

Newborn Screening and Studies of Lysosomal Storage Diseases in CFOH

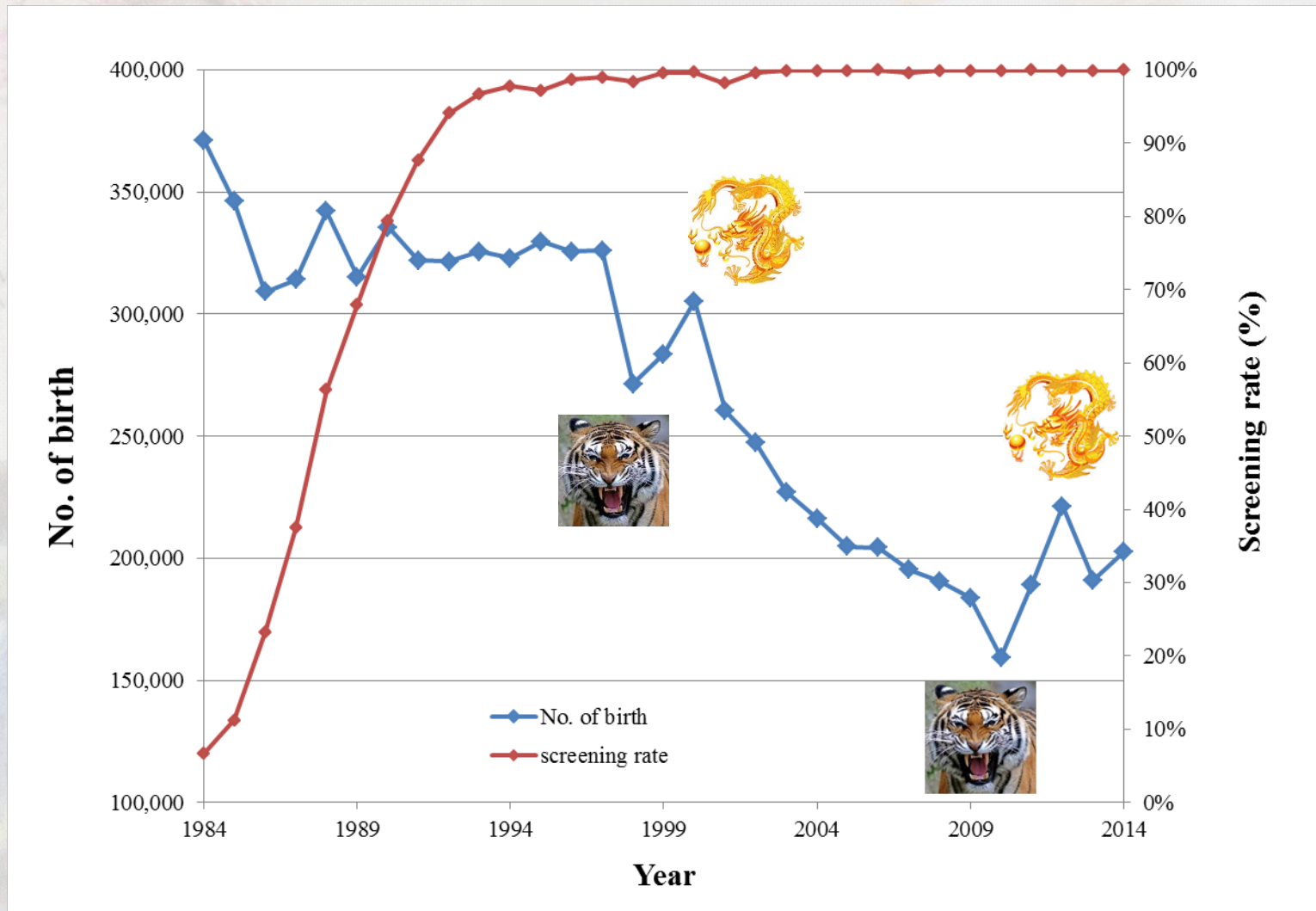


**Chinese Foundation of Health
National Yang-Ming University**



**Director: Dr. Chuan-Chi Chiang
Speaker: Hsuan-Chieh Liao (Joyce)**

Newborn Screening rate in Taiwan



The newborn screening rate reaches **more than 99%** after 2002 in Taiwan.²

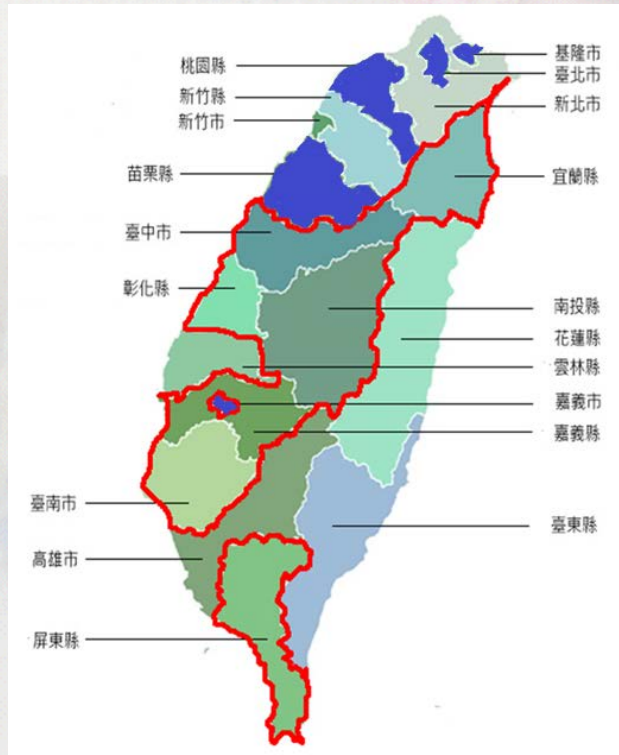
Newborn screening centers in Taiwan

**National Taiwan
University Hospital**

**Taipei Institute of
Pathology**

**The Chinese
Foundation of Health**

***60,000-70,000 cases/year/center** ***Region: 5 Counties, 2 Cities**



	2014	2013
Sample collection (within 3 days)	99.3%	99.2%
Sample delivery (within 2 days)	98.8%	98.4%
Inform positive cases (within 3 days)	100%	100%
Finish all procedures (within 8 days)	99.5%	99.4%

Newborn screening items

Government assign **11** items
NTD 550, subsidy NTD 200

GAL

CHT

HCU

PKU

G6PD

GA I

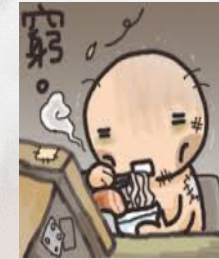
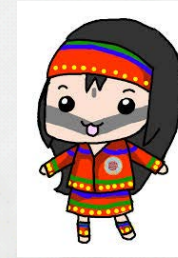
MMA

