Precision Newborn Screening driven by Adjustment of Results for Multiple Covariates

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October 15th, 2018

Outline

• Introduction to CLIR
• Result adjustments for continuous and categorical covariates, & location
• The RUST (the RUSP post-analytical tool)
• Precision newborn screening workflow
• Examples of performance (LSD, CH, CF)

Outline

• Introduction to CLIR

Collaborative Laboratory Integrated Reports (CLIR)

• CLIR is a multivariate pattern recognition software and interactive web tool that was initially developed to support Region 4 Stork (R4S), a federally-funded (2004-2012) collaborative project for laboratory quality improvement of newborn screening by tandem mass spectrometry

World

FINAL Status of R4S (August 31st, 2018)

Countries: 69
Locations (programs): 269
Registered active users: 1,206
Positive cases (94 conditions): 20,938
Final count of website user login: 160,759
Final count of website page views: 1,607,321
Calculated post-analytical tool scores: 411,746,680

Europe

FINAL Status of R4S (August 31st, 2018)

Countries: 69
Locations (programs): 269
Registered active users: 1,206
Positive cases (94 conditions): 20,938
Final count of website user login: 160,759
Final count of website page views: 1,607,321

R4S was sunset on Sep. 25th, 2018

Since 2012, CLIR is supported by institutional funding and has been approved as an official product of Mayo Clinic

https://clir.mayo.edu
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Since 2012, CLIR is supported by institutional funding and has been approved as an official product of Mayo Clinic.

(Mayo letter to NICHD, Sept. 2014) ..... CLIR will remain freely available to all interested users in perpetuity when applications are related to newborn screening, clinical biochemical genetics, and pediatric medicine in general.

Collaborative Laboratory Integrated Reports (CLIR)

CLIR is free.... but contribution of data is required

(Mayo letter to NICHD, Sept. 2014) ..... CLIR will remain freely available to all interested users in perpetuity when applications are related to newborn screening, clinical biochemical genetics, and pediatric medicine in general.

What Does CLIR DO, Exactly?

- Replaces conventional reference ranges
  - With continuous covariate-adjusted %iles
- Replaces analyte cutoff values
  - With condition-specific disease ranges
- Enhances the clinical utility of individual markers
  - With all possible permutation of ratios
- Replaces diagnostic sequential algorithms (“and”)
  - With tool-based parallel algorithms (“or”)

What Does CLIR DO, Exactly?

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  - With continuous covariate-adjusted %iles

Outline

- Introduction to CLIR
- Result adjustments for continuous and categorical covariates, & location

Standard Data Collection

- Date and time (hh:mm) of birth
- Date and time of sample collection

<table>
<thead>
<tr>
<th>Birth Date</th>
<th>Birth Time</th>
<th>Collection Date</th>
<th>Collection Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/13/2018</td>
<td>5:37 AM</td>
<td>10/15/2018</td>
<td>2:45 AM</td>
</tr>
</tbody>
</table>

48h? 72h?
Standard Data Collection

- Date and time (hh:mm) of birth
- Date and time of sample collection

\[
\begin{array}{c|c|c}
\text{Case ID} & \text{Age hr} & \text{Collection Time} \\
18-015-0265 & 339.3833 & 10/27/2018 9:00 AM
\end{array}
\]

= PRECISE Age at collection (hours) 339.3833

Are decimal digits really necessary?

First Submission (Rejected)

Narrow, high density data “stripes” cause significant noise and possibly errors in the calculation of the regression model.

Standard Data Collection

- Date and time (hh:mm) of birth
- Date and time of 2nd sample collection

\[
\begin{array}{c|c|c}
\text{Case ID} & \text{Age hr} & \text{Collection Time} \\
18-015-0265 & 339.3833 & 10/27/2018 9:00 AM
\end{array}
\]

= PRECISE Age at collection (hours) 339.3833

Logical Observation Identifiers Names and Codes

(https://loinc.org)

The universal standard for identifying health measurements, observations, and documents.

Reference labs, healthcare organizations, U.S. federal agencies, insurance companies, software vendors, in vitro diagnostic testing companies, and more than 69,000 registered users from 172 countries use LOINC to move data seamlessly between systems.

