Newborn Screening for Fabry Disease

Dietrich Matern, MD, PhD
Biochemical Genetics Lab
Mayo Clinic

Disclosure of Interests

Relevant financial relationship(s)

Consultant, Mayo Clinic

Research Support

National Institute of Child Health and Development (NICHD)
Newborn Screening Translational Research Network (NBSTRN)
Legacy of Angels Foundation
Hunter's Hope Foundation
GlaxoSmithKline

Off-label usage(s)

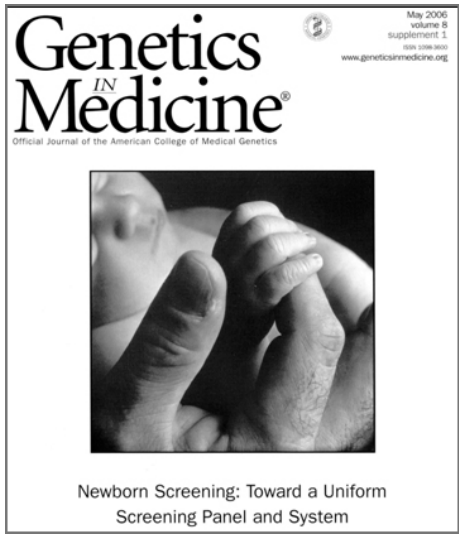
None

Why NBS for LSDs?

- LSDs are historically devastating conditions but now increasingly treatable.
- Prognosis better when treatment started early (NBS!).
- Dried blood spot (DBS) based assays now available.

Dried Blood Spot (DBS) based Assays for LSDs

Method	multiplex	Platform	Complexity
Enzyme Assay (Chamoles et al)	no	Fluorometry	low
Multiplex Enzyme Assay (Gelb/Scott)	yes	MS/MS	high
Multiplex Immune-Quantification Assay (Hopwood et al)	yes	Luminex	low
Digital Microfluidics (Baebies, Inc.)	Yes	*"Fluorometry-on-a-chip"*	low



May 2006 · Vol. 8 · No. 5, Supplement

KDIGO

executive summary

Michael S. Watson, PhD, Marie Y. Mann, MD, MPH, Michele A. Lloyd-Puryear, MD, PhD, Piero Rinaldo, MD, PhD, and R. Rodney Howell, MD, editors

The Maternal and Child Health Bureau commissioned the American College of Medical Genetics to outline a process for the standardization of outcomes and guidelines for state newborn screening programs and to define responsibilities for collecting and evaluating outcome data, including a recommended uniform panel of conditions to include in state newborn screening programs. The expert panel identified 29 conditions for which screening should be mandated. An additional 25 conditions were identified because they are part of the differential diagnosis of a condition in the core panel, they are clinically significant and revealed with screening technology but lack an efficacious treatment, or they represent incidental findings for which there is potential clinical significance. The process of identification is described, and recommendations are provided. ***Genet Med*** **2006:8(5, Supplement): 1S–11S.**

ACMG Panel: final score

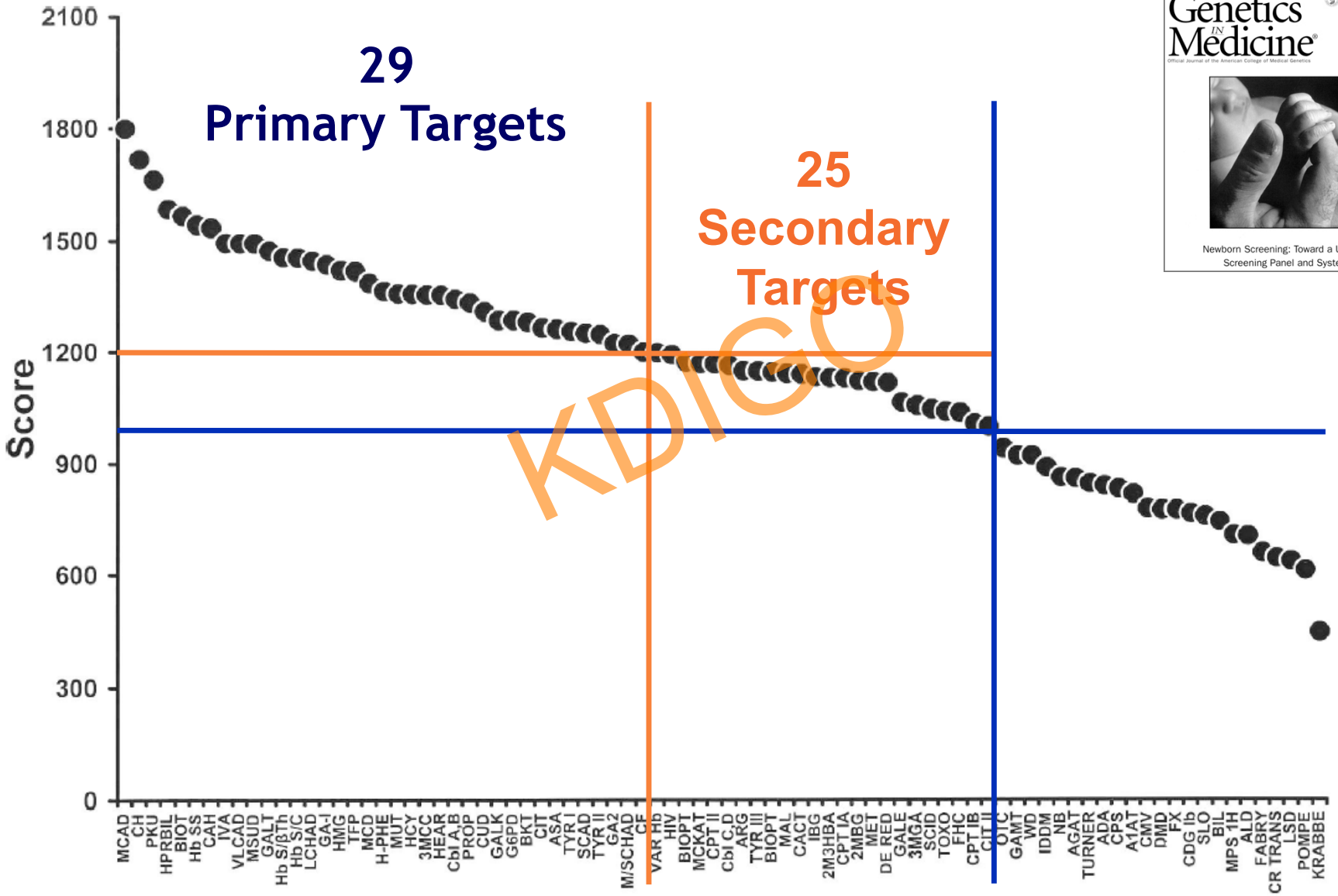


Fig. 1. Scoring by test availability (separates out those conditions that have an acceptable, validated, population-based screening test from those that do not).