IVA

MCAD

MSUD

CAH



Totally free

SCID, NTD 150

Fabry

LSD, NTD 200

*not including Gaucher and MPS

Pompe

Gaucher

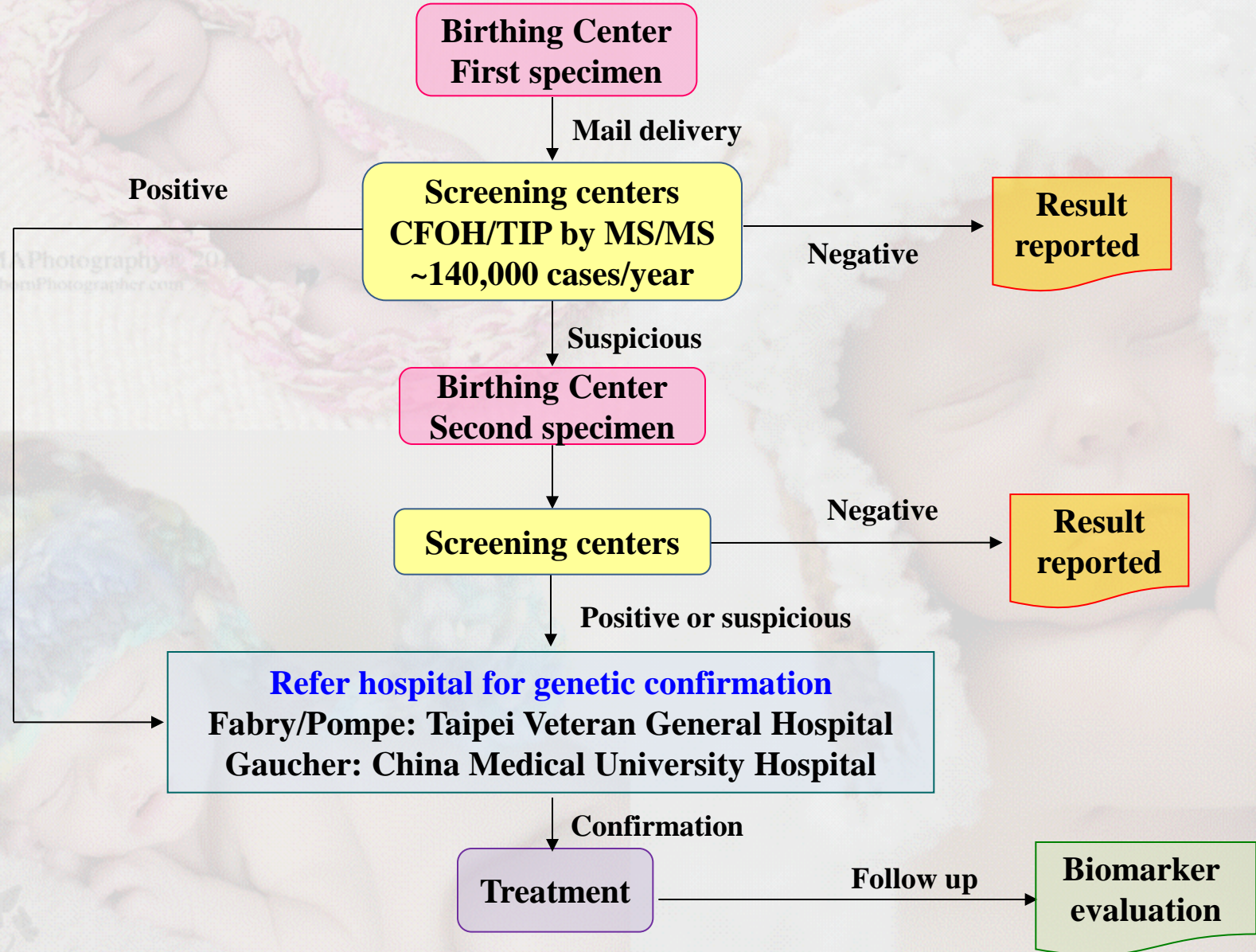
MPS I/II

1983~~~~~1987~~~~~2006~~~~~2008~~~~~2011~~~~~2012~~~~~2015

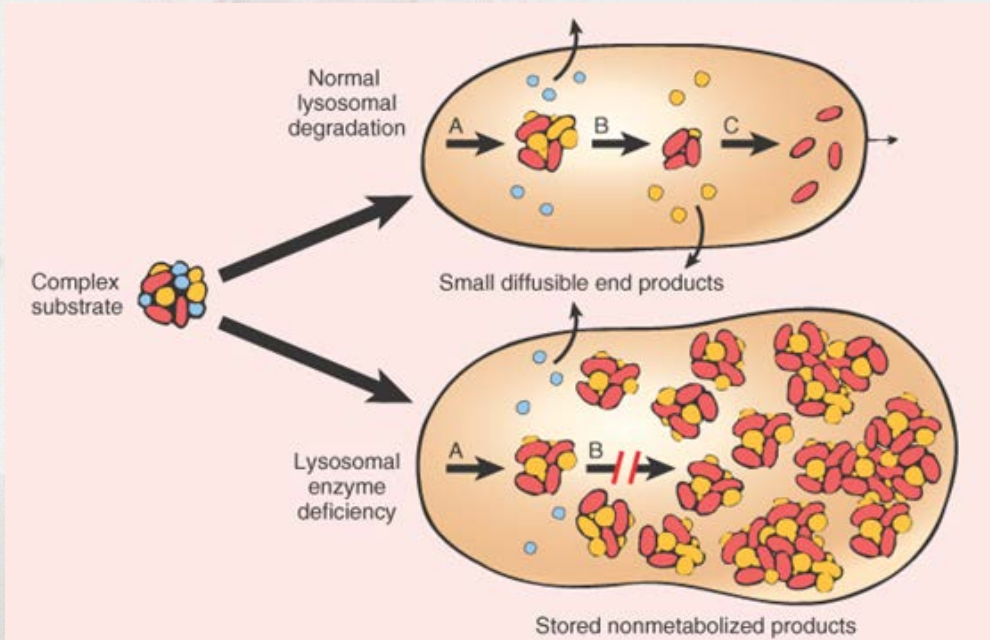
20 MS/MS items (organic/fatty/amino acid), free

Total: NTD700 (USD22)

The Flow Chart of LSD Newborn Screening



Lysosomal Storage Diseases (LSDs)



