Are all variants of uncertain significance created equal? A rough guide.

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What you (hopefully) will learn in this webinar

Genetic testing is one component of a genetic diagnosis

Variants of Uncertain Significance (VUS) represent one category of genetic variants

What you should know about VUS before ordering genetic testing

Pre-test and post-test genetic counseling related to VUS

Understanding a genetic test report with a VUS

Navigating potentially complex decision making when faced with a VUS

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Clinical utility of genetic testing

- Genetic testing is the use of a laboratory test to examine an individual's DNA for variations: **Genetic variants** (also mutations, genetic change).
- The results of a genetic test can be used to confirm or rule out a suspected genetic disease: **Genetic diagnosis**.
- A genetic diagnosis may impact management: **Actionable test results**.
- Results may be used to determine the likelihood of parents passing on a genetic mutation to their offspring: **Recurrence risk**.
- Results may be used to determine the status (affected/unaffected) of other family members at risk: **Cascade testing**.

Applies only to a POSITIVE or NEGATIVE genetic test result.

UNCERTAIN genetic test results do not inform diagnosis, management or recurrence risk and can't be used to assess other family members.

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Clinical utility of genetic test results

POSITIVE or NEGATIVE genetic test result have clinical utility as they inform diagnosis, management or recurrence risk and can't be used to assess other family members.

UNCERTAIN genetic test generally results do not have immediate clinical utility as they do not inform diagnosis, management or recurrence risk and can't be used to assess other family members.

However, understanding an UNCERTAIN test result helps counseling the patient appropriately and navigating the next steps.

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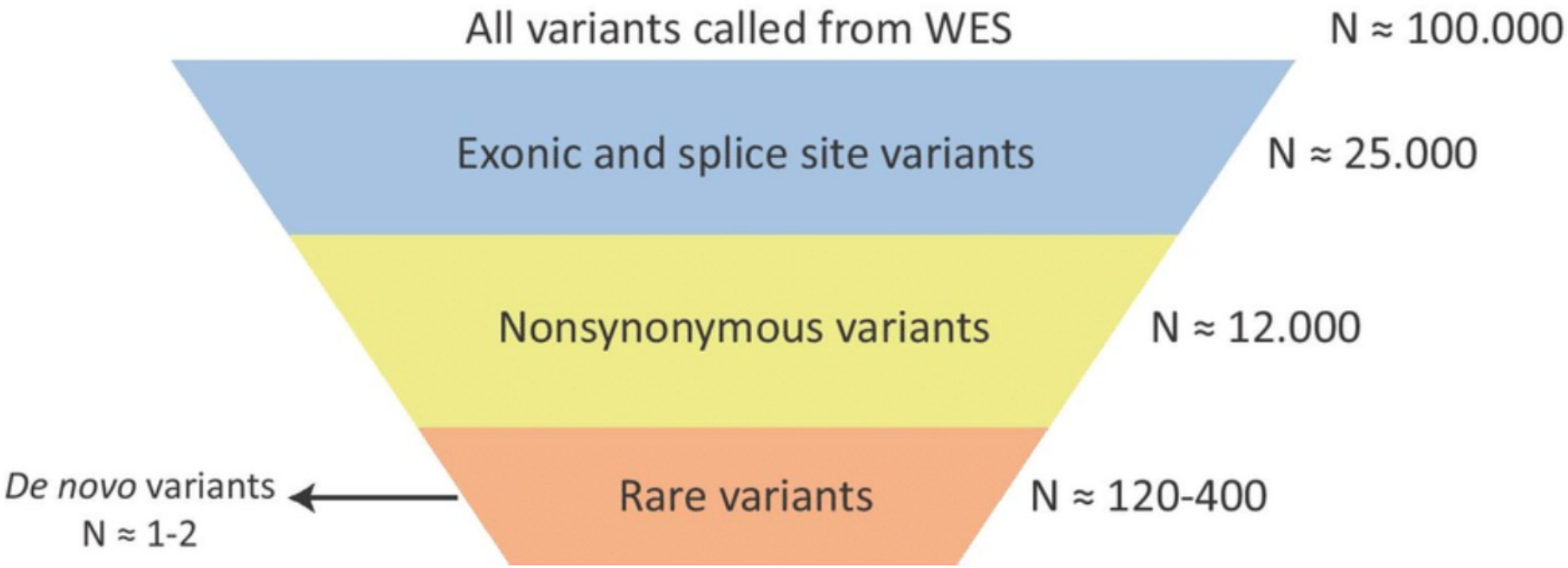
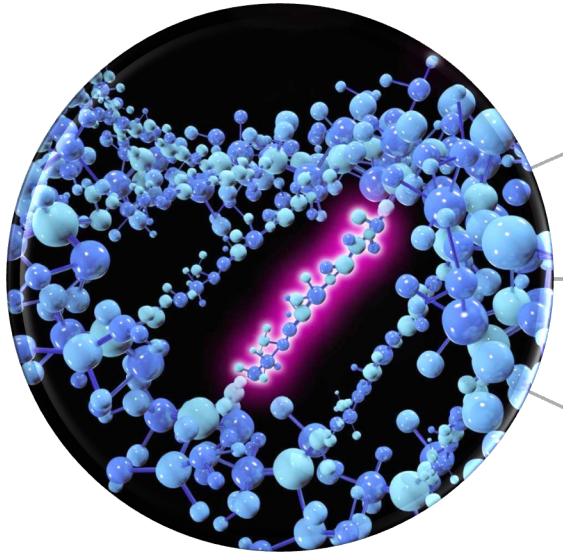
Variants of Uncertain Significance are inevitable in genomic approaches to genetic testing



Vorsteveld EE et al. Clin Rev Allergy Immunol. 2021 Oct;61(2):212-225. PMID: 33666867

Terminology

Genetic Variant



Variant Types

Variant Consequences

Variant Classification

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Variant Types

Variant types

SNV

AGACGCTGTCTTCAG
AGACGCAGTCTTCAG

MNV

AGACGCAGTCCCCAG
AGACGCAGTCTTCAG

Indels

AGACGCAGTC--CAG
AGACGCAGTCTTCAG

AGATCGCAGTCTTCAG
AGACGCAGTCTTCAG

Structural variants/ Copy number variants

Others:

- Nucleotide repeat expansions
- Methylation changes

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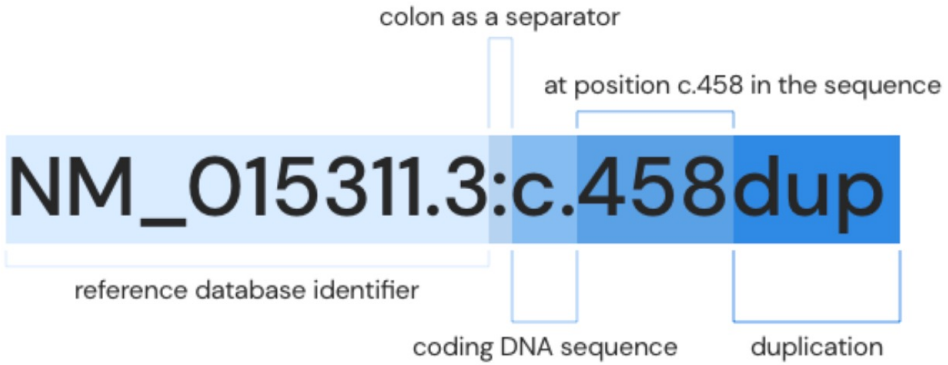
Basurto-Lozada et al. Next Generation Sequencing and Data Analysis. Learning Materials in Biosciences. Springer, Cham.

