Interpreting Genetic Testing results

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Conflicts of interest

- None relevant to this talk
- Scientific consultant to Ultragenyx
- Scientific consultant to Adivo

Why is genetic testing different?

- Genomic data is qualitative
- Needs to be transformed into probabilistic (quantitative) information
- Complications:
  - disruptions in different genes will lead to disease in different ways
  - everyone (yes, including *you*) have thousands of genetic variants
  - genomic technology has blind spots
  - genetic knowledge has blind spots

How nature works

88 5050 80
Oak Hill
Side Chair

IMPORTANT NOTE

Carefully remove all the parts from the carton and put them individually on a soft cloth to prevent scratches or other damages occuring to the wood parts.

We have taken great care in the design of this product and request that you carefully and strictly follow our assembly instructions to ensure a completed product as it was designed.

Home Styles

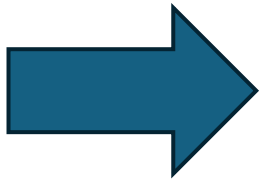
Part List

A. Seat 1 pc.
B. Side Frame 1 pc.
C. Side Frame 1 pc.
D. Front Frame 1 pc.
E. Back Frame 1 pc.

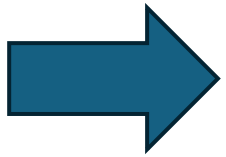
Hardware List

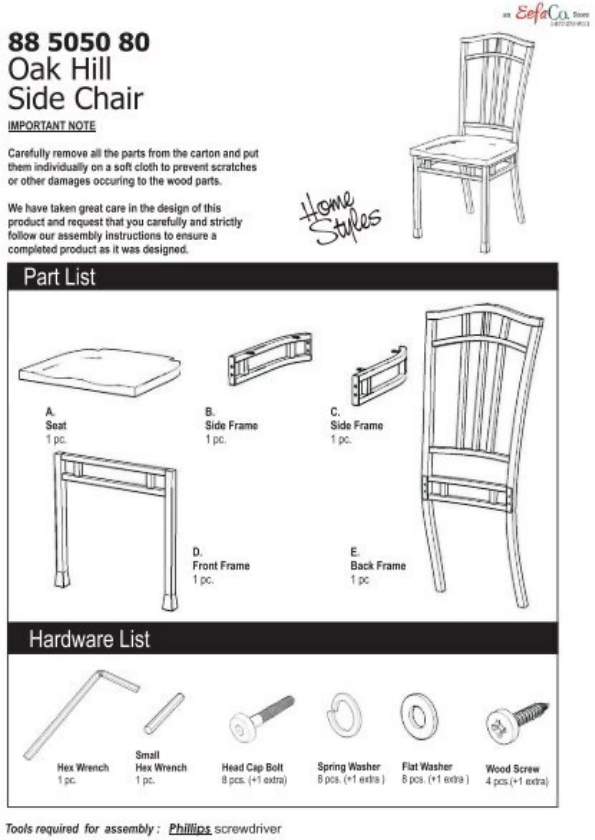
Hex Wrench 1 pc.
Small Hex Wrench 1 pc.
Head Cap Bolt 8 pcs. (+1 extra)
Spring Washer 8 pcs. (+1 extra)
Flat Washer 5 pcs. (+1 extra)
Wood Screw 4 pcs.(+1 extra)

Tools required for assembly : Phillips screwdriver



Genetic testing





In reality

this chair may be wobbly

this is the chair assembly manual. There's a typo in page 7. Would this lead to the chair being wobbly?

How to start?