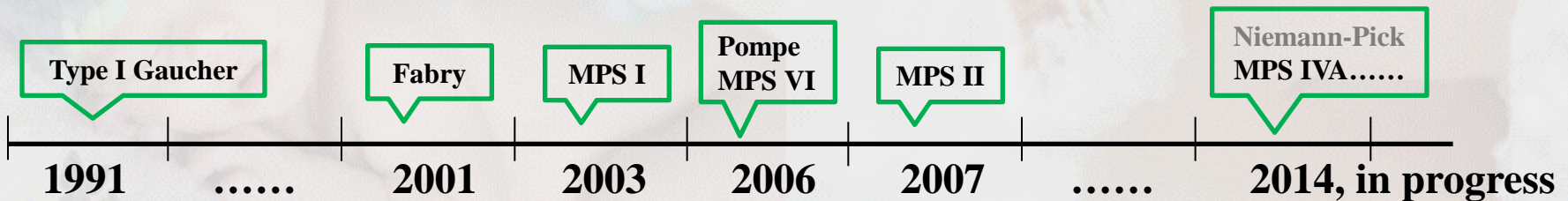
MBBS medicine (Humanity First): Genetic disease

***Newborn screening**
***Biomarker evaluation**









Prof. Y. T. Chen
for Pompe ERT

Timeline for enzyme replacement treatment approved

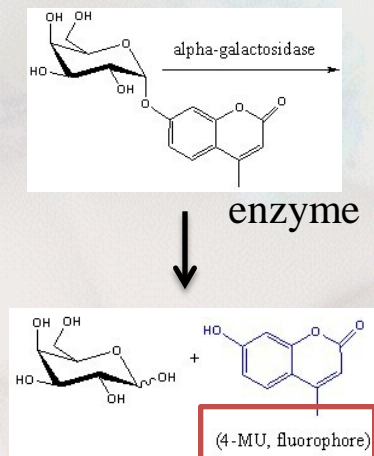


Newborn screening methods for LSDs

Method 1	Method 2
Fluorescence (4-MU)*	HPLC-MS/MS
Single assay	Multiplex 
According to Dr. Chamoles	According to Dr. Gelb
 Cheaper for single assay** Hb interference	More sensitive and specific, less laborious for multiplex  
From 2008-2009 (CFOH) From 2008-2011 (TIP) From 2006-now (NTUH)	From 2010 - now  From 2012 - now  From 2015 (processing?)

*Digital microfluidics was developed from conventional 4-MU substrates.

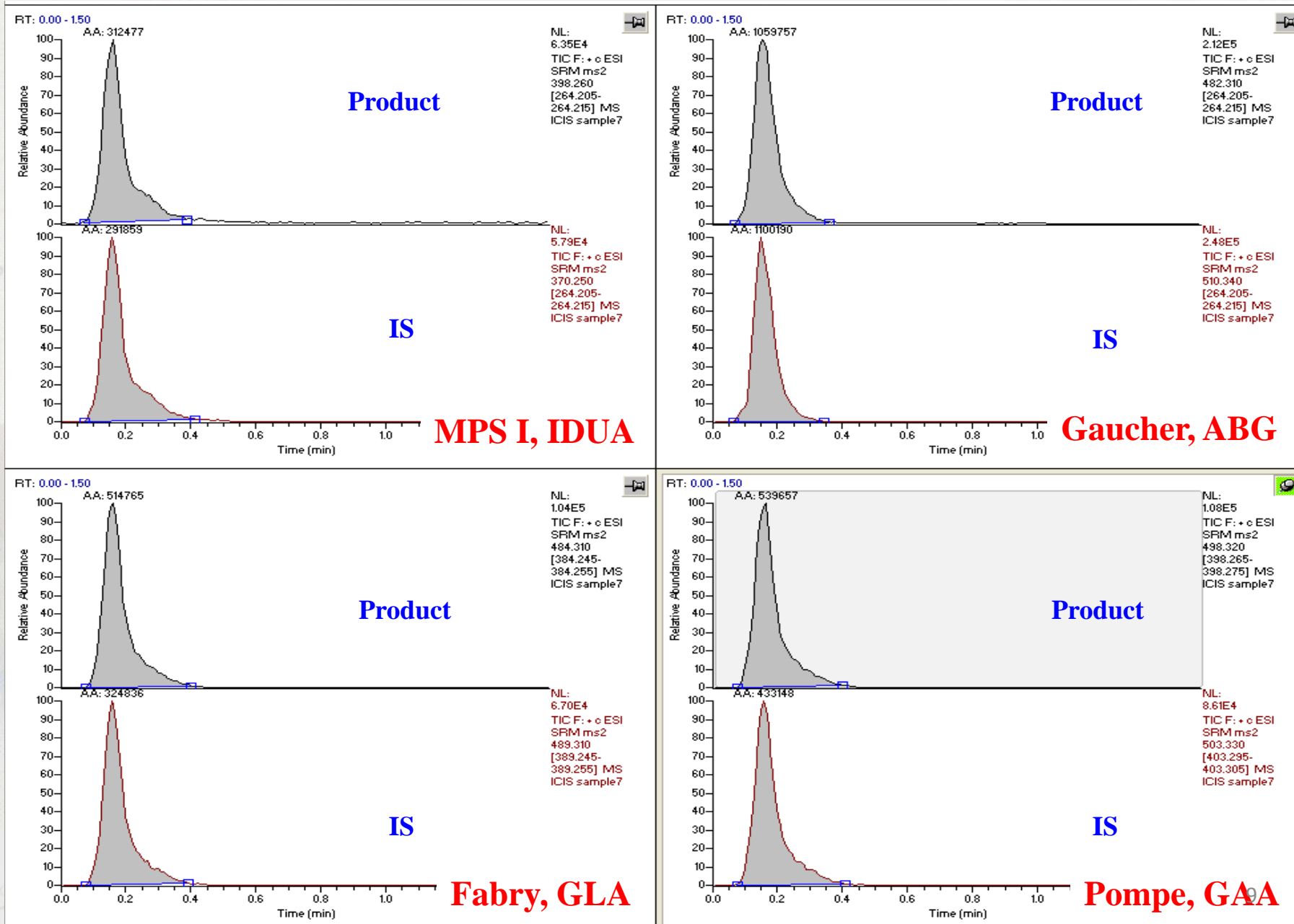
**By traditional 4MU method and own plate reader



