Treatment of Inherited Metabolic Diseases

Dr Dominique Roland

10 January 2014

Institut de Pathologie et de Génétique (IPG)

Center for Inherited Metabolic Diseases
Response to treatment

Number of monogenic disorders

Examples

- $\text{B}_12$-responsive methylmalonic aciduria
- Galactosemia
- Tay-Sachs disease

Total diseases surveyed (n=372)

- Complete response (n=46)
  - 12%
- Partial response (n=200)
  - 54%
- No effective treatment (n=126)
  - 34%
# Treatment of Genetic disease by Metabolic Manipulation

<table>
<thead>
<tr>
<th>Type of metabolic intervention</th>
<th>Substance</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary restriction</td>
<td>Phenylalanine</td>
<td>Phenylketonuria</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>Galactosemia</td>
</tr>
<tr>
<td>Replacement</td>
<td>Biotine</td>
<td>Biotinidase deficiency</td>
</tr>
<tr>
<td></td>
<td>Enzyme Replacement</td>
<td>Lysosomal storage diseases</td>
</tr>
<tr>
<td></td>
<td>therapy</td>
<td></td>
</tr>
<tr>
<td>Diversion</td>
<td>Sodium Benzoate</td>
<td>Urea cycle defect</td>
</tr>
</tbody>
</table>
## Treatment of Genetic disease by Metabolic Manipulation

<table>
<thead>
<tr>
<th>Type of metabolic intervention</th>
<th>Substance</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary restriction</td>
<td>Phenylalanine</td>
<td>Phenylketonuria</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>Galactosemia</td>
</tr>
<tr>
<td>Replacement</td>
<td>Biotine</td>
<td>Biotinidase deficiency</td>
</tr>
<tr>
<td></td>
<td>Enzyme Replacement therapy</td>
<td>Lysosomal storage diseases</td>
</tr>
<tr>
<td>Diversion</td>
<td>Sodium Benzoate</td>
<td>Urea cycle defect</td>
</tr>
</tbody>
</table>
• Autosomal recessive disease
• 1 / 10 000 birth
• PAH tetrameric structure
• PAH gene (chromosome 12)
• Classic PKU is caused by a complete or near-complete deficiency of phenylalanine hydroxylase activity (PAH) in liver.
• PAH deficiency results in intolerance to the dietary intake of the essential amino acid phenylalanine and produces a spectrum of disorders including phenylketonuria (PKU), non-PKU hyperphenylalaninemia (non-PKU HPA), and variant PKU.
Hydroxylase system

Phenylalanine Hydroxylase

Phenylalanine → Tyrosine

Tetrahydrobiopterin (BH₄)

Dihydropteridine Réductase (DHPR)

NAD⁺ → NADH + H⁺
The diagnosis of primary phenylalanine hydroxylase deficiency (PAH deficiency) is based on:

- An elevated plasma phenylalanine (Phe) concentration (most commonly identified by quantitative plasma amino acid analysis) persistently > 120 µmol/L (2 mg/dL) in the untreated state.
- Evidence of normal BH4 (tetrahydrobiopterin) cofactor metabolism, can be confirmed by urine pterin studies by liquid chromatography and dihydropterin reductase (DHPR) measurement.
PKU = first metabolic disease detected through Neonatal Screening - increased PHE levels

Dried blood spot on filter paper:
Guthrie card bacterial inhibition assay

R Guthrie in early 1960s
Newborn screening – dried blood spot (DBS)

Heel prick: Day 3 – 5 of life
Newborn screening and MS/MS

Tandem Mass spectrometry (MS/MS)

> 30 metabolic diseases
> 40 metabolites
Patients management after positive newborn screening on DBS

Fig. 1. Schéma décisionnel recommandé selon le chiffre de phénylalanine obtenu au dépistage à j3.