Variant description & HGVS nomenclature

- ✓ A basic understanding of how rare variants are described helps understanding a genetic test report.
- ✓ HGVS develops and curates rules and definitions for describing genetic variants



A letter prefix indicates the type of reference sequence

- "c." for a coding DNA reference sequence
- "p." for a protein reference sequence
- "g." for a genomic reference sequence
- "m." for a mitochondrial DNA reference sequence

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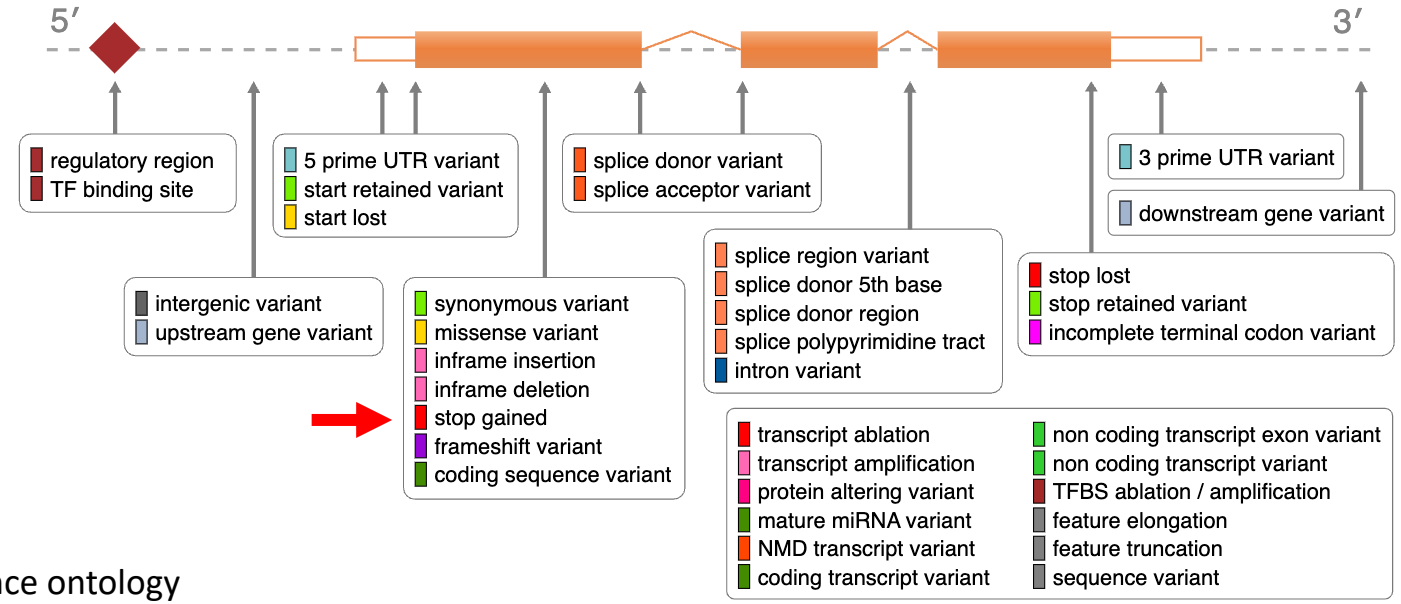
Variant
Consequences

Variant consequences



SO = Sequence ontology

*	SO term	SO description	SO accession	Display term	IMPACT
	splice_acceptor_variant	A splice variant that changes the 2 base region at the 3' end of an intron	SO:0001574	Splice acceptor variant	HIGH
	splice_donor_variant	A splice variant that changes the 2 base region at the 5' end of an intron	SO:0001575	Splice donor variant	HIGH
	stop_gained	A sequence variant whereby at least one base of a codon is changed, resulting in a premature stop codon, leading to a shortened transcript	SO:0001587	Stop gained	HIGH
	frameshift_variant	A sequence variant which causes a disruption of the translational reading frame, because the number of nucleotides inserted or deleted is not a multiple of three	SO:0001589	Frameshift variant	HIGH
	inframe_insertion	An inframe non synonymous variant that inserts bases into in the coding sequence	SO:0001821	Inframe insertion	MODERATE
	inframe_deletion	An inframe non synonymous variant that deletes bases from the coding sequence	SO:0001822	Inframe deletion	MODERATE
	missense_variant	A sequence variant, that changes one or more bases, resulting in a different amino acid sequence but where the length is preserved	SO:0001583	Missense variant	MODERATE
	synonymous_variant	A sequence variant where there is no resulting change to the encoded amino acid	SO:0001819	Synonymous variant	LOW
	5_prime_UTR_variant	A UTR variant of the 5' UTR	SO:0001623	5 prime UTR variant	MODIFIER
	3_prime_UTR_variant	A UTR variant of the 3' UTR	SO:0001624	3 prime UTR variant	MODIFIER
	non_coding_transcript_exon_variant	A sequence variant that changes non-coding exon sequence in a non-coding transcript	SO:0001792	Non coding transcript exon variant	MODIFIER
	intron_variant	A transcript variant occurring within an intron	SO:0001627	Intron variant	MODIFIER

Modified from https://www.ensembl.info/2012/08/06/variation-consequences/

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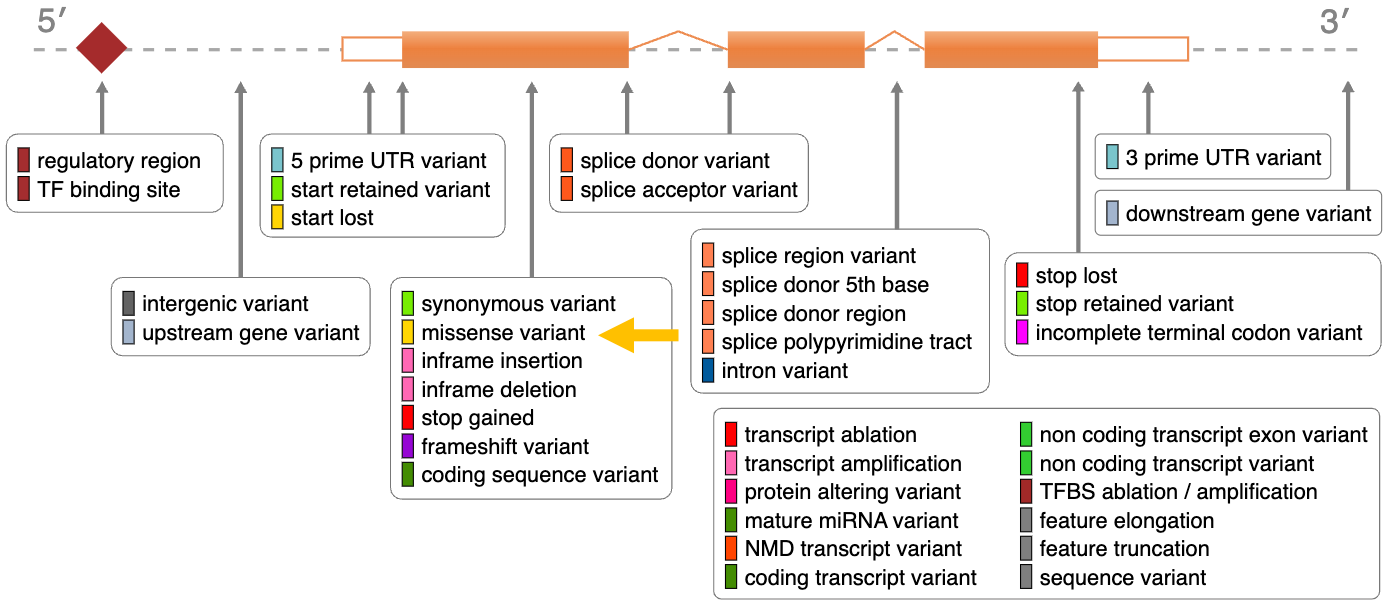
Variant consequences



