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Genetic determination of disease

- Heredity
- Genetic variability (mutations × polymorphism)
- Monogenic × complex diseases

Jiří Navrátil

History

- **Johann Gregor Mendel** was a meteorologist, mathematician, biologist, Augustinian friar and abbot of St. Thomas' Abbey in Brno

  He worked with seven characteristics of pea plants: plant height, pod shape and color, seed shape and color, and flower position and color.

- **Laws of Mendelian genetics**

1. Law of dominance

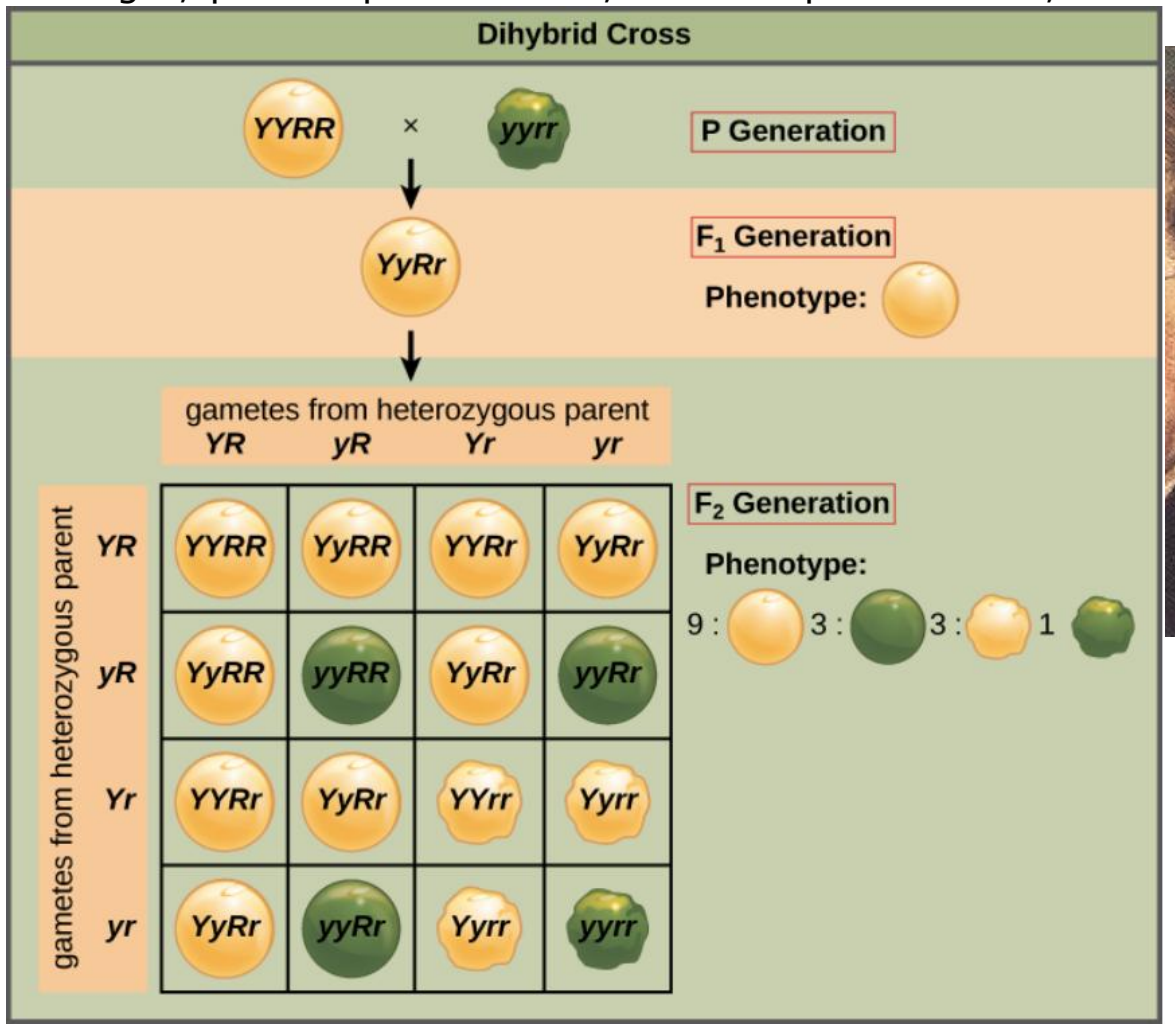
In a heterozygote, one trait will conceal the presence of another trait for the same characteristic. Rather than both alleles contributing to a phenotype, the dominant allele will be expressed exclusively

2. Law of segregation

When an organism makes gametes, each gamete receives just one gene copy, which is selected randomly

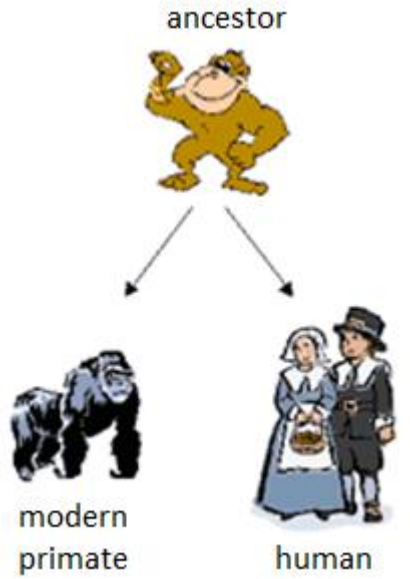
3. Law of independent assortment

The alleles of two (or more) different genes get sorted into gametes independently of one another → the allele a gamete receives for one gene does not influence the allele received for another gene.

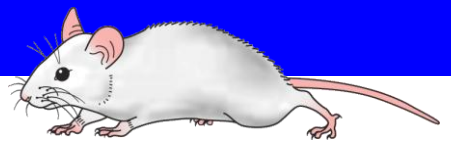


Genetics

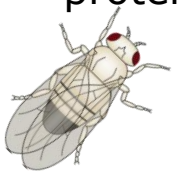
- **Genetics –** specialised field of biology focusing on variability and heritability in living organism
  - **Human genetics**
    - study variability and heritability in human
  - **Clinical genetic**
    - study pathological states, diagnostics, genetic counselling and prevention (family members)
  - **Cytogenetics**
    - chromosome alterations
  - **Molecular genetics**
    - study of the structure and function of isolated genes
  - **Population genetics**
    - study of variability in populations
  - **comparative and evolutionary genetics**
    - inter-species comparisons and evolution of species



Genomics



- study of the structure and function of genomes by means of genetic mapping, sequencing and functional analysis of genes
- aims to understand entire information contained in DNA
    - **structural genomics** = structure of genomes
        - construction of detail genetic, physical and transcriptional maps of genomes with ultimate aim to complete entire DNA sequence
        - (e.g. HUGO project)
    - **functional genomics** = function of genes and other parts of genome
        - understanding of the function of genes; very often using model organisms (mouse, yeast, nematodes, Drosophila etc.) as an alternative to higher organisms (many generations in relatively short time)
    - **Bioinformatics =** concerned with the acquisition, storage, analysis, and dissemination of biological data, most often DNA and amino acid sequences
        - uses computer programs for a variety of applications, including determining gene and protein functions, establishing evolutionary relationships, and predicting the three-dimensional shapes of proteins)



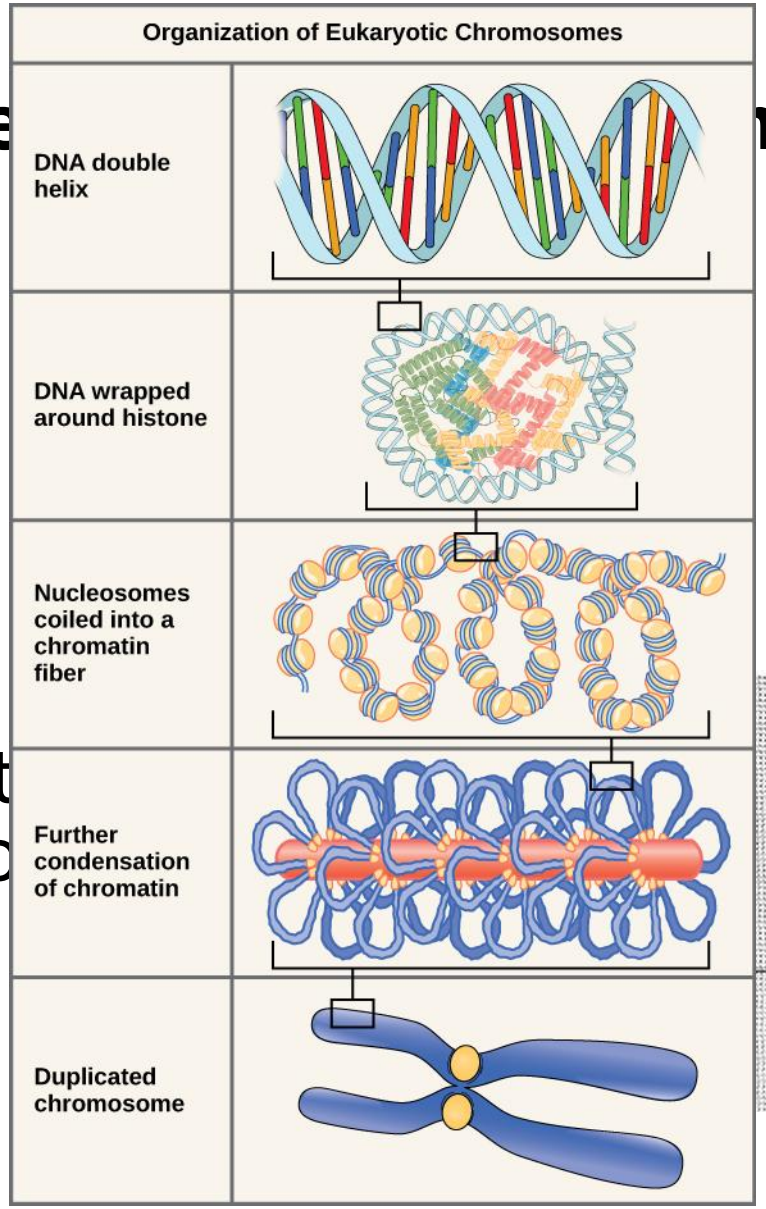
Genetic Terminology

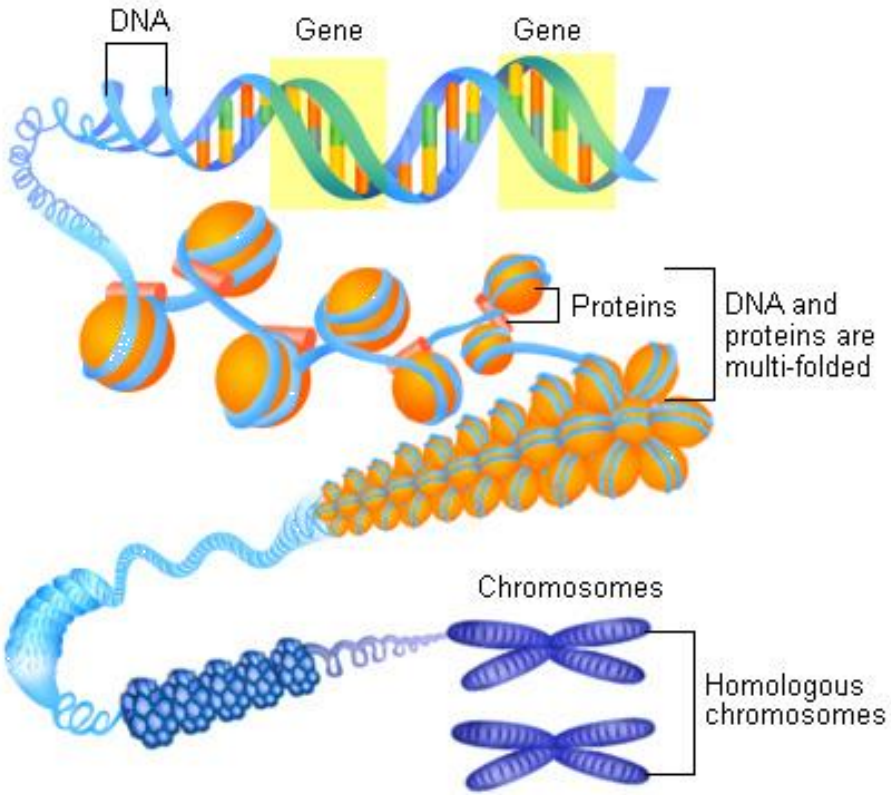
- **gene, alle** **mozome**

- locus
- alleles
- dominant
- recessive

- homozygot
- heterozygo

- genotype
- phenotype





DNAWrapBasic_withFX01.mp4

Chromosomal basis of heredity

- **DNA and RNA**

DNA: is the hereditary material in humans and almost all other organisms containing the instructions needed for an organism to develop, survive and reproduce

It is made of chemical building blocks called nucleotides that are made of three parts:

1. A phosphate group
2. A sugar group

} combined form the repeating 'backbone' of the DNA strands

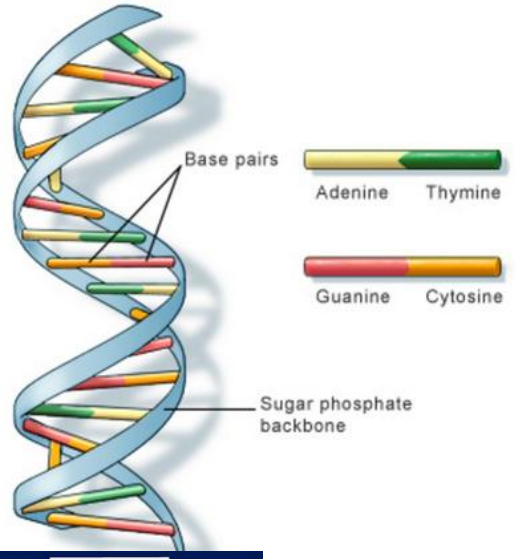
3. A nitrogen base (adenine (A), thymine (T), guanine (G) and cytosine (C))

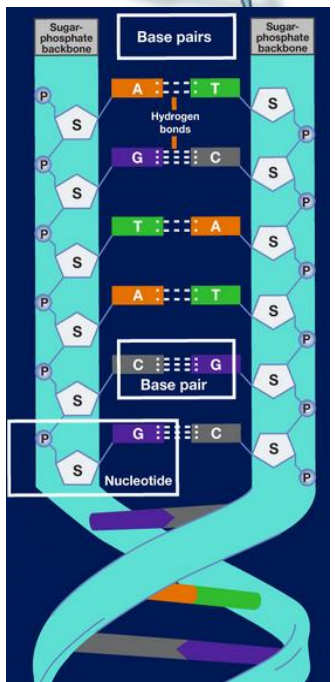
To form a strand of DNA, nucleotides are linked into chains, with the phosphate and sugar groups alternating

- DNA double helix

RNA: molecule similar to DNA. Unlike DNA, RNA is single-stranded

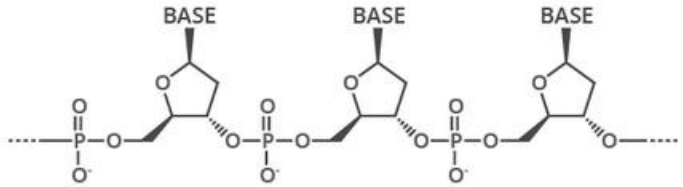
messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA)





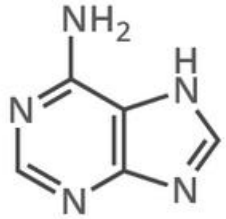
THE CHEMICAL STRUCTURE OF DNA

THE SUGAR PHOSPHATE 'BACKBONE'

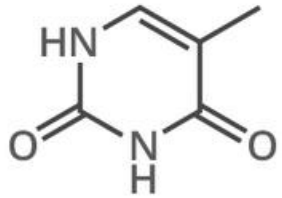


DNA is a polymer made up of units called nucleotides. The nucleotides are made of three different components: a sugar group, a phosphate group, and a base. There are four different bases: adenine, thymine, guanine and cytosine.

