Newborn Screening: Navigating New Conditions and Common Challenges

Jamie L. Fraser, M.D., Ph.D., FACMG

Division of Genetics and Metabolism

Future of Pediatrics 2026

Children's National

Disclosures

- Nothing to disclose

Objectives

- Identify 3 recent conditions added to local NBS panels.
- Identify the next steps required for an abnormal newborn screen
- Locate the ACT sheet for a given disorder

NBS: The First 30 Years

- One test, one disease
  - PKU, Biotinidase, Hypothyroidism, CAH Galactosemia, HgbSS

- Criteria
  - Test is reliable, inexpensive, blood spot
  - Affected children not recognizable at birth
  - Disease has significant burden
  - Early intervention changes outcome

NBS: 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

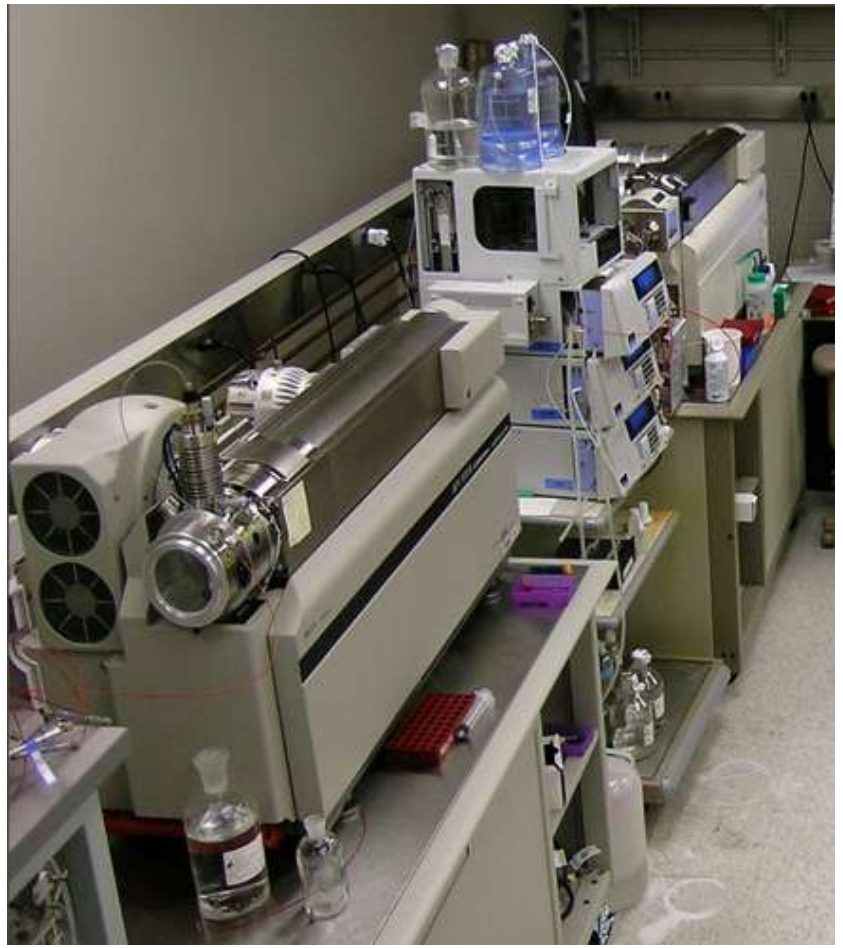
- Clinical characteristics (e.g., incidence, burden of disease if not treated, phenotype in the newborn);
- Analytical characteristics of the screening test (e.g., availability, features of the platform);
- Diagnosis, treatment and management of the condition in both acute and chronic forms (includes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

NBS: Today

- Multi-plexed assays

- MS/MS and LC-MS/MS
- Digital Microfluidics
- Sequencing and Molecular

- Second Tier Testing → Sendouts!



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NBS Is:

- **Public Health Program**
  - Identify individuals in a population who may be at an increased risk of a specific condition
  - Early intervention prevents mortality, morbidity, and disabilities
  - Performed by analysis of diagnostic filter paper blood spots at birth
  - US: State/Territory Dependent with Federal Guidance
  - Paid for by blood spot card charge: $0 (DC), $138 (VA), $163.74 (MD) (USA: $0-$292) [1]

1. NewSteps.org. Fee is per baby regardless of the number of specimens received

NBS Is NOT:

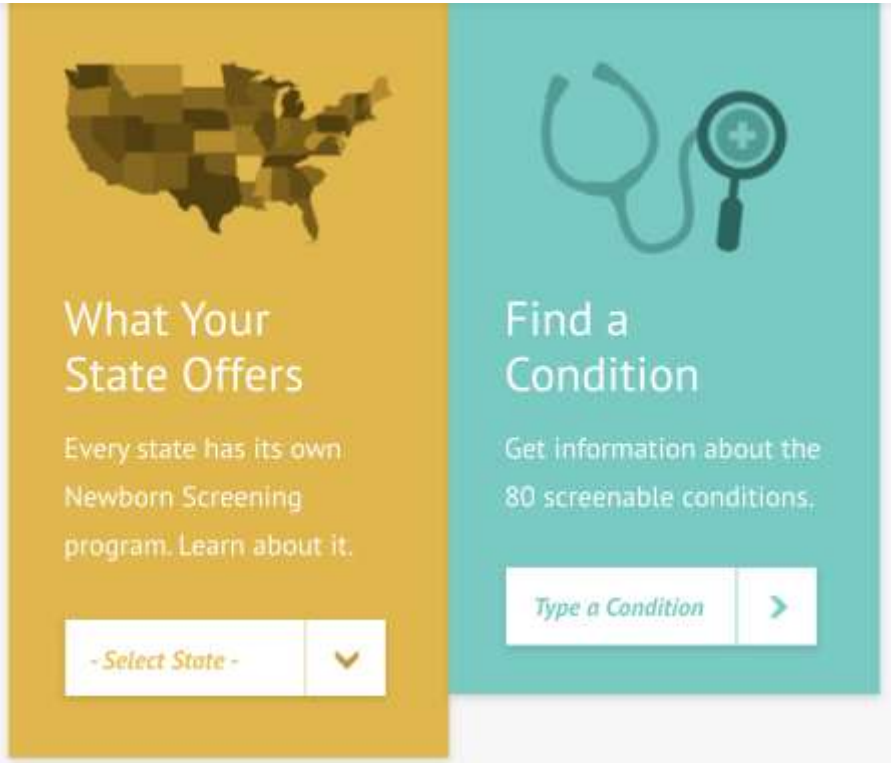
- **The “PKU” Test**

- **Clinical diagnostic program**
  - NBS is a screen
  - False negatives and false positives occur

- **Mechanism to shorten the diagnostic odyssey for all conditions**
  - Not all conditions belong on the NBS (criteria)
  - Not a replacement for early clinical recognition and evaluation

Recommended Newborn Screening Panel

- National *Guideline* for NBS through HRSA
- 36+ core conditions
- 25+ secondary targets
- **http://BabysFirstTest.org**



baby's first test

Recommended Newborn Screening Panel

UNIFORM PANEL				
MS/MS				
Acylcarnitines		Amino acids		
(9) OA	(5) FAO	(6) AA	(3) Hematology	(15) Others

IVA	MCAD	PKU	Hb SS	CH
GA-I	VLCAD	MSUD	Hb S/ß Th	BIOT
HMG	LCHAD	HCY	Hb S/C	CAH
MCD	TFP	TYR I		GALT
MUT	CUD	ASA		HEAR
Cbl A,B		CIT		CF
3MCC				
PROP				
BKT				

