Why? NBS for LSD

Lysosomal Storage Disorders

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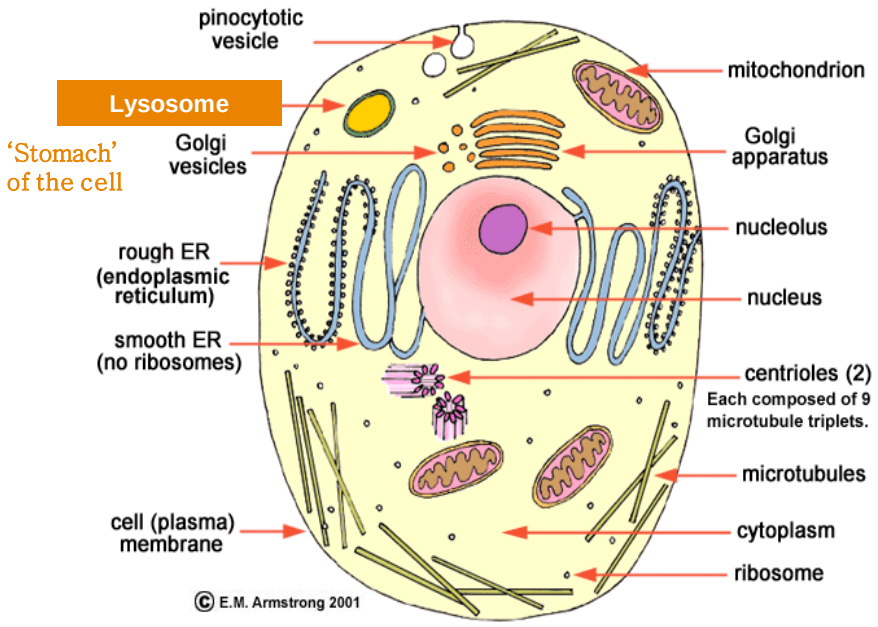
May, 2018

Covers

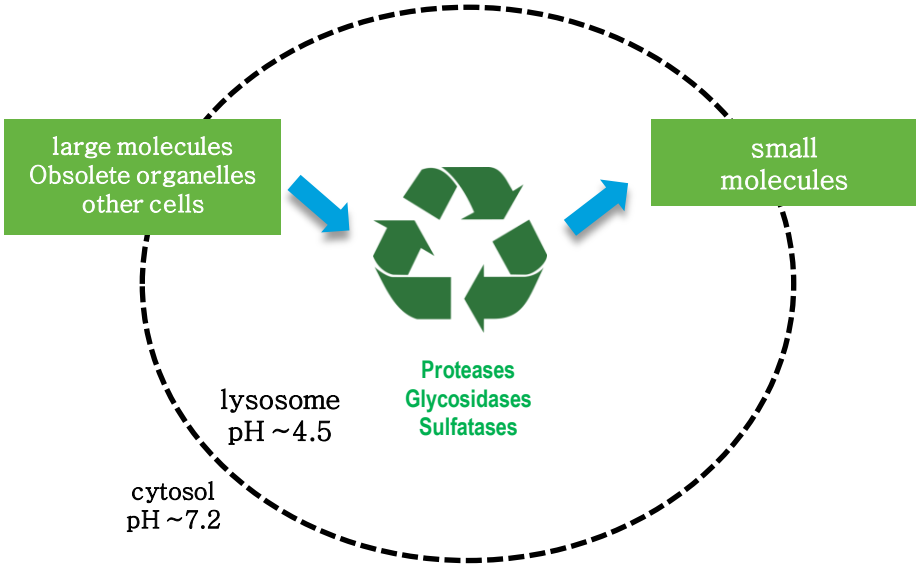
1. Lysosomes are the recycler of the cell
2. What are LSD Lysosomal Storage Disorders?
   - Highlighting Pompe, Fabry, Gaucher
   - Why? NBS for LSD
3. Treatments available
   - Potential LSD Candidates for Newborn Screening
4. Strategies For LSD Screening
5. Experiences and results from labs screening LSD
   - Global LSD Screening Status
6. Summary

What are lysosomes?

Lysosomes are Cellular Organelles



Lysosomes are the 'Recyclers' of the Cell



The end products are either reused by the cell or excreted from the body.

