Metabolic Pathognomonics: Incorporating Disease-Specific Biochemical Data Improves Variant interpretation for Inherited Metabolic Disorders

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Introduction: Variant interpretation

- Clinical symptoms specific to a patient's phenotype are supporting evidence for variant classification when appropriate
- Inherited metabolic disorders: phenotypes include biochemical test results that are highly specific to the condition and, in many cases, diagnostic
- PP4 criterion of ACMG variant interpretation guidelines reflects this practice: "Patient's phenotype or family history is highly specific for a disease with a single genetic etiology"

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SHERLOC

- Developed by Invitae scientists and engineers
- Based on ACMG guidelines (2015). PMID: 25741868

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Open

Sherloc: a comprehensive refinement of the ACMG–AMP variant classification criteria

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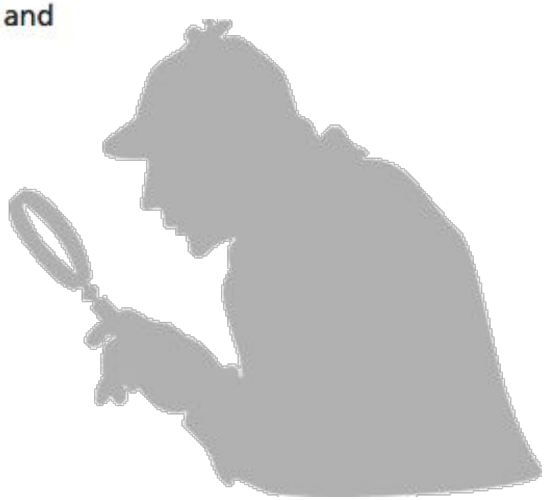
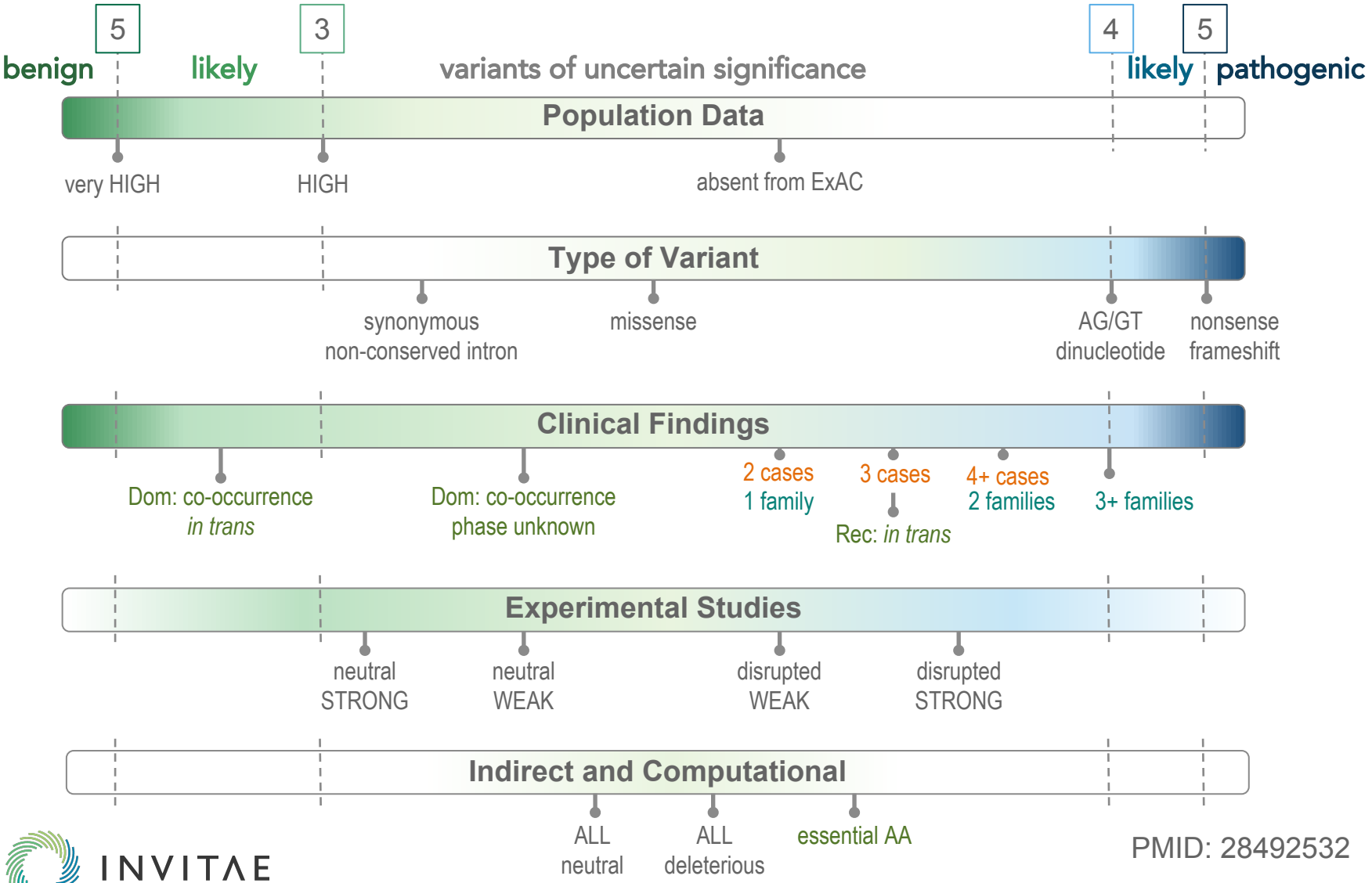


