Second Tier Full Gene Sequencing for Follow Up of Positive Newborn Screening for VLCADD and GA1

Neena Champaigne, MD
Greenwood Genetic Center
South Carolina
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# INTRODUCTION

**VLCADD deficiency**
- Autosomal recessive
- Incidence: 1 in 40-120,000
- Clinical presentation
  - Severe, early-onset form:
    - Cardiomyopathy, hepatomegaly, hypotonia
  - Intermediate form:
    - Hypoketotic hypoglycemia
  - Late-onset form
    - Intermittent rhabdomyolysis, muscle pain and/or exercise intolerance

**Glutaric aciduria, type I (GA-1)**
- Autosomal recessive
- Incidence: 1 in 30-40,000
- Clinical presentation
  - Macrocephaly
  - Hypotonia
  - Acute encephalopathic episode
    - Spasticity
    - Dystonia
    - Dyskinesia
    - Seizures
  - High and LOW excretors
ACMG ACT Sheet VLCADD Algorithm

C14:1 Elevated +/- Other Long-Chain Acylcarnitines

Elevation of C14:1 +/- other long-chain AC

Routine Labs:
- Glucose, electrolytes, blood gas, lactate, ammonia, LFT, CPK

Plasma AC, quantitative

Plasma AC - VLCAD profile

Optional

VLCAD Deficiency

VLCAD gene sequencing

Optional

Fatty Acid Oxidation Probe Assay, Fibroblast Culture

Abbreviations:
- AC = acylcarnitine
- CPK = creatine phosphokinese
- FAO = Fatty acid oxidation
- LFT = liver function test
- VLCAD = very long-chain acyl-CoA dehydrogenase
ACMG ACT Sheet GA-1 Algorithm

**C5-DC Elevated (Isolated)**

- **Isolated Elevation of C5-DC**
  - **Routine Labs:** Glucose, electrolytes, blood gas
  - **Assay:** Plasma AC, Urine QA, Urine AC
    - **Plasma AC:** Normal
      - **Urine QA:** GA1 pred/normal
        - **Urine AC:** C5-DC
          - **Glutaryl-CoA dehydrogenase (GCDH) assay, fibroblasts**
    - **Plasma AC:** Normal
      - **Urine QA:** Normal
        - **Urine AC:** Normal
          - **GCDH activity:** Normal
            - **Glutaric Acidemia Type 1 (GA1)**
              - **Optional Confirmatory Testing:** Glutaryl-CoA dehydrogenase (GCDH) assay, GCDH Gene Analysis
            - **False positive:** No further action required.
          - **GCDH activity:** Low
            - **Glutaric Acidemia Type 1 (GA1)**
              - **Optional Confirmatory Testing:** Glutaryl-CoA dehydrogenase (GCDH) assay, GCDH Gene Analysis
              - **False positive:** No further action required.

**Abbreviations/Key**
- DC = dicarboxylic
- AC = acyl/carnitine
- QA = organic acid
- GA = glutaric acid
- GCDH = glutaryl-CoA dehydrogenase

*When the positive predictive value of screening is sufficiently high and the risk to the baby is high, some initiate diagnostic studies at the same time as the confirmation of screening result is done.*

*Actions are shown in shaded boxes; results are in the unshaded boxes.*
Molecular analysis is often need to follow-up screen positives for VLCAD deficiency and Glutaric aciduria, type 1.

<table>
<thead>
<tr>
<th>South Carolina</th>
<th>2010*</th>
<th>2011</th>
<th>2012</th>
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</thead>
<tbody>
<tr>
<td><strong>Total Births</strong></td>
<td>55,813</td>
<td>54,898</td>
<td>53,691</td>
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<tr>
<td>Elevated C14:1</td>
<td>51</td>
<td>82</td>
<td>61</td>
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<td>Elevated C5DC (D) or C5DC + C6OH (U)</td>
<td>31</td>
<td>49</td>
<td>86</td>
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</table>

*Derivatized method
INTRODUCTION

Implemented 2\textsuperscript{nd} Tier full gene sequencing in April 2013 (in addition to repeat screen):

• VLCAD deficiency (\textit{ACADVL} gene)
  – C14:1 ≥ 0.43 \(\mu\)M

• Glutaric aciduria, Type 1 (\textit{GCDH} gene)
  – C5DC + C6OH ≥ 0.45 \(\mu\)M
METHOD