LYSOSOMAL STORAGE DISORDERS (LSD) ARE…

- **Group of ~50 rare disorders, with a combined prevalence of 1 : 5,000 live births**
- **Recessively inherited genetic disorders**
- **Carriers may have low total enzyme activity, unaffected**
- **Most autosomal, some X-linked (i.e., Fabry)**
- **Mutations affect the severity of the LSD**
- **Can come slowly in adulthood or arrive suddenly and fatally in infancy**
- **Phenotype cannot be predicted from genotype**
- **10 LSDs have drug therapies (others treated by bone marrow transplant), but LSDs with neurological dysfunction are poorly served**
- **Symptoms can include seizures and dementia, enlargement of the spleen and liver, and abnormal bone formation**

Mutation 1 ➡ Unstable enzyme ➡ Partial enzyme activity

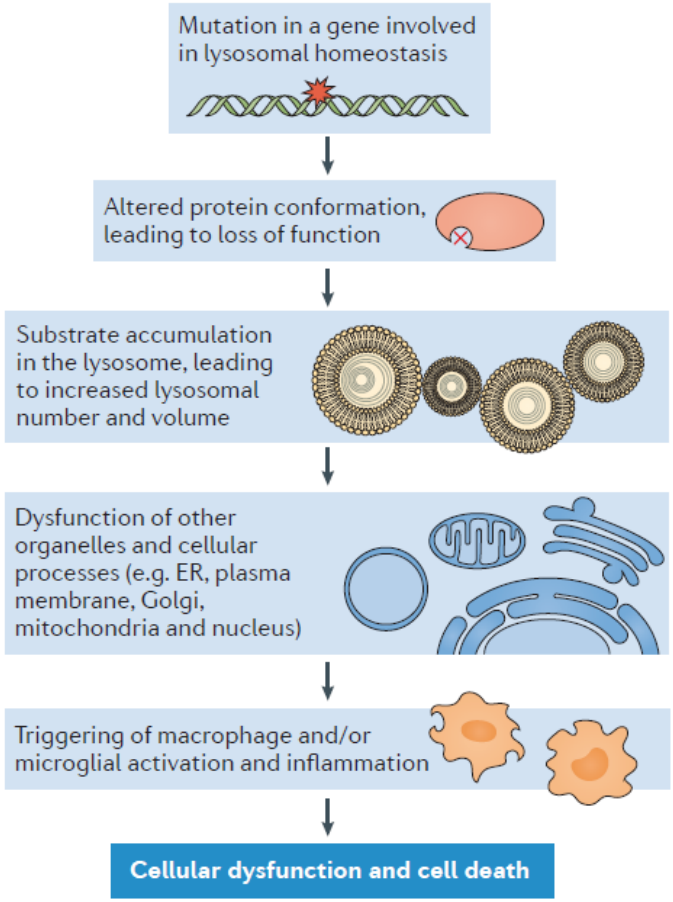
Attenuated (late onset)

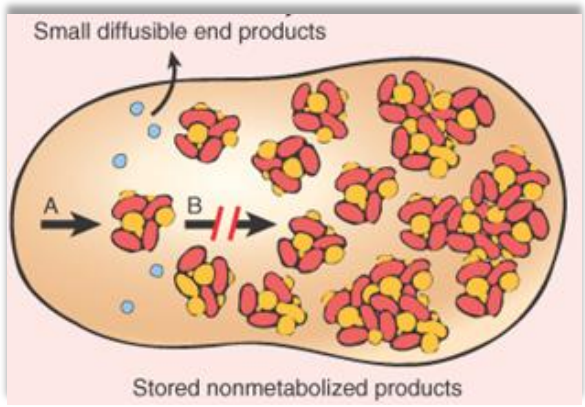
Mutation 2 ➡ Enzyme not transported ➡ No enzyme activity

Severe (immediate)

What are Lysosomal Storage Disorders?