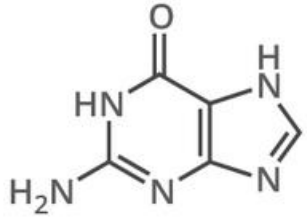
A ADENINE



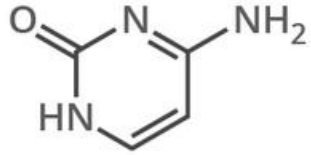
T THYMINE



G GUANINE

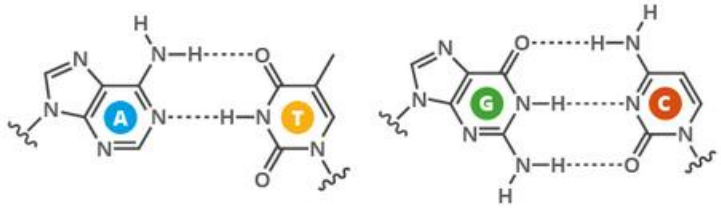


C CYTOSINE



WHAT HOLDS DNA STRANDS TOGETHER?

DNA strands are held together by hydrogen bonds between bases on adjacent strands. Adenine (A) always pairs with thymine (T), while guanine (G) always pairs with cytosine (C). Adenine pairs with uracil (U) in RNA.



FROM DNA TO PROTEINS

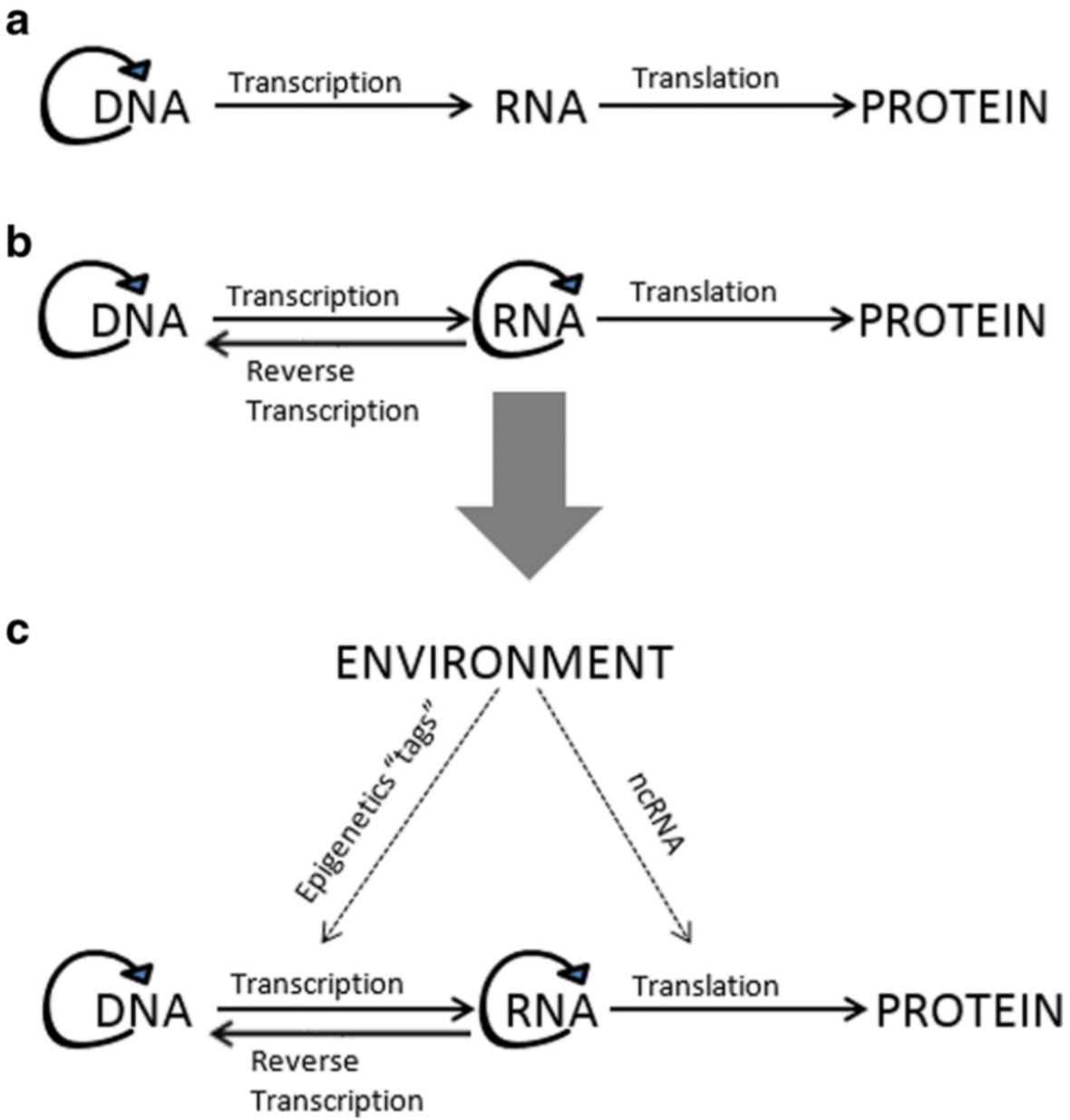
The bases on a single strand of DNA act as a code. The letters form three letter codons, which code for amino acids - the building blocks of proteins.

DNA → (TRANSCRIPTION) → RNA → (TRANSLATION) → PROTEIN

An enzyme, RNA polymerase, transcribes DNA into mRNA (messenger ribonucleic acid). It splits apart the two strands that form the double helix, then reads a strand and copies the sequence of nucleotides. The only difference between the RNA and the original DNA is that in the place of thymine (T), another base with a similar structure is used: uracil (U).

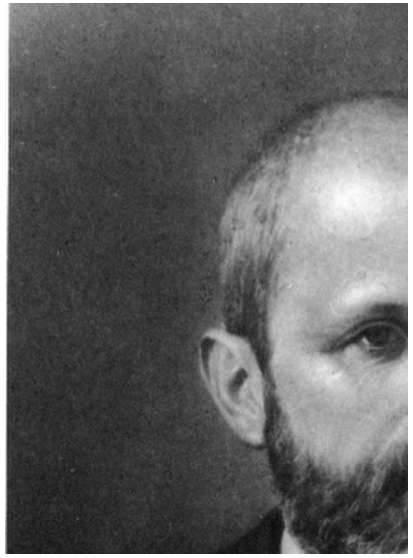
In multicellular organisms, the mRNA carries genetic code out of the cell nucleus, to the cytoplasm. Here, protein synthesis takes place. 'Translation' is the process of turning the mRNA's 'code' into proteins. Molecules called ribosomes carry out this process, building up proteins from the amino acids coded for.

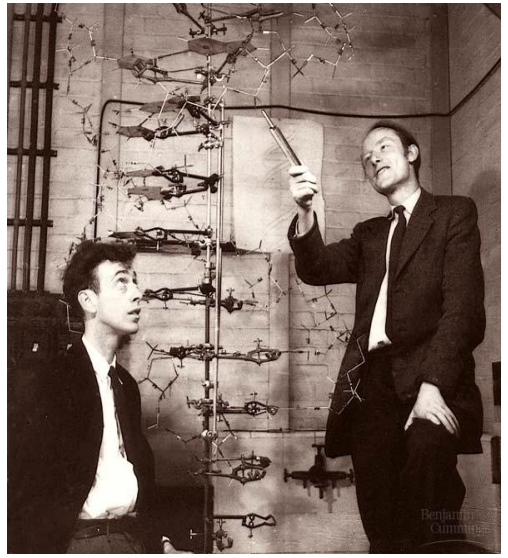
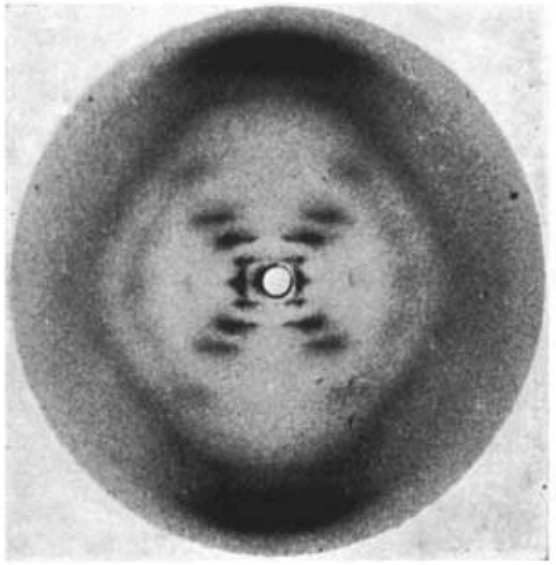
Central dogma of molecular biology



DNA

- DNA was discovered in 1869 by Swiss researcher Friedrich Miescher, who was originally trying to study the composition of lymphoid cells (white blood cells). Instead, he isolated a new molecule he called nuclein (DNA with associated proteins) from a cell nucleus



- In 1953 when James Watson and Francis Crick deduced how nucleotides are organized within DNA.
- 1962 The Nobel Prize in Physiology or Medicine (- Rosalind Franklin)

Gene

basic physical and functional unit of heredity
Genes are made up of DNA
Some genes act as instructions to make molecules called proteins. However, many genes do not code for proteins

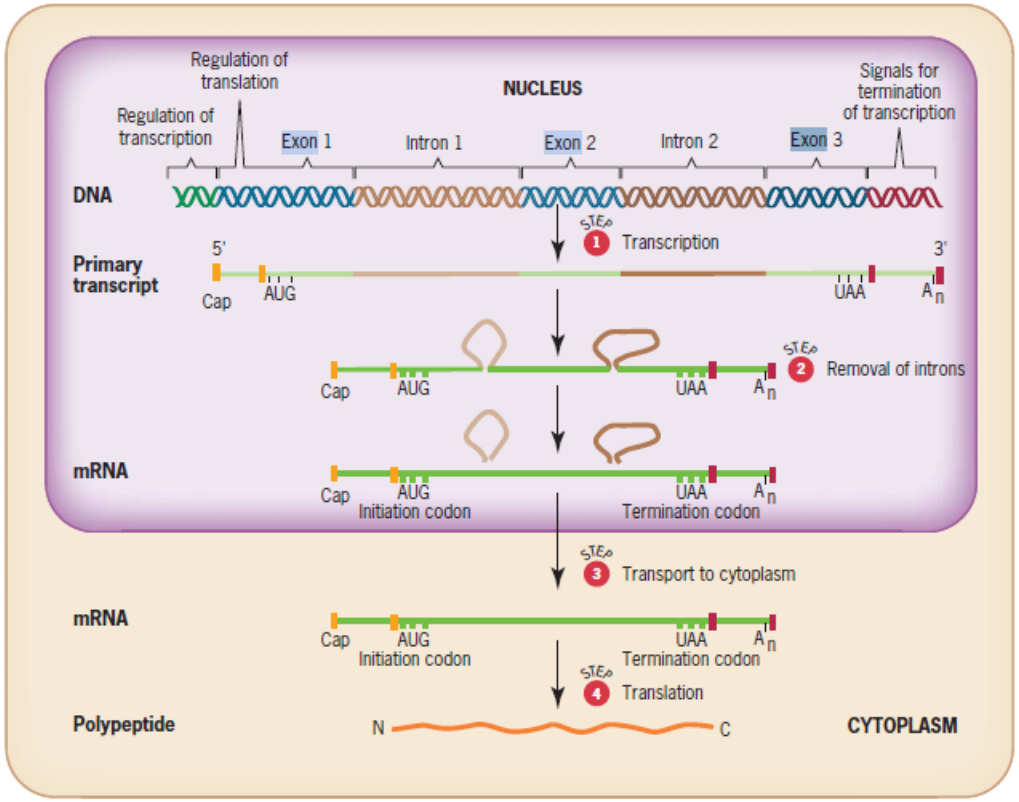
- Structural genes
- Genes for RNA
- Regulatory genes

- **Exon** = portion of a gene that codes for amino acids

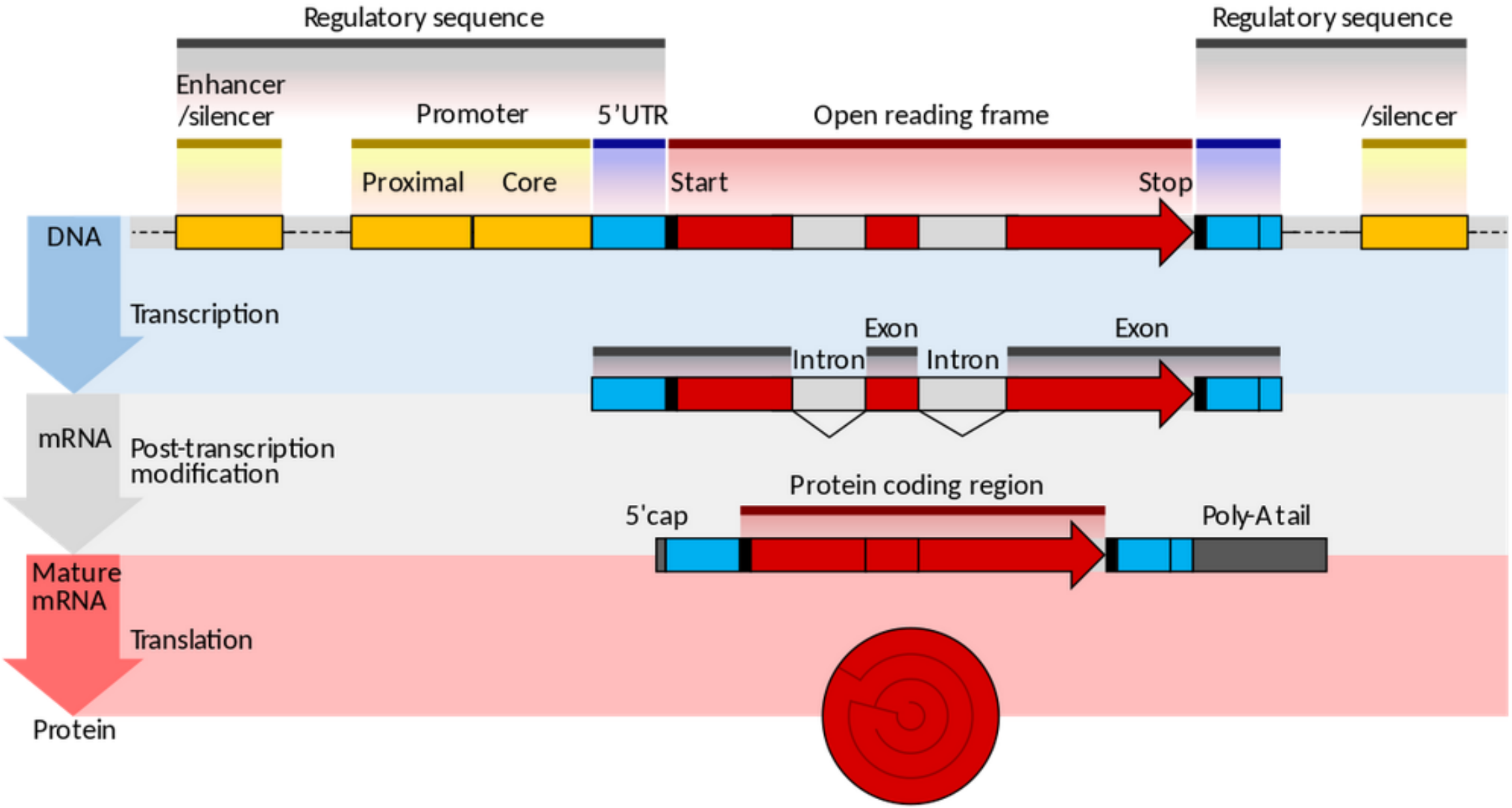
- **Intron** = portion of a gene that does not code for aa

