DNA Analysis in Glycogen storage disease

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Glycogen Synthesis and Breakdown

Glycogen Synthase

- Type 0
- Type IV

UDPGlucose

- Uridine
- Diphosphoglucone
- Pyrophosphorylase

Glucose

- Glucokinase

Glycogen

- Branching Enzyme

Phosphorylase

- Type I
- Type III
- Type V and VI
- Type IX

Glycogen Synthesis and Breakdown

- Alpha-1,4
- Alpha-1,6

Glucose 1-P

- Phosphoglucone Isomerase

Glucose 6-P

- G-6-Phosphatase

Glucose

- Debranching Enzyme

Limit dextrin
Glycogen Synthesis and Breakdown

Glycogen Synthase

UDPGlucose

Glucose 1-P

Alpha-1,4

Alpha-1,6

Glycerogen

Branching Enzyme

Phosphorylase Kinase

Limit dextrin

Debranching Enzyme

UDP Glucose

Glucose

Uruline

Diphosphoglucone

Pyrophosphorylase

Phosphoglucone Isomerase

Glucokinase

G-6-Phosphatase

Type Ia

G6PC gene

Type Ib

SLC37A4 gene
GSD Type I

- First described by von Gierke in 1929
- Approx 1 in 58,000 newborns affected
- Autosomal recessive
- Classification:
  - Ia: Deficiency of glucose-6-phosphatase enzyme
  - Ib/Inon-a: Deficiency of glucose-6-phosphate transporter
- Approx 10% of Type 1 cases are Ib
Mutations identified

• Type Ia
  – *G6PC* gene
    • 5 exons, 13 kb on Chr 17q21
  – >80 mutations reported
  – Common changes:
    • p.Arg83Cys - 32%
    • p.Gln347X - 22%
G6PC gene mutations

Mutations identified

- **Type Ib**
  - *SLC37A4* gene
    - 9 exons, 6 kb on Chr 11q23.3
  - >65 mutations reported
  - Common changes:
    - p.Leu348fs - 28%
    - p.Gly339Cys - 19%
SLC37A4 gene mutations

Genetic Analysis in GSD type I

- Avoids liver biopsy
- Confirms diagnosis - type Ia versus Ib
- No clear genotype/phenotype correlation
Glycogen Synthesis and Breakdown

Glycogen Synthase
- UDPGlucose
- Alpha-1,6

Glycogen
- Alpha-1,4

Branching Enzyme
- Uridine Diphosphoglucose Pyrophosphorylase

Debranching Enzyme
- G-6-Phosphatase

Phosphorylase Kinase
- Type V and VI
- PYGM and PYGL genes

Limit dextrin
- Phosphorylase

Phosphoglucone Isomerase
GSD Type V

• Also known as McArdle Disease
• Deficiency of muscle glycogen phosphorylase
  – cleaves $\alpha$-1,4-glucosidic bonds
• Autosomal recessive
  – $PYGM$ gene
    • 20 exons, 40kb on Chr 11q12-q13.2
• 2.5% of GSDs
Mutations of the *PYGM* gene

- **Common mutations:**
  - p.Arg50X - 32% - 81% of alleles
  - p.Gly205Ser - 0% - 10% of alleles

- **Other mutations**
  - >85 rare mutations

- **Non-sense mediated mRNA decay**
Mutations of the *PYGM* gene
GSD Type VI

- Also known as Hers Disease
- Deficiency of liver glycogen phosphorylase
- Autosomal recessive
  - *PYGL* gene
    - 20 exons, 40kb on Chr 14q21-q22
- Rare
GSD Type VI - Reported Patients

- 11 patients published with 17 mutations
- Majority are missense mutations
  - Clustered in exons 16 and 17

- p.R399X
- [c.1964_1969inv6;c.1969+1_+4delGTAC]
- p.Q13P
- p.V456M
- p.R491C
- p.D634H
- p.K681T
- p.S675L
- p.N632I
- p.E673K
- c.1620+1G>A
- c.1768+1G>A
- c.529-1G>C
- p.M1?
- p.G233D
- p.N339S
- p.N377K
- p.N632I
- p.D634H
- p.E673K
- p.K681T
- p.S675T
GSD Type VI - Screened Patients

- All published patients and 16 patients from clinical service
Glycogen Phosphorylase

Glycogen + $P_i \rightarrow$ Glycogen + Glucose-1-P
Glycogen Synthesis and Breakdown

- **Glycogen Synthase**
  - UDPGlucose
  - Glucose
  - Uridine Diphosphoglucose Pyrophosphorylase