- American College of Medical Genetics (ACMG) 2015 Guidelines

ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

© American College of Medical Genetics and Genomics ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

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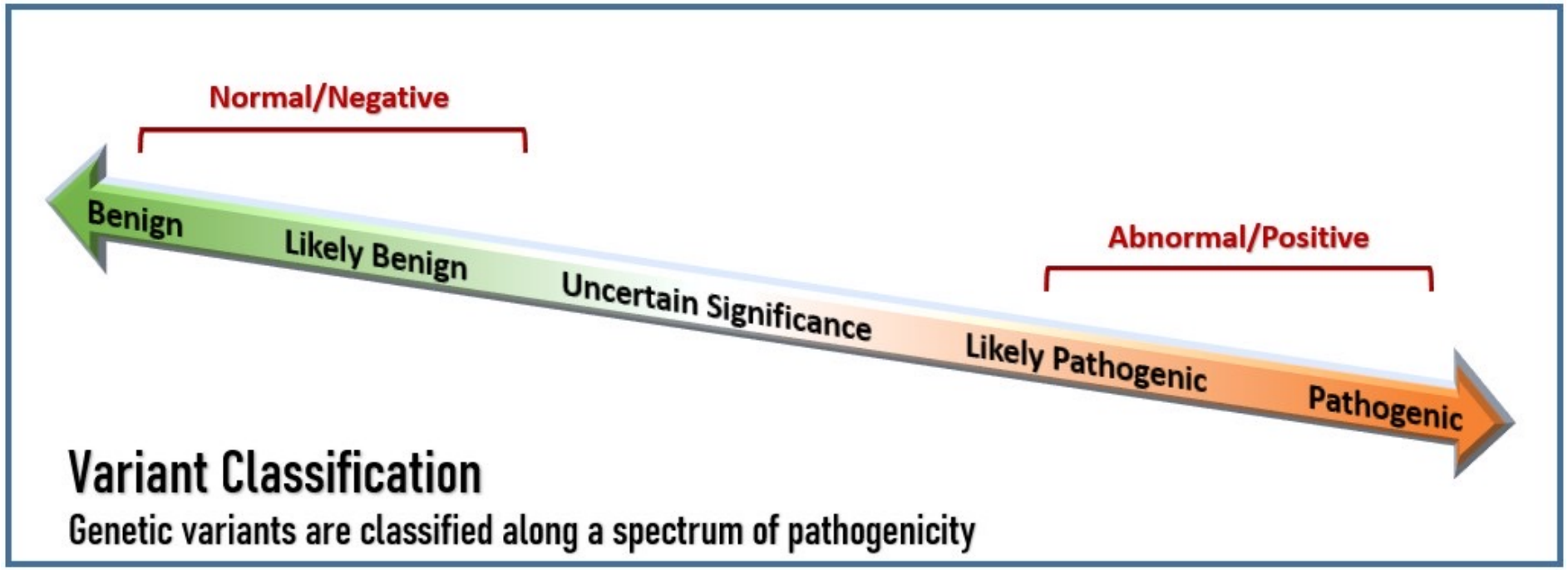
5-tiered system



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Genetics inMedicine | REVIEW ARTICLE

Navigating the nuances of clinical sequence variant interpretation in Mendelian disease

Natasha T. Strande, PhD[1], Sarah E. Brnich, BSc[2], Tamara S. Roman, PhD[2] and Jonathan S. Berg, MD, PhD[2]

		BENIGN CRITERIA		PATHOGENIC CRITERIA			
Strength of evidence		Strong	Supporting	Supporting	Moderate	Strong	Very Strong
Odds of Pathogenicity*		–18.7	–2.08	2.08	4.33	18.7	350.0
Evidence Category and Corresponding ACMG/AMP Codes	Population Data	*BA1*[+] BS1 BS2			PM2	PS4	
	Allelic Evidence & Cosegregation Data	BS4	BP2 BP5	PP1 →	PM3 PM6	PS2	
	Computation & Predictive Data		BP1 BP3 BP4 BP7	PP2 PP3	PM1 PM4 PM5	PS1	PVS1
	Functional Data	BS3				PS3	
	Other		BP6	PP4 PP5			

Vascular Genetics Symposium

What's taken into consideration?

1. Rarity – rare diseases need to be caused by rare variants

Stand-alone criterion: variants >5% in the general population are automatically benign

Variants that are absent from the general population are more suspicious

Take penetrance into account!

