# Carrier detection for Tay-Sachs disease:

## a model for genetic disease prevention


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# Conflict of Interest

- None to declare

# Learning objectives

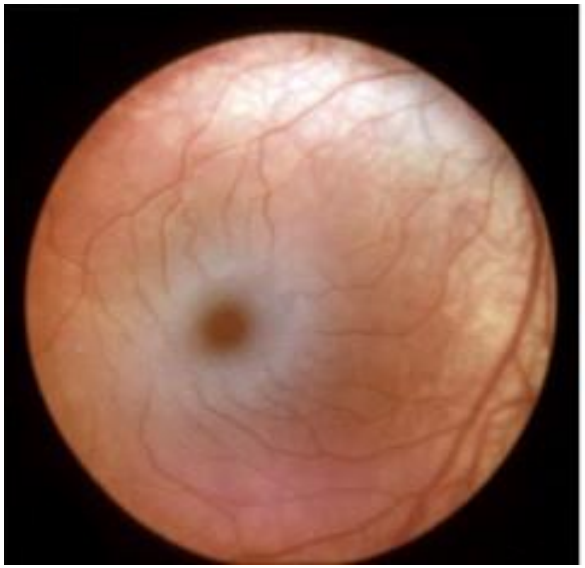
- Review the clinical characteristics and the biochemical features of Tay-Sachs Disease
- Describe the population-based screening for Tay-Sachs disease and its impact on disease incidence
- Explore the unique challenges in carrier testing for Tay-Sachs disease

# Cherry red spot

## Warren Tay

British ophthalmologist

In 1881, he described the **cherry red spot** on the retina of a one-year old child with **mental and physical retardation**



**NOVEL collection**
University of Utah

## Bernard Sachs

Jewish-American neurologist

In 1896, observed the **extreme swelling of neurons** in autopsy tissue of affected children

Also noticed the disease seemed to be of **Jewish origin**

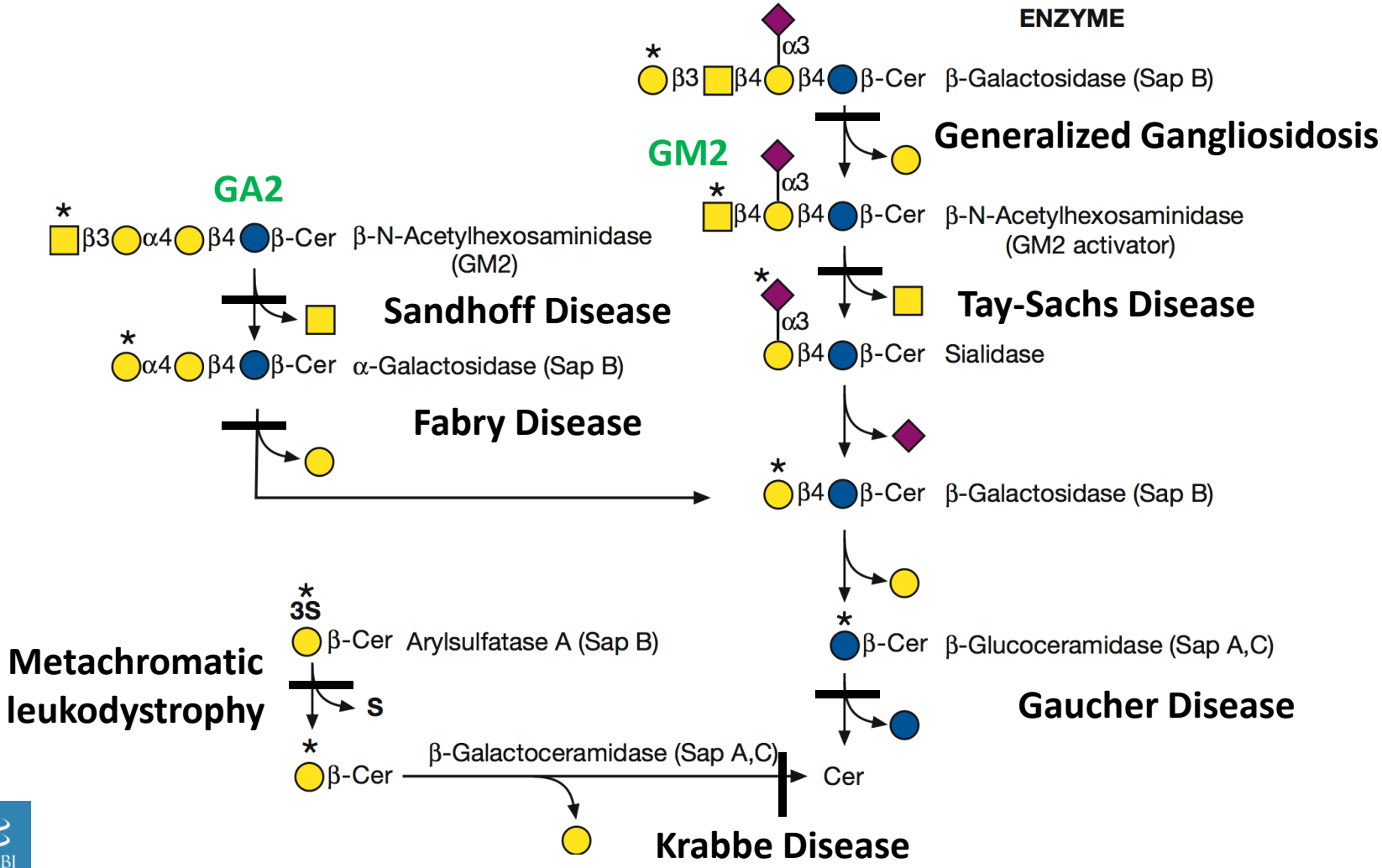
# TSD is a lysosomal storage disease

- The underlying biochemical defect is the profound deficiency of the lysosomal hydrolase β-hexosaminidase A