Pompe GAMT
MPS1, 2 Krabbe*
xALD MLD
SCID DMD

NBS: Challenges

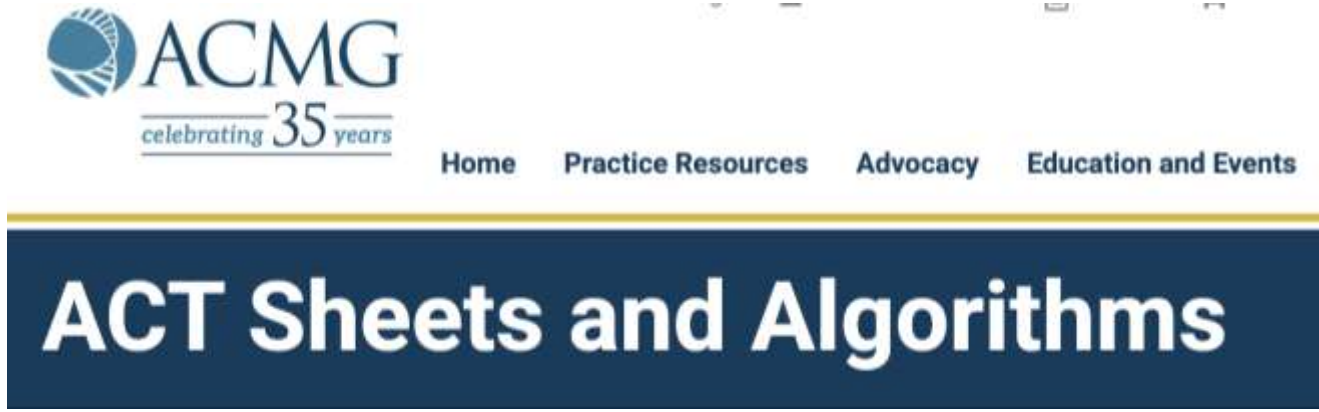
- Critically ill neonates (prematurity, sepsis, etc)
  - Very-low birthweight
  - Total parenteral nutrition
  - Blood transfusions
  - Low WBC in neonates (Enzyme)

- Performance
  - Proper collection of blood spot cards is critical

- Weather/Handling
  - Heat may falsely reduce enzymatic activity
    - Biotinidase, GALT, GALC, ARSA, etc.

NBS: It's abnormal… Now What?

- **Read the report:**
  - Many possible issues and possible recommendations
    - Repeat
    - Refer

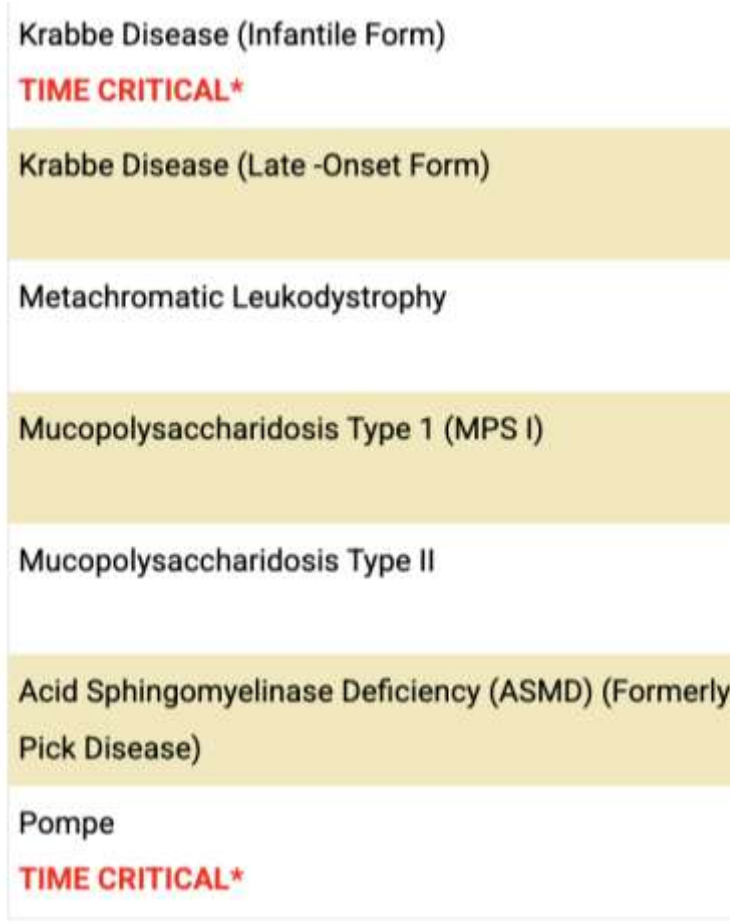


- **ACT Sheets:**
  - For ALL RUSP Disorders + Others
  - Just Google it: ACMG ACT Sheet
  - https://www.acmg.net/ACMG/Medical-Genetics-Practice-Resources/ACT_Sheets_and_Algorithms.aspx

- **Call:** 202-476-5000: Genetics Newborn Screening Pager
  - Or Endocrine (TSH/CAH), Pulmonary (CF), Neurology (SMA/ →DMD), etc.

NBS: It's abnormal… Now What?

- **Don't Panic**
- **Not all Abnormals/Criticals are created equal**
  - Many don't need to be called out in the middle of t night/over the weekend
  - Some DO need to be called out STAT/ASAP:
    - **ACT Sheet! → TIME CRITICAL***
- **Critical ≠ TIME CRITICAL***
- **If you aren't sure, phone a friend!**



NBS: Newer Conditions

- Lysosomal Storage Disorders
  - MPS1, MPSII
  - Pompe Disease
  - Krabbe Disease
  - Metachromatic Leukodystrophy (MLD)
- Peroxisomal Disorder: X-Linked Adrenoleukodystrophy (X-ALD)
- SCID (& other T cell deficiencies)
- Creatine Biosynthesis Disorder: Guanidinoacetate methyltransferase (GAMT) deficiency
- Duchenne/Becker Muscular Dystrophy

X-ALD: Phenotypes

Childhood Cerebral X-ALD (cCALD, 35%) >24-36 months

- Rapid deterioration to complete disability 6-24 months
- Most (90%) have adrenal insufficiency
- **Fatal if undetected/untreated**

Adrenomyeloneuropathy (AMN) (45%) >~20 years

- Spastic paraparesis
- Bowel/bladder/sexual dysfunction
- Many (70%) have adrenal insufficiency
- May progress to aCALD

Addison only (10%)

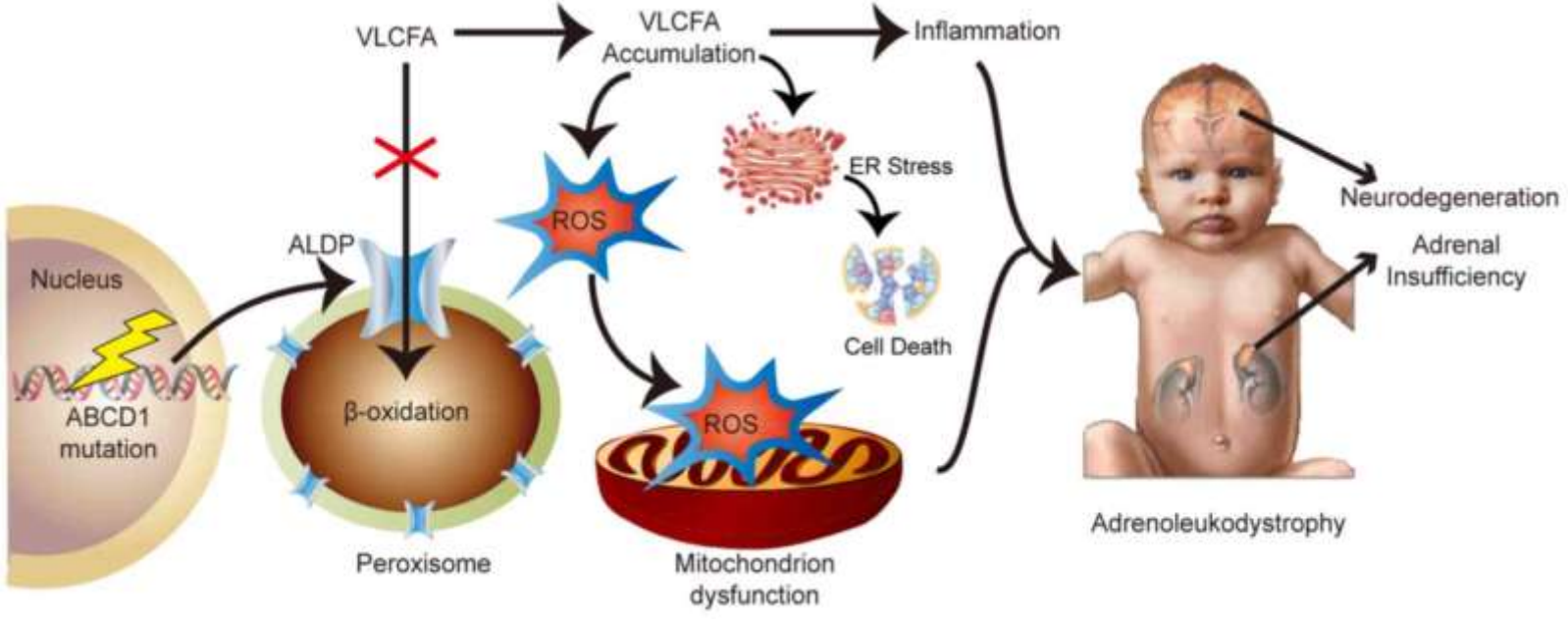
- Adrenal insufficiency without nervous system involvement