Phenylalanine Hydroxylase activity

In vitro

\[ \text{µmol Tyr/gr prot/h} \]

Residual activity

Trefz FK: In vivo residual activities of the phenylalanine hydroxylating system in PKU and variants. *J Inherit Metab Dis* 1981. 4: 101
Hyperphenylalaninemia Classification

- **Normal**: 1 - 3 mg/dl (60 - 180 µmol/l)
- **Hyperphenylalaninemia**: 3 - 10 mg/dl (180 - 600 µmol/l)
- **atypic PKU**: 10 - 20 mg/dl (600 - 1200 µmol/l)
- **Classical PKU**: > 20 mg/dl (>1200 µmol/l)
Hyperphenylalaninemia classification

- **Classical PKU:**
  - PAH activity: 0 - 1%,
  - PHE > 20 mg/dl
  - PHE tolerance: 200 - 350 mg

- **Variant PKU:**
  - PAH activity: 1 - 3 %,
  - PHE 10 - 20 mg/dl
  - PHE tolerance: 350 - 850 mg

- **Non-PKU Hyperphenylalaninemia**
  - PAH activity: 3 - 5 %,
  - PHE 3 - 10 mg/dl
  - PHE tolerance: > 850 mg
Untreated PKU patients

- Mild to severe mental retardation
- Neurologic symptoms
  - Microcephaly
  - Gait instability, tremor
  - Epilepsy
  - Autistic behavior
  - Auto and hetero aggressivity
- Eczema
- Decreased skin and hair pigmentation
  (Blond hair, blue eyes)
- Structural brain changes on MRI
- Musty body odor

*With respect to confidentiality, patients' photographs were removed before online publication*
Brain MRI and white matter abnormalities in adults
First dietetic treatment for an inborn error of metabolism

Horst Bickel (1953)

Phenylketonuria = Low-phenylalanine diet

Lancet, 1953; 2, 812-813
Long term Phenylalanine-restricted diet

- Control of natural protein intake according to PHE tolerance
  - Avoidance of high protein food
    (milk, dairy products, meat, fish, chicken, eggs, beans and nuts,...)
- Phenylalanine free formula (amino acids mixture with vitamins and oligoelements)
- Low protein food (bread, pasta, biscuits, ...)
- No control of protein-free food
PKU Phe-restricted diet

+ AA substitutes

forbidden

limited
International Recommendations for PHE control

Phe mg/dl

France

Germany

USA

GB

Adult

0 5 9 10 11 15 18 (y)
0-12 years:
Each 100µmol/l Phe increase predicted a 1.3 to 3.1 IQ point reduction

Phe is a predictive IQ indicator
Mean SD blood Phe level: 166 µmol/L
Mean lifetime blood Phe level: 412 µmol/L
IQ 116

Mean SD blood Phe level: 325.2 µmol/L
Mean lifetime blood Phe level: 388 µmol/L
IQ 93

Stability of blood phenylalanine levels and IQ in children with phenylketonuria.  
~ 400 mutations worldwide

http://www.pahdb.mcgill.ca
<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>No.</th>
<th>Graph</th>
<th>Statistic by Mutation Type:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missense</td>
<td>308</td>
<td><img src="#" alt="Missense" /></td>
<td>61.85%</td>
</tr>
<tr>
<td>Deletion</td>
<td>66</td>
<td><img src="#" alt="Deletion" /></td>
<td>13.25%</td>
</tr>
<tr>
<td>Splice</td>
<td>52</td>
<td><img src="#" alt="Splice" /></td>
<td>10.44%</td>
</tr>
<tr>
<td>Silent</td>
<td>30</td>
<td><img src="#" alt="Silent" /></td>
<td>6.02%</td>
</tr>
<tr>
<td>Nonsense</td>
<td>26</td>
<td><img src="#" alt="Nonsense" /></td>
<td>5.22%</td>
</tr>
<tr>
<td>Insertion</td>
<td>8</td>
<td><img src="#" alt="Insertion" /></td>
<td>1.61%</td>
</tr>
<tr>
<td>Sil./Splice</td>
<td>3</td>
<td><img src="#" alt="Sil./Splice" /></td>
<td>0.60%</td>
</tr>
<tr>
<td>Splicing</td>
<td>2</td>
<td><img src="#" alt="Splicing" /></td>
<td>0.40%</td>
</tr>
<tr>
<td>Silent ?</td>
<td>1</td>
<td><img src="#" alt="Silent ?" /></td>
<td>0.20%</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td><img src="#" alt="Unknown" /></td>
<td>0.20%</td>
</tr>
</tbody>
</table>