1. Rarity

Population databases:

gnomAD

Genome Aggregation Database

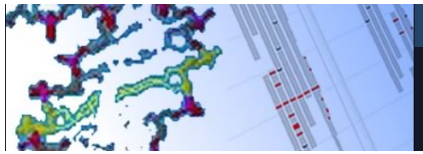
IGSR: The International Genome Sample Resource

Supporting open human variation data

DECIPHER

GRCh38

Ensembl



dbSNP

dbSNP contains human single nucleotide variations, microsatellites, and small-scale insertions and deletions along with publication, population frequency, molecular consequence, and genomic and RefSeq mapping information for both common variations and clinical mutations.

ABraOM: Brazilian genomic variants

1. Rarity

gnomAD
Genome Aggregation Database

COL3A1 c.245G>A, p.(Gly82Glu)

SNV: 2-188984925-G-A(GRCh38) Copy variant ID | Gene page | Dataset: gnomAD v4.1.0

	Exomes	Genomes	Total
Filters	Pass	Pass	
Allele Count	17	1	18
Allele Number	1461034	151912	1612946
Allele Frequency	0.00001164	0.000006583	0.00001116
Grpmax Filtering AF (95% confidence)	0.000008520	—	0.000008780
Number of homozygotes	0	0	0

External Resources
- dbSNP (rs745961376)
- UCSC
- ClinVar (2440294)
- ClinGen Allele Registry (CA75358)
- All of Us

Feedback
Report an issue with this variant

Genetic Ancestry Group Frequencies

gnomAD | HGDP | 1KG | Local Ancestry

Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
Remaining	1	62430	0	0.00001602
European (non-Finnish)	17	1179400	0	0.00001441
African/African American	0	74846	0	0.000
Admixed American	0	59836	0	0.000
Ashkenazi Jewish	0	29562	0	0.000
East Asian	0	44844	0	0.000
European (Finnish)	0	63978	0	0.000
Middle Eastern	0	6078	0	0.000
Amish	0	912	0	0.000
South Asian	0	91060	0	0.000
XX	11	811908	0	0.00001355
XY	7	801038	0	0.000008739
Total	**18**	**1612946**	**0**	**0.00001116**

COL3A1 c.283-45G>T

SNV: 2-188985152-G-T(GRCh38) Copy variant ID | Gene page | Dataset: gnomAD v4.1.0

	Exomes	Genomes	Total
Filters	Pass	Pass	Discrepant frequencies
Allele Count	6072	391	6463
Allele Number	1424452	151990	1576442
Allele Frequency	0.004263	0.002573	0.004100
Grpmax Filtering AF (95% confidence)	0.005111	0.003950	0.005064
Number of homozygotes	17	2	19

External Resources
- dbSNP (rs150919697)
- UCSC
- ClinVar (254966)
- ClinGen Allele Registry (CA75673)
- All of Us

Feedback
Report an issue with this variant

Genetic Ancestry Group Frequencies

gnomAD | HGDP | 1KG | Local Ancestry

Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
European (non-Finnish)	5932	1146324	15	0.005175
European (Finnish)	225	63924	3	0.003520
Remaining	164	61156	1	0.002682
African/African American	69	74152	0	0.0009305
Admixed American	54	59726	0	0.0009041
Ashkenazi Jewish	16	29312	0	0.0005459
Middle Eastern	1	5996	0	0.0001668
East Asian	1	44652	0	0.00002240
South Asian	1	90290	0	0.00001108
Amish	0	910	0	0.000
XX	3402	790958	8	0.004301
XY	3061	785484	11	0.003897
Total	**6463**	**1576442**	**19**	**0.004100**

What's taken into consideration?

