Types of Mutations. Model Monogenic disorders

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Chromosome abnormalities

- Changes are visible in a light microsope
- Numerical and structural abnormalities
- Chromosomal abnormalities are identified in approximately 0,6% of liveborn infants
- More than 50% miscarriages in the first trimester of pregnancy may be due to chromosomal changes

Chromosomal findings in early spontaneous abortions	**frequency (%)**
Trisomy	56
16	15
13,18,21	9
XXX, XXY, XYY	<1
Other	27
X Monosomy	19
Triploidy	18
Tetraploidy	3

Types of chromosomal abnormalities:

- Numerical abnormalities:

- poliploidy: triploidy, tetraploidy
- aneuploidy: trisomy, monosomy

- Structural: translocation, deletion, inversion, duplication, ring, marker

Numerical chromosomal abnormalities:

- **Poliploidy** - occurs when there are more than two paired (homologous) sets of chromosomes (triploidy, tetraploidy).
- **Aneuploidy** - one chromosome is extra (trisomy) or one is lost (monosomy)

Numerical changes can occur within **autosomal** and **sex** chromosomes

Triploidy – 69,XXX 69,XXY 69,XYY

- **Triploidy** - is a rare lethal chromosome abnormality caused by the presence of an extra set of chromosomes
- The most usual cause is two sperm fertilizing a single oocyte (dispermy)
- Sometimes the cause is a diploid gamete
- Triploidis very seldom survive to term
- The condition is not compatible with life

Tetraploidy - 92,XXXX 92,XXYY

- is caused by the presence of two extra sets of chromosomes
- is extremely rare, lethal condition

- it is usually due to failure to complete the first zygotic division

Aneuploidy – trisomy and monosomy

- Trisomy - is an abnormality in which there are three copies of a particular chromosome (e.g. 47,XX,+21)
- Monosomy – occurs when there is only one of a pair of chromosomes (e.g. 45,X)

Aneuploidy cells arise through two main mechanisms:

- Nondisjunction – failure of chromosome pairs to separate properly during cell division or failure of sister chromatids to disjoin (trisomy or monosomy)
- Anaphase lag – delayed movement of chromosome during anaphase (monosomy)

Aneuplidies of sex chromosomes

- Males: 47,XXY 47,XYY
- Females: 45,X 47,XXX

Klinefelter syndrome, 47,XXY

- Children: learning disabilities, delayed speech and language development
- The older child or adolescent may be discovered during an endocrine evaluation for delayed or incomplete pubertal development with eunuchoid body habitus, gynecomastia, and small testes.
- Adults are often evaluated for infertility or breast malignancy

45,X – Turner syndrome

- Short stature
- broad chest
- low hairline
- low-set eares
- webbed neck
- Girls with Turner syndrome typically experience gonadal dysfunction (non-working ovaries), which results in amenorrhea(absence of menstrual cycle) and sterility
- Mental development is normal

Structural changes within chromosome:

- Balanced:

- Translocations
- Inversions

- Unbalanced:

- Duplication
- Deletion
- Insersion
- Marker chromosome
- Ring chromosome

- Structural chromosome abnormalities result form misrepair of chromosome breaks or from malfunction of the recombination system

Structural chromosomal abnormalities:

- Balanced – if there is no gain or loss of chromosomal material

- Unbalanced – if there is gain or loss of chromosomal material
  Unbalanced chromosomal abnormalities have an effect on the phenotype

Reciprocal Translocation

- A type of chromosome rearrangement involving the exchange of chromosome segments between two chromosomes that do not belong to the same pair of chromosomes.
- Carriers of balanced reciprocal translocation are healthy persons but can produce gametes with unbalanced chromosomal material

Robertsonian translocation

- A type of chromosome rearrangement involving the exchange between the proximal short arms of the acrocentric chromosomes: 13, 14, 15, 21 and 22.
- The most common Robertsonian translocation is between chromosomes 13 and 14
- Carriers are asymptomatic but often produce unbalanced gametes that can result in miscarriage (monosomic or trisomic zygote).
- Robertsonian translocation with chromosome 13 or 21 can lead to Patau or Down syndrome.

Inversion

Inversion occurs when the segment between two breakpoints is inverted before rejoining the breaks

Deletion

- Deletion - loss of a segment of the chromosome
- Terminal Deletion - a deletion that occurs towards the end of a chromosome.
- Interstitial Deletion - a deletion that occurs from the interior of a chromosome.

Duplication

- Duplication occurs when a segment of the chromosome is repeated, once or several times.

Marker chromosome

Small supernumerary element in the constitutional karyotype, with or without phenotypic consequences

Isochromosome

Loss of a complete arm, "replaced" by the duplication of the other arm (equivalent to a monosomy for one arm and trisomy for the other)

Ring

- Two ends of the segment between breakpoints are joined to form a circular structure.

