Clinical Presentation and Diagnostic Difficulties of Glycogen Storage Diseases

Matthias R Baumgartner
Division of Metabolism
University Children’s Hospital, Zurich, Switzerland

Glycogen Storage Disorders (GSDs)

- Glycogen synthesis
  - glycogen synthase
  - "branching enzyme"
- Glycogenolysis
  - tissue specific phosphorylases
  - "debranching enzyme"
  - lysosomal glucosidase
- Glycolysis
  - phosphofructokinase
- cumulative incidence 1 : 20,000

Glycogen Structure

- most of Glc units are linked by α-1,4 glycosidic bonds
- approx 1 in 12 Glc residues -α-1,6 glycosidic bond with a second Glc → creation of a branch
- glycogen granules contain both glycogen and the enzymes of glycogen synthesis and degradation
- enzymes are nested between the outer branches of the glycogen molecules and act on non-reducing ends
⇒ the many non-reducing end-branches of glycogen facilitate its rapid synthesis and catabolism

Glycogen Catabolism

Carbohydrate Metabolism
Classification of GSDs

Disturbed Glycogen Synthesis
- Cytosolic Glycogen Storage in the Liver
  "Liver Glycogenosis"
- Cytosolic Glycogen Storage in the Muscle
  "Muscle Glycogenosis"
- Lysosomal Glycogen Storage

Disturbed Glycogenolysis and Gluconeogenesis
- Glycogenolysis defects (GSD III, VI, IX)
- Glycogen synthase deficiency (GSD 0)

Diagnostic Approach to Liver GSDs
- Isolated hepatomegaly (age 3-18 months)
- Additional symptoms and laboratory findings
  - Disturbed Glycogenolysis and Gluconeogenesis
    - (symptomatic) hypoglycemia
    - Short fasting tolerance (2-4 hrs)
    - Urinary ketosis +/-
    - Hyperventilation — lactic acidosis
    - Enlarged kidneys upon US
  - Disturbed Glycogenolysis
    - Mild hypoglycemia
    - Urinary ketosis ++
    - "Mild" forms of GSDs
  - Disturbed Glycogenolysis
    - (with abnormal glycogen)
    - No hypoglycemia
    - Progressive liver dysfunction

Glycogenolysis defects (GSD III, VI, IX)
- Protruding abdomen (large liver), especially in infants and small children
- Mild hypoglycemia and ketosis when fasting
- "Mild" forms of GSDs
- GSD III and IXb also muscle involvement (CK↑)
- Gene defects
  - Amylo-1,6-Glucosidase (GSD III)
  - Phosphorylase in liver (GSD VI), in muscle (GSD V)
  - Phosphorylase b-kinase (GSD IX)

Glycogen synthase deficiency (GSD 0)
- Fasting
- Glycogenolysis
- Gluconeogenesis
- Lactate
- Amino acids
- Ketone bodies ++

Lysosomal Glycogen Storage (lysosomal glycogenosis)
Glycogen synthase deficiency (GSD 0)

- **Fasting**: Hypoglycemia, ketosis und low lactate (and alanine), especially in small children
- **Postprandial**: Hyperglycemia, paradoxically high lactate (and alanine), no ketosis
- Autosomal-recessive, very rare
- Therapy: frequent meals rich in carbohydrates
- Prognosis: good

Glucose-6-Phosphatase deficiency (GSD I)

- Combined defect of glycogenolysis and gluconeogenesis
- Most severe form of GSD
- Protruding abdomen, large liver (and kidneys!)
- Failure to thrive, late puberty
- Intolerance to fasting: hypoglycemia, lactic acidosis, hyperlipidemia, hyperuricemia
- Type Ib (transporter in the ER): with neutropenia
- Therapy
  - frequent (every 2-3 hours!) meals rich in carbohydrates
  - uncooked starch
  - tube feeding at night

Secondary lab changes in GSDs (mainly GSDI)

- Glycogenosis type I
  - Catch up growth thanks to tube feeding at night
Complications in GSDs