ACMG Panel: final score

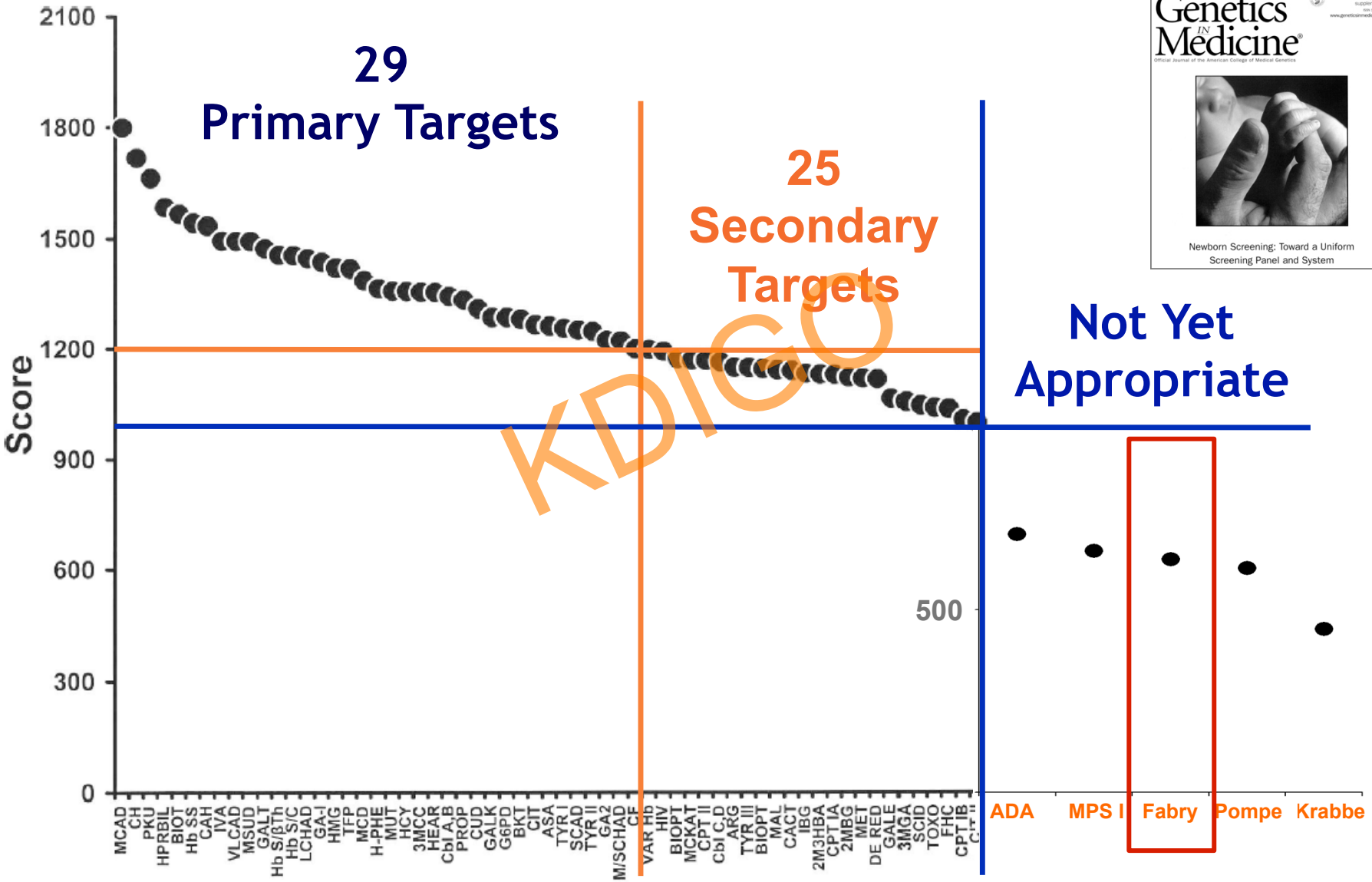
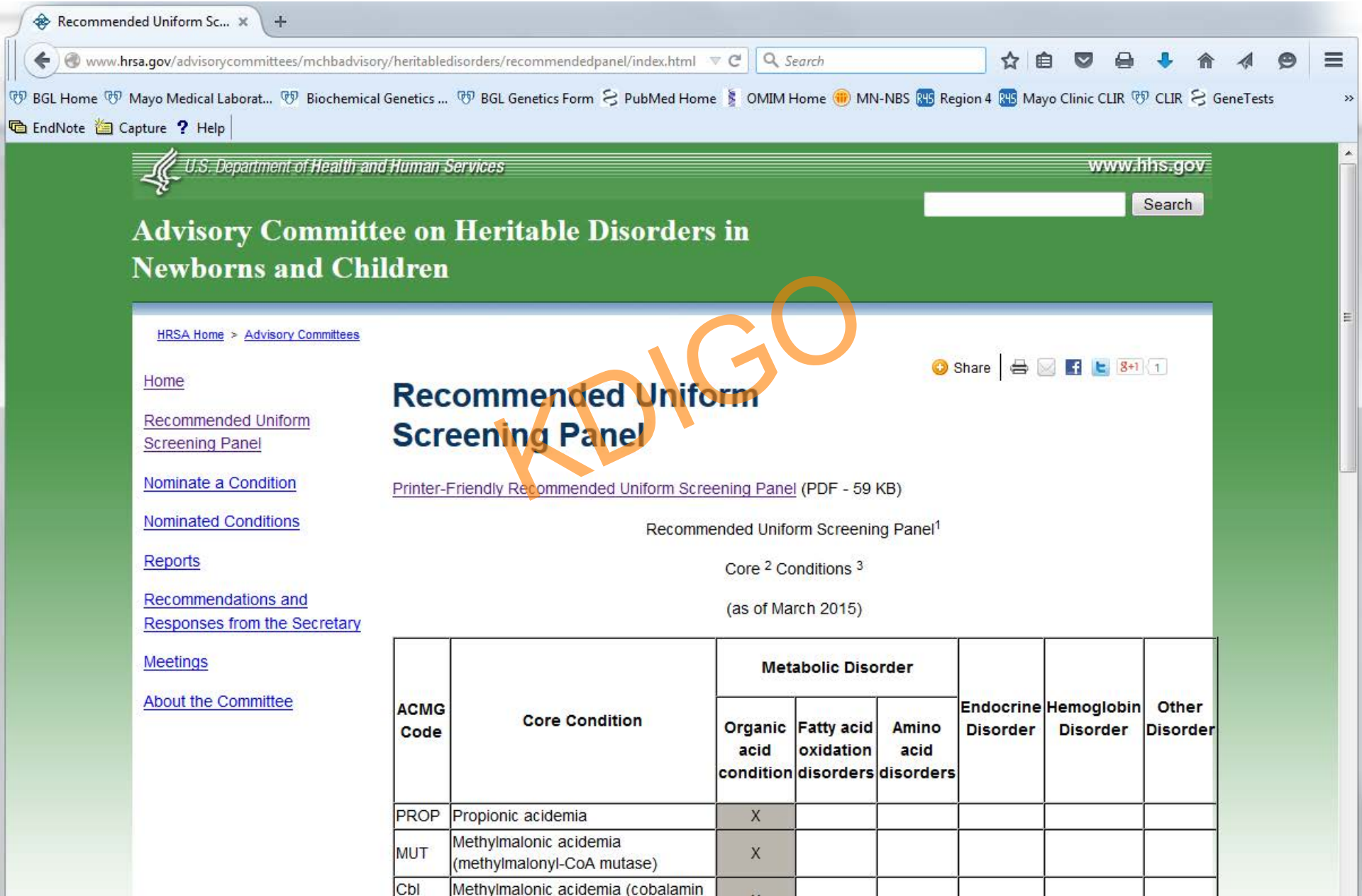


Fig. 1. Scoring by test availability (separates out those conditions that have an acceptable, validated, population-based screening test from those that do not).

HRSA's ACHDNC



Recommended Uniform Sc...

www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendedpanel/index.html

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Recommended Uniform Screening Panel

Printer-Friendly Recommended Uniform Screening Panel (PDF - 59 KB)

Recommended Uniform Screening Panel[1]

Core [2] Conditions [3]

(as of March 2015)

ACMG Code	Core Condition	Metabolic Disorder			Endocrine Disorder	Hemoglobin Disorder	Other Disorder
		Organic acid condition	Fatty acid oxidation disorders	Amino acid disorders			
PROP	Propionic acidemia	X					
MUT	Methylmalonic acidemia (methylmalonyl-CoA mutase)	X					
Cbl	Methylmalonic acidemia (cobalamin						

Why NBS for LSDs

- LSDs are historically devastating conditions but now increasingly treatable
- Prognosis better when treatment started early (NBS!)
- Dried blood spot (DBS) based assays now available
- **Pressure from advocacy groups**