- **Glycogen**
  - Alpha-1,6
  - Alpha-1,4

- **Glucose 1-P**
  - Phosphoglucone Isomerase

- **Glucose 6-P**
  - G-6-Phosphatase

- **Phosphorylase Kinase**
  - PHKA2 gene
  - PHKG2 gene
  - PHKB gene

- **Limit dextrin**
  - Debranching Enzyme

- **Phosphorylase**
  - Type IX
Phosphorylase kinase

• Four copies of each of $\alpha$, $\beta$, $\gamma$, $\delta$ subunits

X-linked GSD Type IX

• Deficiency of liver α subunit (PHKA2 gene)
• Clinical symptoms
  – Hepatomegaly
  – Liver dysfunction
  – Hypoglycaemia
  – Growth retardation
  – Elevated blood cholesterol, triglycerides
  – Mild muscle hypotonia in some cases
X-linked GSD Type IX

- X-Linked Glycogenosis type 1 (XLG1)
  - Reduced PHK activity in RBC and liver

- X-Linked Glycogenosis type 2 (XLG2)
  - Reduced PHK activity in liver only
Case 1

- Symptoms present at 1 year, diagnosed type VI at 7 years
  - Hepatomegaly
  - Normal fasting
  - Raised transaminases
  - Growth retardation
  - WBC Total GP: 1.4 (NR: 1.0-3.2 μmol Pi/mg alb/h)
  - WBC Activated GP: 0.3 (NR: 0.5-2.2 μmol Pi/mg alb/h)
  - RBC PHK: 21.8 (NR: 8.6-45 μmol Pi/min/g Hb)
- No mutation identified in *PYGL* gene – Not GSD type VI
- Analysis of *PHKA2*: p.Arg182Cys – X-linked GSD Type IX
Autosomal GSD Type IX

- Autosomal recessive
- Rarer than X-linked form
- Deficiency of either $\beta$ or liver $\gamma$ subunit
- Mutations in $PHKB$ gene
  - 33 exons, 238kb on Chr 16q12-q13
- or $PHKG2$ gene
  - 10 exons, 9kb on Chr 7p11.2
Autosomal GSD Type IX

- **Deficiency of β subunit**
  - Very mild symptoms
  - Hepatomegaly
  - Hypoglycaemia in rare cases with prolonged fasting
- **Deficiency of liver γ subunit**
  - Severe hepatomegaly
  - Liver dysfunction
  - Recurrent hypoglycaemia
  - Growth retardation
  - Fibrosis of the liver leading to cirrhosis and adenomata
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Genetic Analysis

- Confirms the diagnosis
- Distinguishes GSD type VI from GSD type IX - XLG2
- Identifies the subunit deficient and thus prognosis
- Identifies the inheritance
  - Allowing family studies
Glycogen Synthesis and Breakdown

- **Glycogen Synthase**
  - UDPGlucose
  - Glucose
  - Uridine Diphosphoglucose
  - Pyrophosphorylase

- **Branching Enzyme**
  - Alpha-1,6

- **Phosphorylase Kinase**
  - Limit dextrin

- **Debranching Enzyme**
  - Type III AGL gene

- **Phosphorylase**

- **Guanosine Diphosphatase**
  - G-6-Phosphatase

- **Glucose 1-P to Glucose 6-P**
  - Phosphoglucone Isomerase

- **Glucose**
  - Glucokinase
GSD Type III

• Also known as Cori or Forbes Disease
• Deficiency of glycogen debrancher enzyme
• Autosomal recessive
• Four subtypes:
  – Type IIIa (~85% of patients)
    • Enzyme deficient in both liver and muscle
  – Type IIIb (~15% of patients)
    • Enzyme deficient in liver
  – Type IIIc
    • Loss of glucosidase activity
  – Type IIIId
    • Loss of transferase activity
GSD Type III

- **AGL gene**
  - 35 exons, 85 kb on Chr 1p21
- **Type IIIa**
  - Majority (65%) of mutations are nonsense, frameshift or splice site mutations
  - Common changes
  - Rare changes
    - 118 mutations reported
- **Type IIIb**
  - Exon 3 nonsense mutations
    - c.16C>T, p.Gln6X,
    - c.18_19delGA, p.Gln6HisfsX20
    - c.22C>T, p.Arg8X
GSD Type III – AGL mutations

GSD Type III

Genetic analysis

– Allows distinction between type IIIa and IIIb
– Some indication of severity
  • eg c.4260-12A>G is ‘mild’ mutation
– Correlates with leukocyte enzyme assay
Cases 2 and 3

- Patient, aged 5 years
- Permanent hepatomegaly
- Fasting <3 hours
- Permanent CK elevation
- Cardiomyopathy
- Fatigue not observed