2. Mechanism

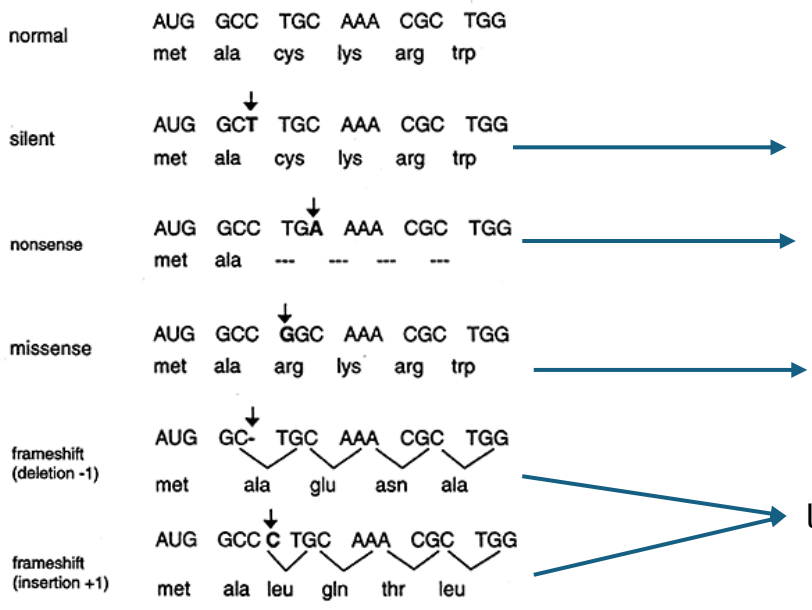
Is the way the variant impacts the gene according to the mechanism of disease for this gene?

Loss of function	Gain of function
The usual function of the protein is abrogated by the variant	The protein either functions at a higher level (i.e. increased enzyme activity) or changes into a new function

2. Mechanism

Types of variants

Is the way the variant impacts the gene according to the mechanism of disease for this gene?