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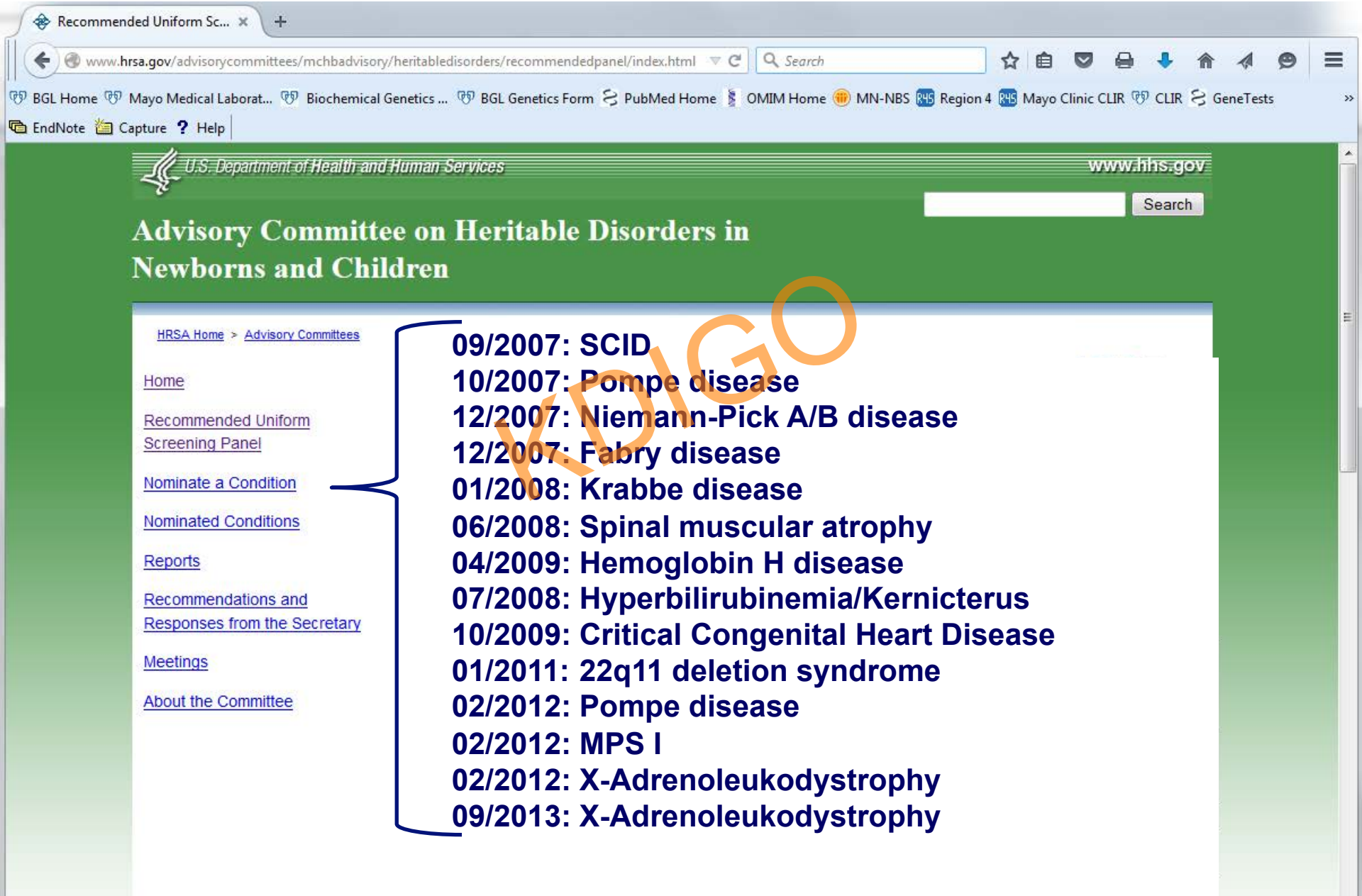
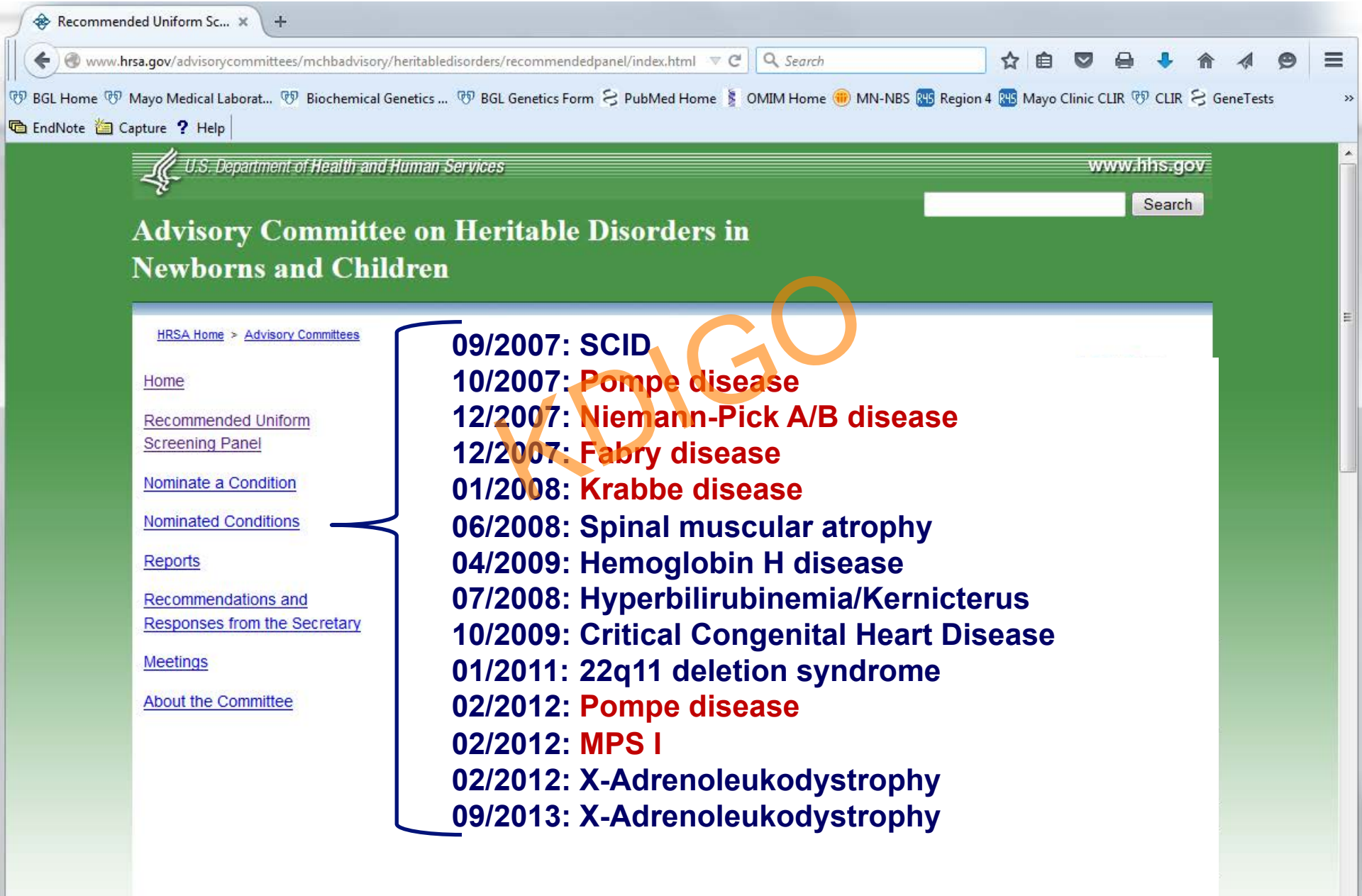
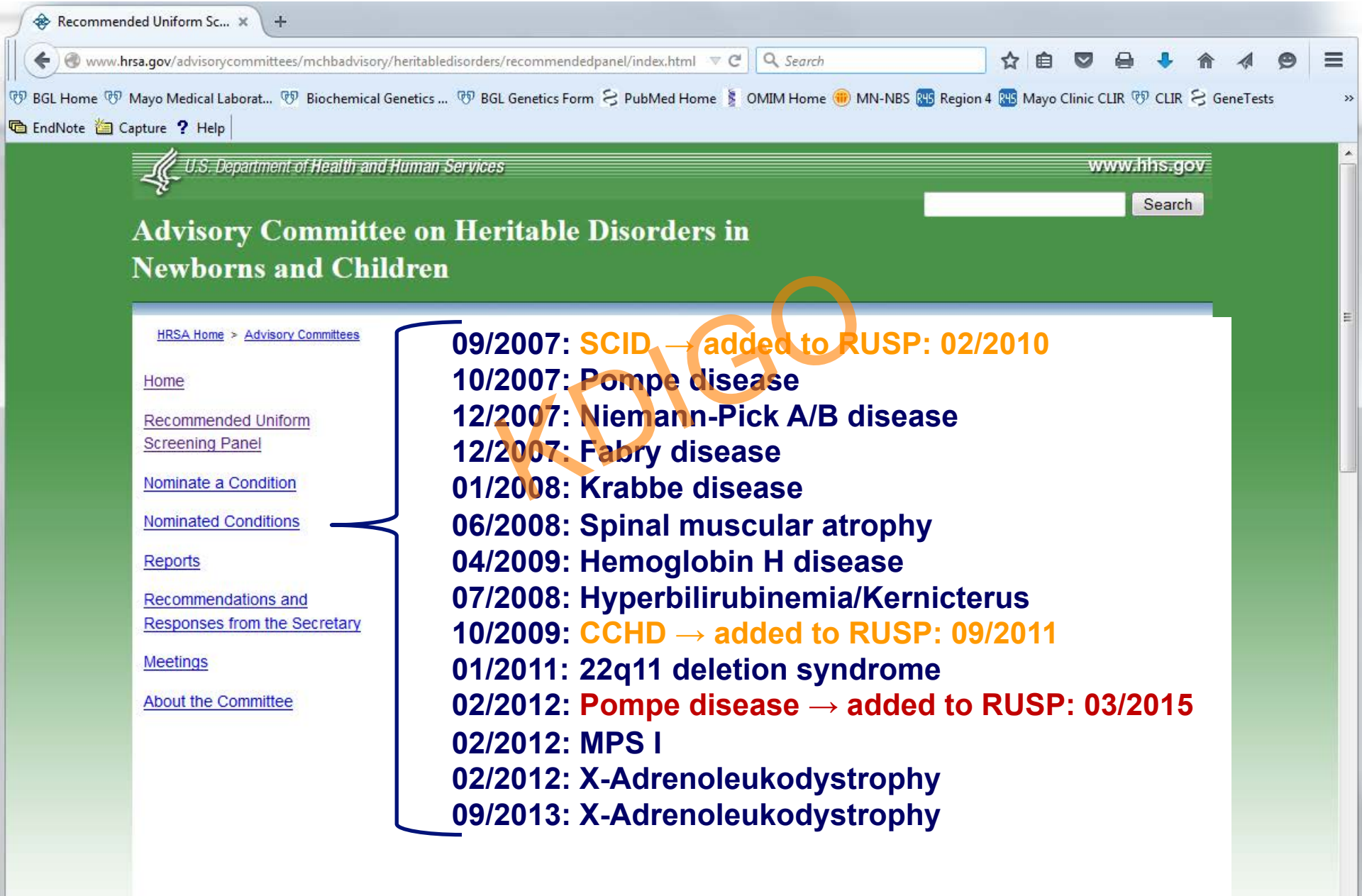
HRSA's ACHDNC