- HexA is necessary for the break-down of the ganglioside GM2, a component of the plasma membrane

Okada *et al*. Science 1969; 165:698-700

# Degradation of glycosphingolipids




*Essentials of Glycobiology*
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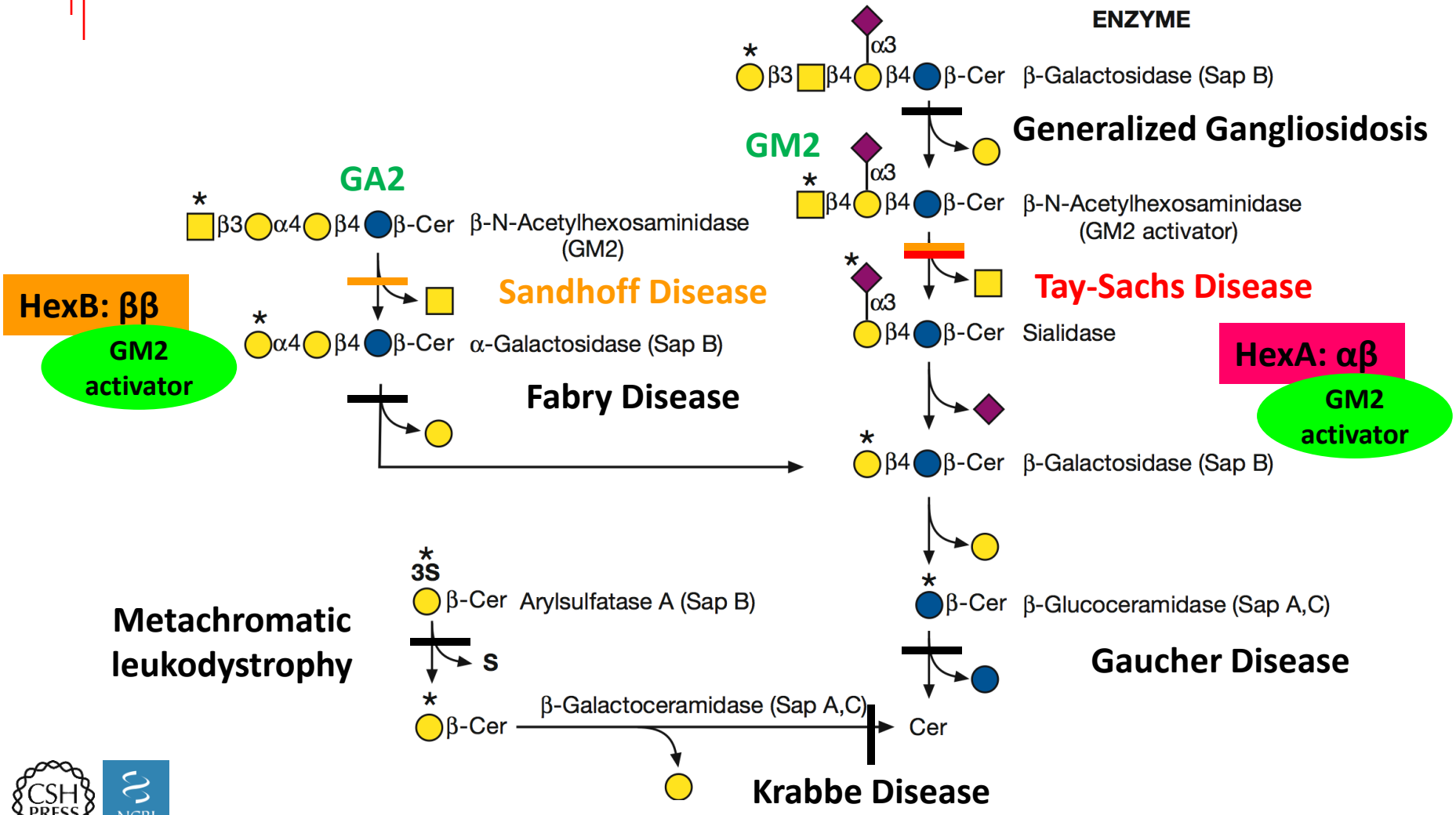
# β-hexosaminidase isoforms: HexA and HexB

