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 diagnostic.

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 database) 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.

Methods (continued)

Evidence-based criteria

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

The usage frequency of criteria in variant classification is as follows:

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

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

3. Horn et al. CNV Analysis of ACMG Somatics Yield in the Molecular Diagnosis of Medium-Chain Acyl-CoA Dehydrogenase Deficiency. Poster # 91, ACMG 2017.