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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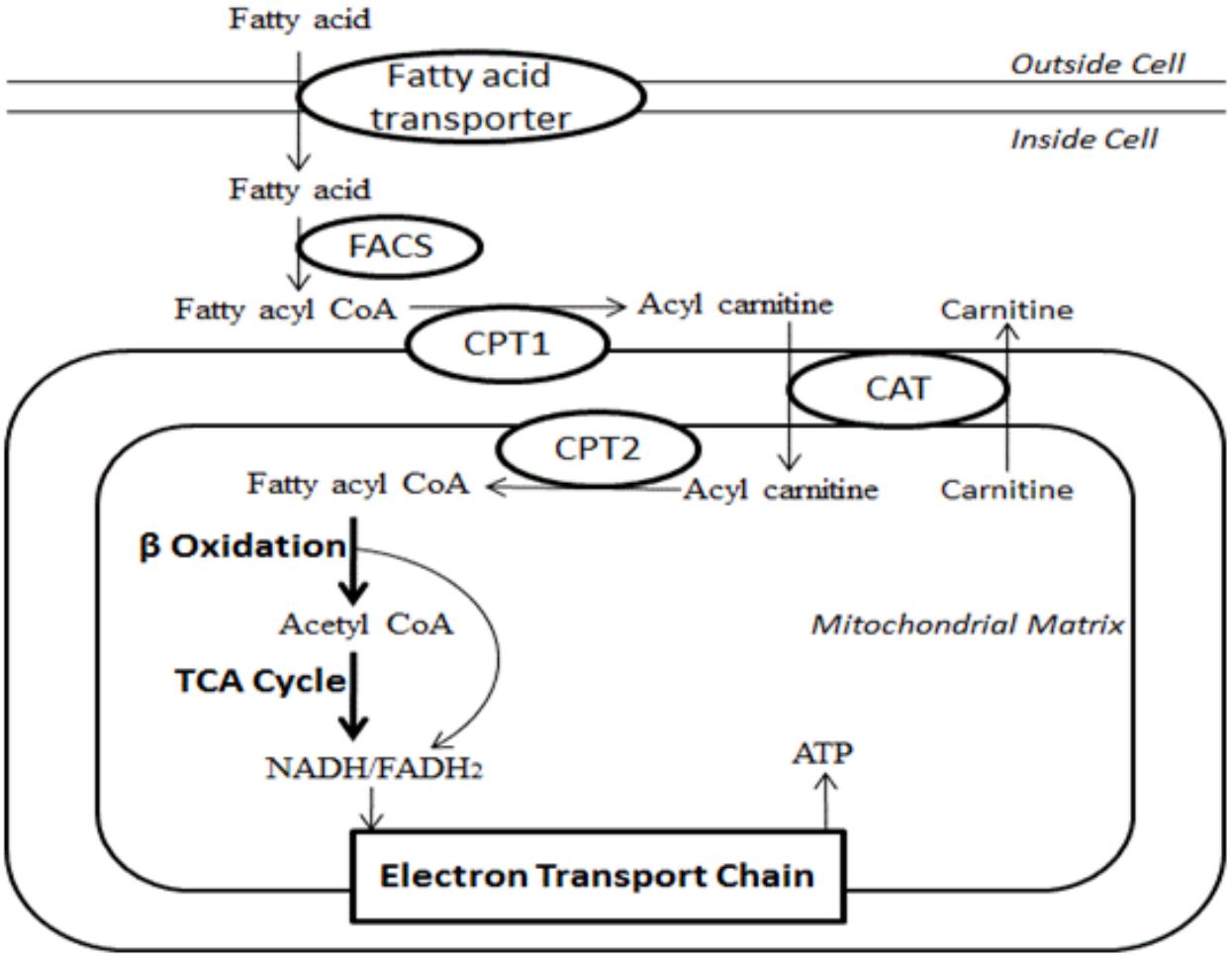
No conflicts of interest to disclose