  - **Intronless genes - single-exon genes**

- **Gene family** = A group of genes that are related in structure and often in function. The genes in a gene family are descended from an ancestral gene. For example, the hemoglobin genes belong to one gene family that was created by gene duplication and divergence

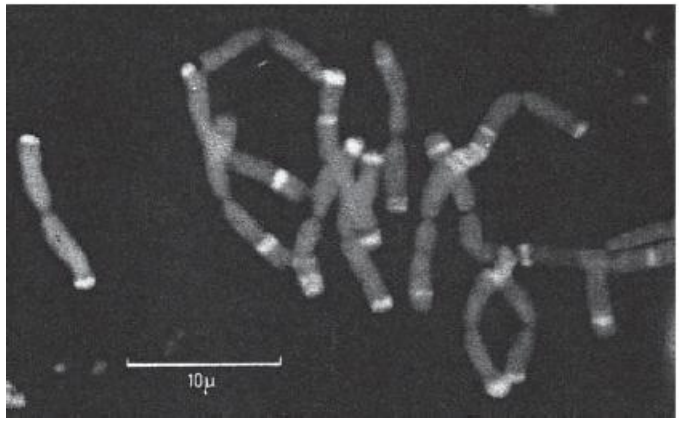


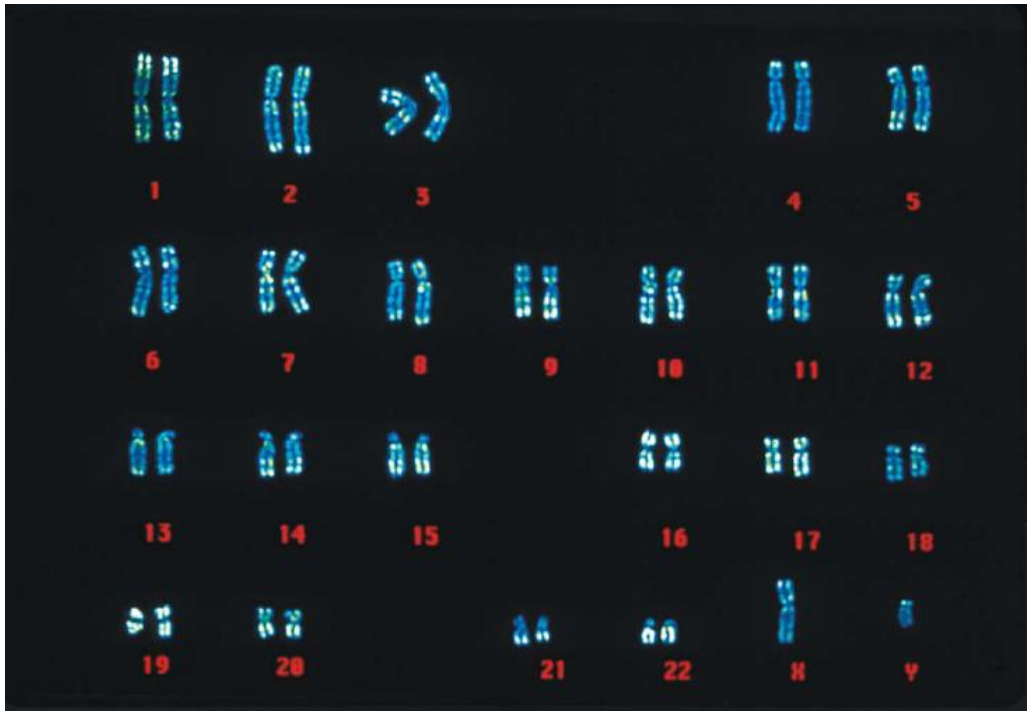
Gene structure

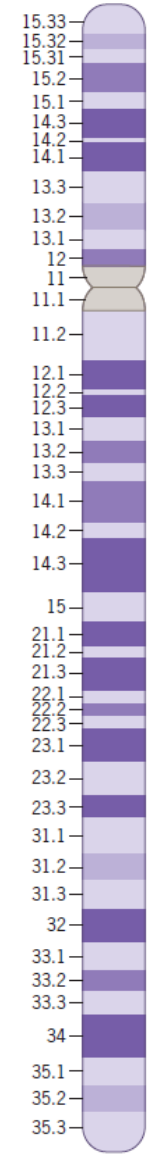


The human karyotype

- A karyotype is an individual's collection of chromosomes → each organism has different number and shape of chromosomes
- mouse 40 chromosomes
- crayfish 200 chromosomes
- fruit flies 8 chromosomes
- Diploid human cells contain 46 chromosomes
  - 44 autosomes
  - 2 sex chromosomes, which are XX in females and XY in males
  - At mitotic metaphase, each of the 46 chromosomes consists of two identical sister chromatids

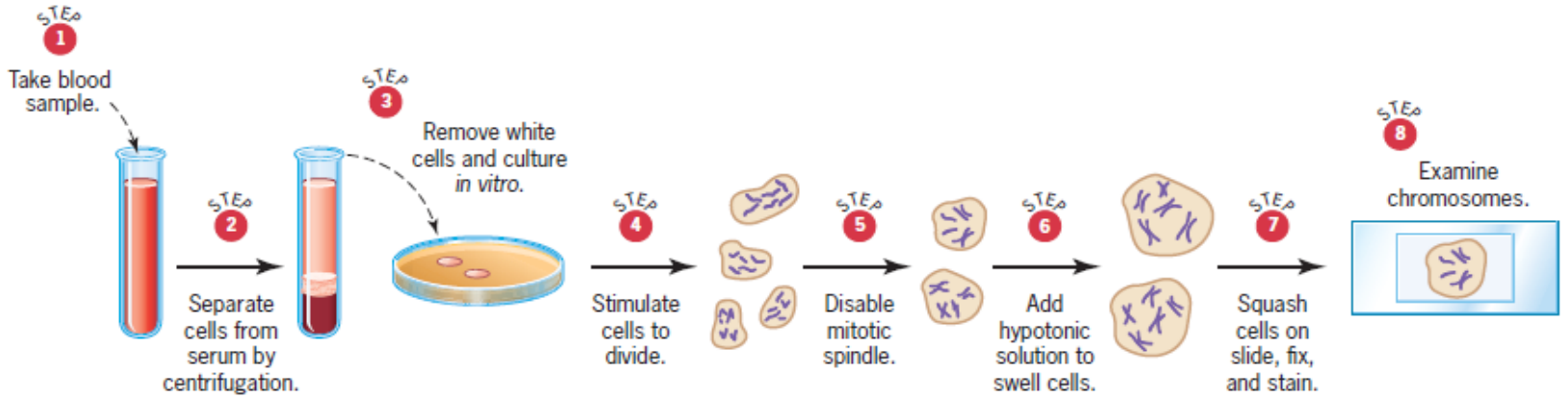


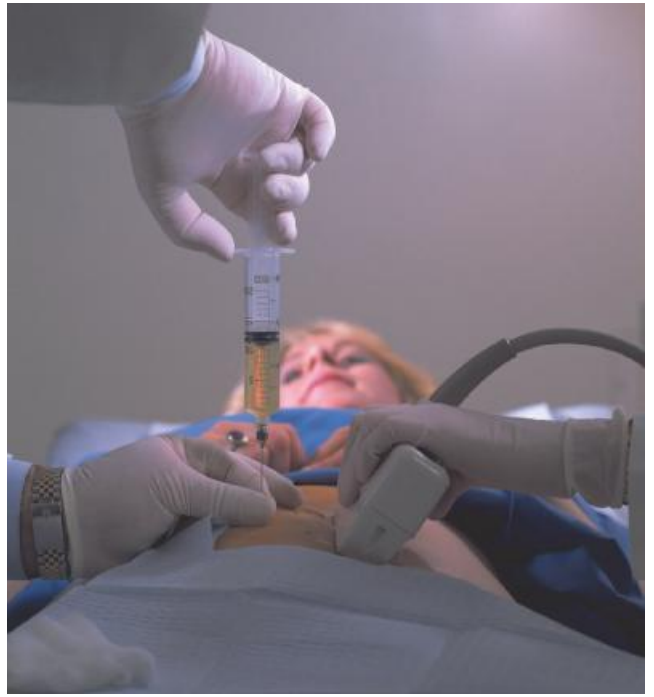




Analysis of mitotic chromosomes

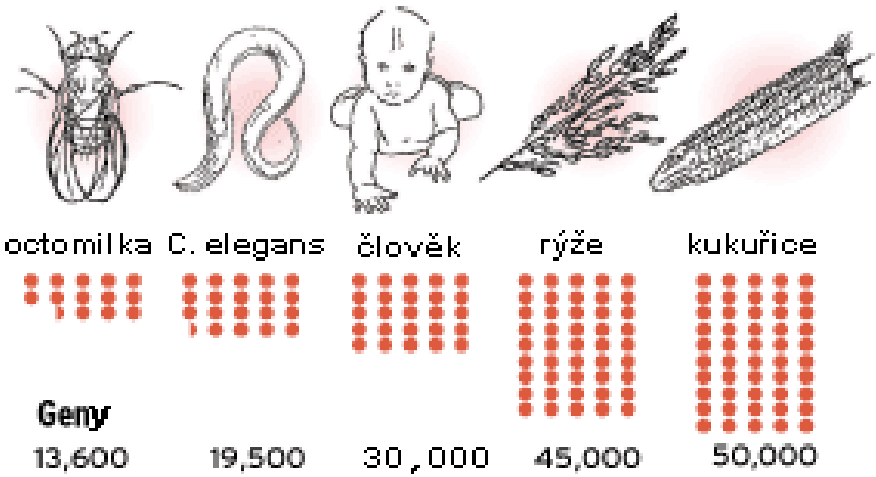
- Staining by dyes (e.g. Giemsa) leads to the characteristic band pattern
- Quinacrine – Ultraviolet irradiation causes some of the quinacrine molecules that have inserted into the chromosome to emit energy. Parts of the chromosome shine brightly, whereas other parts remain dark.
  - This bright-dark banding pattern is highly reproducible and is also specific for each chromosome
- The most advanced technique used by cytogeneticists today is called **chromosome painting.** With this technique, colorful chromosome images are created by treating chromosome spreads with fluorescently labeled DNA fragments that have been isolated and characterized in the laboratory. Such a fragment may, for instance, come from a particular gene.

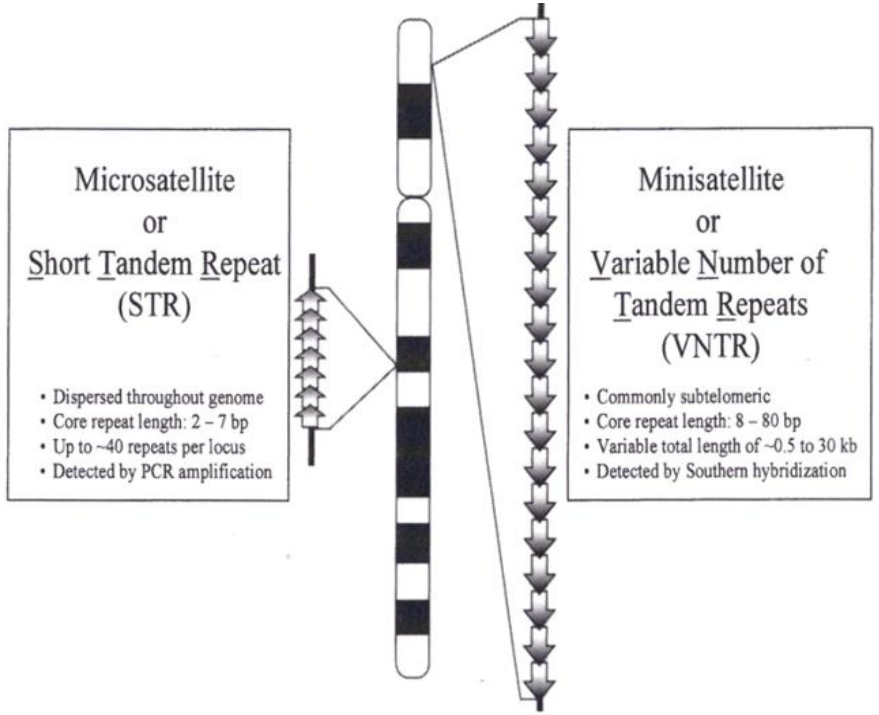




Human genome

- **Human Genome Project (HUGO) 1990 - 2003**
  - ~3.3×10^9 bp in haploid genome
  - **99.9% identical sequence**
  - only **~3% coding sequences**
  - ~30 000 genes expressed in variable periods of life
    - ~25 000 proteins
    - the rest are RNAs and others regulators
  - ~75% formed by unique (non-repetitive) sequence, the rest are repetitions
    - function is not clear, could be structure effects or evolutionary reserve
    - types of repetitions
      - tandem
        - microsatellites
        - minisatellites
      - Alu-repetitions
      - L1-repetitions
- density of genes in and between each chromosome is quite heterogeneous
- mitochondrial DNA
  - several tens of genes coding proteins involved in mitochondrial processes
    - respiratory chain
  - inherited from mother!
- **HapMap project 2003 – 2005**
  - n=269 subjects of 4 ethnicities (Yoruba, Han, Japanese, Caucasian) to asses character of LD and extent of **genetic variability (= 0.1%)**