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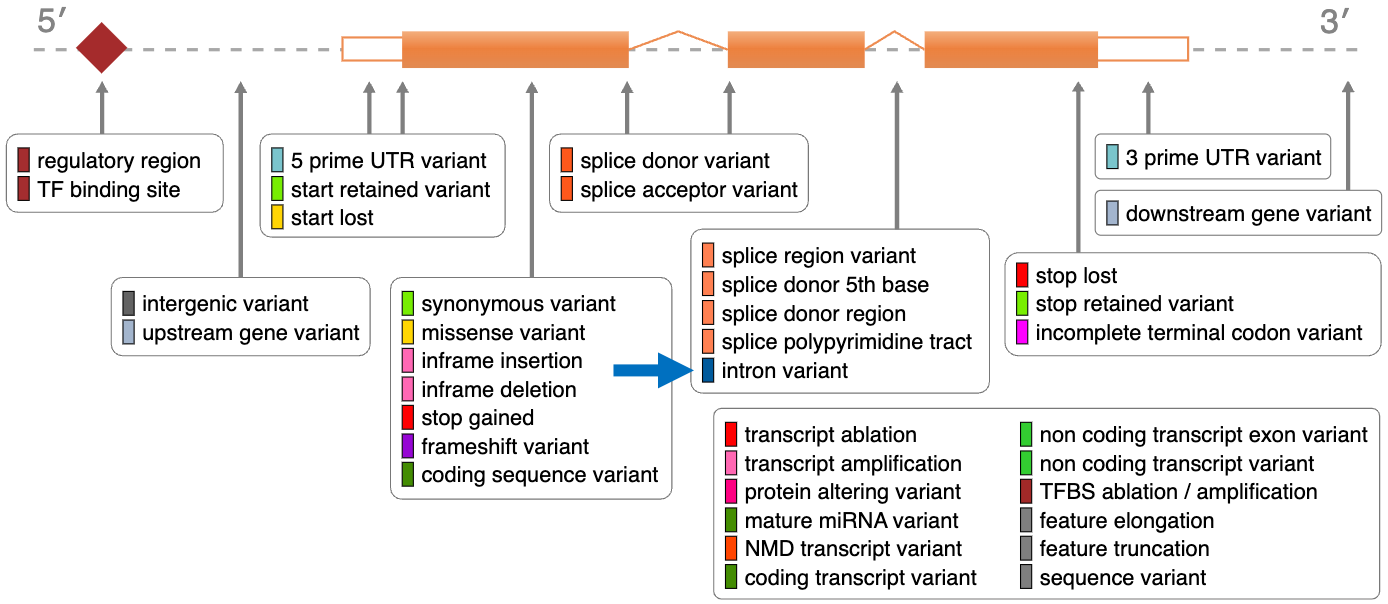
Variant consequences



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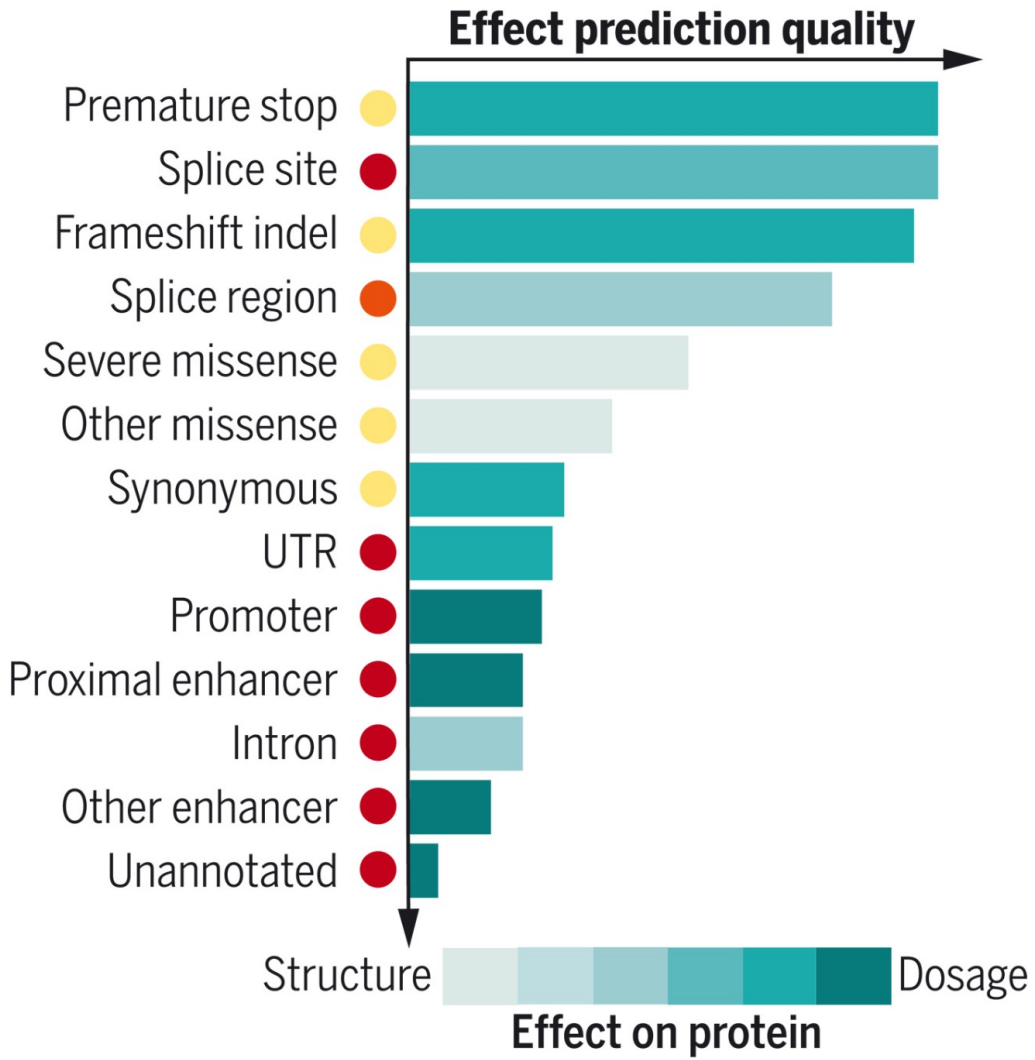
Variant effect predictions by variant consequences



Lappalainen and MacArthur. Science. 2021 Sep 24;373(6562):1464-1468. PMID: 34554789.


