Introduction to biochemical genetics

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Outline

Basic features of IEM, population frequency
History of IEM
Prototype IEM: PKU
Genetic origin of IEM
Pathogenetic mechanisms
Diseases of small molecules and complex molecules
Clinical features of IEM
Biochemical genetics = IEMs

- genetics
- biochemistry
- clinical medicine
Questions for PBCH exam

1. Basic characteristics of IEM.
2. Patogenetic mechanisms of IEM.
3. IEMs of small molecules.
4. IEMs of complex molecules.
Basic features of IEMs
Inborn errors of metabolism

- IEMs ~ 1/10 rare diseases
- About 800 nosological entities
- Cumulative frequency at least 1:500
- Each GP follows at least 1-2 patients with IEM
- About 30% can be managed (about 100 diseases well treatable)


National strategy on RD 2010-2020 (usnesení vlády ČR č. 466 ze dne 14.6.2010)
Inborn errors of metabolism

- Usually AR, GR
- Substrate
- Usually enzyme
- Product
- Clinically variable
Clinical features of IEMs-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 of IEMs-organisms

- **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 skeletal system 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 collects fluid from tissues and returns 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
Clinical features: (non)specific signs

**specific**

**non-specific**

e.g. 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

substrát

produkt

Clinical symptoms/signs
specific
nonspecific
Frequency of IEMs

- newborn screening (10-30 IEMs)
  
  1:800-1:4000

- selective screening (about 800 IEMs):
  
  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
Patients with confirmed diagnosis
(ÚDMP a KDDL VFN, Praha, Dx established in 2010, incl.heterozygotes)

190 patients (26 NS), 42 different diseases (+mito)
Treatment of IEMs
History of biochemical genetics
Trophîme Bigot, 16.-17. století
THE INCIDENCE OF ALKAPTONURIA: A STUDY IN CHEMICAL INDIVIDUALITY

ARCHIBALD E. GARROD

Lancet, vol ii, 1902, 1616-1620
Figura 2 - Depósito de pigmentação ocronótica de coloração azul-enegrecida na pele das mãos.
1859 Boedeker - alkapton in urine
1891 alkapton = homogentisic acid
William Bateson (1861-1926)

http://www.bioinformatics.nl/webportal/background/images/mendelexperiment.gif
Garrod's revolutionary concept

- Chemical individuality is determined by genes

- Inborn errors of metabolism = disturbance of chemical individuality
Garrod 1908: 4 IEMs
Group of authors 2012: about 800 IEMs
Biochemical genetics in the ČR

- 1975: PKU screening incidence 1:8 000
- 70 s – 90 s: Dx of other IEMs-leader Prof. Hyánek
- labs in Prague, H.Králové, Brno, Olomouc, Ostrava
- 2009: expanded newborn screening- 10 IEMs incidence 1:4000
- 2012: selective screening ~ 100 patients/year (frequency at least 1:1000)
Classical IEM:
Phenylketonuria
PAH

Tyrosine → Tyrosine-transaminase → p-Hydroxyphenylpyruvate → p-Hydroxylphenylpyruvate-dioxygenase → Homogentisate → Homogentisate-dioxygenase → Acetocacetate → 4-Fumarylacetocetate → Maleylacetocetate → 4-Maleylacetocetate
Phenylalanine hydroxylase (PAH) mutation map

revised January 31st, 2007 by
Maryjoseph Phammarthi and Dr. Charles Brivio

528 mutations identified to date

- Missense mutation
- Frameshift
- Splice site
- Nonsense
- Translation frame shift

- Significance of missense mutations:
  - Not found
  - Not found (same amino acid change)
  - Missense
  - Missense (same amino acid change)

- Codon
- Normal codon
- Amino acid substitution

- Heterozygous

- Allele specific
  - A to G substitution in codon 5

- Haplotype
- Intron 7, substitution in codon 1

- For more information on PAH mutations, visit:
  - PAHdb
  - PAHdb.ca

http://www.pahdb.mcgill.ca/
Untreated HPA/PKU

- Incidence in Czech Republic 1:8 000
- mental retardation
- behavioral problems
- seizures
- typical smell-mice
- light pigmentation, eczema

- biochemical and clinical sequelae highly variable: mild HPA, PKU
- maternal HPA: fetal damage

http://www.dshs.state.tx.us/newborn/images/PKU_untreated.jpg
Treatment goal = decreased Phe
## Daily intake in mixed western diet

