF	*JAK1*	Chr1	Somatic	Multiple tissues	27%	GOF
Chronic Granulomatous disease		*CYBB*	ChrX	Somatic	Leukocytes	NA	LOF
Hyper IgE syndrome		*STAT3*	Chr11	GS	Multiple tissues	NA	LOF

How we make a diagnosis



What makes IEIs different?

- Infection *susceptibility*
  - Mendelian: Monogenic, causative, highly penetrant
  - Can be inapparent until an infection comes along
  - Mechanism of immune defect dictates *when* and how it will present
    - e.g., adult onset disease due to memory B cells in CVID

- Rare but not that rare
- Variant hierarchy apparent in many of our genes
- Epigenetics (environment!) affects many of our phenotypes

- **The impact: Non-so-rare variants can be pathogenic and can lurk among the populus. Be careful when you look at gnomAD.**

Rareness matters… mostly

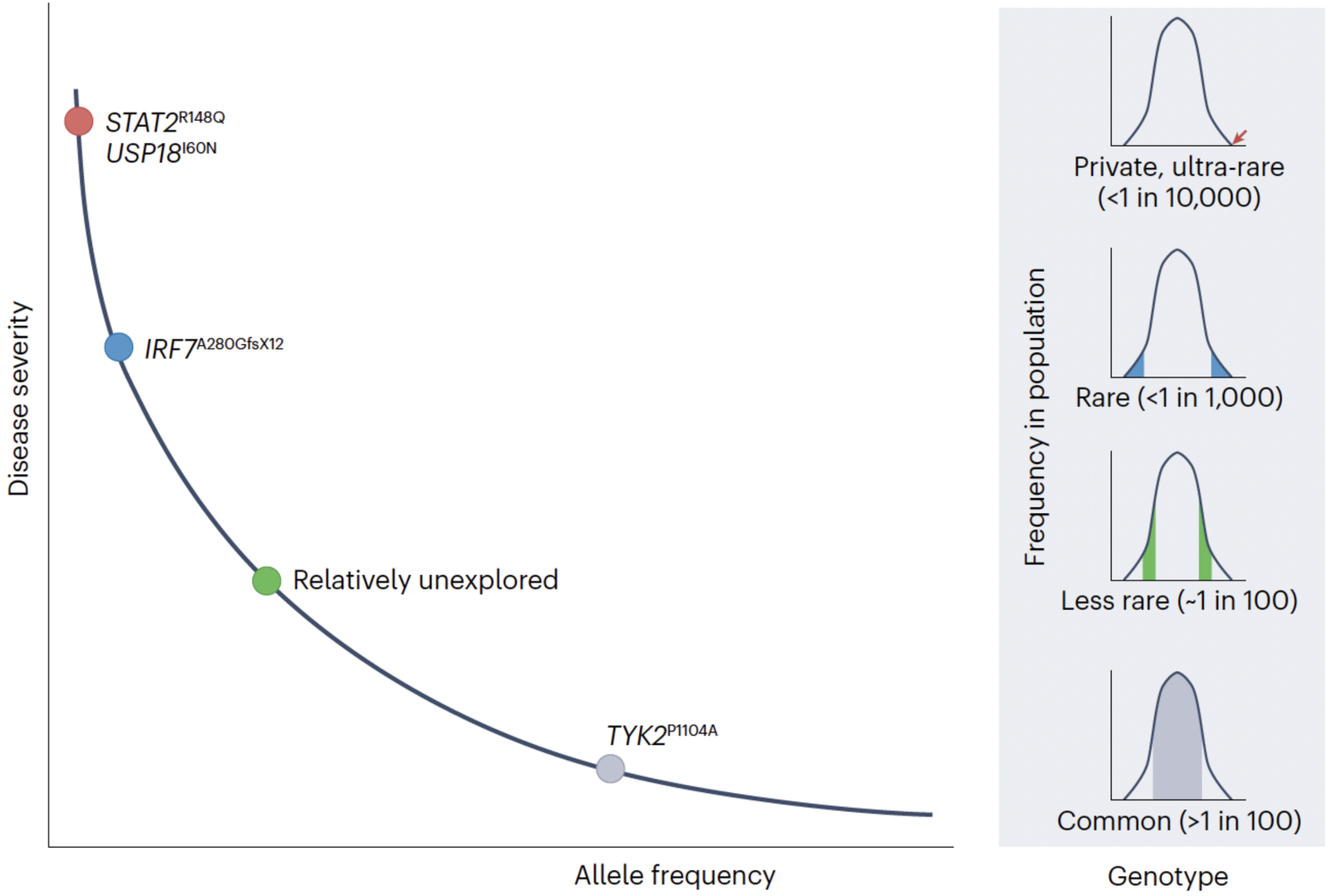
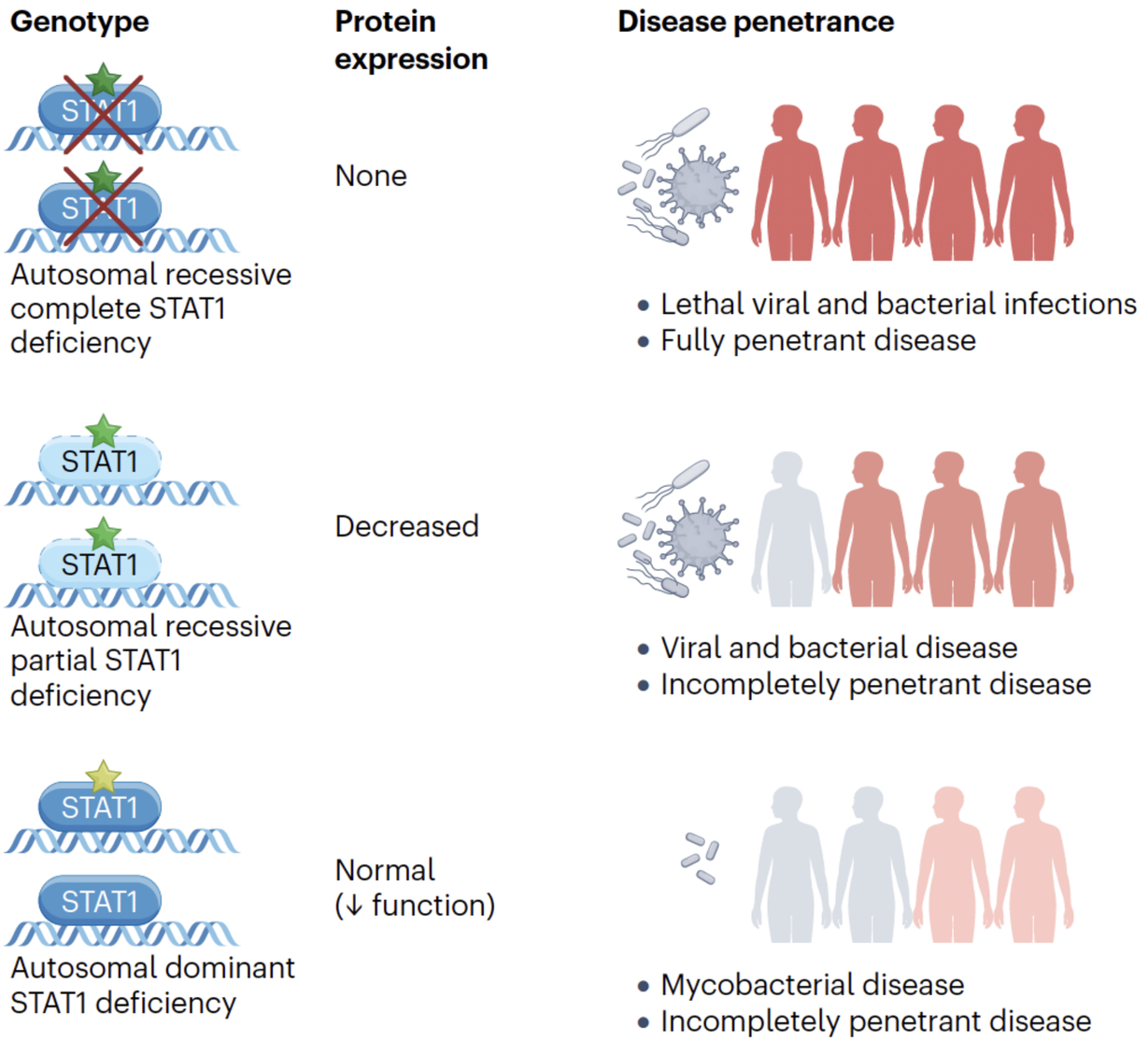