- Liver adenoma
- → hepatocellular cancer
- Osteoporosis
- Renal failure
- Liver cirrhosis (IV)
- Cardiomyopathy (III / IX)

DD: Fanconi-Bickel-Syndrome (FBS) → GLUT2 deficiency

Clinical findings

- Normal at birth
- Failure to thrive with 3-10 months
- Hepato-nephromegaly
- Rickets
- Growth delay
- Rarely cataracts

Fanconi-Bickel-Syndrome (FBS)

<table>
<thead>
<tr>
<th>Patient Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal at birth</td>
</tr>
<tr>
<td>Failure to thrive with 3-10 months</td>
</tr>
<tr>
<td>Hepato-nephromegaly</td>
</tr>
<tr>
<td>Rickets</td>
</tr>
<tr>
<td>Growth delay</td>
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<tr>
<td>Rarely cataracts</td>
</tr>
</tbody>
</table>

Fanconi-Bickel-Syndrome: Diagnosis

1. Fasting hypoglycemia and ketonuria, low lactate
2. Postabsorptive hyperglycemia / galactosemia, high lactate

**Urine**
- Glucose \(\uparrow\uparrow\uparrow\) / Galactose \(\uparrow\uparrow\uparrow\)
- Phosphate \(\uparrow\)
- Calcium \(\uparrow\)
- Amino acids \(\uparrow\)
- Uric acid \(\uparrow\)
- Protein \(\uparrow\)

**Serum**
- Phosphate \(\downarrow\)
- ALP \(\uparrow\), liver enzymes \(\uparrow\)
- Uric acid \(\downarrow\)
- Bicarbonate \(\downarrow\)
- Lipids \(\uparrow\)

Fanconi-Bickel-Syndrome: Therapy

- Vitamin D
- Calcium
- Fluids
- Frequent meals
- Uncooked starch: catch up growth?
- Galactose restriction: avoidance of cataracts?
- Fructose: alternate source of carbohydrates?

How long and how strict??

Diagnostic Approach to Liver Glycogenosis

<table>
<thead>
<tr>
<th>Type I</th>
<th>Type III</th>
<th>Type VI, IX</th>
<th>FBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoglycemia</td>
<td>(+++ \rightarrow +)</td>
<td>(+ + ) (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Lactic acidosis</td>
<td>++</td>
<td>(\emptyset)</td>
<td>(\emptyset)</td>
</tr>
<tr>
<td>Fasting ketosis</td>
<td>(\emptyset \rightarrow +)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Liver enzymes</td>
<td>(\emptyset \rightarrow +)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>CK</td>
<td>(\emptyset)</td>
<td>(\emptyset \rightarrow +)</td>
<td>(\emptyset \rightarrow (+))</td>
</tr>
<tr>
<td>Uric acid</td>
<td>+</td>
<td>(\emptyset)</td>
<td>(\emptyset)</td>
</tr>
<tr>
<td>Renal tubulopathy</td>
<td>(+)</td>
<td>(\emptyset)</td>
<td>(\emptyset)</td>
</tr>
<tr>
<td>Enlarged kidneys</td>
<td>++</td>
<td>(\emptyset)</td>
<td>(\emptyset)</td>
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</table>

Courtesy: Prof. R. Santar
**Phosphorylase System**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Liver</th>
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<tbody>
<tr>
<td>Phosphorylase</td>
<td></td>
</tr>
<tr>
<td>PYGM</td>
<td>GSD V (McArdle disease)</td>
</tr>
<tr>
<td>PHKA1</td>
<td>GSD IX d</td>
</tr>
<tr>
<td>PHKG1</td>
<td>GSD IX e</td>
</tr>
<tr>
<td>PHKB</td>
<td>GSD IX b</td>
</tr>
<tr>
<td>rare</td>
<td>rare</td>
</tr>
<tr>
<td>rare</td>
<td>rare</td>
</tr>
<tr>
<td>♂+♀</td>
<td>♂+♀</td>
</tr>
</tbody>
</table>

