SHOULD WE SCREEN FOR (TREATABLE) LSDs: MPS I, II, IVA, VI, Pompe, Fabry, Gaucher

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EVA PCMA vzw
Cells continually need to digest foreign materials (e.g., bacteria), and damaged or old cellular components.

**Lysosomes** are cell organelles that contain specific enzymes for digestion and degradation of complex waste molecules.

Complex material is broken down by lysosomal enzymes.

Lysosome containing complex material.

Degraded waste material is excreted or can be reused.

Nucleus.

Lysosomal Storage disorders

- Diagnosis on clinical grounds is very difficult:
  - Great clinical variability
  - Genetic heterogeneity
  - Age-dependent clinical symptoms & signs

MPS 1
Age 12 to 34 months
CASE:
Boy, age 5 years
Belgian ancestry
Clinical signs and symptoms:
• Lumbar gibbus
• Motor Dev. retardation
• Obstruction of upper airways
• Sleep apnea
• Growth retardation
• Slight facial dysmorphism
• Hepatosplenomegaly
• Rx hands: dysostosis multiplex

Project: creating awareness to look for clinical signs of MPS in young children
Phase 2: towards neonatal screening

Lymphocyte Arylsulphatase B activity: 0.254/0.54 nmol/mg/min (RV: 2.2-18.6)

GalactoseNac-6-sulfatase: normal

- Urinary GAG: 56 mg/mmol creat
  448 µg/mg creat

1-dimensional electrophoretic separation of GAG species: dermatansulfate, chondroitine sulfate
- Screening method
- Laborious, time-consuming
- Difficult to interpret
2009:
LSD enzymatic analysis in DBS
Technically feasible?
Available Techniques

• Chamoles method (2001):
  - Fluorescence/enzymatic assay
  - Single assay-Single disease model
• Meikle et al (Hopwood)(2004-2006):
  - Multiplexed immune quantification
  - Specific antibodies-two-tier approach
  - Low sensitivity for detection of Pompe & Gaucher
• Gelb/Li et al (2004); Genzyme (Zhang et al)(2008)
  - ESI-MS/MS
  - Analytically multiplex screening
  - MPSI,II,VI, Pompe, Fabry, Gaucher, Niemann-Pick, Krabbe: specific substrates and Internal Standards
  - QC-CDC
• Millington
  - Digital microfluidics platform
  - =multiplex platform of Chamoles method
  - MPSI, VII, Pompe, Fabry
Collaboration:

- **CDC:**
  - QC DBS and calibration reagents
  - Reagents for Gaucher, Fabry, Pompe and MPS-I
- **Dr. Gelb (University of Washington):**
  - Buffer for 4+3 plex and 7 plex assay
  - Reagents for MPS-II (MPS IIIB; MPS VII)
- **Dr. Gelb via BioMarin:**
  - Reagents for MPS-IVA and MPS-VI
- **LC-MS/MS:**
  - LC = Acquity UPLC System (Waters)
  - MS/MS = Xevo TQ (Waters)

• Enzyme assay: blood spot diameter 3 mm
  o 7-plex assay: 3 DBS/3 buffers
    ▪ Fabry, Pompe disease and MPS-I
    ▪ Gaucher's disease (hydrophobic reagents)
    ▪ MPS-II, IVA and VI
  o Assay/incubation duration 18h (overnight)

• UPLC separation:
  o Analyzing all 7 compounds (product and IS) in 1 run
  o Guard column (Xselect CSH; 10 mm x 2.1 mm, 3.5 µm) and analytical column (Xselect CSH; 50 mm x 2.1 mm, 3.5 µm)
  o Linear gradient - constant flow (0.8 ml/min) - total run time 3.2 min/sample

• ESI-MS/MS Selected Reaction Monitoring (SRM):
  o Measured in 3 time blocks
  o Parameters for ion source and mass analyzer optimized

Artificial substrate

MPS-I

MPS-II

MPS-IVA

MPS-VI

Measurement on LS-MS/MS

Artificial MPS-I product

Artificial MPS-II product

Artificial MPS-IVA product

Artificial MPS-VI product
Validation:

- Fabry, Pompe, Gaucher’s disease and MPS-I
- Linear calibration curves
- Column carry-over is almost negligible
- All substrates are well separated from enzymatic product
Validation:

- The method is linear: QC base – low – medium – high

\[
\text{ABG: } y = 0.0258x + 0.7076 \quad R^2 = 0.8755
\]

\[
\text{GAA: } y = 0.0588x + 0.4515 \quad R^2 = 0.9582
\]

\[
\text{GLA: } y = 0.1968x + 2.2783 \quad R^2 = 0.989
\]

\[
\text{IDUA: } y = 0.1374x + 1.9452 \quad R^2 = 0.9991
\]
Validation:

- CV% are < 20 CV%, except for Gaucher (glucocerebrosidase)
- Means and interday CV% in unprocessed cord blood (CDC QC material - high)
- High analytical range (comparison QC high with no blood control (dummy))