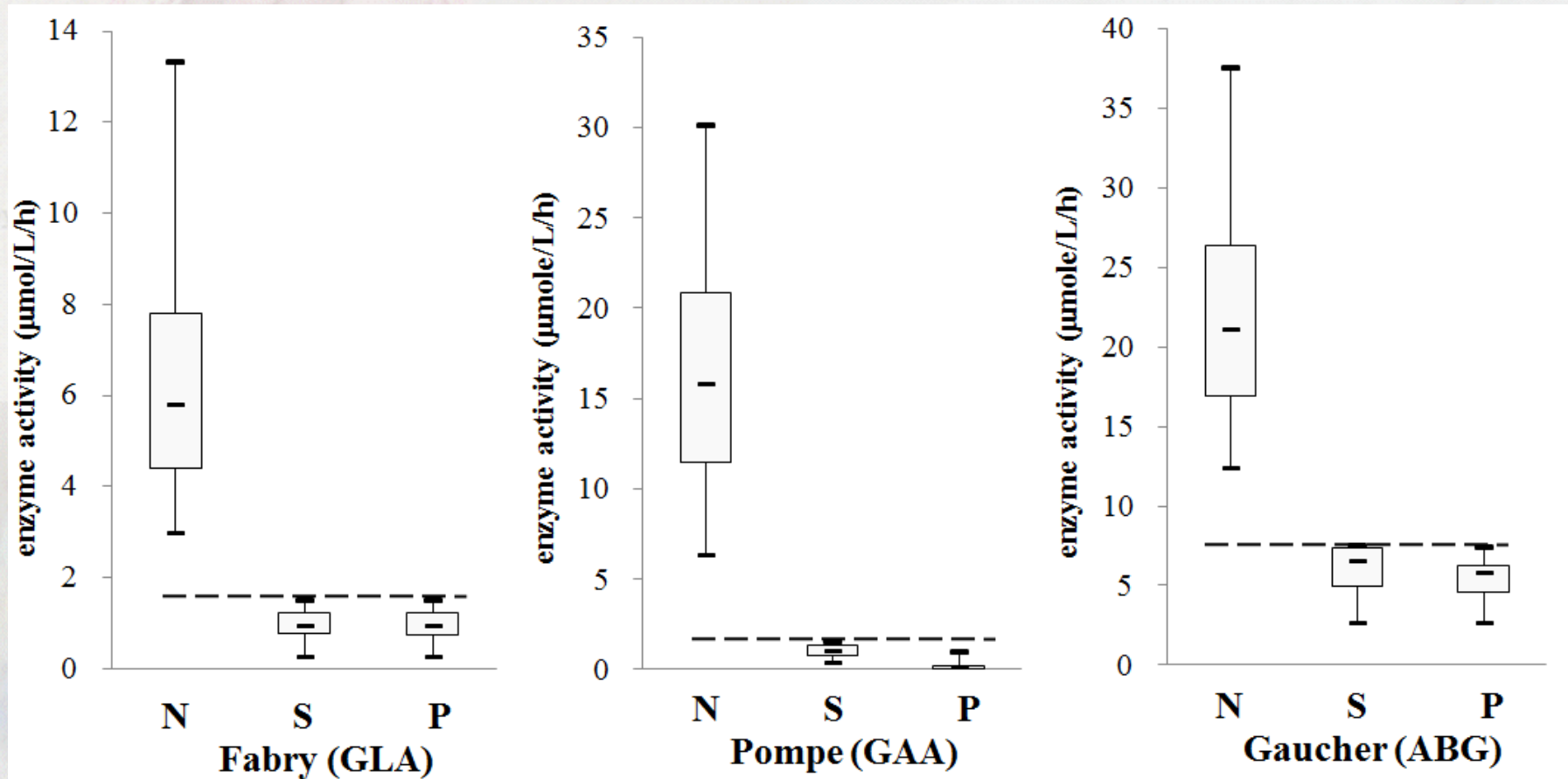
Thermo TSQ Vantage



FIA-MS/MS of LSD screening



Overview of the first DBS enzyme activity by MS/MS method



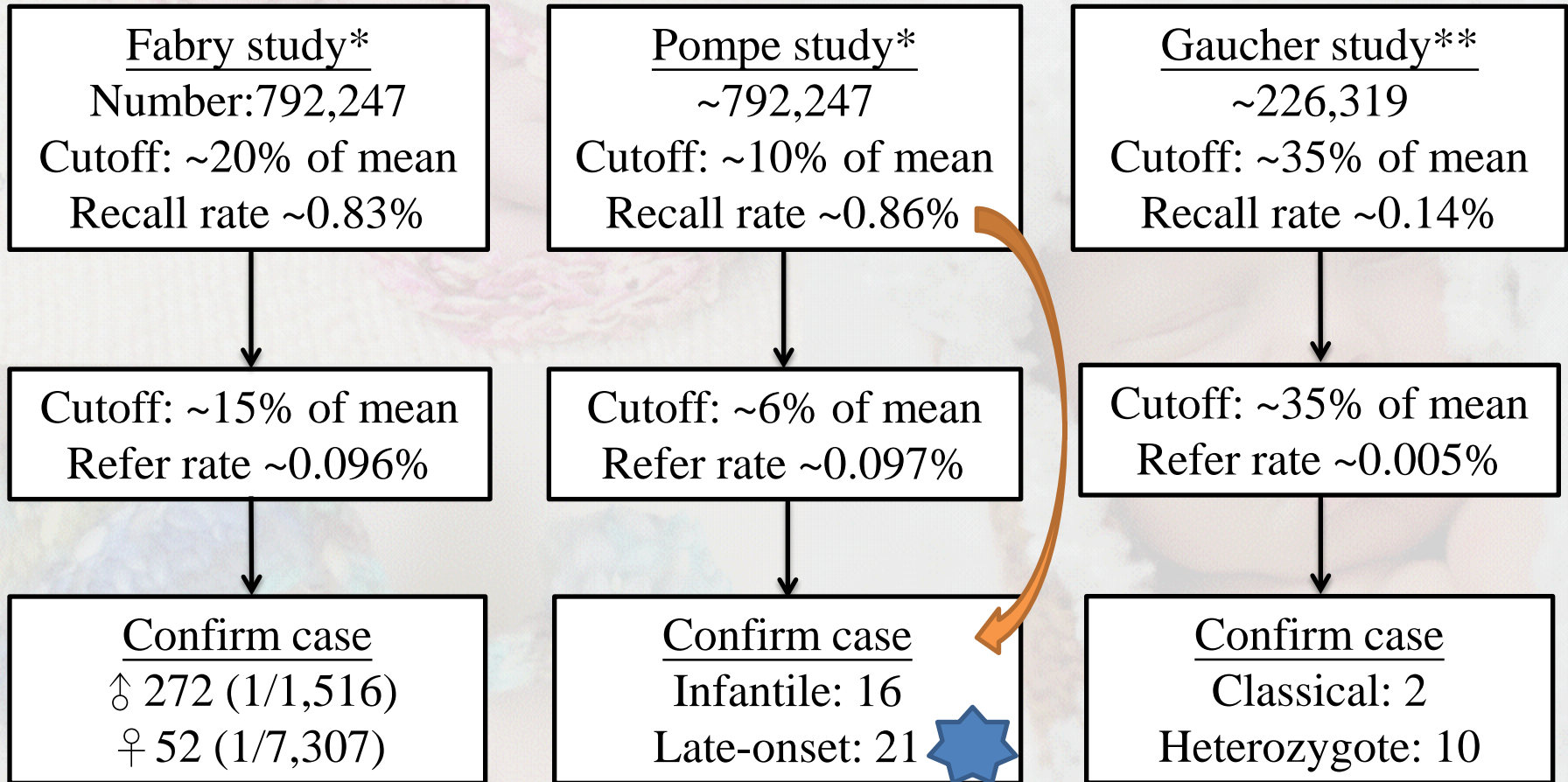
Error bars are 5th and 95th percentiles. Dotted lines are cutoff values for normal activity.