HRSA's ACHDNC



HRSA's ACHDNC



Fabry Disease

Nomination Date	Proponents	ACHDNC vote to send to ERG*	ERG* Final Report	ACHDNC vote to add to RUSP	Secretary approval to add to RUSP
12/2007	Dr. Maryam Banikazemi, NYU	Not approved (8/2008)	-	-	-

*(External) Evidence Review Group

Fabry Disease

- α-galactosidase A (GLA) def.
- X-linked, but most females will become symptomatic
- Accumulation of glycosphingolipids > Disruption of cellular metabolic processes > cell death > inflammation > progressive organ dysfunction
- Not life-threatening in childhood
- Classic vs. later onset cardiac and renal variants

Table 2 Summary of reported clinical manifestations in Fabry patients (newborn–4 years)[2,13,16,27–32,36,40,41,51]

Fabry-related signs and symptoms	Earliest report of symptom
Storage of globotriaosylceramide found in organs on biopsy	Prenatal
Corneal whorls/verticillata	Prenatal/ newborn
Gastrointestinal problems, including nausea, vomiting, diarrhea, constipation, and abdominal pain	1.0 year
Slow growth in boys (mean height/weight <50th percentile)	2.0 years
Intermittent acroparesthesia/neuropathic pain triggered by stress, heat, fatigue, or exercise	2.0 years
Hypohidrosis or anhidrosis	2.5 years
Fabry crises of agonizing neuropathic pain typically begin in the hands and feet and may radiate proximally	2.5 years
Heat, cold, and/or exercise intolerance	3.5 years
Retinal vascular tortuosity	4.0 years
Tinnitus/vertigo	4.0 years
Low glomerular filtration rate	4.0 years
T-wave inversion on electrocardiogram	4.0 years
Trivial cardiac valve disease	4.0 years
Angiokeratoma	4.4 years

Laney DA et al. Fabry disease in infancy and early childhood: a systematic literature review. *Genet Med*. 2015; 17: 323-30

Diagnosis of Fabry Disease

- Clinical and/or (maternal) family history
- Non-specific symptoms >> late or missed diagnoses
- Routine labs: not informative!
- Specialty labs:
  - GLA enzyme assay in DBS or WBC (unreliable for female carriers)
  - Molecular genetic analysis of GLA (esp. females)
  - Urine globotriaosylceramide (Gb3) (informative in newborns?)
  - Plasma lyso-Gb3 (informative in newborns?)
- Newborn screening
  - Currently done in Taiwan, MO and IL by GLA activity assay
  - Several other pilot projects completed/underway (WA, Austria, Hungary, Mayo)
- Treatment:
  - Enzyme replacement therapy (Fabrazyme, Replagal)
  - Chaperone, substrate reduction, and gene therapy trials ongoing

High Incidence of Later-Onset Fabry Disease Revealed by Newborn Screening*

Marco Spada, Severo Pagliardini, Makiko Yasuda, Turgut Tukel, Geetha Thiagarajan, Hitoshi Sakuraba, Alberto Ponzone, and Robert J. Desnick

The classic phenotype of Fabry disease, X-linked α-galactosidase A (α-Gal A) deficiency, has an estimated incidence of

Family studies revealed undiagnosed Fabry disease in affected individuals. In this population, the incidence of α-Gal A deficiency was 1 in ~3,100, with an 11:1 ratio of patients with the later-onset:classic phenotypes.

These results suggest that the later-onset phenotype of Fabry disease is underdiagnosed among males with cardiac, cerebrovascular, and/or renal disease.

Newborn Screening for Fabry Disease

Table 2 – Summary of test methods, results, and performance metrics from screening studies of more than 30,000 newborns for Fabry disease.

Country/state	Taiwan	Taiwan	Taiwan	Austria	Hungary	WA	MO
Condition	Fabry	Fabry	Fabry	Fabry	Fabry	Fabry	Fabry
Method	Fluorometry	Fluorometry	MS/MS	MS/MS	MS/MS	MS/MS	DMF
# of NBS samples	110,027	171,977	191,767	34,736	40,024	108,905	43,701
TP	42 (m); 3(f)	73 (m); 2 (f)	64	6 (m); 3 (f)	– (m); 3 (f)	7 (m)	15 (m)
FP[a]	1052 (m); 512 (f)	565 (m); 323 (f)	315	19	31	6 (m); 3 (f)	13
FPR	1.83% (m); 0.97% (f)	0.63% (m); 0.40% (f)	0.16%	0.05%	0.08%	0.01% (m); 0.006% (f)	0.03%
PPV	3.84% (m); 0.58% (f)	11% (m); 0.62% (f)	17%	32%	9%	54% (m)	54%
Frequency: 1 in	1368 (m)	1237 (m)	2996	3860	13,341	7800 (m)	2913 (m + f)
Follow-up	Clinical	Clinical	Clinical	Mol. genetics[b]	Mol. genetics[b]	Mol. genetics[b]	Clinical
Patients	1 (m) and 42 (m) late	86% (m) late onset	8 (m), 53 late (m), and 3(f)	All late onset; 9 of the FP cases carry SVs	2 Uncertain significance	Mutations suggest late onset	10 (m), 4 late (m), and 1 uncertain (m)
Second tier test	No	Yes (beta-galactosidase/ alpha-galactosidase ratio)	No	No	No	No	No
References	75	9	74	38	39	75	42

DMF, digital microfluidics; f, female; FP, false-positive cases; FPR; false-positive rate; m, male; MS/MS, flow-injection tandem mass spectrometry; PPV, positive predictive value; TP, true positive cases.
[a] False-positive cases (FP) are based on the first DBS sample and include carriers with sequence variants considered non-disease causing by the relevant programs.
[b] Study used de-identified samples.

Matern D et al. Sem Perinatol. 2015; 39: 206-216

Minnesota NBS Performance

209,432 babies screened in 2008, 2009 and 2010

Condition	FPR	PPV	Detection Rate	Unnecessary evaluations/month (100,000 births/yr)
Aminoacidopathies	0.02%	45%	1 : 5,660	2
FAO disorders	0.04%	36%	1 : 5,108	3
Organic acidemias	0.03%	49%	1 : 3,952	2
Biotinidase def.	0.09%	9%	1 : 11,635	7
CAH (with 2nd tier)	0.11%	8%	1 : 11,023	9
Cong. Hypothyroid.	0.21%	27%	1 : 1,232	18
Cystic fibrosis	0.34%	5%	1 : 5,511	28
Galactosemia	0.06%	22%	1 : 6,545	5
Hemoglobinopathies	0.02%	67%	1 : 2,685	2

Fabry Disease

Maryam Banikazemi, M.D.
Neurogenetics
New York University at Rivergate
403 East 34th Street
New York, NY 10016

U.S. Department of Health and Human Services

Advisory Committee on Heritable Disorders in Newborns and Children

8/2008

Dear Dr. Banikazemi:

The Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) received your nomination form and all accompanying materials (nomination package) for Fabry Disease on January 7, 2008. The ACHDNC reviewed the nomination package and reported the results of that review on August 7, 2008. Based on a review of the nomination form and accompanying materials, the decision was made not to send the nomination package forward to the ACHDNC's evidence review group at this time.

Although Fabry Disease is technically detectable in a screening panel for lysosomal storage disorders, the Committee felt that there were several factors that limit its appropriateness for inclusion in the routine newborn screening panel at this time: a) variable and possible late onset (>10 years) of the disease; b) unclear if those at highest risk of serious symptoms can be discerned in newborns; c) the lack of published data of preventive treatment early in life; d) some risk of immunologic response to enzyme replacement therapy; and e) the need for a prospective study of screening and therapeutic intervention to demonstrate the benefit of newborn screening for Fabry Disease. The ACHDNC is available for further discussion of screening newborns for Fabry Disease. The next ACHDNC meeting is October 1-2, 2008. ACHDNC may be contacted at 301-443-1080 if you wish to make public comments at this meeting.

Thank you for your interest and support of newborn screening programs and the ACHDNC's activities.