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<th>Interday CV% (n=30)</th>
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<td>10.8</td>
<td>28.7</td>
<td>42.7</td>
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<td>1373.1</td>
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<td>Fabry</td>
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<td>8.5</td>
<td>738.4</td>
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<td>MPS-I</td>
<td>33</td>
<td>12.2</td>
<td>37.5</td>
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<td>8.7</td>
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Validation:

- CV% are ≤ 20 CV%, except for GBA (Gaucher)
- Means and interday CV% in unprocessed cord blood (CDC QC material - high)
- High analytical range (comparison QC high with no blood control (dummy))

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<td>MPS-IVA</td>
<td>0.55</td>
<td>14.2</td>
<td>52</td>
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<tr>
<td>MPS-VI</td>
<td>0.99</td>
<td>14.6</td>
<td>262</td>
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Stability tests

- Pre-analytic stability test: Enzyme activity on DBS of healthy adults stored at different temperatures (in days of storage)

- Post-analytic stability test - 10 days after enzyme reaction: maximum 10% difference in product/Internal standard ratio
LSD study

- Important to establish LSD reference values for each center (Müller et al. Diagnostic Pathology 2010)
- Prospective study around 20,000 samples are screened for Fabry, Pompe, Gaucher’s disease and MPS-I

<table>
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<tr>
<th>Disease</th>
<th>Number of samples = n</th>
<th>Mean (Ae in µmol/lh)</th>
<th>Low cut-off (Ae in µmol/lh)</th>
<th>% recall</th>
</tr>
</thead>
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<tr>
<td>Gaucher</td>
<td>10716</td>
<td>14.5</td>
<td>4.0</td>
<td>0.103</td>
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<tr>
<td>Pompe</td>
<td>10892</td>
<td>4.9</td>
<td>1.41</td>
<td>0.101</td>
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<tr>
<td>Fabry</td>
<td>10553</td>
<td>6.1</td>
<td>1.63</td>
<td>0.094</td>
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<tr>
<td>MPS-I</td>
<td>10452</td>
<td>27.1</td>
<td>7.4</td>
<td>0.105</td>
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LSD study

- Enzyme activity of ABG, GAA, GLA and IDUA are not normally distributed
LSD study

- Enzyme activity of GAA and GLA is statistically higher in female neonates, compared to male neonates. No statistically different ABG and IDUA activities are observed between the sexes.

- There is a statistically confirmed negative correlation between both GAA and GLA enzyme activity and the neonates weight and gestation age. These correlations are not observed for ABG and IDUA.

- E.g. Weight correlation with GAA en GLA enzyme activity
Method improvements (lower CV%)

- Decreased incubation time: from 16h to 3h
- Increased concentration by lowering analyte volumes
- Omitting guard column
- Increased linear gradient for better peak separation
- Decreased capillary voltage
LSD daily QC low and high

CV% decrease in time after method improvements

3 controls in 1 run ➔ run repeated
Screening method: Take-home messages

• LC-MSMS method provides an effective high-throughput multiplex screening method
  - Quality control of samples
  - Diagnostic yield (e.g. I cell disease)
• The method is robust (except for GBA), fast and cheap as it is performed on the same MS/MS used in the analysis of aminoacids and acylcarnitines
• Enzyme activities are not normally distributed
• Cut-off levels for GAA and GLA are different, depending of:
  - Gender
  - Gestational age and Birth Weight
Infantile-Onset Pompe Disease
## False Positives: e.g. Pompe

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<td>0.82</td>
<td>0.039</td>
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Chien et al. Pediatrics 2009  
# False Positives: e.g. Pompe

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Pompe Screening by MS/MS Discrimination: infantile versus late-onset

Pompe Screening by MS/MS

Discrimination: infantile versus late-onset

Two-tier screening strategy is indicated, second tier: WHAT? DNA?, GAA in leucocytes by MSMS?

LSD incidence:

Current view of LSD incidence underestimated:

- Incidence of Fabry in Italy: 1/3100 births (Spada et al, 2006, Am J Hum Genet)
- Incidence of Pompe in Taiwan: 1/41000 (Chien et al, 2009, Pediatrics)
- Incidence of 1 per 2315 births (3 LSD) (Mechtler et al, 2012, Lancet)
  - Gaucher: 1/17000
  - Pompe: 1/8700
  - Fabry: 1/3900
- Incidence of Fabry, Pompe, and MPS-I is estimated at 1/7500 births (3 LSD) (Scott et al, 2013, J Pediatr)
  - Fabry: 1/7800
  - Pompe: 1/27800
  - MPS-I: 1/35500
Birth prevalence per 100,000

ERT

Newborn screening

90%

1999 2001 2006

Fabry?(Trait)

Fabry disease
Ethics?

It is not about how we will screen, but What and Why we should screen?
Moving forward:
Proposal to our authorities:
start neonatal screening for
MPS I, VI, II, IVA
Evaluation performed in 2014
Towards a stand-still:
Implementation in.....

Thank you!

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