### 3000-4000 mg/day

### Phe tolerance to maintain

- **Children**: 300-400 mg Phe/d
- **Adults**: 800-1200 mg Phe/d

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### Jídla s vysokým obsahem Phe

<table>
<thead>
<tr>
<th>množství</th>
<th>obsah Phe/mg</th>
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<tr>
<td>pstruh na grilu</td>
<td>200 g</td>
</tr>
<tr>
<td>hranolky</td>
<td>100 g</td>
</tr>
<tr>
<td></td>
<td><strong>1110</strong></td>
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<tr>
<td>pečené kuřecí stehno</td>
<td>150 g</td>
</tr>
<tr>
<td>vařené brambory</td>
<td>250 g</td>
</tr>
<tr>
<td></td>
<td><strong>1300</strong></td>
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<tr>
<td>smažený sýr Eldam</td>
<td>140 g</td>
</tr>
<tr>
<td>hranolky</td>
<td>100 g</td>
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<tr>
<td>tatarská omáčka</td>
<td>25 g</td>
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<tr>
<td></td>
<td><strong>1900</strong></td>
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<tr>
<td>smažený veľký rizik</td>
<td>110 g</td>
</tr>
<tr>
<td>vařené brambory</td>
<td>250 g</td>
</tr>
<tr>
<td></td>
<td><strong>1170</strong></td>
</tr>
<tr>
<td>špagety milánské/boloňské</td>
<td>1 porce 330 g</td>
</tr>
<tr>
<td>(se sýrem)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>1320</strong></td>
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</tbody>
</table>

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Hodnoty sú orientačné (průmerné), nikdy nemožné určiť priesnou hodnotu jídla z dôvodu rozdielnych receptur v jednotlivých restaurnácich zařízeních.


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Národní sdružení PKU a jiných DMP vydává

**Miniprůvodce jídelním lístkem s hodnotami Phe**
Classical dietary treatment of PKU

- Decreased Phe intake in natural food (1/5-1/3 of normal intake)
- Supplementation by AA mixture lacking Phe
- Tyr supplementation in AA mixture

Annual costs: 200 tis Kč/pateinet
Increased costs for family 3 tis Kč/month

1,3g B/100 ml
60g B/100 g
10g B/125 ml
10g B/sáček 18,2g

32 mg Phe/100 g
2,1 mg Phe/3/4 hrnku
5,6 mg Phe/56 g
LNAA in PKU treatment

Concept: competition between LNAAAs and Phe for the transporter at BBB

Expected effect: decreased Phe in brain

Clinical utility questionable

Figure 1. Phenylalanine competes with other LNAAAs for the same carrier to pass the blood-brain barrier. High phenylalanine levels, as seen in PKU patients, reduce the brain uptake of other LNAAAs and their availability in the brain.

Enzyme dysfunction in cofactor deficiency

- Severe mutant PAH
- Mild mutant PAH
- Deficiency of BH4

Error bars indicate 95% confidence interval.

Deficient enzyme activity may be due to lack of cofactor

- Genetically determined BH4 deficiency (malignant HPA)
- 1-2% HPA in screening
- More hydroxylases affected (Phe, Tyr, Trp)
- Severe CNS complications despite good dietary control and low Phe levels
- Substitution of neurotransmitter precursors necessary
PAH Mutations Associated with BH4-Responsive HPA

Regulatory domain

Catalytic domain

Tetramerization domain


F39L  L48S  I65T  R68S  A104D  S110C  D129G  Y414C

Only early treatment is efficient= reason why to carry out neonatal screening PKU
### Patients with IEMs in neon.screening

<table>
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<tbody>
<tr>
<td>PKU/HPA</td>
<td>(15-20)</td>
<td>15</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>MCAD deficit</td>
<td>0-1</td>
<td>2</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>LCHAD deficit</td>
<td>0-1</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>GA I</td>
<td>0-1</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Leucinosa</td>
<td>0-1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>VLCAD</td>
<td>0-1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other (IVA, CPTI, CACT, CPTII)</td>
<td>0-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>15-25</strong></td>
<td><strong>20</strong></td>
<td><strong>31</strong></td>
<td><strong>25</strong></td>
</tr>
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</table>
Maternal HPA

- Fetoplacentar quotients 2
- Even mild HPA in mother leads to highly elevated Phe levels in fetus
- Untreated mHPA/PKU: congenital anomalies, mikrocephaly, heart defects, mental retardation
- Strict dietary control in the mother necessary needed
- Concept: mother X fetus interaction
Genetic origin of IEMs
Genes and human diseases