Total: 498

http://www.pahdb.mcgill.ca
Importance of missense mutations

- Enzyme is synthesized but activity is null or decreased
- PKU as a model of « misfolding » +++
- BH4 = Chaperon-like therapy

http://www.bh4.org/biopku.html
Mutations and residual activity

Sapropterin (6R-BH4) synthetic form of tetrahydrobiopterin

- Natural cofactor of aromatic amino acid hydroxylases
- Stabilization of the active tetramer forms of the mutant protein
- Protection from inactivation
- Acts as a chemical chaperon, preventing misfolding
- Orphan drug (FDA and EMEA)
Different responses to BH4 loading test (20mg/kg) according to genotype in PKU patients

BH4 sensitivity is effective if PHE level decrease $\geq 30\%$
Genotype / Phenotype correlation?

BH4 (20 mg/kg)

Patient 1
- No responder

Patient 2
- Responder

Patient 3
- Responder

BH₄ loading with 20 mg/kg bw in patient 1 (■), 2 (▲), and 3 (×), all genotype Y414C/R408W.

M. Lindner¹ Molecular Genetics and Metabolism 73, 104–106 (2001)
Minireview

Optimizing the use of sapropterin (BH₄) in the management of phenylketonuria

Nenad Blau, Amaya Bélanger-Quintana, Mübeccel Demirkol, François Feillet, Marcello Giovannini, Anita MacDonald, Friedrich K. Trefz, Francjaj J. van Spronsen

• About 70% of mild HPA and mild PKU patients proved to have benefits of BH4 therapy.
• About 10% of classical PKU patient respond to BH4

• In PKU patients responsive to BH4, sapropterin treatment could be used as an adjunct to a restrictive low-phenylalanine diet to reduce blood phenylalanine, and might even replace the diet in some instances.

Long term treatment with oral BH4 increase in Phe tolerance

R. Cerone et al. / Molecular Genetics and Metabolism 81 (2004) 137–139
BH4 (sapropterin) oral therapy and adverse effects


<table>
<thead>
<tr>
<th>Any adverse events on or after first dose</th>
<th>Placebo group (n=47)</th>
<th>Sapropterin group (n=41)</th>
<th>Total (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>Events</td>
<td>Patients</td>
<td>Events</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>------</td>
</tr>
<tr>
<td>Any adverse events on or after first dose</td>
<td>34 (72%)</td>
<td>95</td>
<td>21 (51%)</td>
</tr>
<tr>
<td>Adverse events that occurred in 5% or more of patients</td>
<td>7 (17%)</td>
<td>7</td>
<td>20 (23%)</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>13 (28%)</td>
<td>13</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Headache</td>
<td>4 (9%)</td>
<td>4</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4 (9%)</td>
<td>4</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>3 (6%)</td>
<td>3</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>2 (4%)</td>
<td>2</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>3 (6%)</td>
<td>3</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Only adverse events with onset on or after first dose are summarised here. *A patient was counted at most once for a given adverse event. Several events were counted if patients had the same adverse events with different onset dates or times.*

*Table 3: Adverse effects*
Treatment of PKU

« A la carte » treatment :

- Phe restricted diet
- BH4 in association with a Phe restricted diet
- BH4 only
- Therapies under investigation:
  - Large neutral amino acids
  - PEG-PAL (phenylalanine ammonia lyase) enzyme substitution.

PEGylation (conjugation with polyethylene glycol) of PAL has been found to decrease the immune response of the injected PAL. Clinical trials with this protected form of injectable enzyme are currently underway.

Modification of oral PAL to prevent degradation by digestive enzymes is also being investigated.
<table>
<thead>
<tr>
<th>Type of metabolic intervention</th>
<th>Substance</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary restriction</td>
<td>Phenylalanine</td>
<td>Phenylketonuria</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>Galactosemia</td>
</tr>
<tr>
<td>Replacement</td>
<td>Biotine</td>
<td>Biotinidase deficiency</td>
</tr>
<tr>
<td></td>
<td>Enzyme Replacement therapy</td>
<td>Lysosomal storage diseases</td>
</tr>
<tr>
<td>Diversion</td>
<td>Sodium Benzoate</td>
<td>Urea cycle defect</td>
</tr>
</tbody>
</table>
Classical Galactosemia

