Metabolic disorders involving the CNS:
A case-based discussion

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Disclosures

• I have no relevant financial relationships to disclose
Learning Objectives

1. Describe how inborn errors of metabolism present clinically and are diagnosed
2. Identify specific histologic patterns observed in lysosomal storage disorders, organic acidemias, and mitochondrial disorders
3. Outline the appropriate steps for tissue handing in the setting of a suspected metabolic disorder
Outline

• Background
• Amino acid and organic acid disorders
• Storage disorders
• Mitochondrial disorders
• Glycogen metabolism disorders
• Practical considerations
Background: breadth of the topic

• *Metabolic disorder* can encompass anything not considered structural/anatomic and occurs at the intracellular level

• Definition is arbitrary
  – Mitochondrial enzyme vs. respiratory chain deficiency?
  – Disorders of trace metal metabolism? *Wilson’s, Menkes’*
  – Disease secondary to maternal metabolic changes? *hypothyroidism*
  – Acquired or nutritional disorders?
  – Ion channel disorders? *cystic fibrosis, Dravet*

• Today will focus on inborn errors of metabolism (IEM), with a focus on cases encountered in real neuropathology practice
Inborn errors of metabolism (IEM)

• Term first used by Archibald Garrod in 1908
  – Blocks in metabolic pathways
• At least 7,000 hereditary metabolic disorders are known
  – Important to recognize early to treat early
  – Genetic implications for family
• Rare diseases (1:4,000 to 1:250,000)
• Increased awareness and carrier/prenatal screening has lowered incidence of many IEM
  – e.g., Tay Sachs
• Total modern birth prevalence for all IEM is ~50:100,000 live births (Waters et al., J Glob Health 2018)
Mapping out metabolic disorders

- Nearly every author uses a different system to “organize” IEM
- Intoxication
  - Amino acid, organic acidurias
  - Urea cycle disorders
  - Sugar intolerance
- Energy deficiencies
  - Congenital lactic acidemia, fatty acid oxidation disorders, mitochondrial respiratory chain disorders
- Disorders of complex molecules
  - Lysosomal storage, peroxisomal, glycosylation

Dr. Edward Leung/Dr. Yi Xiao
Inborn errors of metabolism

- Inherited biochemical defects
  - Carbohydrates
  - Amino acids
  - Purines/pyrimidines
  - Metals
  - Lipids
  - Mucopolysaccharides
- Each disorder has a very low incidence but cumulatively contribute to morbidity and mortality in pediatrics
- Usually manifest during childhood and often immediately after birth
Newborn Screening (NBS)

- Initiated in 1960s with screening for phenylketonuria (PKU)
  - “Guthrie card”
- Now includes 80 disorders (as of 2022)
- Heel stick blood spots screened by electrospray tandem mass spectrometry
  - More recent use of extracted DNA (e.g., for SMN1)
- High throughput, fast turnaround time
- Expedited diagnosis and initiation of therapy
Tandem Mass Spectrometry (MS/MS)

Ionizer: convert to gas phase ion

Separation by liquid chromatography or gas chromatography

m/z

Intensity, arbitrary unit

Molecule

Ionizer: convert to gas phase ion

select precursor ion based on mass-to-charge ratio ($m/z$)

collision cell break ions apart

select product ion based on mass-to-charge ratio ($m/z$)

Detector

LC-MS/MS at CHLA
Challenges of defining the neuropathologic features of a given metabolic disorder

- Rarity of diseases
- Complexity of illness resulting from IEM
- Difficulty distinguishing early from late changes in patients who survive to older ages
Common (but not universal) features in IEM

• AR inheritance
  – can appear sporadic due to small family size
• “Spongy myelinopathy” – common to AA disorders
  – Vacuoles between myelin lamellae
  – Except urea cycle disorders and homocystinuria
• “catastrophic severe neonatal illness”
• Dysmorphic features
• Manifestations in liver
  – LM and EM usually performed
Phenylketonuria (PKU)  
a.k.a. phenylalanine hydroxylase (PAH) deficiency