N= enzyme activity in health newborn.

S= suspected newborns with decreased enzyme activity and referred to the hospital.

P= newborns confirmed by genetic mutation analysis.

Recall and refer rate of LSD screening



*4MU method from 2008-2009 in CFOH and 2008-2011 in TIP
MS/MS method from 2010-2014 in CFOH and 2012-2014 in TIP

**MS/MS method from 2010-2014 in CFOH

Confirmed cases enrolled from Fabry newborns screening

Mutation type	Mutation site	Number from newborn screening
Cardiac type	IVS4+919G>A	302 (85%)
Classical type	6 mutations	7 (2%)
	c.[394 G>A], p.G132R	1
	c.[1034 C>G], p.S345X	1
	c.[1066 C>T], p.R356W	1
	c.[1081 G>T], p.G361X	2
	c.[1087 C>T], p.R363C	1
	c.[1228 A>G], p.T410A	1
Non-classical/Novel	20 mutations	47 (13%)
	c.[1078 G>T], p.G360C	8
	c.[157 A>G], p.N53D	5
	c.[1722 A>C], p.K391T	5

Total		356

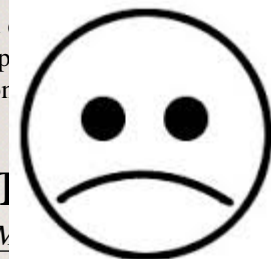
*Enzyme based screening can only identify a subset of mutation-positive patients

* ♂ 1/875; ♀ 1/399

Comparison of 4-MU fluorescence and MS/MS methods in Fabry and Pompe studies in CFOH

Method	Fabry		Pompe	
	4-MU	MS/MS	4-MU	MS/MS
Period	2008.1 ~ 2009.12	2010.2 ~ 2013.1	2008.1 ~ 2009.12	2010.2 ~ 2013.12
Number of screening newborn	122,890	191,767	122,937	247,611
% of total newborn ^a	94.1	95.5	94.1	95.5
Positive in first DBS	2,104	379	1,613	1,144
% of screening newborn ^b	1.71	0.20	1.31	0.46
Suspected newborn ^c	127	79	135	308
% of screening newborn ^d	0.10	0.04	0.11	0.12
Reject newborns ^e	22	12	10	11
Confirmed newborn ^f	64	64	4	22
Positive predict value % ^g (95% CI) ^h	61.0 (50.9-70.3)	95.5 (87.5-99.1)	3.2 (0.8-7.4)	7.4 (4.5-10.6)

a. Newborns enrolled in LSDs study/ total routine newborns screening conducted at CFOH(%) b. Newborns with decreased enzyme activity in DBS and referred to hospitals c. Newborns with decreased enzyme activity in DBS and referred to hospitals d. Suspected newborns enrolled in LSDs study (%) e. Newborns who rejected to confirm f. Newborns confirmed by genetic mutation analysis g. Correlation of enzyme activity in DBS and confirmed newborns- newborns who rejected to confirm (%) h. CIs were calculated by Clopper-Pearson confidence method



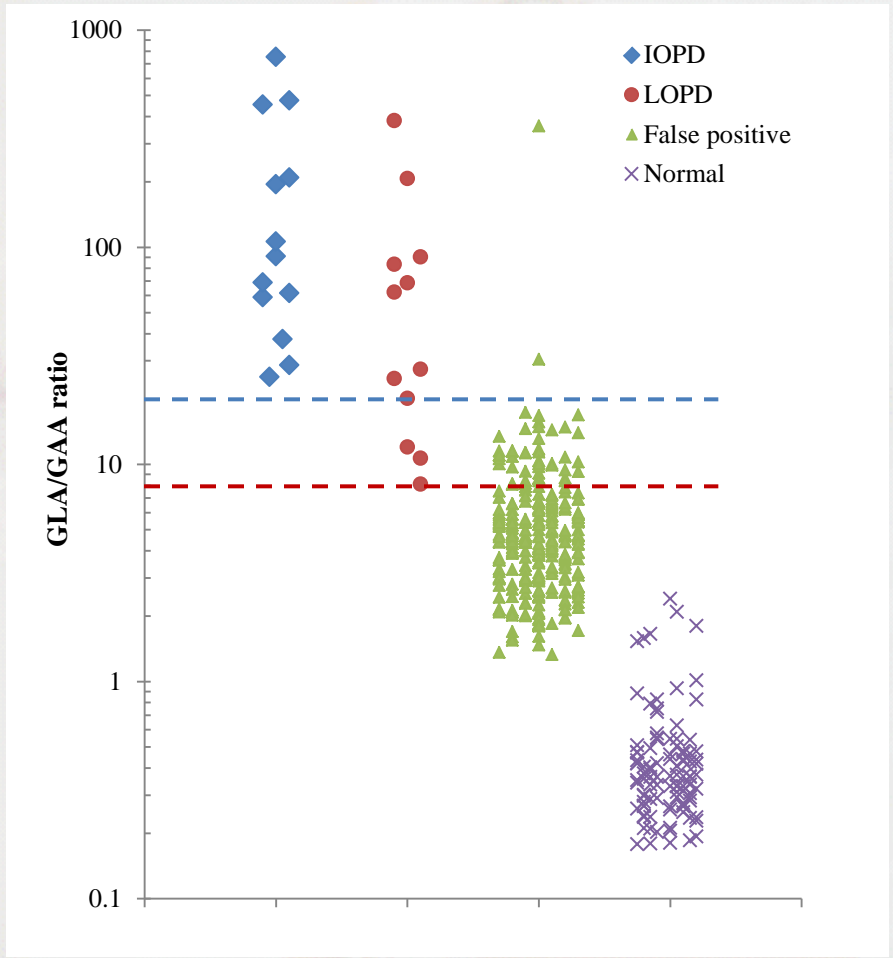
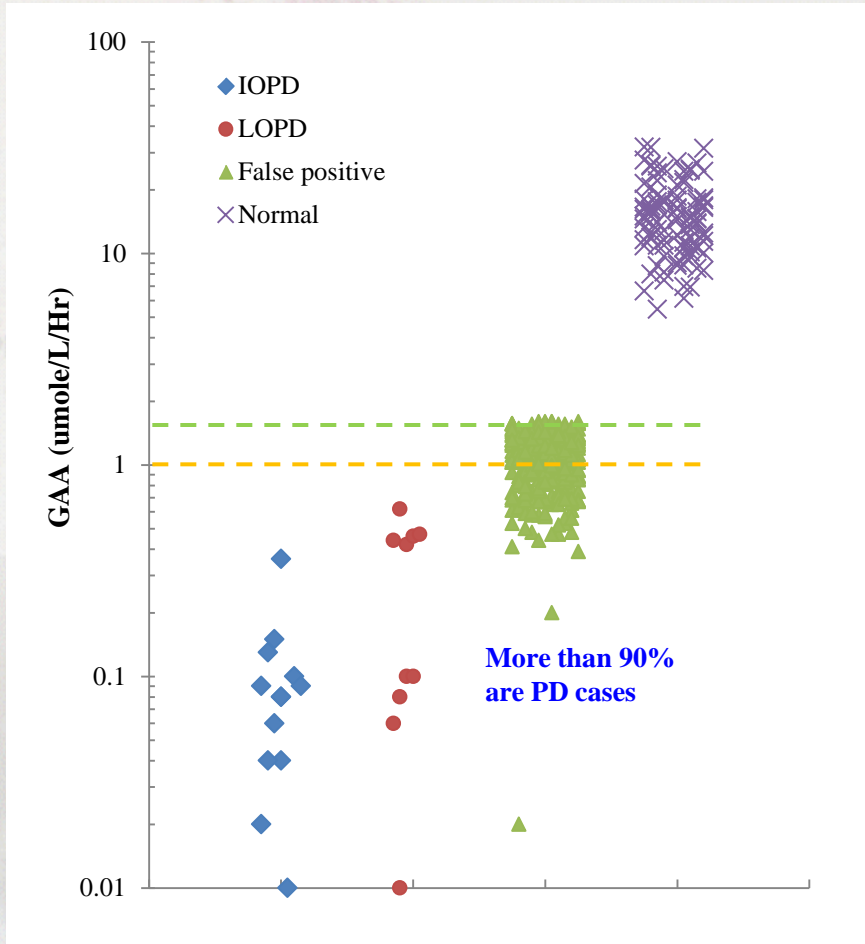
*High prevalence (14.5%) of pseudodeficiency allele p.G576S in the T

