Hereditary metabolic disorders/Genetic background

Introduction

- **inborn disorders of metabolism** = biochemical human diseases; disease states in which a specific genetically determined enzyme defect causes a metabolic block with pathological consequences
- most inborn errors of metabolism are linked to the X chromosome; some are AD Hereditary - probably affect regulators of metabolic processes (membrane receptors and enzymes that catalyze steps dependent on the amount of product); their disorder leads to feedback damage, mutation and subsequent changes in protein structure

- mutations can manifest as enzymopathy, but they can also affect other regulatory mechanisms
- most metabolic diseases are caused by an "enzyme defect" - a defective enzyme most often has a "reduced enzyme activity" compared to its normal counterpart; sometimes the activity is completely absent − reduced activity is possibly the result of reduced affinity to the substrate or cofactor or instability of the enzyme molecule; sometimes **increased protein activity**, prolonged or shortened biological half-life may be present; other times, some changes in the nucleotide sequence can lead to "'excessive formation of a fully functional protein **(disorder of regulation of gene expression or translation) - protein disorders are therefore both qualitative and quantitative in nature**
- metabolism takes place step by step through a series of reactions, each step being catalyzed by a different enzyme; mutation of one enzyme of the metabolic process leads to the accumulation of the precursor and a decrease in the amount of the product; both can have pathological consequences
- examples:

defect type	examples of disabilities
enzyme defect	PKU, galactosemia, adenosine deaminase deficiency
receptor defect	testicular feminization, hypercholesterolemia
molecular transport defect	cystic fibrosis, hypertension
cell structure defect	Duchenne and Becker muscular dystrophy
defect homeostasis	antihemophilic globulin, immunoglobulins
growth and differentiation regulation defect	sex determination, X chromosome inactivation, tumor suppressors
intercellular communication defect	insulin, growth hormone, sex differentiation
defect mitochondria	Leber optic atrophy

Enzymopathy

- a total of more than 200 enzyme dysfunctions have been described
- **phenylketonuria** (PKU), **hyperphenylalaninemia'** (HPA)
  - disorders of amino acid metabolism
  - PKU = AR hereditary disorder of phenylalanine metabolism with a frequency in the Czech Republic of approx. 1:6000
  - is manifested by the gradual development of mental retardation, epilepsy and little pigmentation
  - high level of phenylalanine in the blood, manifested by phenylpyruvic acid in the urine
  - is caused by a mutation in the gene for phenylalanine hydroxylase (PAH), which catalyzes the conversion of phenylalanine to tyrosine
  - phenylalanine is part of all dietary proteins and if not metabolized to tyrosine by PKU patients, it accumulates in body fluids and damages the myelination of developing nerve fibers
  - part of the phenylalanine is converted by the phenylalanine aminotransferase into phenylpyruvic acid, which is excreted to an increased extent in the urine and gives it the smell of mice; there is less tyrosine in body fluids and less products of its metabolism in the body
  - manifests itself only after birth
  - during pregnancy, the excess of phenylalanine fetus is removed by the placenta; with the first doses of milk drunk, the level of phenylalanine in the blood rises newborn
  - a diet with a very low content of phenylalanine and the addition of tyrosine can influence the level of phenylalanine in the blood and ensure almost normal psychomotor development of the child
  - the basis of successful treatment is early diagnosis
  - affected children are born to healthy parents
  - the diagnosis can be established by screening examination: blood is taken on the 5th day of life; if PKU is suspected, the newborn is hospitalized immediately
  - for women with PKU, another critical period of their life is motherhood; during pregnancy, the placenta multiplies the concentration of phenylalanine in the blood of the fetus - if the woman does not follow a strict diet, the level of phenylalanine will harm the development of the fetus, regardless of its genotype; the consequence of so-called maternal PKU is mental retardation, microcephaly, congenital heart defects

