Diagnostic Yield for Neurological and Neuromuscular Disorders Testing via High-Depth Multi-Gene Panel Analysis with Integrated Sequence and Copy Number Detection

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An Argument for Universal Del-Dup Testing for Neuromuscular Disorders
Disclosures

All authors are employees of Invitae Corporation
Historically, genetic testing was limited by cost.
Variants by size – what NGS can do

- Single nucleotide variants
- Small indels (<30bp)
- Large indels (>30bp, <full exon)
- Exon-level copy number variants
- Whole gene copy number variants
- Micro-deletions/duplications
- Aneuploidies

NGS, SANGER, MLPA, targeted array, aCGH, karyotypes
Variants by size – what NGS can do

- Single nucleotide variants
- Small indels (<30bp)
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- Exon-level copy number variants
- Whole gene copy number variants
- Whole chromosome copy number variants
- Micro-deletions/duplications
- Aneuploidies

Methods:
- SANGER
- MLPA, targeted array
- NGS
- aCGH, karyotypes
Why does CNV detection matter?

In >143,000 patients, del/dup events are seen:
- In 1.9% of all tested patients
- In 9.8% of patients with any pathogenic variant
  ➢ A disproportionate fraction of del/dups are pathogenic
Read depth (a.k.a. coverage)

- Coverage
- NGS reads
- Exon targets

Relatively high coverage region
Relatively low coverage region
Copy number detection by NGS

- Read depth varies between targets, due to both the target chemistry and NGS itself
- Depth profile *is non-uniform* but *reproducible*
Deviations with respect to baseline samples

Deletion
Deviations with respect to baseline samples

Duplication
Validity of method: retrospective cohorts

  - 43 CNVs among 391 patients
    - 100% concordance using NGS methods

  - 29 CNVs among 1,105 patients
    - 100% concordance using NGS methods
Prevalence of CNVs in neuromuscular disorders

- Referrals to a commercial lab
- Sole criterion: *physician ordered*
- Diagnostic (not carrier)
- Typically limited clinical/lab data

CNVs account for 39% of positive results
7,092 unrelated individuals tested

<table>
<thead>
<tr>
<th>test panel</th>
<th>no. of genes</th>
<th>no. of requests</th>
<th>no. of mol dx</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuromuscular disorders</td>
<td>104</td>
<td>1073</td>
<td>251</td>
<td>(23)</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>70</td>
<td>2741</td>
<td>364</td>
<td>(13)</td>
</tr>
<tr>
<td>Myopathy</td>
<td>50</td>
<td>352</td>
<td>53</td>
<td>(15)</td>
</tr>
<tr>
<td>Muscular dystrophy</td>
<td>46</td>
<td>399</td>
<td>154</td>
<td>(39)</td>
</tr>
<tr>
<td>Charcot-Marie Tooth</td>
<td>42</td>
<td>1040</td>
<td>308</td>
<td>(30)</td>
</tr>
<tr>
<td>LGMD</td>
<td>30</td>
<td>174</td>
<td>46</td>
<td>(26)</td>
</tr>
<tr>
<td>HSP</td>
<td>43</td>
<td>843</td>
<td>133</td>
<td>(16)</td>
</tr>
<tr>
<td>Dystonia</td>
<td>16</td>
<td>535</td>
<td>61</td>
<td>(11)</td>
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<tr>
<td>DMD</td>
<td>1</td>
<td>187</td>
<td>103</td>
<td>(55)</td>
</tr>
<tr>
<td>SMN1</td>
<td>1</td>
<td>246</td>
<td>155</td>
<td>(63)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7590</strong></td>
<td><strong>1628</strong></td>
<td></td>
<td>(23)</td>
</tr>
</tbody>
</table>
CNV rates by panel

- Neuromuscular: 14%
- Neuropathy: 48%
- Myopathy: 0%
- Muscular dystrophy: 17%
- CMT: 47%
- LGMD: 13%
- HSP: 13%
- Dystonia: 15%
- DMD: 65%
- SMN1: 100%

Total number of molecular diagnostics: 1628
Total number of CNVs: 637
39%
CNV rate by gene

- SMN1
- PMP22
- DMD
- PARK2
- LAMA2
- GCH1
- SPG11
- SPAST
- MFN2

CNVs in positive cases (%)
Case for universal CNV analysis

For DMD, how many ‘CNV-positive’ cases occur among multi-gene panel tests?

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>LGMD (%)</th>
<th>MD (%)</th>
<th>NMD (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNV</td>
<td>5/46 (11)</td>
<td>24/154 (16)</td>
<td>13/251 (5)</td>
<td>42/451 (9)</td>
</tr>
</tbody>
</table>
Summary

- NGS data can be used for highly sensitive del/dup analysis
- CNVs are prevalent in neuromuscular disorders
- Universal CNV detection may increase diagnostic rate
Questions?
Age at diagnosis

- 0-19: 45 cases (DMD only)
- 10-19: 18 cases (DMD only), 23 cases (Panels)
- 20-29: 12 cases (DMD only), 4 cases (Panels)
- >30: 7 cases (DMD only), 27 cases (Panels)
Validity of method: prospective cohorts

  - 130 CNVs among 2375 patients
  - 106 confirmed by alternate methods (FP=1%)

- Invitae
  - 4,028 CNVs among 227,022 patients
  - 3,910 confirmed by alternate methods (FP=0.05%)
What do real data look like?

Duplication

Deletion