It's free, but invaluable.
Sex (Male/Female)

Gestational age (weeks)

Birth weight (grams)

2957 39.1

NBS Demographic Information

Case ID  BW  GA (wks)  Sex
181015-0265  57.1333  2957  Female
181027-0030  339.3833  2957  Female

Second Submission (Accepted)

Reluctantly.......

Improving newborn screening laboratory test ordering and result reporting using health information exchange

Second Submission (Accepted)

Reluctantly......

Covariate Density Plot of Congenital Hypothyroidism

Propionylcarnitine (C3) as Model Marker

- 62 contributing locations (5k to 575k)
- Cumulative data count: 3,209,067

Propionylcarnitine (C3) as Model Marker

- 62 contributing locations (5k to 575k)
- Cumulative data count: 3,209,067
- Outliers (excluded): 16,602
Marker vs. Covariate Plot

Continuous Moving Percentiles?

Continuous Moving Percentiles?

Marker vs. Covariate Plot (LOG)

Continuous Moving Percentiles?

Marker vs. Covariate Plot (LOG)

Continuous (Unadjusted) Moving Reference Interval

Using same cutoff for repeat samples?
Continuous Age- and Sex-Adjusted Reference Intervals of Urinary Markers for Cerebral Creatine Deficiency Syndromes: A Novel Approach to the Definition of Reference Intervals

Does Propionylcarnitine (C3) Need an Adjustment for SEX?
Male median ± ¼ SD vs. Female median ± ¼ SD
If the two bands overlap for the whole range of the covariate (no separation) there is no need to establish separate reference intervals by sex
The Gowans' Plot of 17-Hydroxy Progesterone (17OHP)

N=1,199,877

Age at collection 12-36 hours

Age in hours (log)

More later, now back to C3

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C3 Adjustments (Age)

How do you get there?

(prepared by Stephanie Stoway, CLIR system manager)

C3 Degree of Overlap (DOL) between Reference and Disease-Specific Ranges

Unadjusted

DOL

1%

DOL

4%

DOL

21%

DOL

0%

DOL

1%

DOL

4%

DOL

5%
What Difference Does it Make?

The price of being wrong

Newborn screening saves babies, but lives can be shattered when state labs ignore science and common sense.

Dec. 9, 2016

What Difference Does it Make?

The price of being wrong

Newborn screening saves babies, but lives can be shattered when state labs ignore science and common sense.

Jonathan needed more from a new blood sample. By now, he was two weeks old. The baby seemed healthy, but his mom brought him to the doctor to have more blood collected. A week later his pediatrician received the follow-up report:

Norm

C3 Results of Jonathan Page

C3 Adjustments (Age or BW)
C3 Adjustments (Age or BW)

What does it mean?

How does it look?

Ideal status is achieved when
90\text{th} is = 1.28
50\text{th} is = 0.00
10\text{th} is = -1.28

These parameters mean that
80\% of the data are within
± 1 SD (std. dev.)
Reference Ranges for 17OHP Adjusted for AGE & BW & SEX

Reference Ranges for 17OHP Adjusted for AGE/BW/SEX/LOC

Continuous Moving Reference Range of C3 Adjusted for AGE and BW

Continuous Moving Reference Range of 17OHP Adjusted for AGE and BW and SEX

Continuous Moving Reference Range of 17OHP Adjusted for AGE & BW & Location

Reference Ranges for 17OHP Adjusted for AGE/BW/SEX/LOC
Continuous Moving Reference Range of C3 Adjusted for AGE & BW & Location

Propionylcarnitine (C3) Adjusted for Age / BW / Location

What Difference Does It Make?
What Difference Does It Make?

