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

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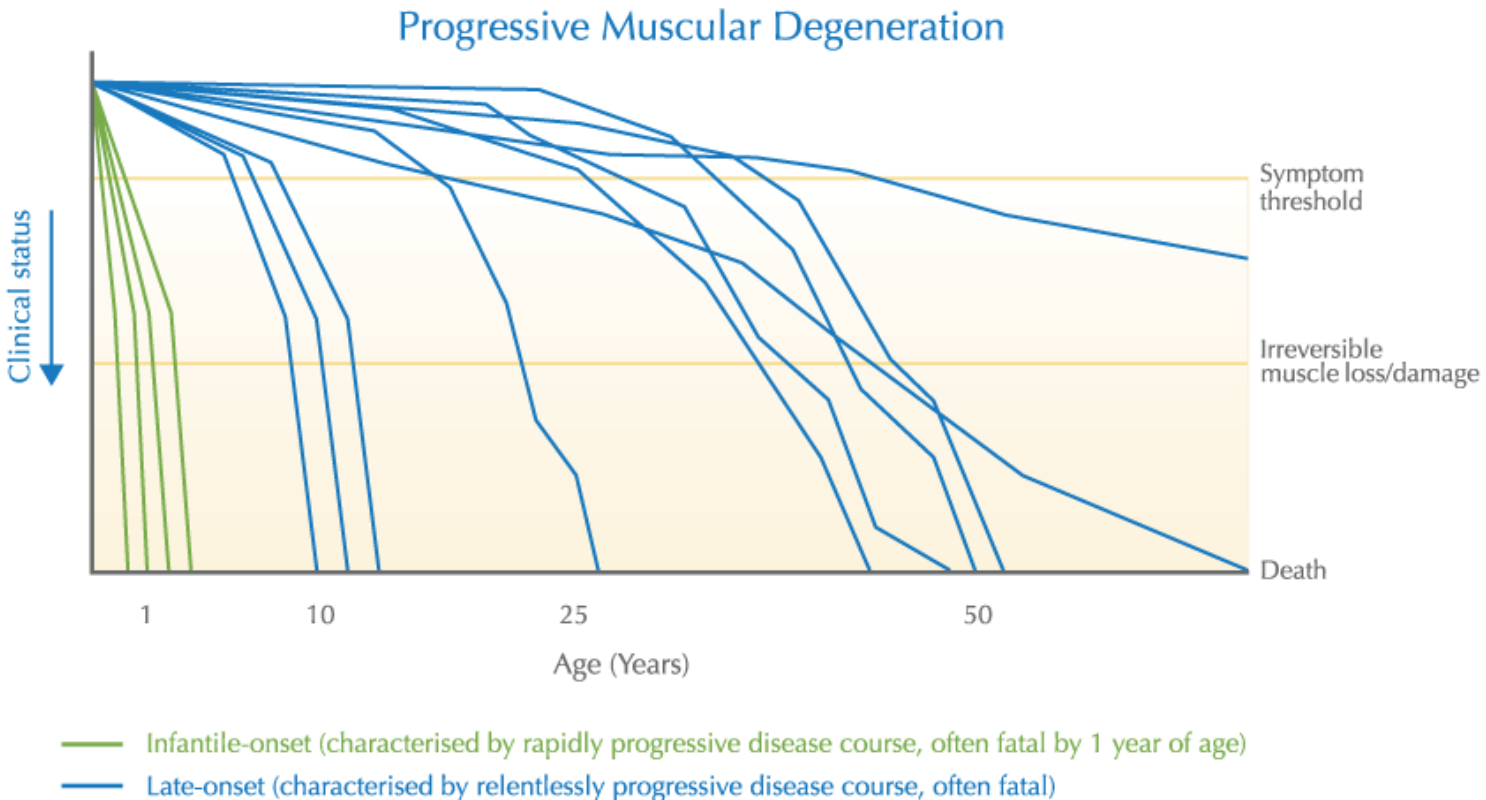
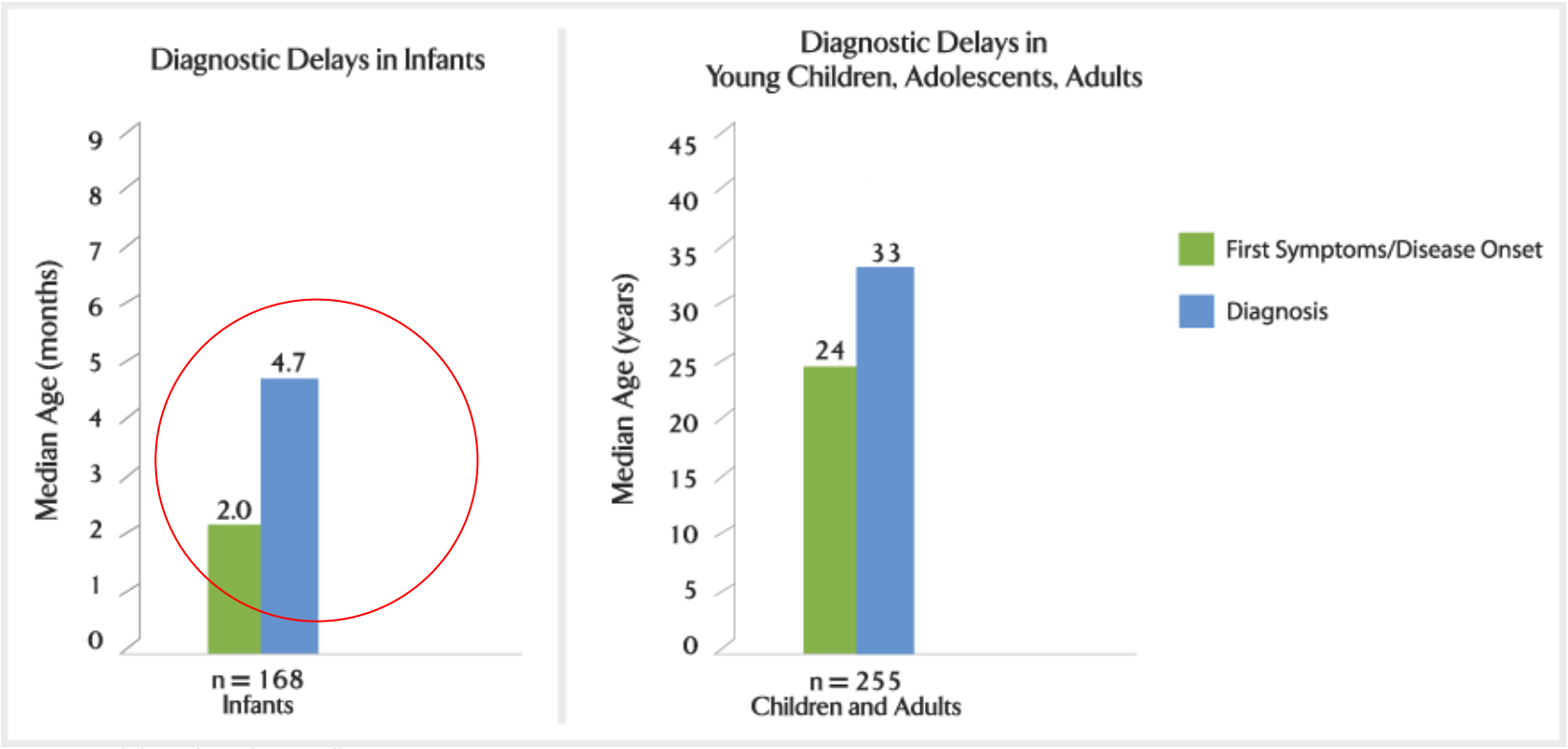
Pompe Disease