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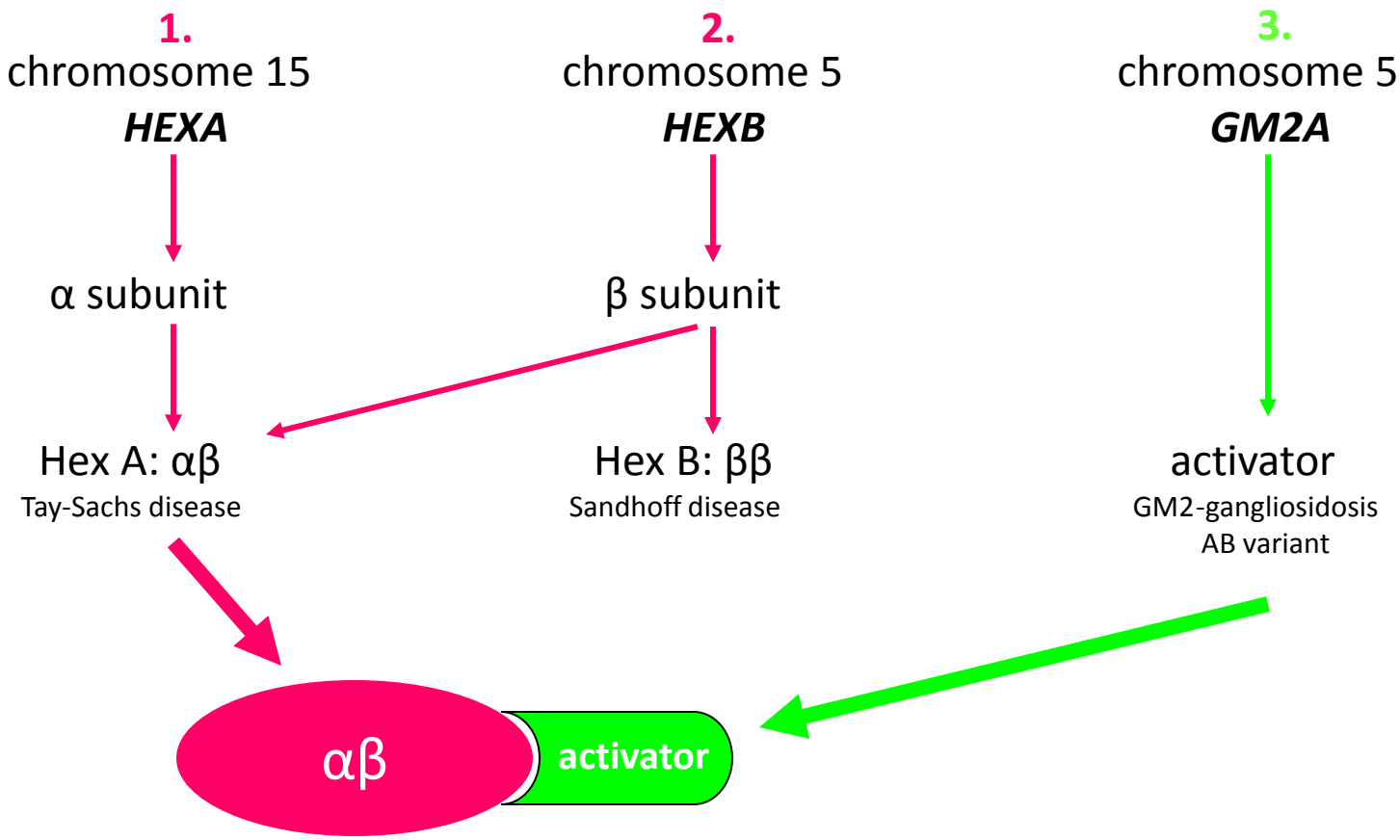
# Three gene system required for HexA activity



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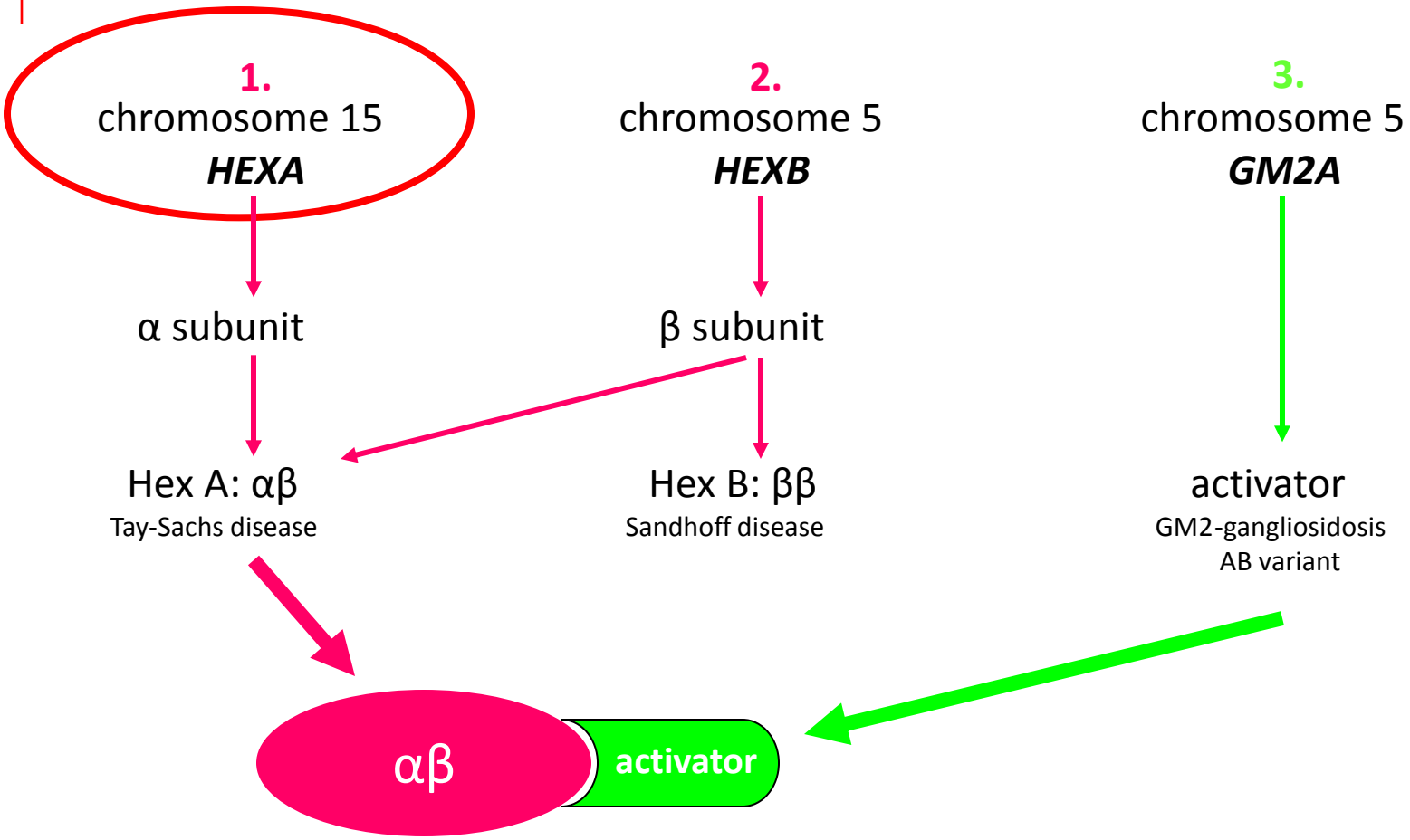