- gene PAH was localized to chromosome 12q24.1 using mRNA probes isolated from liver cells; it is 90 kb long and has 13 exons
- more than 170 different mutations of the PAH gene have been described so far; 4 prevail in the Czech Republic, the most common being R408W (60%); mutation changed codon 408 in exon 12 so that instead of arginine (R) it codes for tryptophan (W)
  - the highest frequency of the mutation is in Belarus - it is therefore considered a Slavic mutation; the next most represented mutations are R158Q (aRginine – glycine (Q)) – a mutation frequent in the Mediterranean and R261Q in the 5th and 7th exons
  - other type is the second most common IVS12nt1 mutation; affects 12th intron and changes the editing point; the mutation is most abundant in Denmark
- most individuals with PKU are compound heterozygotes; the severity of the clinical manifestation is related to the combination of mutagenic alleles in the genome; eg R408W, IVS12nt1 and R158Q mutations cause classic PKU with virtually zero PAH activity, but R261Q mutation causes benign hyperphenylalaninemia (HPA); PAH activity is partially preserved and the blood phenylalanine level is significantly lower than in classic PKU (less than 1mM); the psychological development of children with HPA is adequate even without diet, but the fetus of women with HPA without diet during pregnancy is damaged in the same way as the fetus of women with classic PKU
- DNA analysis enables the detection of heterozygotes as well as prenatal examination of the fetus of heterozygous parents, examination of the fetus is performed from trophoblast cells or amniotic fluid; with DNA analysis, a presymptomatic diagnosis of PKU can be established, but also the severity of the disease and the method of treatment can be determined
- PKU is one of the candidates for gene therapy; the PAH gene was successfully transferred into mouse hepatocytes
- in some newborns with a higher level of phenylalanine in the blood, the condition later improves and they do not need a diet = the so-called transient form of PKU with delayed expression of the PAH gene in liver cells
- diet treatment is not effective in 1-3% of children; PKU caused by mutation of the genes for the synthesis of the PAH cofactor – tetrahydrobiopterin (BH1) – for dihydrobiopterin synthetase and dihydropteridine reductase – more complicated treatment was proven

Receptors and their disorders

- receptor dysfunction - proven as one of the causes of familial hyperlipidemias (*hypercholesterolemia*, FH) = increased level of cholesterol, triglycerides or both of these substances in the plasma; hyperlipidemia plays a significant role in the development of atherosclerosis and subsequently MI
- fats taken in food are absorbed in the small intestine; much more cholesterol is synthesized de novo in the liver
- fats are not soluble in water and blood and are therefore transported and distributed in the body in association with proteins
- lipoproteins = spherical formations composed of apolipoproteins that solubilize fats and bind specifically to cell receptors from fats; there are 9 types of apolipoproteins: from the intestine, fats are transported by chylomicrons (CM) to liver cells and tissues
- CM mainly contain apolipoprotein B-48; in tissues, lipase breaks down triacylglycerols into glycerol and fatty acids, which cells use as a source of energy
- in liver cells, fats from CM are metabolized and, together with cholesterol synthesized de novo, incorporated into VLDL with apolipoprotein B-100 and E and transported to tissues
- in the tissues, lipases cleave triacylglycerols from VLDL and change them to LDL, which mainly carries cholesterol
- LDLs can enter cells via LDL receptors on the cell surface
- cells use cholesterol as a precursor to other metabolites; intake of LDL by LDL-receptors of liver cells inhibits cholesterol neosynthesis; in excess, cholesterol is transported from the tissues to the liver as HDL
- bound by HDL receptors of liver cells and metabolized

- FH = incompletely dominantly inherited disease with elevated plasma cholesterol level, heterozygotesi have 7-15 mmol/l (norm around 5 mmol/l); incidence 1:500; homozygousi have more than 20 mmol/l and may be affected by MI already in childhood and adolescence; the most common cause of FH are mutations of the LDL receptor (LDLr)
- the LDLr gene is located on chromosome 19q13.3 (it has 45 kb, 18 exons and a 5.3 kb mRNA transcript), mutations in different exons have different phenotypic manifestations; mutations can completely block LDLr, disrupt mRNA splicing, disrupt the binding of the receptor in the membrane or the transport of the complex into the cytoplasm and its processing in lysosomes
- another type of FH – conditioned by mutation of the **APOB'** ***gene for*** *apoB-100* (chromosome 2p23, the gene is 43 kb long, has 29 exons, 14 kb mRNA - a very long transcript compared to the length of the gene) • changes in affinity of apoB-100 to LDLr due to mutation
- special expression of the APOB gene is the so-called editing (editing) of mRNA • apoB-100 is produced in the liver • in intestinal cells, 6666 bases of the APOB gene transcript are deaminated • changing the codon from CAA to UAA (stop codon) results in apoB-48 typical for chylomicrons
- another example of an APOB mutation is the codon 2488 mutation, causing an AMK swap that causes low blood cholesterol
- another hereditary change in fat metabolism is caused by, for example, AR hereditary deficiency of cholesterol transferase – it transfers cholesterol between different carriers (apolipoproteins) • a decrease in the level of HDL and an increase in the level of LDL and VLDL • a greater risk of atherosclerosis