Fig. 2 | The relationship between allele frequency and disease severity for causal genetic lesions of inborn errors of immunity. The classic view is that private, ultra-rare and rare gene variants (such as variants of *STAT2*, *USP18* and *IRF7*) cause severe disease, whereas common gene variants (such as variants of *TYK2*) cause mild disease. The notion that less rare variants may cause inborn errors of immunity (IEIs) remains relatively unexplored, and advances in next-generation sequencing (NGS) are likely to uncover new variants belonging to this category. Examples of IEI gene variants that are common ($TYK2^{P1104A}$; ~1 in 20 individuals of European ancestries)[37], rare ($IRF7^{A280GfsX12}$; ~1 in 5,000 or ~1 in 1,400 individuals of Swedish or Finnish ancestries, respectively)[136], ultra-rare ($USP18^{I60N}$; ~1 in 250,000 individuals)[137] or private ($STAT2^{R148Q}$)[138] are indicated.

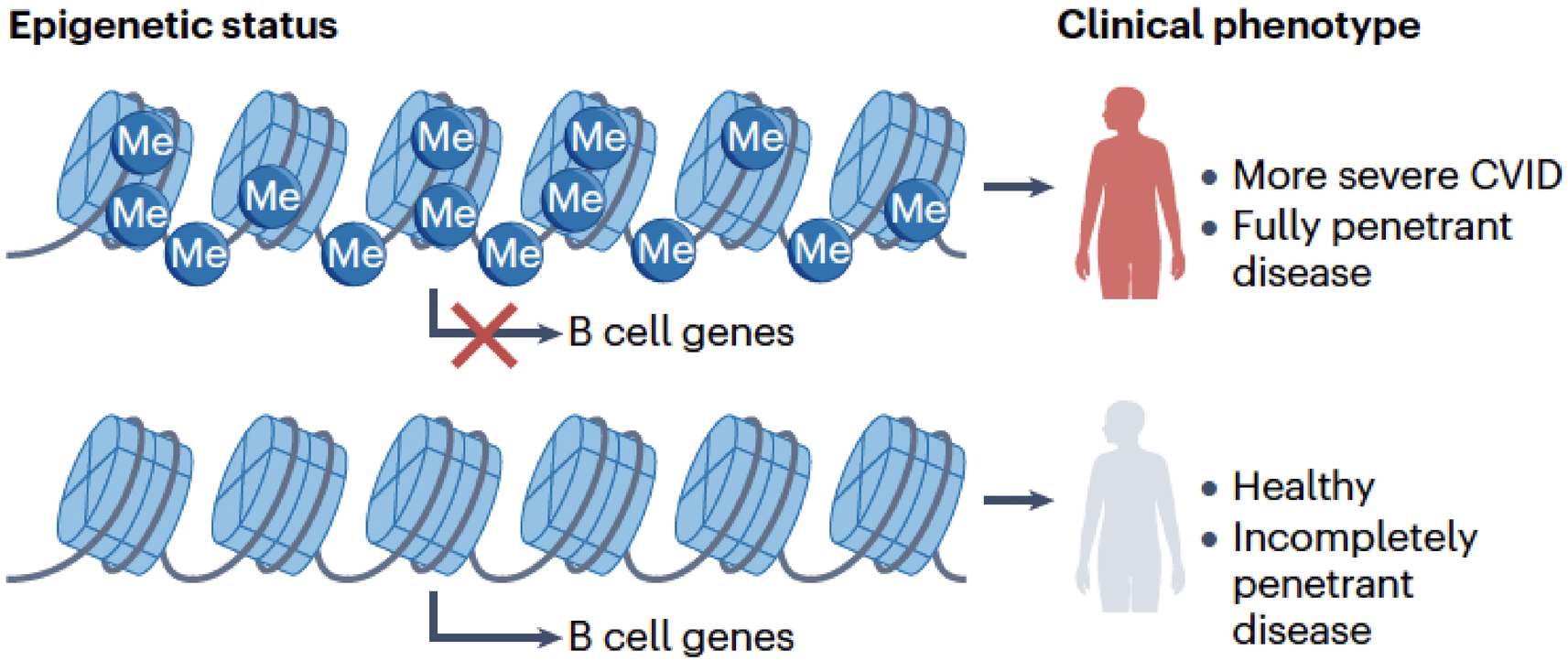
Akalu and Bogunovic, Nat Rev Gen 2024

Variant hierarchy affects clinical phenotype



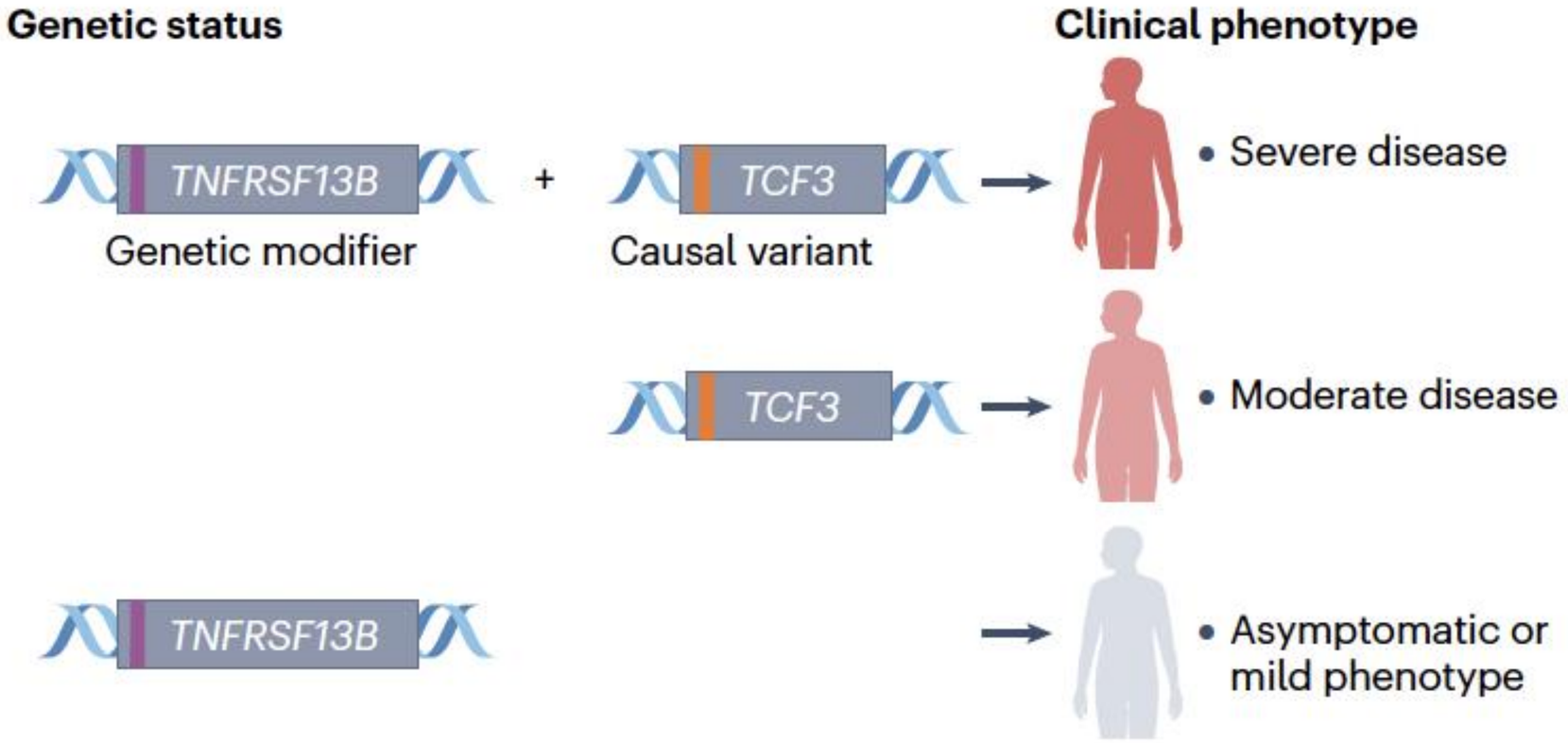
Akalu and Bogunovic, Nat Rev Gen 2024

Epigenetics affects clinical phenotype



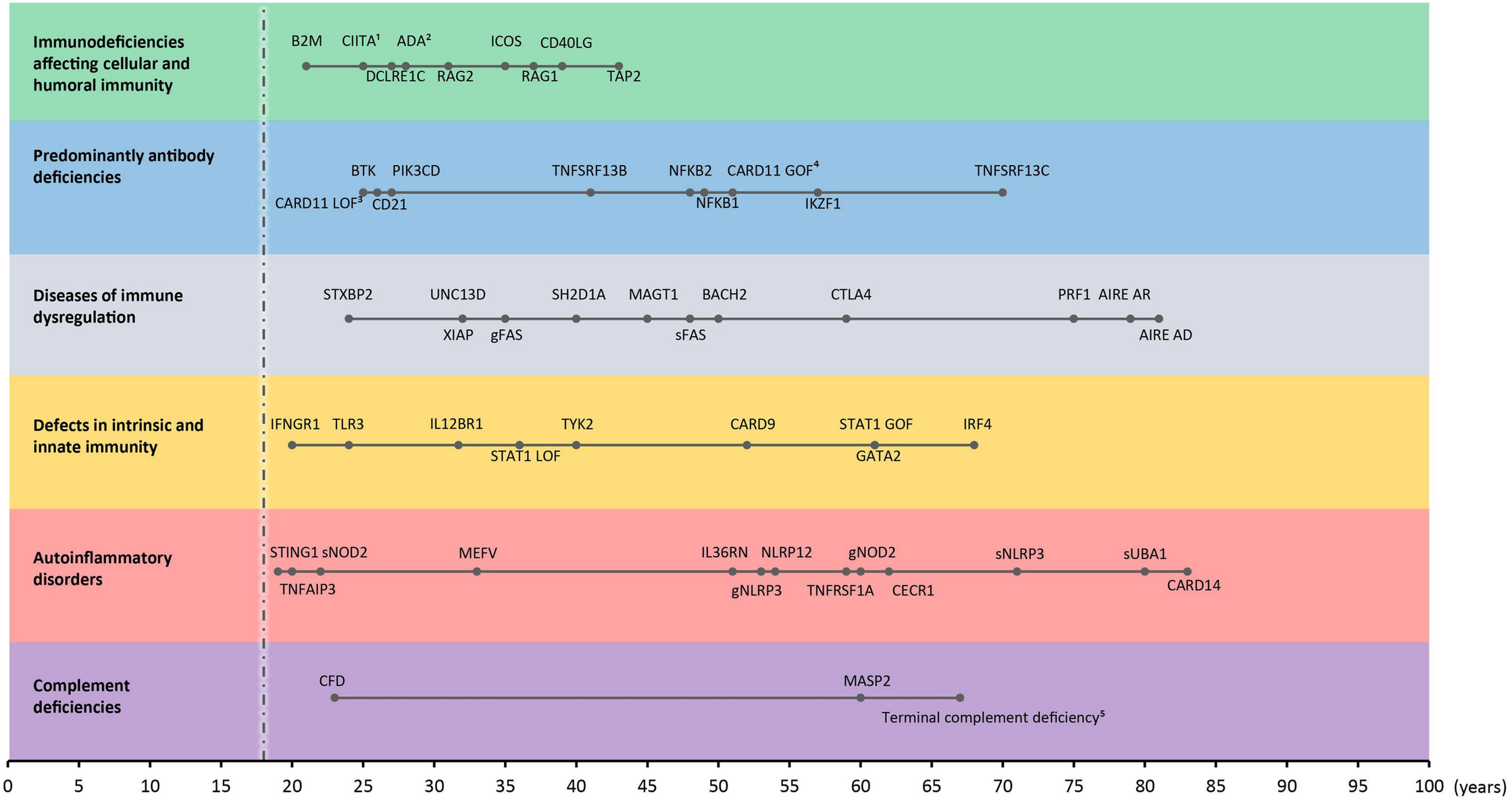
Akalu and Bogunovic, Nat Rev Gen 2024

Variant modifiers affect clinical phenotype



Akalu and Bogunovic, Nat Rev Gen 2024

Inborn…but not only in children



