Second-tier Sequencing Analysis for VLCAD Deficiency in Texas: The First 14 Months

D’Andra Luna, M.P.H.
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Objectives

• Brief overview of TX NBS testing
• Brief overview of VLCAD Sanger sequencing methodology
• Brief overview of pathogenicity determination
• Evaluate molecular findings
• Assess utilities of 2nd-tier VLCAD DNA testing
• Lessons learned
Texas NBS Program

Testing Performed for 53 Disorders

• Two screens:
  • 1\textsuperscript{st} screen: 24-48 hours of age
  • 2\textsuperscript{nd} screen: 1-2 weeks of age

• June 2017-July 2018: Received 867,690 specimens (~446,709 newborns)
  • ~2,350 specimens per day (6 days per week)

• 270 reported abnormal/borderline for VLCAD deficiency
  • 265 reflexed to DNA sequencing
  • 15 diagnosed cases with incidence of ~ 1:29,800
Molecular Testing in TX NBS Program

- SCID qPCR: TREC & RNaseP
- GALT ARMS-PCR: 4 common variant panel
- MCAD RT-PCR (allelic discrimination): 4 common variant panel
- Hb RT-PCR (allelic discrimination) and Sanger Sequencing of the β-globin gene
- VLCAD Sanger Sequencing of the ACADVL gene
- CF Luminex: 60 mutation panel
VLCAD DNA Testing

- Implemented June 1, 2017
- Testing performed 5 days per week
- Abnormal and Borderline specimens reflexed for DNA testing
- Only one screen DNA tested
- Sanger sequencing
- Expected turnaround time: 2 weeks
VLCAD Sanger Sequencing Methodology

- NBS DNA extraction
  - QIAGEN Generation DNA Purification
- Sequencing PCR
  - ABI BigDye Direct Cycle Sequencing kit
- Post-cycle clean up
  - ZR-96 DNA Sequencing Clean-up kit
- Sequencing instrument
  - ABI 3130 Genetic Analyzer
- Variant analysis
  - Mutation Surveyor V5.1.0
Variant Pathogenicity Determination

- Pathogenicity categories:
  - Pathogenic, Likely Pathogenic, Uncertain Significance, Likely Benign, Benign, No variant identified
- Pathogenicity determination:
  ACMG guidelines (PMID 25741868)
- Three reviewers for each variant:
  - Each reviewer will fill out worksheet
  - Discrepancy requires discussion
  - Time requirement: ~4-5 hours/variant
## Variant Assessment Worksheet

<table>
<thead>
<tr>
<th>Condition</th>
<th>1st Review</th>
<th>2nd Review</th>
<th>3rd Review</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variant Name</td>
<td>Evidence</td>
<td>Category</td>
<td>1st Review</td>
</tr>
<tr>
<td>Evidence</td>
<td>Category</td>
<td>1st Review</td>
<td>2nd Review</td>
</tr>
</tbody>
</table>

1. Condition specific site: Evidence Category
   - Evidence Category
   - Evidence Category
   - Evidence Category

2. Broad Exome Aggregation Database (ExAC):
   - Evidence Category
   - Evidence Category
   - Evidence Category

3. Genome:
   - Evidence Category
   - Evidence Category
   - Evidence Category

4. ASNL:
   - Evidence Category
   - Evidence Category
   - Evidence Category

5. ClinVar:
   - Evidence Category
   - Evidence Category
   - Evidence Category

6. Public Sites:
   - Evidence Category
   - Evidence Category
   - Evidence Category

### 7. Active Sites

<table>
<thead>
<tr>
<th>Hb</th>
<th>VLCAO</th>
<th>X-LAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 8. Literature Review:

<table>
<thead>
<tr>
<th>Evidence</th>
<th>Category</th>
<th>Summary of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 9. Multiple Lines of Computational Evidence:

- UCSC: [http://genome.ucsc.edu](http://genome.ucsc.edu)
- PolyPhen: [http://genetics.bwh.harvard.edu](http://genetics.bwh.harvard.edu)
- SIFT: [PROVANT](http://genetics.bwh.harvard.edu) [PROVEAN Prediction](http://genetics.bwh.harvard.edu)

### 10. Additional Categories

<table>
<thead>
<tr>
<th>Evidence Category</th>
<th>Defines</th>
<th>Summary of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

4/10/2019
Result Reporting

4 Clinical Significance Statements

- The results suggest Very Long Chain Acyl-CoA Dehydrogenase Deficiency. If you have not already done so, consultation with a metabolic specialist within 24 hours is strongly recommended.