- AR disorder of phenylalanine (Phe) metabolism
  - Pathogenic variants in PAH cause lack of enzymatic function
  - “hyperphenylalaninemia” (HPA)
  - Deamination of Phe forms phenylketone bodies readily excreted in the urine
- Profound intellectual disability/brain dysfunction
- One of the earliest identified IEM, strong intervention effect
  - Dietary restriction of phenylalanine (1953) – low protein diet
  - Untreated/late-treated children often required permanent institutional care (Weller et al. 1983)
  - Treated kids were as healthy at age 7 as their unaffected siblings
- In 1960s transition to blood screening was recommended
  - First disease taken to population-based neonatal screening

Presentation of PKU

- Incidence in US 1:8,000-15,000
- Normal appearance at birth, first signs @ months
  - Musty/mousy odor from skin and urine
  - Fair skin/hair (lack of Tyr => lack of melanin)
  - Eczema, seizures, tremors
- Lab: Elevated blood Phe, decreased Tyr
- Genetics: >1000 mutations can result in PKU, cause protein misfolding/instability
  - Spectrum of severity
  - Most patients are compound heterozygotes
- Treatment: lifelong restrictive diet with target blood Phe range

https://www.sciencehistory.org/distillations/on-the-scent-the-discovery-of-pku
Neuropathology of PKU

- Hypothesized to be due to toxic effects of Phe
- Limited human neuropathology studies
  - Small brain (80%)
  - Abnormal myelination of late-myelinating structures
    • “spongy change”, pallor
  - ?Cortical developmental delay

Where PKU fits on the map

• Amino acid disorder
• PAH fails to convert Phe to Tyr
  – One gene, one protein
<table>
<thead>
<tr>
<th>Class of error</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid disorders</td>
<td>PKU, homocystinuria</td>
</tr>
<tr>
<td>Organic acid disorders</td>
<td>Propionic aciduria, methylmalonic aciduria</td>
</tr>
<tr>
<td>Fatty acid disorders</td>
<td>Short/Medium chain acyl coenzyme A dehydrogenase deficiency, carnitine deficiency</td>
</tr>
<tr>
<td>Lysosomal disorders</td>
<td>Fabry, Farber, Gaucher, Niemann-Pick</td>
</tr>
<tr>
<td>Carbohydrate metabolism disorders</td>
<td>Galactosemia, Pompe, Lafora</td>
</tr>
<tr>
<td>Urea cycle disorders</td>
<td>Citrullinemia, argininemia</td>
</tr>
<tr>
<td>Mitochondrial disorders</td>
<td>Leigh</td>
</tr>
<tr>
<td>Peroxisomal disorders</td>
<td>Zellweger, Refsum</td>
</tr>
</tbody>
</table>

Adapted from Waters D et al. *J Glob Health* 2018 PMID 30479748
Amino acidemias/urias and white matter impact

“During the past decade or two, a new group of hereditary diseases with amino-acidemia or amino-aciduria of various types has become recognized... Relatively few have been subjected to neuropathological examination... but one observation is relevant to the purposes of this chapter – that the white matter appears to bear the brunt of the pathological process during the period of active myelination, and further, that the changes in the white matter are nonspecific.”

– Roizin, Haymaker, and D’Amelio, Disease states involving the white matter of the CNS, in Histology and Histopathology of the Nervous System (Haymaker and Adams), vol. 1 1982.
Case 1

- 18 month old F presents with progressive dystonia and seizures
- Found to have macrocephaly and metabolic acidosis
- Diagnosed with glutaric acidemia type 1
- Sibling with same disease
- GCDH gene mutation
  - Loss of glutaryl-coA dehydrogenase function
  - Inability to metabolize lysine, hydroxylysine, and tryptophan
  - Accumulation of glutaric acid, 3-OH-glutaric acid, and glutaconic acid
Case example, glutaric acidemia type 1, continued

• Treated with dietary restriction of lysine and tryptophan
• Supplementation of carnitine and riboflavin
• Experienced urinary tract infection and consequent metabolic decompensation
• Progressive neurologic deficits and death at age 14

Urine organic acid by GC-MS. From Stanley Lo et al., Clin Appl of Mass Spectrometry in Biomolecular Analysis, 2022