- Autosomal, recessive disorder (ca. 1:50 000)
- Deficiency of acid α-glucosidase
- Accumulation of glycogen



Pompe Disease



Kishnani et al. *J Pediatr* 2006; 148:671-676

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

Sample Types

- EDTA-blood / leukocytes
- Dried blood spots

} Interference by maltase glucoamylase

- EDTA-blood/ lymphocytes

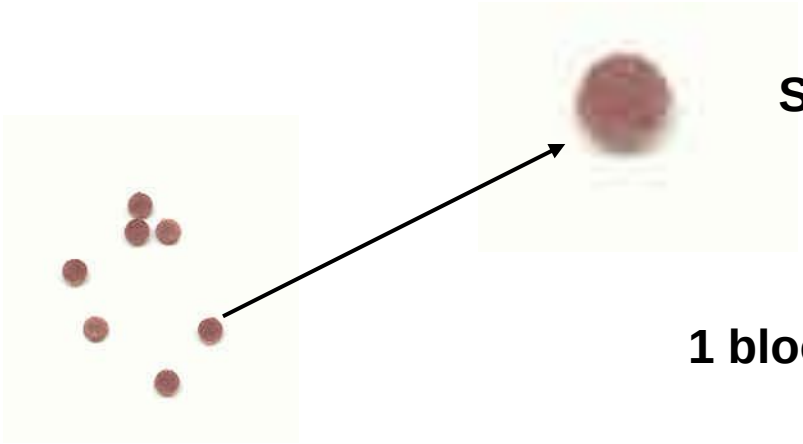
} has to be prepared immediately

- 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 ?

