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

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

- Tay-Sachs Disease
- Generalized Gangliosidosis
- Gaucher Disease
- Sandhoff Disease
- Fabry Disease
- Metachromatic leukodystrophy
- Krabbe Disease
- Gaucher Disease

**Enzymes:**
- β-Galactosidase (Sap B)
- β-N-Acetylhexosaminidase (GM2 activator)
- Sialidase
- β-Glucocerebrosidase (Sap A,C)
- β-Galactocerebrosidase (Sap A,C)
\( \beta \)-hexosaminidase isoforms: HexA and HexB

**Tay-Sachs Disease**
- \( \alpha \beta \) GM2 activator
- \( \beta \)-Galactosidase (Sap B)

**Generalized Gangliosidosis**
- \( \alpha \beta \) GM2 activator
- \( \beta \)-Galactosidase (Sap B)

**Sandhoff Disease**
- \( \beta \beta \) GM2 activator
- \( \beta \)-Galactosidase (Sap B)

**Fabry Disease**
- \( \alpha \beta \) GM2 activator
- \( \alpha \)-Galactosidase (Sap B)

**Metachromatic leukodystrophy**
- \( \beta \)-Cer
- Arylsulfatase A (Sap B)

**Gaucher Disease**
- \( \beta \)-Glucocerebrosidase (Sap A,C)
- \( \beta \)-Galactocerebrosidase (Sap A,C)

**Krabbe Disease**
- \( \beta \)-Cer
- \( \beta \)-Glycerocerebrosidase (Sap A,C)
Three gene system required for HexA activity

1. chromosome 15
   \(HEXA\)
   \(\alpha\) subunit
   Hex A: \(\alpha\beta\)
   Tay-Sachs disease

2. chromosome 5
   \(HEXB\)
   \(\beta\) subunit
   Hex B: \(\beta\beta\)
   Sandhoff disease

3. chromosome 5
   \(GM2A\)
   activator
   GM2-gangliosidosis
   AB variant
Three gene system required for HexA activity

1. chromosome 15
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   GM2-gangliosidosis
   AB variant

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

<table>
<thead>
<tr>
<th>3 - 6 mo</th>
<th>6 - 10 mo</th>
<th>After 10 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Excessive Startling</td>
<td>• Gradual loss of vision</td>
<td>• Complete blindness</td>
</tr>
<tr>
<td>• Twitchy eye movement</td>
<td>• Gradual deafness</td>
<td>• Strong seizures</td>
</tr>
<tr>
<td>• Reverse maturation (i.e. failure to walk)</td>
<td>• Loss of motor skills</td>
<td>• Dementia</td>
</tr>
<tr>
<td></td>
<td>• Macrocephaly</td>
<td>• Unresponsive, vegetative state</td>
</tr>
<tr>
<td></td>
<td>• Hypotonia</td>
<td>• Death due to bronchopneumonia between ages 2-5</td>
</tr>
</tbody>
</table>
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
  o infantile TSD: 0% - 5% residual activity
  o juvenile or chronic adult-onset TSD: < 15% residual activity

✓ Molecular testing
  o Confirm diagnosis: mutations in the HEXA gene
  o Exclude pseudodeficiency alleles
  o 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
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%

<table>
<thead>
<tr>
<th>Carrier of Tay-Sachs disease</th>
<th>↓</th>
<th>%HexA</th>
<th>↓/N Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with Tay-Sachs disease</td>
<td>↓↓</td>
<td>%HexA</td>
<td>↓↓ Total</td>
</tr>
<tr>
<td>Carrier of Sandhoff disease</td>
<td>↑</td>
<td>%HexA</td>
<td>↓  Total</td>
</tr>
<tr>
<td>Patients with Sandhoff disease</td>
<td>↑↑</td>
<td>%HexA</td>
<td>↓↓ Total</td>
</tr>
<tr>
<td>Pregnant Women</td>
<td>↓/N</td>
<td>%HexA</td>
<td>↑↑↑ Total</td>
</tr>
</tbody>
</table>

Hex A: αβ  Hex B: ββ
Tay-Sachs disease  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.