Staels, …, Schrijvers. Front Imm 2021

What types of genetics testing are available?

- Sanger (**single gene**) testing
  - ONLY if you have a familial variant
- Consider **gene panels** (i.e. SCID)
  - Quick and relatively inexpensive
- **Whole exome sequencing** (WES)
  - Will go away soon
- **Whole genome sequencing** (WGS)
- **Chromosomal microarrays**

Recent CIS review for background and insurance purposes: Heimall et al, JoCI, 2018

How does genetic testing help IEI?

- **Ends the diagnostic odyssey**
  - Relief!
  - Avoid unnecessary testing

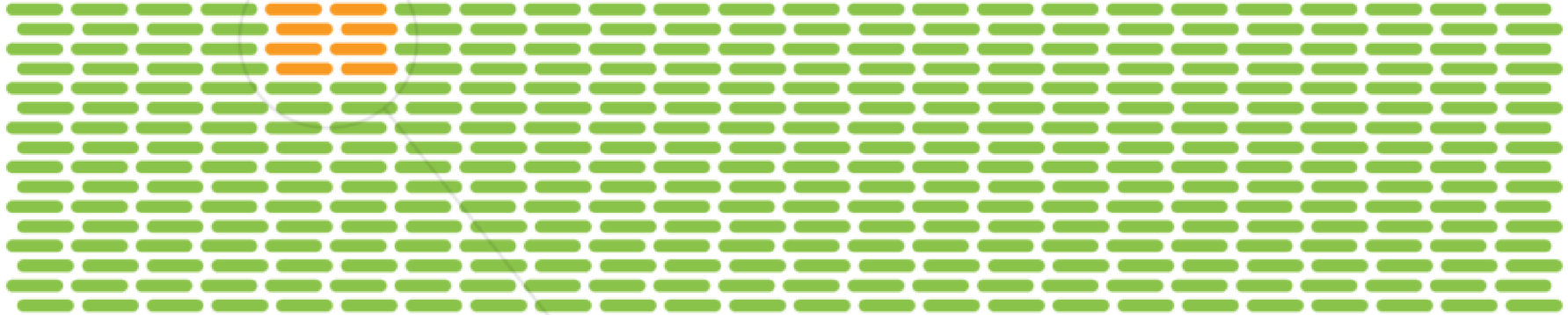
- **Gives you an ace card** to play against your Payor
  - for immunoglobulin or other treatments