Mol Genet M

ulation

2816

GAA activity and GLA/GAA ratio in Pompe patients



- *GAA 10% → 6% of mean, PPV 7%→17%
- *GAA 10% of mean & GLA/GAA>8, PPV 7% →41%
- *GAA 10% of mean & GLA/GAA>20, PPV 7% →93%

GAA activity and GLA/GAA ratio in Pompe screening

- **Different number of white cells or protein quantity, the GAA and other LSD enzymes would both go up or down together**
- **Do NOT require any additional instrumentation, procedure, or sample collection**
- **The cost and manpower is minimal**
- **Reduce the false positive rate significantly**
- **Also being used in the other newborn screening center (TIP)**
- **Be careful about LOPD and IVS4 patients**

Infantile-onset Pompe Disease (IOPD)

*Effective diagnostic protocol:

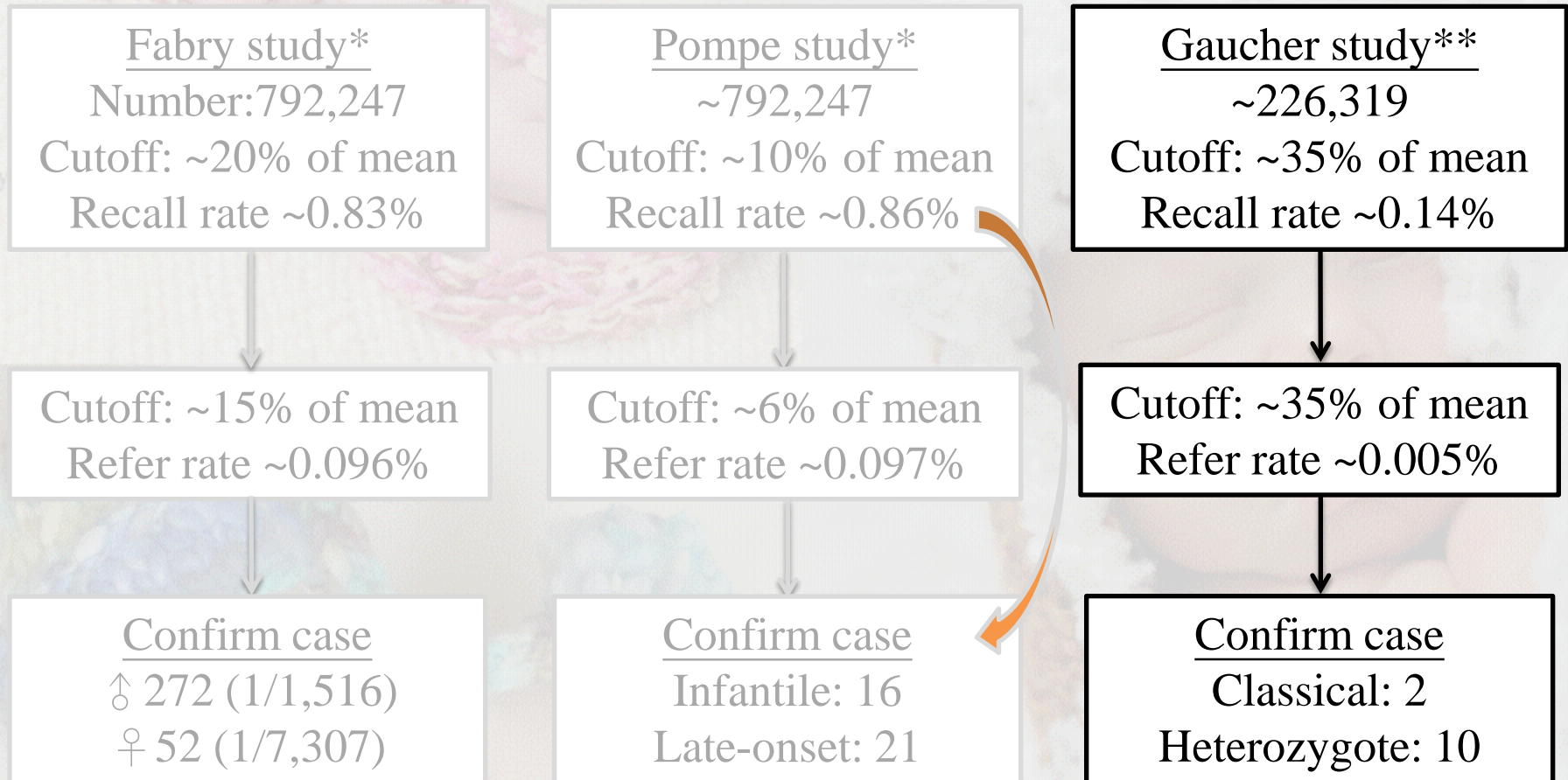
[Am J Med Genet A. 2014 Jan;164A\(1\):54-61.](#)

- 1. Extremely low GAA DBS enzyme activity by MS/MS method**
- 2. Hypotonia**
- 3. Elevated CK (>250U/L)**
- 4. Elevated LVMI (>80g/m^{2.7})**

Case 1, **1st ERT 15-day-old**
c.1935 C>A, p.D645E, homozygous
c.1726 G>A, p.G576S, homozygous
CK: **542** u/L
GAA: **0.36** umol/L/hr)
LVMI: **154.5**g/m^{2.7}