Dr. Edward Leung/Dr. Yi Xiao
Glutaric acidemia, type 1 – gray/white junction, temporal lobe
Glutaric acidemia, type 1 – white matter, temporal lobe
Brain findings – glutaric acidemia type 1

• Simplified gyral pattern/broad gyri
• Macrocephaly
• Spongy myelin particularly in temporal stem and striatum
• Small basal ganglia (may be a common feature of this dx*)
• Neuronal loss and gliosis
Glutaric acidemias

• Type I (case shown), can present with encephalopathy
  – deficient metabolism of L-lysine, L-OH-lysine and L-tryptophan
  – 1:110,000

• Type II, “multiple acyl coA dehydrogenase deficiency” (MADD)
  – Enzyme primarily active in mitochondria
  – Deficient metabolism of fatty acids, amino acids, and choline
Putting glutaric acidemias on the map

- Loss of function of glutaryl-coA dehydrogenase
  - Key step in lysine and tryptophan metabolism
  - Glutaryl CoA dehydrogenase operates within the mitochondria
  - Feeds forward to Krebs Cycle
- Shows the close relationship between
  - amino acid disorders
  - organic acid disorders
  - mitochondrial disorders
Canavan disease (aspartoacyclase/ASPA deficiency)

- 1:6,000-14,000 among Ashkenazi Jewish community
- 1:100,000 in general population
- progressive psychomotor delay
- progressive epileptic encephalopathy
- macrocephaly
- leukodystrophy with spongiform appearance, predilection for subcortical U-fibers
  - “mass of lacy oedema with glia cells fairly well preserved, but not in excess”
  - Dr. Myrtelle M. Canavan, Arch NeurPsych 1931

Fig. 4.—Horizontal sections of the occipital lobes. Note the complete softening of the white matter as evidenced by its retraction from the cortex.

Fig. 8.—Weigert section showing loss of myelin sheaths at the periphery of the white matter and preservation within.
MRS in diagnosis of Canavan disease

- N-acetylaspartate (NAA) accumulates due to the absence of aspartoacylase (ASPA), the enzyme that breaks down NAA.
  - MRS: prominent NAA in both GM and WM
Putting Canavan on the map (aspartate is circled)

- NAA is particularly important in CNS and is transferred from neurons to glial cells
- Key enzyme in myelin formation by oligodendrocytes

Proposed model for NAA synthesis and degradation (Madhavarao et al., 2005)
Case 2

- 8 month old girl with developmental delay and failure to thrive
- Hepatosplenomegaly
- Progressive deterioration
Gaucher disease

- Absence of lysosomal acid β-glucosidase (glucocerebrosidase)
- Accumulation of glucocerebroside
- Accumulation in reticuloendothelial system
- Large numbers of membrane-bound inclusions containing tubular structures
Gaucher Lymph node, PAS-D

Image from Dr. Bruce Pawel
Case 3

- 4 year old girl with hepatomegaly
- Decreased strength and motor delay
- Difficulty sustaining upward gaze

- Virtual slide: spinal cord
Niemann-Pick type C

• 1:150,000
• Failure of transport of cholesterol and lipids
  – Lysosomal storage disorder
• Rapid neonatal form recognized
• Most cases detected in childhood and lead to early death
• Hepatosplenomegaly common presenting sign
• Impaired vertical eye movements
  – ?dorsal midbrain involvement

Beedasy et al., Afr Vision Eye Health 2019
Niemann-Pick type C

- Genetics: Mutation of *NPC1* or *NPC2*
- Visceral pathology: accumulation of foamy macrophages
- CNS pathology: cerebral atrophy, expanded neurons  
  - PAS-negative or weakly positive  
  - Loss of Purkinje cells with axonal spheroids
- Ultrastructure: pleomorphic inclusions with electron-lucent vacuoles, electron-dense curved short membranous structures
Note that Niemann Pick type C (NPC) is distinct from A and B types, which are acid sphingomyelinase deficiencies.