- Allows **family planning** and genetic counseling
  - Preimplantation genetic diagnosis

- Directs specific ("**targeted**") treatments
  - gene therapy
  - specific inhibitors for autoimmunity / inflammation

Whole Genome Sequencing

~6,400,000,000 bases (100% of human genome)

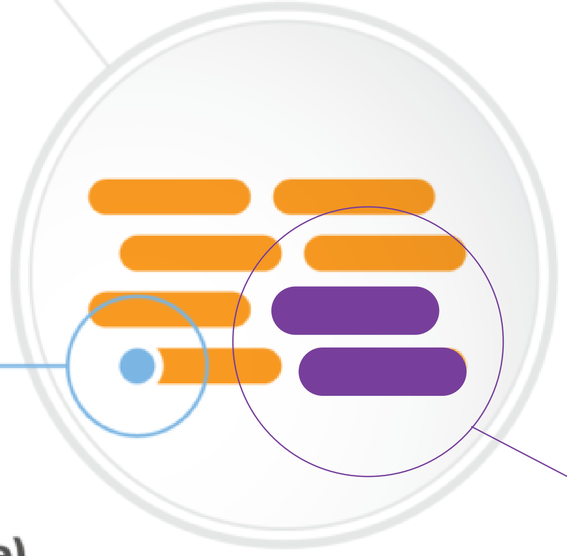


Whole Exome Sequencing

~60,000,000 bases
(~2% of human genome)

Large Scale Genotyping

~1,000,000 bases
(~0.03% of human genome)



23andMe is for entertainment & ancestry, NOT for rare disease diagnosis

Gene Panels

Exons of ~500 IEI genes

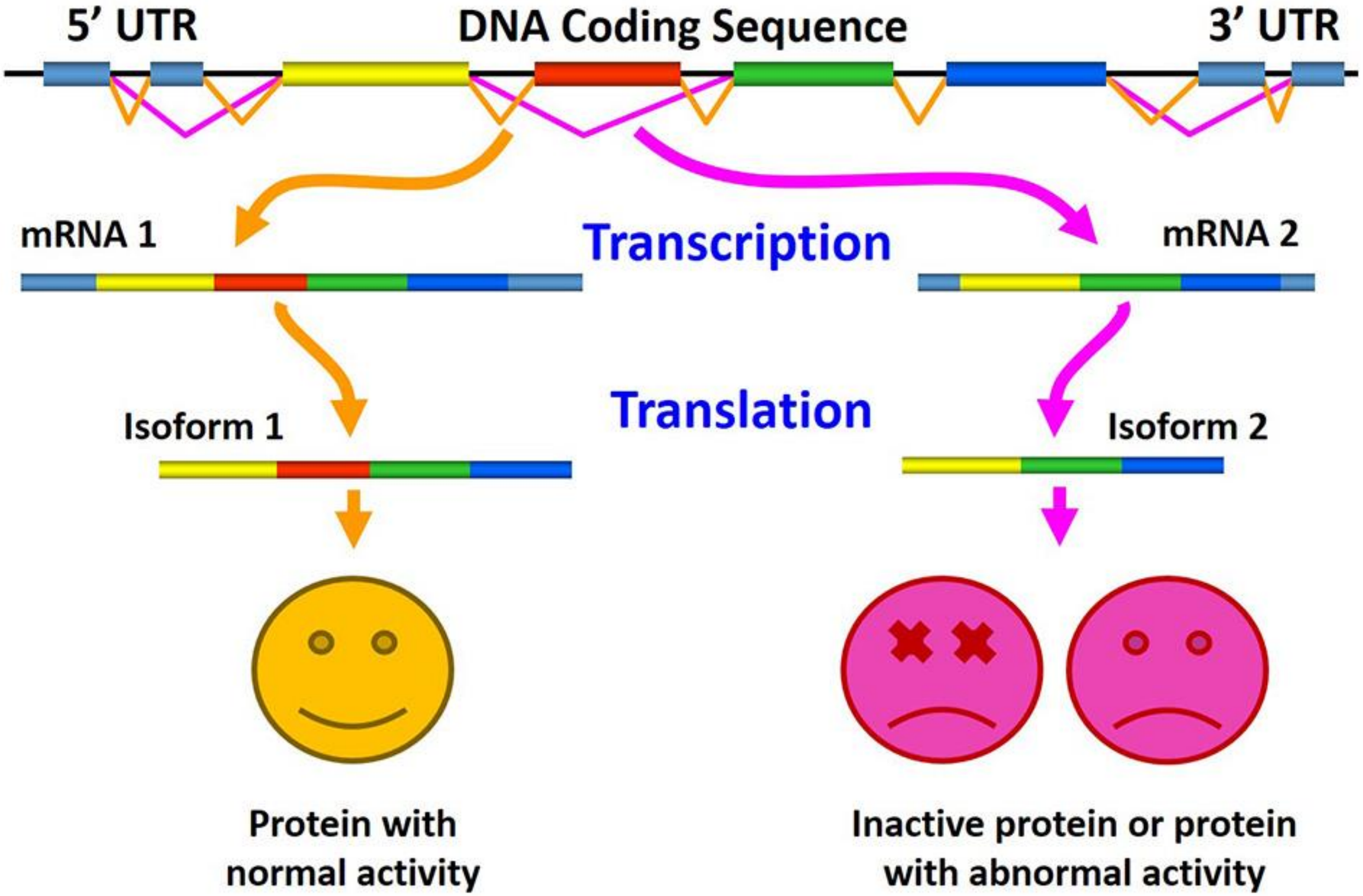
Caveats to WES

- Beware of low-cost, fly-by-night WES companies
- WES is useful for finding most variants (85+%)

(Botstein and Risch, 2003)

- WES *does not* look at all 20,000 genes
- **Not useful** for
  - Certain locations: Introns, regulatory regions
  - Types of variants: Not large deletions or large insertions

with well-selected patients, success rate of 20-40%



Uversky VN, Frontiers Mol Bio 2014

What does a WES miss?