Case 2, **1st ERT 9-day-old,**
c.1935 C>A, p.D645E, homozygous
c.1726 G>A, p.G576S, homozygous
CK: **766** u/L
GAA: **0.04** umol/L/hr)
LVMI: **191.5** g/m^{2.7}

Recall and refer rate of LSD screening



*4MU method from 2008-2009 in CFOH and 2008-2011 in TIP
MS/MS method from 2010-2014 in CFOH and 2012-2014 in TIP

**MS/MS method from 2011-2014 in CFOH

Analytical range for MS/MS and 4MU LSD assays

Disease	Ratio of normal mean/ patient mean		Ratio of normal mean/ no blood (blank)	
	UW 2014 MS/MS	4MU	UW 2014 MS/MS	4MU
Fabry	28	6.1	109	--
Gaucher	67	3.7	216	--
Pompe	63	5.0	367	12
MPS-I	168	7.4	230	20
MPS-II	60	3.9	80	11
Krabbe	27	--	85	--
Niemann	26	--	104	--

***Data from Prof. Michael Gelb's PPT at APHL and online video**

Pilot study of MPS screening in Taiwan

	Alive	Total
Type I	11	16
Type II	29	112
Type III	25	37
Type IV	17	32
Type VI	9	15
Total	91	212

Data from MPS society 2014/10



<http://www.mpssociety.org.tw/>

「協會的人白都知道，我是裡頭的協理；經病！愈難的事我都要做。」一句自語的玩話，點出黏多醣症協理手長蔡瓊瑋的辛酸史，與打不走的怨氣。

為兒童獻化小兒班大愛

卅年前，周道的出生讓家裡如獲珍寶，但他從小常生病，小感冒變成肺炎屢見不鮮，智力發展遲緩，直到五歲確診為黏多醣症，那時蔡瓊瑋天蠶盡，甚至一度尋短。

正所謂為母則強，蔡瓊瑋選擇陪孩子一起成長。無論是周道被小朋友欺負，掉入陷阱裡出不來；或是罹病過程中手術十多次；甚至因為一口痰卡住而瀕臨死亡，靠著地獄般的急救送醫救治。這一點一滴的累積，讓蔡瓊瑋從無助的家庭主婦，蛻變成打不走的交戰工。

「我知道周道的生命有限，但若能幫助同樣疾病家庭，成立黏多醣協會，並推動篩檢臨牀家族遺傳，減少悲劇發生，也能讓周道的背影永存不滅。」蔡瓊瑋說。

說來容易做來難，到處找尋黏多醣家庭的個夫妻頻頻碰壁，在朋友幫忙下，媒體報導了黏多醣症兒一周道的故事。透過媒體力量，才發現現在台黏多醣病童不算少，「那時

永緬愛兒 煮婦蔡瓊瑋 蛻變女戰士

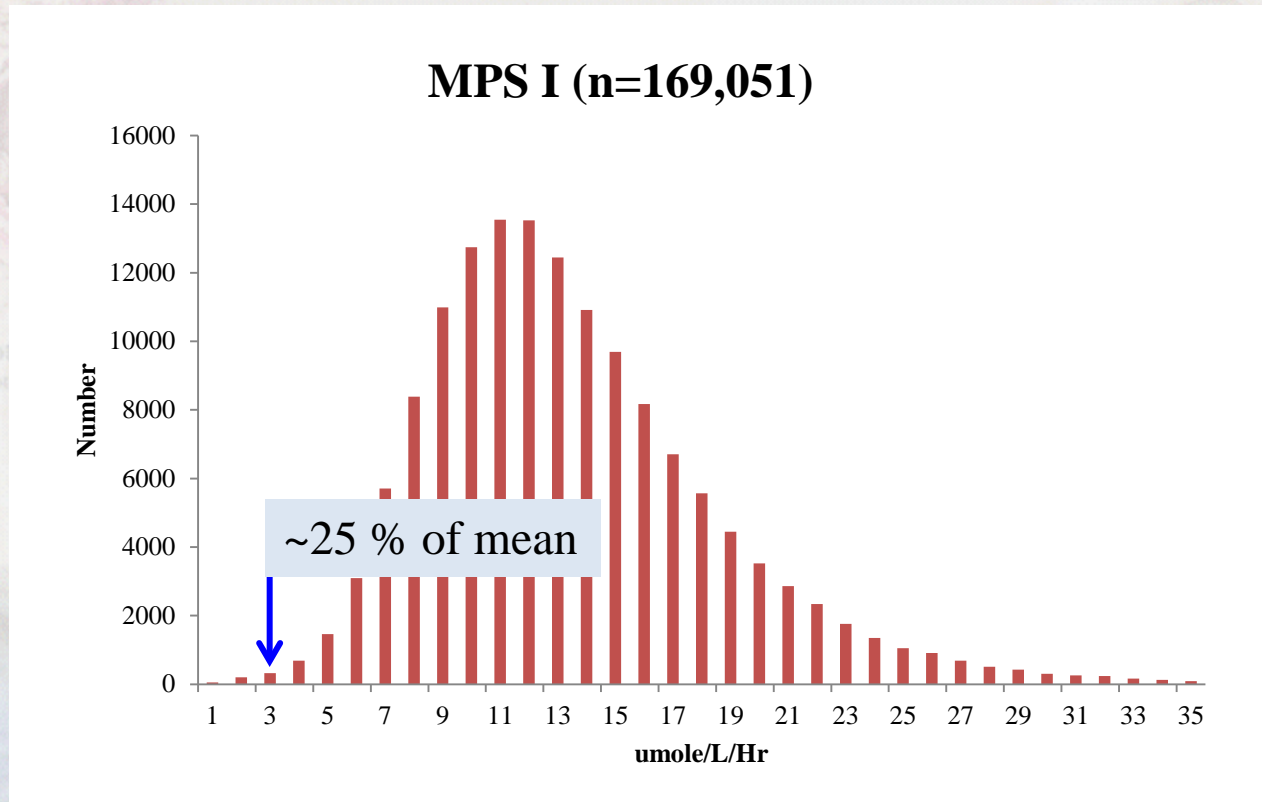
蔡瓊瑋，一位平凡的家庭主婦，從兒子周道確診為黏多醣症的那一天起，披上鋼鐵的外衣，帶著兒子向全世界求診；創立黏多醣協會幫助其他家庭，與黏多醣結緣三十年，她，靠的是想念兒子的復仇。

社團法人中華民國台灣黏多醣症協會 大事紀

- 1997年 協會成立
- 1998年 國際扶輪社總會「關懷兒童傑出服務獎」
- 2008年 加入國際黏多醣症團體組織 舉辦亞太區第一屆黏多醣會議
- 2010年 經濟部發展局升計劃-國際會議推動小組顧問
- 2014年 簽署「海峽兩岸黏多醣互助聯盟」 黏多醣國際會議獲頒「終身成就獎」



