Expanded genetic testing for primary immunodeficiencies: Findings from a 207-gene next-generation sequencing panel

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BACKGROUND

- Many primary immunodeficiencies (PIDs) share overlapping presentations, complicating the clinical diagnosis.
- Expanded next-generation sequencing panels are valuable in facilitating the diagnosis of patients with PIDs due to their ability to test many genes at once.
- We developed a 207-gene next-generation sequencing panel inclusive of copy number variation analysis for the clinical diagnostic testing of patients with PIDs (Figure 1).

METHODS

- NGS testing was performed as previously described, and variant interpretation was carried out based on an expansion of the ACMG guidelines.
- De-identified results of patients tested between April and October of 2017 were reviewed and categorized by variants identified and their classifications:
  - Negative: no reportable variants identified
  - Positive: Pathogenic/Likely Pathogenic variants (P/LP)
  - Uncertain: Variants of Unknown Significance (VUS)
  - Of note, increased risk alleles, such as common NOD2 alleles associated with Crohn’s disease, were excluded.
- Positive results were further categorized as follows:
<table>
<thead>
<tr>
<th>Likely genetic diagnosis</th>
<th>Carrier status</th>
<th>Genetic result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous P/LP allele in AD or XL gene</td>
<td>1 heterozygous allele in AR gene</td>
<td>1 heterozygous allele in XL gene in female</td>
</tr>
<tr>
<td>Heterozygous P/LP allele in AR gene</td>
<td>1 heterozygous allele in AR gene in female</td>
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<td>Homozygous P/LP allele in AR gene</td>
<td>1 heterozygous allele in AR gene</td>
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<tr>
<td>Homozygous P/LP allele in AR gene &amp; male</td>
<td>1 heterozygous allele in AR gene &amp; male</td>
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</tbody>
</table>

RESULTS

- During the studied period, 260 patients underwent genetic testing with the 207-gene PID panel.
- The average turnaround time from test requisition to return of results was 18 days.
- The vast majority (96%) of patients had variants identified in one or more genes tested (Figure 2).
- P/LP variants were identified in 24% of patients (63) (Figure 2).
- Multiple variants of uncertain significance were identified in most patients (Figure 3).
- Seventy-four pathogenic or likely pathogenic (P/LP) variants were identified, over 10% of which were copy number variations (Table 1, Figure 4).
- 63 patients had positive findings (Figure 5):
  - 35% (22) were heterozygous carriers of AR conditions
  - 38% (24) were heterozygous for variants in genes with AR and AD inheritance patterns, in which the positive finding may or may not explain the patient’s phenotype
  - 30% (19) had likely genetic diagnoses (see methods)
- 6 patients had results in more than one category.

CONCLUSIONS

- Expanded next-generation sequencing panels offer an effective tool to aid in the rapid molecular diagnosis of patients with PIDs.
- Detection of copy number variations with multigene panels is critical, as CNVs represented 10% of pathogenic variants identified by our panel.
- In total, 24% of patients had positive findings, and a likely genetic diagnosis was made in 30% of these cases. Importantly, 63% of conditions diagnosed are treatable with HSCT. Of note, the diagnostic yield observed here is likely lower than it would be in an unselected population of patients with PIDs; individuals with clear phenotypes may have been tested with single gene assays or targeted panel tests.

REFERENCES


DISCLOSURES: All of the authors are stockholders in and employees of Invitae.