Educational Goals

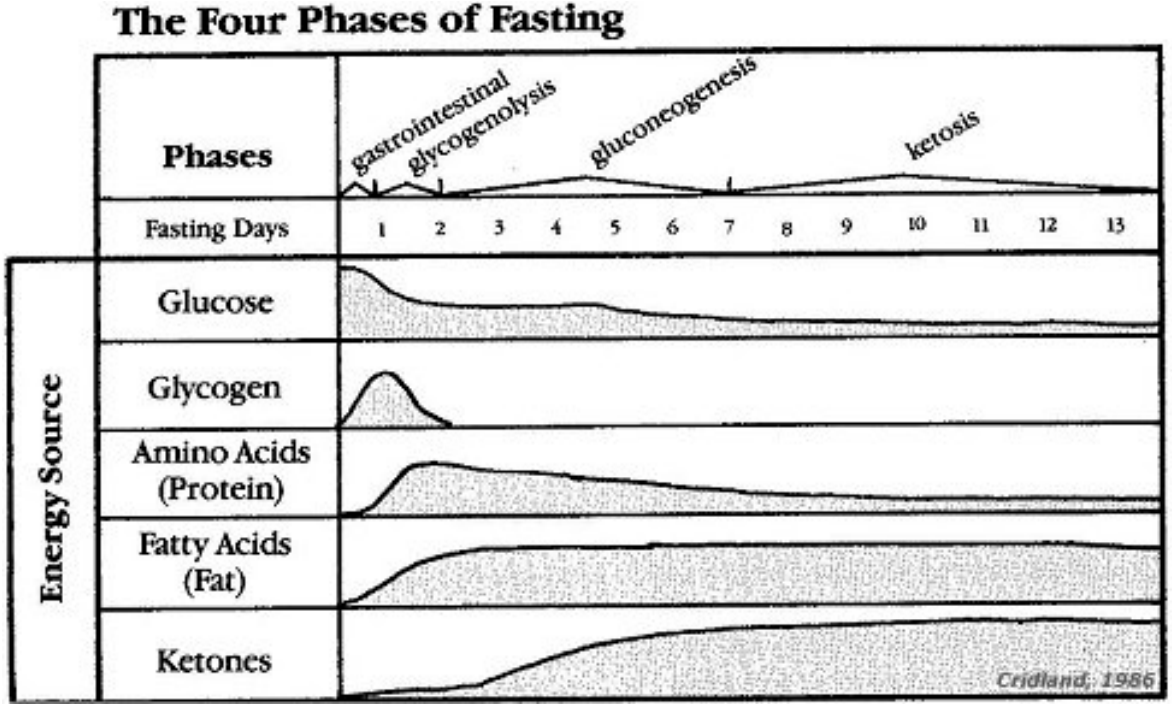
- Present information obtained from the Iowa Newborn Screening Program for long chain fatty acid oxidation disorders between 2008-2013.
- Present the performance of the Region 4 laboratory assessment tool
- Discuss strength, weaknesses and limitations of this assessment
- Provide recommendations to improve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

Overview


Fatty acid
Fatty acid transporter
Outside Cell
Inside Cell
Fatty acid
FACS
Fatty acyl CoA
Acyl carnitine
Carnitine
CPT1
CAT
CPT2
Fatty acyl CoA
Acyl carnitine
Carnitine
β Oxidation
Acetyl CoA
Mitochondrial Matrix
TCA Cycle
NADH/FADH2
ATP
Electron Transport Chain

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

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

Primary Markers

C12:1 > 0.5
C14 >0.7
C14:2 > 0.15
C14/C16 > 0.2
C14:1/C12:1 > 5

Secondary Markers

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

Email:

Password:

Login

Reset your password

Request Access

MS/MS COLLABORATIVE PROJECT

Region4 Genetics Collaborative

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MS/MS **Welcome: Alvaro Serrano-Russi**

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

MS/MS COLLABORATIVE PROJECT

Region4 Genetics Collaborative

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.

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All Conditions Post-Analytical Tool

Create PDF

Condition Type: ☑ Amino Acid ☑ Fatty Acid Oxidation ☑ Organic Acid ☑ False Positives ☑ Other

