Organisation of Biochemical Genetic Testing in Estonia

Katrin Õunap
Department of Pediatrics, University of Tartu
Department of Genetics, Tartu University Hospital

Basel, 09th May 2008
ESTONIA

- 1,35 million inhabitants
- Approximately 300,000 children
- ~15,000 births per year
Genetic service in Estonia

- In 1990 medical genetic service was opened at Tartu Children’s Hospital
  - For counselling children with rare inherited diseases (incl. metabolic diseases)
  - For prenatal testing
- In 1996 molecular diagnostic laboratory
  - For newborn screening (PKU, hypothyreosis)
  - For DNA testing of inherited diseases (over 30 different tests)
- Tallinn Children’s Hospital
  - Genetic counselling
  - Official genetic centre in 2004
At the present moment

• Two genetic centres for counselling and investigating patients with metabolic diseases:
  – In Tartu University Hospital, Department of Genetics
  – In Tallinn Children’s Hospital
Following analyses are available:

- ammonia, lactate, uric acid
- simple urinary screening tests:
  - Tests for mucopolysaccharidoses (MPS)
  - Reducing substances (Benedickt reaction)
  - Sulfites
  - Ketones (DNPH test)
- phenylalanine from dried blood (newborn screening since 1993)
- homocysteine
- total serum sialotransferrine
Following analyses are available:

- In cooperation with Central Laboratory of Chemistry of Health Protection Inspectorate in Tallinn:
  - Amino acid analysis (HPLC) since 1992
  - Sugars (HPLC) since 1992
  - Organic acid GC/MS since 2003
  - Very long chain fatty acid in serum since 2005
  - Creatine and guanidinoacetate analysis since 2007
  - MPS analysis, SAICAR test since 2007
### No of quantitative biochemical analyses in 2007

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Tartu</th>
<th>Tallinn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>453</td>
<td>279</td>
<td>174</td>
</tr>
<tr>
<td>Sugars HPLC</td>
<td>64</td>
<td>26</td>
<td>38</td>
</tr>
<tr>
<td>Quantitative MPS</td>
<td>14</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Organic acid GC/MS</td>
<td>439</td>
<td>246</td>
<td>193</td>
</tr>
<tr>
<td>GUAA/creatinine</td>
<td>108</td>
<td>98</td>
<td>10</td>
</tr>
<tr>
<td>VLCFA</td>
<td>23</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>
Following analyses are available:

- In Department of Genetics of Tartu University Hospital DNA tests for:
  - Phenylketonuria (PKU, 6 common mutations)
  - Classical galactosemia (Q188R, sequencing)
  - The main mutation of MCAD deficiency (985A>G)
  - The main mutation of LCHAD deficiency (G1528C)
  - CLN type III
Specific metabolic tests and investigations done elsewhere:

- Over 700 patients during 1990-2007:
  - Urinary organic acid GC/MS 154
  - Tandem MS screening (acylcarnitines) 172
  - MPS, oligosaccharides, sialic acid in urine 136
  - Specific enzymatic tests for LSD 100
  - Enzyme/DNA analysis for mitochondrial disease 34
  - The confirmation of classical galactosemia 17
  - VLCFA in serum 26
  - Isoelectric focusing of sialotransferrines 21
  - 7-dehydrocholesterol 26
Tests developed out locally:

- Organic acid GC/MS
- VLCFA
- GAA/creatine
- Galactosemia
- GAG/oligosaccharides/sialic acid
Training possibilities

• Biochemist K. Kall studied in Amsterdam MC in 2005 January (3w)
  → organic acid GC/MS, GUAA/creatine, VLCFA

• Biochemist K. Krabbi studied in Rotterdam Erasmus MC in 2006 (5m)
  → urinary MPS, oligosaccharides
Tests to work out in the near future:
Tandem MS analysis

- 3200 Q-TRAP LC MS/MS mass spectrometer (Applied Biosystems, USA)
- In Department of Biochemistry of Tartu University
- Dr. K. Joost (ERNDIM scholarship) and biochemist K. Kilk will study in Amsterdam MS in May 2008
To continue ordering from abroad

![Graph showing enzyme analysis and DNA analysis trends from 1995 to 2007.]

