Metabolic pathognomonics: incorporating disease-specific biochemical data improves variant interpretation for inherited metabolic disorders

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Abstract

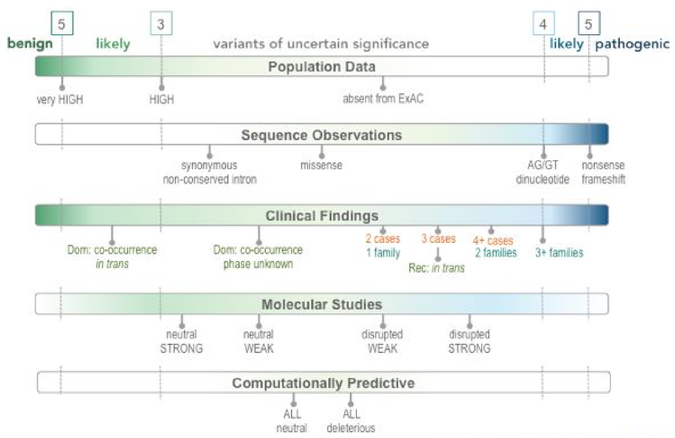
Commercial laboratories routinely use patient clinical information provided by ordering physicians so that clinical symptoms specific to a patient's phenotype can be applied as supporting evidence for variant classification when appropriate. The PP4 criterion of the American College of Medical Genetics and Genomics (ACMG) variant interpretation guidelines reflects this practice: "Patient's phenotype or family history is highly specific for a disease with a single genetic etiology." However, little guidance is provided for implementing this criterion. To augment this guideline, we developed more detailed criteria to be applied in variant interpretation, combining distinctive phenotypic data with specific gene-level information. The likelihood that any variant causes disease depends on the distinctiveness of the phenotype, the degree of locus heterogeneity, the fraction of locus heterogeneity accounted for by the tested genes, and the prevalence of phenocopies in the population. Therefore, we developed a new category of evidence and incorporated it into Sherloc, our evidence-based system for variant interpretation.

Inherited metabolic disorders are unique in that the phenotypes of affected individuals include biochemical information that is highly specific to the condition and, in many cases, diagnostic. Therefore, our systematic approach to variant interpretation incorporates these condition-specific phenotypic and biochemical data. Our approach is adapted from the ACMG guidelines and uses a points-based system for variant classification. As done for functional data, we incorporate results from clinical biochemical testing by granting points during the interpretation process.

In addition, when published literature shows that the diagnostic yield for a disorder is >75%, we consider these biochemical results to be pathognomonic. Cases that meet these more stringent biochemical criteria and have the expected genotype (e.g., two rare variants identified in a gene that causes an autosomal recessive disorder) are weighted with additional points toward a pathogenic (P) classification. Notably, however, this criterion alone is insufficient for reaching a likely pathogenic (LP) classification and is considered among multiple lines of evidence incorporated into our variant interpretation process. For autosomal recessive inherited metabolic disorders, we combine this systematic method of assessing phenotypic data with variant phasing information, which provides a powerful approach for the interpretation of novel variants. Using this approach, we have been able to fine-tune our classifications and identify multiple likely LP/P rare variants that would be classified as variants of uncertain significance (VUS) with approaches that ignore biochemical data. In some cases, this result has provided a patient with a positive instead of a negative diagnosis. We have used this approach successfully for several disorders, such as medium-chain acyl-CoA dehydrogenase deficiency, dihydropteridine reductase deficiency, phenylalanine hydroxylase (PAH) deficiency, citrullinemia, very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD), glutaryl-CoA dehydrogenase deficiency, propionic acidemia, and maple syrup urine disease (MSUD).

Methods

- Our Sherloc framework for variant interpretation uses a point system based on ACMG guidelines[1,2]:



Methods (continued)

Evidence-based criteria

Inheritance	Path points	Description
AR, XR	2	Homozygous or hemizygous variant in pathognomonic gene
AR, XR	1.5	Rare heterozygous variant co-occurring w/ LP/P variant in pathognomonic gene
AR, XR	1	Rare heterozygous variant co-occurring w/ another rare heterozygous variant in pathognomonic gene
AR, AD, XR, XD	1	Lab tier 1
AR, AD, XR, XD	0.5	Lab tier 2
AR, XR	1	In trans with an LP/P variant in an affected individual

The new set of evidence-based criteria must meet the following:

1. Diagnostic yield **>75%** for the gene(s) tested.
2. Clinical features described in a given patient (literature or Invitae patient) must be so specific that they are essentially pathognomonic for the disorder.
3. The patient's genotype must match the expected inheritance of the disease.