Heterozygote Females

- >20% may develop spastic paraparesis/AMN
- Executive dysfunction

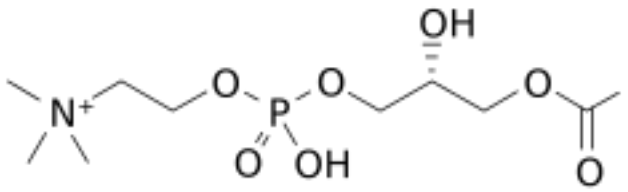
X-ALD: Mechanism

- *ABCD1* encodes peroxisomal binding protein: ALDP transporter
- Expressed primarily in brain, adrenal gland, testes
- Elevated very long chain fatty acids
  - C26
  - C24.0:C22.0
  - C26.0:C22.0



X-ALD: NBS & Beyond

- **Primary Analyte:** C26LysoPC



C26

- **Confirmatory Testing**
  - VLCFA, C26LysoPC
    - Diagnostic!
  - *ABCD1* gene sequencing → Expansive sequencing for negative cases
    - VUS
    - Grey Zone Project
- **Cascade Testing of Family**
  - HSCT donors
- **Surveillance protocols** for multidisciplinary management → Myelin Disorders Program @ CNH
- **Treatment:** HSCT > Gene Therapy

X-ALD: NBS False Positive and Incidental Findings

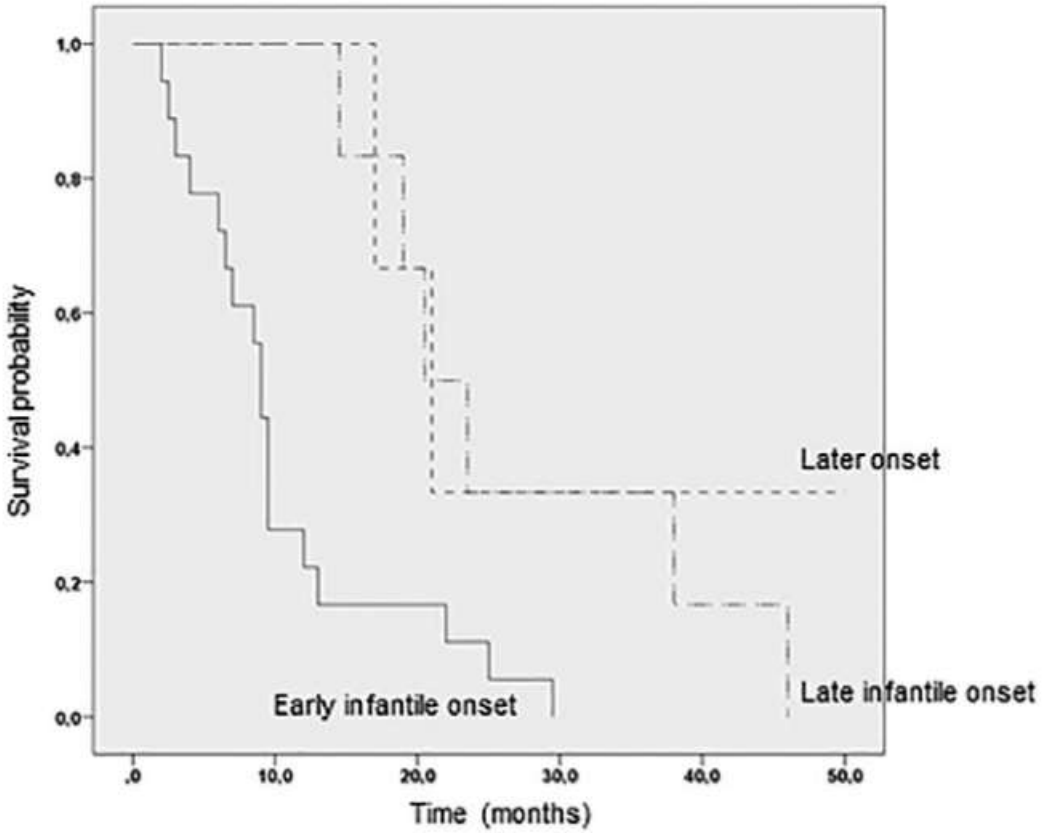
- **Incidental Diagnoses**
  - Zellweger Spectrum Disorders
  - D-Bifunctional Protein Deficiency
  - Aicardi-Goutières Syndrome

- **Maternal Lupus**

- **Maternal consumption of common high-fat foods**
  - Peanut Butter
  - Coconut Oil

Krabbe Disease: Phenotypes

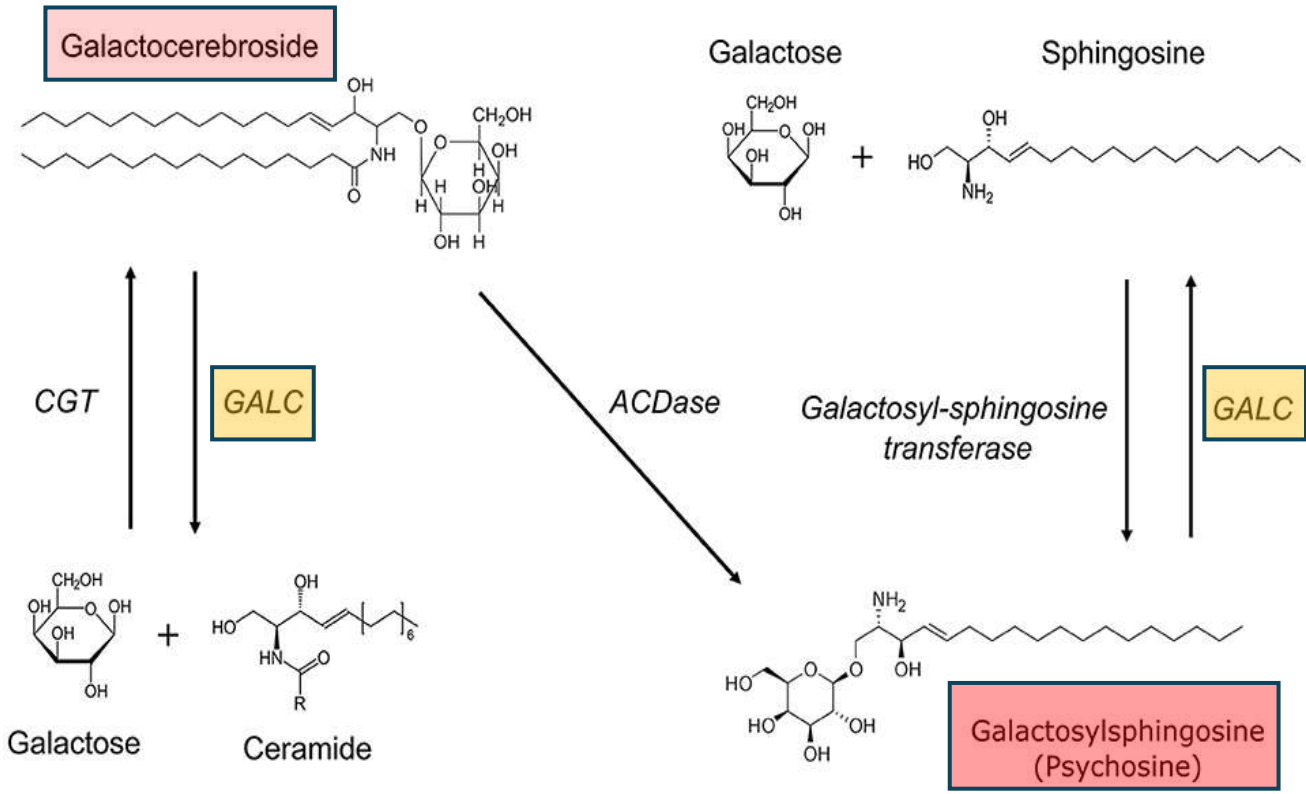
- **Infantile form (onset ≤ 12 mo)**
  - Death by 2
  - Hyperirritability, hypersensitivity, spasticity, seizures, demyelinating polyneuropathy, optic atrophy
- **Late infantile (onset 1 yr – 3 yrs)**
- Similar to infantile, but longer life expectancy
- **Juvenile (onset 3 yr – 8 yrs)**
  - Vision loss, spasticity, ataxia, gait disturbance, cognitive impairment
- **Adolescent-adult form (onset ≥ 10 yrs)**
  - Spastic paraparesis, weakness, vision loss, neuropathy
- **CNS and PNS**
- **Enzyme activity: phenotype**
- **Genotype: Phenotype**