- Autosomal recessive disease
- 1/40,000 to 1/60,000 birth (in western countries)
- Galactose-1-phosphate-uridyl-transferase (GALT) deficiency
- GALT gene locus 9p13.13 (11 exons)
- Neonatal revelation – intoxication type disease
- Urgent diagnostic with DBS - Urgent treatment
- Pronostic is reserved
Classical galactosemia

Galactose
Classical galactosemia

- First symptoms, first weeks of life
- Gastrointestinal problems, feeding difficulties, failure to thrive, lethargy
- Hepatomegaly
- Severe hepatic insufficiency (jaundice, bleeding tendency, hypoglycemia) and death if not promptly treated
- E.Coli infection

- Diagnosis:
  - Galactose, GALT activity on dried blood spot test
  - Total red blood cell (RBC) Gal-1-P concentration
  - Galactitol on urine

- Urgent treatment: removal of lactose and galactose from diet (soy or lactose free formula) \(\rightarrow\) rapid recovery
Homozygotes for the classic galactosemia (G) allele (G/G): GALT enzyme activity < 5% of control values.

Heterozygotes for the classic galactosemia allele and a normal (N) allele (G/N): GALT enzyme activity +/- 50% of control values.

**Duarte variant galactosemia**

- The Los Angeles (LA) (D1) (p.Asn314Asp) variant produces no change in GALT enzyme activity in the erythrocyte and has normal promoter activity.
- The Duarte (D2) (p.Asn314Asp) allele produces bioinstability to the GALT enzyme complex and has reduced promoter expression.
- Newborns who are G/D heterozygotes may have a positive newborn screening and require further clinical, biochemical, and molecular genetic testing.
- Risk: cataract
- Annual follow up
- Under strict galactose restricted diet, pronostic is good
- Theoretical risk at adolescence when diet is released
- Resolves with dietary treatment
Psychomotor development and IQ

BUT .......

- Despite good compliance to diet
- Gal-1-P concentration maintained within normal range through ages.

- At 2 y: 80% patients with IQ > 80
- At 12 y: 80% patients with IQ < 80
Psychomotor development

- Mild growth retardation
- Delayed speech development
- Verbal dyspraxia
- Difficulties with spatial orientation
- Decreased concentration ability
- Reading difficulties
- Abnormal brain white matter

Despite galactose restricted diet started soon after birth

Despite compliance to diet

Despite Gal-1-P concentration within N range
Puberty and fertility in girls with classical galactosemia

- In general puberty is normal but may be delayed
  - Secondary Amenorrhea due to premature ovarian insufficiency (81%)
  - Hormonal substitution needed
  - Infertility regularly constant
Erythrocyte Galactose-1-Phosphate < 5 mg/dl

Classical galactosemia and variants

Alternative pathway

Residual activity

Berry et al  Mol Genet Metab 2004; 82, 130
<table>
<thead>
<tr>
<th>Type of metabolic intervention</th>
<th>Substance</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary restriction</td>
<td>Phenylalanine Galactose</td>
<td>Phenylketonuria Galactosemia</td>
</tr>
<tr>
<td>Replacement</td>
<td>Biotine Enzyme Replacement therapy</td>
<td>Biotinidase deficiency Lysosomal storage diseases</td>
</tr>
<tr>
<td>Diversion</td>
<td>Sodium Benzoate</td>
<td>Urea cycle defect</td>
</tr>
</tbody>
</table>
Vitamin-responsive Inborn Errors of Metabolism
Classical Homocystinuria
Cystathionine-β-synthase (CBS) deficiency

50% of patients responsive to B6
Marfanoid habitus, tall
Ectopia lentis, myopia, glaucoma, cataract
Osteoporosis, scoliosis, genu valgum, pectus excavatum or carinatum
Arachnodactyly
Restricted joint mobility
Developmental delay and mental retardation 60%
Seizure, psychiatric disturbances 50%

Main complication: thromboembolic events (veins and arteries)

With respect to confidentiality, patients' photographs were removed before online publication.
Pyridoxine trial in CBS deficiency

Vitamin B6 trial
+ folates and vitamin B12

B6 responsive 25-50 %

B6 non-responsive

Betaïne (methyl donor)
Avoid oral contraceptive
Antiaggregant treatment

Strict low protein diet (methionine restriction)
+ methionine free amino acid supplements