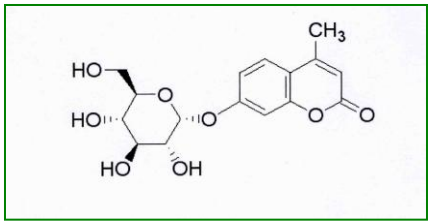


Standardized dried blood spot

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

Contains:		Activity
Plasma	1.5 µL	+
Erythrocytes	1.5 µL	-
Leukocytes	ca. 20,000	+

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-gluco[illegible]e (MGA), active pH ~3 – 8
    - Activity o[illegible] the [illegible]ctivity of GAA
    - Interferes [illegible] assay

reference enzyme

acarbose

DBS Assay - Fluorometry

360 µL dem. water
for elution

40 µL each well

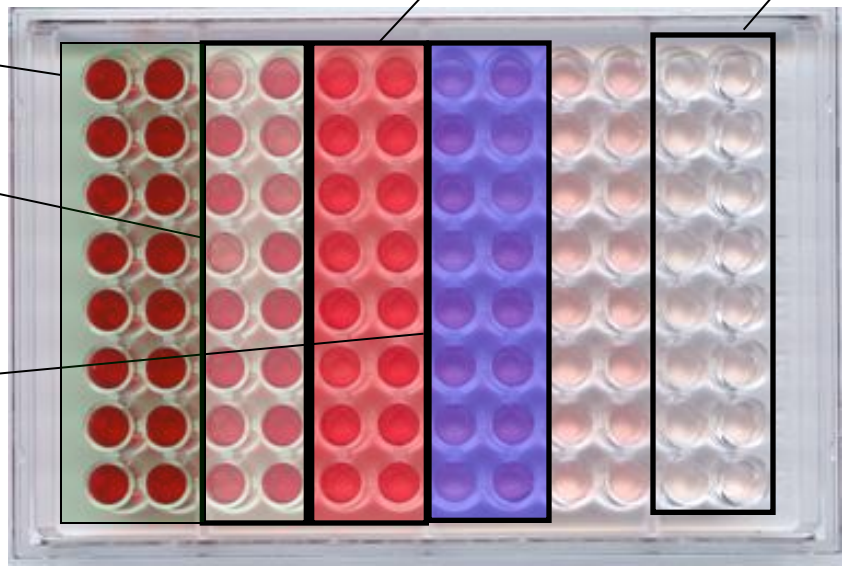
blank (added later)

Std

substrate buffer
pH 3.8

substrate buffer
pH 7.0

substrate buffer
pH 3.8 / acarbose



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

Fluorometry - Equipment

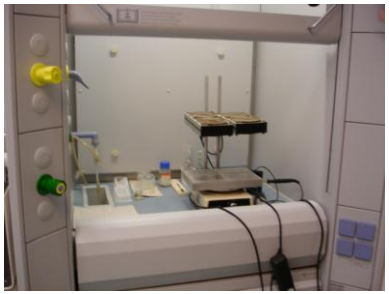
e.g. Victor D2 or F (Perkin Elmer)

but evaluation of results requires experience !

DBS Assay – Mass Spectrometry

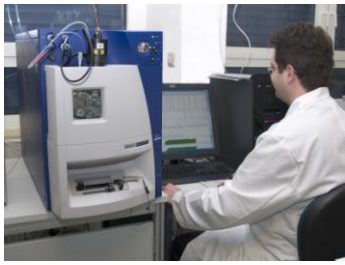
Incubation (20 h)