LSD affects Normal Functioning of Cells





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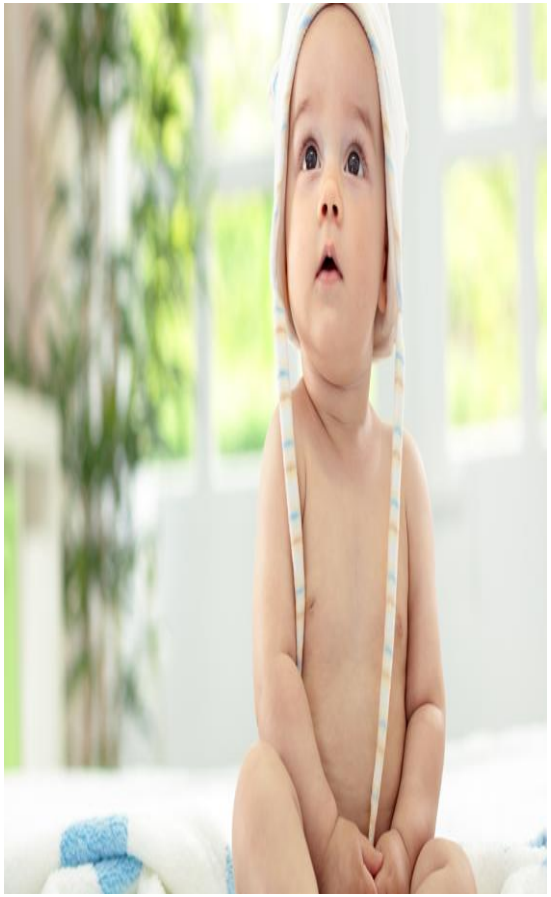
Highlighting LSD Candidates For Newborn Screening

Pompe 1:40,000	• Glycogen storage disease type II, also called Pompe disease, is an autosomal recessive metabolic disorder which damages muscle and nerve cells throughout the body. • Symptoms: Floppy Baby (of muscle weakness, poor muscle tone), Difficulty breathing, Trouble feeding / failure to thrive, Respiratory infections • ERT, 2006
Fabry 1:40,000*	• It is inherited in an X-linked manner. • Mutation in the GLA gene cause deficient a-Galactosidase A enzyme activity which lead to progressive globotriaosylceramide (GL-3) accumulation • Symptoms: Enlarged heart, Heart murmur, Unknown cause of kidney failure, Fabry crises (pain in particularly in hands and feet), Stomach pain, nausea, and vomiting • ERT, 2003
Gaucher 1:57,000	• One of the most common lysosomal storage disorders • Type 1, 2, 3 • Not enough enzyme glucocerebrosidase (GCase), which breaks down a certain lipid, or fat, in the body's cells called glucocerebroside • Symptoms: enlarged spleen and liver, which are often present at birth; liver malfunction; bone deformities, pain or crises; severe neurologic complications • ERT, 1991 and SRT, 2002