Sherloc: ACMG guidelines into a point-based system



PMID: 28492532

What about novel variants?

Challenging, and a common error to conclude that a novel VUS found in a patient with a disease must be causative. The opposite is also not beneficial for the patient when a novel variant is left as a VUS.

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Aim

Because the presence of a distinctive phenotype in a patient can provide a powerful line of evidence for variant classification, we set out to develop pathognomonic criteria, a new category of evidence integrating unique phenotypic data with more detailed, gene-level guidelines, with more weight than regular case reports, and incorporate it into Sherloc.

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Methods: Pathognomonic criteria

Must meet the following:

1. Diagnostic yield >75% for the gene(s) tested, given the specific phenotype feature(s).
2. Clinical features must be so specific that they are essentially pathognomonic for the disorder.
3. Patient's genotype must match the expected inheritance of the disease.

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Methods: Pre-curation for pathognomonic criteria

Example of the pre-curation of a test given the genes being tested and the unique features of the disorder that must be present to assure that our diagnostic yield meets an appropriate threshold

Test	Genes	Diagnostic yield	Diagnostic guidelines (aka minimum REQUIRED features)	Reference
Elevated C14:1, C14 test VLCAD deficiency test	*ACADVL*	Scenario 1: 70%–80% Scenario 2: 77.8%	Scenario 1: Plasma C14:1 at least 2 times the upper limit of normal range Scenario 2: VLCAD activity of ≤0.64 times the lower limit of normal range	PMID: 19327992 Describes required features and clinical sensitivity

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Methods: New set of evidence-based criteria

Description	Path points	Inheritance
Homozygous or hemizygous variant in pathognomonic gene	2	AR, XR
Rare heterozygous variant co-occurring with LP/P variant in pathognomonic gene	1.5	AR, XR
Rare heterozygous variant co-occurring w/ another rare heterozygous variant in pathognomonic gene	1	AR, XR
Rare heterozygous variant in pathognomonic gene	1	AD, XD
In trans with an LP/P variant in an affected individual	1	AR, XR