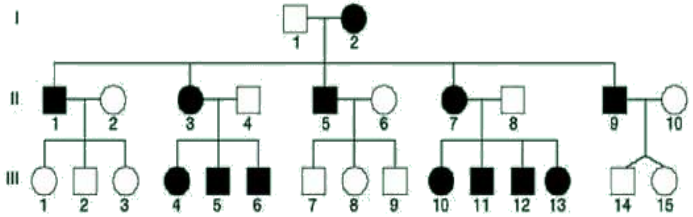


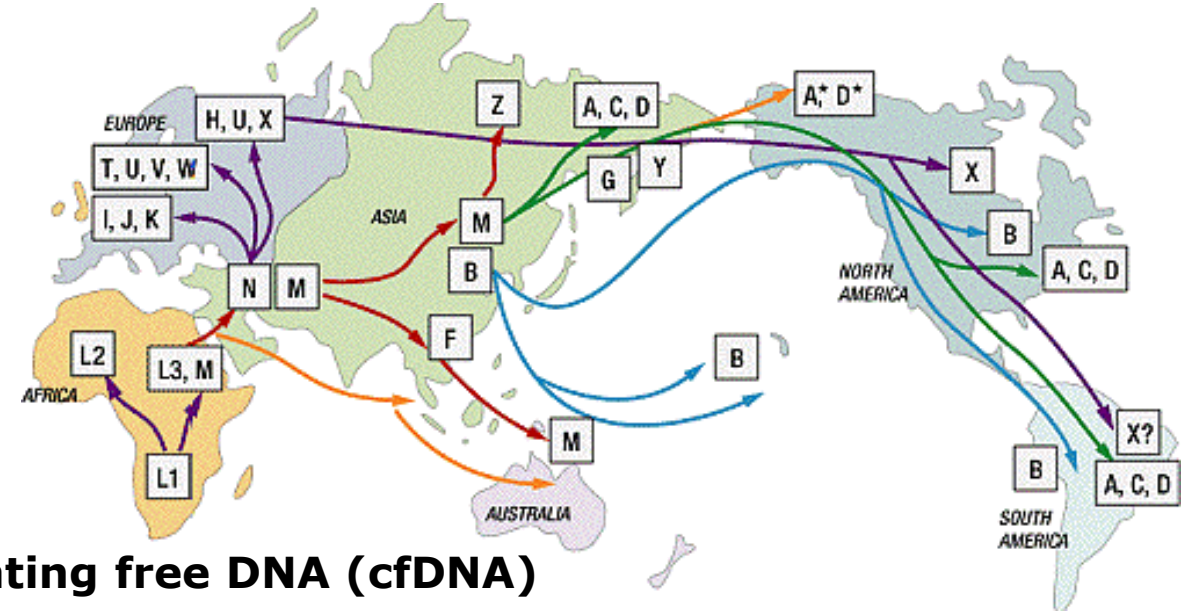


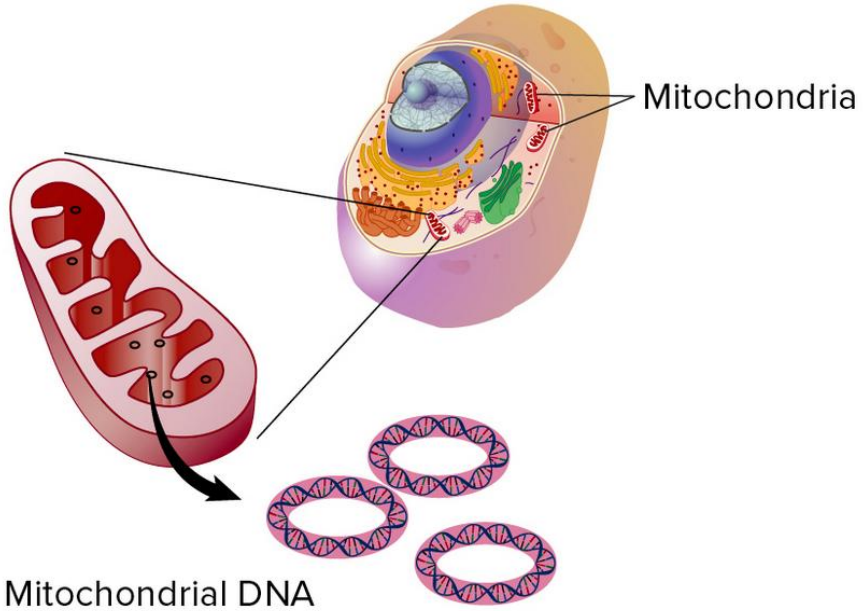
Non-nuclear DNA

Mitochondrial DNA (mtDNA)

- origin: alfaproteobacteria fused by eukaryotickou buňkou
- During evolution, many mitochondrial genes were transferred to the nucleus (proterins coded by these genes and very similar to genes coded by bacteria)
- In humans, for example, the mtDNA consists of 16,571 base pairs
- 37 distinct genes are packed into a 16- to 17-kb circle
  - 2 for ribosomal RNAs
  - 22 for transfer RNAs
  - 13 for polypeptides involved in oxidative phosphorylationmaternální dědičnost





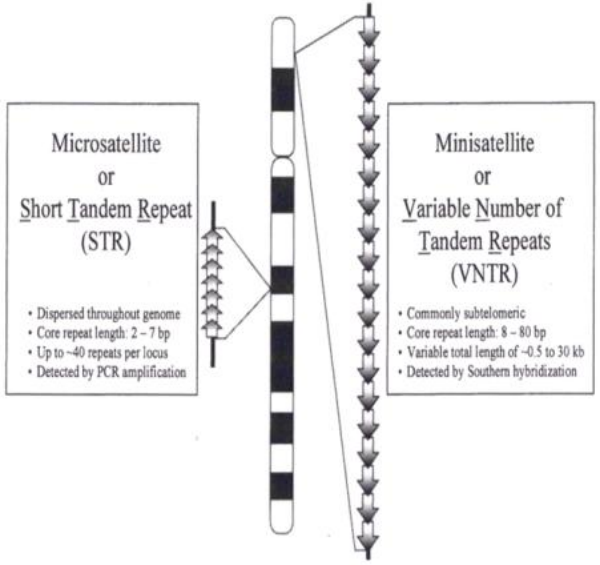


Circulating free DNA (cfDNA)

Repetitive sequences

Repetitive sequences = sequences that are repeated many times over

- concentrated in centromeres, which anchor spindle fibers to chromosomes during mitosis, and telomeres, which are special structures at the ends of chromosomes.
- necessary to format expression of unique coding sequence files and to organise additional functions essential for genome replication and accurate transmission to progeny cells
- possible evolutionary reserve

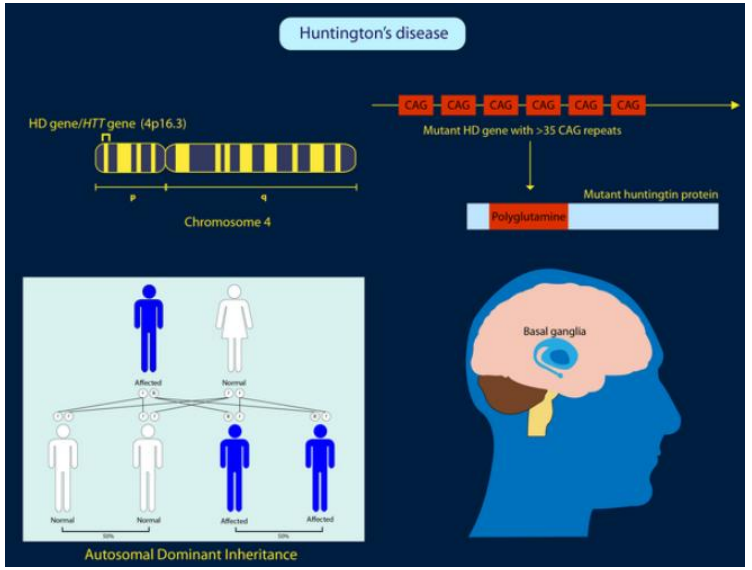


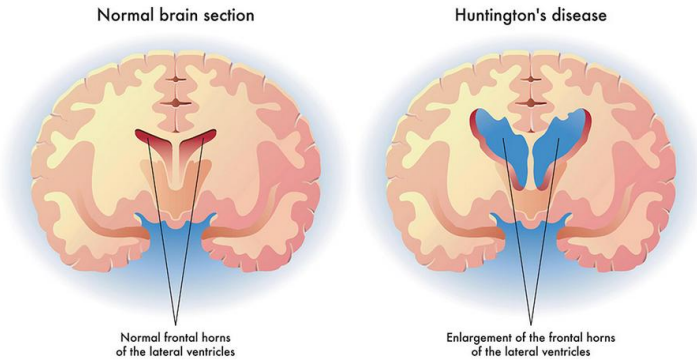
- **Interspersed repeats**
  - DNA transposomes (eg. „Sleeping Beauty" – gene therapy)
  - retrotranspozony
- LTR - Identical or nearly identical DNA sequences at opposite ends of an integrated retrovirus or a retroviruslike element.
- Long-Interspersed Elements (LINEs)
- Short-Interspersed Elements (SINEs) – (Alu family in humans)

- **Tandem repeats** = za sebou jdoucí identické repetice, VNTR
  - Short tandem repeat (STR) (**microsatellite**) - A highly polymorphic tandem repeat of a sequence only two to five nucleotide pairs in length.
  - Variable number tandem repeat (VNTR) (**minisatellite**) - A highly polymorphic tandem repeat of a sequence of 10 to 80 nucleotide pairs in length.

Repetitive sequences - clinical examples

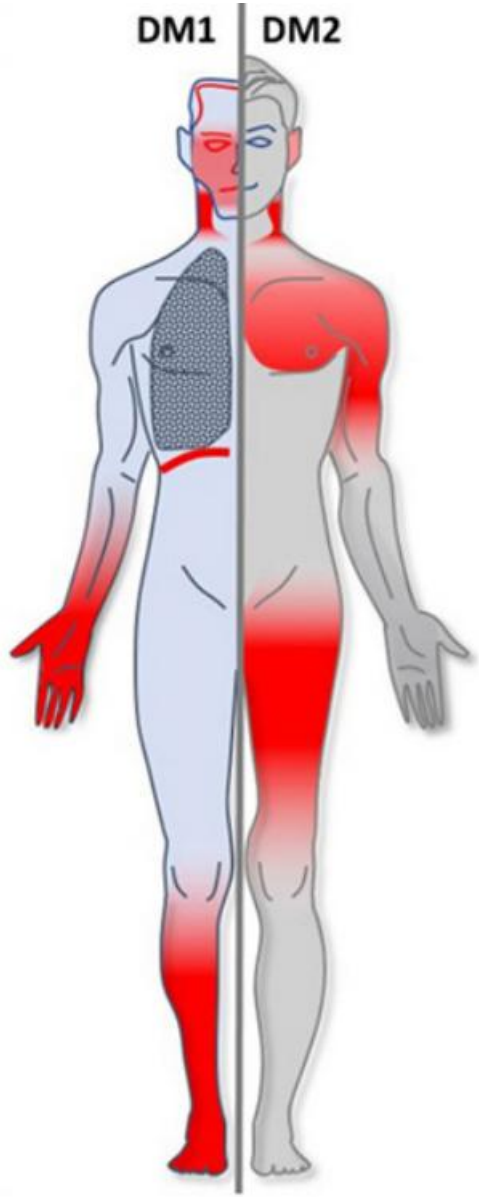
- **Huntington disease –** progressive brain disorder that causes uncontrolled movements, emotional problems, and loss of thinking ability (cognition).
  - Caused by the mutation in the *HTT* gene. The *HTT* gene provides instructions for making a protein called huntingtin. Although the function of this protein is unclear, it appears to play an important role in nerve cells (neurons) in the brain.
  - The HTT mutation that causes Huntington disease involves a DNA segment known as a CAG trinucleotide repeat. This segment is made up of a series of three DNA building blocks (cytosine, adenine, and guanine) that appear multiple times in a row. **Normally, the CAG segment is repeated 10 to 35 times** within the gene. In people with Huntington disease, the CAG segment is repeated 36 to more than 120 times. **People with 36 to 39 CAG repeats may or may not develop the signs and symptoms of Huntington disease**, while people with **40 or more repeats almost always develop the disorder**.
  - An increase in the size of the CAG segment leads to the production of an abnormally long version of the huntingtin protein. The elongated protein is cut into smaller, toxic fragments that bind together and accumulate in neurons, disrupting the normal functions of these cells.





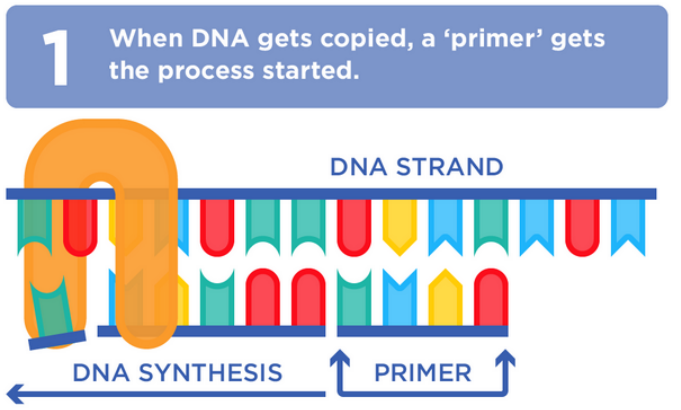
Repetitive sequences - clinical examples

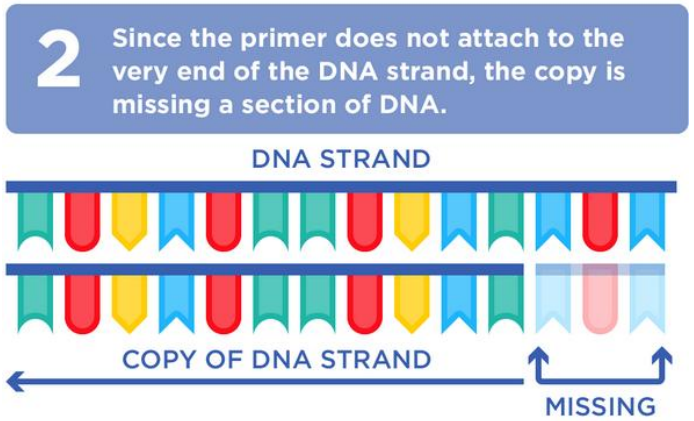
- **Myotonic dystrophy –** part of a group of inherited disorders called muscular dystrophies. It is the most common form of muscular dystrophy that begins in adulthood
  - Myotonic dystrophy is characterized by progressive muscle wasting and weakness. People with this disorder often have prolonged muscle contractions (myotonia) and are not able to relax certain muscles after use. For example, a person may have difficulty releasing their grip on a doorknob or handle. Also, affected people may have slurred speech or temporary locking of their jaw.
  - Myotonic dystrophy type 1 is caused by mutations in the DMPK gene, while type 2 results from mutations in the CNBP gene
  - In each case, a segment of DNA (trinucleotide CTG on 3′) is abnormally repeated many times, forming an unstable region in the gene
  - The gene with the abnormal segment produces an unusually long messenger RNA, which is a molecular blueprint of the gene that guides the production of proteins. The unusually long messenger RNA forms clumps inside the cell that interfere with the production of many other proteins.