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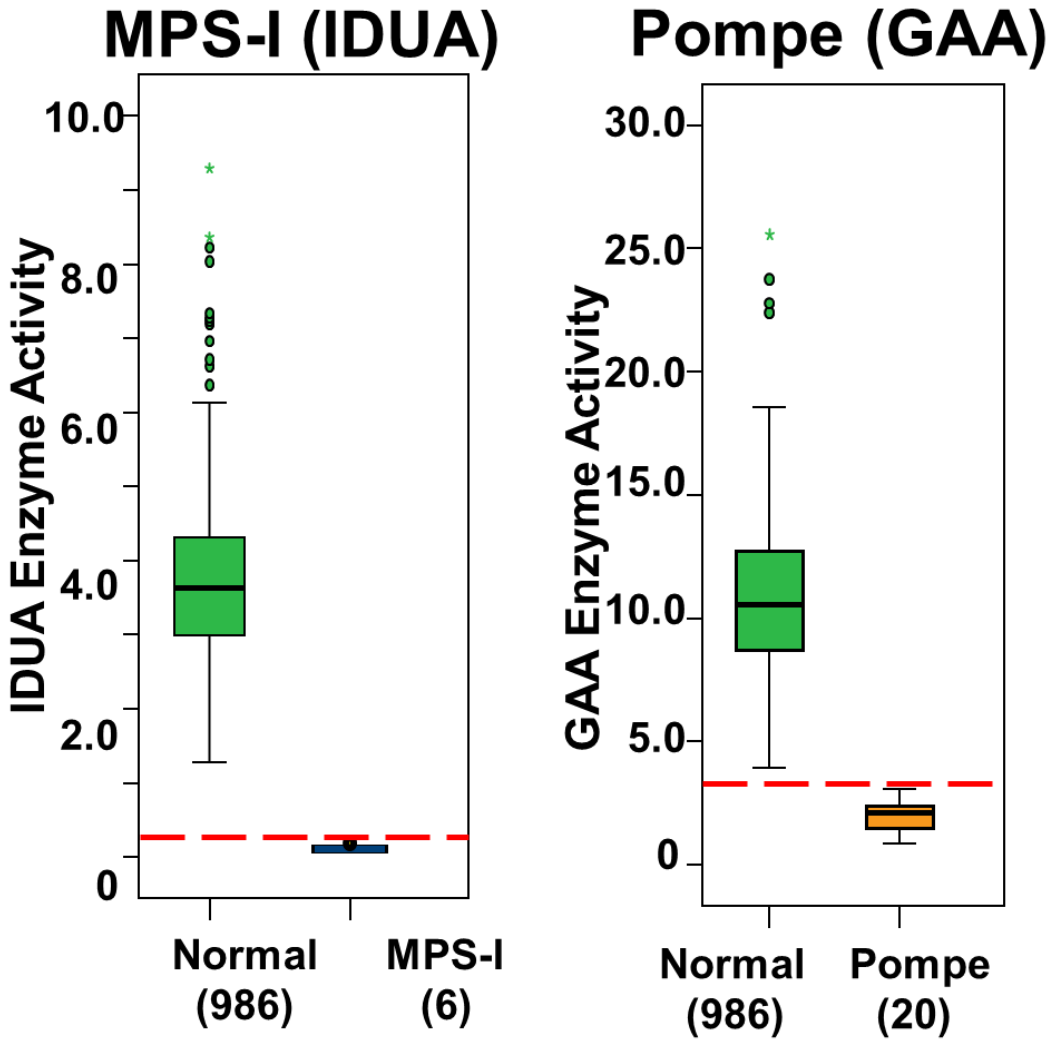
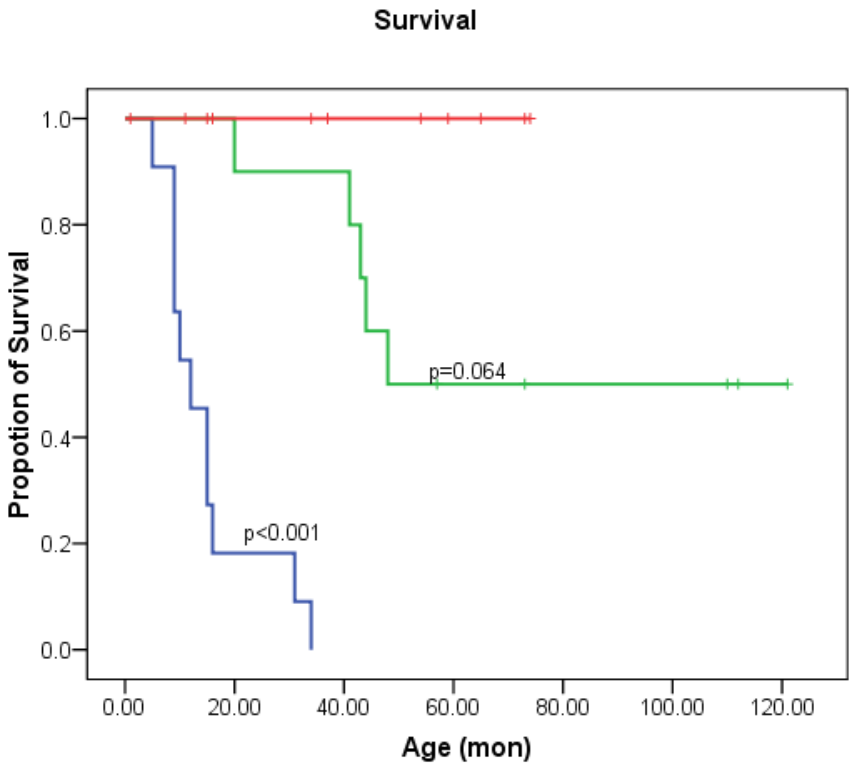
MPS-I & Pompe – Normal vs- Affected



A good separation between normal and affected groups is prerequisite for screening purposes.