## TSD mode of inheritance: autosomal recessive

# TSD clinical phenotype varies widely

- **Infantile TSD**
  - most prevalent
  - usual onset at 6 months
- **Juvenile TSD**
  - extremely rare
  - onset between ages of 2 and 10 years
- **Late Onset TSD**
  - rare
  - signs and symptoms present in late 20's and early 30's

# Infantile Tay-Sachs Disease

✓ Relentless deterioration of mental and physical abilities beginning around six months of age, and resulting in death by age 5

**3 -6 mo**

- Excessive Startling
- Twitchy eye movement
- Reverse maturation (i.e. failure to walk)

**6 - 10 mo**

- Gradual loss of vision
- Gradual deafness
- Loss of motor skills
- Macrocephaly
- Hypotonia

**After 10 mo**

- Complete blindness
- Strong seizures
- Dementia
- Unresponsive, vegetative state
- Death due to bronchopneumonia between ages 2-5

# Late-onset Tay-Sachs Disease (LOTS)

✓ Juvenile

- Ataxia (beginning at 2-10 years of age)
- Cognitive decline
- Spasticity and seizures
- Loss of vision
- Early death

✓ Chronic adult-onset

- Psychosis, depression, bipolar symptoms
- Progressive dystonia, choreoathetosis, ataxia
- Cognitive dysfunction and dementia

# Diagnostic confirmation for a symptomatic patient

- ✓ **β-hexosaminidase A (HexA) enzymatic activity** in serum or white blood cells using synthetic substrates
  - infantile TSD: 0% - 5% residual activity
  - juvenile or chronic adult-onset TSD: < 15% residual activity

- ✓ **Molecular testing**
  - Confirm diagnosis: mutations in the *HEXA* gene
  - Exclude pseudodeficiency alleles
  - Identify specific disease-causing mutations in at-risk family members and for prenatal diagnosis

# Tay-Sachs Disease Management

Tragically, there is no cure

Affected children can only be made as comfortable as possible

- Adequate nutrition and hydration (feeding tubes)
- Manage infectious disease
- Respiratory care
- Anti-convulsion medications to control seizures
- Antipsychotic or antidepressant therapy (adult-onset TSD)

# Novel Treatments?

- Hematopoietic stem-cell transplantation

**No benefit for neurodevelopmental symptoms, and potential harm for overall survival (Bley et al. 2011)**

- Substrate reduction therapy

**No measurable benefits in late-onset TSD with Miglustat [inhibitor of glycosphingolipids synthesis] (Shapiro et al. 2009)**

- Recombinant beta-hexosaminidase A

***Work in progress*. Difficult to deliver across the blood–brain barrier**

- Pharmacological chaperones

**HexA selective inhibitors, *work in progress* (Rountree 2009)**

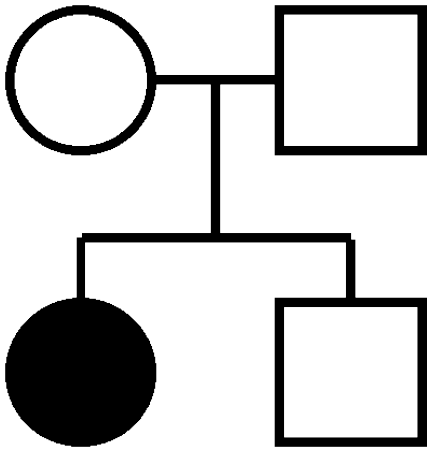
**Possible benefits in late-onset TSD using Pyrimethamine [antimalarial drug that enhances HexA activity] (Osher et al. 2011)**

# Most common in Ashkenazi Jews

- Most common in Eastern Europeans of Jewish descent (Ashkenazi Jews), French Canadians and members of the Cajun community in Louisiana
  - **1:30 carrier frequency**
  - **1:3,600 disease frequency (Infantile Type)**
  - **1:67,000 disease frequency (Adult type)**

- General population
  - **1:300 carrier frequency**
  - **1:320,000 disease frequency (Infantile Type)**

# The importance of being tested



**Carrier testing**

- Screening programs for *at-risk* populations
- Individuals with a positive family history

**ACOG/ACMG guidelines:** *TSD carrier screening should be offered to individuals and couples at high-risk, including those of Ashkenazi Jewish, French-Canadian, or Cajun descent and those with a family history consistent with TSD, as part of routine obstetric care*

ACOG Committee on Genetics committee opinions #318, 2005
ACMG Practice Guidelines. Genet Med 2008; 10(1):54 –56

# TSD carrier screening started in 1971

The screening program for Tay-Sachs Disease started at Johns Hopkins (Dr. Michael Kaback) in 1971