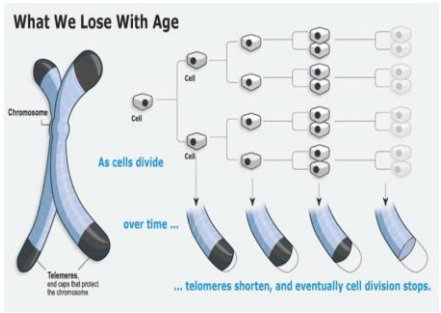


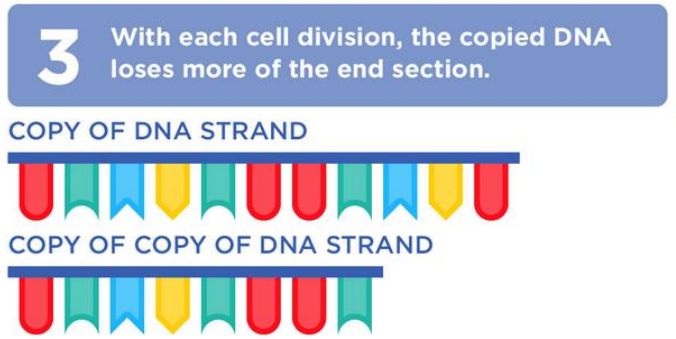
Telomere function

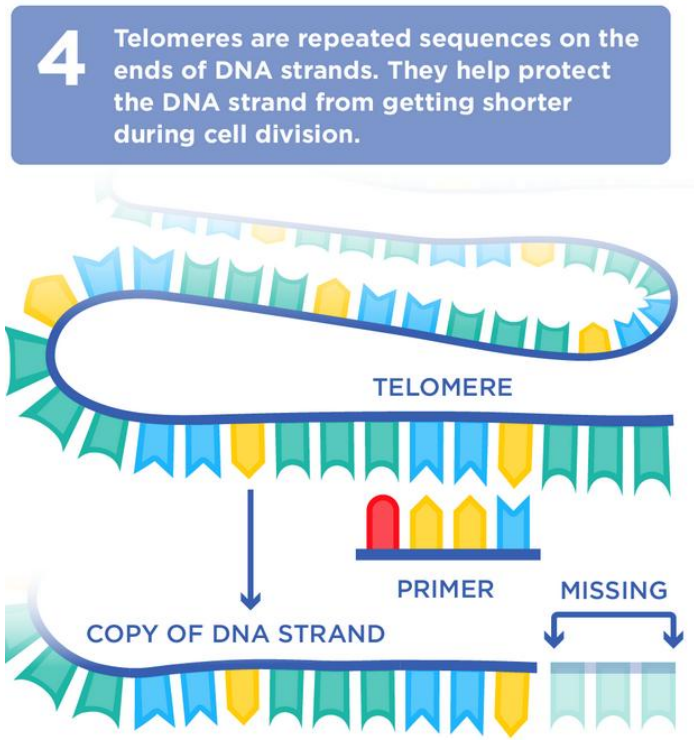
- **Telomeres –** distinctive structures found at the ends of our chromosomes
  - made of repetitive sequences of non-coding DNA that protect the chromosome from damage
  - In humans the telomere sequence is TTAGGG → repeated about 3 000 times
  - Each time a cell divides, 25-200 bases are lost from the ends of the telomeres on each chromosome
- **Hayflick limit –** number of times a normal cell population divides before entering the senescence phase
  - In humans 40 to 60 times
  - Telomerase enzyme
    - Embryonic stem cells
    - Cancer cells





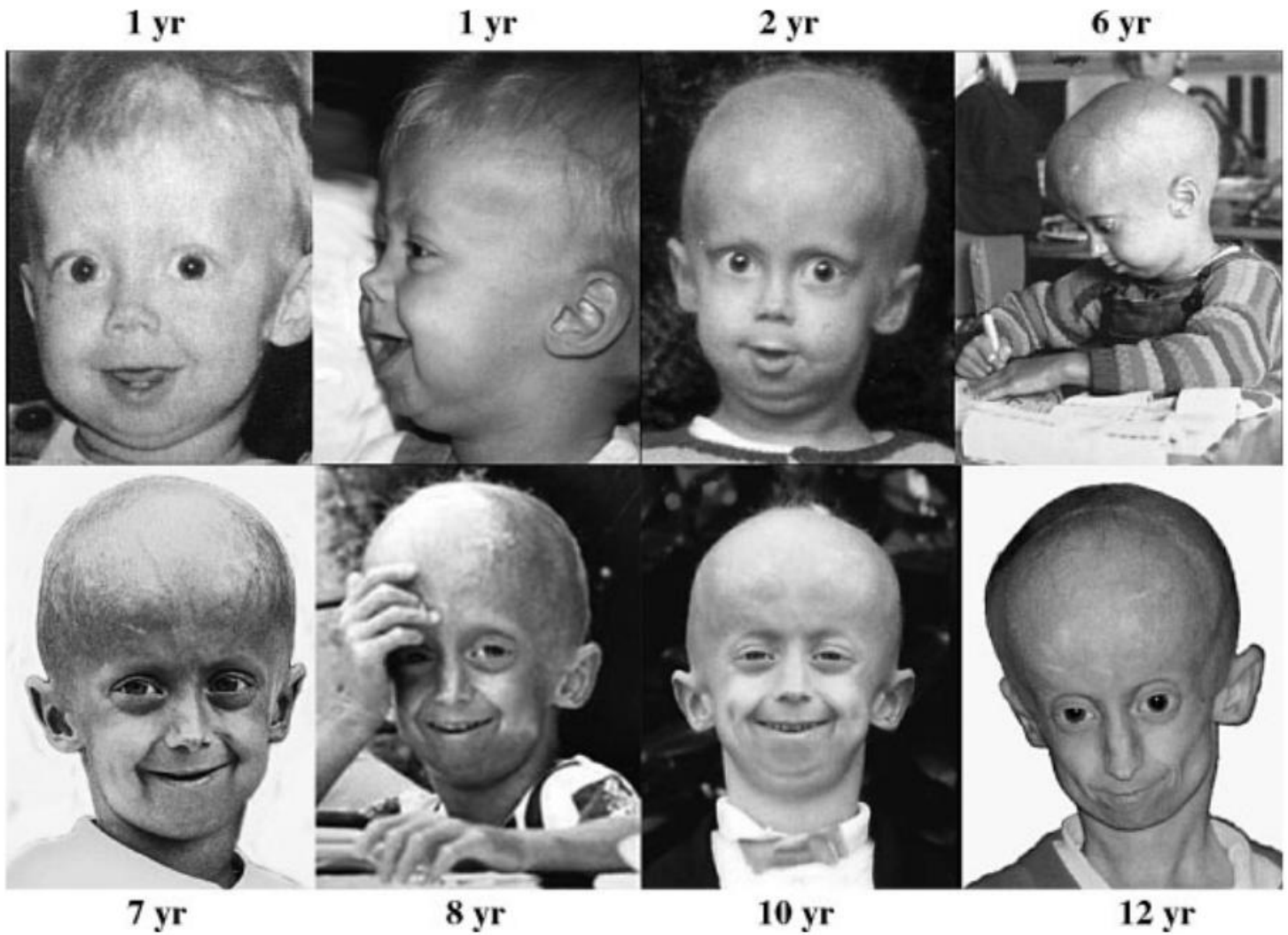






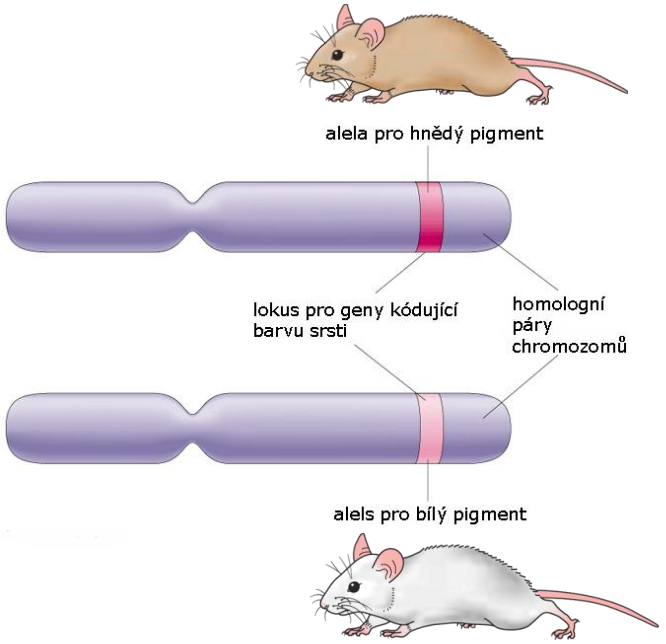
Repetitive sequences - clinical examples

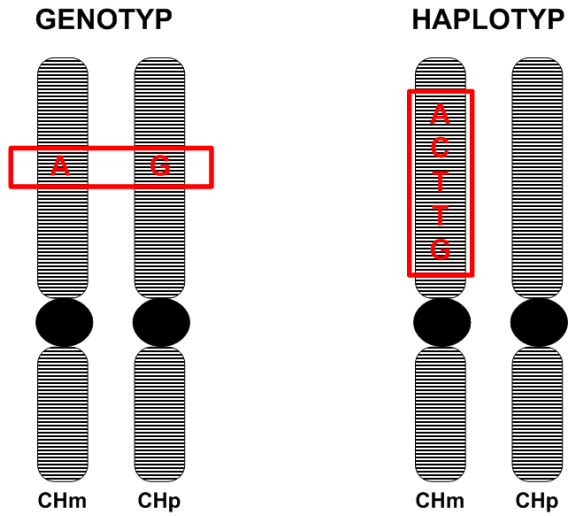
- **Hutchinson-Gilford Progeria Syndrome (HGPS) –** rare premature aging disorder caused by mutations in the gene LMNA, which encodes the nuclear matrix protein lamin A.
  - **Average telomere length in fibroblasts from HGPS patients is shorter than in age-matched controls.**
  - How mutations in lamin A lead to shortened telomere lengths is not known nor is the contribution of individual chromosome ends to the low average length understood
  - Shorter telomeres are symptom not cause of the disease!



Gene × allele × genotype × phenotype

- **gene** – basic unit of heritability
  - **gene families**
    - sequence evolution similarity among genes formed e.g. by duplication
    - hemoglobin chains, immunoglobulins, some isoenzymes, …
  - **pseudogenes**
    - similar to functional genes by non-functional
- each gene occupies particular site in the chromosome = **locus** (e.g. 12q21.5)
  - localization of genes in the same in species but sequence is not!
- **allele** – sequence variant of gene
  - vast majority of genes in population has several variants (= alleles) with variable frequency = **genetic polymorphism**
- **genotype** – combination of alleles in a given locus in paternal and maternal chromosomes in diploid genome
- **haplotype** – linear combination of alleles in a single ch. of homologous pair
- **phenotype** – expression of genotype
  - trait –measurable, very often continuous variable
    - QTL – quantitative trait locus (e.g. weight, height, …)
  - phenotype – set of traits
  - intermediate phenotype – similar to trait but not always continuous



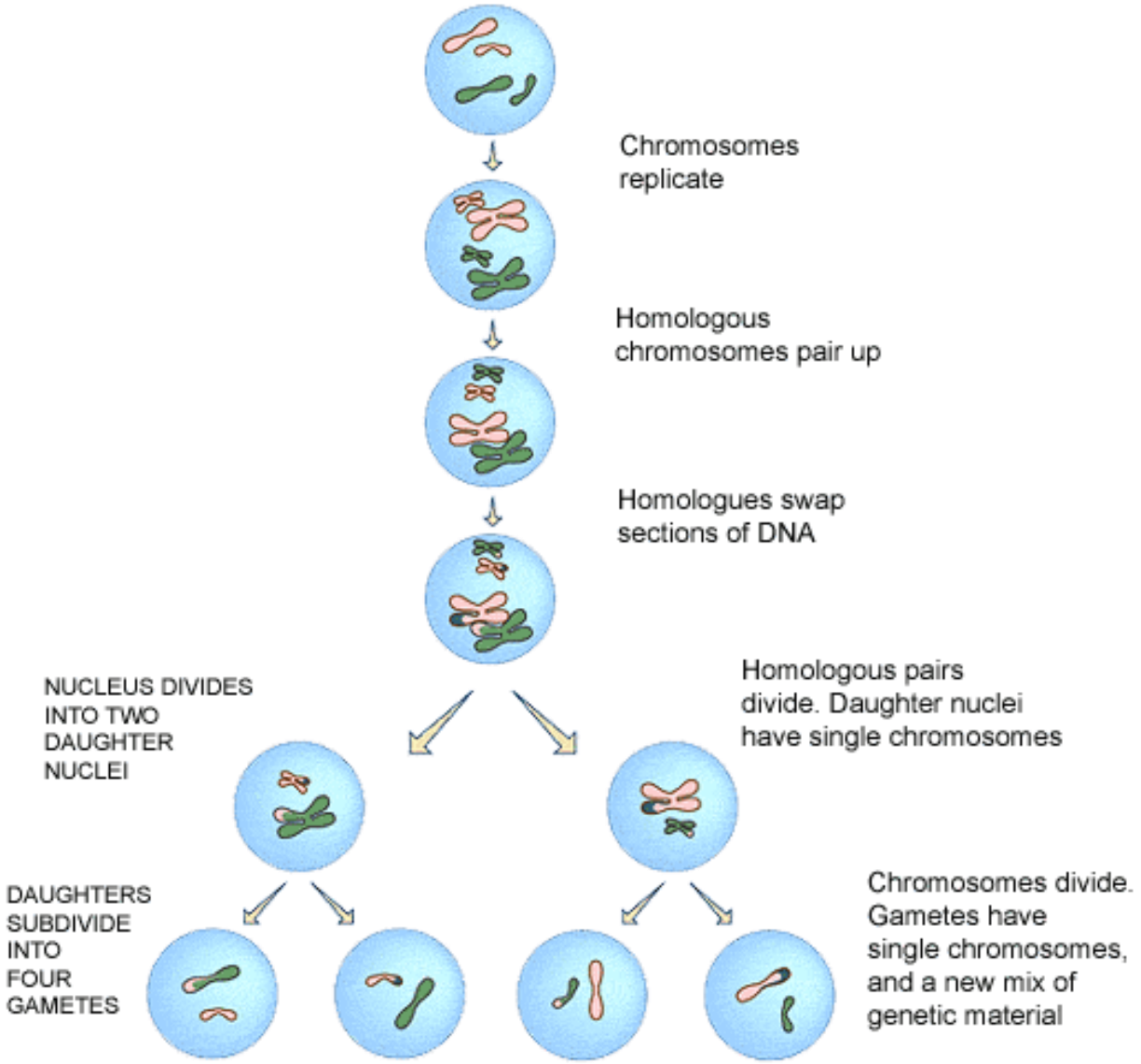


How big difference in DNA amount between banana and human?

- 50% - 60%
- Most DNA is involved in production of proteins, enzymes for creating or breaking down sugars, for building cellular structures and processes, etc. etc.
- Structure of the hemoglobin pigment (a protein) has a lot of common code with the structure of the chlorophyll pigment.
- the same basic molecule structure was basically readapted for two very different functions
- lot of DNA has no function called "junk DNA."
- It's this junk DNA that's really a tell-tale sign of commo ancestry. Why else would there be common sequences of base-pairs in the DNA, that are present in both species, but serves no function in either species?