Variant
Classification

Variant classification

CLASS	DESCRIPTION
Pathogenic	Strong evidence that variant is disease causing
Likely Pathogenic	Evidence to support at least 90% certainty that variant is disease causing
Variant of Uncertain Significance	Variant does not fulfill criteria for benign or pathogenic, or the evidence for benign and pathogenic is conflicting
Likely Benign	Evidence to support at least 90% certainty that variant is *not* disease causing
Benign	Strong evidence that variant is *not* disease causing

Modified from Richards et al. 2015 and Brnich et al. 2018.

Variant classification

CLASS	DESCRIPTION	RESULT
Pathogenic	Strong evidence that variant is disease causing	POSITIVE
Likely Pathogenic	Evidence to support at least 90% certainty that variant is disease causing	POSITIVE
Variant of Uncertain Significance	Variant does not fulfill criteria for benign or pathogenic, or the evidence for benign and pathogenic is conflicting	UNCERTAIN
Likely Benign	Evidence to support at least 90% certainty that variant is *not* disease causing	NEGATIVE
Benign	Strong evidence that variant is *not* disease causing	NEGATIVE

Modified from Richards et al. 2015 and Brnich et al. 2018.

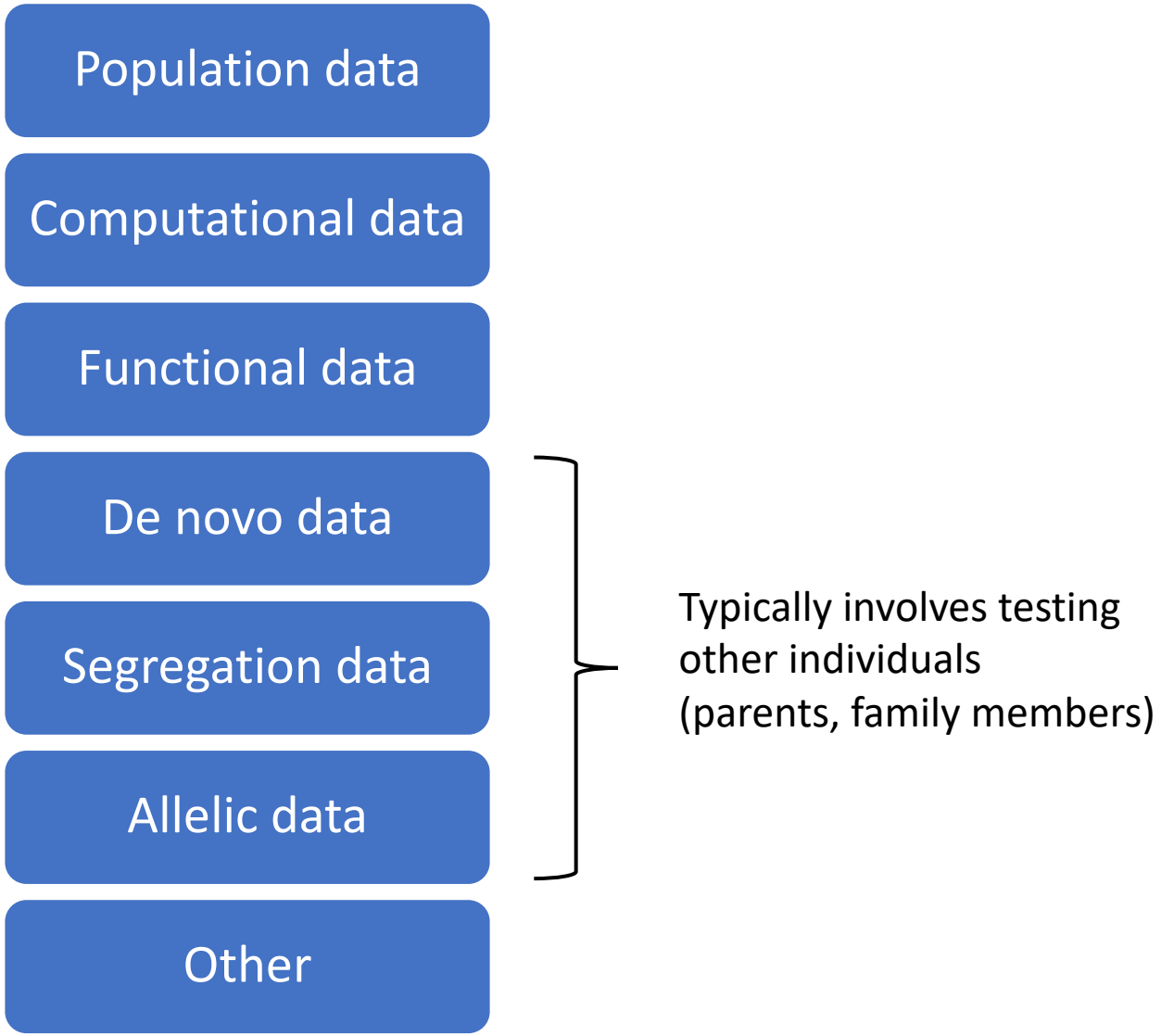
Variant classification

Evidence Code Strength
- Very strong pathogenic
- Strong pathogenic
- Moderate pathogenic
- Supporting pathogenic
- Supporting benign
- Strong benign
- Stand alone benign

EVIDENCE COMBINATIONS	CLASS	DESCRIPTION	RESULT
PS PVS PM PM PVS PP PM PVS PP PP PVS PS PS PM PM PM PS PP PP PM PM PS PP PP PP PP PM PS	Pathogenic	Strong evidence that variant is disease causing	POSITIVE
PM PVS PM PM PS PM PS PP PP PS PM PM PM PP PP PM PM PP PP PP PP PM	Likely Pathogenic	Evidence to support at least 90% certainty that variant is disease causing	POSITIVE
Insufficient evidence to reach a pathogenic or benign classification OR Mix (PP, PM, PS, or PVS) AND (BP or BS)	Variant of Uncertain Significance	Variant does not fulfill criteria for benign or pathogenic, or the evidence for benign and pathogenic is conflicting	UNCERTAIN
BP BS BP BP	Likely Benign	Evidence to support at least 90% certainty that variant is *not* disease causing	NEGATIVE
BA BS BS	Benign	Strong evidence that variant is *not* disease causing	NEGATIVE

Modified from Richards et al. 2015 and Brnich et al. 2018.

