Retrospective Analysis of the Region 4 Post Analytical Tool and Confirmatory Testing for Long Chain Fatty Acid Oxidation Disorders Screened in the State of Iowa

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No conflicts of interest to disclose
Educational Goals

• Present information obtained from the Iowa Newborn Screening Program for long chain fatty acid oxidation disorders between 2008-2013.
• Present the performance of the Region 4 laboratory assessment tool
• Discuss strength, weaknesses and limitations of this assessment
• Provide recommendations to improve research in this area.
Overview
Long Chain Fatty Acid Oxidation Disorders

- VLCADD, LCHAD, SCHAD, CPT2/CACT, TFP and CPT1 deficiency.
  - Hereditary disorders that impair the metabolism of long chain fatty acids.

http://naturalhygienesociety.org/images/fast-phases.jpg
Fatty Acid Oxidation Disorders

• Clinical manifestations
  – Somnolence
  – Vomiting
  – Lethargy
  – Myopathy
  – Cardiomyopathy
  – Liver disease
  – Coma
Clinical evaluation

• Clinical evaluation
  – May be initially asymptomatic
  – Initial decompensation may be the last

• Laboratory evaluation
  – Combination of elevation of specific acylcarnitines depending on the defect
  – Dicarboxylic aciduria
  – Hypoketotic hypoglycemia
Current Screening Strategy

Iowa State Hygienic Lab

TMS

Cut-off

Primary Markers
- C14:1 > 0.67 (1.78)
- C16 > 7.47 (16.54)
- C16OH > 0.15 (0.74)
- C18 > 1.8
- C18:1 > 2.5

Secondary Markers
- C12:1 > 0.5
- C14 > 0.7
- C14:2 > 0.15
- C14/C16 > 0.2
- C14:1/C12:1 > 5
Current Screening Strategy

• Notification to Short Term Follow up office
• Elevation of primary markers are referred to clinic for evaluation. (Normalization of second tier tests may happen in a well fed state)
• Collection of second tier testing ASAP
• Completion of skin biopsy to complete fatty acid oxidation studies
• DNA testing is completed if second tier testing is persistently abnormal or there are symptoms or evidence of end organ involvement
Disadvantages of Current Strategy

- Parental concerns
- Unnecessary testing
- Increased costs
Profile Oriented Testing

LABORATORY QUALITY IMPROVEMENT OF NEWBORN SCREENING

Email: [Input field]
Password: [Input field]

Login

Reset your password
Request Access
EDUCATIONAL PRESENTATIONS

Hot Topics NEW! A 2013 series of 7 presentations recorded by Mayo Medical Laboratories describing the products and clinical tools of the R4S collaborative project

POST-ANALYTICAL INTERPRETIVE TOOLS

Post-Analytical Tools Calculate a condition-specific score for a case based on all clinically significant analytes and ratios

CURRENT DATA POSTED BY YOUR LABORATORY

Cutoff Values Normal Percentiles
True Positives Performance Metrics

Last Update

COMPARE YOUR LABORATORY DATA WITH OTHER PARTICIPANTS

Cutoff Values Comparison Percentiles Comparison
Performance Metrics Comparison Disease Range Disease Range (MoM)
Analyte Comparison Profile Comparison
Post-Analytical Tools

One Condition
These tools generate a score and suggest interpretation guidelines for a specific condition

Two Conditions
These tools generate a score and suggest interpretation guidelines for a specific condition, and a direct comparison with a second related condition

Dual Scatter Plot
This tool shows the distribution of score pairs for the same case calculated with two of the tools (two conditions) above. The plot provides a visual report of likelihood to be one or the other condition

Multiple Conditions
These tools generate a score and suggest interpretation guidelines for a specific condition, and a direct comparison with multiple other conditions

All Conditions
This tool generates a score for all conditions for which the complete set of required results is available (uploaded either manually or by .csv file). A separate plot sorts the scores of the case under evaluation based on the %ile rank among true positive cases for each condition.
NOTE: The one-condition tools available to all users are applied as default. If site-specific one-condition tools are available, they will replace the general tools automatically

Tool Runner
This tool takes an entire batch of data and calculates a score for all existing tools, with a tabular output.
Excel File with coded metabolites as LOINC codes
Case Score

41  60  281
All  United States  Iowa

View Calculations

%ile Rank of all VLCAD Scores:

4 %  6 %  0 %
All  United States  Iowa

Count of VLCAD Scores

352  205  5
All  United States  Iowa

Score Interpretation Guidelines

Score is \( \geq 111 \)
Condition is very likely VLCAD.

Score is \( \geq 59 \) and \( < 111 \)
Condition is likely VLCAD.

Score is \( \geq 38 \) and \( < 59 \)
Condition is possibly VLCAD.

Score is \( < 38 \)
Profile is not informative for VLCAD.

Score Comparison Plot
Number of cases in the time period

2008  27,016  (May thru Dec)
2009  39,482
2010  38,381
2011  37,772
2012  38,197
2013  38,521

Total screenings: 219,369
Performance Analysis

• Single state, retrospective analysis
• Samples between May of 2008 and December of 2013
• Newborns with increased C12, C14, C14:1, C16, C18 and C18:1. N=93
• Tandem mass spectrometry data was analyzed using the Region 4 post analytical tool and the newborn screen database was reviewed for final diagnostic results and clinical outcomes followed by performance analysis of the Regions 4s tool and cost analysis.
Gender Distribution

Total referrals identified: 93
Population Information

• The average birth weight was 3242 gr (Median of 3256 gr)

• Average gestational age of 38.7 weeks. (Median of 39 weeks)