Krabbe: Mechanism

β-galactocerebrosidase Enzyme (GALC)

- Expressed by all brain cells
- Oligodendrocytes
- Schwann cell dysfunction



- Accumulation galactosylsphingosine “psychosine”
  - Toxic
  - Demyelination → severe white matter injury → loss
    - Regression → Neuropathy → Autonomic Dysregulation → Death

Krabbe: Long-Term Management and Therapies

- Hematopoietic stem cell transplantation

- ~~Gene therapy clinic trials~~

FORGE BIOLOGICS
A member of Ajinomoto Bio-Pharma Services

January 28, 2025

Update on Forge Biologics' FBX-101 Clinical Program

Dear Krabbe Community,

We are writing to share an update regarding Forge Biologics' clinical program, FBX-101, for patients with Krabbe disease. After a holistic and strategic review, we have made the difficult decision to discontinue our development of the FBX-101 program. This was not an easy decision, and we recognize the impact this news will have on the Krabbe community and all those searching for an effective treatment for this devastating disease.

Through careful evaluation, we determined that Forge is no longer optimally positioned to progress and support the additional development needed to ensure the success of FBX-101. Advancing it further would require resources and expertise beyond our current scope as we focus on our core mission of advancing gene therapy manufacturing.

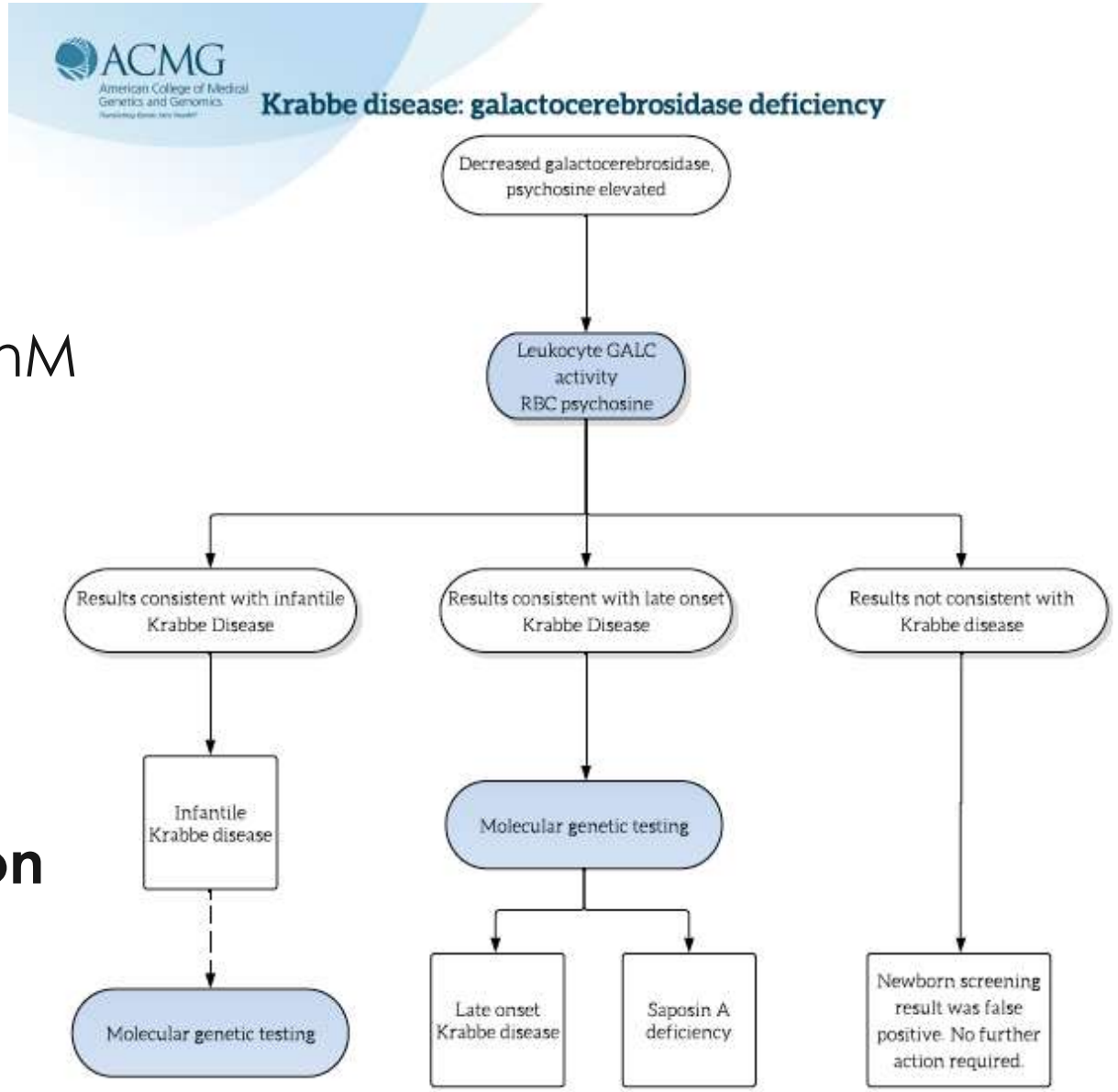
Krabbe Disease: Genetics

- > 140 disease-associated variants *GALC*
  - 30-kb deletion (also called 502T/del)
    - Most common in populations of European ancestry
    - ~40% of infantile-onset KD alleles
  - Two missense variants (p.T513M and p.Y551S) ~10–15% of the remaining infantile KD alleles in Europeans.
  - p.G270D common in later-onset KD

- Pseudodeficiency:
  - Three common polymorphisms (p.R168C, p.D232N, p.I546T)
  - Attenuate GALC activity
  - Low in vitro enzyme activity
  - Do not cause disease independently

Krabbe: NBS & Beyond

- **First Tier:** GALC Activity (<20% MDA)
- **Second Tier:** Psychosine*
  - RUSP: >10nM
  - Clinical Reference Labs report >1-2 nM
- **Confirmatory Testing**
  - GALC Activity (WBC)
  - Psychosine (Blood, CSF)
  - GALC, PSAP sequencing (SAP-A)
    - GALC VUS
- **Protocols for multidisciplinary evaluation and management**



Krabbe: Long-Term Management and Therapies

Table 3
Recommended clinical follow-up schedules for asymptomatic individuals at-risk for LOKD.

Follow-up pathway for individuals at high-risk for onset of LOKD in early childhood

	By 2 mo	4 mo	6 mo	8 mo	10 mo	12 mo	14 mo	16 mo & 18 mo	20 mo	22 mo & 24 mo	26 mo	2.5 yrs	3 yrs	Annual until age 12	Every 2–5 yrs from 12–18 yrs
History	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
PE	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
MRI brain	√		√		√		√		√		√	√	√	√	√
NCS	√		√		√		√		√		√	√	√	+/−	+/−
Psy	√		√		√		√		√		√	√	√	√	+/−
BAER	+/−		+/−		+/−		+/−		+/−		+/−	+/−	+/−	+/−	+/−
LP	+/−		+/−		+/−		+/−		+/−		+/−	+/−	+/−	+/−	+/−
VEP	+/−		+/−		+/−		+/−		+/−		+/−	+/−	+/−	+/−	+/−

Follow-up pathway for individuals at low-risk for onset of LOKD in early childhood

	By 6 mo	12 mo	18 mo	24 mo	Annual until age 12	Every 2–5 yrs until age 12	Every 2–5 yrs from 12–18 yrs
History	√	√	√	√	√	N/A	√
PE	√	√	√	√	√	N/A	√
MRI brain			√			√	√
Psychosine			√			+/−	+/−
NCS			√			+/−	+/−
BAER			+/−			+/−	+/−
LP			+/−			+/−	+/−
VEP			+/−			+/−	+/−

PE = physical examination, MRI = magnetic resonance imaging, NCS = nerve conduction study, Psy = psychosine, BAER = brainstem auditory evoked potential, LP = lumbar puncture, VEP = visual evoked potentials.