**Genetic diagnosis of hepatic GSDs**

<table>
<thead>
<tr>
<th>Type I</th>
<th>Type III</th>
<th>Type VI, IX</th>
</tr>
</thead>
<tbody>
<tr>
<td>genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G6PC</td>
<td>G6PT</td>
<td>AGL</td>
</tr>
<tr>
<td>PYGL</td>
<td>PHKA2</td>
<td>GLUT2</td>
</tr>
<tr>
<td>PHKB</td>
<td>PHKG2</td>
<td></td>
</tr>
<tr>
<td>number of coding exons</td>
<td>1 1 1</td>
<td></td>
</tr>
<tr>
<td>5 8 33</td>
<td>20 33 31</td>
<td></td>
</tr>
<tr>
<td>number of common mutations</td>
<td>none none none</td>
<td></td>
</tr>
<tr>
<td>Cave: IX a-2 diagnosis not possible in blood</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Diagnostic difficulties in not typical patients…**

- SN, female, *02/2003
- healthy, not related Swiss parents
- normal pregnancy and birth
- aunt with Crohn’s disease

Admission with 18 months because of gastroenteritis
- ASAT 164, ALT 72, γGT 104 U/l
- Hepatomegaly (US) 15 cm in MCL
- Parainfectious Hepatopathy
- DD metabolic disease
- Did not show up for follow up

**Admission at 6 years**

- Microcytic anemia: Hb 62, MCV 68.2
- Thrombocytosis 1.014.000
- CRP 20.1 mg/dl
- Weight 13 kg, length 103 cm, BMI 12.3 (all <<P3)
- Glucose 5.3 mmol/l
- ASAT 73 U/l, ALAT 23 U/l
- γGT 76 U/l
- Endoscopy: Ulcers throughout the colon
- chronic infectious bowel disease?

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**Muscle Glycogenosis**

Muscle cell

- GSD V (McArdle disease)
- GSD VII (Tarui disease)

- Muscle Phosphorylase
- Phosphofructokinase

- Symptoms earlier
- exercise intolerance
- muscle pain, muscle stiffness, - cramps
- acute crises (rhabdomyolysis)

- Hemolysis
- Icterus

- CK
- EMG
- forearm-ischaemia-test
- histochemistry
- molecular genetics

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**Diagnostic difficulties in not typical patients…**

- At 5 ¾ years
  - Abdominal pain
  - Diarrhea
  - Fever
  - Not eating well
- At 6 years
  - Rotavirus enteritis
Some findings not fitting and irritating…

- Liver ultrasound: hepatomegaly (18 cm MCL)
  ⇒ storage disorder?
- Screening for GSD and lysosomal storage disorders
- Biotinidase: 12.6 mU/ml (N 7.0–10.6)
- Chitotriosidase: not done after asking back
- Phosphorylase b-Kinase: 7.1 E/g Hb (N 3.5–6.5)
- Amylo-1,6-Glucosidase: 2.3 E/g Hb (N 0.9-4.2)
- Catabolism Limit Dextrin and Glycogen: normal
- Suspicion for GSD, type III and IX ruled out
  ⇒ ask for better history

After asking specifically…

- Discrete enlargements of both kidneys
- Lactic acid 5.1 mmol/l
- Uric acid (↑)
- Triglycerides ↑↑ (8.6 mmol/L)
- Doll face, large abdomen, thin legs

Nutritional history
- „the child is always hungry and is eating all the time“
- no defined main meal times
- Small feedings spread over the entire day
- „I can’t get rid of her demand for milk during the night“

⇒ longest fasting time: 4.5 hours

Summary extended history / findings….

