Two years of high-risk population screening for Pompe disease in Europe – An alternative to newborn screening?

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Pompe Disease

- Autosomal, recessive disorder (ca. 1:50 000)
- Deficiency of acid $\alpha$-glucosidase
- Accumulation of glycogen
Pompe Disease

Diagnostic Delays in Infants

- Median Age (months)
- n = 168 Infants
- First Symptoms/Disease Onset: 2.0
- Diagnosis: 4.7

Diagnostic Delays in Young Children, Adolescents, Adults

- Median Age (years)
- n = 255 Children and Adults
- First Symptoms/Disease Onset: 24
- Diagnosis: 33

Winkel et al. J Neurol 2005; 252:875-84
Sample Types

- EDTA-blood / leukocytes
- Dried blood spots

Interference by maltase glucoamylase

- EDTA-blood/ lymphocytes

has to be prepared immediately

- fibroblasts
- muscle biopsy

invasive procedures

Pompe disease remained frequently undiagnosed.
At the Hamburg Metabolic Laboratory (Pompe diagnostics only):
Number of samples ca. 10 years ago: < 10 samples/year
Number of samples 2009: 759 samples/year
Where does the activity come from?

1 blood spot (3 mm) consists of:
ca. 3 μL whole blood

Contains:

<table>
<thead>
<tr>
<th>Activity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>1.5 μL</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>1.5 μL</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>ca. 20,000</td>
</tr>
</tbody>
</table>

Standardized dried blood spot
Development of the DBS-Assay

- **Substrate**
  - 4 - methylumbelliferyl α- D-glucopyranoside (4-MUG)

- **Blood contains four α-glucosidases that recognize 4-MUG**
  - Lysosomal α-glucosidase (GAA) – active pH 3.5 – 6
    - The enzyme deficient in Pompe disease
  - Two neutral α-glucosidases - optimum pH ~7.5
    - Do not have significant activity in acid conditions
    - Do not interfere in the GAA assay, can be used as a control enzyme
  - Maltase-glucosidase (MGA), active pH ~3 – 8
    - Activity overlaps the activity of GAA
    - Interferes in the GAA assay

**Reference enzyme**: acarbose
DBS Assay - Fluorometry

360 µL dem. water for elution

substrate buffer
pH 3.8

substrate buffer
pH 7.0

substrate buffer
pH 3.8 / acarbose

40 µL each well

blank (added later)

Time for assay: 23 h
Manual working time: 1-2 h
Fluorometry - Equipment

e.g. Victor D2 or F (Perkin Elmer)

but evaluation of results requires experience!
DBS Assay – Mass Spectrometry

Incubation (20 h)

Liquid-liquid extraction with solvent

Simple solid phase extraction

Time for the assay: ca. 28 h
Manual working time: ca. 6 h

Li et al, 2004
- Each test must contain a positive / negative control

Acceptance criteria for each test must be established
## Comparison DBS / Lymphocytes (Fluorometry)

<table>
<thead>
<tr>
<th>No</th>
<th>Onset</th>
<th>pH 3.8</th>
<th>Dried Blood Spots pH 3.8 +Acarbose nmol/spot*21 h</th>
<th>pH 7.0</th>
<th>Inhib. [%]</th>
<th>pH Ratio</th>
<th>Lymph. [nmol/mg *min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>infantile</td>
<td>0.81</td>
<td>0.09</td>
<td>2.79</td>
<td>92</td>
<td>2</td>
<td>0.03</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.36</td>
<td>0.09</td>
<td>12.96</td>
<td>86</td>
<td>1</td>
<td>0.02</td>
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<tr>
<td>3</td>
<td>juvenile</td>
<td>0.90</td>
<td>0.09</td>
<td>3.51</td>
<td>88</td>
<td>3</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.54</td>
<td>0.09</td>
<td>6.57</td>
<td>84</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>5</td>
<td>adult</td>
<td>0.90</td>
<td>0.09</td>
<td>2.88</td>
<td>92</td>
<td>2</td>
<td>0.01</td>
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<tr>
<td>6</td>
<td></td>
<td>1.62</td>
<td>0.27</td>
<td>10.40</td>
<td>83</td>
<td>3</td>
<td>0.01</td>
</tr>
<tr>
<td>7</td>
<td>carrier</td>
<td>2.97</td>
<td>0.99</td>
<td>6.03</td>
<td>65</td>
<td>17</td>
<td>0.14</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>1.35</td>
<td>0.63</td>
<td>3.78</td>
<td>49</td>
<td>18</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Comparison Fluorometry/MSMS (DBS)

<table>
<thead>
<tr>
<th>No</th>
<th>pH 3.8</th>
<th>Dried Blood Spots pH 3.8 +Acarbose nmol/spot*21 h</th>
<th>pH 7.0</th>
<th>MSMS + Acarbose [pmol/spot*20 h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.36</td>
<td>0.09</td>
<td>6.89</td>
<td>17.41</td>
</tr>
<tr>
<td>2</td>
<td>1.62</td>
<td>0.27</td>
<td>7.38</td>
<td>31.02</td>
</tr>
<tr>
<td>3</td>
<td>0.81</td>
<td>0.14</td>
<td>4.37</td>
<td>56.68</td>
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<tr>
<td>4</td>
<td>1.08</td>
<td>0.27</td>
<td>6.35</td>
<td>93.30</td>
</tr>
<tr>
<td>5/carrier</td>
<td>1.58</td>
<td>0.54</td>
<td>5.49</td>
<td>140.61</td>
</tr>
<tr>
<td>6/carrier</td>
<td>1.31</td>
<td>0.36</td>
<td>5.67</td>
<td>128.80</td>
</tr>
</tbody>
</table>
Some thoughts to Newborn Screening

- Multiplexing necessary for reasonable incidence
- Ideally only one pre-incubation (optimization necessary)
- Automatization necessary (process has to be adapted)
- Evaluation of false positive in a population (pseudodeficiency?)