Target: ☑ Primary ☑ Secondary ☑ Other

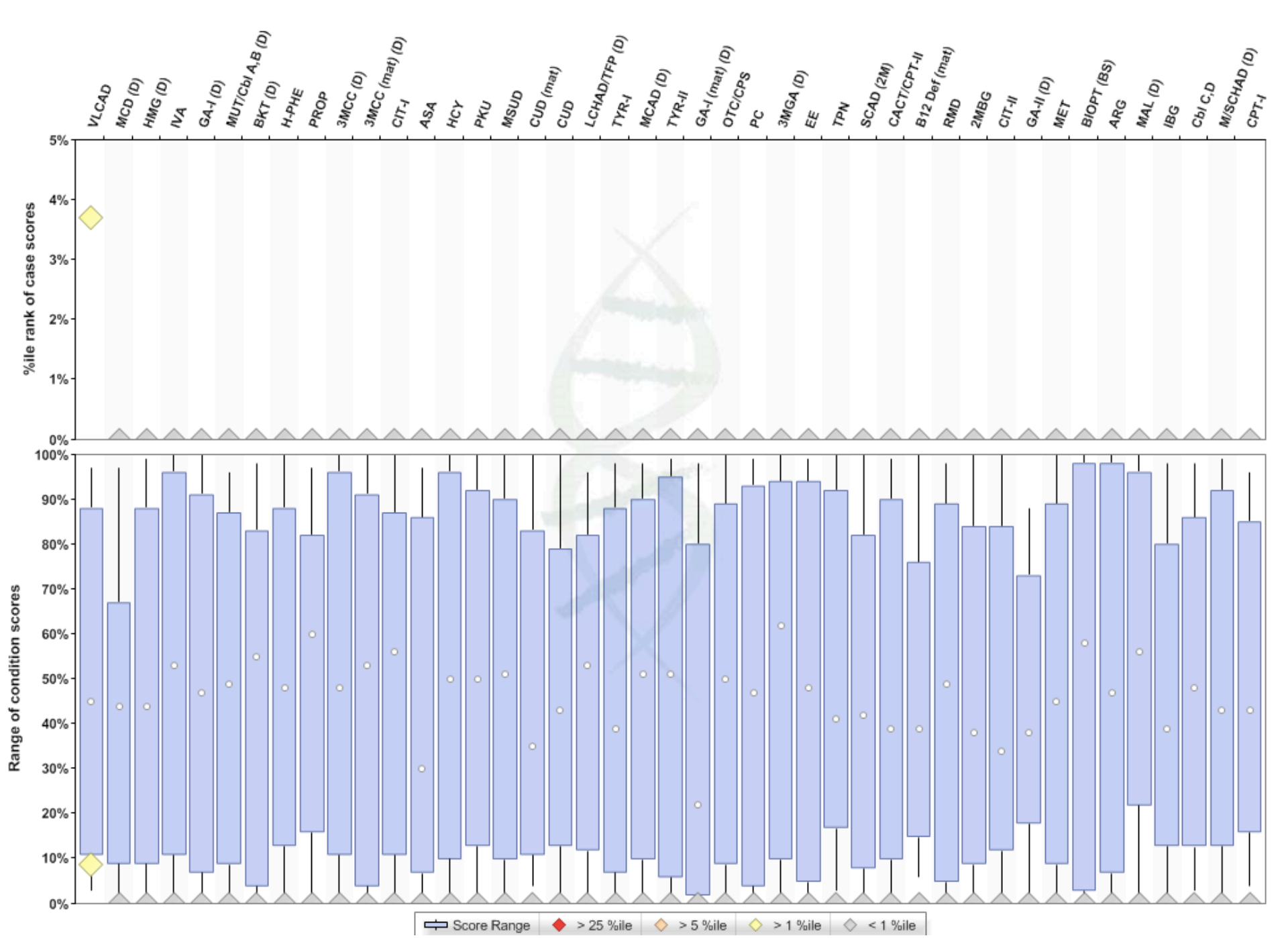
Score Type: ◉ MinMax ○ Regular ○ Z-Score

