Genetic testing for lysosomal storage disorders in a commercial laboratory: Use of pathognomonic criteria in variant interpretation

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BACKGROUND

- Genetic testing in lysosomal storage disorders (LSDs) developed for diagnostic and confirmatory purposes
- Detailed criteria are needed to be applied in variant interpretation, combining distinctive phenotypic data with specific gene-level information. This leads to the importance of pathognomonic criteria.
- Sherloc[1], our in-house point-based framework adapted from ACMG VI guidelines, implements these criteria and aids in attaining positive results for patients tested for LSDs.

METHODS

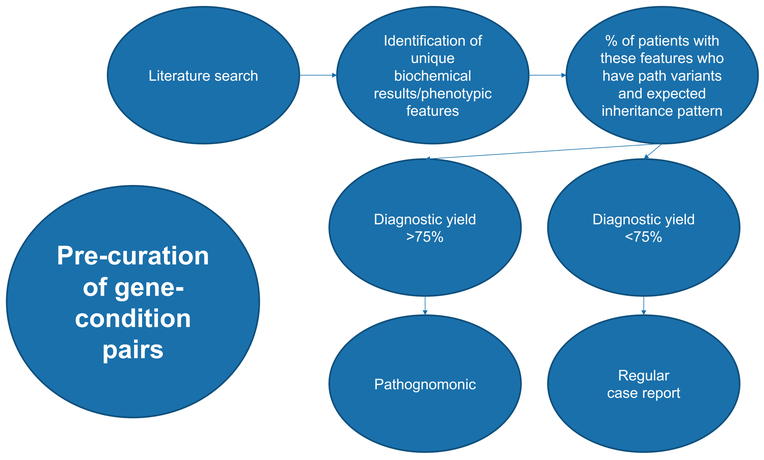


Table 1. New evidence-based criteria

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

RESULTS

- Cases that meet these more stringent biochemical and/or unique features criteria and have the expected genotype are weighted with additional points toward a pathogenic classification (**Table 1**).
- Notably, this criterion alone is insufficient for reaching a likely pathogenic classification and it is always considered in addition to multiple lines of evidence incorporated into our variant interpretation process. **Table 2** shows the application of these criteria in Invitae patients.

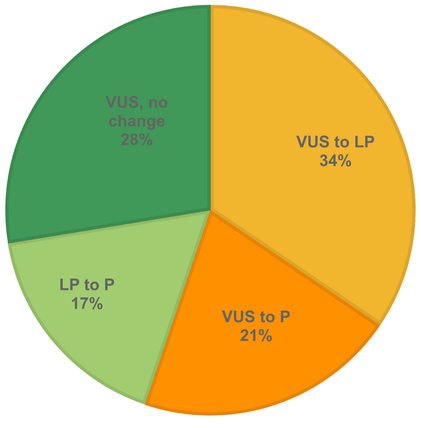
Table 2. Case examples

			New evidence criteria			
Test	**Clinical features**	**Gene, variant, zygosity**	**Hom/ hemi**	**Rare VUS with LP/P**	**In trans with LP/P variant**	**Interpretation**
Epilepsy panel	Biliary atresia s/p liver transplant; convulsive seizures, daily staining spells, speech delay, toe walking. Very low TPP1 enzymatic activity	*TPP1* c.617G>A (p.Arg206His) Het				P
		TPP1 c.1266G>C (p.Gln422His) Het		1.5	1	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
Epilepsy and Glycine encepha -lopathy panels	Seizures, global developmental delay, microcephaly, myoclonic; abnormal PPT1 enzyme assay	*PPT1* c.364A>T (p.Arg122Trp) Hom	2			P

RESULTS

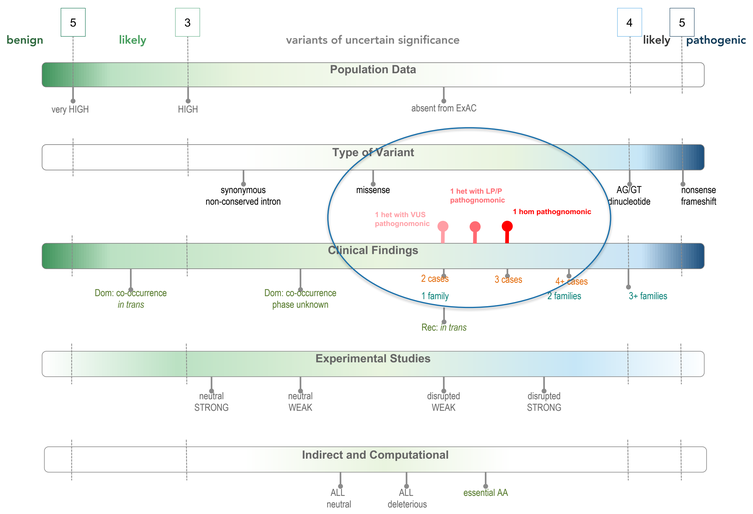
- We have applied new criteria to 32 unique variants in LSD genes from 37 individuals.
  - 16 unique variants were reclassified as LP or P that would have otherwise stayed in the purgatory of Variants of Uncertain Significance (VUS).
  - 5 unique variants were reclassified as P that would have otherwise remained as LP.
  - For 8 unique variants, these criteria were applied during the variant interpretation, but the variant remained a VUS. For 3 unique variants, new criteria applied to the case, although the interpretation was already P.
- 12 patients received a positive diagnosis, which in the case of autosomal recessive conditions means two LP or P variants proved to be in opposite chromosomes.

Figure 2. Unique variants reclassified with new criteria



72% unique variants were reclassified with the use of pathognomonic criteria

Figure 1. Pathognomonic criteria in Sherloc



CONCLUSIONS

- **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/disorder pairs is necessary, including the required distinctive phenotypes along with the diagnostic yield of the gene/panel.**
- **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.**

References: 1) Sherloc: a comprehensive refinement of the ACMG–AMP variant classification criteria. Genet. Med. Nykamp *et al*. 2017

Disclosures: LM, RH, YH, SW, DB, HW, TW, BT are stockholders and employees of Invitae.