- 90% identity in mammals
- **99,9% identity between peoble**

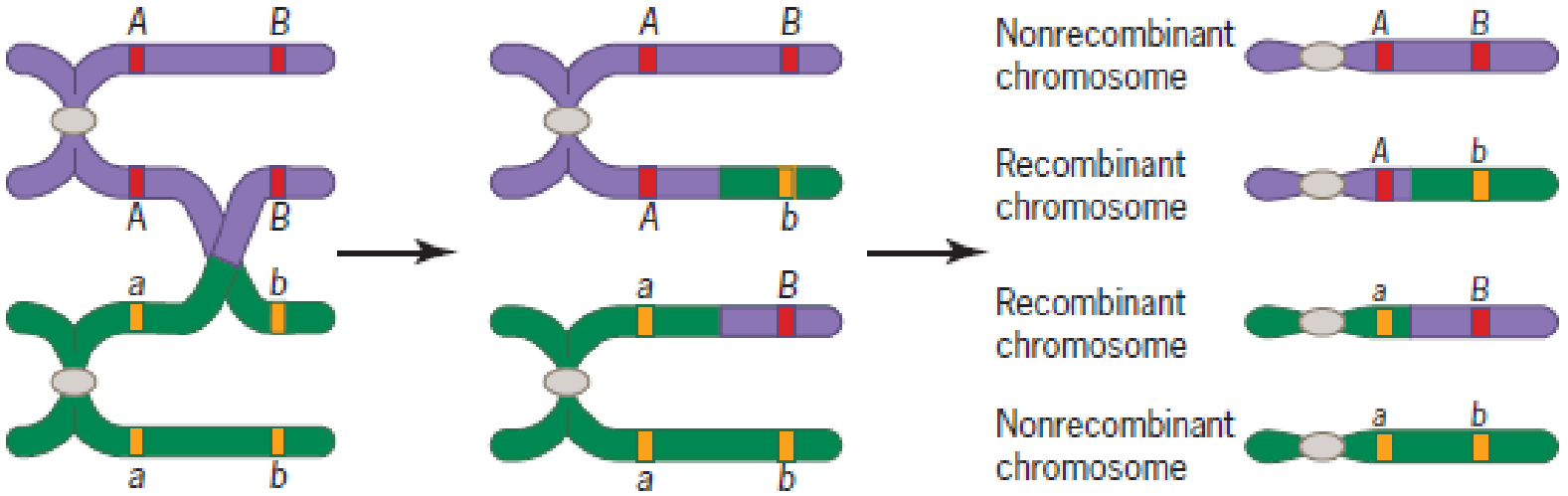
Genetic variability (~0.1%)



- DNA sequence of coding as well as non-coding regions of genome is variable in each individual
- **genetic variability** = v existence of several variants (alleles) with various frequency for a given gene in population
- sources:
  - 1) sexual reproduction
  - 2) recombination (meiotic crossing-over)
  - 3) mutations *de novo*
    - "errors" during DNA replication
      - proof-reading of DNA polymerase is not 100%
    - effect of external mutagens
  - 4) effects on the population level (evolution) – *Hardy/Weinberg law*
    - natural selection = adaptive (reproductive success)
    - genetic (allelic) drift = random selection of alleles (entirely from chance)
      - "founder" effect

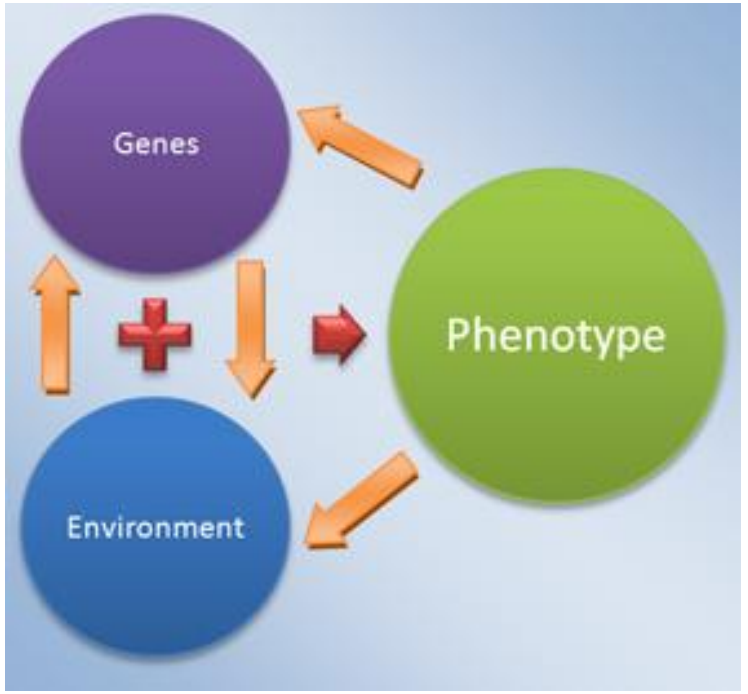
Crossing-over and recombination

- each gamete formed receives **randomly** 1 ch. of the homologous pair of chromosomes - paternal (CHp) or maternal (CHm)
  - given 23 ch. pairs there is theoretically 2^{23} possible combinations (= **8,388,608** different gametes)
- in fact, each gamete contains a mixture of homologous CHm and CHp due to the process during 1st meiotic division = **crossing-over** and **recombination**
    - thus alleles originally coming from different grandparents can appear in one chromosome
  - creates much greater number of combinations than 8 millions
- however, **probability of recombination** is not the same in all parts of DNA, it depends on the distance (*linkage disequilibrium / haplotype block*)
  - the closer the genes are, the lesser is the probability of recombination
    - such length is expressed in centiMorganes (1cM = 1% probability of recombination)

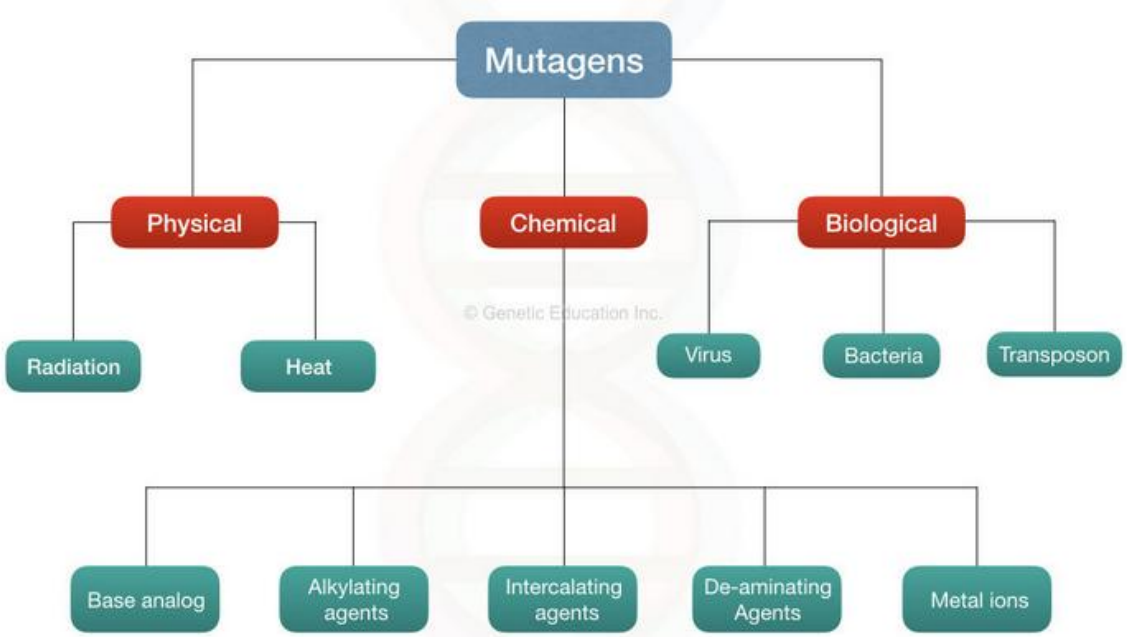


Gene environment interaction

- **Gene environment interaction –** an influence on the expression of a trait that results from the interplay between genes and the environment. Some traits are strongly influenced by genes, while other traits are strongly influenced by the environment. Most traits, however, are influenced by one or more genes interacting in complex ways with the environment.
  - Epigenetic changes
  - Malignant transformacy



- **Mutagens –** biological physical or phenomenon that promotes errors in DNA.



Genetic variability

- Source of individual variability (new combinations of alleles are created):
  - Segregation of alleles
  - Recombination during crossing-over
  - Origin of new spontaneus combinations during fertilization

Mutations

- process during which the number of alleles is changes (affects quality and quantity of genes)
- **mutagenesis** = The process of inducing mutations
- mutation = change in the DNA at a particular locus in an organism

Types of mutations

- Induces mutations – results from the exposure of an organism to a chemical or physical agent that causes changes in the structure of DNA or RNA
- Spontaneous mutations – mutation that occurs without a known cause (, approx. ~10-7, repair by polymerase and p53):

- **Gene mutations**
- **Chromosomal aberations**
- **Genome mutations**

Rare mutations and polymorfism

- **Rare variants (mutations) -** alternative forms of a gene that are present with a minor allele frequency (MAF) of less than 1%
  - They are often cause of death or are relatively new
- **Polymorphism –** common mutation, these mutations are source of interindividual variability, existence of two or more variants in a population of individuals, with at least two of the variants having frequencies greater than 1 percent

  - **SNP** = Single-nucleotide polymorphism → A single base pair in the DNA that varies in a population.

  - **Microsatellite and Minisatellite** → multiallelic

Germinal and Somatic mutations

- **Germinal mutation –** mutation that occurs in the reproductive cells (germ-line cells) of the body and is transmitted to progeny (often cause of abortion),
  - in each cell of the offspring, have effect on evolution, success rate 1:100000
- **Somatic mutation –** mutation that occurs in the nonreproductive cells (somatic cells) of the body and is not transmitted to progeny

Gene (variant) mutation

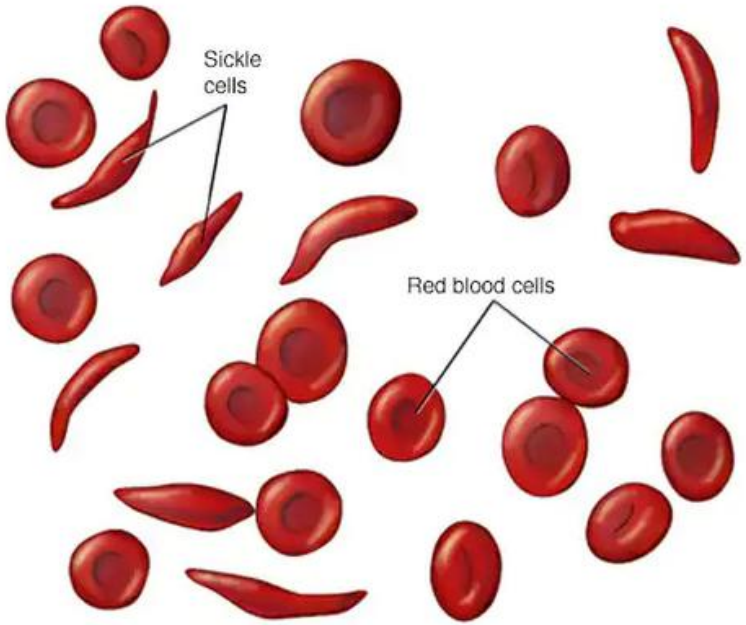
- Change of individual genes (alleles), typically induced
- DNA structure is altered but integrity of chromosome is intact
- In somatic cells and gametes

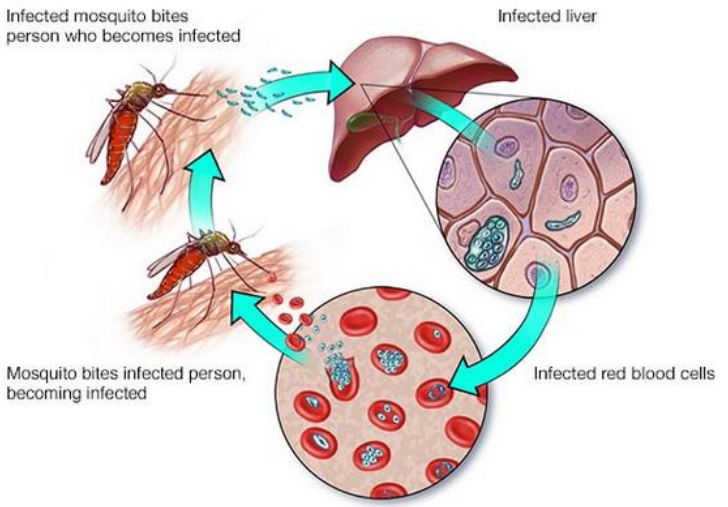
- **Point mutation – substitution** = replaces one DNA building block (nucleotide) with another. Substitution variants can be further classified by the effect they have on the production of protein from the altered gene
  - Missense → amino acid change may alter the function of the protein
  - Nonsense → shortened protein that may function improperly, be nonfunctional, or get broken down
- **Deletion** = changes the DNA sequence by removing at least one nucleotide in a gene.
- **Insertion** = changes the DNA sequence by adding one or more nucleotides to the gene. As a result, the protein made from the gene may not function properly.

Gene mutations - clinical examples

- **Sickle cell disease (SCD) –** group of inherited red blood cell disorders that affects hemoglobin
  - In people with sickle cell disease, abnormal hemoglobin molecules - hemoglobin S - stick to one another and form long, rod-like structures. These structures cause red blood cells to become stiff, assuming a sickle shape
  - Caused by a mutation in the hemoglobin-Beta gene found on chromosome 11.
  - Sickle-shaped red blood cells carry oxygen much worse. Therefore, people with sickle cell disease have more red blood cells than ordinary people. This gives them a big advantage over malaria, giving them a better chance of surviving.

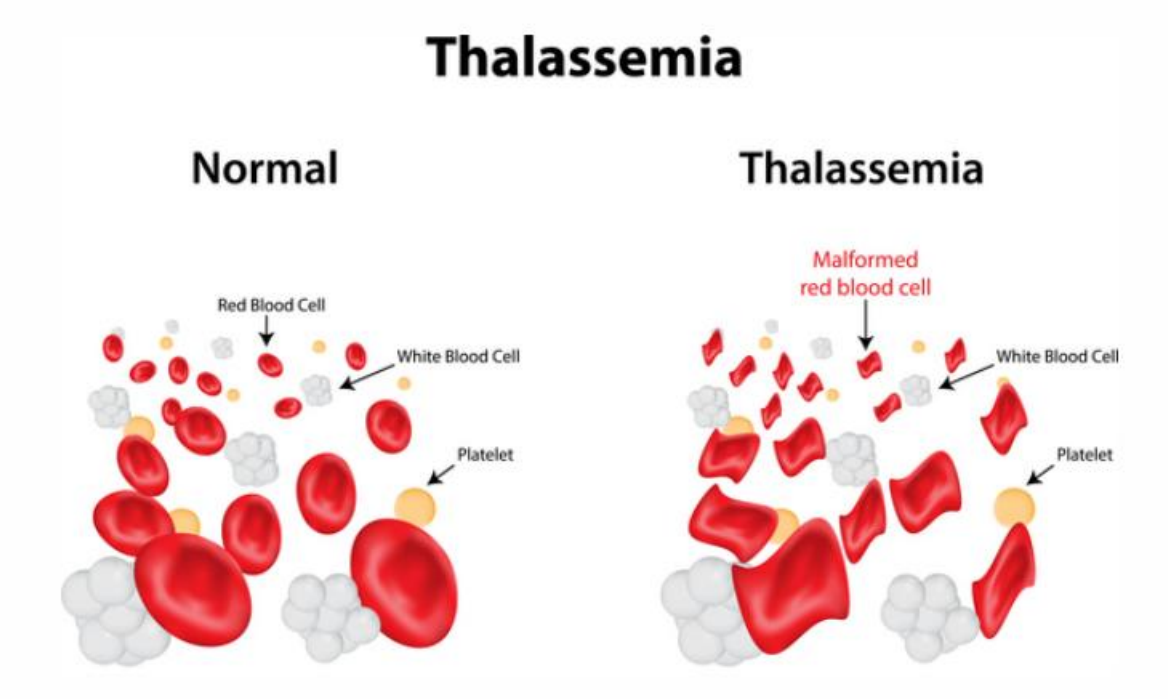
	ancestral	mutant
DNA	-CTC-	-CAC-
mRNA	-GAG-	-GUG-
AA	-glu-	-val-