NPC is a cholesterol trafficking defect
Krabbe disease (globoid cell leukodystrophy)

- “brain sclerosis” described 1916 by Knud Krabbe: small, firm brain
- Metabolic disorder of lipids (neural membranes)
  - Galactocerebrosidase-beta-galactosidase deficiency
    - Lipids accumulate in globoid macrophages
- Demyelination phenotype
- EM: crystalline clear inclusion bodies
- Genetics: $GALC$ deletion/point mutation
Krabbe disease

- 1-2:100,000 live births
- Rapidly progressive infantile CNS disease with peripheral nerve involvement
  - Delayed nerve conduction
- Lab: elevated CSF protein, decreased GBG activity (definitive)
- MRI: white matter abnormalities
CNS findings

- Brain macrophages filled with PAS-positive material
- Perivascular aggregates
- Demyelination with gliosis
- Neuronal loss
Krabbe – ultrastructural findings (sural nerve, 1 yo F)
Krabbe ultrastructural findings (sural nerve, 1 yo F)

Elongated clear crystalline inclusions in Schwann cells (galactocerebroside)
Another lysosomal leukodystrophy: Metachromatic Leukodystrophy

- 1:40,000
- Deficiency of arylsulfatase A (ARSA gene)
  - Inability to degrade sulfated glycolipids
  - Sulfatides stain brown on cresyl violet (metachromasia)
- Causes diffuse demyelination
  - Usually central to peripheral, sparing U fibers
- MRS: lipids and lactate elevated
  - Consistent with acute demyelination
• MCL: breakdown of cerebroside sulfatase (no galactocerebroside generated)
• Krabbe: breakdown of galactocerebroside (no ceramide generated)
Case 4

- 8 year old girl, child of third cousins
- Younger sibling with history of “encephalitis”
- Small for age, problems with balance/coordination
- Started losing milestones at age 3.5, poor fine motor skills
- Admitted at age 8 with weakness and respiratory distress, MRI showed T2 hyperintensity in putamen/globus pallidus, and midbrain
- Terminal pneumonia and sepsis
- Mitochondrial \( ATP6 \) gene mutation (codon 8993 T>C)
  - 90% in blood and 95% in muscle
Virtual slides

• Midbrain
Leigh syndrome

- Subacute necrotizing encephalomyelopathy
  - Described 1951 by Denis Leigh
- 1:36,000-40,000 live births
- Deficiency in mitochondrial production of ATP
  - Electron transport chain
- Focal bilaterally symmetric spongy lesions
  - Particularly in brain stem and thalamus
- Treatment limited, supportive
- Survival poor (20% at 20 years)
Leigh syndrome

• Symptoms between 3 months – 2 years
• Progressive deterioration (loss of milestones), vomiting, seizures
• Lab: high CSF lactate, sometimes aciduria
• Genetics: Defects of mitochondrial energy production
  – Pyruvate hydrogenase deficiency
  – Pyruvate decarboxylase deficiency
  – Respiratory chain complex deficiency
  – May be from nuclear DNA or mitoDNA (heteroplasmy)
    • MT-ATP6 mutation is associated with the majority of LS cases (seen in this case)
  – Inheritance pattern varies – AR, X-linked, maternal
## More frequent mitochondrial disease “classic syndromes”

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Relative frequency</th>
<th>Features</th>
<th>Inheritance</th>
<th>Genetic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kearns-Sayre Syndrome</td>
<td>Frequent</td>
<td>Ocular myopathy (ptosis, ophthalmoparesis)</td>
<td>Sporadic</td>
<td>Single large-scale deletion of mtDNA</td>
</tr>
<tr>
<td>Leber hereditary optic neuropathy</td>
<td>Very frequent</td>
<td>Optic neuropathy</td>
<td>Maternal</td>
<td>Various mtDNA mutations</td>
</tr>
<tr>
<td><strong>Leigh syndrome</strong></td>
<td>Frequent</td>
<td>Severe encephalopathy</td>
<td>AR, XL, maternal</td>
<td>Various nuclear or mtDNA mutations</td>
</tr>
<tr>
<td>Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS)</td>
<td>Frequent</td>
<td>Stroke-like episodes</td>
<td>Maternal</td>
<td>mtDNA mutation (m.3243A&gt;G)</td>
</tr>
<tr>
<td>Myoclonic encephalopathy with ragged red fibers (MERRF)</td>
<td>Frequent</td>
<td>Myoclonus</td>
<td>Maternal</td>
<td>m.8344A&gt;G</td>
</tr>
<tr>
<td>Nonsyndromic hearing loss (NSHL)</td>
<td>Frequent</td>
<td>Hearing loss</td>
<td>Maternal</td>
<td>m.1555A&gt;G</td>
</tr>
<tr>
<td>Progressive external ophthalmoplegia (PEO)</td>
<td>Very frequent</td>
<td>Ocular myopathy</td>
<td>AD, AR, maternal, sporadic</td>
<td>Various nuclear genes with secondary mtDNA deletions/mutations</td>
</tr>
</tbody>
</table>