• All were orally fed at the time of collection.
Population Information

Marker

- C14:1: 38%
- C16 and C18:1: 31%
- C16: 21%
- C18:1: 10%

Legend:
- C14:1
- C16 and C18:1
- C16
- C18:1
Region 4s Display

- VLCADD
- VLCADD/GAII/LCHAD, MCD
- VLCADD/GAII
- VLCADD/GAII/CPT2/CACT
- VLCADD/GAII/CPT2/LCHAD
- VLCADD/GAII/LCHAD
- VLCADD/GAII/CPT2/CACT
- VLCADD/GAII/CPT2/CACT
- VLCADD/GAII/CPT2/CACT
- VLCADD/GAII/CPT2/CACT
- VLCADD/GAII/CPT2/CACT
Population Information

• 56 of 93 had fatty acid oxidation analysis on skin fibroblasts

• 44 of 93 did not show a suggestive screen with the Region 4s tool.
  – Of these; 28 had fatty acid oxidation analysis
  – 18 of the 28 negative.
  – 9 showed SCADD and 1 was not specific

• 8 total cases did not have confirmatory testing and their outcome is unknown.
CPT1 deficiency cases

- CPT1 enzyme activity reduced in 2
- Both showed no flags on the Region 4s
- First case
  - (41 wks, 2500 gr; Male); (Region 4s=0); NBS (55 hrs): C0= 59.35; C14: 0.74 (0.7); C16= 8.42 (7.47); C18:1= 2.92(2.5); C18= 1.63. C0/(C16+C18)= 5.9
- ACP: elevated C18; repeat normal. CPT1 enzyme activity 11%. DNA negative. At 3 yo, no issues, on MCT oil.
CPT1 deficiency cases

• Second case

• (39 wks, Male, 2960 gr, Breast fed); (Region4s = 0) NBS (29 hrs): C0= 60.59 (60); C14=0.73 (0.7); C16=9.31 (7.47); C18: 1.87 (1.8); C0/(C16+18)= 5.4. CPT1 activity modestly reduced. Repeat screen normal (70 hrs). ACP C16, C18 elevations. Normalized in 2 repeats. CPT1a/CPT2/CACT DNA negative. FAOP compatible with SCADD. Had neonatal hypoglycemia (11 mg/dL on transition). At 5 yo, asymptomatic. No other episodes.
VLCAD Deficiency cases

- (39 wks; 3432 gr, female; breast fed) C12:1 0.78; C14:1 0.77. FAOP compatible with 75% enzyme activity; compatible with late onset VLCADD. DNA showed only one mutation. Region 4s score: 52. Discharged. (Region 4s: VLCADD: 40; Cat 2)

- (36wks; 2520gr, Female ;Breast fed); C12:1: 0.78; C14:1: 0.77. Followed in Nebraska as a real VLCADD. Unknown mutations, Unknown FAOP, unknown outcome. (Region 4s: VLCADD : 52 (cat 2) Repeat screen is negative. Initial sample at 44 hours, Repeat sample at 147 hours.

- (39 wks; female; 3580 gr; breast fed) C14:0.41; C10: 033, C10:1: 0.28; C12:1: 0.62; C14:2.14; C14:1: 3.14. Second tier test compatible. No FAOP, DNA showed, two mutations. C.520G>A and c.1316G>A (Exon 7, Exon 13) (Region 4s: VLCADD/GAII; VLCADD : 373 (CatIV); GAII: 124 (cat IV) Behavioral problems, sleep apnea, multiple preventive admissions with no major morbidity. 2 yo no cardiomyopathy.
Indeterminate case

• Birth at 41 wks, Male, 4360 gr, Breast fed
• C2: 74.06, C16: 7.64 (7.47), C18: 1.98 (1.8)
• UOA, ACP: neg.
• Region 4s score: 0
• Fatty acid ox probe (CHOP) showed impaired oxidation of Myrastate and palmitate. Combined medium and long chain FAO disorder.
• DNA testing for CPT2 and CACT negative.
• No episodes of hypoglycemia. At 3 yo patient on regular diet., clinically well.
## Performance Analysis

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<th></th>
<th>D+</th>
<th>D-</th>
<th>Total</th>
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<tbody>
<tr>
<td>R4 +</td>
<td>3</td>
<td>46</td>
<td>49</td>
</tr>
<tr>
<td>R4 -</td>
<td>3</td>
<td>41</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>87</td>
<td>93</td>
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Non Specific: 1 case  
VLCAD: 3 cases  
CPT1: 2 cases  
CPT2: 0 cases  
LCHAD: 0 cases
Performance Analysis

• Sensitivity of 50%
• Specificity of 47%
• Positive predictive value: 6%
• Negative predictive value: 93%
Cost Analysis

• The average diagnostic workup per infant was $3,200 USD.

• Cost savings of $44,000 in fatty acid oxidation analysis and $166,760 in DNA testing without adversely affecting the clinical outcome and sparing the families of the psychological burden of a possibly affected child at risk of clinical complications.
Conclusion

- Region 4 Newborn screening tool has a very good negative predictive value in our case series.
- Region 4 had sub optimal sensitivity and positive predictive value due to the lack of definite positive cases in our state that had newborn screening completed at birth.
Conclusions

• Weakness of the study includes inconsistency on the final confirmatory methods or “case definition”

• C0 was not included due to the high variability and special circumstances (prematurity, carnitine supplementation with TPN)

• Results of the confirmatory testing sometimes discloses a disorder different from the one that the original screen suggested.
Recommendations

• Compare our data with other more extensive programs in the country that may have identified more definite positive cases. (Involvement with the IBEMC collaborative)

• A conclusive negative predictive value of 100% would be compelling to adopt a strategy to filter out cases that do not flag with the Region 4 tool.

• This assessment has to be expanded for the rest of the inborn errors of metabolism.
Questions