Gene Characterization: A Scientific Approach To Multi-gene Panel Testing Design

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**BACKGROUND**

- Multi-gene panel tests (MGPT) have grown in use with advances in sequencing technology.
- Higher gene content on MGPTs can increase the rate of variants of uncertain significance (VUS).
- Inclusion of genes of uncertain significance (GUS) on MGPTs can lead to uninformative clinical impact for patients and complicate results disclosures for clinicians.\(^1\)\(^2\)\(^3\)
- Proper clinical validity aims to assess relevant information to identify genes with clinically significant gene-disease relationships.
  - May maximize clinical impact for patients
  - May minimize the VUS rate

**METHODS**

- Retrospective analysis of the data from 3,524 cases tested for as many as 106 genes from cardiovascular MGPTs, and 245 cases tested for either 11 Diamond-Blackfan anemia (DBA) genes or 7 dyskeratosis congenita (DKC) genes.
- Clinical validity scoring was performed on all genes using a system based on a recently published paper by Smith et al., 2017\(^4\)
  - Point-based clinical validity scoring assessing Mendelian gene-disease relationships.
  - Data was assessed to determine how many mutations/VLPs and VUSs were identified per gene per clinical validity category.

**RESULTS**

**Fig 2. Distribution of Clinical Validity Scores for Multi-Gene Testing Panels**

- Cardio Panel (N=106 genes): 13% Definitive, 22% Strong, 28% Moderate, 39% Limited
- DBA/DKC Panels (N=18 genes): 22% Definitive, 22% Strong, 17% Moderate, 42% Limited

**Fig 3. Mutation/VLP and VUS Rates Per Panel**

- Cardio Panel (N=106 genes): 19% Mutation/VLP, 81% VUS
- DBA/DKC Panels (N=18 genes): 41% VUS, 59% Mutation/VLP

**Fig 4: Mutation/VLP and VUS rates of Combined Panels Per Clinical Validity Category**

- Limited: 0.5% Limited, 3.7% Moderate, 10.1% Strong, 39.2% Definitive
- Moderate: 99.5% Limited, 96.4% Moderate, 90.9% Strong, 71.8% Definitive
- Strong: 71.8% Limited, 90.9% Moderate, 99.5% Strong, 39.2% Definitive
- Definitive: 39.2% Limited, 71.8% Moderate, 90.9% Strong, 99.5% Definitive

**Table 1: Distribution of Genes, VUS, Mutations/VLPs per clinical validity category**

<table>
<thead>
<tr>
<th>Clinical Validity</th>
<th>Type</th>
<th>DKC/DBA</th>
<th>Cardio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limited</td>
<td>Genes (N)</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>VUS (N)</td>
<td>2</td>
<td>191</td>
</tr>
<tr>
<td></td>
<td>Mutation (N)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>Genes (N)</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>VUS (N)</td>
<td>5</td>
<td>510</td>
</tr>
<tr>
<td></td>
<td>Mutation (N)</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Strong</td>
<td>Genes (N)</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>VUS (N)</td>
<td>0</td>
<td>348</td>
</tr>
<tr>
<td></td>
<td>Mutation (N)</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>Definitive</td>
<td>Genes (N)</td>
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<td>44</td>
</tr>
<tr>
<td></td>
<td>VUS (N)</td>
<td>20</td>
<td>1405</td>
</tr>
<tr>
<td></td>
<td>Mutation (N)</td>
<td>31</td>
<td>528</td>
</tr>
</tbody>
</table>

**TAKE-HOME POINTS**

- When designing MGPTs, a proper clinical validity process may improve detection rates.
- Genes in all clinical validity categories contribute to VUS rates therefore, VUS rates of MGPTs are likely to increase as their gene content increases.
- Rates of clinically significant findings tend to increase with inclusion of genes with higher clinical validity scores but are not impacted by inclusion of genes with limited clinical validity.
  - Mutations and VLP made up nearly 40% of the total calls for genes in the Definitive clinical validity category.
  - Only 1 VLP was identified among genes with limited clinical validity.
- The data herein highlight the importance of clinical validity assessment to molecular laboratories.

**REFERENCES**