Aim of treatment: Plasma Total Homocysteine < 50 µmol/L
## Treatment of Genetic disease by Metabolic Manipulation

<table>
<thead>
<tr>
<th>Type of metabolic intervention</th>
<th>Substance</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary restriction</td>
<td>Phenylalanine Galactose</td>
<td>Phenylketonuria Galactoseemia</td>
</tr>
<tr>
<td>Replacement</td>
<td>Biotine Enzyme Replacement therapy</td>
<td>Biotinidase deficiency Lysosomal storage diseases</td>
</tr>
<tr>
<td>Diversion</td>
<td>Sodium Benzoate</td>
<td>Urea cycle defect</td>
</tr>
</tbody>
</table>
Sphingolipids synthesis

Gal, galactose; GalNAc, N-acetyl-D-galactosamine; Glc, glucose; NeuAc, N-acetyleneuraminic acid
Glucosylceramide accumulates in the lysosomes of certain cells, primarily tissue macrophages.
## Classification of Gaucher Disease

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Non-neuronopathic (type 1)</th>
<th>Acute neuronopathic (type 2)</th>
<th>Sub-acute neuronopathic (type 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>Childhood</td>
<td>Infancy</td>
<td>Childhood</td>
</tr>
<tr>
<td>Visceral involvement</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hematologic abnormalities</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bone disease</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Neurodegenerative course</td>
<td>-</td>
<td>+++</td>
<td>++ (progressive)</td>
</tr>
<tr>
<td>Survival (y)</td>
<td>6-80</td>
<td>&lt; 2</td>
<td>20-40</td>
</tr>
<tr>
<td>Frequency</td>
<td>1/40.000-60.000</td>
<td>&lt; 1/100.000</td>
<td>&lt; 1/100.000</td>
</tr>
</tbody>
</table>

Gaucher Disease Type 1
A Multisystemic Disorder

Heterogeneity in clinical presentation
Clinical diagnosis at every age

- Bone pain (63%)
- Bone crisis (33%)
- Hepatomegaly (79%)
- Splenomegaly (87%)
- Anemia (64%)
- Thrombocytopenia (56%)
- Erlenmeyer flask deformity (46%)

General symptoms:
- Fatigue
- Easy bruising/bleeding
- Menorrhagia
- Decreased appetite
- Abdominal pain
- Growth retardation
- Slow pubertal development

Pathologic fracture (15%)
Fibrose pulmonaire interstitielle
Joint collapse (8%)
Osteonecrosis (25%)
Osteopenia (42%)
Bone marrow infiltration (40%)

• Gaucher disease-related manifestations are progressive
• Skeletal tissue necrosis is irreversible and may lead to joint replacement
• Demineralisation can lead to osteopenia/osteoporosis and pathological fractures

Bone manifestations

- Demineralization and osteonecrosis - Femur
- Bone Marrow infiltration and Erlenmeyer flask deformity - Femur
- Demineralization and osteonecrosis - Humerus
- Pathologic fracture - Humerus
Patients with Gaucher disease can have a spectrum of symptoms, ranging from mild to severe neurological effects. The classic categories of types 1, 2 and 3 have blurry edges along this continuum.
About 95% - Type 1 Gaucher disease

About 5% - Types 2 and 3
Neuronopathic Gaucher

- Type 2
- Start at +/- 6 months - death < 2 ans
- Bilateral and fixed strabism
- Isolated horizontal supranuclear gaze palsy
- Trismus
- Cognitive impairment
- Bulbar signs
- Progressive spasticity
- Choreaathetotic movements
- Myoclonic epilepsy
- Hepatosplenomegaly
- Pulmonar infiltration
- Dermatologic changes

With respect to confidentiality, patients' photographs were removed before online publication.
Glucocerebrosidase (acid β glucosidase)

- Can be done on **leukocytes** (peripheral blood) or **cultured fibroblasts** (skin biopsy)
- Enzyme activity levels
  - Adults: usually **10% to 30%** of normal
  - Children (severe cases): < **10%** of normal
- Residual activity does not predict clinical outcome

**DNA analysis**
- Reliable way to test carriers among relatives at risk
- Genotype does not predict clinical phenotype
- N370S associated with mild disease/L444P with severe disease

Bases of treatment of Lysosomal Storage Diseases

- Increase degradation:
  - ERT (Enzyme replacement therapy)
  - EET (Enzyme Enhancement therapy)
- Reduce synthesis of substrat (SRT)

Synthesis → Degradation

Stockage
Response to ERT in Gaucher Disease

With respect to confidentiality, patients' photographs were removed before online publication.