- Things we think we're properly testing…but aren't
  - Exome baits can miss unknown exons or poorly mapped areas or GC rich regions (often the 5' exon)
- Things we know we're missing…and are
  - You will miss intronic regions (IL7R, IL2RG, ZAP70 intronic mutations have been seen) and other non-coding regions
  - We will only catch things that have been seen before (even if variants are present, won't be included in a clinical report unless it matches the phenotype and has been published)
    - Most often companies only report genes that are implicated in human disease (some with related phenotypes in animal models)

What to do when a WES is unrevealing

- Call the lab/company
  - Discuss the HPO (human phenotype ontology) terms and how the *phenotype* was used for filtering
    - If recurrent bacterial infections vs. viral infections – say that
    - If there is lymphopenia or neutropenia – say that
    - other associated symptoms or signs, give more details
    - Learn about HPO terms **https://hpo.jax.org/app/**

- Confirm read depth for any candidate genes

- Ask about research-level variants not included in the report

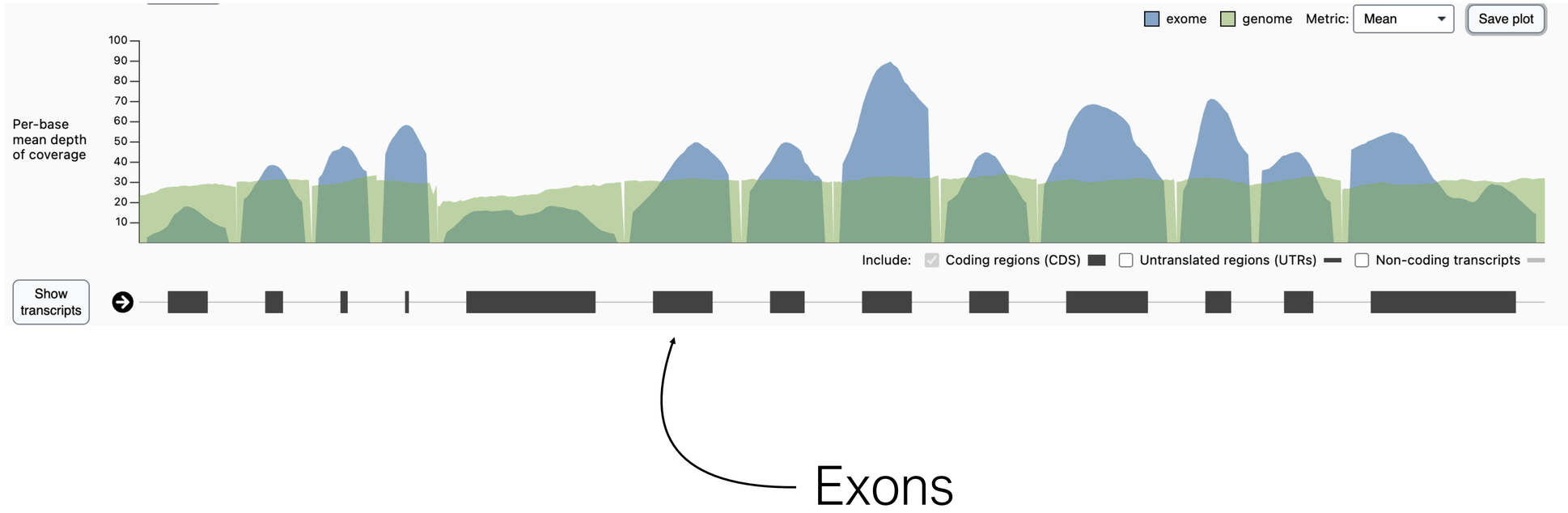
- Consider a WGS!

What does a WGS add beyond a WES?

- Generally more even and better coverage
  - No use of DNA baits to capture exons
  - Regulatory regions can be assessed
  - Deep intronic regions can be assessed

WGS more coverage than WES

RELB



IEI genes improved with WGS

Poor WES and Poor WGS									
Gene	Chr	WES %BP	WGS %BP	ΔWGS %	Gene	Chr	WES %BP	WGS %BP	ΔWGS %
CFHR1	1	69.8%	0.0%	-69.8%	FAM105B	5	83.4%	82.4%	-1.0%
CFHR3	1	70.2%	0.1%	-70.1%	RELB	19	48.6%	82.9%	34.3%
C4B	6	10.7%	5.5%	-5.2%	SPPL2A	15	80.0%	84.2%	4.2%
C4A	6	10.4%	7.5%	-2.8%	MYSM1	1	89.3%	85.6%	-3.7%
IKBKG	X	19.4%	26.8%	7.4%	UBE2T	1	88.2%	85.8%	-2.4%
NCF1	7	30.5%	30.3%	-0.2%	IRAK1	X	80.4%	86.0%	5.6%
TBX1	22	49.2%	48.2%	-1.0%	PMS2	7	83.7%	86.4%	2.7%
IRF2BP2	1	40.5%	61.2%	20.7%	CFHR2	1	84.9%	86.7%	1.7%
BCL11B	14	45.0%	66.1%	21.1%	SMARCD2	17	64.6%	87.0%	22.4%
GFI1	1	45.5%	71.9%	26.4%	CCBE1	18	89.2%	87.6%	-1.7%
ORAI1	12	84.1%	73.5%	-10.6%	CD55	1	66.8%	88.6%	21.8%
IFNGR2	21	87.3%	73.7%	-13.6%	TBK1	12	85.1%	88.9%	3.8%
USP18	22	64.1%	74.4%	10.3%	RFXAP	13	29.9%	89.1%	59.2%
NFKBIA	14	82.6%	79.3%	-3.4%	UNC93B1	11	24.1%	89.4%	65.3%
POLE2	14	82.6%	81.5%	-1.2%	RAD51	15	89.4%	89.4%	0.0%
PTEN	10	80.0%	82.2%	2.2%					