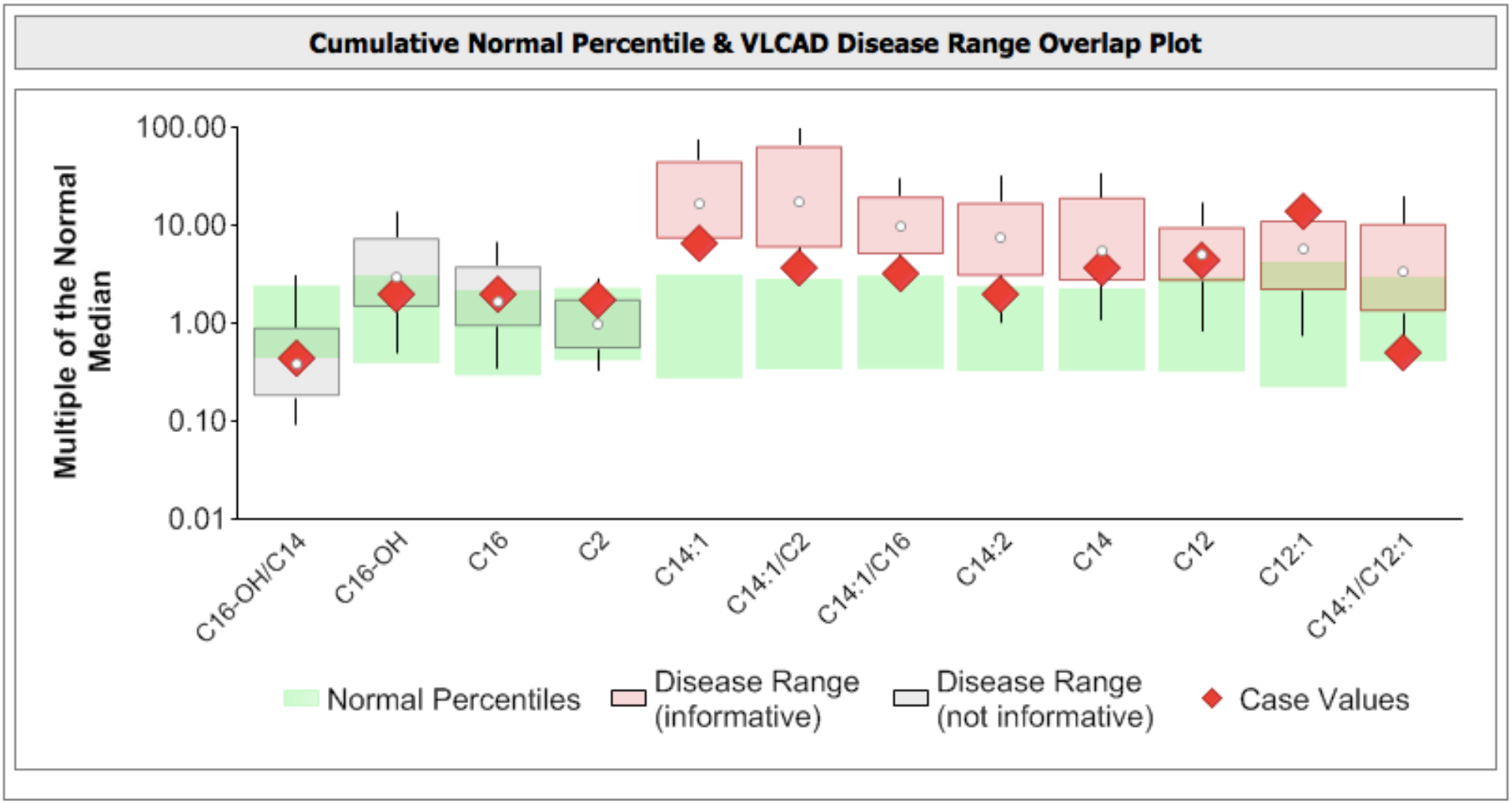
Choose File no file selected

Excel File with coded metabolites as LOINC codes

Click to Show Analytes...

Run Tools


VLCAD
MCD (D)
HMG (D)
IVA
GA-I (D)
MUT/Cbl A,B (D)
BKT (D)
H-PHE
PROP
3MCC (D)
3MCC (mat) (D)
CIT-I
ASA
HCY
PKU
MSUD
CUD (mat)
CUD
LCHAD/TFP (D)
TYR-I
MCAD (D)
TYR-II
GA-I (mat) (D)
OTC/CPS
PC
3MGA (D)
EE
TPN
SCAD (2M)
CACT/CPT-II
B12 Def (mat)
RMD
2MBG
CIT-II
GA-II (D)
MET
BIOPT (BS)
ARG
MAL (D)
IBG
Cbl C,D
M/SCHAD (D)
CPT-I
%ile rank of case scores
5%
4%
3%
2%
1%
0%
Range of condition scores
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Score Range
> 25 %ile
> 5 %ile
> 1 %ile
< 1 %ile


Cumulative Normal Percentile & VLCAD Disease Range Overlap Plot
Multiple of the Normal Median
100.00
10.00
1.00
0.10
0.01
C16-OH/C14
C16-OH
C16
C2
C14:1
C14:1/C2
C14:1/C16
C14:2
C14
C12
C12:1
C14:1/C12:1
Normal Percentiles
Disease Range (informative)
Disease Range (not informative)
Case Values