Evidence used in variant classification



Based on ACMG/AMP variant interpretation guidelines (Richards et al. 2015)

Evidence from population data

Absent from population databases (e.g. gnomAD)

- Moderate criterium supporting pathogenic classification (PM2)

Allele frequency in population too high for disorder

- Strong criterium supporting benign classification (BS1)

Allele frequency significantly increased in affected individuals

- Strong criterium supporting pathogenic classification (PS4)

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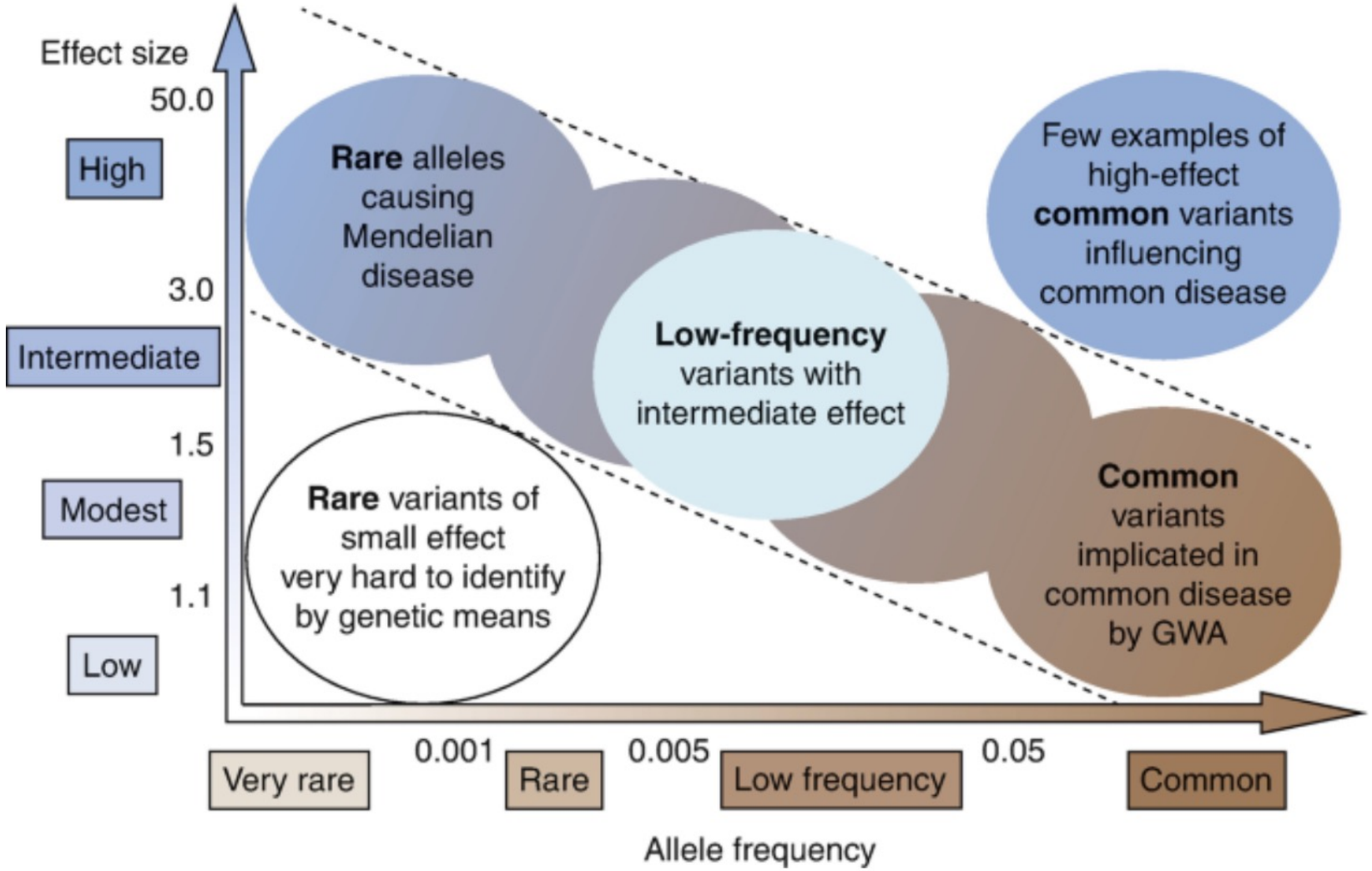
Population allele frequency and variant effect size



Modified from Antonorakis et al. 2010, Nature Reviews Genetics 11.

Evidence from computational data

Loss of function in a gene where LoF is a disease mechanism

- Very strong criterium supporting pathogenic classification (PVS1)

Same amino acid change as established pathogenic variant

- Strong criterium supporting pathogenic classification (PS1)

Multiple lines of computational evidence supporting deleterious effect (PP3)

- Supporting criterium supporting pathogenic classification (PS4)

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Evidence from testing other individuals

De novo: not detected in parents

- Strong (parentage confirmed) or moderate (parentage not confirmed) criterium supporting pathogenic classification (PVS2 and PM6, respectively)

Segregation data: co-segregation with disease in affected

- Moderate to strong evidence supporting pathogenicity (PP1)

Allelic data: For recessive disorders, detected in trans with a pathogenic variant

- Moderate to evidence supporting pathogenicity (PM3)

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Limitations of the 2015 ACMG guidelines:

- Some criteria imply subjective judgement
- Some criteria could be double counted without appropriate guidance
- No guidance how to combine pathogenic and benign criteria

	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			

Richards S, et al. Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5. PMID: 25741868.

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

Clinical validity and other aspects in variant classification

- Gene-disease association: how well is a particular gene established in relation to a particular disease? (clinical validity of a genetic test)
- Genetic heterogeneity: can a particular phenotype be caused by variation in more than one gen? (e.g., Noonan syndrome)
- Allelic disorders: can different variants in the same gene cause different phenotypes? (e.g.SCN1A LoF variants causing Dravet syndrome versus SCN1A GoF variants causing familial hemiplegic migraine)
- Is the predicted variant consequence consistent with the known disease mechanism? (e.g. a rare SCN1A GoF variant detected in an infant with infantile epileptic encephalopathy (Dravet syndrome)
- Does the genetic test detect the variant type associated with the disease in question? (clinical validity of a genetic test: e.g., short read NGS does not detect repeat expansion disorders or methylation disorders)
- Use of public databases and curated information sites (e.g. GnomAD, ClinVar, ClinGen, OMIM, Genereviews)

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Global *versus* Disease-specific Tests

Global Tests

- Chromosomes/Karyotype
- Chromosome microarray
- Next Generation Sequencing
  - Gene panels
  - Whole exome sequencing
  - Whole genome sequencing

Disease-specific Tests

- FISH
- Repeat expansion disorders
  - Fragile X testing
  - Huntington Disease
  - Myotonic Dystrophy
- Spinal Muscular Atrophy
- Methylation Disorders
  - Angelman/Prader-Willi Syndromes
  - Beckwith-Wiedemann Syndrome
  - Russell-Silver Syndrome
  - Temple syndrome/Kagami-Ogata S.
- Uniparental disomy
- X-chromosome inactivation
- Sanger sequencing/specific mutations: Hemochromatosis, Factor V Leiden, Prothrombin, MTHFR