a.k.a "synonymous" – tolerated unless affects splicing

a.k.a "stop-gain" – leads to loss of function unless at the end of the protein

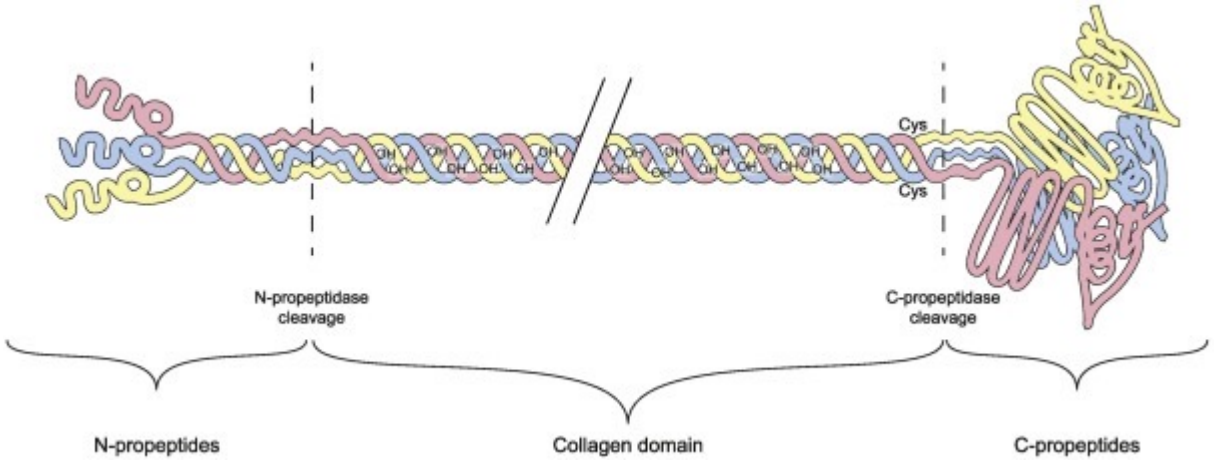
most variants – variable effect

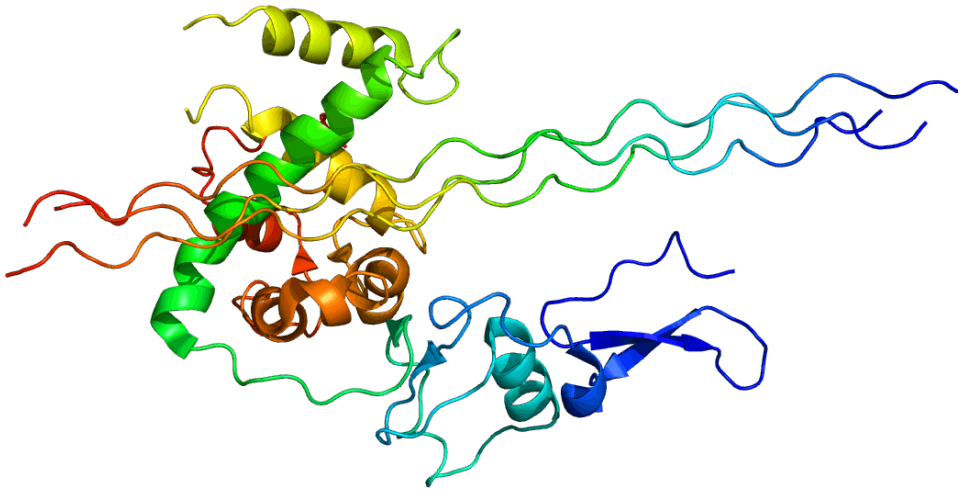
leads to loss of function unless proven otherwise

2. Mechanism

Assessing missense variants – geneticists' job security

- where in the protein is this change?
  - hotspot domains, active sites, important structural components
- what are the characteristics of the amino acids that are being substituted?
- does it also affect splicing?





Kuivaniemi H and Tromp G, *Gene*, 2019

Example (real case)

COL3A1 c.1024G>A, p.(Gly342Arg)

SNV: 2-188992914-G-C(GRCh38) Copy variant ID | Gene page | Dataset: gnomAD v4.1.0

	Exomes	Genomes	Total
Filters	Pass	No variant	
Allele Count	1	0	1
Allele Number	1461740	152098	1613838
Allele Frequency	6.841e-7		6.196e-7
Grpmax Filtering AF (95% confidence)	—		—
Number of homozygotes	0		0

External Resources

- UCSC
- All of Us

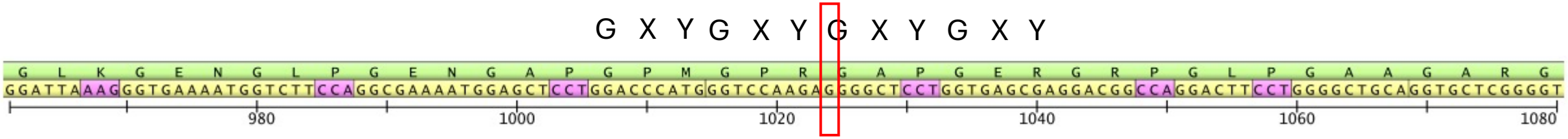
Feedback

Report an issue with this variant

Genetic Ancestry Group Frequencies

Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
South Asian	1	91062	0	0.00001098
African/African American	0	74990	0	0.000
Admixed American	0	59992	0	0.000
Ashkenazi Jewish	0	29604	0	0.000
East Asian	0	44826	0	0.000
European (Finnish)	0	63998	0	0.000
Middle Eastern	0	6058	0	0.000
European (non-Finnish)	0	1179908	0	0.000
Amish	0	910	0	0.000
Remaining	0	62490	0	0.000
XX	1	812312	0	0.000001231
XY	0	801526	0	0.000
Total	**1**	**1613838**	**0**	**6.196e-7**

Frequency is very low in gnomAD
Disrupts a G-X-Y helical motif (known mechanism)



Kuivaniemi H and Tromp G, *Gene*, 2019

2. Mechanism

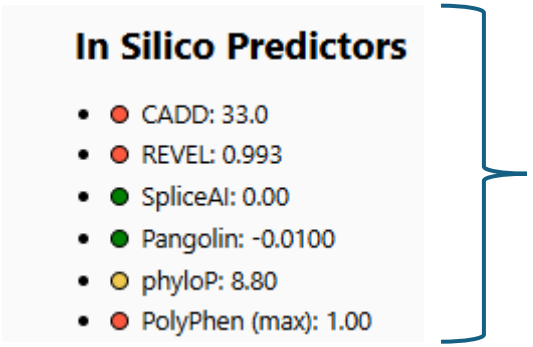
Assessing missense variants – geneticists' job security

In silico predictors – use with caution!

