Introduction to biochemical genetics

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Outline

Aims of the course
Basic features of IEM, population frequency
History of IEM
Genetic origin of IEM
Pathogenetic mechanisms
Small molecules and complex molecules
Clinical features of IEM
Aims of the course on biochemical genetics
Biochemical genetics = IEMs
Case 1-Isovaleric aciduria

- failure to thrive,
- vomiting
- repeatedly altered consciousness
- ketoacidosis
- sweaty feet odour
Case 2-adult cystinosis

Case 3- MPS I

http://eyepathologist.com/images/KL1771.jpg
http://deti.msk.ru/plaxin_eger.jpg
http://myweb.lsbu.ac.uk/dirt/museum/margaret/438-1811-2640151.jpg
Case 4-cystinuria

http://www.nature.com/ki/journal/v73/n8/images/5002790f1.jpg
Basic features of IEMs
Inborn errors of metabolism

usually AR, GR

usually enzyme

product

clinically variable
Clinical features of IEMs-age
Clinical features of IEMs-organs

http://universe-review.ca/110-82-organs.jpg
Clinical features: (non)specific signs

- **specific**
- **non-specific**

*Examples: NH₃, uric acid*

http://gatsome.com/images/iq.gif
http://www.saratogaschools.org/AcademicServices/MiddleSchool
Clinical features—multisystemic involvement
Diagnostic procedures in IEMs

DNA/RNA  Enzymes  Metabolites

| Clinics
| specific-e.g.
| smell
| urine color
| nonspecific-e.g.
| coma
| PMR
| dysmorphic features
| hepato/myopathy
| other
Frequency of IEMs

- newborn screening 1:1000-1:4000
- selective screening at least 1:500-1:1000
- frequency of heterozygotes at least 1:15
- population specific examples
  - higher incidence in imbred populations (PKU Turkey, organic acidurias Middle East)
  - tyrosinemia type I- Quebec
  - aspartylglykosaminuria- Finland
  - lysosomal storage disorders- Izrael
IEMs in the Czech Republic

incidence ~ 1:1000
so far ~150 different diseases

ČR, 2005, n=127
Treatment of IEMs
History of biochemical genetics
1857-1936

http://www.historiadelamedicina.org/imagenes/garrod.jpg
Trophîme Bigot, 16.-17.století
1857 L. Pasteur demonstrates microbial origin of fermentation, R. Wirchow describes mitosis
1867 dynamit
1874 jeans
1886 automobil = car
1890 underground
1895 cinematograf
1902 periskop, el. typewriter; Nobel prize for discovery of malaria transmission
1908 Nobel prize for discovery of immunity
1936 tape recorded; Nobel prize for neurotransmitter research
THE INCIDENCE OF ALKAPTONURIA: A STUDY IN CHEMICAL INDIVIDUALITY

ARCHIBALD E. GARROD

Lancet, vol ii, 1902, 1616-1620
Expansion of the field

1908
- 5 diseases (alkaptonuria, cystinuria, porphyria, pentosuria, congenital steatorhea)
- 1909 first textbook
- Incidence unknown
- Dx: 4 metabolites in urine
- Rx none

cca 1960
- Ca 40-50 diseases
- Analytical methods
- Incidence of PKU
- PKU-1st screening program (R. Guthrie)
- PKU-1st treatable IEM (H. Bickel)

2009
- At least 500 diseases
- Medline 122,797 papers (28/9/2009)
- Textbook-web based
- Incidence at least 1:1,000
- Dx: metabolites, enzymes, DNA
- Screening MS-MS
- Rx for ~ 1/3 diseases
Biochemical genetics in the ČR