Enzyme Activity (NYS Experience)

- Lysosomal Diseases Testing Laboratory at TJU
- 2006 to 2011, risk stratified based on GALC activity analysis
  - high ≤0.15
  - moderate = 0.16–0.29
  - low = 0.3–0.5
  - no risk >0.5
- 2012
  - high ≤0.15
  - moderate = 0.16–0.29
    - (or 2 potentially disease-associated variants with GALC 0.3–0.5)
  - no risk >0.3

Krabbe Disease: NY State Experience

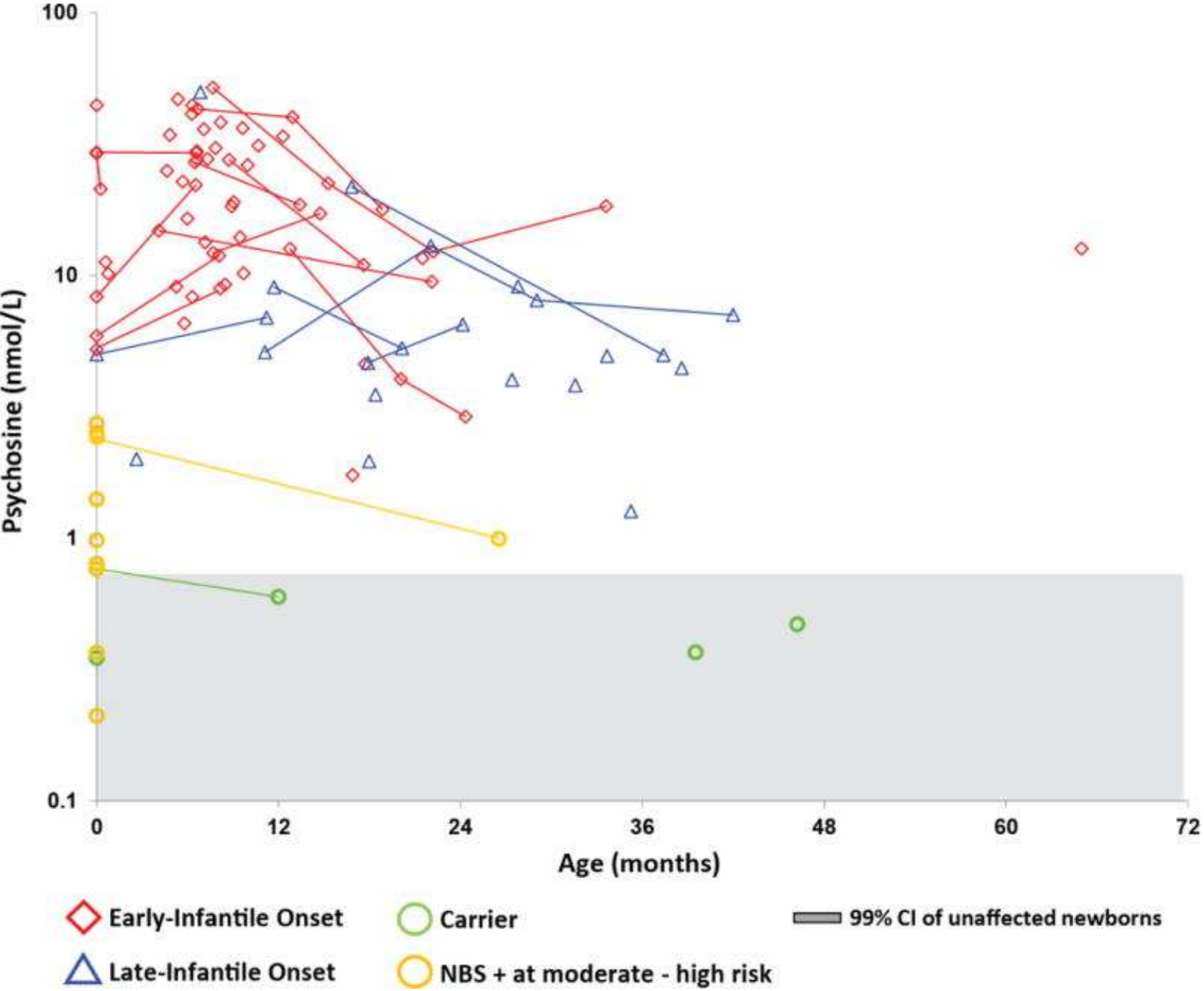
- Infants at high risk for KD
  - (combination of enzyme activity and genotype)
- 14 infants with high risk for KD
  - five confirmed EIKD
    - at least one copy of the 30-kb deletion
    - HSCT
    - 2 died from transplant-related complications
  - 9 asymptomatic infants

Risk category[b]	Case #	Gender	Age at last contact	Allele 1[c,d,e]	Allele 2[c,d,e]	Average newborn screening GALC activity μmol/hour/l (% DMA)	Diagnostic testing GALC activity nmol/hour/mg[c]
High risk, infantile KD ($N = 5$)	1	M	8 y	30-kb del+p.R168C	c.-335G>A+p.D94=+p.I546T+p.*670Qext42	0.41 (9.9%)	0.01
	2	M	3 m (d)	30-kb del+p.R168C	30-kb del+p.R168C	0.43 (10.9%)	0.05
	3[f]	M	18 m (d)	30-kb del+p.R168C	30-kb del+p.R168C	0.34 (7.6%)	0.02
	4	F	5 y	30-kb del+p.R168C[g]	c.-335G>A+**p.G360Dfs*2#**[g]	0.22[h] (5.6%)	0.12
	5[f]	F	2.5 m (d)	30-kb del+p.R168C	30-kb del+p.R168C	0.20[h] (4.3%)	0.05
High risk ($N = 9$)	6	M	8 y	p.A5P+p.D232N+p.Y303C	p.A5P+p.D232N+p.Y303C	0.26 (6.1%)	0.06
	7	M	7 y	p.A5P+p.D232N+p.Y303C	p.I546T+**p.D556fs*1#**	0.31 (8.3%)	0.12
	8	F	4 y	p.H375Qfs*3+p.I546T	**c.-348C>T#**+p.A5P+p.D232N+p.Y303C	0.36 (9.6%)	0.07
	9	M	6 m	c.-128_-123delATCAGC+p.L618S	p.L618S	0.37 (9.1%)	0.12
	10	M	5 y	p.M101V+c.1786+5C>G+p.A625T	p.M309V+p.I546T	0.20[h] (4.7%)	0.03
	11	M	4 y	c.147G>C/p.G49=+p.I546T	**p.K83E#**+p.I546T	0.25 (6.2%)	0.05
	12	M	6 m	p.T452I	p.A5P+p.D232N+p.Y303C	0.21[h] (4.9%)	0.05
	13	F	2 y	p.R63C+p.I546T	p.R111*	0.45 (10.8%)	0.09
	14	F	13 m	c.-335G>A+p.I546T+p.R380W	c.-128_-123delATCAGC+p.L618S	0.48 (8.0%)	0.07