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)
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)

<table>
<thead>
<tr>
<th>Status</th>
<th>Percentage</th>
<th>(N =)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-carrier</td>
<td>66%</td>
<td>104</td>
</tr>
<tr>
<td>TSD Carrier</td>
<td>7%</td>
<td>11</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>11%</td>
<td>18</td>
</tr>
<tr>
<td>Carrier with ↑↑ total activity</td>
<td>15%</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>N = 158</td>
<td>(86M, 72F)</td>
</tr>
</tbody>
</table>

![Graph showing total activity in plasma/serum]
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 GM₂ Gangliosidoses*

<table>
<thead>
<tr>
<th>Form of Disease</th>
<th>Mutation</th>
<th>Ethnic Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>“B₁ Variant”</td>
<td>Arg₁₇₈ → His</td>
<td>Portuguese, European</td>
</tr>
<tr>
<td>(late infantile or juvenile onset)</td>
<td>Arg₁₇₈ → Cys</td>
<td>Czech</td>
</tr>
<tr>
<td></td>
<td>Arg₄₉₉ → His</td>
<td>Scotch, Irish</td>
</tr>
<tr>
<td></td>
<td>Arg₅₀₄ → His</td>
<td>Assyrian, Armenian</td>
</tr>
<tr>
<td></td>
<td>Gly₂₅₀ → Asp</td>
<td>Lebanese</td>
</tr>
<tr>
<td>Juvenile GM₂</td>
<td>Gly₂₆₉ → Ser</td>
<td>Ashkenazi Jewish, diverse</td>
</tr>
<tr>
<td></td>
<td>Lys₁₉₇ → Thr</td>
<td>Dutch</td>
</tr>
</tbody>
</table>

*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

HexA% activity in leukocytes
(May 2013 hotline – August 2014)

- Around 10% of results are outside normal range but higher than observed in Tay-Sachs disease
- Carrier status should be excluded
Targeted mutation analysis greatly improves detection in at-risk populations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>AJ</th>
<th>Not- AJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1278insTATC</td>
<td>~82%</td>
<td>~8-30%</td>
</tr>
<tr>
<td>IVS12+1</td>
<td>~10-15%</td>
<td>0</td>
</tr>
<tr>
<td>G269S</td>
<td>~ 2%</td>
<td>~ 5%</td>
</tr>
<tr>
<td>c.1073+1G&gt;A</td>
<td>0</td>
<td>~ 15%</td>
</tr>
<tr>
<td>Pseudo-alleles</td>
<td>2%</td>
<td>4-32%</td>
</tr>
<tr>
<td>7.6-kb del</td>
<td>French Canadian</td>
<td>~ 99% Ashkenazi Jews Mutations</td>
</tr>
</tbody>
</table>
“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

<table>
<thead>
<tr>
<th>HEXA 7 mutations Panel</th>
<th>N</th>
<th>Non-carrier</th>
<th>TSD Carrier</th>
<th>Pseudodeficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>742</td>
<td>98%</td>
<td>2%</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>(67M, 675F)</td>
<td>(N = 724)</td>
<td>(N = 14)</td>
<td>(N = 4)</td>
<td></td>
</tr>
</tbody>
</table>

➢ Tay-Sachs Disease (*HEXA*) 7 Mutations (0051428)
Towards an ethnicity-independent TSD carrier screening

Molecular Genetics & Genomic Medicine

METHOD

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

Jodi D. Hoffman¹, Valerie Greger², Erin T. Strovel³, Miriam G. Blitzer³, Mark A. Umbarger², Caleb Kennedy², Brian Bishop², Patrick Saunders², Gregory J. Porreca², Jaclyn Schienda⁴, Jocelyn Davie², Stephanie Hallam² & Charles Towne²

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²Good Start Genetics Inc., Cambridge, Massachusetts
³Division of Genetics, Department of Pediatrics, University of MD School of Medicine, Baltimore, Maryland
⁴Dana 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
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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