A **karyotype** is the number and appearance of chromosomes in the nucleus of an eukaryotic cell

- The term is often used to mean an image showing the chromosomes of a cell sorted in order and arranged in pairs

Mosaic karyotype

- When we have two or more genetically different cell lineages within one individual
- In mosaic karyotype we have a proportion of normal cells and a proportion of abnormal cells
- This anomaly results from mitotic nondisjunction or structural rearrangement

Autosomal Trisomies

- Down Syndrome
- Patau Syndrome
- Edwards Syndrome

Down Syndrome: Trisomy 21

- **1 in 1000**
- **60%** embryos and fetuses die in utero
- Full trisomy **– 95%**
- Unbalanced Robertsonian translocation**– 3- 4%**
- Mosaic trisomy – **1-2%**

Down syndrome 47,XY+21; 47,XX+21

- flat face
- short neck
- small ears
- small nose
- large tongue
- Epicanthus - a small normal fold of skin covering the inner corner of the eye
- Brushfield spots -small white spots on the periphery of the iris in the human eye
- Hypertelorism - an abnormally increased distance between the eyes
- short stature
- obesity
- mental retardation – mild (IQ 50-70) to moderate (35-50)
- high infertility rate
- life expectancy – approximately 50 years

Patau Syndrome: **Trisomy 13**

- 1 in 5000
- Over 95% of liveborn infants die in the first year of life
- 95% embryos and fetuses die in utero
- Full trisomy – 75%
- Unbalanced Robertsonian translocation – 20%
- Mosaic trisomy – 5%

Patau syndrome 47,XX, +13; 47,XY, +13

- small head (microcephaly)
- - cleft lip and/or cleft palate
- - small eyes (microphthalmia)
- - hypotelorism (abnormally close eyes)
- - absent or malformed nose
- - malformed ears

- - deformed feet
- - scalp defect
- - polydactyly (extra fingers)
- - clenched hands

Edwards Syndrome: **Trisomy 18**

- 1 in 3000
- Over 95% of liveborn infants die in the first year of life
- 95% embryos and fetuses die in utero
- Full trisomy – 99%

Edwards syndrome 47,XX, +18

- microcephaly (small head)
- - prominent back portion of the head (occiput)
- - small face
- - abnormal, low-set ears
- - small eyes
- - small mouth
- - recessed chin
- - ovelapping digits with clenched hands
- clubfeet

Microdeletion syndromes

- Diagnostics: prometaphase chromosomal analysis (HRBT – high resolution banding technique), fluorescent *in situ* hybridization (FISH)
- Phenotype: dysmorphism, congenital malformations, mental retardation
- Examples:

- Prader-Willi syndrome 15q11-1
- Angelman syndrome 15q11-12
- Langer-Giedion syndrome 8q24
- Miller-Dieker syndrome 17q13

Prader-Willi syndrome

In infancy, this condition is characterized by:

- weak muscle tone (hypotonia)
- feeding difficulties
- poor growth
- delayed development.

Beginning in childhood, affected individuals develop an insatiable appetite, which leads to chronic overeating (hyperphagia) and obesity. Some people with Prader-Willi syndrome, particularly those with obesity, also develop type 2 diabetes mellitus (the most common form of diabetes).

Monogenic disorders

- Changes are submicroscopic
- Only methods of molecular biology will allow to see changes
- Mutations affect only a single gene
- About 1% of the general population is affected by monogenic disorders
- 16 000 different monogenic diseases is known

Mutation - a permanent structural alteration in DNA.
A **mutation** occurs when a DNA gene is damaged or changed in such a way as to alter the genetic message carried by that gene.
- spontaneous: Caused by replication mistakes
- induced: Induced by exposure to a variety of mutagenes

Mutations:
- Somatic cells - the mutation is not passed along to the next generation
Cancer tumours are a unique class of somatic mutations.
- Germinal cells - germ cells give rise to gametes, some gametes will carry the mutation and it will be passed on to the next generation
Typically germinal mutations are not expressed in the individual containing the mutation.

Main classes of DNA mutations:
1 Big genomic rearrangement (insertions, deletions, duplications)
2 **Point mutations** (point deletions, insertions, duplications..)
3 **Splice site mutations** – create or destroy signals for exon-intron splicing
4 **Dynamic mutations** – are tandem repeats that often change size on transmission to children

1. Big genomic rearrangements
deletions – loss of a piece of DNA, deletion of a gene or part of a gene can lead to a disease or abnormality.
example: Duchenne muscular dystrophy (DMD), alpha-thalassemia

duplications - production of one or more copies of any piece of DNA, including a gene or even an entire chromosome. example: DMD

insertions – additional DNA sequence is inserted into a gene, disrupting the normal structure and function of that gene. example: hemophilia, neurofibromatosis

Duchenne muscular dystrophy (DMD) deletion example
- starts 3-8 year of life
- progressive deterioration of muscle tissue and resultant weakness
- lumbar hyperlordosis
- gnome's calf

Deletion of a small fragment of exon 44 dystrophin gene causes a severe form of DMD, whereas a large deletion, which occupies more than half of the entire gene, causes a milder disease – BMD (Becker muscular dystrophy).