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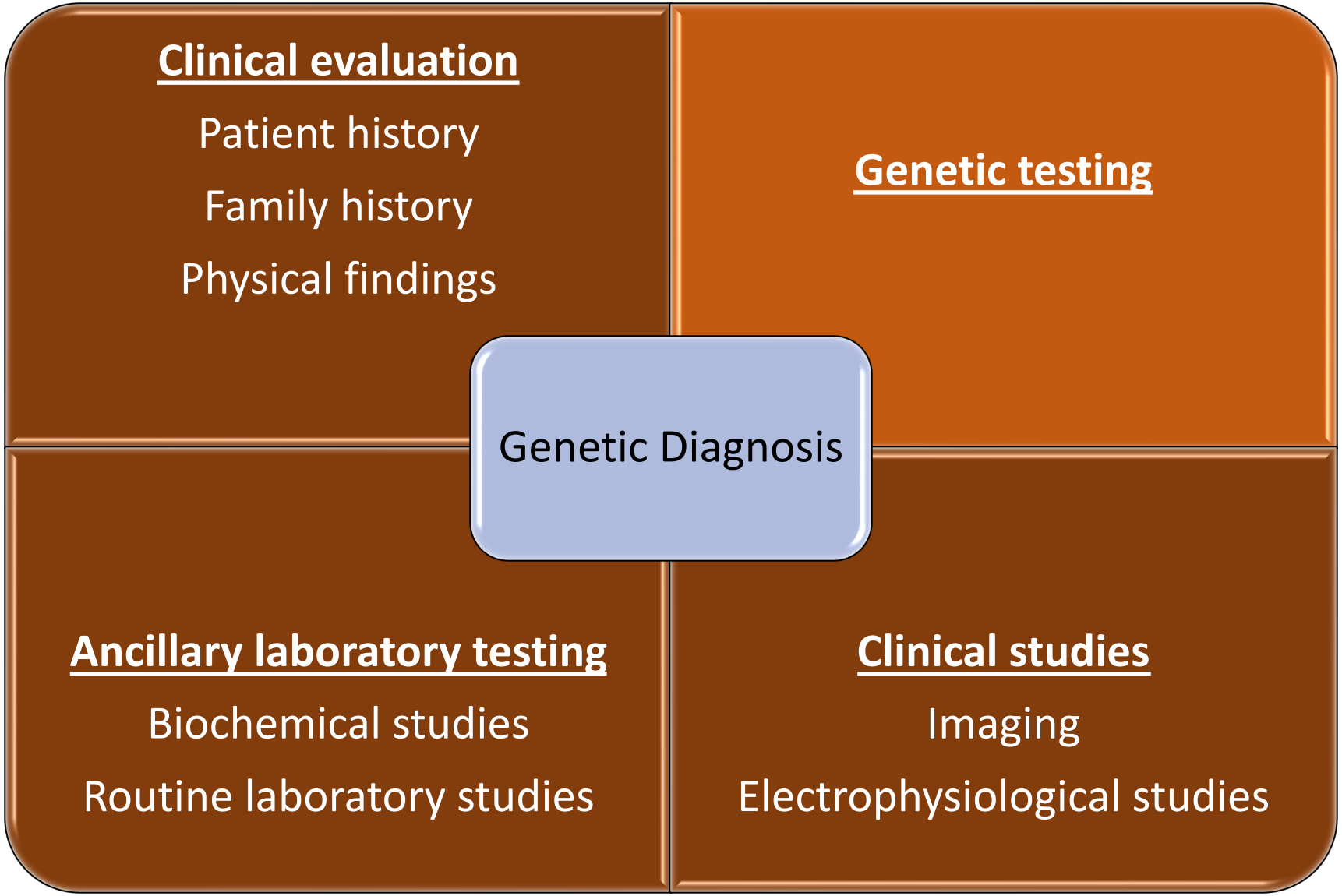
Components of a genetic diagnosis



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Case Examples

9-month-old male with red cell aplasia

Provider ordered a 149 gene bone marrow failure panel

Gene	Genomic coordinates	Sequence variant	Protein variant	Zygosity
RPL5	1:93300444	NM_000969.5:c.298T>C	NP_000960.2:p.Cys100Arg	Heterozygous

RPL5 is associated with Diamond-Blackfan anemia 6 (MIM #612561,DBA6), an autosomal dominant disorder characterized by normochromic macrocytic anemia, reticulocytopenia, and nearly absent erythroid progenitors in the bone marrow → Excellent phenotypic overlap

Variant classified as VUS based on ACMG criteria:

- Absent in population database (PM2)
- Not cited in ClinVar or affected individuals
- Missense variant with multiple lines of computational evidence supporting deleterious effect (PP3)
- Unknown whether inherited or de novo (PM6 or PS2 cannot be applied)
- Communication with provider: clinical evaluation strongly suggestive of Diamond-Blackfan Anemia (PP4)

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Table 5 Rules for combining criteria to classify sequence variants

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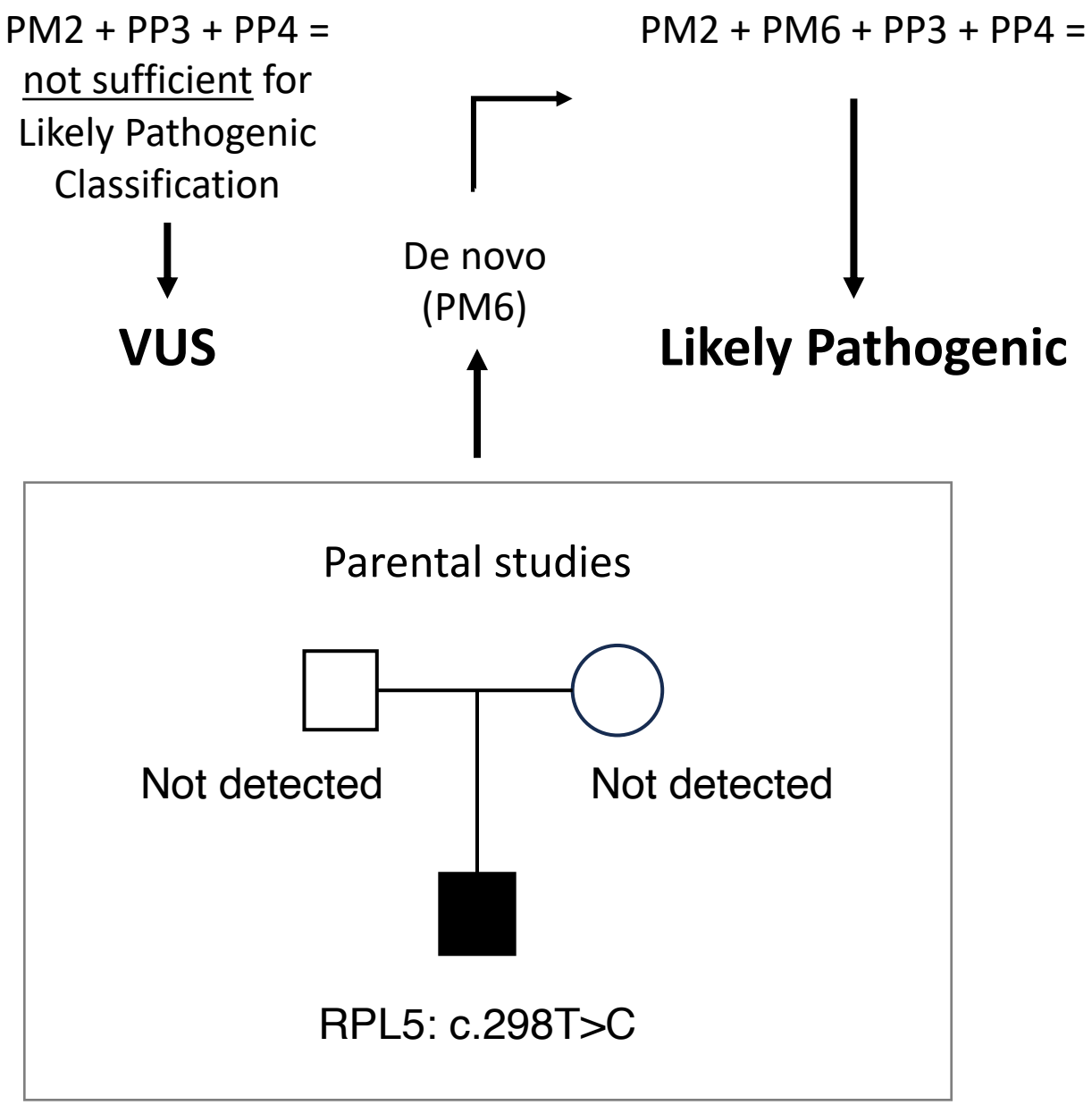