Early Detection is Critical - POMPE



NBS
Clinical comparators
Untreated historical group

Screening benefits:

- Identify affected as early as possible
- Provide reproductive choices for preventive actions for additional babies

Chien YH, et al. J Pediatr. 2015; 166:985-991

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LSD Therapy – Better outcome when treated early in life

ERT	• Enzyme Replacement Therapy - Intraveneous delivery of deficient enzymes
SRT	• Substrate Reduction Therapy - oral intake of molecules to reduce excess substrates
HSCT	• Hematopoietic stem cell transplatation
GT	• Gene Therapy

Potential LSD Candidates For Newborn Screening

Disorder	Prevalence	Approved Therapy	Therapy in Clinical Trial
Pompe	1 : 40,000	ERT, 2006	GT, phase I/II
MPS-I	1 : 100,000	ERT, 2003	
Fabry	1 : 40,000*	ERT, 2003	HSCT, phase I/SRT, phase II
Gaucher	1 : 57,000	ERT, 1991/SRT, 2002	GT, phase I
Krabbe	1 : 100,000		*HSCT ?*
Niemann-Pick A/B	1 : 250,000		ERT, phase III
MPS-II	1 : 136,000	ERT, 2006	GT, phase I/II
MPS-IVA	1 : 250,000	ERT, 2014	
MPS-VI	1 : 300,000	ERT, 2005	

*male births

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Strategies For LSD Screening

Direct measurement of lysosomal enzymatic activity

- MSMS or fluometric methods

Direct measurement of lysosomal enzymatic abundance

- Misses cases where enzyme is folded but inactive (e.g. 10-20% of MPS-II cases)

LSD biomarker quantification

- Measurement of substrates that accumulate due to a deficient lysosomal enzyme
- Substrates may not accumulate within 1-3 days after birth
- Promising for 2nd tier tests

Sequencing of lysosomal proteins

- Pathogenic mutation not known
- Too slow and expensive
- Poor knowledge of genotype-phenotype relationship

Direct measurement of lysosomal enzymatic activity using tandem mass spectrometry and fluorometric methods

- Disadvantage of fluorescent is lack of multiplexing

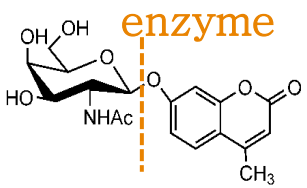
  This will be problematic because the number of LSDs entering in NBS arena is increasing

- Advantage of MS/MS is multiplexing and high dynamic range

  Multiple enzymes can be analyzed in a single DBS punch

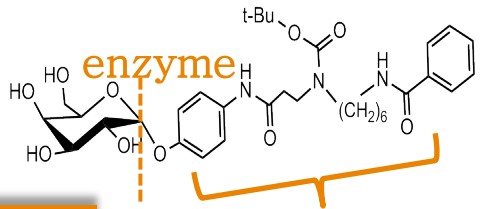
- Not all lysosomal enzymes can be assayed by fluorometric method

  Ex. All fluorometric substrates for Niemann-Pick-A/B lead to False-negative due to a mutation that causes high reading of enzymatic activity for fluorogenic substrates compared to the natural sphingomyelin substrate



fluorescence currently can't multiplex

all substrates produce the same fluorescent enzyme product



Fabry

Structure diversity allows multiplexing

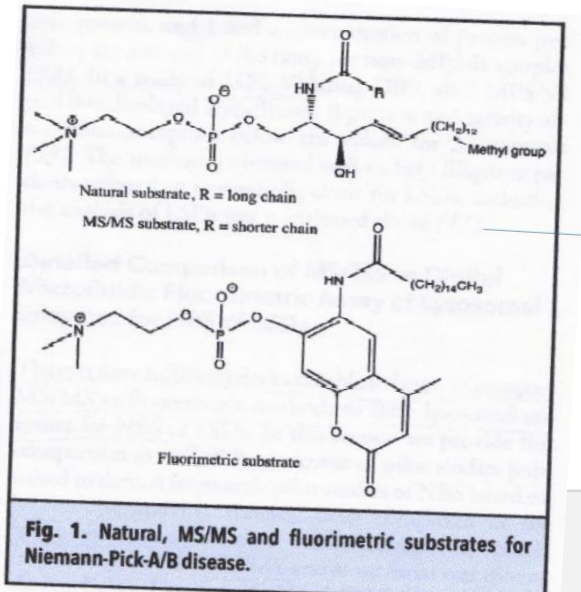


Fig. 1. Natural, MS/MS and fluorimetric substrates for Niemann-Pick-A/B disease.

the MS/MS substrate is nearly identical t natural, difference in the length of the fatty acyl chain.