Effect of mutation does not depends on the size of the deletion , but whether this deletion disrupts open reading frame (ORF) or not.
Open reading frame (ORF) is a DNA sequence that does not contain a stop codon.

Mutations affecting the open reading frame (ORF) produce a complete lack of protein and an acute form of the disease - DMD
Mutations that retain open reading frame (ORF) will result in protein abridgement and (depending on the amount and degree of changes in the protein molecule) causes varying degrees of severity of BMD.

Insertions - incorporation of additional DNA sequences (example: hemophilia, cystic fibrosis). Within inserted fragment stop codon may appears and reduces the protein length (causing lack of functionality).

Hemophilia – insertion example

- Inheritance: X-recessive
  Incidence: 1/10.000 births
- Blood coagulation disorder associated with the lack or deficiency of coagulation factor VIII
- excessive bleeding spontaneous or induced by minor injuries. Bleeding usually occurs next to the joint, causing pain, swelling and surrounding muscle spasms.
- Repeated episodes which lead up to permanent damage of the joints

2. Point mutations

- Mutations that occur within coding DNA can be grouped into two classes:

▸ **synonymous (silent) mutations** do not change the sequence of the gene product. Causes a codon change but does not result in an altered amino acid because of the *degenaracy* of the genetic code.

▸ **nonsynonymous mutations** change the sequence of the gene product, which may be a polypeptide or functional noncoding (=untranslated) RNA.

Point mutations - mutation resulting from a change in a single base pair in the DNA molecule (point insertion, point deletion, substitution)
<u>Single base substitutions</u>:

- **missense mutations** – replace one amino acid with another in gene product. These are nonsynonymous substitutions occur in coding region and changes the triplet codon for different amino acid
- **nonsense mutations** – replace an amino acid codon with a stop codon. Point mutation, which converts the normal codon to UAA, UGA, UAG, creating a premature STOP codon is called.
- **frameshift mutation** - Insertion or deletion of a small number of nucleotides (different than multiplication of three) into a coding region, which alter the reading frame of translation from that point.

- **Missense mutations can be classified into two subgroups**:

▸ **conservative substitutions** result in the replacement of an amino acid by another that is chemically similar to it. Often the effect of such substitutions on protein is minimal

▸ **nonconservative substitutions** result in the replacement of an amino acid by another with a dissimilar side chain

Subsitution:

- transition - exchange purin-purin or pirymidyn-pirymidin
- transversion - exchange purin-pyrimidin

Substitution in FGFR3 (Fibroblast Growth Factor Receptor 3) gene, Achondroplasia, Autosomal dominant disorder

- long, narrow trunk
- short extremities, particularly in the proximal (rhizomelic) segments
- a large head with frontal bossing
- hypoplasia of the midface
- trident configuration of the hands

transition - exchange purin-purin or pirymidyn-pirymidin – G->A -98% individuals affected with achondroplasia
transversion - exchange purin-pyrimidin G->C - 1% individuals

3. Splice site mutations

Splice site mutations – mutations altering sequences that are important for **splicing** (process that removes introns and joins exons in a primary transcript)

Concequenses:

- Exon skipping
- Intron retention

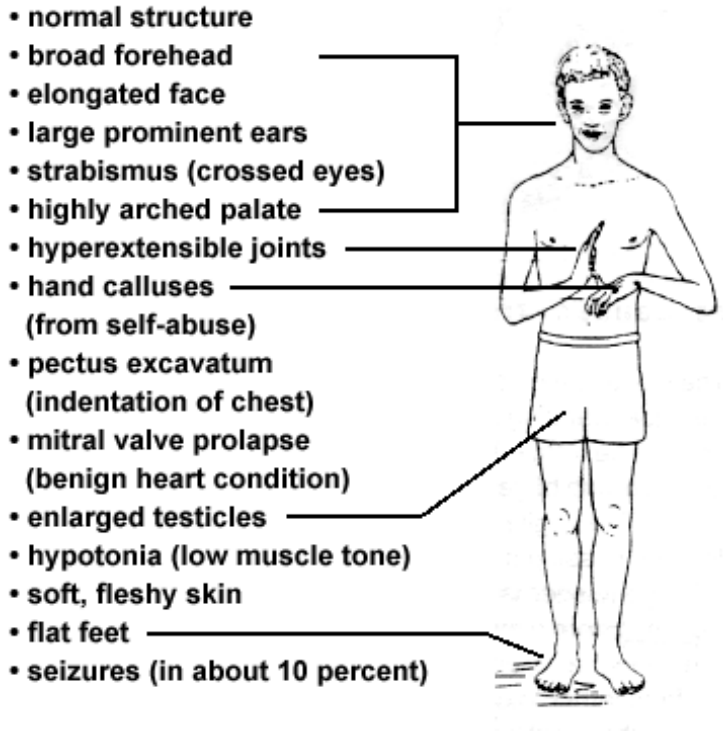
4. Dynamic mutations

- Dynamic mutation is caused by the expansion of trinucleotide repeats within the genome
- Trinucleotide repeat units lies within or adjacent to a disease-associated gene, there is a tendency for the tract to become progressively larger by expansion at meiosis, it becomes „unstable" by reaching a certain treshold size.
- Anticipation – the tendency for the severity of a condition in successive generations

Huntington's disease- example of dynamic mutation

- HD is a rare neuredegenerative disorder of the central nervous system characterized by unwanted choreatic movements, behavioral and psychiatric disturbances and dementia.
- CAG repeats expansion in IT15 gene - which adds a string of glutamines (Gln) to the encoded protein called **huntingtin**

Fragile X syndrome - example of dynamic mutation



Normal - The genetic code for the FMR-1 gene usually contains a limited repetition of CGG sequences. The normal range is 5-50 repeats.

Premutation - Carriers are not usually affected by fragile X syndrome, but they are at risk of having affected children.

Full mutation - If the number of repeats exceeds 200, usually this disrupts the code and prevents the production of the FMR protein. These individuals are usually affected by fragile X syndrome

Mitochondrial DNA mutations

- The mutation rate of mtDNA is higher than that of nuclear DNA. It is assumed to be the result of:
- the lack of DNA repair system
- lack of histones
- great amount of free radicals

Mutations in densely packed mitochondrial genome are associated with broad spectrum of degenerative diseases including central nervous system, heart, muscle, endocrine system, kidney and liver. Cells contain many mtDNA molecules
Polyplasmy – 1-10000 mtDNA in a cell

Homoplasmy – all copies of mtDNA are identical within coding region

Heteroplasmy – the presence of normal and mutated mtDNA within a cell (the proportion of mutant mtDNA molecules determines both the penetrance and severity of expression of some diseases)

DNA repair:
DNA repair usually involves cutting out and resynthesizing a whole area of DNA surrounding the damage.
Extracellular agents causing DNA damage:

- Ionizing radiation – gamma rays, X-rays can cause single and double-strand breaks in DNA
- UV light - causes cross-linking between adjacent thymines on a DNA strand
- Environmental chemicals: hydrocarbons, some plant and microbial products (alfatoxins), chemicals used in cancer therapy

Endogenous agents causing DNA damage:

- Depurination – approximately 5000 adenine or guanines are lost every day from each nucleated human cell
- Deamination – about 100 cytosines spontaneously deaminate per day in each nucleated human cell to produce uracil
- Mistakes in DNA replication – incorrect proofreading results in incorporation of mismatched bases e.g. Uracil is often incorrectly inserted instead of thymine into DNA
- Mistakes in recombination – cause strand breaks to be left in DNA

DNA repair:

- DNA repair seldom involves simply undoing the change that caused the damage (**direct repair**)
- Almost always a stretch of DNA containing the damaged nucleotide(s) is excised and the gap filled by resynthesis (**excision repair**)

The importance of effective DNA repairs is highlighted by the approximately **130** human genes participating in DNA repair system

Defects in those genes cause severe diseases:
Xeroderma pigmentosum
Nijmegen breakage syndrome

Xeroderma pigmentosum - severe sensitivity to all sources of UV radiation (especially sunlight). Defect in ultraviolet radiation induced **DNA repair** mechanisms.
Wide range of symptoms:

- premature aging of skin, lips, eyes, mouth and tongue; increased incidence of cancer in these areas
- blindness resulting from eye lesions or surgery for skin cancer close to the eyes
- progressive neurological complications including:

- developmental disabilities
- mental retardation
- high frequency hearing loss, progressing to deafness

NIJMEGEN BREAKAGE SYNDROME- autosomal recessive condition of chromosomal instability DNA double-strand breaks (on chromosome 8q21) can be induced by ionizing radiation

Clinical features:

- microcephaly with loss of cognitive skills
- distinct facial appearance
- short stature
- immunodeficiency
- radiation sensitivity
- strong predisposition to lymphoid malignancy.