- Originally done by enzyme assay

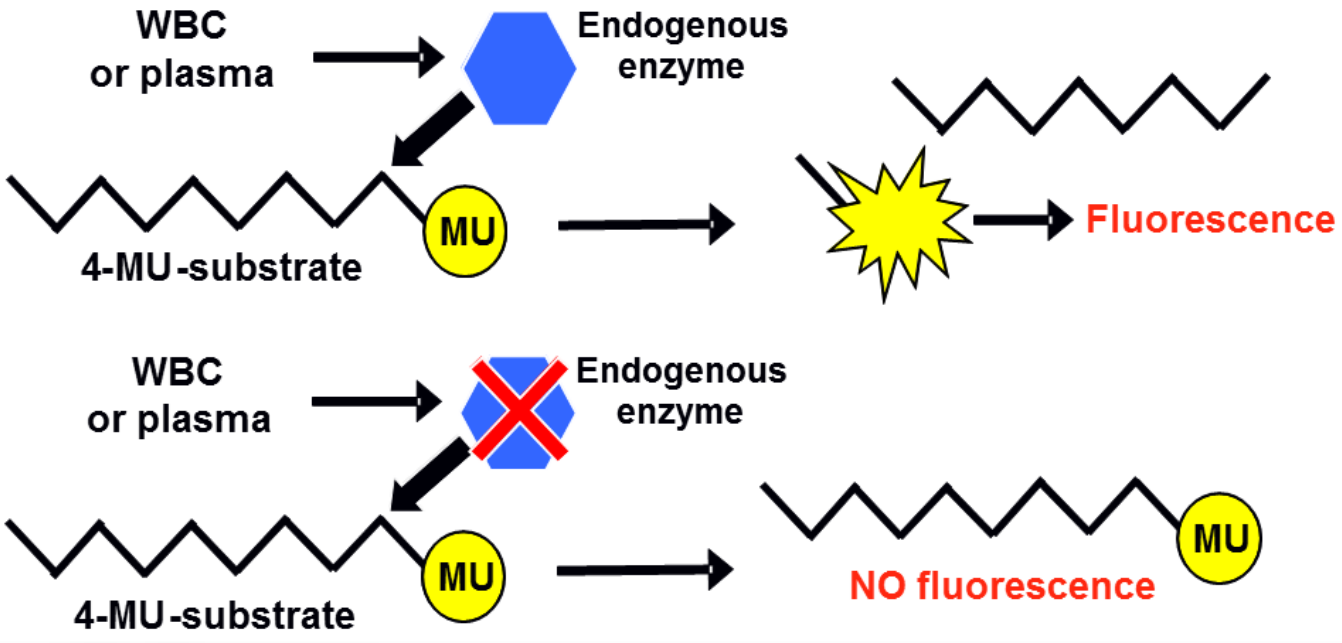
**Rationale**

➢ TSD occurs predominantly in a defined population (Ashkenazi Jews)

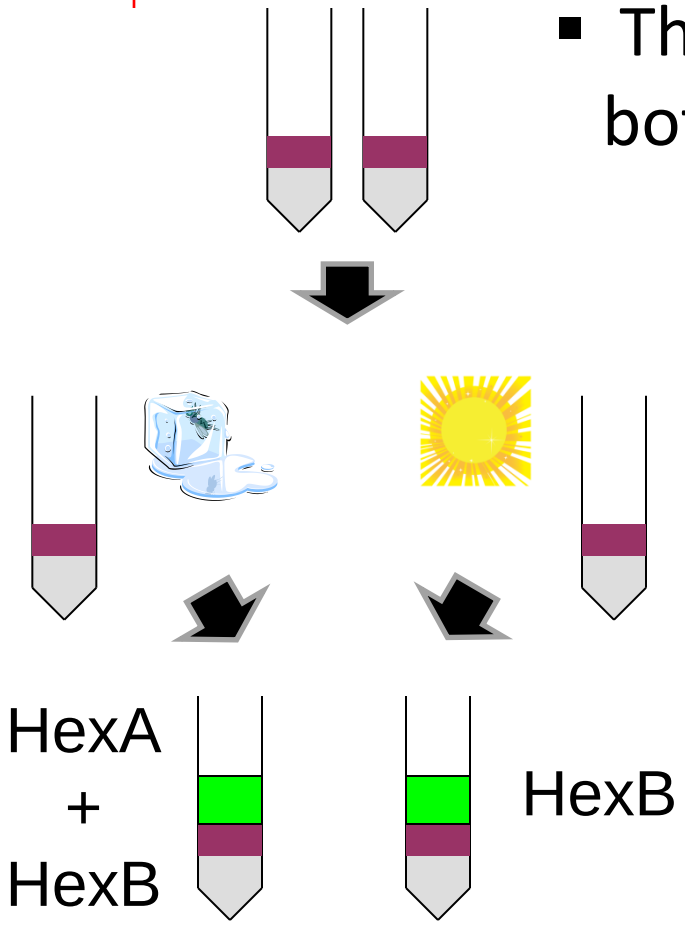
➢ Availability of a simple, inexpensive carrier detection test (serum and/or WBC HexA activity)

# β-hexosaminidase A (HexA) enzymatic assay

- Uses enzyme-specific artificial 4-MU-conjugated substrate
- 4-MU released is measured using a fluorometer



# Measurement of HexA activity




- The fluorogenic substrate measures both the HexA and Hex B activities
  - HexA + Hex B = total activity
- Hexosaminidase A is heat labile
- Heat-inactivation allows to quantify HexA activity as a ratio of total activity

# Carrier status is established by HexA%

| | | |
|---|---|---|
| Carrier of Tay-Sachs disease | ↓ %HexA | ↓/N Total |
| Patients with Tay-Sachs disease | ↓ ↓ %HexA | ↓ ↓ Total |
| Carrier of Sandhoff disease | ↑ %HexA | ↓ Total |
| Patients with Sandhoff disease | ↑↑ %HexA | ↓ ↓ Total |
| Pregnant Women | ↓/N %HexA | ↑↑↑ Total |