Gene mutations - clinical examples

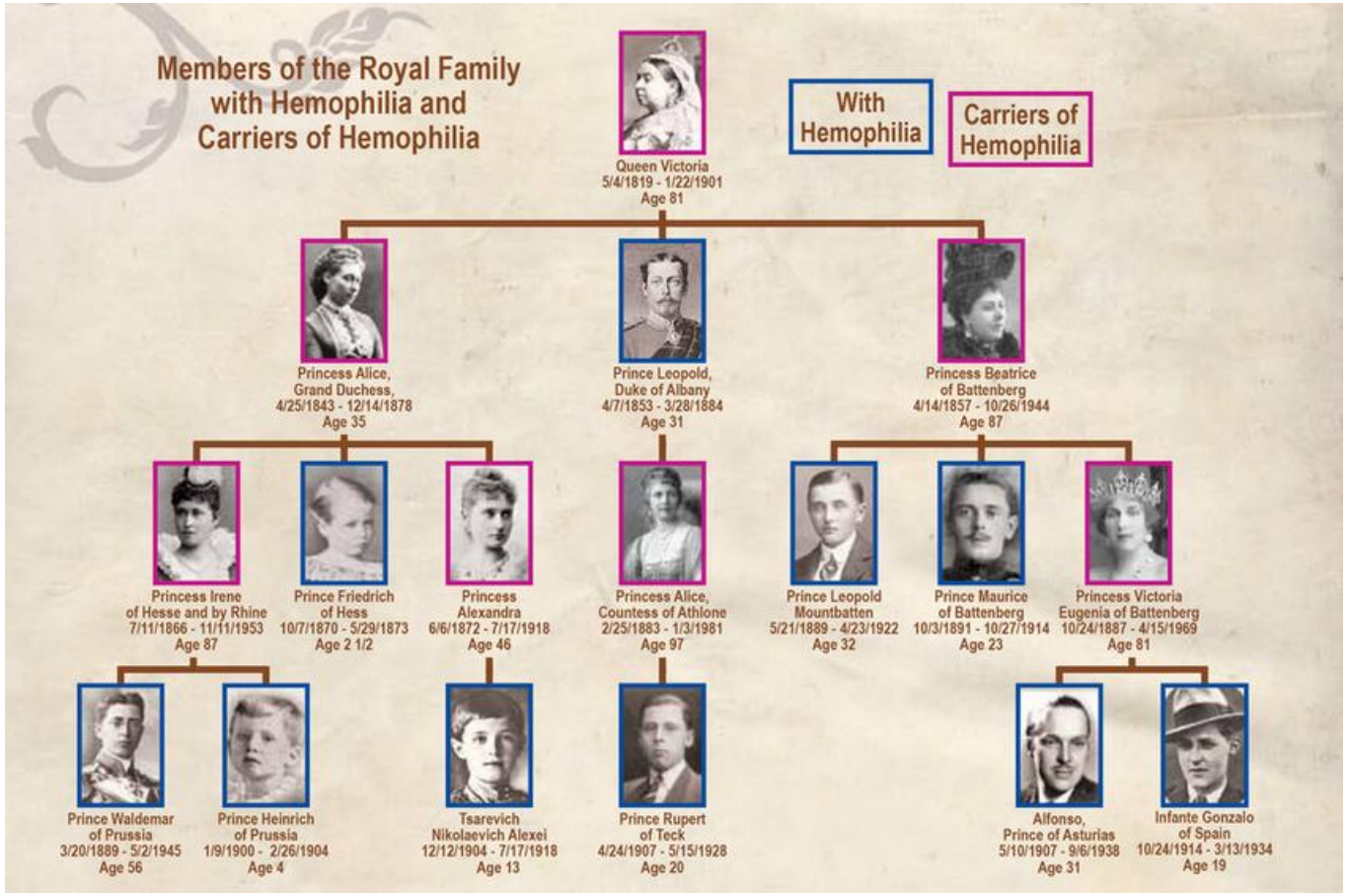
- **Beta thalassemia –** blood disorder that reduces the production of hemoglobin
  - Mutation in *HBB* gene → instructions for making a protein called beta-globin
  - The absence of beta-globin is referred to as beta-zero (β^0) thalassemia
  - In people with beta thalassemia, low levels of hemoglobin lead to a lack of oxygen in many parts of the body.
  - People with beta thalassemia are at an increased risk of developing abnormal blood clots.



	ancestral	mutant
DNA	-AGT-	-ACT-
mRNA	-UCA-	-UGA-
AA	-ser-	-STOP

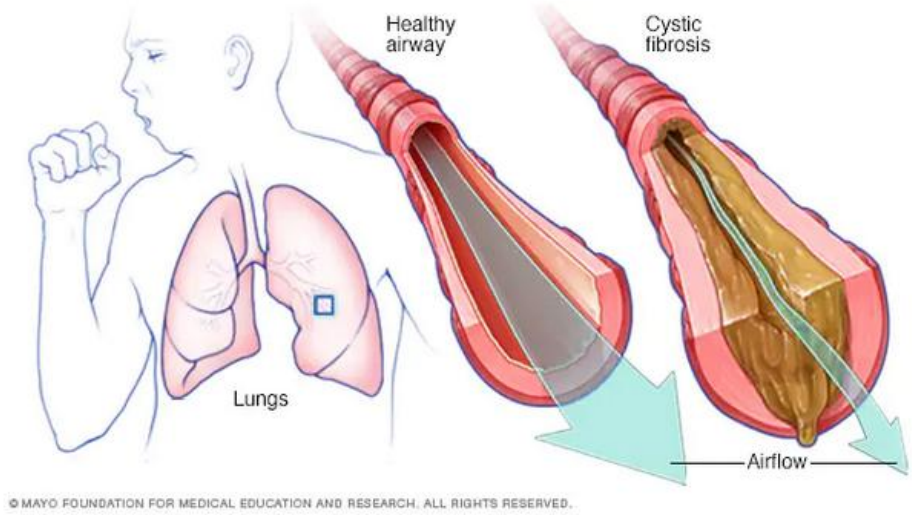
Gene mutations - clinical examples

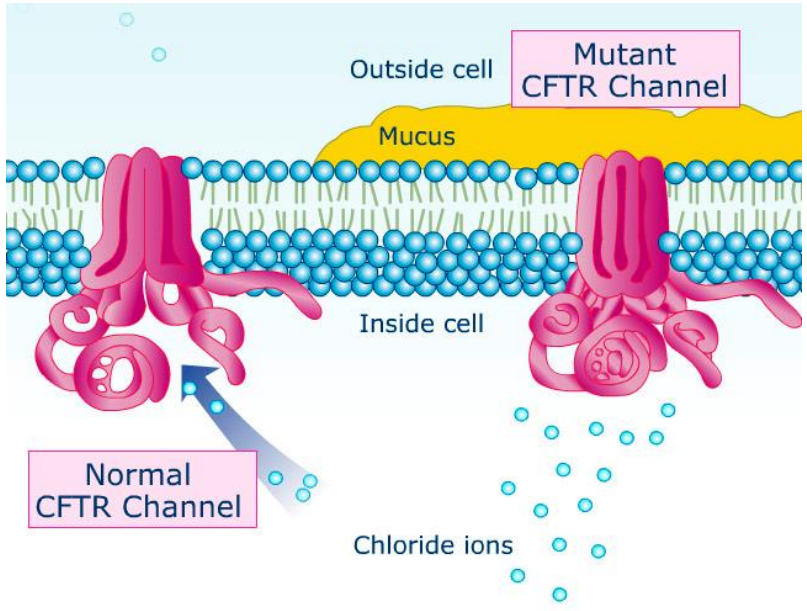
- **Hemophilia A –** hereditary bleeding disorder caused by a lack of blood clotting factor VIII → without enough factor VIII, the blood cannot clot properly to control bleeding.
  - Caused by an inherited X-linked recessive trait, with the defective gene located on the X chromosome.
  - Insertion of 3000 bp → changes in *F8* gene
  - If a woman has a defective factor VIII gene, she is considered a carrier (two copies of X chromosome)
  - Males have only one X chromosome. If the factor VIII gene is missing on a boy's X chromosome, he will have hemophilia



Gene mutations - clinical examples

- **Cystic fibrosis (CF) –** inherited disorder that causes severe damage to the lungs, digestive system and other organs in the body.
  - In cystic fibrosis, a defect (mutation) in a gene — the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene — changes a protein that regulates the movement of salt in and out of cells. The result is thick, sticky mucus in the respiratory, digestive and reproductive systems, as well as increased salt in sweat.
  - Deletion of 3 bp from position 1652 to 1655 in exon 10 (deletion of phe in codon 508)

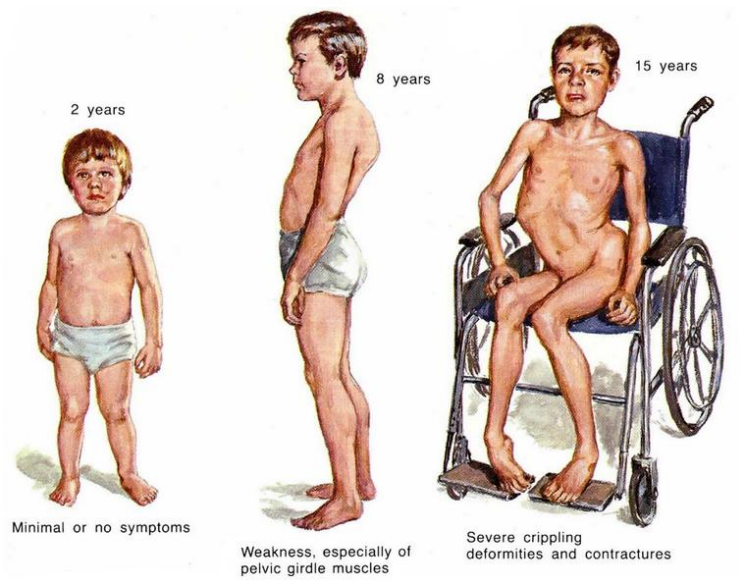


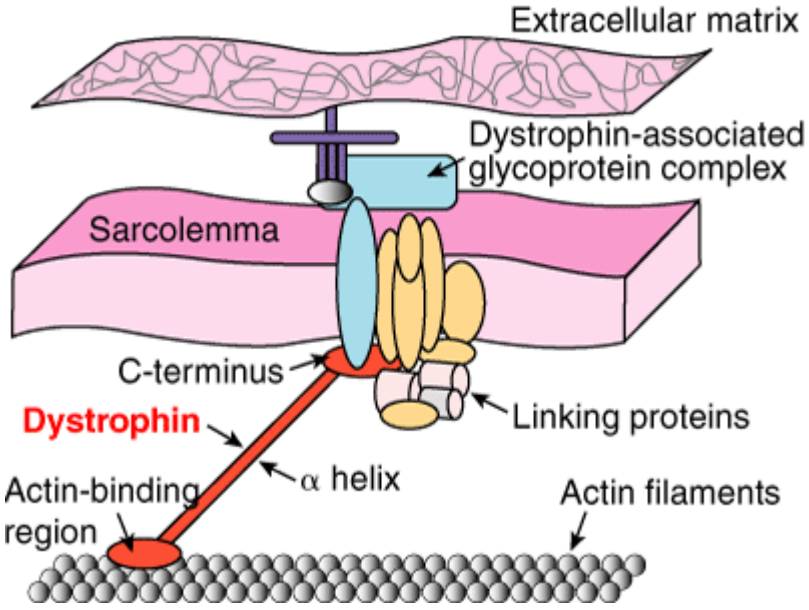


	ancestral	mutant
DNA	-TAG-AAA-CCA-	-TA A-CCA-
mRNA	-AUC-UUU-GGU-	-AUU-GGU-
AA	-ile-phe-gly-	-ile-gly-

Gene mutations - clinical examples

- **Duchenne Muscular Dystrophy (DMD) –** is a genetic disorder characterized by progressive muscle degeneration and weakness.
  - caused by mutations in *DMD* (encoding dystrophin) that prevent the production of the muscle isoform of dystrophin. Mutations in DMD can also cause Becker muscular dystrophy (BMD).
  - In DMD, frameshifting mutations or nonsense cause premature truncation of protein translation, leading to non-functional and unstable dystrophin

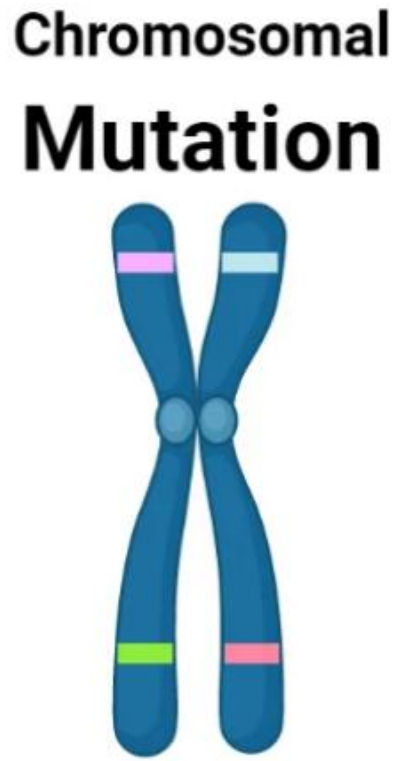


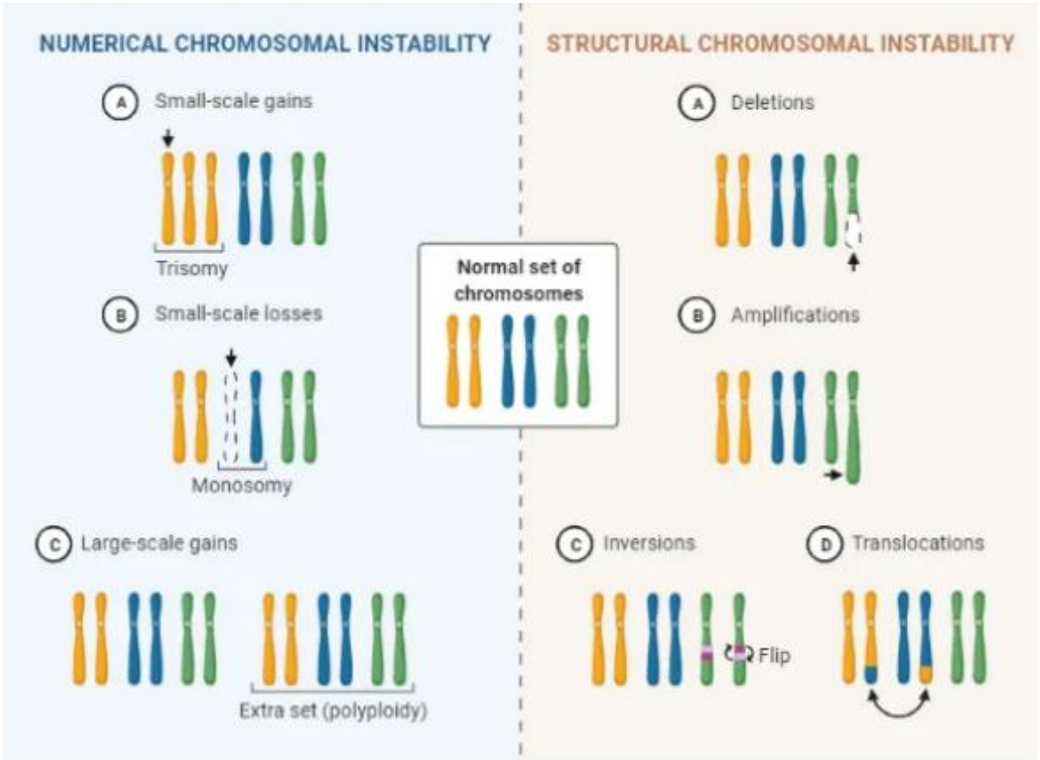


	ancestral	mutant
DNA	-CAC-TGT	-CAC-TTG-T..-
mRNA	-GUG-ACA-	-GUG-AAC-U..-
AA	-val-thr-	-ile-gly-

Chromosomal mutations – aberations

- A chromosome mutation is an unpredictable change that occurs in a chromosome.
- Chromosome mutations can result in **changes in the number of chromosomes** in a cell or **changes in the structure of a chromosome**.
- Unlike a gene mutation which alters a single gene or larger segment of DNA on a chromosome, chromosome mutations change and impact the entire chromosome.