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

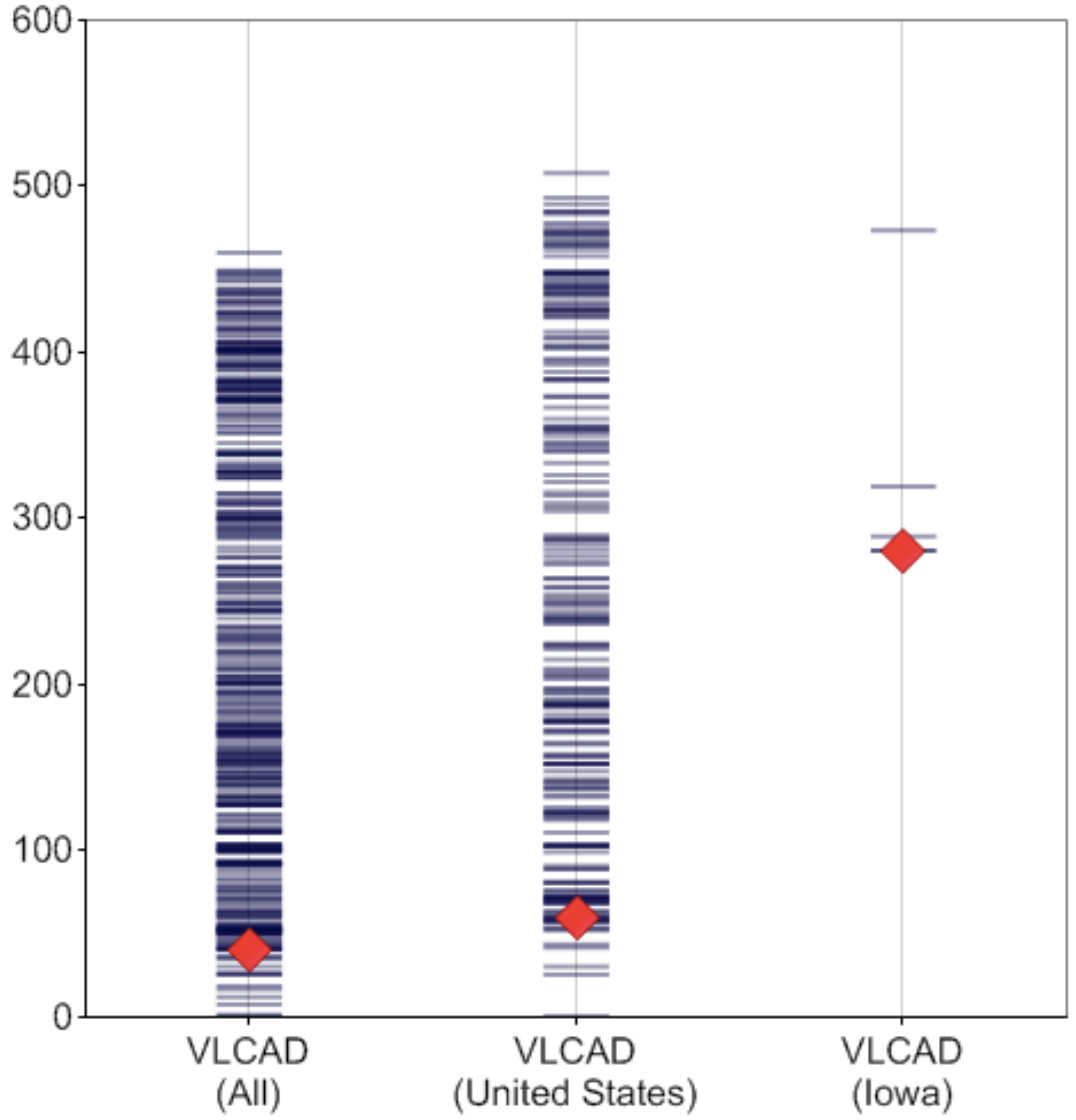
Score is >= 111
Condition is very likely VLCAD.

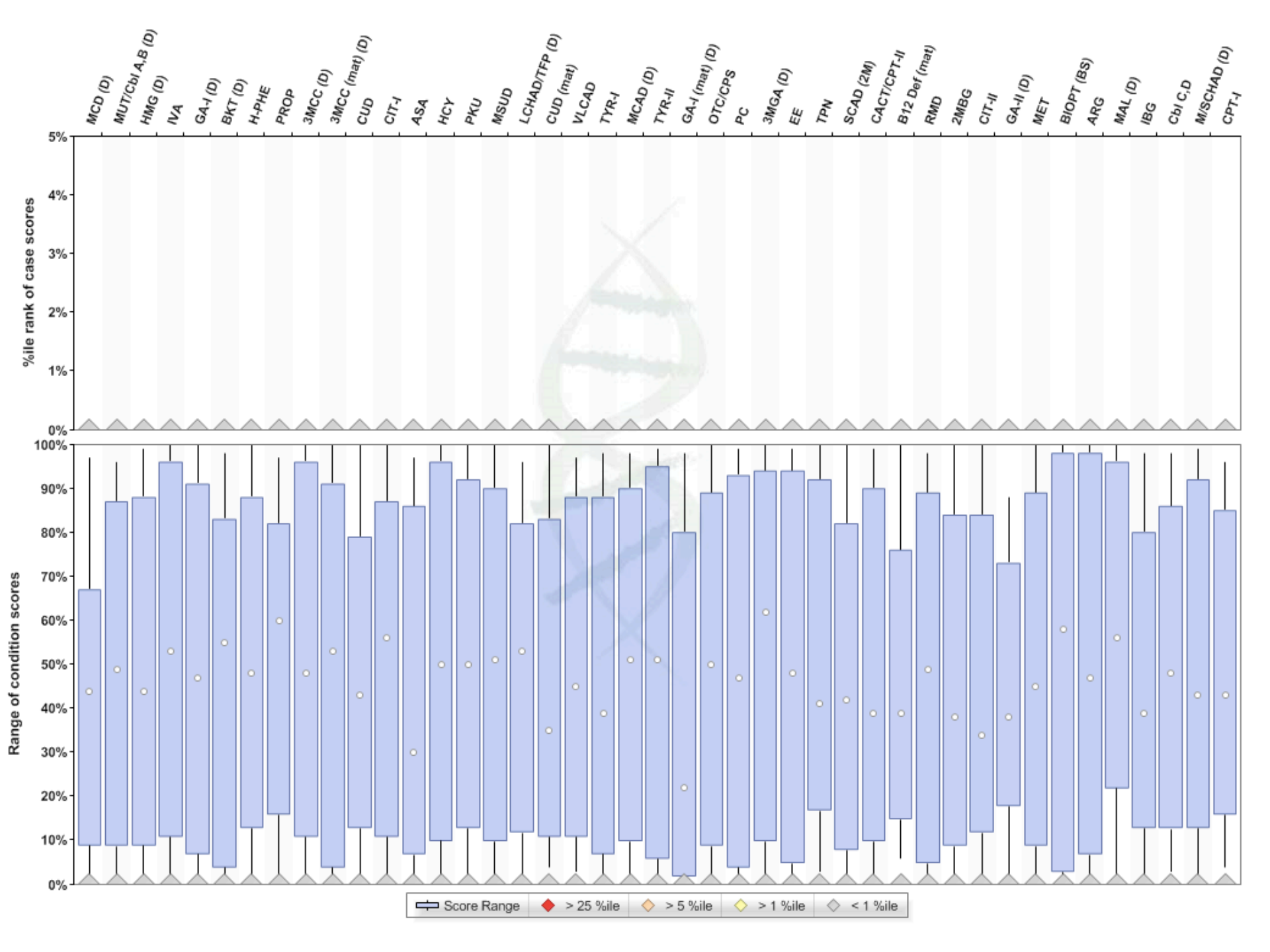
Score is >= 59 and < 111
Condition is likely VLCAD.