4-year-old girl with bilateral high frequency hearing loss

Provider ordered a 224 gene hearing loss panel

Gene	Sequence variant	Protein variant	Zygosity	Classification
MYO15A	c.6177+2T>A (Splice donor)	N/A	Heterozygous	Likely Pathogenic
MYO15A	c.4769A>G	p.Tyr1590Cys	Heterozygous	Uncertain

Phase of the two *MYO15A* variants is unknown.

MYO15A is associated with autosomal recessive deafness-3 (DFNB3). Initially, most MYO15A variants were documented in patients with severe to profound congenital sensorineural deafness phenotypes from consanguineous families in the Middle East and Southeast Asia. Since then, a milder phenotype with postlingual predominantly high-frequency HL has been recognized.

<u>MYO15A c.6177+2T>A</u>

- Absent in population databases (PM2)
- Not cited in ClinVar or affected individuals
- Affects a donor splice site in intron 28 of the MYO15A gene expected to disrupt RNA splicing (PVS1)

<u>MYO15A c.4769A>G (p.Tyr1590Cys)</u>

- Present in population databases (gnomAD 0.02%)
- ClinVar has 4 citations with classification of Uncertain (Variant ID: 228956)
- Not reported in affected individuals
- Missense variant with multiple lines of computational evidence supporting deleterious effect (PP3)

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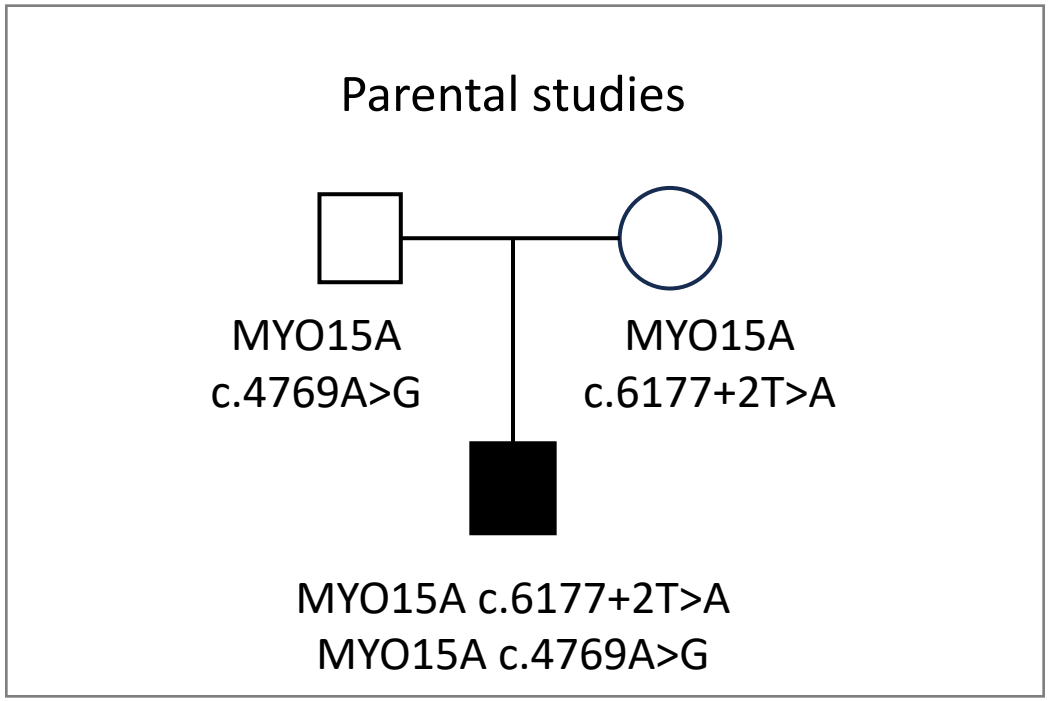

Parental testing revealed biparental inheritance of the two variants and prompted re-classification of the MYO15A c.4769A>G variant from UNCERTAIN to LIKELY PATHOGENIC.

DIAGNOSIS: MYO15A-related autosomal recessive deafness-3 (DFNB3)

12-month-old boy with congenital bilateral profound SNHL

Provider ordered a 224 gene hearing loss panel

Gene	Sequence variant	Protein variant	Zygosity	Classification
GJB2	*c.365A>T*	*p.Lys122Ile*	Homozygous	Pathogenic
USH2A	*c.4016T>G*	*p.Val1339Gly*	Heterozygous	Pathogenic
USH2A	*c.11168T>G*	*p.Leu3723Trp*	Heterozygous	Uncertain
LOXHD1	*c.4740+17A>G (Intronic)*	N/A	Heterozygous	Uncertain
PCDH15	*c.4314_4322dup*	*p.Pro1441_Pro1443dup*	Heterozygous	Uncertain
TSPEAR	*c.1001G>A*	*p.Arg334His*	Heterozygous	Uncertain

1. What is the primary diagnosis for this patient?
2. What other diagnosis needs to be considered?
3. What is the next step to address the concern for the possible second diagnosis and why is it important to address this concern?
4. Can some of the VUS be dismissed?

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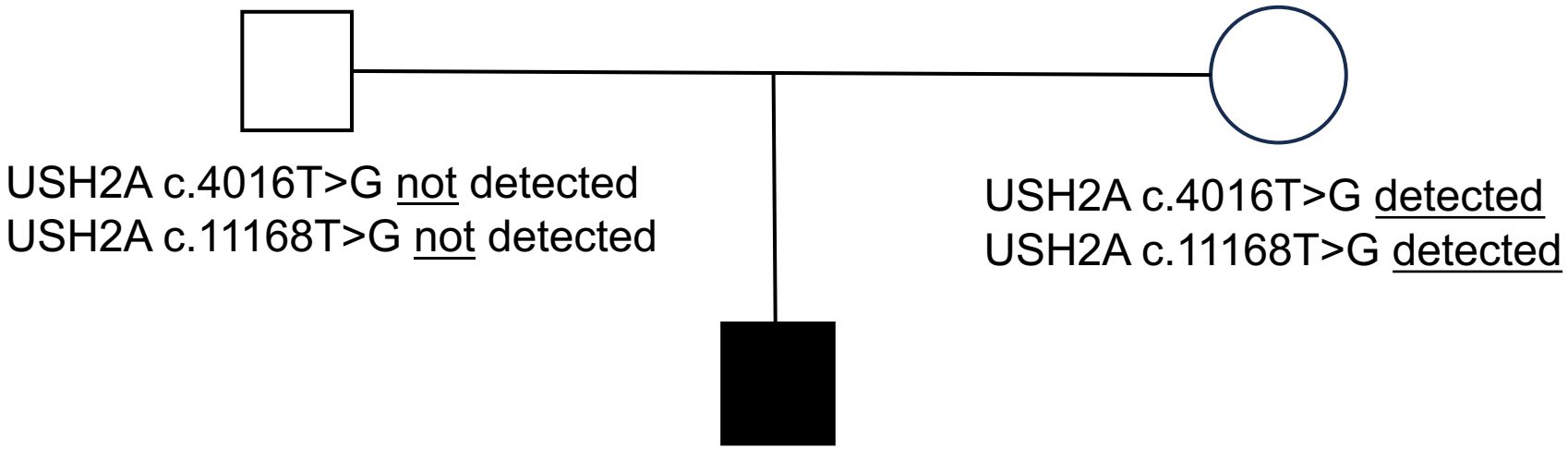
Parental studies