Liquid-liquid
extraction with solvent



Simple solid phase extraction

Evaporation



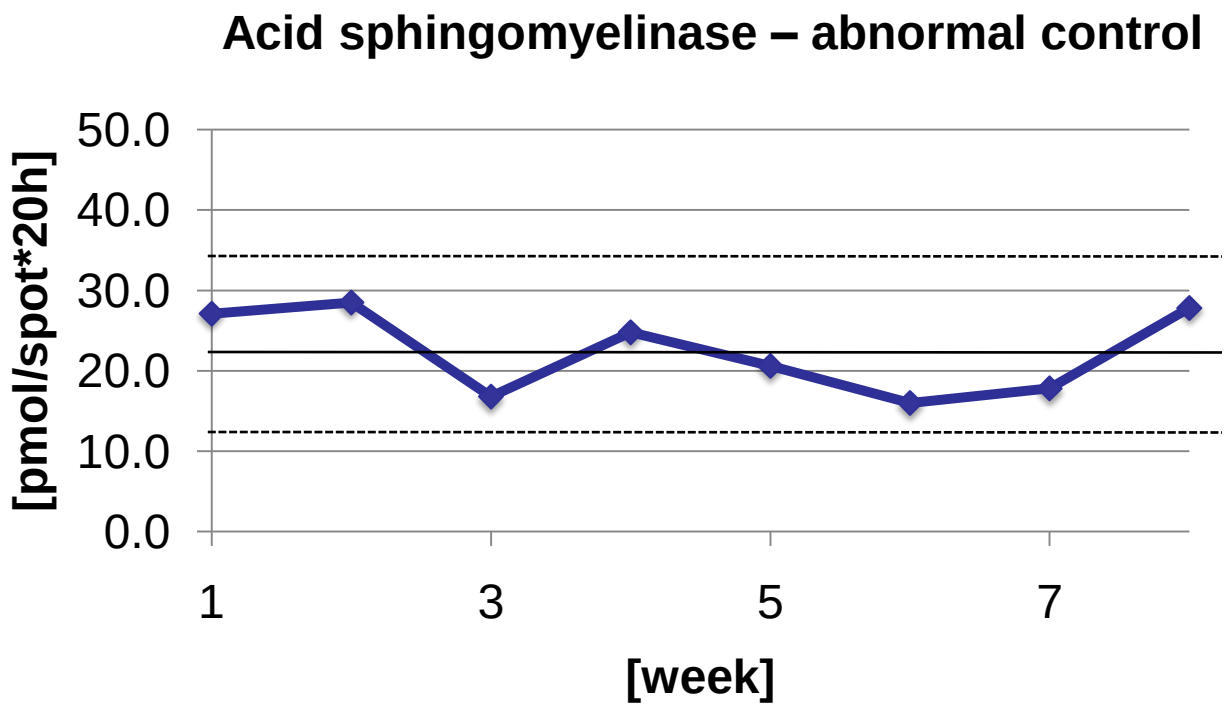
Measurement

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

Li et al, 2004

Quality Control / Quality Assurance

- Each test must contain a positive / negative control



Acceptance criteria for each test must be established

Comparison DBS / Lymphocytes (Fluorometry)

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

Comparison Fluorometry/MSMS (DBS)

No	Dried Blood Spots nmol/spot*21 h			MSMS + Acarbose [pmol/spot*20 h]
	pH 3.8	pH 3.8 +Acarbose	pH 7.0	
1	0.36	0.09	6.89	17.41
2	1.62	0.27	7.38	31.02
3	0.81	0.14	4.37	56.68
4	1.08	0.27	6.35	93.30
5/carrier	1.58	0.54	5.49	140.61
6/carrier	1.31	0.36	5.67	128.80

Some thoughts to Newborn Screening

Newborn Screening for Pompe Disease

Clinical Chemistry 54:10
1624–1629 (2008)

Newborn Screening for Pompe Disease by
Measuring Acid α-Glucosidase Activity

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-Hsiu 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

Reviews on LSD screening

American Journal of Medical Genetics Part C (Seminars in Medical Genetics) 157:63–71 (2011)

ARTICLE

Newborn Screening for Lysosomal Storage Disorders

KIMITOSHI NAKAMURA,* KIYOKO HATTORI, AND FUMIO ENDO

THE JOURNAL OF PEDIATRICS • www.jpeds.com

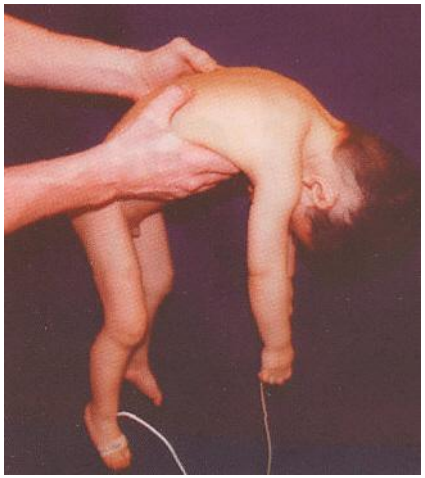
MEDICAL PROGRESS

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)

CK-Study - Heterozygotes

No	Symptoms	Mutation	Activity (Fl.) [nmol/spot*21 h] > 0.9	Activity (MS) [pmol/spot*20 h] > 200
1	CK 1566 U/L, LGMD, mild unspecific myopathy	c1942A>G	0.54	140.8
2	CK up to 1500 U/L mild LGMD	c.-45T>G	0.36	128.8
3	LGMD Type 2I	c.664G>A	0.63	184.5
4	Mother Pompe/CK	na	0.59	302.9
5	Mother Pompe/CK	na	0.68	336.3
6	Severe dyspnoe	Del exons 3, 10 and 14	0.46	172.9
7	Family affected/CK	na	0.36	286.2
8	Family affected/CK	na	0.50	320.5

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

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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