Adapted from Orsucci et al., J Clin Med. 2021
Case 5

- 8 week old girl with failure to thrive
- Elevated liver enzymes, lactate, and ammonia
  - Ketogenic diet did not improve clinical course
- MRI not performed
- Died with respiratory failure and acidosis
- Compound heterozygous EARS2 mutations
  - Combined OXPHOS deficiency 12
  - Leukoencephalopathy with ragged red fibers
Virtual slide

• Medulla
Different pattern of mitochondrial encephalopathy: LTBL

• Leukoencephalopathy with thalamus and brainstem involvement and high lactate
• Symmetric white matter abnormalities
• Infantile onset, rapidly progressive
• Associated with EARS2 mutations
EARS2-associated mitochondriopathy - ultrastructure

- Markedly increased number of mitochondria particularly in subsarcolemmal space
- Inclusions in mitochondria noted despite poor preservation from autopsy
Mitochondrial disorder

Courtesy of Dr. Mika Warren
Where mito disorders fit on the map

- Defects of energy production
- Numerous genes implicated
- Variable degrees of severity
- Variable patterns of inheritance
Pompe disease (Glycogen storage disease type II)

- Glycogen accumulation disorder (lysosomal aggregates)
- Acid alpha-glucosidase deficiency ("GAA," lysosomal enzyme)
  - a.k.a. acid maltase
  - AR inheritance, GAA pathogenic variants
- 1:40,000 births
- Infantile and late-onset types
  - Onset age associated with severity of deficiency
  - White matter hyperintensity and hypertrophic cardiomyopathy common in infantile type
  - Slowly progressive myopathy typical of late-onset type
- Enzyme replacement therapy available
CNS Neuropathology features in Pompe

- Glycogen accumulation in brainstem, cortex, dentate nuclei, Purkinje cells (case reports)
- Glycogen accumulation in anterior horn cells (common)
- Neuronal loss with areas of gliosis

DeRuisseau et al. PNAS 2009
Glycogen storage diseases that affect muscle

- Type II (Pompe, GAA)
- Type V (McArdle, phosphorylase)
- Type VII (Tarui, phosphofructokinase)
  - Muscle involvement only rarely
- Type IX (phosphorylase b kinase)
- Type X (Dimauro, phosphoglycerate mutase)
Case 6

- 14 year old F with intractable epilepsy
- Axillary biopsy (target eccrine duct cells)
- \textit{NHLRC1} mutation (homozygous)
Lafora disease

- Estimated prevalence 0.4:100,000
- Glycogen metabolism disorder
- Accumulation of polyglucosan (poorly branched glycogen)
- Progressive myoclonic epilepsy, starting in teenage years
- Genetics: mutations of
  - \textit{EPM2A} laforin, glycogen phosphatase
  - \textit{NHLRC1} (\textit{EPM2B}) malin, E3 ubiquitin ligase (ubiquitinates glycogen metabolism enzymes)
  - Either result in excess of hyperphosphorylated glycogen with disrupted glucose chain branching
• Note that apocrine glands can normally have prominent PAS-positive inclusions in their apical regions
  – Arrowheads – normal
  – Full arrows – Lafora bodies
• Diagnostic inclusions should be basal/myoepithelial
• Some advise avoiding axillary skin for this reason
Where Pompe and Lafora fit on the map