- If you have not already done so, consultation with a metabolic specialist within 24 hours is recommended.

- If clinically indicated, consultation with a metabolic specialist within 72 hours is recommended. As appropriate, recommend genetic counseling within 30 days.

- Notify family of test results. As appropriate, recommend genetic counseling within 30 days.
## Variant Distribution Among Abnormal and Borderline Specimens

<table>
<thead>
<tr>
<th>Category</th>
<th>Abnormal</th>
<th>Borderline</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st screen</td>
<td>2nd screen</td>
<td>1st screen</td>
</tr>
<tr>
<td>Two or more Pathogenic/Likely Pathogenic/VOUS</td>
<td>20</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>One Pathogenic/Likely Pathogenic/VOUS</td>
<td>77</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Likely Benign or Benign variant only</td>
<td>33</td>
<td>28</td>
<td>19</td>
</tr>
</tbody>
</table>
| No variants                                  | 3          | 5           | 3          | 2           | 13     

Total Presumptive Positives reflexed to DNA: 265
## Diagnosed Cases
### June 2017-July 2018

<table>
<thead>
<tr>
<th></th>
<th>Abnormal</th>
<th></th>
<th>Borderline</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st screen</td>
<td>2nd screen</td>
<td>1st screen</td>
<td>2nd screen</td>
<td></td>
</tr>
<tr>
<td>Two or more Pathogenic/ Likely Pathogenic/ VOUS</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>One Pathogenic/ Likely Pathogenic/ VOUS</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Total Diagnosed Cases: 15
Most Prevalent Variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Exon</th>
<th>Reported Pathogenicity</th>
<th>Allele Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.848T&gt;C</td>
<td>9</td>
<td>Pathogenic</td>
<td>22</td>
</tr>
<tr>
<td>c.1322G&gt;A</td>
<td>13</td>
<td>Pathogenic</td>
<td>6</td>
</tr>
<tr>
<td>c.1375C&gt;T</td>
<td>14</td>
<td>Pathogenic</td>
<td>6</td>
</tr>
<tr>
<td>c.829_831delGAG</td>
<td>9</td>
<td>Likely Pathogenic</td>
<td>5</td>
</tr>
<tr>
<td>c.1066A&gt;G</td>
<td>10</td>
<td>VOUS</td>
<td>5</td>
</tr>
<tr>
<td>c.343delG</td>
<td>6</td>
<td>Pathogenic</td>
<td>4</td>
</tr>
<tr>
<td>c.1096C&gt;T</td>
<td>11</td>
<td>Pathogenic</td>
<td>3</td>
</tr>
<tr>
<td>c.1376G&gt;A</td>
<td>14</td>
<td>Pathogenic</td>
<td>3</td>
</tr>
<tr>
<td>c.1531C&gt;T</td>
<td>15</td>
<td>Likely Pathogenic</td>
<td>3</td>
</tr>
<tr>
<td>c.950T&gt;C</td>
<td>10</td>
<td>Likely Pathogenic</td>
<td>3</td>
</tr>
<tr>
<td>c.751A&gt;G</td>
<td>8</td>
<td>VOUS</td>
<td>3</td>
</tr>
</tbody>
</table>

4/10/2019 Total: 143 pathogenic, likely pathogenic, and VOUS variants identified. Most are single alleles.
Average Age at Diagnosis

Prior to June 1, 2017

After June 1, 2017
Lessons Learned

1. Better able to anticipate needs for X-ALD Sanger sequencing
   a. Variant classification
   b. More robust database
   c. Increase capacity of sequencers
   d. Process automation
   e. Expansion of LIMS reporting functionality

2. Universal coverage of sequencing: supplemental information to assist specialists for case prioritization and quicker diagnosis

3. Necessity of reporting Benign/Likely Benign
Newborn DNA Analysis Team

- Raven Hardcastle
- Yan Sun, Ph.D.
- Shirley Hammond, M.S.
- Dagoberto Martinez, M.S.
- Joyce Cen, M.S.
- Stephanie Thompson, M.P.H.
- Kendra Tucker-Jerkic, D.V.M.
- Rachel Lee, Ph.D.
Thank you

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