<table>
<thead>
<tr>
<th>Condition: B12 Def (mat)</th>
</tr>
</thead>
</table>

**Available Covariates:** CL-IMS

<table>
<thead>
<tr>
<th>Available</th>
<th>Brief</th>
<th>Date and Time</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjustment</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Set All Adjustments**

- Set All Adjustments to No Adjustment
- Set All Adjustments to Preferred
- Set All Adjustments to Smallest Overlap

**Filter Covariates**

Include:
- Age
- BW
- GA (wk)
- Sex

Outline

- Introduction to CLIR
- Result adjustments for continuous / categorical covariates, and location

The RUST (the RUSP post-analytical Tool)

Recommended Uniform Screening Panel

- **35** primary conditions
  - 20 IEM detected by MS/MS (AA, FAO, OA)
  - 2 endocrine disorders (CH, CAH)
  - 3 Hemoglobinopathies (S/S, S/βThal, S/C)
  - 2 Lysosomal disorders (Pompe, MPS I)
  - 8 others (BIOT, CF, GALT, HEAR, SCID, CCHD, X-ALD, SMA)

- **27** secondary targets
  - 22 IEM detected by MS/MS (AA, FAO, OA)
  - 1 Hemoglobinopathy (many variants counted as 1)
  - 4 others (GAL-epimerase, GAL-kinase, other T-cell def., DPB)

Primary Markers of 29 Target Conditions

- **Amino acids** (5)
  - Cit, Xle, Met, Phe, Tyr

- **Acylcarnitines** (8)
  - C0, C3, C5, C5OH, C8, C5DC, C14:1, C16OH

- **Lysosomal enzymes** (2)
  - GAA, IDUA

- **Other markers** (7)
  - 17OHP, BIOT, C26, IRT, GALT, TRECS, TSH

Recommended Uniform Screening Panel

- **29** primary conditions
  - 20 IEM detected by MS/MS (AA, FAO, OA)
  - 2 endocrine disorders (CH, CAH)
  - 2 Lysosomal disorders (Pompe, MPS I)
  - 5 others (BIOT, CF, GALT, SCID, X-ALD)

Additional Markers of Target Conditions

- **Amino acids** (1)
  - Val

- **Acylcarnitines** (3)
  - C2, C10, C16

- **Lysosomal enzymes** (0)

- **Other markers** (2)
  - C24, T4
RUSP Primary Target Conditions with Single Informative Marker

- AA disorders (3/6, 50%)
  - CIT-I, HCY, PKU
- FAO disorders (1/5, 20%)
  - CUD
- OA disorders (6/9, 66%)
  - 3MCC, Cbl AB, GA-I, IVA, MUT, PROP
- Lysosomal disorders (2/2, 100%)
  - Pompe, MPS I
- Other conditions (4/5, 80%)
  - BIOT, CAH, CF, GALT

Primary RUSP Markers in Cystic Fibrosis

- 10/20
- 50%
- Others 6/7
- 86%

Primary RUSP Markers in Cystic Fibrosis

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Recommended Uniform Screening TOOL for Cystic Fibrosis (IRT + 26 Ratios)
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USA-CA CF Single Condition Tool (SCT)
What Do You Do with >4,000 FP Cases?

Precision NBSE Workflow

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- The RUST (the RUSP post-analytical tool)
- Precision newborn screening workflow
Location Configuration of the Tool Runner

Post-Analytical Tools

Single Condition Tools
All Conditions Tool
Tool Runner
Dual Scatter Plots
Dual Scatter Plot Runner

Location Configuration of the Tool Runner

Preparation of .csv File

• 175 cases (ISNS TR test file)
Preparation of .csv File
- 175 cases (ISNS TR test file)
  - 4 covariates (700 data points)
  - 81 markers (14,350 data points)
  - 6,480 ratios (1,134,000 data points)

The RUST panel (27/81, 33%)

Tool Runner (SCT)
**Tool Runner (DSP)**

Dual Scatter Plot Runner: VCLD vs VCLD (test)

- Reference Range Values
- User: [User Name]
- Filter Cases
  - Assay: [Assay Name]
  - Include All

**2nd Tier Tests**

Reduction of the false-positive rate in newborn screening by implementation of MS/MS-based second-tier tests: The Mayo Clinic experience (2004-2007)

- A cost effective approach to improve specificity when reference and disease ranges overlap considerably
- Same specimen, no additional patient contact
- Normal results overrule primary screening

- CAH
- MS/MS (unpublished)
- MS/MS
- X-ALD
- LSD

2nd Tier Test for Pompe Disease

Genet Med 2018;8:840-846

2nd Tier Test for Pompe Disease

12-plex Pompe Tool

Simultaneous Testing for 6 Lysosomal Storage Disorders and X-Adrenoleukodystrophy in Dried Blood Spots by Tandem Mass Spectrometry

Sivka Tortorelli, Coleman T. Torgerson, Dimitar K. Gavrilov, Devin Ophulsse, Kimyo M. Raymond, William H. Altshuler, and Dietrich Mataro
Near 0% FPR for Pompe, MPS I, and X-ALD is achievable without additional patient contact and NO molecular testing.