- PKU screening 1975-Doc. Blehová, Prof. Hyánek, Ing. Mrskoš
- Dx of other IEMs-leader Prof. Hyánek
- Labs in Prague, H. Králové, Brno, Olomouc, Ostrava
- In 2009:
  - Selective screening ~ 100 pacientů/rok (incidence 1:1000)
  - Newborn screening- 10 IEMs since October 1 2009, expected incidence 1:4000
First IEM: Alkaptonuria
Figura 2 - Depósito de pigmentação ocronótica de coloração azul-enegecida na pele das mãos.
1859 Boedeker- alkapton in urine
1891 alkapton=homogentisic acid
Pedigree for Alkaptonuria
William Bateson (1861-1926)

http://www.bioinformatics.nl/webportal/background/images/mendelexperiment.gif
Garrodův revolutionary concept

- Chemical individuality is determined by genes

- Inborn errors of metabolism = disturbance of chemical individuality
Alkaptonuria in 2009

- retrospective research of mummy HARWA 1500 př.n.l.
- patients known before Garrod
- 1902 Garrod
- 1909 tyrosine degradation pathway described
- 1958 enzyme defect in alkaptonuria established
- 1996 HGO gene description
- 2003 treatment with nitisone
Coxarthrosis

Valvular involvement

Urolithiasis
Liver HGO deficiency

Incidence 1:250 000 až 1:1 000 000

High incidence Slovakia

Late complications frequent

Rx inefficient
Novel treatment in alkaptonuria

nitison (NTBC)

http://www.natuurlijkerwijs.com/english/b4f4ca00.gif
Genetic origin of IEMs
Genetic origin of IEM

- Monogenic Mendelian inheritance
  - AR inheritance
  - GR/GD inheritance
  - rarely AD inheritance

- Mitochondrial inheritance
- Epigenetic changes
- X-inactivation
- Epsistasis
- Gene x environment interaction
- other
Common types of mutations

- Normal gene
- Point mutation
- Deletion
- Insertion
- Frame Shift
Molecular consequences of mutations

- **Protein amounts**
  - decreased
  - same
  - increased

- **Protein properties**
  - changes of isolated functions
  - global changes
  - misfolding

- **Nonprotein gene products**
  - siRNA
Role of mutations in evolution

- **Source of variability**
  (less important than meiotic recombination and combination of gametes)

- **Favourable mutations**
  are rare and already contained in genomes

- **Genetic diseases** = tip of iceberg (of genetic variability)
Mutations affect different domains

- e.g. PAH
- cytosolic enzyme
- homotetramer
- BH4 as cofaktor
- complicated mechanism of catalysis

Cellular consequences of mutations (e.g. CFTR)

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<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
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<tr>
<td></td>
<td>No synthesis</td>
<td>Block in processing</td>
<td>Block in regulation</td>
<td>Altered conductance</td>
<td>Reduced synthesis</td>
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<tr>
<td>Nonsense</td>
<td>G542X</td>
<td>Missense N1303K</td>
<td>Missense G551D</td>
<td>Missense R117H</td>
<td>Missense A455E</td>
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<tr>
<td>Frameshift</td>
<td>394 del TT</td>
<td>AA deletion ΔF508</td>
<td>RR317P</td>
<td>Alternative splicing</td>
<td>3849+10 kbc → T</td>
<td></td>
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http://student.biology.arizona.edu/honors97/group7/Halick1.gif
Genotype-phenotype (M. Gaucher)

Phenotype

- **severe**
  - splenomegaly
  - anemia
  - bone disorder
  - CNS

- **mild**
  - splenomegaly
  - anemia

- **unclear**

http://www.nature.com/embor/journal/v4/n7/thumbs/embor873-f4.gif
Health-disease threshold

- Environment
- Genes

Clinical manifestation
Preclinical test
Genetic test
IEM treatment - modifying environment

- Clinical manifestation
- Preclinical test
- Genetic test

- Environment
- Genes
Gene x environment interaction (PKU)

http://www.pkunews.org/adults/image005.gif
Disease modification by environment (alpha1-antitrypsin)
Current genetics is not Mendelian