Hex A: αβ
Tay-Sachs disease

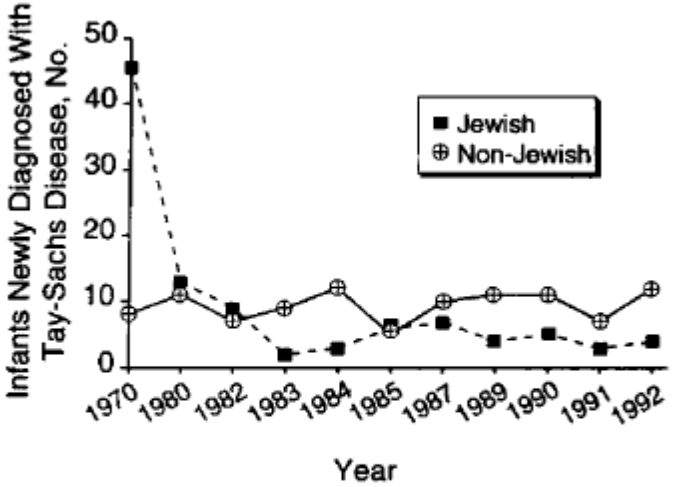
Hex B: ββ
Sandhoff disease

# Prototype for ethnic-based carrier screening

Before population carrier screening the incidence of Tay-Sachs disease was 1:3,600 for Ashkenazi Jewish births

Table 1.—Tay-Sachs Disease Heterozygote Screening, 1971 Through 1992

| Country | No. Tested | Carriers | At-Risk Couples |
|---|---|---|---|
| United States | 712 818 | 27 150 | 657 |
| Israel | 159 544 | 4229 | 263 |
| Canada | 55 922 | 2922 | 57 |
| South Africa | 11 638 | 1286 | 36 |
| Europe | 10 927 | 725 | 21 |
| Brazil, Mexico | 1682 | 96 | 20 |
| Australia | 473 | 10 | 2 |
| **Total** | **953 004** | **36 418** | **1056** |




After implementation of screening, the incidence was reduced by greater than 90%

Kaback M & the International TSD Data Collection Network (JAMA. 1993;270:2307-2315)

"Tay-Sachs Disease represents a prototypic effort in the coordination of adult public education, voluntary carrier testing, and comprehensive genetic counseling directed to the prospective prevention of an unbeatable and uniformly fatal childhood disease"

Kaback M & the International TSD Data Collection Network
(JAMA. 1993;270:2307-2315)

# TSD Biochemical Genetics Testing at ARUP

- Hexosaminidase A Percent and Total Hexosaminidase in Plasma or Serum (2008121)
  - Confirm diagnosis of Tay-Sachs disease
  - Carrier screening in males or non-pregnant females

- Hexosaminidase A Percent and Total Hexosaminidase in Leukocytes (2008125)
  - Carrier status in women who are pregnant or taking oral contraceptives
  - Individuals with inconclusive serum results

- Hexosaminidase A Percent and Total Hexosaminidase in Plasma with Reflex to Leukocytes (2008129)

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# Is it really that simple?

✓ Limitations of the HexA enzymatic test

**False positives**

- Alternative hexosaminidase isoforms
- Pseudodeficiency alleles

**False negatives**

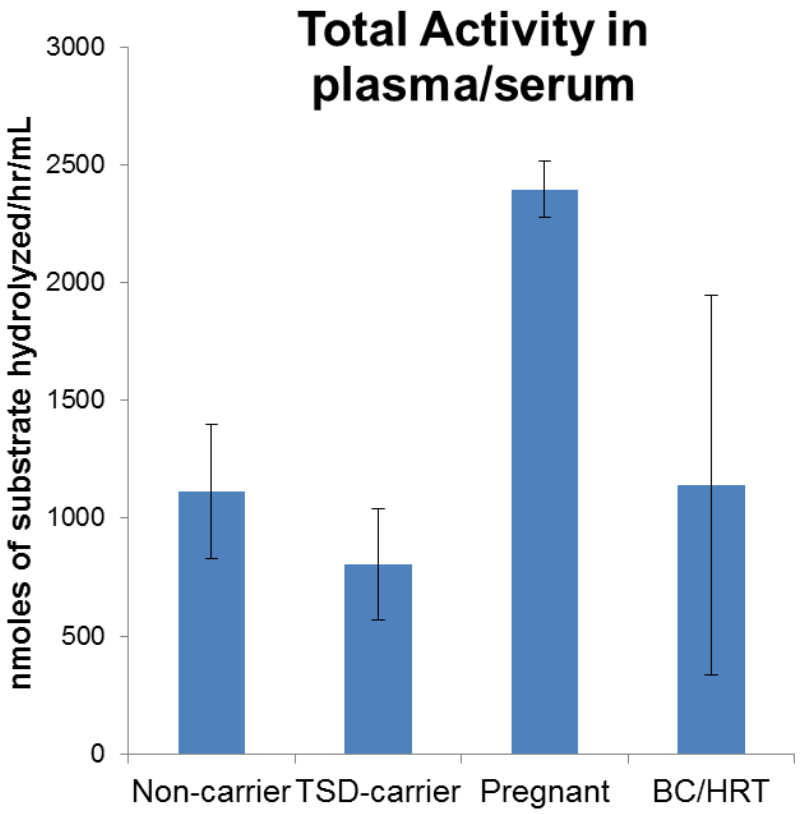
- B1 variant

**Inconclusive results**

# Increases in plasma/serum total hexosaminidase cause false positive

## HexA enzymatic test in plasma/serum
**(May 2013 hotline – August 2014)**

| | |
|---|---|
| Non-carrier | 66% (N = 104) |
| TSD Carrier | 7% (N = 11) |
| Inconclusive | 11% (N = 18) |
| Carrier with ↑↑ total activity | 15% (N = 24) |
| Total | N = 158 (86M, 72F) |