Results

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 for the defined disorder (must be ≥75%)	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

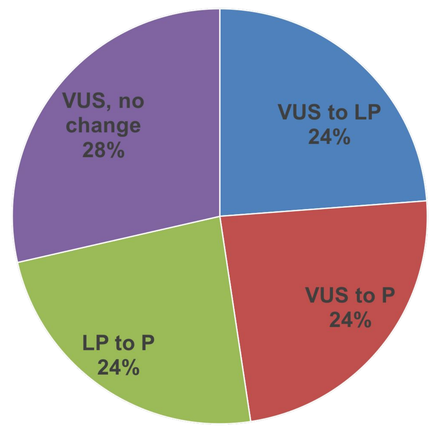
Case examples

					New evidence criteria					
Test ordered	**Clinical features**	**Gene**	**Variant**	**Zyg**	**Hom/ hemi**	**Rare VUS with LP/P**	**2 Rare VUS**	**Lab tier 1/2**	**In trans with LP/P variant**	**Criteria effect/ interpretation**
Organic Acidemias Panel	Intellectual disability, elevated Thr, Leu, Ile, where Leu is >3x normal upper limit, clinical diagnosis of MSUD	*BCKDHB*	c.995C>T (p.Pro332Leu)	Hom	X					VUS to P
Krabbe Disease Panel	Positive lyosomal studies, very low GALC activity	*GALC*	c.1541T>C (p.Phe514Ser)	Het				X		VUS to LP
		GALC	Exons 11-17 deletion	Het						P
Hyperphe Panel	Positive for hyperphe, elevated phenylalanine levels	*PAH*	c.835C>G (p.Pro279Ala)	Het		X				VUS to LP
		PAH	c.1042C>G (p.Leu348Val)	Het						P
ARSA Gene	Clinical diagnosis of adult-onset metachromatic dystrophy. Seizures, abnormal brain imaging, sulfatides in urine, and low leukocyte arylsulfatase A are diagnostic.	*ARSA*	c.746T>C (p.Phe249Ser)	Het		X			X	VUS to LP
		ARSA	c.542T>G (p.Ile181Ser)	Het						P

Results (continued)

- With the aid of this new set of criteria, as of March 3, 2017, we have interpreted **139** variants in 114 patients (101 unique variants). For an example of including biochemical data in CNV interpretation, see Invitae poster #91.
  - 40 unique rare variants were classified as LP/P that would have otherwise stayed in the purgatory of VUS.
  - 20 unique rare variants were classified as P variants that would have otherwise remained LP variants.
  - These interpretation criteria were used to interpret 24 unique rare variants that remained VUS. New criteria applied to 17 additional unique rare variant, for which the interpretation was already P.
- **29** patients have received a positive genetic diagnosis (which in the case of recessive diseases would mean that two LP or P variants proved to be in opposite chromosomes).

Unique variants reinterpreted with new criteria



Usage frequency of criteria in variant classification

	Total variants	Unique variants
Homozygous or hemizygous variant in pathognomonic gene	18	13
Rare heterozygous variant co-occurring w/ LP/P variant in pathognomonic gene	39	32
Rare heterozygous variant co-occurring with another rare heterozygous variant in a pathognomonic gene	6	6
Lab tier 1	34	27
Lab tier 2	3	3
In trans with an LP/P variant in an affected individual	63	42

Conclusions

- Developing a systematic framework for the inclusion of highly distinctive phenotypic information is necessary for variant interpretation in phenotypically distinct disorders.
- 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. Population frequencies, functional studies, and other clinical findings are also 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.

References

1. Richards, S, *et al.* 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. *Genet Med.* 2015; 17(5):405-24.
2. Invitae. Invitae's method of variant classification. Available at https://www.invitae.com/en/variant-classification/. Accessed March 14, 2017.
3. Ho et al. CNV Analysis of ACADM Enhances Yield in the Molecular Diagnosis of Medium-Chain Acyl-CoA Dehydrogenase Deficiency. Poster # 91, ACMG 2017.