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, 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

- Literature search
- Identification of unique biochemical results/phenotypic features
- % of patients with these features who have path variants and expected inheritance pattern
- Diagnostic yield >75%
- Diagnostic yield <75%
- Pathognomonic
- Regular case report

Table 1. New evidence-based criteria

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

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Clinical features</th>
<th>Gene, variant, zygosity</th>
<th>Hom/ hemi</th>
<th>Rare VUS with LP/P variant</th>
<th>In trans with LP/P variant</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epilepsy panel</td>
<td>Biliary atresia s/p liver transplant; convulsive seizures, daily staring spells, speech delay, toe walking. Very low TPP1 enzymatic activity</td>
<td>TPP1 c.817G&gt;A (p.Arg266His) Het</td>
<td>1.5</td>
<td>1</td>
<td>VUS to LP</td>
<td></td>
</tr>
<tr>
<td>ARSA gene</td>
<td>Clinical diagnosis of adult-onset metachromatic leukodystrophy. Low leukocyte arylsulfatase A, which is diagnostic in itself.</td>
<td>ARSA c.746T&gt;C (p.Phe249Ser) Het</td>
<td>1.5</td>
<td>1</td>
<td>VUS to LP</td>
<td></td>
</tr>
<tr>
<td>Epilepsy and Glycine encephalopathy panels</td>
<td>Seizures, global developmental delay, microcephaly, myoclonic, abnormal PPT1 enzyme assay</td>
<td>PPT1 c.364A&gt;T (p.Arg122Trp) Hom</td>
<td>2</td>
<td></td>
<td>P</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Pathognomonic criteria in Sherloc

Figure 2. Unique variants reclassified with new criteria

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

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.


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