Each predictor has its own algorithm and they may be widely discordant – seek consensus!

- Some aggregator software may synthesize different predictors and provide an aggregated score

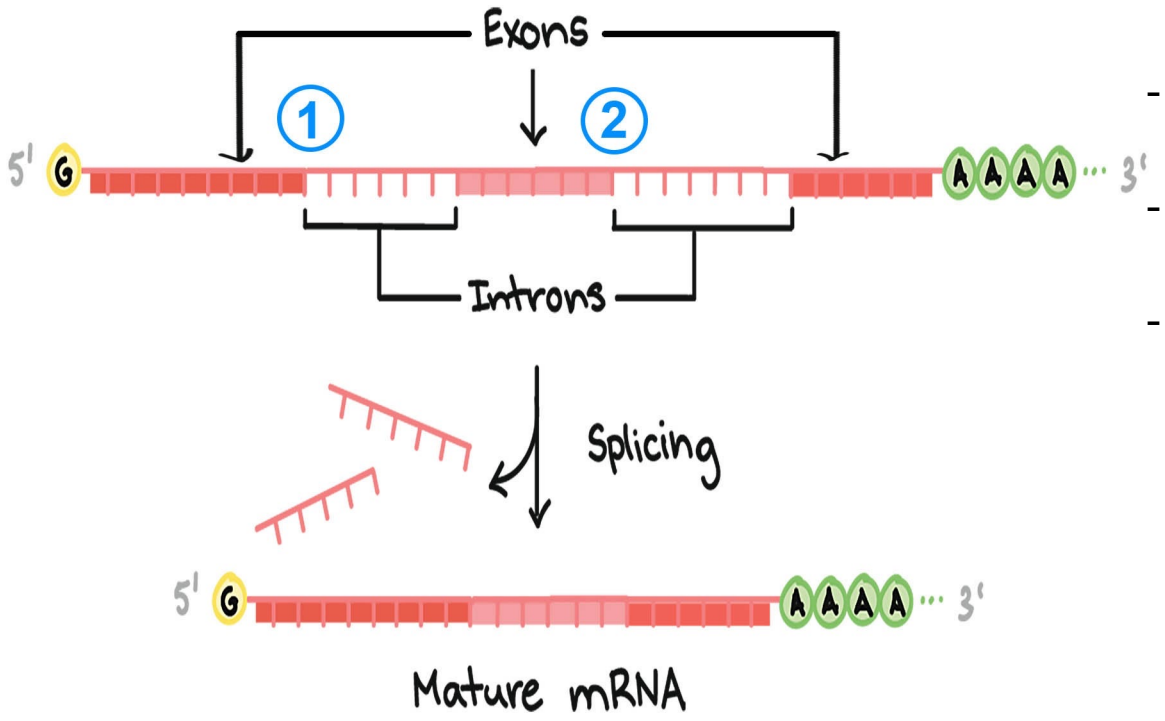
COL3A1 c.1024G>A, p.(Gly342Arg)



SpliceAI and Pangolin (*in silico* splice variant predictors) are not applicable to this case. Other predictors reached consensus

2. Mechanism

Don’t forget about splicing!