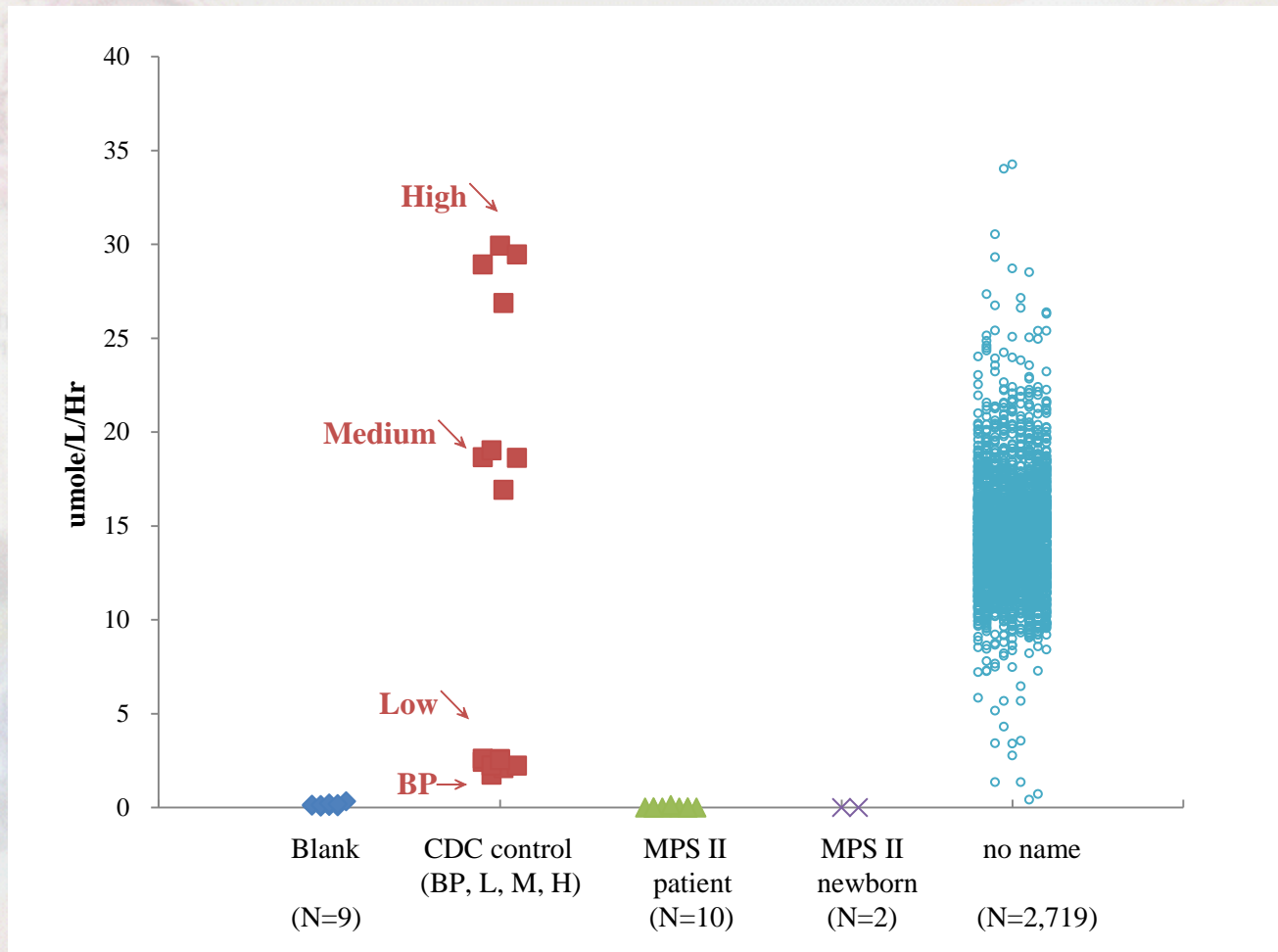
Overview of DBS enzyme activity in MPS I assay by MS/MS method



*Recall rate would be 0.08% (136). Incidence: $\sim 1/42,263$

Case 1	c.571G>C, c.1093C>G
Case 2	c.535A>T, c. 1643C>T
Case 3	c.76G>A, c. 911delT
Case 4	c.571G>C, c.1093C>G

Overview of DBS enzyme activity in MPS II assay



*All the MPS II could be distinguished clearly from normal by MS/MS method.

*Substrate: **from Prof. Michael Gelb, will be commercialized by PE in near future**

*The pilot study of large screening in WA and CFOH NBS lab

Analysis of enzyme activity and metabolite of LSD in CFOH

Disease	Item	Enzyme activity		Metabolite / Biomarker
		4-MU	MS/MS	
Fabry	α -Galactosidase	V	V	
	GB-3 (urine/plasma)			V (MS/MS)
	Lyso GB-3 (urine/plasma)			V (MS/MS)
Pompe	α -Glucosidase	V	V	
	Glc 4 (urine)			V (MS/MS)
Gaucher	β -Glucocerebrosidase	V	V	
	CCL18 (plasma/DBS)			V (ELISA)
	Chitotriosidase			V (4-MU)
Niemann-Pick A/B	Acid sphingomyelinase		V	
Krabbe	Galactosylceramidase		V	
MPS I	α -L-Iduronidase	○	V	○ (MS/MS for Dermatan sulfate, Heparan sulfate, Chondroitin sulfate Keratan sulfate)
MPS II	Iduronate-2-sulfatase	○	V	
MPS IIIA	Sulfamidase	○	△	
MPS IVA	Galactose 6-sulfatase	○	△	
MPS VI	Arylsulfatase B	○	△	
All MPS	GAG (urine/plasma)			V (Alcian blue)

V : Have been done and workable ○ : Can be done △ : Developing

Future work for LSD screening in CFOH

***Newborn screening:**

--MPS II, IVA, and VI, following MPS IIIA and IIIB

***Biomarker analysis**

--LysoGb3 for Fabry disease

--CK for Pompe disease

--Glucosylsphingosine for Gaucher disease

--Sulfatides for MLD

--C26-LPC for X-ALD

***This may be permit enzyme activity and biomarkers to be quantified in a single, first-tier newborn screening run lasting <2 min per sample**

****LSD video one line from Prof. Gelb**

Acknowledgement

- **Taipei Institute of Pathology**
 - MS/MS data collect
- **Taipei Veteran General Hospital**
 - Fabry/Pompe confirmation, F/U
- **Chinese Medicine University Hospital**
 - Gaucher confirmation, F/U
- **Prof. Michael H. Gelb**
 - Technical advice



The website of Chinese Foundation of Health

www.cfoh.org.tw

為下一代的健康把關。

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THANK YOU FOR YOUR ATTENTION!

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新生兒篩檢中心

相信每一個成員的努力，一定能有更優於今日之豐碩成果，為新生兒把好第一關！

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