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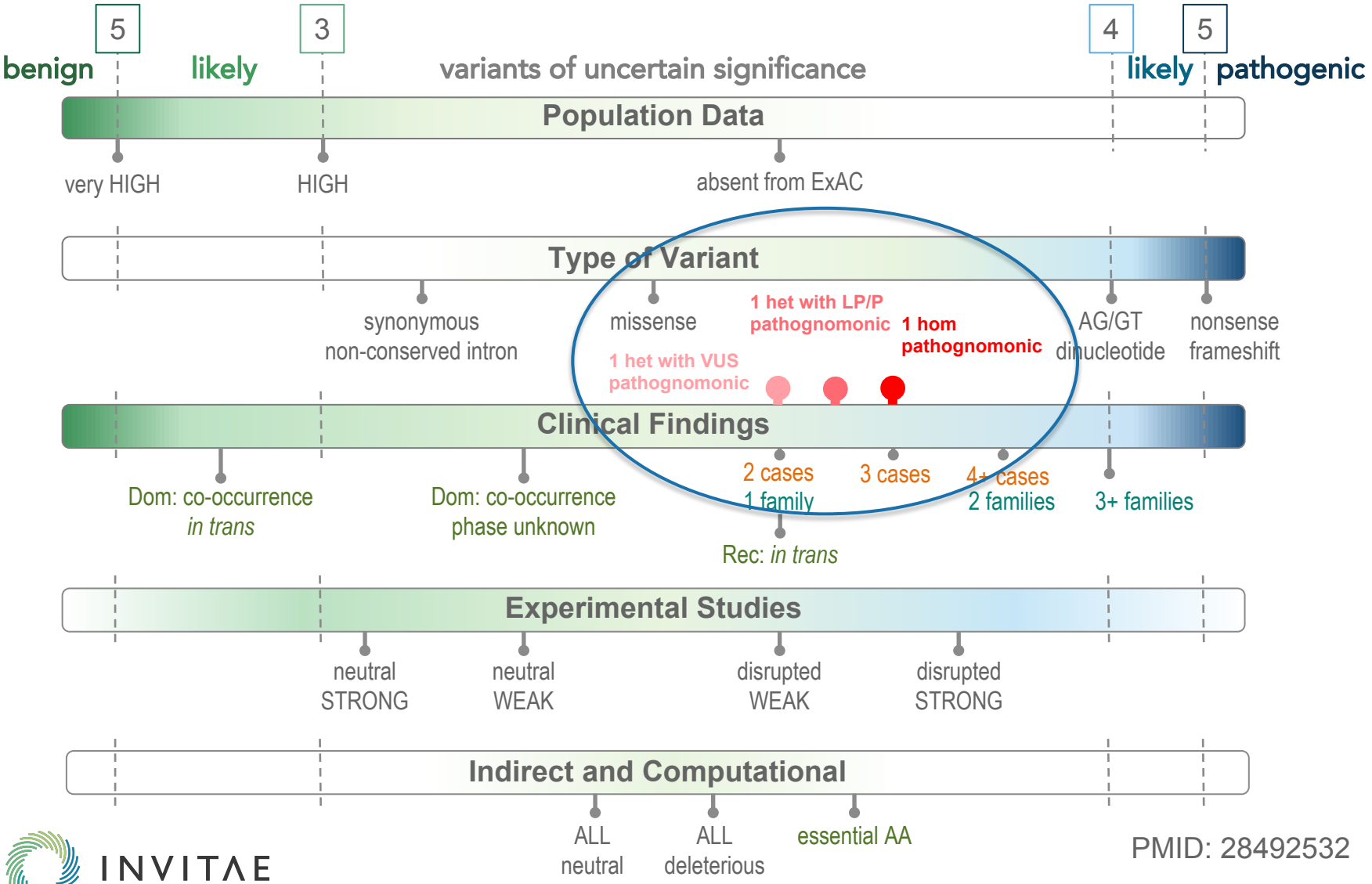
Pathognomonic criteria in Sherloc



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PMID: 28492532

Results

GENE	No change: VUS	VUS->LP	VUS->P	LP->P	No change: P
ACADM	2	1	4	1	4
ACADVL	6	1	2	3	2
ALDH7A1			1		
AMT	1				
ARG1				1	
ARSA		1			
ASL	2		1		
ASPA					1
ASS1	2			2	2
ATP7B				2	2
BCKDHB	1	1	1		
BTD	2	1	4	1	
CBS	2				1
CFTR					1
CPT2		1			1
GALC	1	1			1
GALT			1		2
GCDH	3	2		1	
GLA			1		
GLDC				2	1
HEXA			1		
IKBKAP					1
MCCC2				1	
MMAA			1		
MUT				1	1
NPC1	1			2	
OAT		1			
OTC					1
PAH	1	3	5	10	11
PCCA		2			
PCCB		1			
PGM1	1				
PHKB	1				
PMM2				1	1
PTS	1	1		1	
PYGM				1	
QDPR			1		
SEPN1		1			
SLC22A5	4		1	2	
SMPD1					1
TPP1		2	3		
TOTAL	32	21	27	32	34

←Table. Number of variants by gene reclassified with the use of the new criteria

- **220** variants in 182 patients (146 unique variants) interpreted
- **43** patients received a positive genetic diagnosis (for recessive diseases this means that two LP or P variants proved to be in trans)