Technical
- Consensus in society for neonatal screening
- Adequate information (time? who?)

Parent
- Physicians have to be trained to deal with new patients
- Adequate health care facilities (ERT or SCT/BMT)
- Costs have to be covered

Health Care
Newborn Screening for Pompe Disease

Diagnostic efficacy of the fluorometric determination of enzyme activity for Pompe disease from dried blood specimens compared with lymphocytes—possibility for newborn screening

Zoltan Lukacs ∙ Paulina Nieves Cobos ∙ Eugen Mengel

Early Detection of Pompe Disease by Newborn Screening Is Feasible: Results From the Taiwan Screening Program
Yin-Hsiiu Chien, Shu-Chuan Chiang, Xiaokui Kate Zhang, Joan Keutzer, Ni-Chung Lee, Ai-Chu Huang, Chun-An Chen, Mei-Hwan Wu, Pei-Hsin Huang, Fu-Jen Tsai, Yuan-Tsong Chen and Wuh-Liang Hwu
Pediatrics 2008;122;e39-e45; originally published online Jun 2, 2008; DOI: 10.1542/peds.2007-2222
Newborn Screening for Lysosomal Storage Disorders

KIMITOSHI NAKAMURA,* KIYOKO HATTORI, AND FUMIO ENDO

Newborn Bloodspot Screening for Lysosomal Storage Disorders

Hui Zhou, MD, PhD, Paul Fernhoff, MD, and Robert F. Vogt, PhD
High-Risk Population Screening for Pompe Disease

- Cardiomyopathy (infantile patients)
- Neuromuscular Diseases
  - unclear CK-elevations
  - unclear limb girdle dystrophy
CK-Study / Prevalence Study

- Study to assess the prevalence of Pompe disease among
  - patients with unexplained CK-elevations
  - patients with limb girdle muscle dystrophy of unknown origin
  - infantile patients with cardiomyopathy (extended part)

For the European study:

Austria, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, Germany, Israel, Latvia, Lithuania, Portugal, Romania, Russia, Serbia, Slovakia, Spain, Turkey
CK-Study / Prevalence Study - Results

Time: May 2009-May 2011 (open end)

CK Study:  Total number of samples: 1320 samples
Patients found: 21
Most patients from Germany
Mean age at diagnosis: 39 years

Prevalence Study:  Total number of samples: 1578 samples
Patients found: 72
Most patients from Turkey, Israel and Spain
Mean age: 30 years (excluding infantile onset)
Mean age: 18 years (with infantile patients)

Total: 3.2% of samples have been positive
(probably 3.7 million babies have to be screened to find similar number of patients)
<table>
<thead>
<tr>
<th>No</th>
<th>Symptoms</th>
<th>Mutation</th>
<th>Activity (Fl.) [nmol/spot*21 h] &gt; 0.9</th>
<th>Activity (MS) [pmol/spot*20 h] &gt; 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CK 1566 U/L, LGMD, mild unspecific myopathy</td>
<td>c1942A&gt;G</td>
<td>0.54</td>
<td>140.8</td>
</tr>
<tr>
<td>2</td>
<td>CK up to 1500 U/L mild LGMD</td>
<td>c.-43T&gt;G</td>
<td>0.36</td>
<td>128.8</td>
</tr>
<tr>
<td>3</td>
<td>LGMD Type 2I</td>
<td>c.664G&gt;A</td>
<td>0.63</td>
<td>184.5</td>
</tr>
<tr>
<td>4</td>
<td>Mother Pompe/CK</td>
<td>na</td>
<td>0.59</td>
<td>302.9</td>
</tr>
<tr>
<td>5</td>
<td>Mother Pompe/CK</td>
<td>na</td>
<td>0.68</td>
<td>336.3</td>
</tr>
<tr>
<td>6</td>
<td>Severe dyspnoe</td>
<td>Del exons 3, 10 and 14</td>
<td>0.46</td>
<td>172.9</td>
</tr>
<tr>
<td>7</td>
<td>Family affected/CK</td>
<td>na</td>
<td>0.36</td>
<td>286.2</td>
</tr>
<tr>
<td>8</td>
<td>Family affected/CK</td>
<td>na</td>
<td>0.50</td>
<td>320.5</td>
</tr>
</tbody>
</table>
Summary

- High-risk population screening has been shown to be successful for the identification of, esp. adult-onset patients

- It provides an excellent cost-benefit-ratio

- In regions where neonatal screening cannot be introduced for fiscal or ethical/political reasons, high-risk screening is a valid alternative

- It can lay the groundwork for future neonatal screening by answering many scientific questions and educating physicians about these rare diseases
Thank you!

Genzyme
  Joan Keutzer
  Stefaan Sansen
  and many others

Munich
  Prof. Schoser
  Prof. Müller-Felber

Halle
  PD Dr. Deschauer
  Dr. Hanisch

Copenhagen
  Prof. Visser
  Dr. Preisler

Genetics
  Dr. Gläser
  Prof. Santer

.... and all other people who send samples to our laboratory