Two – 3 mm punches

Quiagen EZ1 Advanced DNA Tissue Kit
50 µl of DNA
1-5 ng/ µl

1 µl DNA/PCR Reaction

ACADVL gene: 20 exons
GCDH gene: 12 exons
ACADVL Results by C14:1

N = 109

Pathogenic: 0 0 1 0 0 2 1
VUS: 0 1 0 0 2 1
ACADVL Results by C14:1/C2

N = 109
ACADVL Results by Region 4 Score

N = 109
<table>
<thead>
<tr>
<th>Exon/Intron</th>
<th>Nucleotide change</th>
<th>AA change</th>
<th>Interpretation</th>
<th>Exon/Intron</th>
<th>Nucleotide change</th>
<th>AA change</th>
<th>Interpretation</th>
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<tr>
<td>2</td>
<td>c.128G&gt;A</td>
<td>p.G43D</td>
<td>VUS</td>
<td>12</td>
<td>c.1246_1248delGCC</td>
<td>Pathogenic</td>
<td></td>
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<tr>
<td>9</td>
<td>c.848T&gt;C</td>
<td>p.V283A</td>
<td>Pathogenic</td>
<td>14</td>
<td>c.1405C&gt;T</td>
<td>p.R469W</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>10</td>
<td>c.1066A&gt;G</td>
<td>p.I356V</td>
<td>VUS</td>
<td>16</td>
<td>c.343delG</td>
<td>Pathogenic</td>
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<tr>
<td>10</td>
<td>c.1064T&gt;C</td>
<td>p.I355T</td>
<td>VUS</td>
<td>16</td>
<td>c.1591C&gt;T</td>
<td>p.R531W</td>
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<td>10</td>
<td>c.1066A&gt;G</td>
<td>p.I356V</td>
<td>VUS</td>
<td>16 In</td>
<td>c.1605+3A&gt;G</td>
<td>VUS</td>
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<tr>
<td>10</td>
<td>c.1077_1077+1delGGinsCAC</td>
<td>Pathogenic</td>
<td></td>
<td>17 In</td>
<td>c.1678+3_6delAAGT</td>
<td>VUS</td>
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<tr>
<td>10</td>
<td>c.1064T&gt;C</td>
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<td>p.S583W</td>
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<tr>
<td>11 In</td>
<td>c.1182+1G&gt;A</td>
<td>Pathogenic</td>
<td></td>
<td>20</td>
<td>c.1913C&gt;T</td>
<td>p.S638F</td>
<td>VUS</td>
</tr>
</tbody>
</table>
GCDH Results by C5DC+C6OH

C5DC+C6OH (Initial NBS)

Pathogenic VUS

N = 69
Cutoff 0.45

N = 69
GCDH Results by Region 4 Score

Region 4 Score

Pathogenic: 0 0 0 2
VUS: 0 1 0

N = 69
## GCDH Mutations

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<tr>
<td>8</td>
<td>c.640A&gt;G</td>
<td>p.T214A</td>
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<tr>
<td>9</td>
<td>c.862G&gt;A</td>
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<td>VUS</td>
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<tr>
<td>9</td>
<td>c.937C&gt;T</td>
<td>p.R313W</td>
<td>Pathogenic</td>
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<tr>
<td>11</td>
<td>c.1085C&gt;A</td>
<td>p.A362D</td>
<td>SNP (&lt;1%)</td>
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TURN AROUND TIMES

- ≤7 days
- 8-14 days
- 15-21 days
- >22 days

VLCADD: 83% ≤14 days
GA1: 97% ≤14 days
FOLLOW-UP

• **Short-term Follow-up (DHEC):**
  – Communicate results to Primary Care Provider and Metabolic Geneticist:
    • Initial Screen
    • Repeat Screen
    • Molecular Report

• **Metabolic Geneticist (Greenwood Genetic Center):**
  – Reviews all abnormal AC profiles and molecular testing
  – Carriers:
    • Families offered genetic counseling via telephone or in person.
  – Indeterminate/Affected:
    • Seen and followed in Metabolic clinic.
Gene Sequencing Performed for VLCADD and GA-1

APRIL 2014: Changes to the testing algorithm

C14:1: 2nd Tier Gene Sequencing
- Cutoff increased to <0.50 µM
- Added C14:1/C2 ratio of <0.02
- 0.50 – 0.74; If C14:1/C2 is ≥0.02
- ≥ 0.75 µM

C5DC +C6OH: 2nd Tier Gene Sequencing
- 0.45 – 0.74 µM; If Region 4 Tool ≥ 80
- ≥ 0.75 µM
# Status

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<td>82</td>
<td>61</td>
<td>82</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>VLCADD</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
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<td>86</td>
<td>69</td>
<td>45</td>
<td>3</td>
</tr>
<tr>
<td>GA-1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
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*Derivatized method

**Preliminary
FUTURE PLANS

2\textsuperscript{nd} Tier Gene Sequencing for Carnitine Uptake Defect, in progress:

\textit{SLC22A5} gene sequencing if:

\[ C_0 \leq 8.00 \, \mu \text{M} \quad \text{and} \quad C_3 + C_{16} \leq 2.00 \]

Potential to add full gene sequencing for other disorders:

– Galactosemia
– Biotinidase deficiency
– CPT1A
– MCAD deficiency
– Lysosomal storage disorders (when screened)
Acknowledgements

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  Laura Pollard, PhD

Molecular Laboratory