Score is >= 38 and < 59
Condition is possibly VLCAD.

Score is < 38
Profile is not informative for VLCAD.

Score Comparison Plot




MCD (D)
MUT/Cbl A,B (D)
HMG (D)
IVA
GA-I (D)
BKT (D)
H-PHE
PROP
3MCC (D)
3MCC (mat) (D)
CUD
CIT-I
ASA
HCY
PKU
MSUD
LCHAD/TFP (D)
CUD (mat)
VLCAD
TYR-I
MCAD (D)
TYR-II
GA-I (mat) (D)
OTC/CPS
PC
3MGA (D)
EE
TPN
SCAD (2M)
CACT/CPT-II
B12 Def (mat)
RMD
2MBG
CIT-II
GA-II (D)
MET
BIOPT (BS)
ARG
MAL (D)
IBG
Cbl C,D
M/SCHAD (D)
CPT-I
%ile rank of case scores
5%
4%
3%
2%
1%
0%
Range of condition scores
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Score Range
> 25 %ile
> 5 %ile
> 1 %ile
< 1 %ile

Number of cases in the time period

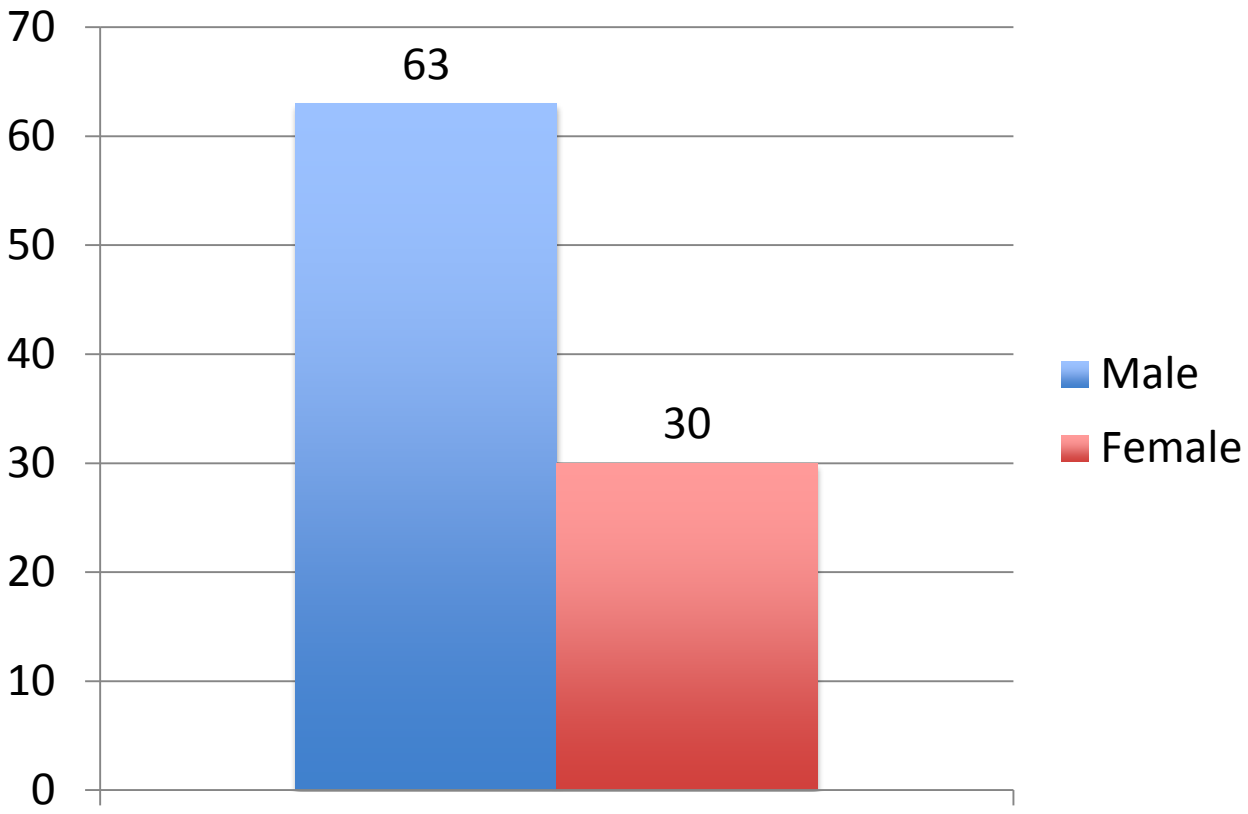
2008	27,016	(May thru Dec)
2009	39,482	
2010	38,381	
2011	37,772	
2012	38,197	
2013	38,521	