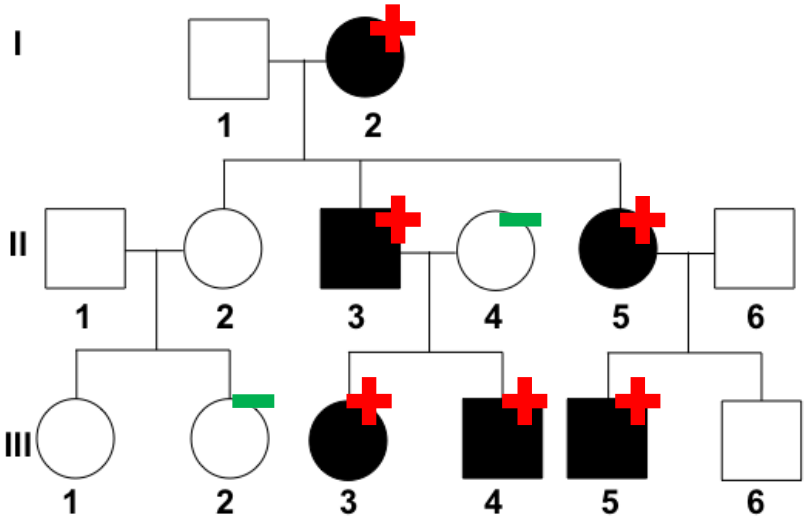


- “canonical” splice site variants (+-2) are usually very deleterious!
- other variants adjacent to the splice site may or may not cause abnormal splicing
- some variants are hiding deep in the introns but may also cause abnormal splicing

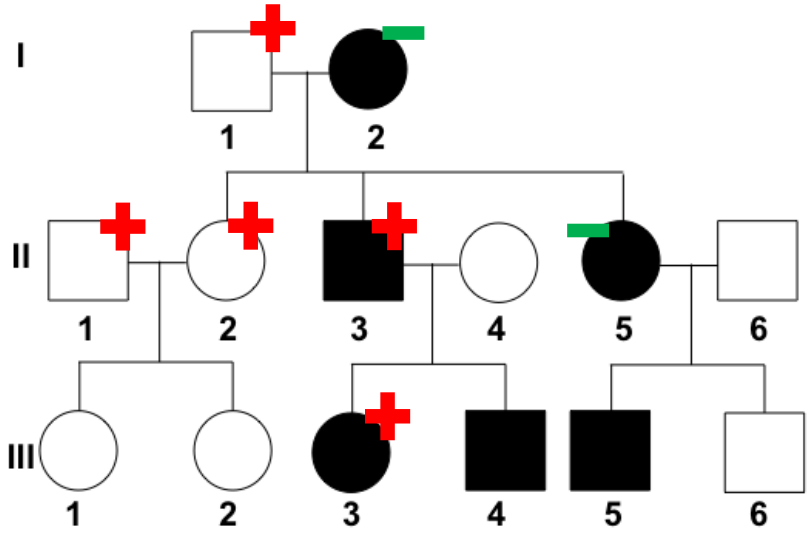
What's taken into consideration?

2. Allelism and segregation

Does the variant *segregate* with the phenotype/disease?



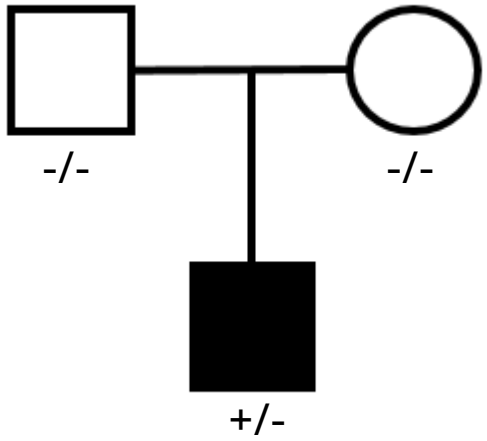
perfect segregation



lack of segregation

3. Allelism and segregation

De novo/germline mosaicism



- *De novo* variants have not been filtered out by generations of natural selection
  - Increased probability of pathogenicity
- Any *de novo* variant may be germline mosaic (parent's gonad has the variant, but not other tissues)
  - Implications for recurrence risk

What's taken into consideration?

4. Phenotype

Does the phenotype match what is expected from a variant in this gene?
Is it a *specific* match?