- The patient's mother carries BOTH USH2A variants detected in the patient *in cis*.
- This indicates that the patient is not at risk to develop Usher syndrome due to the variants he carries.
- The patient and his mother are unaffected carriers for Usher syndrome type A.

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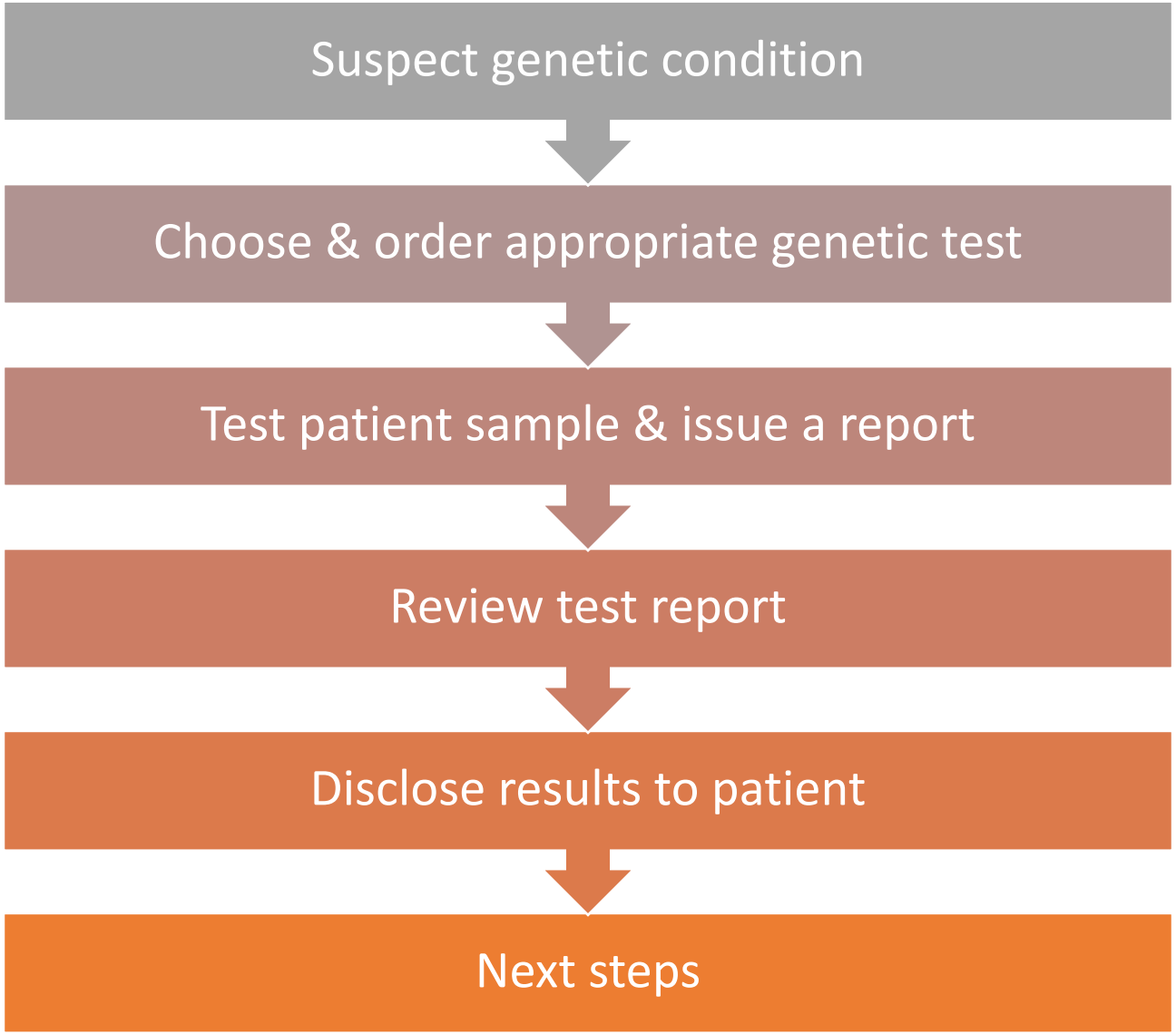
Genetic testing discussion



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SUMMARY - Variants of Uncertain Significance (VUS)…

- …are an inevitable consequence of genomic approaches to genetic diagnosis
- …do not meet criteria for benign or pathogenic, or the evidence for benign and pathogenic is conflicting
- …do not inform diagnosis, management or recurrence risk and can't be used to assess other family members
- …may be re-classified after additional variant information becomes available
- …should be clinically appraised in the context of phenotype
- …may be resolved through appropriate family studies

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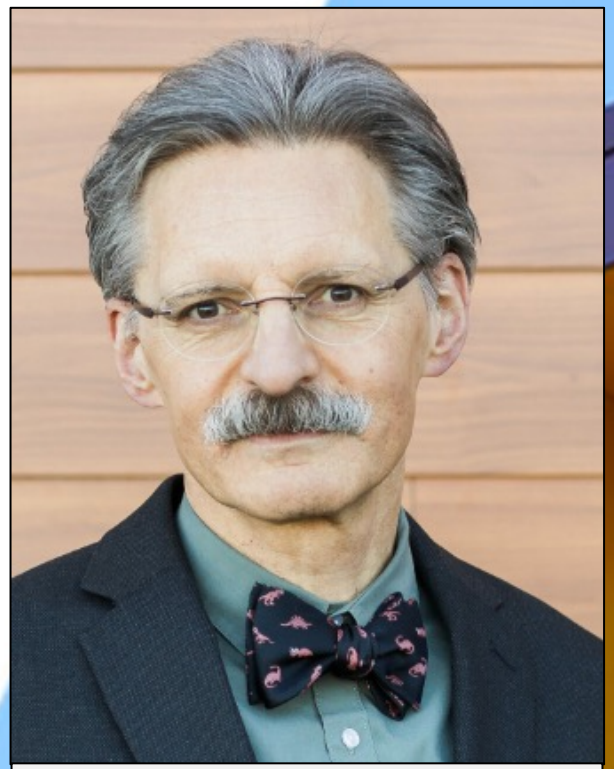
Thank you!



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