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A supporting publication

Clin Chem. 2017 Jul;63(7):1271-1277. doi: 10.1373/clinchem.2016.269027. Epub 2017 Apr 27.

Mass Spectrometry but Not Fluorimetry Distinguishes Affected and Pseudodeficiency Patients in Newborn Screening for Pompe Disease.

Liao HC[1,2], Chan MJ[3], Yang CF[4,5], Chiang CC[3], Niu DM[2,4], Huang CK[4], Gelb MH[6].

Author information

Abstract

BACKGROUND: Deficiency of the lysosomal enzyme acid α-glucosidase (GAA) causes Pompe disease. Newborn screening for Pompe disease is ongoing, and improved methods for distinguishing affected patients from those with pseudodeficiency, especially in the Asian population, would substantially reduce the number of patient referrals for clinical follow-up.

METHODS: We measured the enzymatic activity of GAA in dried blood spots on newborn screening cards (DBS) using a tandem mass spectrometry (MS/MS) assay. The assay displayed a relatively large analytical range compared to the fluorimetric assay with 4-methylumbelliferyl-α-glucoside. DBS from newborns confirmed to have infantile-onset Pompe disease (IOPD, n = 11) or late-onset Pompe disease (LOPD) (n = 12) and those from patients bearing pseudodeficiency alleles with or without Pompe mutations, or Pompe disease carriers (n = 230) were studied.

RESULTS: With use of the MS/MS GAA assay in DBS, 96% of the pseudodeficiency newborns and all of the Pompe disease carriers were well separated from the IOPD and LOPD newborns. The fluorimetric assay separated <10% of the pseudodeficiencies from the IOPD/LOPD group.

CONCLUSIONS: The relatively large analytical range MS/MS GAA assay but not the fluorimetric assay in DBS provides a robust approach to reduce the number of referrals and should dramatically facilitate newborn screening of Pompe disease.

Other Publications

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Pilot study of newborn screening for six lysosomal storage diseases using Tandem Mass Spectrometry☆

Susan Elliott [a], Norman Buroker [a], Jason J. Cournoyer [b], Anna M. Potier [b], Joseph D. Trometer [b], Carole Elbin [b], Mack J. Schermer [b], Jaana Kantola [e], Aaron Boyce [a], Frantisek Turecek [c], Michael H. Gelb [c,d,**], C. Ronald Scott [a,*]

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Newborn screening for lysosomal storage disorders by tandem mass spectrometry in North East Italy

Alberto B. Burlina [1] · Giulia Polo [1] · Leonardo Salviati [2,3] · Giovanni Duro [4] · Carmela Zizzo [4] · Andrea Dardis [5] · Bruno Bembi [5] · Chiara Cazzorla [1] · Laura Rubert [1] · Roberta Zordan [2,3] · Robert J. Desnick [6] · Alessandro P. Burlina [7]

Experiences and results from labs screening LSD

Global LSD Newborn Screening Status

S = Screening
P = Pilot
D = Diagnostic

Terriotory	Region
	Georgia, US
	Illinois, US
	Kentucky, US
	Michigan, US
	Missouri, US
	North Carolina, US
	New Jersey, US
	New York, US
	Ohio, US
	Pennsylvania, US
	Tennessee, US
	Washington, US
	Wisconsin, US
NA	Mexico
	Taiwan
	Japan
	Korea
APAC	China
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EMEA	Russia

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Summary

- NBS for LSDs is successful by direct measurement of enzyme activities in DBS.

- Newborn screening for LSDs is taking off in the US and some countries in Europe and Asia. From Asia, Taiwan is leading and screening 4 LSDs when Myozyme is available in 2005 (for Pompe treatment under country's health reimbursement).

- MSMS enzyme assays have a much higher analytical range than fluorimetric assays, leading to a lower number of screen positives, as shown by large-scale pilot studies using equivalent cutoffs.

- PerkinElmer NeoLSD™ is CE-IVD product, available and used by Newborn Screening facilities.

Visit our booth.
See you and Thank you.

Grace Chua

Reproductive Health

Product Manager

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