Disorders of molecular transport

- **cystic fibrosis** (CF) = AR hereditary disease and incidence in newborns 1:1600 –1:2500
- CF affects the function of exocrine glands; insufficient secretion of pancreatic digestive enzymes is the cause of stool thickening and intestinal obstruction (meconium ileus) in newborns and indigestion in children; there is a higher concentration of chlorides in sweat; mucus in the respiratory tract is viscous, difficult to cough up and this is the cause of repeated chronic infections of the respiratory tract and lungs; men tend to be yourilly and in women there is reduced fertility
- death in 20-30 years due to changes in lung tissue after repeated infections and heart failure due to increased pulmonary blood flow resistance
- the CF gene - CFTR - was located on chromosome 7q31, it is expressed in epithelial cells
- the product is a protein with 1480 AMK with all domains duplicated; CF is a real candidate for gene therapy
- 50-80% of CFTR gene mutations are due to the deletion of 3 bases in exon 10, coding for the 1st ATP binding domain, the mutation causes the deletion of phenylalanine = the deltaF508 mutation causes a severe form of CF
- CFTR is already degraded in the ER and is not incorporated into the plasma membrane at all
- patients with CF are often compound heterozygotes
- symptomatic treatment is focused on the substitution of exocrine secretion of the pancreas, on the liquefaction of the secretion of the glands of the respiratory tract, on the prevention and therapy of respiratory tract infections

Cell structure defect

- **Duchenne muscular dystrophy** (DMD) and **Becker muscular dystrophy** (BMD) - X-linked recessive inheritance diseases affecting skeletal muscles and, to a lesser extent, cardiac muscle and smooth muscle
- patients with DMD after birth without problems, weakness of the muscles of the lower limbs manifests itself during childhood, **calves are hypertrophic'** (fatty pseudohypertrophy), CNS involvement manifests itself in a decrease in IQ by an average of 20 points, muscle weakness gradually worsens, so that in adolescence patients are confined to a wheelchair and die by the age of 20 from heart or respiratory failure; the level of serum creatine kinase is increased in the serum of those affected; color changes in the muscle structure are demonstrable in a muscle biopsy
- in female carriers, the manifestation is affected by inactivation of the X chromosome - they may have mild muscle problems, an increased level of serum creatine kinase and histologically demonstrable involvement of some muscle fibers
- DMD in women is rare, it affects women with karyotypes 45.X (Turner's syndrome) possibly. 46,XY (testicular feminization) or with deletion of the short arms X –chromosome
- BMD is milder, with a later onset of clinical manifestations, slower progression, and allows living to an older age; the population frequency is around 1:3500, of which 10–15% is maintained by a high frequency of new mutations; a third of cases are due to new mutations
- the DMD gene is the largest known human gene - located on Xp21 - 2.3 kb, more than 75 short exons, introns make up 99% of the gene length
- most mutations are caused by deletions of one or more exons - most of these deletions are accumulated at the 5' end of the gene and in the 44-50 region. exon; DMD and BMD do not differ in the location of the mutations; the more severe course of DMD is conditioned by a frameshift, in BMD the deletion affects entire exons or triplets and there is no frameshift
- the gene transcript is mRNA longer than 11kb and the translation product is dystrophin with 3685 AMK, dystrophin is located in the cytoplasmic membrane of muscle cells, stabilizes the membrane and anchors actin molecules in the cytoskeleton