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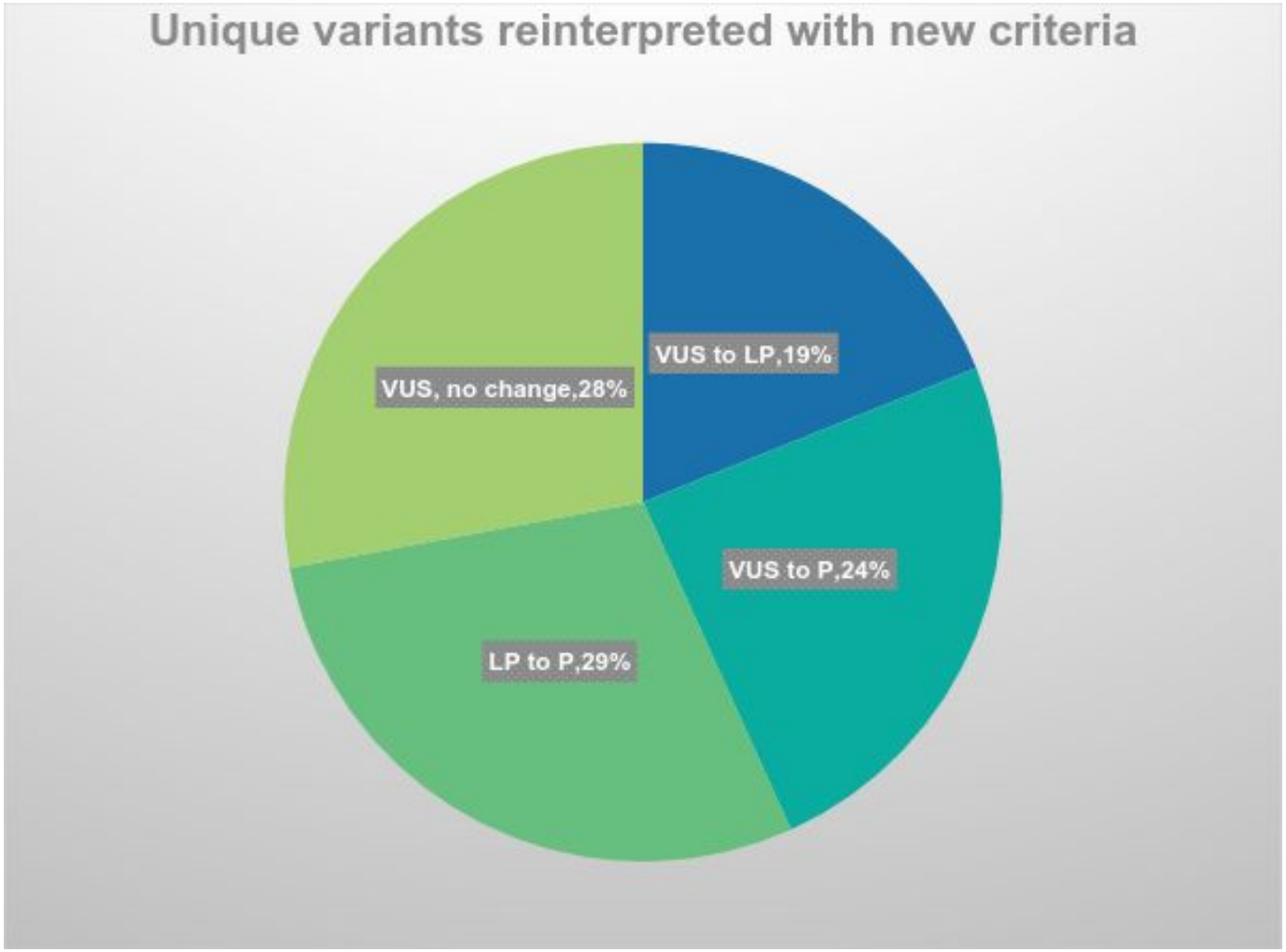
Results



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Case examples

Test ordered	Clinical features	Gene	Variant	Zyg.	New evidence criteria: Hom/hemi	New evidence criteria: Rare VUS with LP/P	New evidence criteria: In trans with LP/P variant	Reinterpretation
OAT gene	Clinical diagnosis of gyrate atrophy	*OAT*	c.722C>T (p.Pro241Leu)	Hom	2			VUS to LP
ARSA gene	Clinical diagnosis of adult-onset metachromatic leukodystrophy. Low leukocyte arylsulfatase A, which is diagnostic in itself.	*ARSA*	c.746T>C (p.Phe249Ser)	Het		1.5	1	VUS to LP
		ARSA	c.542T>G (p.Ile181Ser)	Het				P

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Case examples

					New evidence criteria			
Test ordered	Clinical features	Gene	Variant	Zyg.	Hom/hemi	Rare VUS with LP/P	In trans with LP/P variant	Reinterpretation
Hyperphe. Panel	Positive for NYS NBS. DHPR deficiency diagnosed by confirmatory testing	*QDPR*	c.344C>T (p.Ser115Leu)	Hom	2			VUS to P

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Conclusions

- Developing a systematic framework for the inclusion of highly distinctive phenotypic information is necessary for variant interpretation in phenotypically distinct disorders.
- Inclusion of biochemical test results is specific to pathognomonic criteria.
- Careful curation of the gene/disorders for which these criteria can be used is necessary, including the required distinctive phenotypes along with the diagnostic yield of the gene/panel.
- Each of the new evidence types on its own is insufficient to reach an LP interpretation if the variant has only been seen in one affected individual. A second case, population frequencies, functional studies, and other clinical findings are necessary to reach an LP classification.
- This framework provides a mechanism to account for the increased prior probabilities in diagnostic genetic testing for rare disorders with highly distinctive phenotypes to provide accurate results in genetic testing.

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