Before treatment: Girl of 8 y and 8 months

After treatment: Girl of 10 y and 10 months
Enzyme Replacement Therapy in Gaucher Disease

Effect on Hemoglobin concentration

![Graph showing the effect of enzyme replacement therapy on hemoglobin concentration over time.](image-url)
Enzyme Replacement Therapy in Gaucher Disease

Visceral organ and hematologic responses to Imiglucerase treatment in children

Andersson et al, Pediatrics 2008;122(6):1182-1190S
Enzyme replacement therapies available in LSD

- Gaucher type 1
  - Imiglucerase - Cerezyme TM / CHO cells/Genzyme
  - Velaglucerase alpha - VIPRIV TM/ Shire Human Genetic
  - Taliglucerase alpha - Elelyso TM/carrot cell/Protalix

- Fabry
  - agalsidase alfa - Replagal TM / human cells / Shire Human Genetic
  - agalsidase beta - Fabrazyme TM / CHO cells / Genzyme

- MPS I (H/S, S) (extension MPS I < 5 ans)
  - CHO cells : Aldurazyme TM / Genzyme

- MPS II
  - CHO cells : Elaprase TM/ Shire Human Genetic

- MPS VI
  - CHO cells : Naglazyme TM/ BioMarin

- GSD II infantile ( + juvenile / adult)
  - CHO cells : Myozyme TM/ Genzyme
ERT: frequent immune response

<table>
<thead>
<tr>
<th>Maladie</th>
<th>rh-enz</th>
<th>Nbr</th>
<th>% Ab</th>
<th>% patients with reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaucher</td>
<td>Cerezyme</td>
<td>1322</td>
<td>15 %</td>
<td>13,8 %</td>
</tr>
<tr>
<td>MPS I</td>
<td>Aldurazyme</td>
<td>55</td>
<td>91%</td>
<td>32 %</td>
</tr>
<tr>
<td>MPS II</td>
<td>Elaprase</td>
<td>55</td>
<td>11%</td>
<td>55 %</td>
</tr>
<tr>
<td>MPS VI</td>
<td>Aryplase</td>
<td>10</td>
<td>100%</td>
<td>5 %</td>
</tr>
<tr>
<td>Fabry</td>
<td>Fabrazyme</td>
<td>58</td>
<td>89 %</td>
<td>52 %</td>
</tr>
<tr>
<td></td>
<td>REPLagal</td>
<td>55</td>
<td>55 %</td>
<td>10 %</td>
</tr>
<tr>
<td>Pompe</td>
<td>Myozyme</td>
<td>3</td>
<td>66 %</td>
<td>66 %</td>
</tr>
</tbody>
</table>

Limitations to E R T

- Immune response
- No brain access - tried in Gaucher type II (neurologic form)
- Limited results on bone
Substrate reduction therapy

Efficient if residual degradation activity

- Possible application on glycosphingolipids synthesis
- Application only with Gaucher disease
Sphingolipids synthesis

Gal, galactose; GalNAc, N-acetyl-D-galactosamine; Glc, glucose; NeuAc, N-acetyleneuraminic acid
- **Imminosucre N- butyldésoxynojirimycine (NB-DNJ)** (analogue of glucose) inhibits glucosylcéramide-synthase

- But inhibits also other enzymes:
  - α-glucosidases I et II
  - Explains adverse effects as diarrhea

- **Avantages**
  - Cross the blood-brain barrier
Other possible utilisation of Miglustat

- Neurologic Gaucher (NIH)
- GM2 Gangliosidosis (New York)
- GM1 Gangliosidosis

Need of a residual activity
Trials

- Fabry
Sphingolipids synthesis

Gb3 (globotriaosylceramide)

Gal, galactose; GalNAc, N-acetyl-D-galactosamine; Glc, glucose; NeuAc, N-acetyleneuraminic acid
Fabry Disease