Sincerely yours,

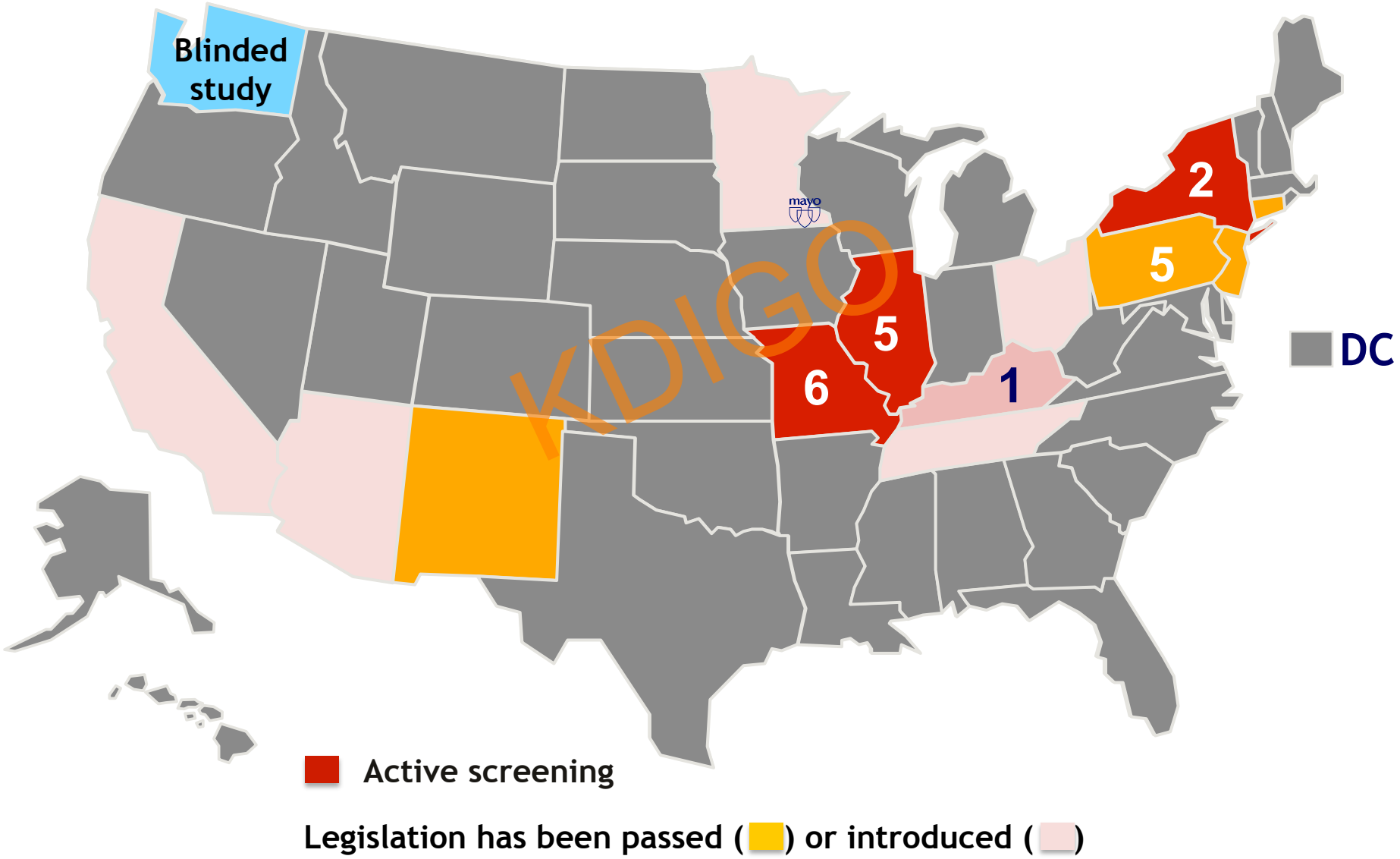
/s/

R. Rodney Howell, M.D.
Chairperson

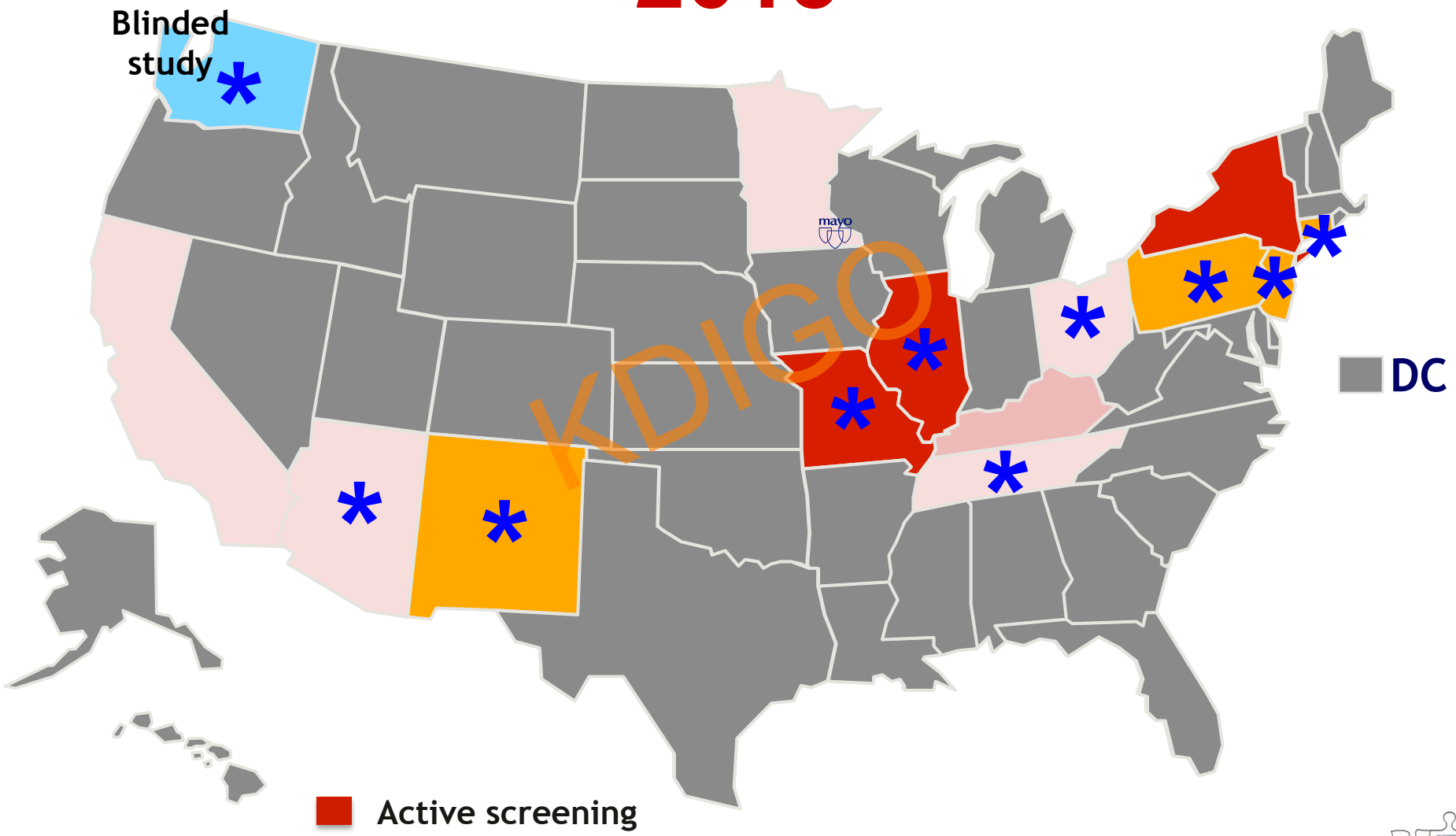
Rejected because of uncertainties about:

- **variable/late onset of disease,**
- **sensitivity of screening test,**
- **effectiveness of and immunologic response to treatment, and**
- **lack of prospective NBS and treatment studies**

NBS for LSDs in the USA 2015



NBS for Fabry Disease in the USA 2015



NBS for Fabry Disease

Some considerations:

- Cost of screening (1st tier, 2nd tier)
- Cost of follow up
- Differentiation between classic disease vs. milder variants vs. pseudodeficiency
- Cost of treatment
- Short/long term efficacy of treatment
- Acceptance by society vs. affected families
- No FDA approved NBS or confirmatory assay (not really a problem but an issue)

Approaches to NBS for LSDs

Method	multiplex	platform	ease of use	performance
Enzyme Assay (Chamoles et al.)	no	Fluorometry	low	poor
Multiplex Enzyme Assay (Gelb/Scott)	yes	FIA-MS/MS → LC-MS/MS → FIA-MS/MS	moderate → high → moderate	(?)
Multiplex Immune-Quantification Assay (Hopwood et al)	yes	Luminex	low	?
Digital Microfluidics (Baebies, Inc.)	yes	*Fluorometry*	low	(?)

Which one should be used?