- **enzyme analysis for LSD**
- **DNA analysis for mitochondrial diseases**
- **enzyme analysis for mitochondrial diseases**
- **neurotransmitters**
- **biotinidase**
- **7-dehydrocholesterol**
Problems in ordering from abroad

- It takes time!
- The price list of our Health Insurance Fund (HIF) for BGT is ~3-4x lower than in developed EU countries
- Our HIF does not give us E112 form, but they give the guarantee letter, if we send
  - The application from MD
  - The application from parents
  - Answer within 2-4 weeks
Results during 1990-2007:

• In 123 patients the diagnosis of inherited metabolic disease was confirmed:
  – PKU 38 cases (31%)*
    • 24 during newborn screening program since 1993
    • 13 late diagnosed PKU cases
    • 1 prenatally diagnosed PKU case

* There are altogether 77 PKU cases in the register (1979-2007)
PKU screening programme

- No of live births
- No of tested newborns
- Diagnosed PKU cases

Graph showing trends from 1993 to 2006.
Lysosomal storage disorders:

- 35 patients (28%) during 1990-2007
- Mucopolysaccharidoses (MPS) 14 cases
  - 8 patients with MPS II (Hunter syndrome)
  - 4 patients with MPS IIIA (Sanfilippo syndrome)
  - 2 patients with MPS VI (Maroteaux-Lamy s.)

- Others 21 cases
The livebirth incidence of MPS in Estonia 1:24,154

We excluded last 5 years (possible diagnostic time)
Other LSD

- Fabry disease 5 (2 families)
- GM1 gangliosidosis 3 (2 families)
- Gaucher disease 3 (2 families)
- CLN type 2 2
- CLN type 1 1
- CLN type 3 2 (1 family)
- Tay-Sachs disease 1
- Metachromatic leukodystrophy 1
- Wolman disease 1
- Niemann-Pick disease 1
Classical galactosemia:

- 10 patients (8%), Birth prevalence is ~1:20,000
Mitochondrial disorders:

10 patients (8%) with mitochondrial disease:

- **Leigh syndrome** (2 patients)
  - Respiratory chain complex I deficiency (mtDNA mutation T10191C in ND3 gene)
  - Respiratory chain complex I and IV deficiency (mutations in SCO2 gene)
- **Mitochondrial myopathy in a girl and her mother** – respiratory chain complex I and IV deficiency
- **PDH deficiency** (2 patients)
- **MELAS syndrome**
- **Kearns-Sayre syndrome** (2 patients)
- **LHON**
Urea cycle disorders:

- 7 patients (6%) with urea cycle disorders:
  - 1 boy with hemizygous ornitine transcarbamylase (OTC) deficiency
  - 5 females with heterozygous OTC deficiency
  - 1 patient with argininemia
Fatty acid oxidation defects:

• **LCHAD**
  – One family with 2 children – genotype 1528G>C/IVS16-2A>G/A (Olsen et al.)
  – One family with 2 children – genotype 1528G>C/1528G>C

• **MCAD**:
  – We have not found any MCAD deficiency cases!
  – The frequency of possibly affected homozygotes 1 out of 193 000 (Lilleväli et al. 2000)
Other metabolic diseases:

- Alkaptonuria 2
- Tyrosinemia type I 1
- Lysinuric protein intolerance 2
- Maple syrup urine disease 1
- Hyperornithinemia/gyrate atrophy (HOGA) 2
- Dihydropteridine reductase (DHPR) deficiency 1
- Aromatic L-amino acid decarboxylase (AADC) deficiency 2
- Hereditary fructose intolerance 1
- CDG Ia 1
Many thanks!

- **Charite Virchow Klinikum**
  - Prof. E. Mönch
- **Gothenburg University**
  - Dr. E. Holme
- **Rotterdam Erasmus University**
  - Dr. O.P. van Diggelen, Dr. J. Huijmans
- **Amsterdam Free University**
  - Prof. C. Jakobs, Dr. G. Salomons
Many thanks!

• Tallinn Childrens Hospital
  – Dr. R. Zordania, Dr. K. Joost

• Central Laboratory of Chemistry
  – K. Kall, K. Krabbi, T.-M. Laht

• Tartu University Hospital
  – Dr. T. Reimand, Dr. K. Muru, K. Varb
Thank you for your attention!