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

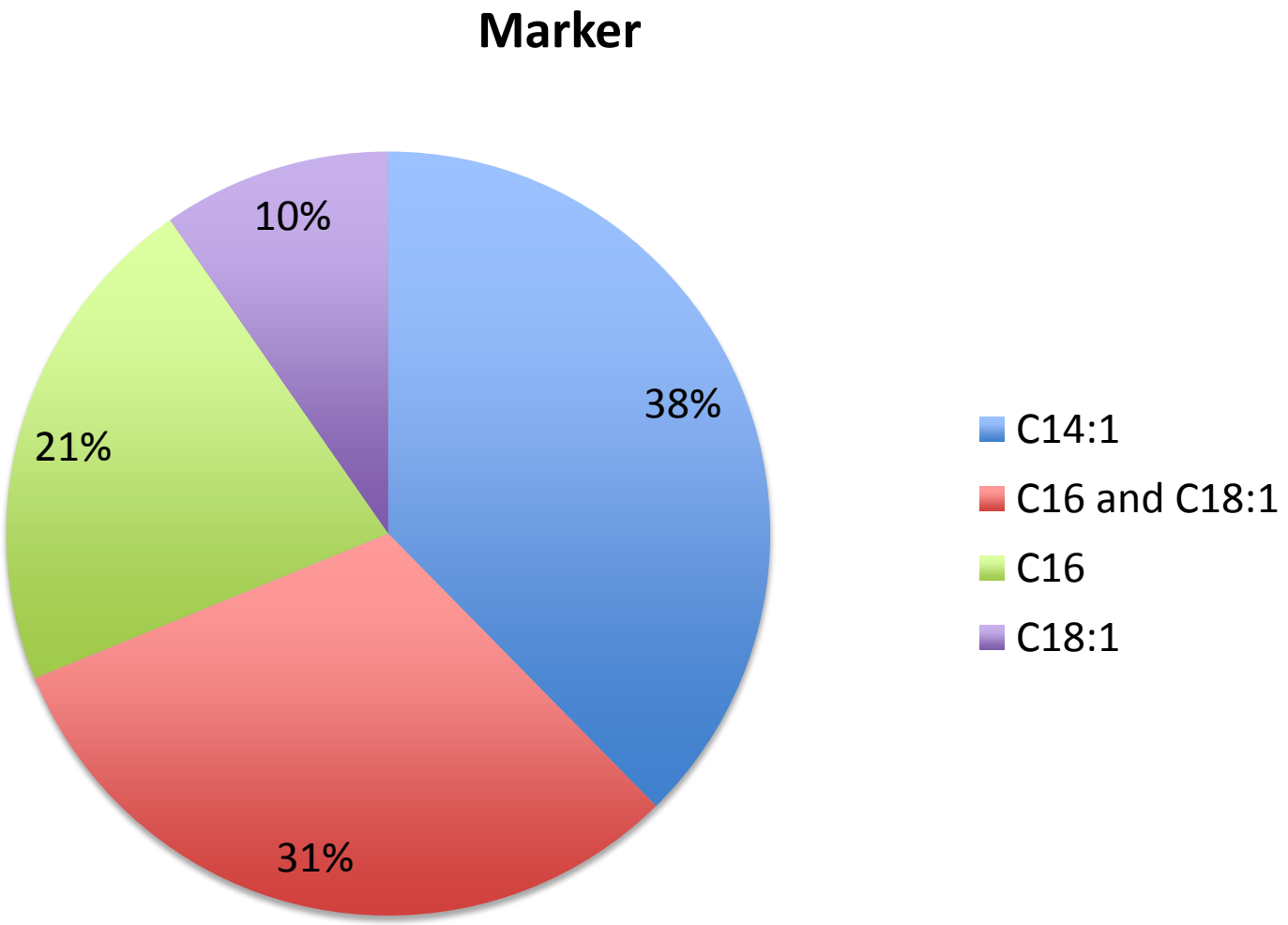


Total referrals identified: 93

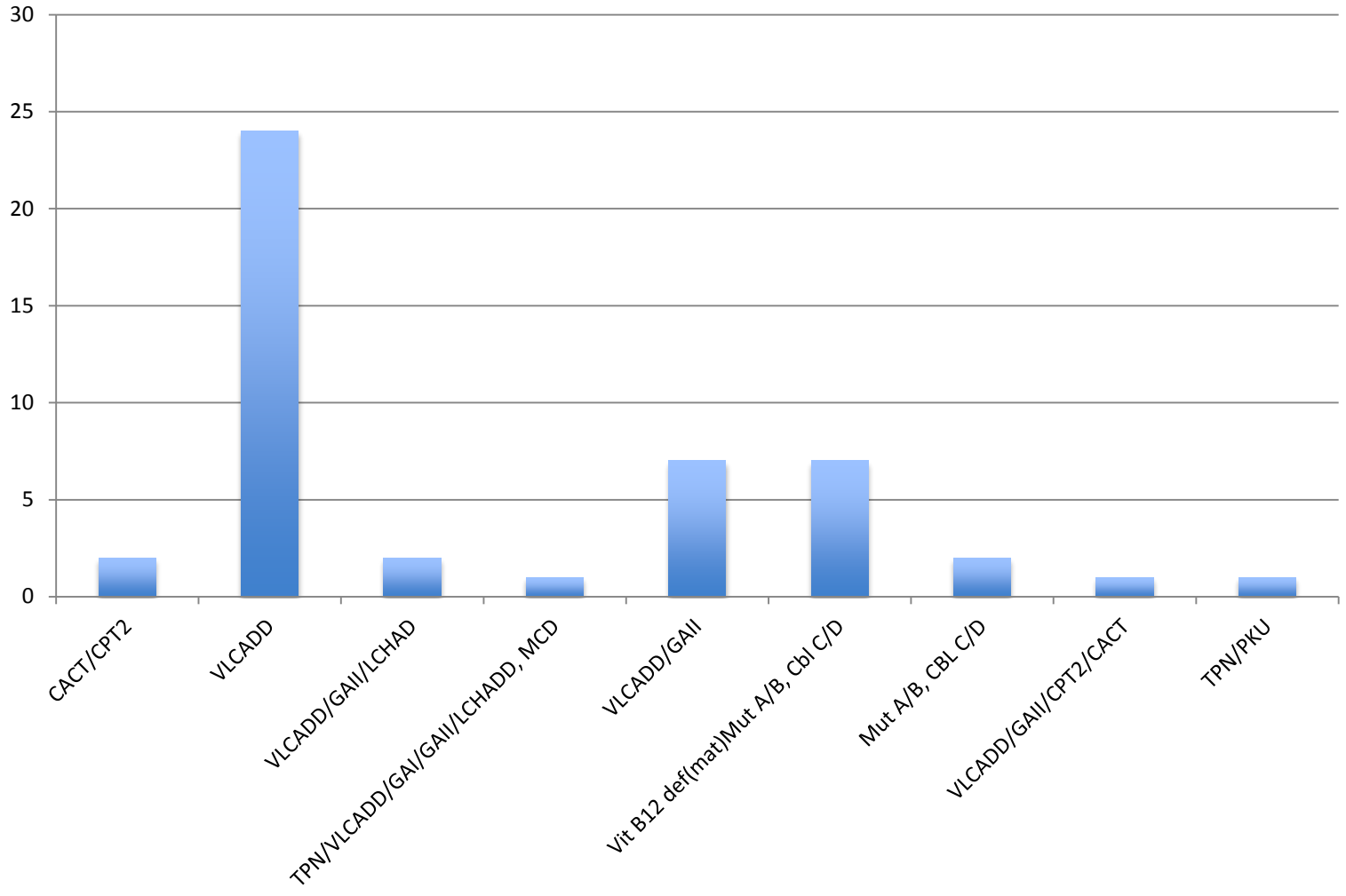
Population Information

- 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



Region 4s Display



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

	D+	D-	Total
R4 +	3	46	49
R4 -	3	41	44
Total	6	87	93

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