Mayo's Comparative Effectiveness Study

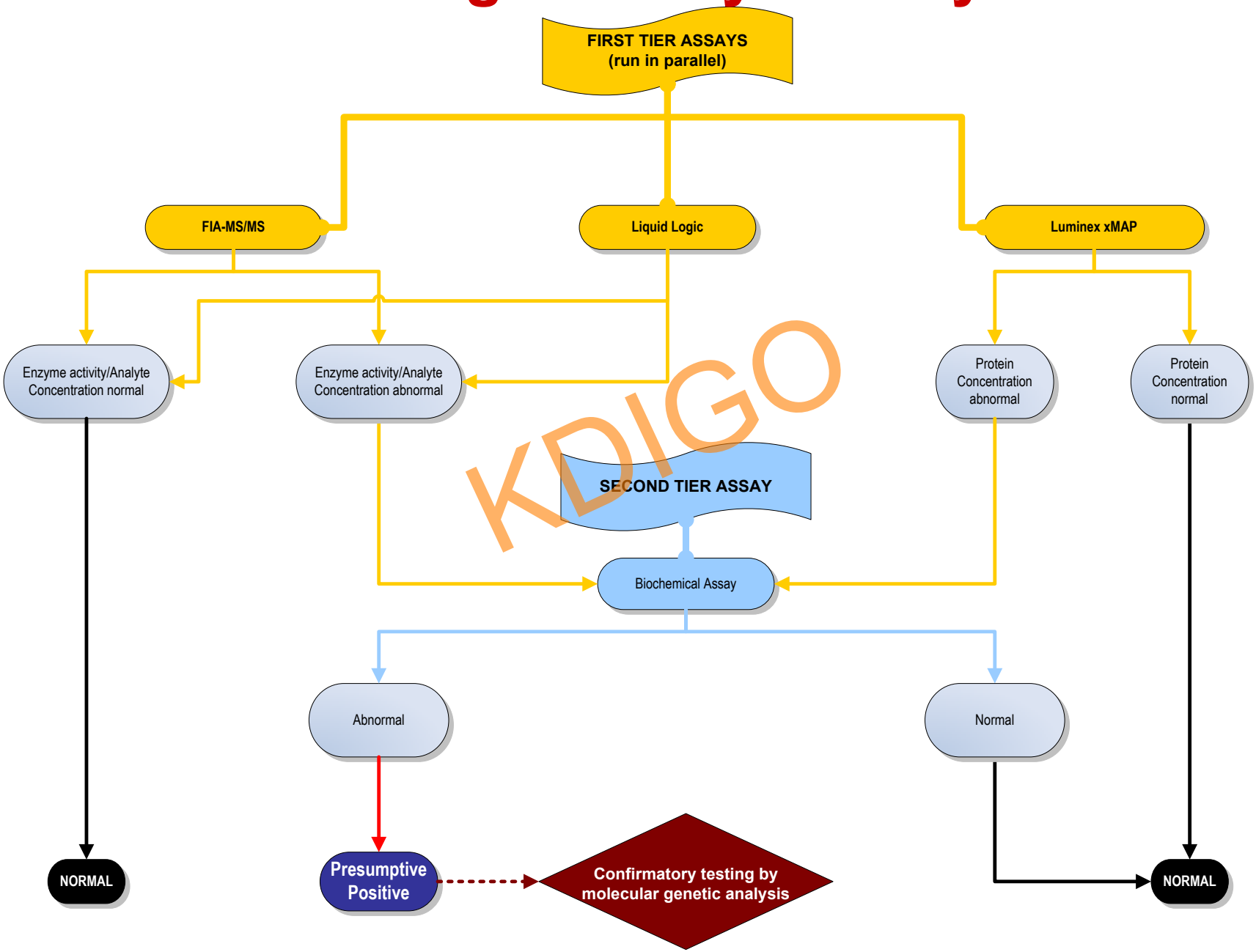
Prospective analysis of 100,000 de-identified NBS samples from the California Department of Public Health (CDPH)

FIRST TIER ASSAYS
(run in parallel)

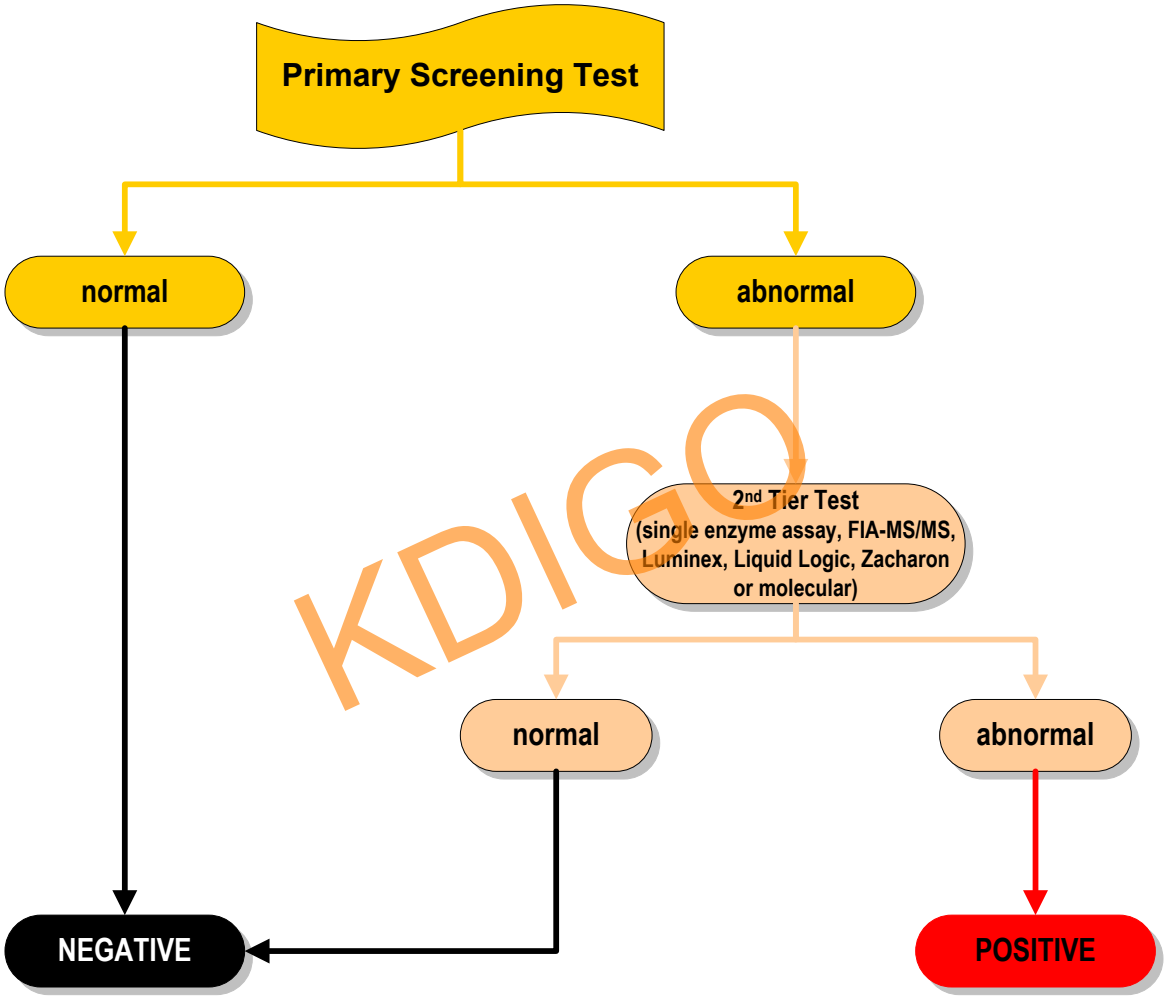
- FIA-MS/MS
- Liquid Logic
- Luminex xMAP

DISORDER	MS/MS	Immunocapture	Dig. Microfluidics
Fabry disease	+	+	+
Gaucher disease	+	+	+
Krabbe disease	+	+	
MLD		+	
MPS I	+	+	+
MPS II		+	(+)
MPS IIIA		+	
MPS IIIB		+	
MPS VI		+	
Mucolipidosis II/III		+	
MSD		+	
Niemann-Pick A/B	+	+	
Pompe Disease	+	+	+
Wilson disease		+	
Aceruloplasminemia		+	
Menkes disease		+	
Friedreich Ataxia		+	
X-Adrenoleukodystrophy	+		
Zellweger spectrum dis.	+		
Acyl-CoA oxidase def.	+		
Bifunctional protein def.	+		

Design of Mayo Study



Goal of Mayo Study



To identify an effective and efficient testing approach.

NOT to determine which condition should be screened for!!!

What We Found

- The high-throughput assays we tested were well received by the technologists and sufficiently robust
- All assays seem sensitive.
- No assay seems sufficiently specific on its own.
- 2nd tier testing:
  - Genotyping improves specificity … but may not be cost effective (+ too many variants of uncertain significance)
  - Only few other potentially helpful biomarkers (PSY, GPSY, LSM, lysoGb3, GAGS, LSM)
- Somewhat surprising prevalence findings (means what?).