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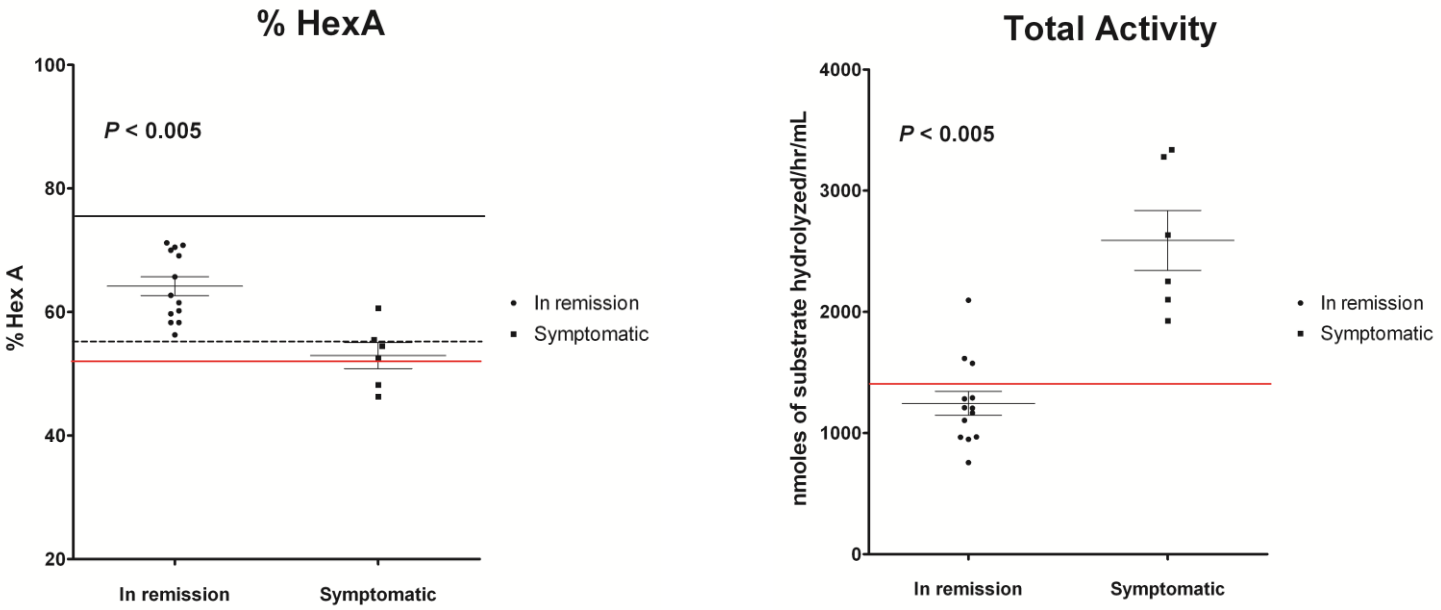
# Alternative heat-resistant forms of Hexosaminidase

Several conditions increase total hexosaminidase activity in serum/plasma, but NOT in leukocytes




**%HexA and total activity in a cohort of patients with symptomatic liver disease or in remission**

# Pseudodeficiency alleles

- General population carrier frequency: 1:300
- General population carrier frequency **by enzyme***: 1:170

## ✓ p.Arg247Trp and p.Arg249Trp

- not associated with disease
- reduce HexA enzymatic activity toward synthetic substrates when activity is determined [the naturally occurring GM2 ganglioside is not stable and not available]
- Molecular genetic testing can be used to clarify

  About 35% of non-Jewish individuals and 2% of Jewish individuals (identified as carriers by HEX A enzyme-based testing) are carriers of a pseudodeficiency allele

*** Triggs-Raine et al. 1992**

# B1 variant

Table 4.—Mutations Associated With Later-Onset Forms of Hexosaminidase-A–Deficient $GM_2$ Gangliosidoses*

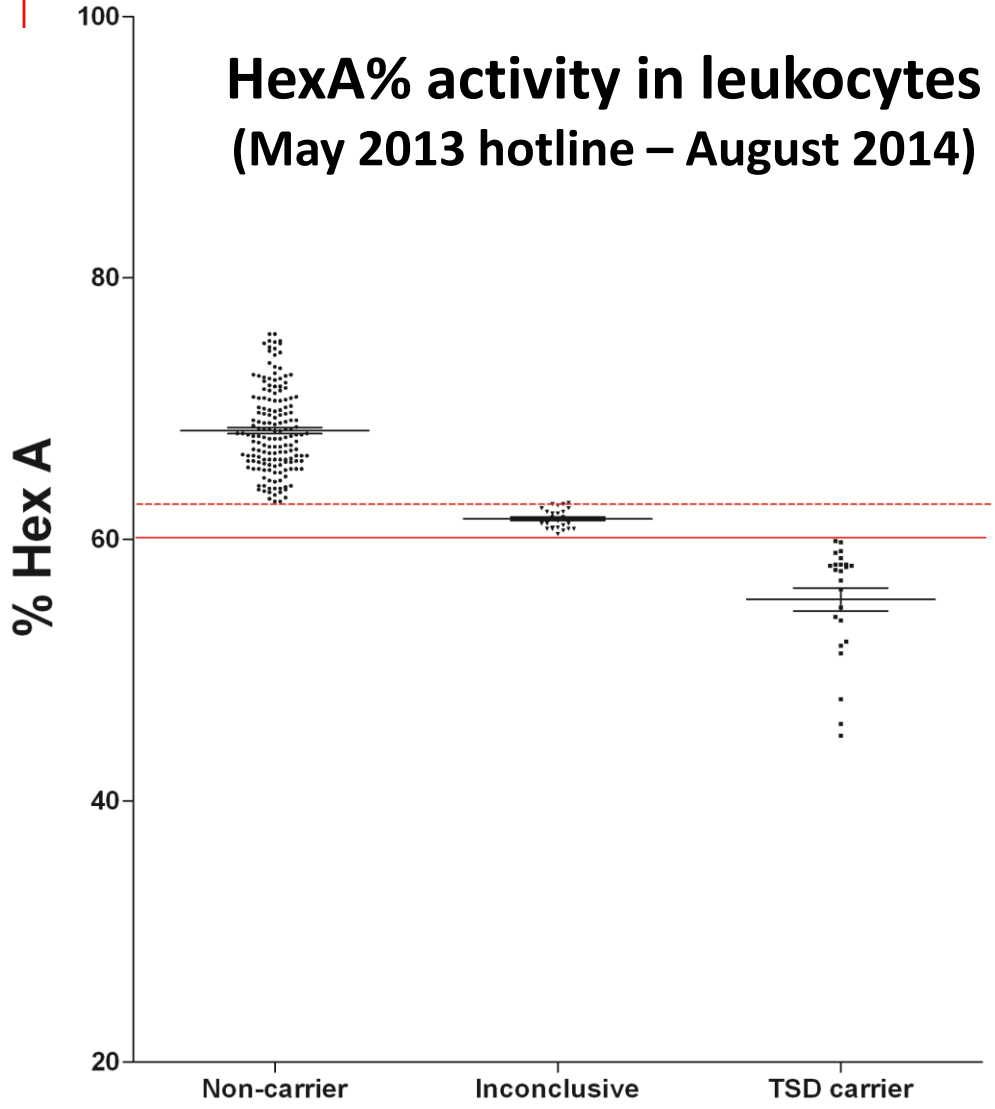
| Form of Disease | Mutation | Ethnic Group |
|---|---|---|
| "$B_1$ Variant" (late infantile or juvenile onset) | Arg178 → His | Portuguese, European |
| | Arg178 → Cys | Czech |
| | 5 other mutations reported | Diverse other |
| Juvenile $GM_2$ | Arg499 → His | Scotch, Irish |
| | Arg504 → His | Assyrian, Armenian |
| | Gly250 → Asp | Lebanese |
| Chronic or adult $GM_2$ | Gly269 → Ser | Ashkenazi Jewish, diverse other |
| | Lys197 → Thr | Dutch |

