DETECTION OF NF1 VARIANTS BY NGS PANELS WITH SNV AND CNV IDENTIFICATION EXCEEDS PUBLISHED ESTIMATES AND IMPROVES CLINICAL UTILITY

B. Monica Bowen, Nila Patil, Rachel Lewis, Shan Yang, Darlene Ho Reithmaier, Michael Anderson, Daniel Pineda-Alvarez, Swaroop Aradhya, Ian Wilson, Karen Ouyang

Invitae Corporation, San Francisco, CA

BACKGROUND

- Neurofibromatosis type 1 (NF-1) is an autosomal dominant condition due to loss-of-function mutations in the neurofibromin 1 (NF1) gene. NF-1 affects individuals of all ethnicities and has a prevalence of 1 in 3,500.
- Nearly half of all NF-1 cases are caused by de novo sporadic mutations in the NF1 gene. The variant landscape of pathogenic NF1 variants includes missense and nonsense substitutions, splice variants, small insertions and deletions, sub-genic copy number variants (CNVs), as well as whole-gene deletions.
- Next-generation sequencing (NGS) panels have enabled dramatic improvements in the detection of nearly all of these variants simultaneously. Current published literature estimates that sequencing genomic DNA (gDNA) can detect NF1 variants in 60-90% of individuals, while combined cDNA and gDNA sequencing enables detection of >95% of NF1 variants.
- Current recommendations for NF1 variant detection appear to undervalue the clinical utility of gDNA analysis in NGS panels and overstate the relative importance of cDNA analysis.

METHODS

- We conducted a literature review of published studies assessing the NF1 variant landscape and found that 97.5% of the variants reported in NF1 patients would be detectable by NGS sequencing using gDNA exclusively and analyzing exonic and canonical splice site intronic regions. The only variants not detectable by gDNA analysis but detected by cDNA analysis were deep intronic variants, which comprised just 2.5% of all NF1 variants reported to date and were shown or predicted to create novel splice sites (Table 1).
- We also performed an analysis of NF1 variants identified among 490 patients referred for NF-1 testing at Invitae. We detected an NF1 variant in all patients described by their ordering physicians. The only variant in all patients with NF-1 features was detected in all patients with NF-1 features.
- We used our Sherloc algorithm for semi-quantitative variant classification, based on ACMG guidelines, to interpret detected variants.
- Given that 50% of NF-1 cases are sporadic, we performed targeted family variant testing (FVT) to resolve the clinical significance of any variants of uncertain significance (VUS). Of 16 families that enrolled in FVT, 13 were for suspected de novo, and 3 were for suspected segregation. In 2 families, only 1 parent was available for testing and de novo status was not confirmed.

RESULTS

- An NF1 variant was detected in all patients with NF-1 features.
  - 69.38% of variants identified were pathogenic (P), 13.28% were likely pathogenic (LP), and 17.34% were variants of uncertain significance or VUS (Figure 1).
  - Among the detected NF1 variants, 94% were in coding regions or the consensus splice site, with the remaining 6% in adjacent intronic regions (Figure 2).
  - Most VUS in NF1 were missense variants (>75%), while intronic variants near intron-exon junctions comprised 13%. In-frame indels, silent variants, and sub-genic duplications comprised under 10% of VUS combined (Figure 3).

Table 1. Literature review of published variants in NF-1 cases with both gDNA and cDNA sequenced.

<table>
<thead>
<tr>
<th>PMID</th>
<th>Cohort size</th>
<th>Total variants detected</th>
<th>Variants in CDS</th>
<th>Intronic +/- 10 bp of exon</th>
<th>Deep intronic</th>
<th>Sub-genic CNV</th>
<th>Whole-gene CNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>23913538</td>
<td>565</td>
<td>546</td>
<td>432</td>
<td>101</td>
<td>13</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>23154044</td>
<td>105</td>
<td>93</td>
<td>79</td>
<td>2</td>
<td>5</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>27322474</td>
<td>361</td>
<td>348</td>
<td>262</td>
<td>13</td>
<td>9</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>1,031</td>
<td>987</td>
<td>773</td>
<td>116</td>
<td>24</td>
<td>34</td>
<td>55</td>
</tr>
<tr>
<td>Percentage</td>
<td>78.32%</td>
<td>11.75%</td>
<td>2.43%</td>
<td>3.44%</td>
<td>5.57%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS

- NGS-based NF1 gDNA sequencing with both SNV and CNV detection identifies > 95% of variants reported in the literature.
- Intronic and deep intronic variants outside the typical reportable range of exonic and canonical splice site regions account for 2.5% of NF1 variants reported in the literature.
- While RNA/cDNA analyses provide additional functional data, this information alone is generally unlikely to alter variant classification. NGS gDNA panels offer nearly comparable detection to cDNA approaches.
- Missense variants comprise a greater proportion of NF1 VUS than intronic variants, and allow for the classification of more actionable variants by FVT at scale.
- Among individuals with NF-1 and negative family history, FVT confirmed de novo status and resolved VUS in 11/13 or 85% of patients.
- Family studies for segregation and de novo status provide powerful evidence that Invitae NGS panels are a strong clinical option for NF1 variant testing.

Figure 1. NF1 variant classification in 490 patients with NF-1 clinical features.

Figure 2. Effect of all gDNA NF1 variants detected.

Figure 3. Effect of NF1 VUS detected.

Figure 4. Variant classification before and after family variant testing (FVT).

Disclosures: All authors are stockholders and employees of Invitae. Prepared for presentation at ACMG 2018