• Glycogen to G-1-P cycle errors
  – Classical GSD
• Glycogen branching errors
  – Lafora disease
<table>
<thead>
<tr>
<th>Disease</th>
<th>Category</th>
<th>Gene(s)</th>
<th>Molecule(s) accumulated</th>
<th>Neuropathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKU</td>
<td>Amino acid disorder</td>
<td>PAH</td>
<td>Phenylalanine</td>
<td>Small brain, spongy WM</td>
</tr>
<tr>
<td>GA-1</td>
<td>Amino acid/organic acid disorder</td>
<td>GCDH</td>
<td>glutaric acid, 3-OH-glutaric acid, glutaconic acid</td>
<td>Small striatum, spongy WM</td>
</tr>
<tr>
<td>Canavan</td>
<td>Amino acid disorder</td>
<td>ASPA</td>
<td>NAA</td>
<td>Spongiform WM, U-fibers involved</td>
</tr>
<tr>
<td>Gaucher</td>
<td>Lysosomal storage</td>
<td>GLU1/2</td>
<td>glucocerebroside</td>
<td>Perivascular storage PAS+ Mφ</td>
</tr>
<tr>
<td>NPC</td>
<td>Lysosomal storage</td>
<td>NPC1/2</td>
<td>cholesterol, lipids</td>
<td>Cerebral atrophy, neurons with storage material (PAS+/-)</td>
</tr>
<tr>
<td>Krabbe</td>
<td>Lysosomal storage</td>
<td>GALC</td>
<td>Galactocerebroside</td>
<td>Small firm brain, demyelination</td>
</tr>
<tr>
<td>MCL</td>
<td>Lysosomal storage</td>
<td>ARSA</td>
<td>Sulfatides</td>
<td>Diffuse demyelination, U-fibers spared</td>
</tr>
<tr>
<td>Mito (LS, LTBL)</td>
<td>Energy deficiency</td>
<td>Various</td>
<td>Symmetrical subacute focal necrosis (with vascular proliferation)</td>
<td></td>
</tr>
<tr>
<td>Pompe</td>
<td>Glycogen metabolism/Lysosomal storage</td>
<td>GAA</td>
<td>Glycogen</td>
<td>Myopathy</td>
</tr>
<tr>
<td>Lafora</td>
<td>Glycogen metabolism</td>
<td>EPM2A, NLHRC1</td>
<td>Hyperphosphorylated glycogen</td>
<td>Intracellular inclusions</td>
</tr>
</tbody>
</table>
Outline

- Background
- Amino acid and organic acid disorders PKU, GA1
- Storage disorders Gaucher, Niemann-Pick type C, Krabbe
- Mitochondrial disorders Leigh syndrome, LTBL
- Glycogen metabolism disorders Pompe, Lafora
- Practical considerations
Clinical suspicion for metabolic disorder

- History of parental consanguinity
- History of unexplained death, multiple spontaneous abortions
- Critical illness with nonspecific findings refractory to normal treatments
  - Dehydration
  - Acidosis
  - Vomiting
  - Seizures
- Acute encephalopathy
- Hydrops fetalis or placental abnormalities
Biopsies for metabolic disorders

• Liver, muscle, intestinal, skin biopsies more common
• Any tissue should be divided for
  – morphology
  – biochemical analysis
  – probably EM
  – skin for fibroblast culture
Metabolic autopsy” (Ernst et al., J. Peds 2006)

• Rapid tissue collection required
• Suggested procurement:
  – Snap frozen tissue (liver, muscle, heart, kidney, brain)
  – Tissue in glutaraldehyde for EM
  – Blood
  – Urine
  – Vitreous fluid
  – Bile
  – Skin
Emergence of rapid exome/genome sequencing for NICU (2012-present)

• Can be fast but not yet cheap
• Raises ethical challenges not present in traditional mass spec screening
  – How to manage uncertain or unrelated genetic findings
  – Status of parental consent in medical crisis
  – Whether to use result to redirect/withdraw care
• Could shorten time to definitive diagnosis
• Shift towards genomic diagnosis for **newborn screening** is being studied in many countries; cost and variant calling are issues
Learning Objectives

1. Describe how inborn errors of metabolism present clinically and are diagnosed
2. Identify specific histologic patterns observed in lysosomal storage disorders, organic acidemias, and mitochondrial disorders
3. Outline the appropriate steps for tissue handing in the setting of a suspected metabolic disorder
Acknowledgments

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References

Q&A