*Identified in both homozygous and compound heterozygous states. Arg indicates arginine; His, histidine; Cys, cystine; Gly, glycine; Asp, aspartic acid; Ser, serine; Lys, lysine; and Thr, threonine.

- Associated with juvenile and chronic hexosaminidase A deficiency
- Able to cleave the artificial substrate, but NOT GM2

Kaback M & the International TSD Data Collection Network
(JAMA. 1993;270:2307-2315)

# Inconclusive results using the enzymatic test




- Around 10% of results are outside normal range but higher than observed in Tay-Sachs disease
- Carrier status should be excluded

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# Targeted mutation analysis greatly improves detection in at-risk populations

| Mutation | AJ | Not- AJ |
|---|---|---|
| 1278insTATC | ~82% | ~8-30% |
| IVS12+1 | ~10-15% | 0 |
| G269S | ~ 2% | ~ 5% |
| c.1073+1G>A | 0 | ~ 15% |
| Pseudo-alleles | 2% | 4-32% |
| 7.6-kb del | French Canadian | |

**~ 99% Ashkenazi Jews Mutations**

# "Next generation" TSD carrier screening Challenges

- ✓ Targeted mutation analysis identified 92 – 99% of carriers in a **homogeneous** AJ population
  - ➢ **AJ population tested by our labs is probably NOT homogeneous**

**Jan 2011 – Dec 2013**

| *HEXA* 7 mutations Panel | | | |
|---|---|---|---|
| N | Non-carrier | TSD Carrier | Pseudodeficiency |
| 742 (67M, 675F) | 98% (N = 724) | 2% (N = 14) | < 1% (N = 4) |

- Tay-Sachs Disease (*HEXA*) 7 Mutations (0051428)

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# Towards an ethnicity-independent TSD carrier screening

Molecular Genetics & Genomic Medicine

Open Access

METHOD

## Next-generation DNA sequencing of *HEXA*: a step in the right direction for carrier screening

Jodi D. Hoffman[1], Valerie Greger[2], Erin T. Strovel[3], Miriam G. Blitzer[3], Mark A. Umbarger[2], Caleb Kennedy[2], Brian Bishop[2], Patrick Saunders[2], Gregory J. Porreca[2], Jaclyn Schienda[4], Jocelyn Davie[2], Stephanie Hallam[2] & Charles Towne[2]

[1]Division of Genetics, Department of Pediatrics, Floating Hospital for Children, Tufts Medical Center, Boston, Massachusetts
[2]Good Start Genetics Inc., Cambridge, Massachusetts
[3]Division of Genetics, Department of Pediatrics, University of MD School of Medicine, Baltimore, Maryland
[4]Dana Farber Cancer Institute, Boston, Massachusetts

- Full gene analysis limits false-positive and false-negative results compared to traditional enzyme and genotyping methodologies
- CAVEAT: variant of unknown significance still require functional studies

# ARUP performs full gene sequencing

- Tay-Sachs Disease (HEXA) Sequencing and 7.6kb Deletion (2009298)

Identify causative *HEXA* gene mutation(s) in individual with abnormal level of HEX A enzyme

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# Conclusions

- Tay-Sachs disease population-based carrier screening is a good model for genetic disease prevention
- Best sensitivity is achieved combining enzyme and molecular testing
- Access to inexpensive sequencing methodologies is necessary for pan-ethnic carrier screening

# Unresolved issues

- ✓ Current recommendations is to offer carrier screening to members of *at-risk* populations
  - ➢ **TSD has been reported in children of all ethnic, racial, and religious groups**
- ✓ Preventing the births of affected children is a less-than-ideal method of disease control
  - ➢ **We need a cure!!**

?

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