- 1 gene = more diseases
- 1 gene = diseases with different types of inheritance
- several genes = same disease
- digenic inheritance
- transgenerational transmission of epigenetic marks
- other ……

classical genetics

1 gene=1 disease
Genetics has been and will be full of suprises
Patophysiological mechanisms in IEMs
Patophysiology

precursor

substrate

byproduct

product
Patophysiology

precursor -> substrate -> byproduct

Examples:
- Phe & Phe-derivatives
- ammonia
- cystine in cystinosis
- cystine in cystinuria
- mucopolysaccharides
Patophysiology

Examples:
- glucose in GSD
- ketone bodies in beta-oxidation defects
- plasmalogens in peroxisomal diseases
- cysteine in CBS deficiency
- AdoMet in RM
- ATP in mitochondrial diseases
Local vs. systemic consequences

Local consequence, e.g.:
- MPS-bones, tendons, spleen, liver, CNS
- cystinuria-urinary tract
Local vs. systemic consequences

Distant consequences-e.g.
- urea cycle disorders- coma
- organic acidurias-encephalopathy
- CBS deficiency- thrombosis, connective tissue disturbances
Disorders of small and complex molecules
Small molecules in biochemical genetics

- definition: < 1500 Da
  - gases, inorganic ions
  - amino acids
  - organic acids
  - saccharides
  - polyols
  - simple lipids
  - purines, pyrimidines
  - vitamins
  - oligomers: peptides up to 5-10 AA, oligosaccharides

- cytosol, mitochondrial stroma
- blood, urine
Diseases of small molecules

- usually dependent on exogenous supply
- manifestation: (repeated) acute toxicity, usually with encephalopathy/coma
- hepatopathy common
- common disturbances in routine labs-ammonia, Astrup, ketone bodies, glycemia, uric acid...
- symptoms develop due to specific type of food, fasting, catabolism
- chronic course possible (if toxicity low)
- usually good therapeutic response to diet and/or vitamins
Isovaleric aciduria

- FTT, vomiting, Kussmaul breathing
- consciousness: coma within 24-48 h after onset of symptoms
- metabolic acidosis, ketonuria
- sweaty feet syndrome

Cystinuria

http://www.nature.com/ki/journal/v73/n8/images/5002790f1.jpg
Complex molecules in biochemical genetics

- definition: > 1500 Da
  - glykolipids
  - sphingolipids
  - plasmalogens
  - neutral polysaccharides (glycogen)
  - mucopolysaccharides
  - (other polymers: proteins, nucleic acids...)

- usually associated with membranes

- concentrations in blood/urine rather low, exceptions exist (x MS/MS technologie)
Diseases of complex molecules

- Disease progresses usually regardless of any exogenous sources from food
- Typical course is progressive (± symptom-free period)
- Dysmorphosis at birth possible
- Frequent involvement of nervous system and musculature
- Organomegaly due to storage in lysosomal storage disorders
- Usually untreatable by diet or vitamins
Mukopolysaccharidosis type I

http://deti.msk.ru/plaxin_egor.jpg

http://myweb.lsbu.ac.uk/dirt/museum/margaret/438-1811-2640151.jpg

http://eyepathologist.com/images/KL1771.jpg

http://deti.msk.ru/plaxin_egor.jpg
<table>
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<th><strong>Origin</strong></th>
<th>&lt; 1500 Da</th>
<th>&gt; 1500 Da</th>
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<tbody>
<tr>
<td>Acute toxicity</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Chronic progression</td>
<td>±</td>
<td>Y</td>
</tr>
<tr>
<td>Localization</td>
<td>cytosol, ECT</td>
<td>membranes</td>
</tr>
<tr>
<td>Impact on structure</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>Dx</td>
<td>blood,urine</td>
<td>tissues (U)</td>
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<tr>
<td>Origin</td>
<td>exogenous</td>
<td>endogenous</td>
</tr>
<tr>
<td>Rx-diet, vitamins</td>
<td>efficious</td>
<td>inefficious</td>
</tr>
</tbody>
</table>
Patophysiology IEM

substrate

1. <1500 Da
2. >1500 Da
3. product
Clinical manifestation of IEMs
Clinical picture-age

http://www.hrr.co.uk/acatalog/crocodile_toddler.jpg
http://www.co.shasta.ca.us/html/DSS/images/FosterParentingAdopt/infant.jpg
http://markandrich.googlepages.com/Old-woman.jpg/Old-woman-full.jpg
Clinical features-organs