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Example of performance (LSD, CH, CF)

MS/MS Performance (Interpretive Tools w/o cutoff values)

<table>
<thead>
<tr>
<th>System</th>
<th>R4S</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBS test</td>
<td>MS/MS</td>
</tr>
<tr>
<td>Conditions</td>
<td>RUSP</td>
</tr>
<tr>
<td>From (date)</td>
<td>01/01/13</td>
</tr>
<tr>
<td>To (date)</td>
<td>12/31/13</td>
</tr>
<tr>
<td>State</td>
<td>MN</td>
</tr>
<tr>
<td>Newborns tested</td>
<td>71,207</td>
</tr>
<tr>
<td>True positive cases</td>
<td>38</td>
</tr>
<tr>
<td>False positive cases</td>
<td>17</td>
</tr>
<tr>
<td>False positive rate (FPR)</td>
<td>0.024%</td>
</tr>
<tr>
<td>Pos. predictive value (PPV)</td>
<td>67%</td>
</tr>
</tbody>
</table>

Precision Newborn Screening

<table>
<thead>
<tr>
<th>Conditions</th>
<th>From (date)</th>
<th>To (date)</th>
<th>State</th>
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<td>71,207</td>
<td>38</td>
<td>17</td>
<td>0.024%</td>
<td>67%</td>
</tr>
</tbody>
</table>

Precision Newborn Screening

Table 1 Count of cases requiring a repeat analysis and/or second-tier tests

<table>
<thead>
<tr>
<th>Conditions</th>
<th>MPS I</th>
<th>Pompe disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases requiring a repeat analysis and a second-tier test</td>
<td>11</td>
<td>57</td>
</tr>
<tr>
<td>Cases information by repeat analysis and/or second-tier test and reported as screen positive</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Confirmed true positive</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Confirmed false positive</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Genetic false positive</td>
<td>155,131</td>
<td>16,331</td>
</tr>
<tr>
<td>False positive rate</td>
<td>0%</td>
<td>3.02%</td>
</tr>
<tr>
<td>Pos. predictive value</td>
<td>100%</td>
<td>50%</td>
</tr>
</tbody>
</table>
Summary and Conclusions

• It's all about the bacon (t-shirt available)

• Quantity (millions), quality (granularity), and curation (removal of outliers) of submitted data is an absolute necessity

• Precision newborn screening is achievable rapidly by recycling data already available

• Everybody is needed, new bacon lovers are welcome (but you have to contribute data)

• New releases (new tools!) of CLIR are scheduled to the end of 2019 with a special emphasis on computational performance
Why Computational Performance?

• It takes 10-20’ to complete one complex adjustment

• A marker may need 5 or more adjustments to find the lowest degree of overlap to be used clinically

• 1-2 hours per markers, thousands of markers.....

Global Implementation of Precision Newborn Screening

• For every current and future marker compile
  – Up to 10,000,000 ref. profiles with detailed covariates
  – or... >1,000 cases per sex per hour of life (0-1 yr)

• Generate reference moving %iles adjusted for
  – 2 continuous covariates
  – 1 categorical covariate
  – (location)n

• For every target condition compile
  – >1,000 confirmed cases with detailed covariates
  – >10,000 false positive cases (10:1 ratio)

• Screening for 100+ conditions with a cumulative FPR <0.1% (<0.01% per test), sustained by 2nd tier tests
Grateful Acknowledgment of CLIR Team & External Collaborators

CLIR workshop - Rochester (MN), May 7-11, 2018

and Thank YOU for Your Attention

“Today the only thing that is permanent is change”

Charles H. Mayo, MD (1919)