- Leuk Debrancher: 5.7
  Normal range: 26.8-105 nmol glu/mg protein/hour
- RBC Glycogen: 565
  Normal range: 5.7-135 µg/g Hb
- p.Arg408X homozygote

- Patient, aged 20 years
- Hepatomegaly till 16 years
- Normal feeding
- Normal CK
- No Cardiomyopathy
- Periodic fatigue

- Leuk Debrancher: 0.69
  Normal range: 26.8-105 nmol glu/mg protein/hour
- RBC Glycogen: 726
  Normal range: 5.7-135 µg/g Hb
- p.Arg8X and c.4260-12A>G
Glycogen Synthesis and Breakdown

- Branching Enzyme
  - Type IV
  - GBE gene

- Glycogen Synthase
  - UDPGlucose
  - Glucose
  - Uridine Diphosphoglucone Pyrophosphorylase
  - Phosphoglucone Isomerase
  - Glucokinase

- Glucose 1-P
  - Alpha-1,6
  - Alpha-1,4

- Glucose 6-P
  - Phosphoglucone Isomerase

- Glycogen

- Limit dextrin
  - Debranching Enzyme

- Phosphorylase
  - Kinase

- G-6-Phosphatase
  - Glucose
GSD Type IV

- Also known as Anderson Disease
- Deficiency of glycogen branching enzyme
- Autosomal recessive
  - $GBE1$ gene
  - 16 exons, 262 kb on Chr 3p14
- Rare
GSD Type IV

- 20 Patients reported
  - 34 unique mutations
  - Some phenotype/genotype correlation
    - Wide range of phenotypes
    - Congenital presentation to polyglucosan body disease

- Genetic analysis allows prenatal diagnosis
  - Identification of carriers
GSD Type 0

- Deficiency of glycogen synthase
- Autosomal recessive
  - GYS2 gene
    - 16 exons, 69kb on Chr 12p12.2
- Rare
GSD Type 0

- Clinical symptoms
  - Ketotic hypoglycaemia
  - Post-prandial hyperglycaemia and hyperlactataemia
  - Low activity in liver biopsy
- Molecular analysis avoids liver biopsy
GYS2 Mutations

New mutations

N39S
72 base deletion
R5X
G+1/T Splice
Q183X
A339P
G-1T/CT
R246X
T445M
R582K
D668N

Previously reported mutations

M491R
S483P
P479Q
H446D

Glycogen Synthesis and Breakdown

- **Glycogen Synthase**
- **UDP-Glucose**
- **Glucose 1-P**
- **Glucose 6-P**
- **G-6-Phosphatase**
- **Pyrophosphorylase**
- **Branching Enzyme**
- **Alpha-1,4**
- **Alpha-1,6**
- **Phosphoglucomutase**
- **Glucokinase**
- **Phosphorylase Kinase**
- **Phosphorylase**
- **Limit dextrin**
- **Debranching Enzyme**

**Key Reactions:**
- Conversion of UDP-Glucose to Glucose 1-P
- Conversion of Glucose 1-P to Glucose 6-P
- Conversion of Glucose 6-P to Glucose 1-P
- Conversion of Glucose 1-P to UDP-Glucose

**Enzymes:**
- Glycogen Synthase
- UDP-Glucose Pyrophosphorylase
- Glucokinase
- Phosphoglucomutase
- Phosphorylase Kinase
- Phosphorylase
- Debranching Enzyme
- G-6-Phosphatase
Glycogen Synthesis and Breakdown

Uridine Diphosphoglucose Pyrophosphorylase
Glucose → Glucose 1-P → Glucose 6-P
Glucokinase
Phosphoglucone Isomerase

Alpha-1,4
Debranching Enzyme

G-6-Phosphatase
Glucose
Glycogen Synthesis and Breakdown

- Uridine Diphosphoglucose Pyrophosphorylase
- Glucokinase
- Phosphoglucone Isomerase
- Type VII
- Phosphofructokinase
- Fructose-1,6-bisphosphatase
- FBP1 deficiency

- Glucose 1-P
- Glucose 6-P
- Fructose 6-P
- Fructose-1,6-bisphosphate
- Pyruvate
- G-6-Phosphatase
- Glucose Phosphate Isomerase
- Alpha-1,4 Debranching Enzyme
GSD Type VII

- Also known as Tarui disease
- First identified in 1965
- Deficiency of muscle phosphofructokinase
  - Homo-tetramer
  - Three isoforms muscle, liver and platelet types
- Autosomal recessive inheritance
  - $PFKM$ gene
    - Located on 12q13
    - 24 exons covering 30kb
- Rare
GSD Type VII