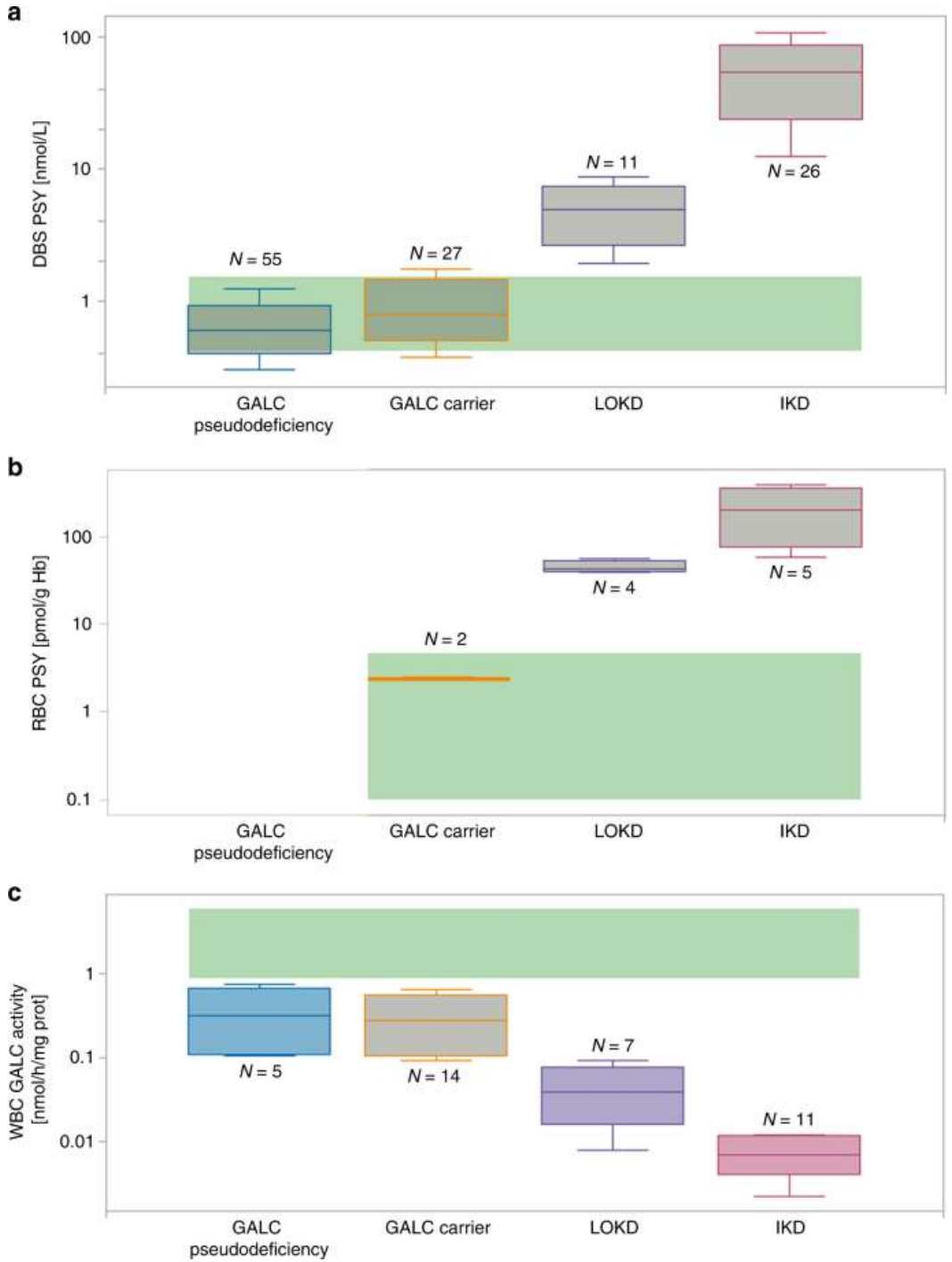
(d), age at death; GALC, galactocerebrosidase; m, months; NCBI, National Center for Biotechnology Information; y, years.

[a]One infant who was initially classified as being at high risk (GALC = 0.09) was reclassified to the moderate risk group after repeat testing at age 4 years old revealed GALC activity of 0.21 is not included in this table. Details for this infant can be found in **Supplementary Table S4** online. [b]Risk category determined from diagnostic testing, as described in Materials and Methods (high risk GALC ≤0.15). [c]Genotype phase estimated from parental data, where available. [d]Synonymous variants including p.G9=, p.L117=, p.S434=, p.Q312=, p.T524=, and p.V550= and noncoding variants including c.-196T>C and c.-7G>C that are not predicted to affect protein function are not included in table. All alleles with p.A5P also carried c.-196T>C and c.-7G>C. [e]Variants are numbered using traditional nomenclature (downstream initiator as codon 1). Variants not previously reported and not catalogued in dbSNP (NCBI), ClinVar (NCBI), EmVClass (Emory Genetics Laboratory), or the ExAC database (Exome Aggregation Consortium) are indicated by # and shown in bold. [f]Cases 3 and 5 are siblings. [g]Only one parent available for testing. [h]Values are estimates and are below the LOD of 0.24 μmol/hour/l.

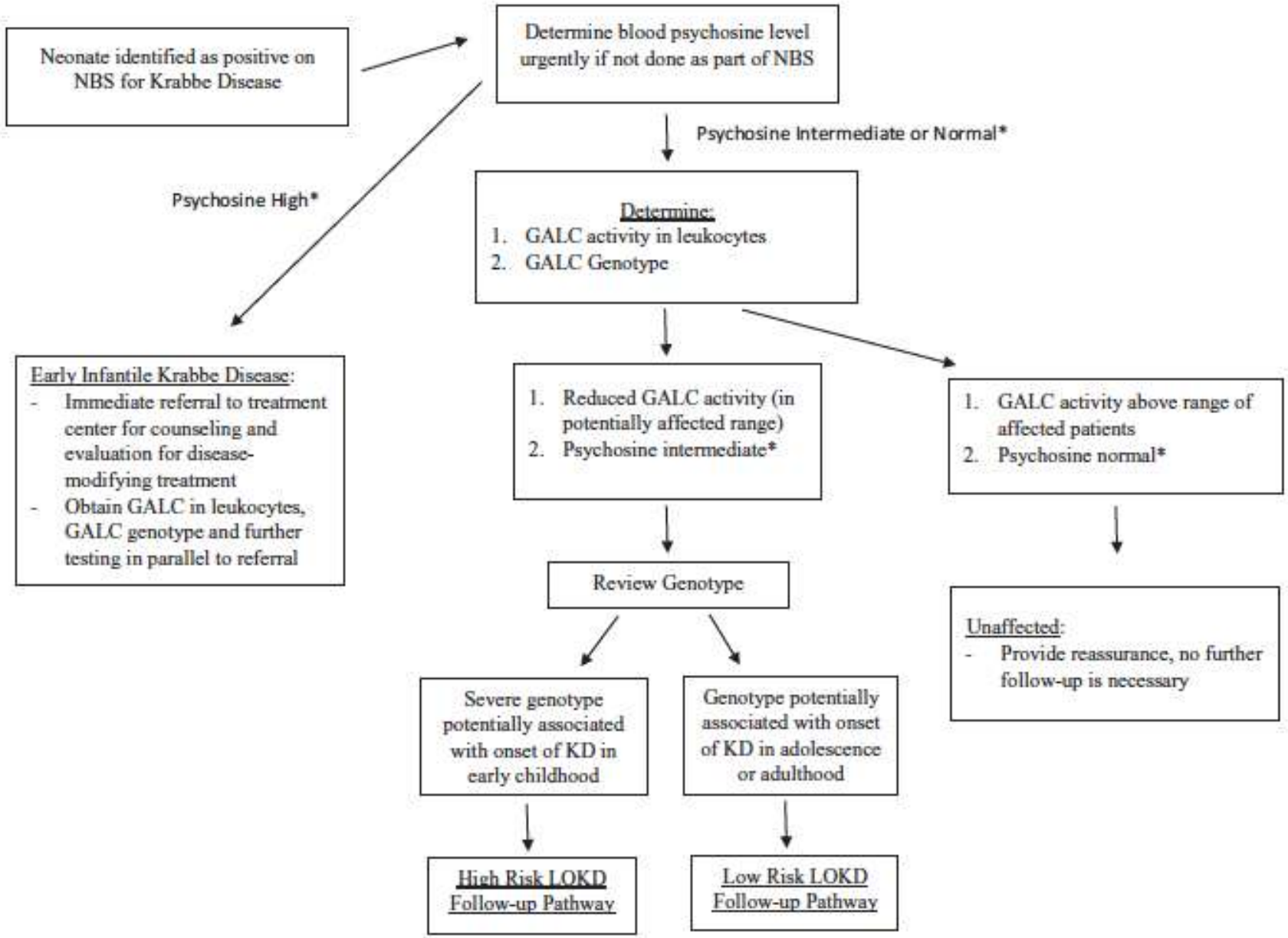
Psychosine


100
10
1
0.1
Psychosine (nmol/L)
0
12
24
36
48
60
72
Age (months)
Early-Infantile Onset
Late-Infantile Onset
Carrier
NBS + at moderate - high risk
99% CI of unaffected newborns