- **MUSCULAR SYSTEM**: The muscular system consists of layers of muscles that cover the bones of the skeleton, extend across joints, and can contract and relax to produce movement.

- **SKELETAL SYSTEM**: The skeleton is a strong yet flexible framework of bones and connective tissue. It provides support for the body and protection for many of its internal parts.

- **CIRCULATORY SYSTEM**: This system consists of the heart and a network of vessels that carry blood. It supplies oxygen and nutrients to the body's cells and removes waste products.

- **NERVOUS SYSTEM**: The nervous system is the body's main control system. It consists of the brain, the spinal cord, and a network of nerves that extend out to the rest of the body.

- **LYMPHATIC (IMMUNE) SYSTEM**: This system is a network of vessels that collect fluid from tissues and return it to the blood. It also contains groups of cells that protect the body against infection.

- **RESPIRATORY SYSTEM**: The respiratory system is centered on the lungs, which work to get life-giving oxygen into the blood. They also rid the body of a waste product, carbon dioxide.

- **ENDOCRINE SYSTEM**: Many body processes, such as growth and energy production, are directed by hormones. These chemicals are released by the glands of the endocrine system.

- **DIGESTIVE SYSTEM**: The digestive system takes in the food the body needs to fuel its activities. It breaks the food down into units called nutrients and absorbs the nutrients into the blood.

- **EXCRETORY SYSTEM**: The body's cells produce waste products, many of which are eliminated in urine. The job of the urinary system is to make urine and expel it from the body.

- **REPRODUCTIVE SYSTEM**: The male and female parts of the reproductive system produce the sperm and eggs needed to create a new person. They also bring these tiny cells together.

http://universe-review.ca/I10-82-organs.jpg
Selected common situations with high risk of IEM

- **Small molecules**
  - acutely ill newborn
  - (repeated) attack of long-term unconsciousness
  - failure to thrive

- **Complex molecules**
  - progressive CNS and musculature involvement
  - facial dysmorphism
  - organomegaly (liver, spleen, heart)
Food and IEMs (small molecules)

- (sub)acute toxicity
  - milk (lactose)-hepatopathy
  - saccharose/fructose/sorbitol- hepatopathy and hypoglycemia
  - excess protein- vomiting, lethargy, coma (urea cycle disorders, organic acidurias)
Fasting and IEMs

- hypoglycemia in GSD
- hypoglycemia with decreased production of ketone bodies (beta-oxidation defects)
- acidosis, ketonuria and metabolic encephalopathy in prolonged fasting (organic acidurias)
- respiratory alkalosis and encephalopathy (urea cycle disorders)
Abnormal urinary smell and color

- **smell (small volatile molecules):**
  - sweaty feet-isovalerate
  - maple syrup-branched ketoacids
  - boiled cabbage-methionine oxid
  - fish-trimethylamine
  - blackcurrant- organic acids
  - mouse-phenylacetate

- **color**
  - orange-urate
  - black upon oxidation-homogentisate
  - blue-odoxyl derivaties
  - green-4-OH-butyrate
Common labs in IEMs

Blood
- glycemia
- cholesterol
- TG
- uric acid
- MAc
- hyperammonemia, RAlk
- ALT,AST
- CK
- anemia/pancytopenia

Urine
- ketone bodies
- uric acid
- crystaluria
- myoglobinuria