- Type VIIa ‘Classical’
  - Exercise intolerance
  - Muscle cramps and pain after exercise
- Type VIIb ‘Late onset’
  - Mean age of onset 55 years
  - Progressive fatigue and weakness
- Type VIIc ‘Infantile’
  - Floppy and hypotonic babies
  - Die within 1 year
- Type VIIId ‘Haemolytic’
  - No muscle symptoms
  - Severe haemolysis
PFKM gene and Mutations

- 15 mutations reported
- Genetic analysis
  - ? avoid muscle biopsy
  - Prenatal diagnosis

Glycogen Synthesis and Breakdown

- **Glucose**
  - Glucokinase
- **Glucose 1-P**
  - Phosphoglucone Isomerase
  - Alpha-1,4
  - Alpha-1,6
- **Glucose 6-P**
  - G-6-Phosphatase
  - Glucose Phosphate Isomerase
- **Fructose 6-P**
  - Phosphofructokinase
  - Fructose-1,6-bisphosphatase
- **Fructose-1,6-bisphosphate**
- **Pyruvate**

- **Debranching Enzyme**
  - Uridine Diphosphoglucone Pyrophosphorylase

- **FBP1 deficiency**
Fructose-1,6-bisphosphatase

• Clinical Symptoms
  – Hypoglycaemia
  – Lactic acidosis
  – Glyceroluria
  – Hepatomegaly

• Autosomal Recessive
  – $FBP1$ gene
    • 7 exons, 31 kb on Chr 9q22.3
**FBP1 Gene Mutations**

- 18 point mutations
- Exon 1 deletion
Fructose-1,6-bisphosphatase

• Molecular analysis
  – Avoids liver biopsy
  – Diagnosis when enzymology not available

• No clear genotype/phenotype correlation
Case 4

- Presented at 2 years with hypoglycaemic coma following intercurrent illness
  - Mild hepatomegaly
  - Slight hypertransaminasemia (135 U/l) with lactic acidosis
  - Lactic aciduria with ketonuria and slight excretion of glycerol
  - Normal FBPase activity in leukocytes
  - Decreased activity of FBPase in liver (2.1, NV: 22 and 26 mol/min/mg ptn)
  - Decreased glycogen content in liver (15.1 mg/g of tissue; NR: 20-50).

- Genetic analysis of FBP1 gene:
  - c.618delA, p.G207fs homozygote
GSD Type II

- Also known as Pompe Disease, acid α-glucosidase deficiency or acid maltase deficiency
- Autosomal recessive
- Lysosomal storage disease - accumulation of glycogen in all tissues
- 1 in 40,000 live births
- Treatment - Enzyme replacement therapy Myozyme (Genzyme)
GSD Type II

• Infantile onset (1 month)
  – Cardiomegaly, cardiomyopathy, hepatomegaly, weakness and hypotonia
  – Death due to cardiorespiratory failure in first year

• Late or adult onset (20-60 years)
  – Slowly progressive myopathy affecting skeletal muscle
    • Involvement of diaphragm and accessory muscle of respiration leads to respiratory failure
  – Cardiomyopathy generally absent

• Symptoms correlate with residual enzyme activity
GAA gene Mutations

• Common Mutations
  – c.-45T>G - Mild phenotype
  – c.525del - severe phenotype

• Rare mutations
  – >150 listed on mutation database at: http://www.pompecenter.nl/
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| 0      |     |     |     |     |     |     |      |     | 1      |
| Ia     |     |     |     |     |     |     |      |     | 2      |
| Ib     |     |     |     |     |     |     |      |     | 3      |
| III    |     |     |     |     |     |     |      |     | 1      |
| VI     |     |     |     |     |     |     |      |     | 1      |
| IX X-linked |   |     |     |     |     |     |      |     | 2      |
| IX Autosomal | |     |     |     |     |     |      |     | 1      |

|        |     |     | PHKG2 |     | PHKB | PHKG2 | PHKB | PHKB |
| 0      |     |     |       |     |      |       |      |      |
| Ia     |     |     |       |     |      |       |      |      |
| Ib     |     |     |       |     |      |       |      |      |
| III    |     |     |       |     |      |       |      |      |
| VI     |     |     |       |     |      |       |      |      |
| IX X-linked |   |     |       |     |      |       |      |      |
| IX Autosomal | |     |       |     |      |       |      |      |
Conclusions

• Genetics analysis can:
  – Provide a definitive diagnosis
  – Replace liver or muscle biopsy
  – Allow carrier testing and prenatal diagnosis
  – Change diagnosis and inheritance patterns
  – In some cases indicate prognosis