- 1/117,000 male live births → 1/40,000 → 1/4,000 (Austria)
- X-linked inherited Lysosomal Storage Disorder
- Hemizygous male are symptomatic
- Heterozygous female may be as severely affected as hemizygous male
- (asymptomatic carrier?)
Alpha-galactosidase A gene (GLA gene) in Fabry disease

GLA: 7 exons
1290 nucleotides
429 AA

X- chromosome
Multi-organ Involvement in Affected Children and Adults

- Left ventricular hypertrophy
- Peau d'orange
- Angiokeratoma
- Head/cold intolerance
- Gastrointestinal pain
- Recurrent fever
- Deafness/vertigo/tinnitus
- Psychosocial problems
- Progression of kidney failure
- TIA/CVA’s
- Peripheral neuropathy

Early symptoms

Adapted from Zarate et al; Lancet 2008;372:1427-1435.
Progressive storage in Fabry Disease

- Early Symptoms:
  - Acroparesthesia
  - Early deafness
  - Deregulation of the autonomic nervous system

- Late Complications:
  - TIA, Stroke
  - Proteinuria, ESRD
  - Arhythmia, LVH
  - Organ Failure

- Organs involved:
  - Artery
  - Renal
  - Tissue
  - Autonomic nervous system

- Cellular GL-3 Storage

- Time progression

Modified from Wanner, Clin Ther. 2007; 29: 2-5
Typical distribution of angiokeratomas in Fabry disease

Angiokeratoma corporis diffusum

Courtesy of Dr Thomas Jansen, Bochum, Germany
Cornea verticillata: the most typical ocular sign

Increased vessel tortuosity in the conjunctiva and retina of the eye

Present in 70% of females
Present in 94% of males

Associated with loss of function mutations and predictors of disease severity in the absence of renal and cardiac manifestations

Reduction in the innervation of sweat glands
(right picture)

Hypohidrosis in FD

Lauria et al. BMJ, 2007

Untreated Fabry patient: significantly reduced sweat output

1 - iontophoresis onset
2 - QSART latency
3 - iontophoresis end

Courtesy M. Hilz
Clinical findings in young male and female patients with Fabry disease

- Acroparaesthesiae: Males 67%, Females 65%
- Hypohidrosis: Males 25%, Females 93%
- Cornea verticillata: Males 73%, Females 70%
- Angiokeratomas: Males 30%, Females 53%
- Gastrointestinal problems: Males 20%, Females 40%
- Cardiac abnormalities: Males 13%, Females 20%
- Low creatinine clearance/proteinuria: Males 13%, Females 15%
- Neurological/psychological findings: Males 25%, Females 67%

Adapted from Ries et al., Eur J Pediatr 2003;162:767–72 with permission. © Springer
Renal Manifestations, 19-year-old male

GL-3 accumulation in the kidney leads to irreversible damage

Images courtesy of B. Thurberg

Lipid deposition in the kidneys begins in the glomerulus

FSGS, focal segmental glomerulosclerosis

Renal Manifestations, 19-year-old male
Progression of Fabry Disease
Renal Complications

- Progressive glomerulosclerosis
- Tubular atrophy
- Renal tissue fibrosis

(Overt) Proteinuria
- Progressive decline in GFR
- Hypertension
- End-stage renal disease

Cardiac complications in FD

Hypertrophic cardiomyopathy

Typical electrocardiogram

magnetic resonance

Lidove et al., Am J Roentgenol 2006;186:1184–91
## Progression of Fabry Disease
### Cardiac Complications

<table>
<thead>
<tr>
<th>Condition</th>
<th>Males %</th>
<th>Age at first event</th>
<th>Females %</th>
<th>Age at first event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any cardiovascular events</td>
<td>19.3</td>
<td></td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>2.4</td>
<td>46</td>
<td>1.7</td>
<td>55</td>
</tr>
<tr>
<td>Significant cardiac procedure</td>
<td>7.7</td>
<td>46</td>
<td>4.7</td>
<td>53.5</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>11.3</td>
<td>40.5</td>
<td>7.1</td>
<td>47</td>
</tr>
<tr>
<td>Angina</td>
<td>3.4</td>
<td>43</td>
<td>4.3</td>
<td>45</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>3.8</td>
<td>45</td>
<td>2.9</td>
<td>54</td>
</tr>
<tr>
<td>LVH (echo)</td>
<td>21.6</td>
<td>42</td>
<td>18.2</td>
<td>51</td>
</tr>
</tbody>
</table>