Regulation of differentiation

- male gender is determined by the combination of heterochromosomes XY
- in the case of the presence of more than one X chromosome, the second and next X chromosome is in the nucleus 15-16. the day after fertilization spiralized (forms the sex-chromatin mark) and most of its genes are inactivated, thereby compensating for the imbalance in the number of X chromosomes in females and males
- in women with a translocation of part of the X chromosome on autosomes, only one of its parts is inactivated - studies have shown an X inactivation center on the X chromosome
- the gene is located in the Xq13.2 region = the so-called XIST – it is 17 kb long; the transcript can be detected in the nuclei of cells with more than one X chromosome; the transcript never enters the cytoplasm and therefore is never translated
- for male sex determination: the SRY gene was located in close proximity to the pseudoautosomal part of the Y chromosome, the gene is 2.1kb and is highly conserved; its translocation to the X chromosome conditions the findings of 46,XX in men with phenotype Klienefelter syndrome
- SRY is homologous to non-histone nuclear proteins, binds to the promoter of the cytochrome-P450-aromatase gene, which converts testosterone to the female hormone estradiol
- inactivation of the SRY gene in the embryo determines male sex
- SRY protein also binds to the promoter of the Muller inhibitory substance gene, its inactivation in the male embryo causes testes differentiation and regression of female organs
- the SRY gene product acts as a transcription regulator; it contains no introns and terminates the sequence for the polyadenyl terminus of the mRNA

Mitochondrial diseases

- mitochondria contain circular DNA (mtDNA) without nucleosomes and a nuclear membrane; they obtain energy through oxidative phosphorylation of sugars and fats and transfer it to cellular metabolism in the form of macroergic phosphate bonds of ATP
- each mitochondrion contains multiple copies of DNA
- it is actually the 24th chromosome of the human genome
- arise by autoreplicationand during mitosis they divide into daughter cells; the zygote is equipped only with maternal mitochondria; sperm does have one mitochondrion, but it is destroyed after fertilization
- mtDNA mutations are inherited matrilineally and their manifestations become more pronounced with age
- mtDNA mutations affect oxidative phosphorylation and are therefore manifested mainly in tissues sensitive to energy deficiency
- **Leber optic atrophy**, bilateral blindness in adulthood
- defects in the structure and function of mitochondria can also be conditioned by nuclear DNA mutations and be inherited in the classic mendelian way

Genes with an as yet unknown mechanism of action

- **fragile X syndrome** (fra-X), mental retardation of varying degrees; frequency in men 1:1250, characteristic facial appearance – protruding long earlobes, elongated rough face and large testes, fragile spot on the X chromosome in Xp27.3 location – FMR1 gene; it is inherited recessively linked to the X chromosome, 30% of female carriers are slightly mentally retarded, but without somatic changes, 20% of men with cytogenetically demonstrable fra-X are carriers without clinical symptoms, the daughters of these carriers are healthy, but the sons and grandsons of these daughters they may be mentally disabled = the so-called Sherman paradox

- in healthy men – carriers with cytogenetically demonstrable fra-X, the number of CGG triplets is 52 – 200 = so-called premutation, if an increase in triplets (premutation) has occurred, further increase in triplets and the emergence of a full mutation with clinical manifestations is very likely; when the mutation is transmitted by women, the emergence of a full mutation, the multiplication of CGG triplets up to 4000 - near the described fragile site, another sequence of CGG triplets was discovered with the possibility of multiplication and manifestations of fragility of the X chromosome and oligophrenia = the so-called FRAXE - it is 600 kb away from FRAXA (FMR1) - in the gene for myotonic dystrophy (19q13.2-3), a polymorphism in the number of GCT triplets was found, in Kennedy's disease (spinal and bulbar muscular atrophy) and in spinocerebellar ataxia, a polymorphism of CAG triplets; the number of CAG triplets decides who and when will be affected by Huntington's disease (HD) - AD (4p16.3), at the beginning of the gene for HD (IT15) we find 40 or more than 100 CAG triplets (norm 11 – 34) - deviations from the basic rules of monogenic inheritance apply to all these diseases, during transmission from generation to generation there is anticipation - in subsequent generations, the trait manifests earlier and to genomic imprinting - when transmitted from a parent of the same sex, the manifestation is earlier than when transmitted from a parent of the opposite sex

Links

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- Inherited metabolic disorders

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- MASOPUST, Jaroslav – PRUŠA, Richard. *Pathobiochemistry of metabolic pathways.* 1. edition. Prague : Charles University, 1999. 182 pp. pp. 211–212. ISBN 80-238-4589-6.