- Hepatomegaly, nephromegaly
- Elevated lipids, lactate and uric acid
- Failure to thrive, delayed growth
- Large abdomen, doll face
- Unusual eating habits
- Infectious bowel disease (Crohn’s disease like)

⇒ Glykogenosis type Ib?
  Neutropenia and functional disturbance of neutrophils because of defective glucose transport in ER

But: normal white blood and neutrophils count

Further diagnostic steps

Liver biopsy
- Glycogen content: 10.5g/100g (N 2.4-6.4)
- Glucose-6-Phosphatase: 0.4 U/g liver (N 3.7-9.6)
- Phosphorylase and phosphorylase b-kinase normal

Mutation analysis: G6PC D38V / G188R
  ⇒ GSD la

But: how does this fit to infectious bowel disease?
  ⇒ G188R homozygotes with GSD Ib like phenotype have been reported (Weston et al. Ped Res 2000)

Conclusions from this case

- Always ask for a complete history
- Ask the right questions
- Stepwise approach
  - good history
  - basic lab tests including glucose, lactate, uric acid, lipids, CK and transaminases
    → screening test → „easy“ enzymes in blood
    → liver biopsy and/or mutation analysis

⇒ Screening test: biotinidase useful?
Elevated Serum Biotinidase Activity in Hepatic GSDs: How specific is this phenomenon?

- Elevated serum biotinidase activity in patients with GSD type Ia reported previously
  - Wolf et al. (JIMD 2003), Bufoff et al. (JIMD 1996),
  - Talib et al. (Am J Gastroent 2000), Hug et al. (Ped Res 1994)

Goals of study

- Expand to other types including GSD type Ib, III, VI and IX
- 68 patients with different GSDs
  - 21 GSD Ia
  - 4 GSD Ib
  - 13 GSD III
  - 3 GSD VI
  - 22 GSD IX
  - 5 patients with Fanconi-Bickel Syndrome

Serum Biotinidase Activity in Controls and Patients with Hepatic Storage Disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>n</th>
<th>SD</th>
<th>Range</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>26</td>
<td>± 1.0</td>
<td>7.8 - 10.6</td>
<td></td>
</tr>
<tr>
<td>GSD Ia</td>
<td>21</td>
<td>± 3.9</td>
<td>11.4 - 24.8</td>
<td>100</td>
</tr>
<tr>
<td>GSD I non-a</td>
<td>4</td>
<td>± 5.6</td>
<td>14.6 - 26.0</td>
<td>100</td>
</tr>
<tr>
<td>GSD III</td>
<td>13</td>
<td>± 3.6</td>
<td>7.8 - 19.1</td>
<td>62</td>
</tr>
<tr>
<td>GSD VI</td>
<td>3</td>
<td>± 2.0</td>
<td>14.1 - 17.7</td>
<td>100</td>
</tr>
<tr>
<td>GSD IX</td>
<td>22</td>
<td>± 3.8</td>
<td>7.5 - 21.6</td>
<td>77</td>
</tr>
<tr>
<td>Fanconi-Bickel</td>
<td>5</td>
<td>± 3.7</td>
<td>11.0 - 19.4</td>
<td>100</td>
</tr>
</tbody>
</table>

Biotinidase activity expressed in mU/ml

- Elevated serum biotinidase activity in sera from patients with
  - GSD Ia and Ib
  - GSD III
  - GSD VI
  - GSD IX
  - Fanconi-Bickel Syndrome (FBS)
- High sensitivity for GSD type I, VI and FBS
- Somewhat less sensitivity for GSD III and IX

Elevated serum biotinidase activity ⇒ convenient biomarker for hepatic GSDs

Influence of Blood Sample Storage on Biotinidase Activity

Blood samples from one healthy control stored at room temperature
Measurements were done in replicates; mean values are shown

Biotinidase Activity in Hepatic GSDs: Conclusions

- Biotinidase activities are significantly elevated in sera from patients with
  - GSD Ia and Ib
  - GSD III
  - GSD VI
  - GSD IX
  - Fanconi-Bickel Syndrome (FBS)
- High sensitivity for GSD type I, VI and FBS
- Somewhat less sensitivity for GSD III and IX

Elevated serum biotinidase activity ⇒ convenient biomarker for hepatic GSDs

Patricie Paesold
Johannes Häberle
Beat Steinmann

René Santer
Hamburg