Psychosine


a
DBS PSY [nmol/L]
100
10
1
N = 55
N = 27
N = 11
N = 26
GALC pseudodeficiency
GALC carrier
LOKD
IKD
b
RBC PSY [pmol/g Hb]
100
10
1
0.1
N = 2
N = 4
N = 5
GALC pseudodeficiency
GALC carrier
LOKD
IKD
c
WBC GALC activity [nmol/h/mg prot]
1
0.1
0.01
N = 5
N = 14
N = 7
N = 11
GALC pseudodeficiency
GALC carrier
LOKD
IKD

Krabbe: Post-NBS Care



Krabbe: Post-NBS Care

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NCS	√		√		√		√		√		√	√	√	+/−	+/−
Psy	√		√		√		√		√		√	√	√	√	+/−
BAER	+/−		+/−		+/−		+/−		+/−		+/−	+/−	+/−	+/−	+/−
LP	+/−		+/−		+/−		+/−		+/−		+/−	+/−	+/−	+/−	+/−
VEP	+/−		+/−		+/−		+/−		+/−		+/−	+/−	+/−	+/−	+/−

Follow-up pathway for individuals at low-risk for onset of LOKD in early childhood

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PE	√	√	√	√	√	N/A	√
MRI brain			√			√	√
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VEP			+/−			+/−	+/−

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Metachromatic Leukodystrophy (Arylsulfatase A deficiency)

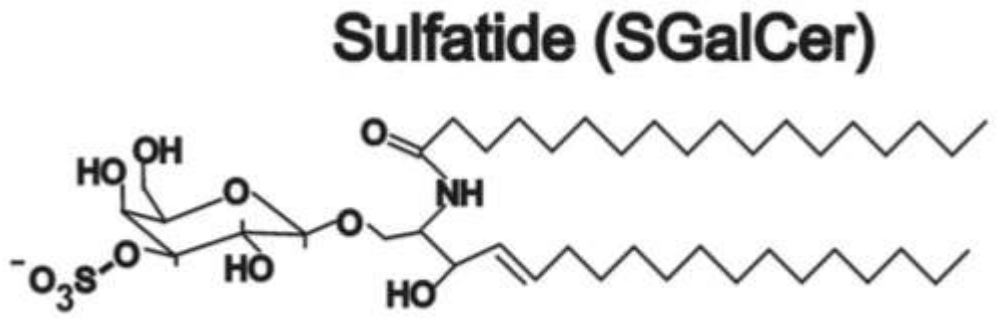
- MLD
- AR, *ARSA and PSAP* genes
- Arylsulfatase A deficiency leads to sulfatide accumulation
- Diagnosis: ARSA enzyme activity, urine sulfatides, molecular
- PSAP: SAP-B (SAPOSIN-B) = co-factor to ARSA
- Other features: peripheral neuropathy, gallbladder polyps

Metachromatic Leukodystrophy: Phenotypes

	Late infantile	Juvenile	Adult
Onset	< 30 months	< 16 yr	> 16 yr
Symptom	Motor decline	School decline, gait difficulty	Psychiatric, behavior change
Prognosis	< 5 years	< 20 years	variable

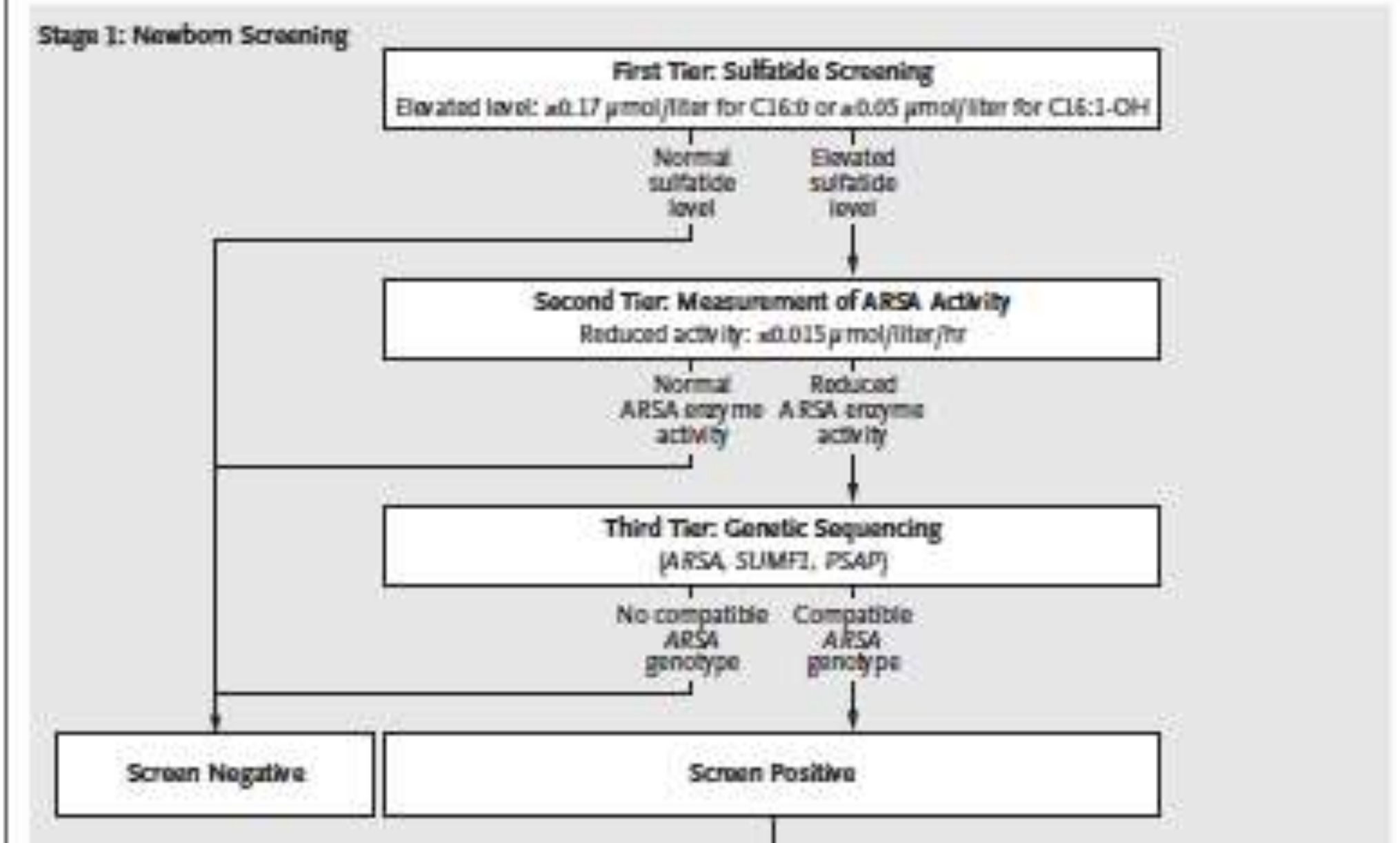
MLD: NBS

- Primary Analyte: Sulfatide
- Second Tier: ARSA enzyme activity (<20% MDA)