- Humans - 23,000 genes estimated
- Knowns diseases with genetic component: 7,000 nemocí
- Causative gene known in 3,300 diseases
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)
Autosomal recessive inheritance in IEMs

- Typical for majority of IEMs
- Only apparent if more sibs affected or in consanguineous marriages
- Family history usually not helpful
- Heterozygote detection at metabolite or enzyme level usually not possible
X-linked inheritance of IEMs

- Less frequent in IEMs (OTC, Fabry, MPS II..)
- Typically- hemizygotes are affected
- Cave: heterozygotes may be symptomatic due to inactivation of the wild type X-chromosome (e.g. 30% OTC gene carriers are symptomatic, heterozygotes for Fabry disease are frequently symptomatic with later onset and milder course)
- Counselling consequences quite important
Maternal inheritance

- mtDNA transfer almost exclusively by oocyte cytoplasm
- Random distribution of mutant and wild type mtDNA (heteroplasmy)
- Variable clinical course in offspring
- Varied time of onset and varied organ involvement
- Prenatal and preimplantation dx not possible

http://www.ncbi.nlm.nih.gov/books/NBK22019/bin/ch21fb1.jpg
Genotype vs. phenotype

- Monogenic diseases = abstract construct
- Majority of traits (incl. diseases) are oligogenic/polygenic with environment interactions
- Effect such as X-inactivation, imprinting, epigenetic changes etc.
- Continuum of phenotypes based on localization and mutation severity

http://web.pdx.edu/~newmanl/ContinuousDiscont.GIF
Gene x environment

- injury
- PKU
- cancer
- lab.abnormal
- healthy

Clinical manifestation
Preclinical test
Genetic test
Gene x environment interaction (PKU)

![Image](http://www.pkunews.org/adults/image005.gif)
Genotype X environment (diet)

Irene
Dx 3 days of life

Irene's sister
Dx late in 1 year of life (seizures, cognitive impairment)

Irene's daughter following good dietary control in Irene's pregnancy

http://www.pahdb.mcgill.ca/
Patophysiological mechanisms in IEMs
Patophysiology IEM

substrate

1. <1500 Da
2. >1500 Da

product

1 2 3
Patophysiology

precursor → substrate → byproduct

product
Patophysiology

- precursor
- substrate
- byproduct

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

Examples:
- glucose in GSD
- ketone bodies in beta-oxidation defects
- plasmalogens in peroxisom.diseases
- cysteine in CBS deficiency
- AdoMet in RM
- ATP in mitochondrial diseases
- glycoproteins in CDG
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

substrát

produkt
Disorders of small and complex molecules
<table>
<thead>
<tr>
<th></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>
</tr>
<tr>
<td>Origin</td>
<td>exogenous</td>
<td>endogenous</td>
</tr>
<tr>
<td>Rx-diet, vitamins</td>
<td>efficious</td>
<td>ineffectual</td>
</tr>
</tbody>
</table>
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
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)
Mucopolysaccharidosis 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
Defects in protein glycosylation

Deficit fosfomanomutasy 2

Dědičné mnohočetné exostosy
Patophysicsology IEM

substrate

1. <1500 Da
2. >1500 Da
3. Product
## Categories of IEMs-examples

<table>
<thead>
<tr>
<th></th>
<th>Small molecule</th>
<th>Complex molecule</th>
</tr>
</thead>
</table>
| **Substrate accumulation** | • Aminoacidopathies  
• Hyperammonemias  
• Org. acidurias | • Lysosomal storage diseases |
| **Product deficiency**  | • Glycogenoses  
• FAO  
• Creatine synthesis defects | • CDG syndromes  
• Generalised peroxisomal diseases |
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
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[Image: http://universe-review.ca/l10-82-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 dysmorphology
  - organomegaly (liver, spleen, heart)
Hints of the possibility of IEM

- Family history: consanguinity or typical family tree, similar diseases in relatives, unexplained death in relatives
- Illness considered originally a common disease does not respond adequately to treatment
- Multisystemic involvement
- External factors/food influencing the course
  - catabolism
  - Fasting
  - Proteins or sugars (galactose, fructose) aggravate diseases
- Unexplained routine lab tests

Courtesy - Dr. D. Behulová
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- indoxylic 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