Rishi R. Goel et al, unpublished

Outcomes of the WES/WGS

1. **A clear answer**
   - Known pathogenic variant in a known disease-causing gene that matches your patient
2. **A potential answer**
   - Novel variant in a known gene causing human disease
     - Functional outcome is unclear, ranging from LOF to GOF (e.g., STAT1)
   - Novel variant in a gene without known link to human disease but that makes biological sense
   - Compound heterozygote mutations in a single pathway where each gene usually requires homozygous mutations
3. **No relevant findings**

Now what?

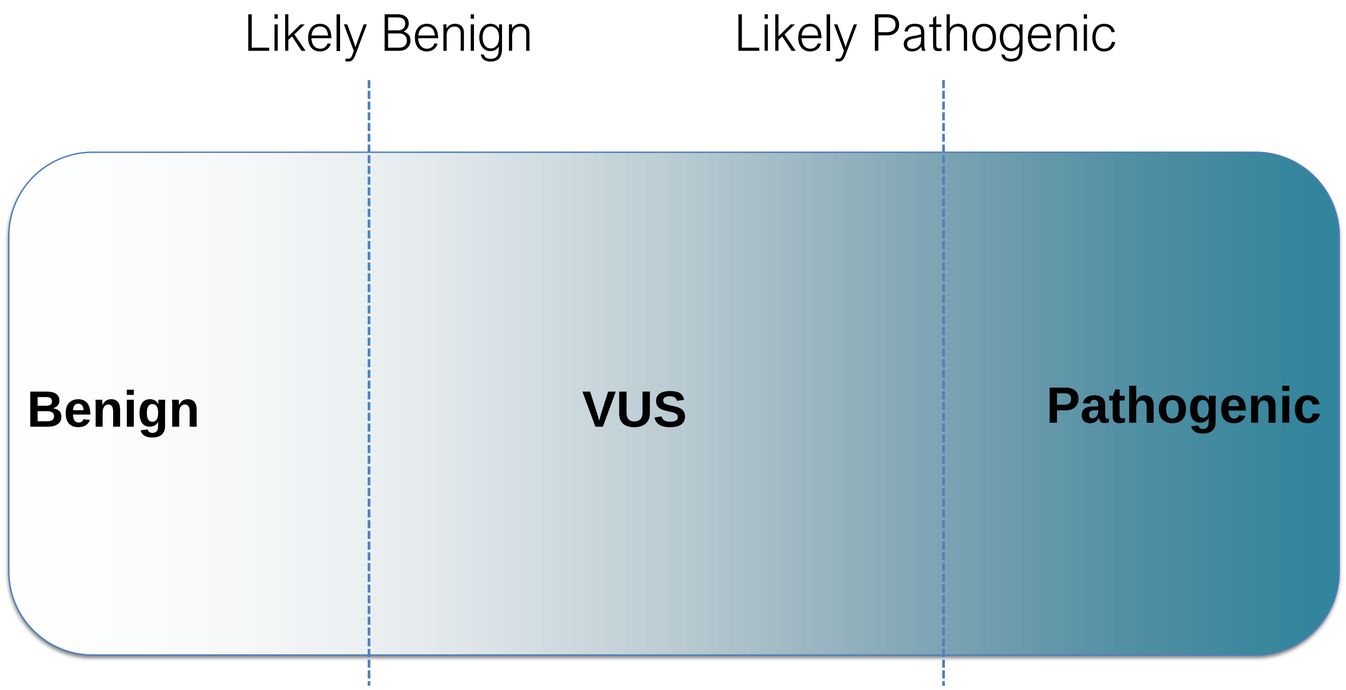
Key points of Genetics of IEI

- IEI is (largely) caused by monogenic variants in the genome that alter the function of immune development, homeostasis or responses.
  - everyone with IEI should have a genetic diagnosis
  - Only 20-30% of the cases are we successful

- If we say that a single genetic variant causes rare phenotypes like IEI, then the **variant ought to be rare** in the population.

- We do not believe that one gene ⇔ one disease anymore
  - multiple phenotypes are possible
  - Genetic testing is necessary for IEI

Second, variant classification



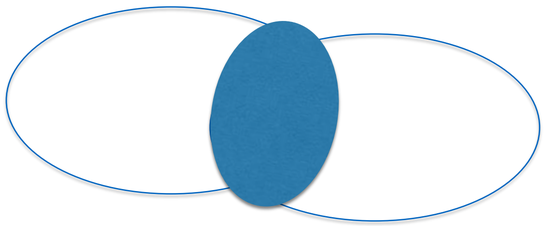
“Uncertain significance” means *don’t ignore it*

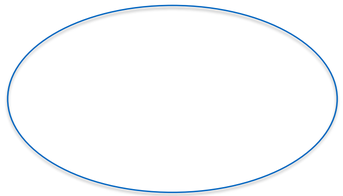
(Adapted from Richards et al, Genetics in Medicine, 2015)

Multiple VUS

- Which variant do you focus on first?
- Prioritize those genes that
  - The clinical symptoms overlap with the gene function