*Wilcox et al 2008; Mol Genet Metab; 93:112-28 – Fabry Registry*
Stroke in Fabry Disease

Possible pathogenetic mechanisms causing stroke in Fabry disease:
- Arterial remodeling
- Progressive small vessel stenosis
- Endothelial dysfunction
- Thrombosis
- Compromised cerebral blood flow and cerebral autoregulation
- Cardiac embolism (cardiomyopathy)
- Hypertension (renal failure)

Stroke in Fabry disease frequently occurs before diagnosis and in the absence of other clinical events

Sims et al, Stroke 2009;40;788-794
Other symptoms of Fabry disease

- Hearing loss (85% of males and 75% of females over 60 years of age)
- Tinnitus
- Fever without explanation
- Impact on quality of life
- Psychiatric problems
Diagnosis

- Alpha-galactosidase A enzyme activity (on DBS or leucocytes): decreased in males – may be normal in females (not reliable for diagnosis)
- Gene sequencing

958del4

<table>
<thead>
<tr>
<th>Male</th>
<th>Hemizygous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>
## Enzyme Replacement Therapy

Two available preparations with proven efficacy

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Dose</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese Hamster Ovary (CHO) cell-derived recombinant human $\alpha$-Gal A</td>
<td>$0.2 \text{ mg/kg/2 weeks in 40 min}$</td>
<td>Shire Human Genetic Therapy Inc., Cambridge</td>
</tr>
<tr>
<td>Agalsidase $\alpha$</td>
<td>$1 \text{ mg/kg/2 weeks at a rate of 15 mg/h}$</td>
<td>Genzyme corp., Cambridge</td>
</tr>
</tbody>
</table>

Amino acid sequence identical to that of native $\alpha$-galactosidase A
Trafficking of the enzyme α-galactosidase A to cellular lysosomes

ERT, enzyme replacement therapy; M6P, mannose-6-phosphate; TGN, trans-Golgi network

Reproduced from Beck & Ries, *Fabry disease: clinical manifestations, diagnosis and therapy*. Oxford: OCC Europe Ltd; 2001 with permission
Current guidelines for starting ERT vary from one country to another.

Time of initiation still debated, especially in heterozygous females and children.

Current guidelines for starting ERT in Fabry disease patients:

<table>
<thead>
<tr>
<th>Fabry population</th>
<th>Guidelines for starting ERT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult males &gt; 16 y</td>
<td>At time of diagnosis of Fabry</td>
</tr>
<tr>
<td>Paediatric males</td>
<td>At time of development of significant symptoms † or If asymptomatic, consider at 10-13 years old</td>
</tr>
<tr>
<td>Females (all ages)</td>
<td>Monitor, institute if significant symptoms or evidence of progression of organ involvement</td>
</tr>
</tbody>
</table>

† Significant symptoms: pain, hypohydrosis, GI symptoms, development of proteinuria.

Improvements in acroparesthesia after 2 years of treatment with ERT

Figure 4  Improvement in Brief Pain Inventory scores after 1 and 2 years of treatment with agalsidase alfa in patients with Fabry disease (n = 20; 4 women, 16 men). *p<0.05 compared with baseline.
Improvement in both the severity and frequency of abdominal pain.

In a majority of patients, alleviation of these symptoms was evident after 3 months of treatment (agalsidase-α).

Dehout et al., *J Inherit Metab Dis* 2004;27:499–505
Reduction of left ventricular mass (LVM) after 2 years of treatment with ERT

17 patients with LV hypertrophy at baseline
(LVM indexed to height > 50 g/m².7)

Change in LV radial peak systolic strain rate of the posterior wall during 3 years of ERT according to the degree of myocardial fibrosis.
ERT stabilizes estimated glomerular filtration rate in patients with Stage I and Stage II chronic kidney disease.

24 patients receiving ERT

Adapted from Schiffmann et al., 2006; with permission from the European Renal Association–European Dialysis and Transplant Association.