<u>Non-specific phenotypes:</u>
- joint hypermobility
- skin hyperextensibility
- mild ascending aortic aneurysm
- pectus excavatum
- "Marfanoid" habitus

<u>Specific phenotypes:</u>
- early-onset aortic rupture
- idiopathic viscus perforation
- ectopia lentis (lens subluxation)

Beware!
If you are using a panel, selecting a panel is already a phenotypic filter, and only *very* specific phenotypes should be applied for further variant classification!

Example (real case, cont'):

COL3A1 c.1024G>A, p.(Gly342Arg)

Patient: 64 y.o. F with:
- early-onset bilateral carotid dissections
- multiple spontaneous tendon ruptures
- uterine prolapse
- acrogeria

Family history:
- daughter (27 y.o.) with ascending aortic aneurysm and carotid dissection
- daughter (25 y.o.) with ascending aortic aneurysm
- both positive for same variant

This case meets:
- PM1 (hotspot variant)
- PM2 (absent/essentially absent from healthy population)
- PP1 (segregation with a few family members)
- PP3 (*in silico* consensus for deleteriousness)
- ?PP4 (specific phenotype)

→ ACMG criteria → likely pathogenic

Disclaimer

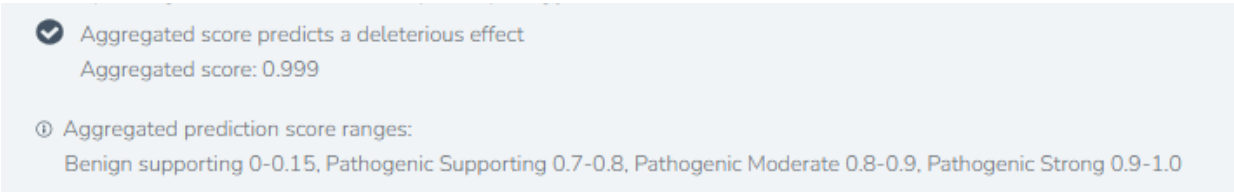
- There are other relevant criteria that have not been discussed at this presentation
  - Ex.: whether a variant has already been reported as pathogenic
- ClinGen: working groups that refine the overall criteria for specific genes
- Trust the lab, but verify! (Especially with large commercial labs!)

Your turn!

Case: 43 y.o. F
- spontaneous common iliac rupture
- splenic artery aneurysm requiring splenectomy

FH:
- mother deceased at 42 y.o. from ruptured renal artery
- maternal grandfather deceased in 50s of aortic rupture

Genetic testing:
- NW GAAP Panel: *COL3A1* c.2770G>A, p.(Gly924Ser)
  - variant disrupts G-X-Y motif
  - variant is absent from gnomAD



Summary of the criteria – click on the ones that this case matches, and it will compute them for you

Your turn!

Case: 26 y.o. M
- ascending aortic aneurysm (Z = +3.1)
- joint hypermobility

FH:
- father with coronary artery disease requiring stent at 56 y.o.
- sister with joint hypermobility

Genetic testing:
- Direct-to-consumer test: *COL3A1* c.130G>A, p.(Val44Ile)

Aggregated score: 0.382

ⓘ Aggregated prediction score ranges:
Benign supporting 0-0.15, Pathogenic Supporting 0.7-0.8, Pathogenic Moderate 0.8-0.9, Pathogenic Strong 0.9-1.0

Summary of the criteria – click on the ones that this case matches, and it will compute them for you

Genetic Ancestry Group Frequencies

gnomAD | HGDP | 1KG | Local Ancestry

Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
African/African American	377	74976	1	0.005028
Remaining	11	62464	0	0.0001761
Admixed American	6	59914	0	0.0001001
East Asian	3	44842	0	0.00006690
European (non-Finnish)	9	1179376	0	0.000007631
Ashkenazi Jewish	0	29554	0	0.000
European (Finnish)	0	64036	0	0.000
Middle Eastern	0	6056	0	0.000
Amish	0	912	0	0.000
South Asian	0	91076	0	0.000
XX	228	811964	1	0.0002808
XY	178	801242	0	0.0002222
Total	**406**	**1613206**	**1**	**0.0002517**

Thank you!

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