Gene1 function



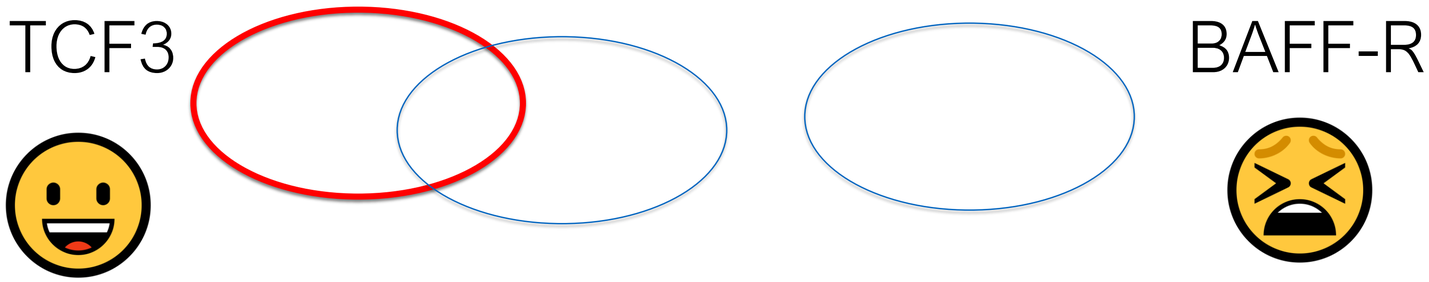


Gene2 function

Patient clinical phenotype

Multiple VUS

- For example



Which VUS to prioritize?

- Is the **gene expressed in the immune system**?

  – Use Immgen.org
  – Use google scholar

- Is the **variant likely to affect the function** of the protein?

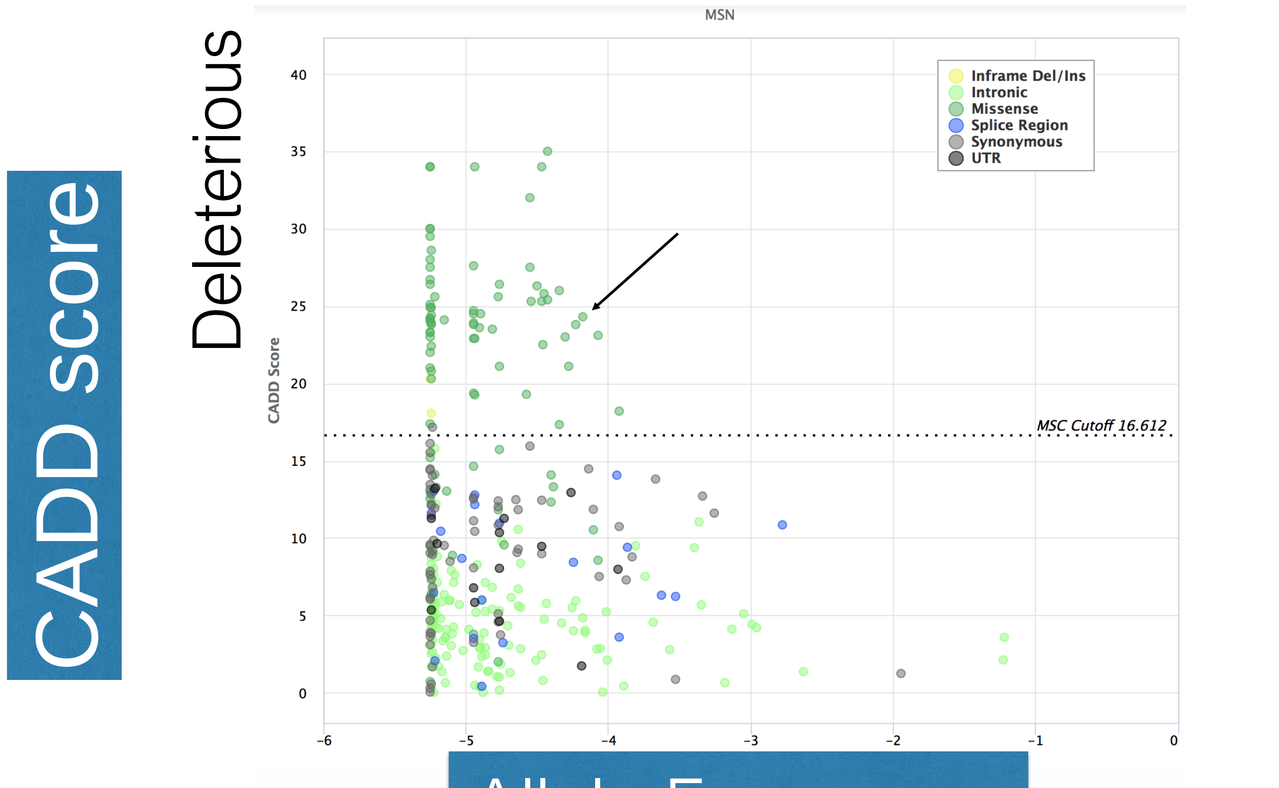
  – Does it hit a conserved domain?

How likely is your variant to be deleterious?

- Try a few useful metrics:
  - **CADD** score: a way of measuring the likelihood that a variant is *deleterious* (Kirchner, Nat Gen, 2014)
    - >20 in the top 1% of deleterious variants. >30 in the top 0.1% of deleterious variants.
  - **MAF**: minor allele frequency
    - The frequency in a population of the second most common allele (i.e. not the major allele)
  - Rare in healthy controls
    - https://gnomad.broadinstitute.org/

Easiest to visualize the CADD and MAF together

- Use“PopViz” (http://shiva.rockefeller.edu/PopViz/)



Zhang, *Bioinformatics*, 2018