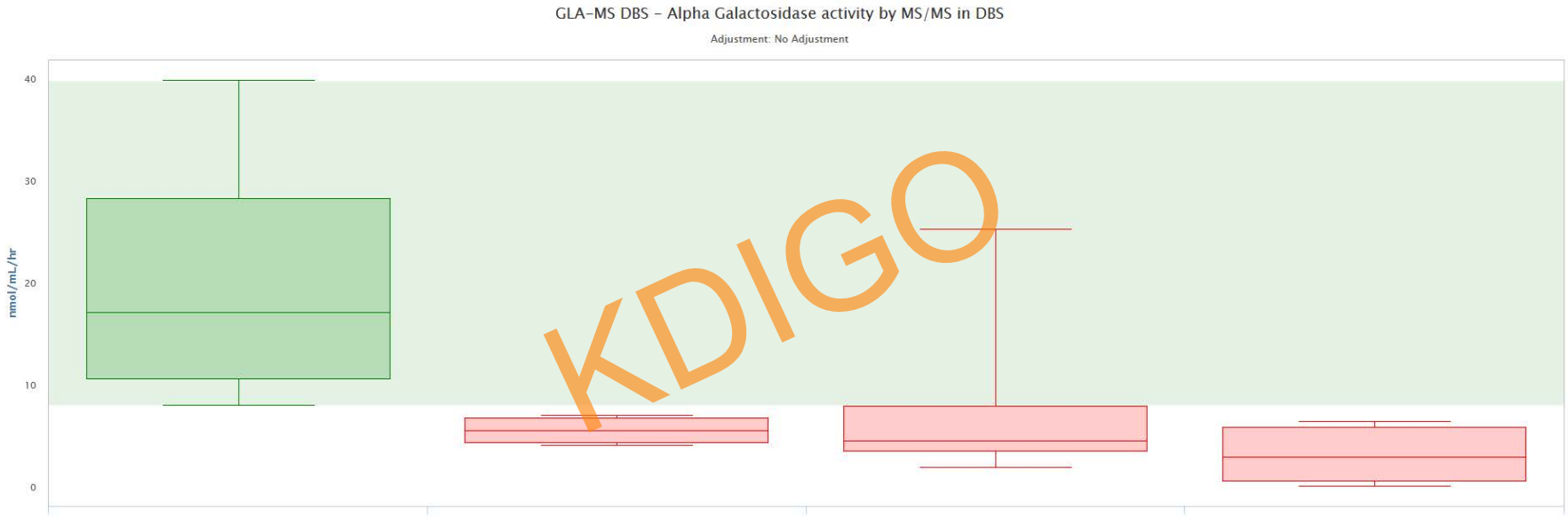
What could help NBS for Fabry Disease?

- Do not use a simple cut off, but consider ‘disease’ AND ‘normal’ ranges
- Do not use GLA as the only screening marker
- Measure other enzymes simultaneously as internal controls
- Measure other enzymes simultaneously and use enzyme activity ratios as additional markers

NBS Data Project

GLA Activity

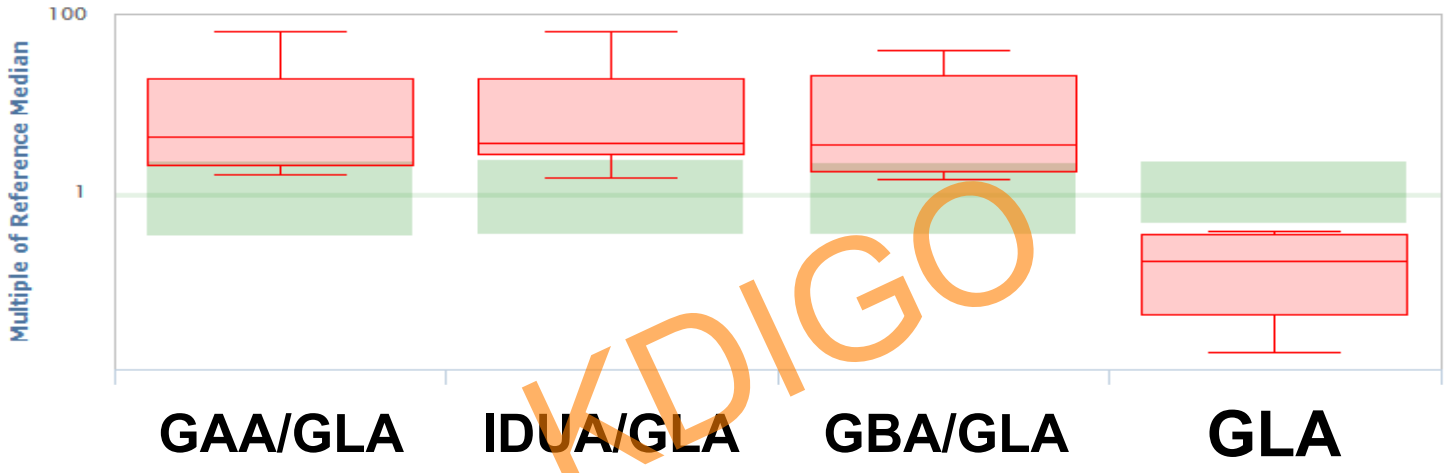
MS/MS



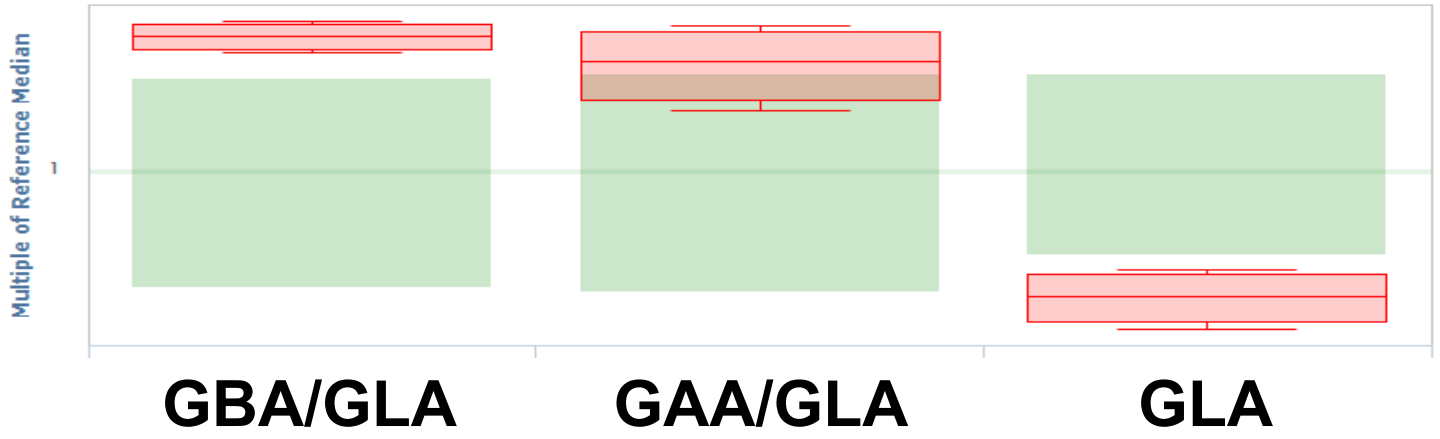
Controls | **Fabry (female)** | **False Positive** | **Fabry (male)**

NBS for Fabry Disease

MS/MS



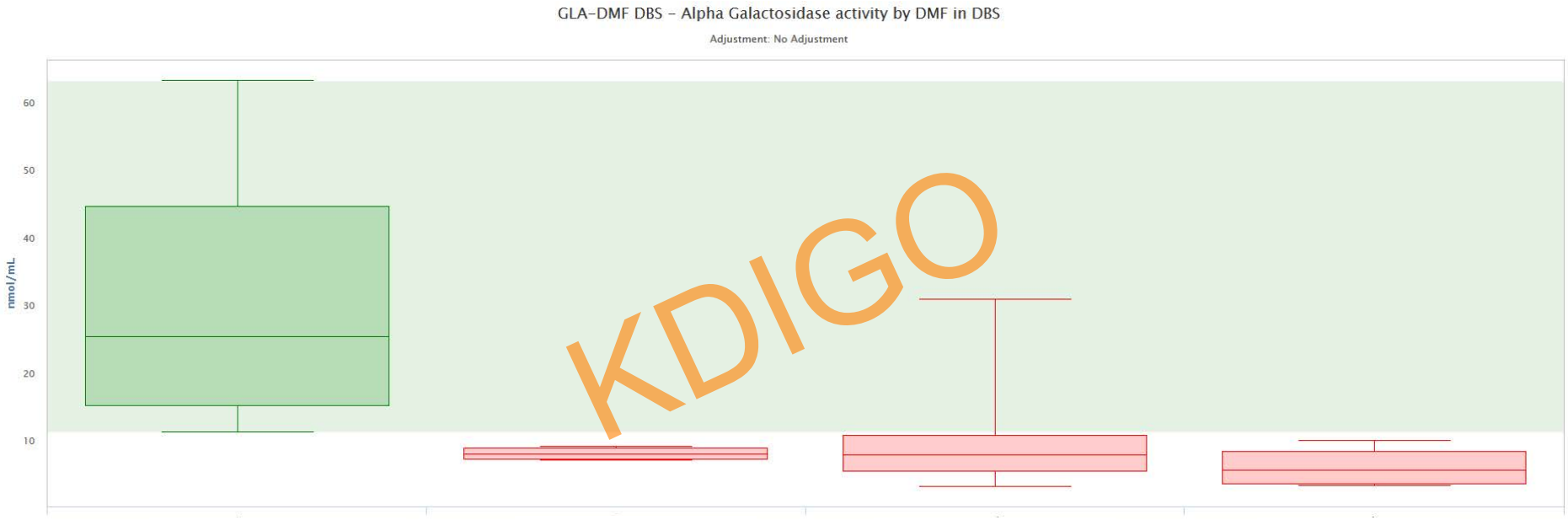
Fabry (male)



Fabry (female)