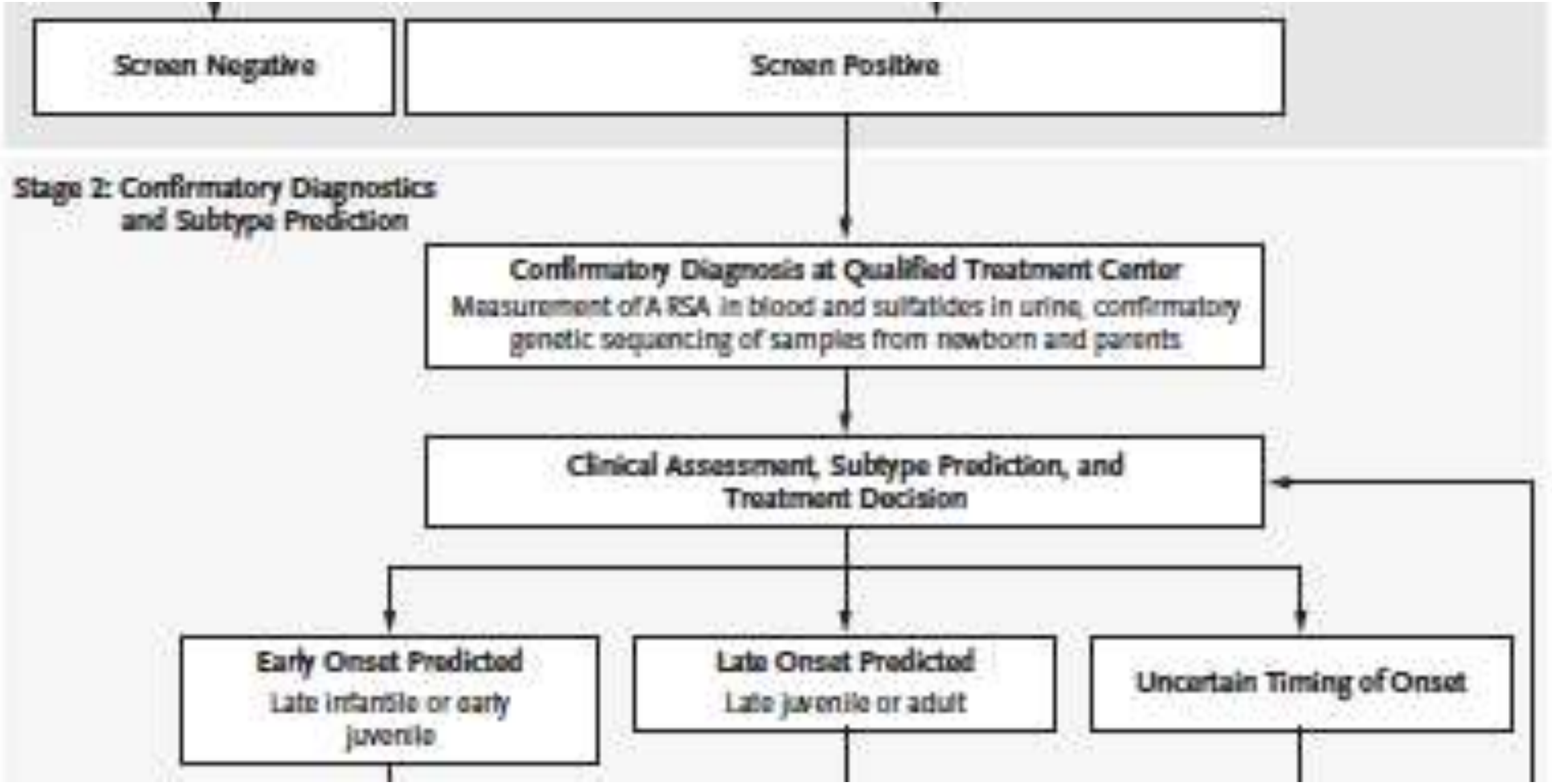


- Confirmatory Testing
  - Sulfatides
  - GALC Activity
  - *ARSA, PSAP, SUMF* sequencing
    - VUS
    - ARSA variants – active research for enzyme effects
- Surveillance protocols for multidisciplinary management
- Treatments!
  - Autologous ex vivo gene therapy
  - HSCT

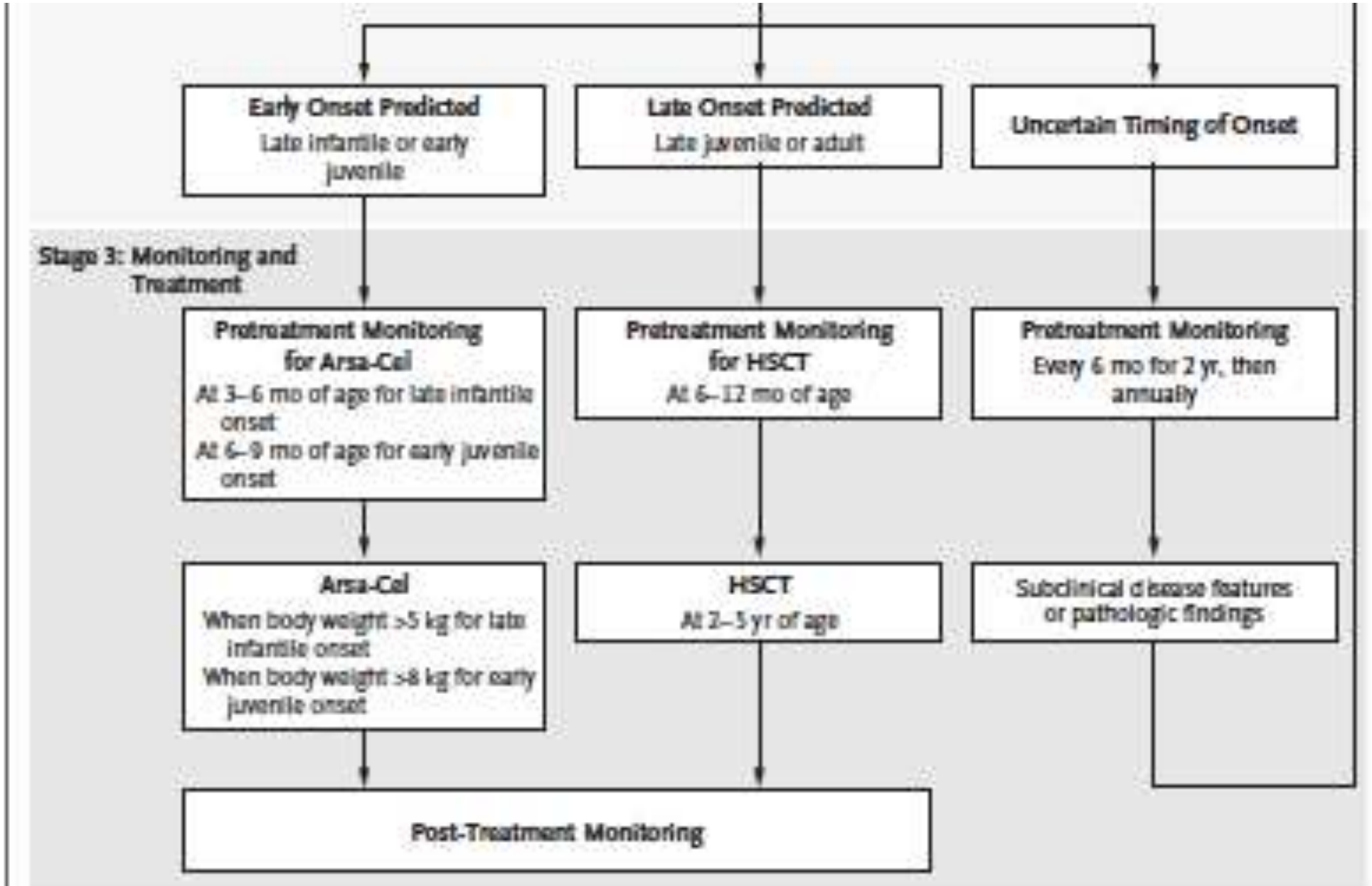
MLD: NBS


Stage 1: Newborn Screening
First Tier: Sulfatide Screening
Elevated level: ≥0.17 μmol/liter for C16:0 or ≥0.05 μmol/liter for C16:1-OH
Normal sulfatide level
Elevated sulfatide level
Second Tier: Measurement of ARSA Activity
Reduced activity: ≤0.015 μmol/liter/hr
Normal ARSA enzyme activity
Reduced ARSA enzyme activity
Third Tier: Genetic Sequencing
(ARSA, SUMF1, PSAP)
No compatible ARSA genotype
Compatible ARSA genotype
Screen Negative
Screen Positive

MLD: NBS → Diagnosis



MLD: NBS → Long Term Management



MLD NBS: Incidental Diagnoses

- **Incidental Diagnoses**
  - PSAP Deficiency
  - Multiple Sulfatase Deficiency

Leukodystrophy NBS Summary

- X-ALD (DC, VA, MD)
- Krabbe (MD)
- MLD (MD ETA 10/2026)

NBS Is NOT Fool-Proof!

- **Just because a condition is on your local NBS, your patient may not have been screened**
  - Birthplace
  - Birth year
  - Parental opt-out
  - Ex: X-ALD (2015)
    - DC: 2017
    - VA: 2019
    - MD: 2024
- **Always maintain clinical suspicion if the phenotype fits**
  - False Negatives
  - Later-onset disease

References

- Baby's First Test (http://www.babysfirsttest.org/)
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