GLA Activity

Digital Microfluidics



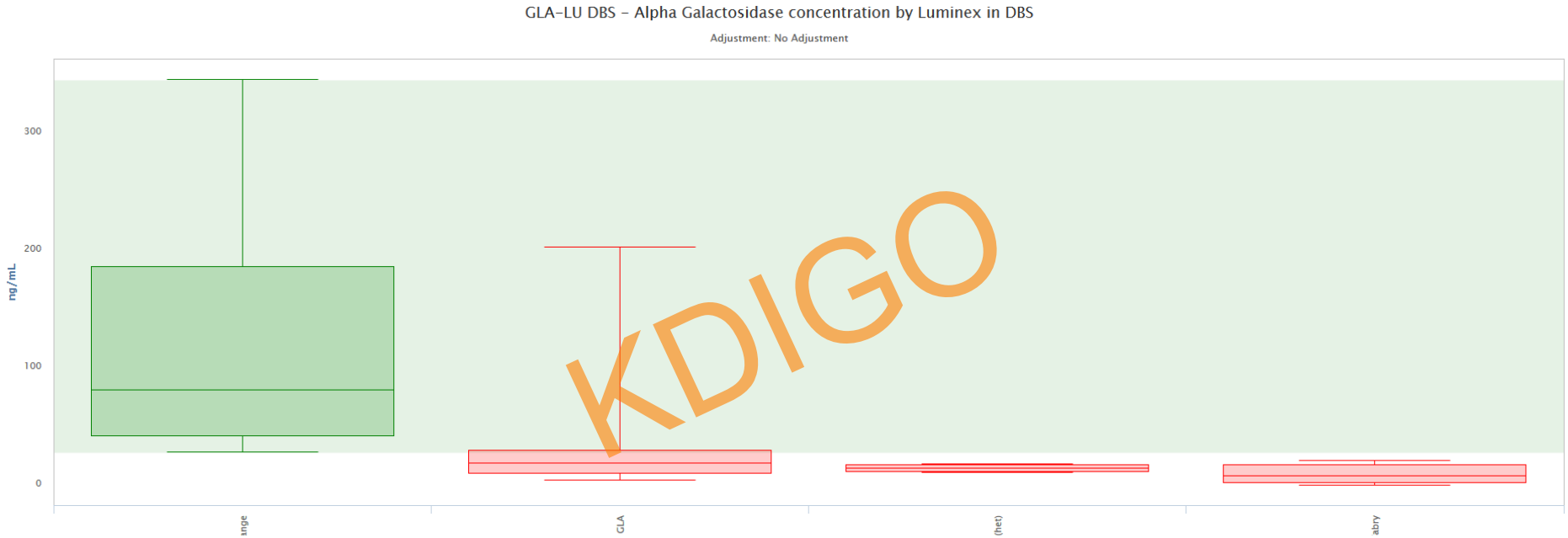
Controls | **Fabry (female)** | **False Positive** | **Fabry (male)**

NBS for Fabry Disease

Digital Microfluidics

GLA Activity

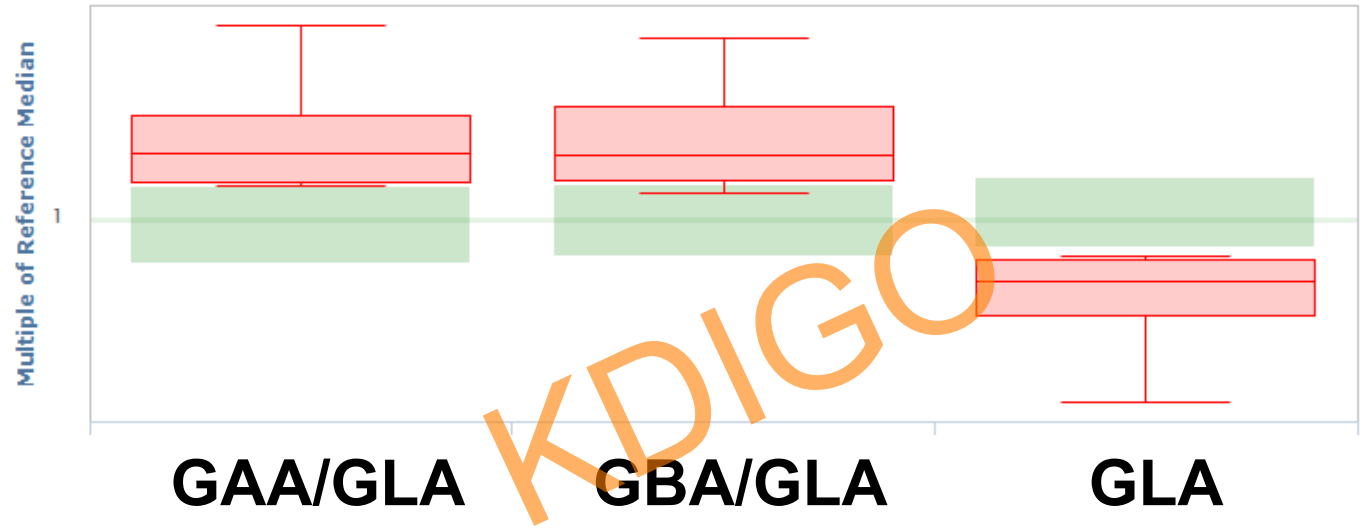
Immunocapture



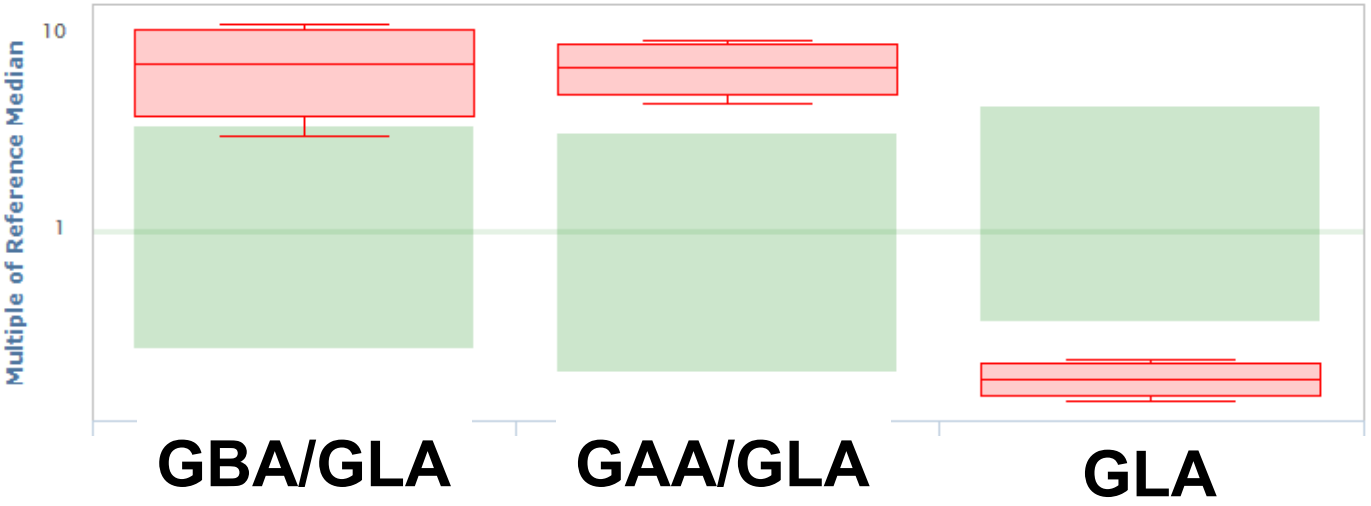
Controls | **Fabry (female)** | **False Positive** | **Fabry (male)**

NBS for Fabry Disease

Immunocapture



Fabry (male)



Fabry (female)

What else could help NBS for Fabry Disease?

- Do not use a simple cut off, but consider 'disease' AND 'normal' ranges
- Do not use GLA as the only screening marker
- Measure other enzymes simultaneously as internal controls
- Measure other enzymes simultaneously and use enzyme activity ratios as additional markers
- **Find another marker, even as a 2nd tier test, to increase specificity**

Closing Thoughts

If we want Newborn Screening for LSDs, incl. Fabry disease

- **Need better definitions of what we want to identify through Newborn Screening:**
  - **Conditions affecting the baby only?**
  - **Conditions affecting the baby only early in life (classic phenotypes)**
- **Need to agree if identification of unknowing family members (mother, uncles) is an appropriate reason for Newborn Screening.**

Would "Child Screening" be more appropriate for late onset conditions?

What is the impact of increasing prenatal/-conception genetic screening?

Acknowledgments

mayo

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Hunter's Hope Foundation

Krabbe ~ Leukodystrophies ~ Newborn Screening

Women's & Children's Hospital Foundation

the legacy of angels foundation

John Hopwood

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Screening Criteria

1968 - WHO (Wilson & Jungner)

- Treatable illness
- Detectable in newborn period
- Presymptomatic initiation of treatment is beneficial
- Available resources for diagnosis/treatment/follow-up
- Availability of a simple method for sample collection
- Evidence of substantial public benefit & acceptance
- Suitable and simple test methods
- Acceptable costs

2006 - ACMG Criteria

- Clinical characteristics (e.g., incidence, burden of disease if not treated, phenotype in the newborn);
- Analytical characteristics of the screening test (e.g., availability, features of the platform);
- Diagnosis, treatment and management of the condition in both acute and chronic forms (includes the availability of health professionals experienced in diagnosis, treatment, and management).