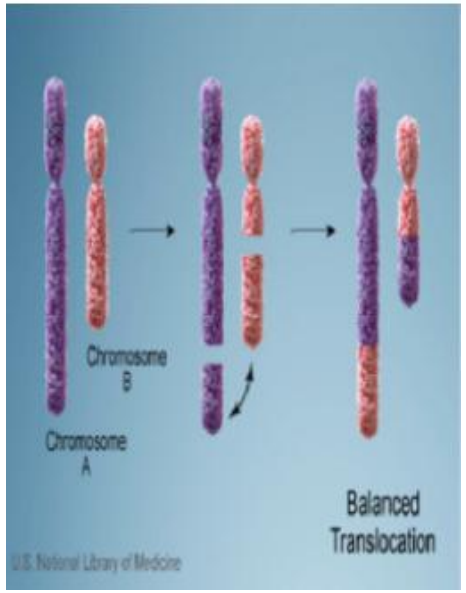


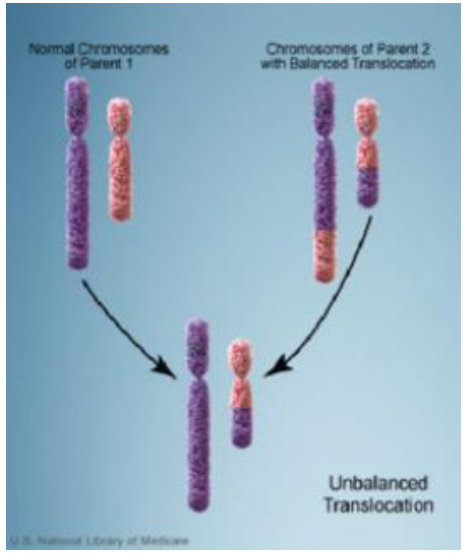
Chromosomal mutations – aberations

Structural chromosomal aberations

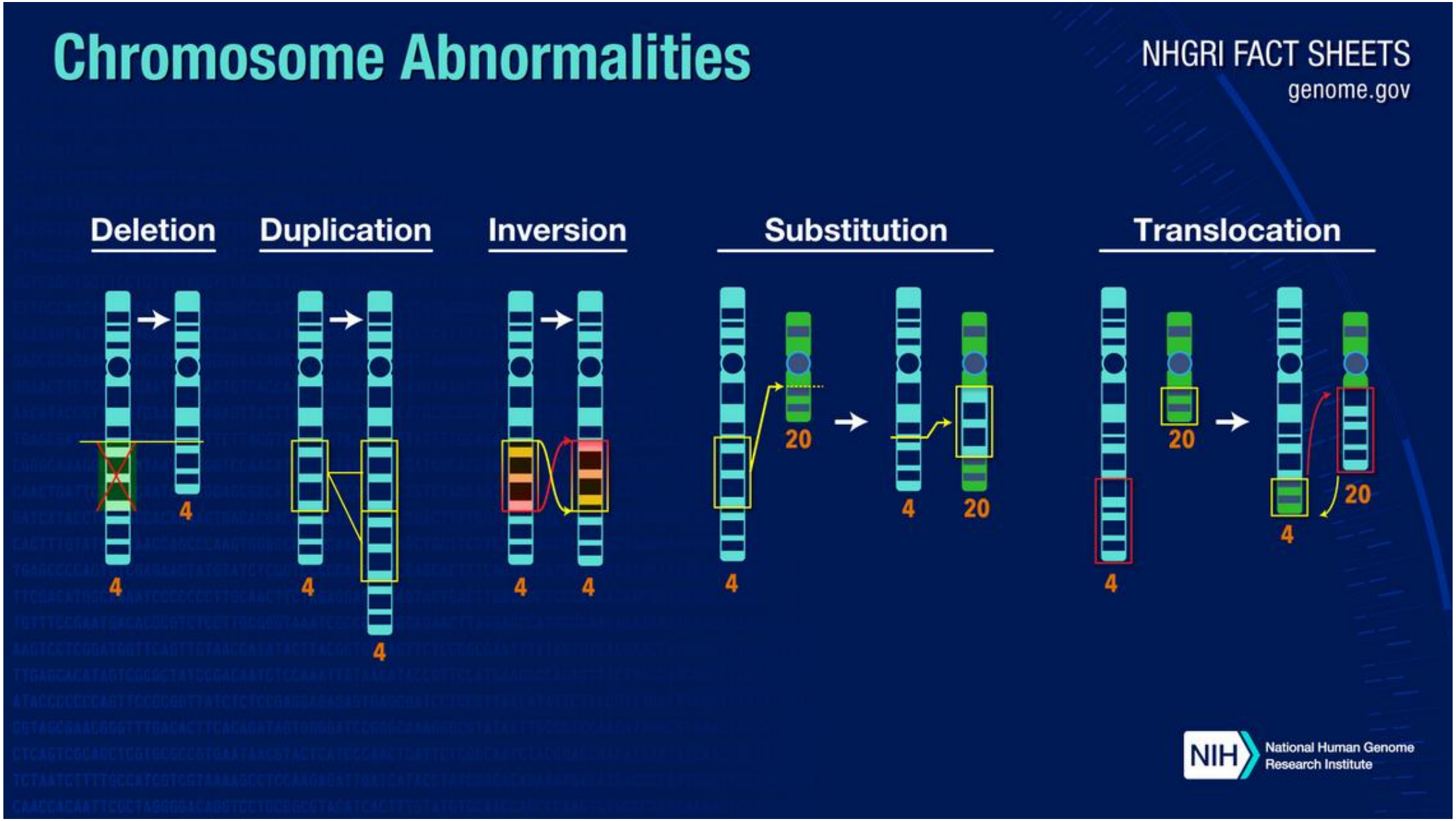
- Chromosome structure changes are often harmful to an individual leading to developmental difficulties and even death. Some changes are not as harmful and may have no significant effect on an individual. There are several types of chromosome structure changes that can occur.
- They can be divided into two groups: balanced (amount of genetic materiál is same) and unbalanced (genetic material is missing or reside).

- **Deficience** = external part of the chromosome is missing or has been deleted.
- **Deletion** = internal part of the chromosome is missing or has been deleted.
- **Duplication** = part of the chromosome has been duplicated, resulting in extra genetic material.
- **Inversion** = part of the chromosome has broken off, turned by 180° and reattached.
- **Translocation** = part of one chromosome is transferred to another chromosome → new trait can be created.
- **Fragmentation** = disintegration into small parts.



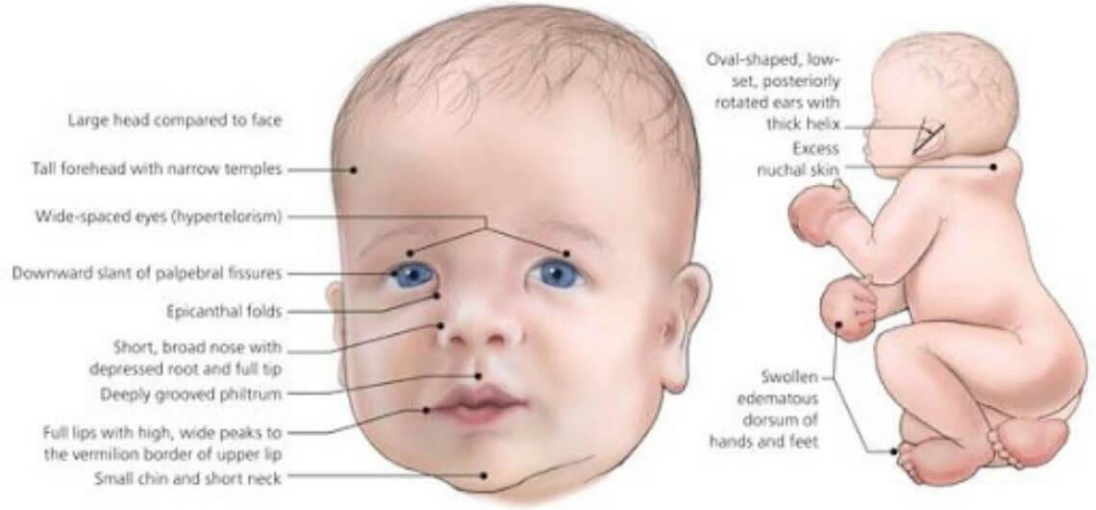


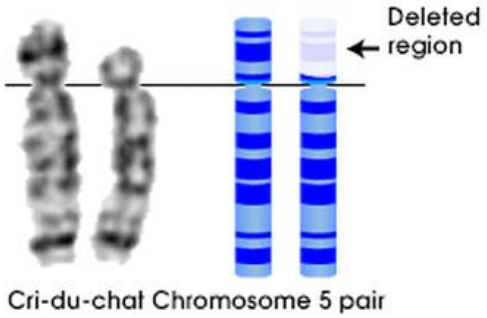
Chromosomal mutations – aberations



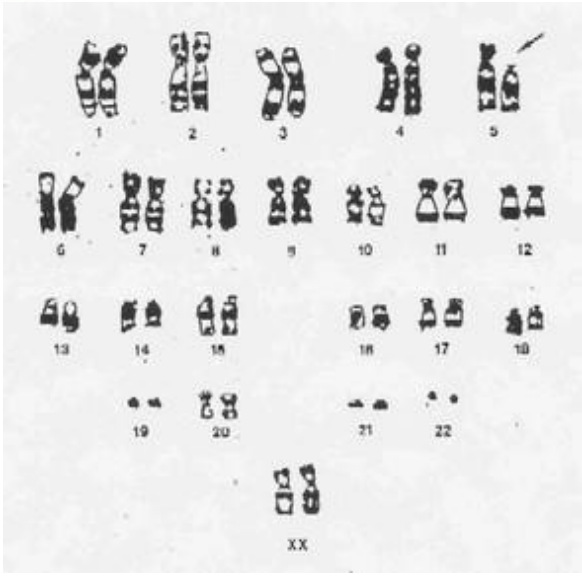
Chromosomal mutations - clinical examples

- **Cri Du Chat (46,XX/XY, del(5)(p?) –** chromosomal deletion
  - Cri du chat syndrome is a rare chromosomal disorder caused by a deletion of genetic material on part of chromosome 5.
  - Other names for the condition are cat cry syndrome and 5p- syndrome.
  - Symptoms can vary depending on the size and area of the deletion of chromosome 5.
  - The most common symptom is a shrill, cat-like cry that newborns make.
  - There's no cure for cri du chat. But, with prompt diagnosis and early intervention, child may be capable of reaching their fullest potential and leading a meaningful life.



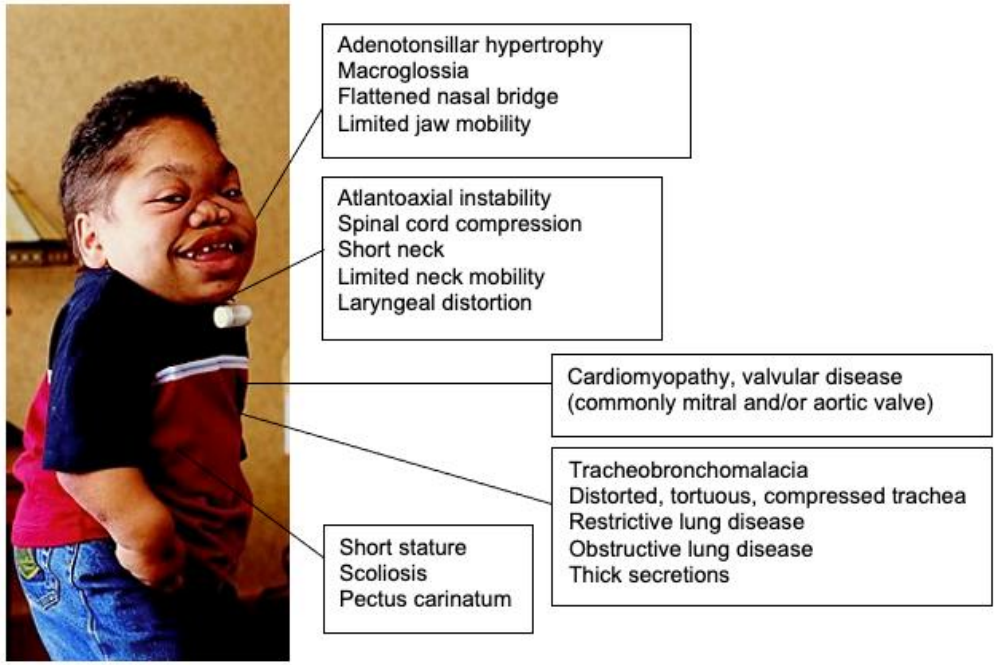


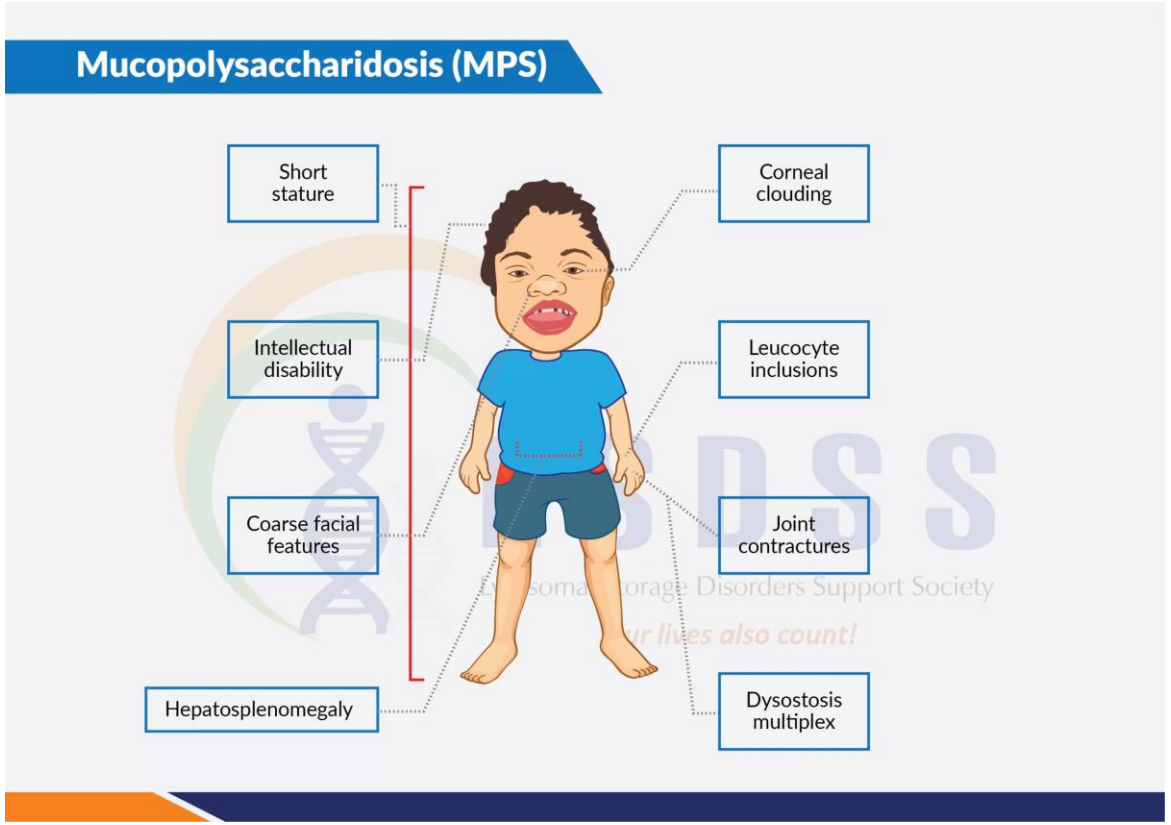
Cri-du-chat Chromosome 5 pair



Chromosomal mutations - clinical examples

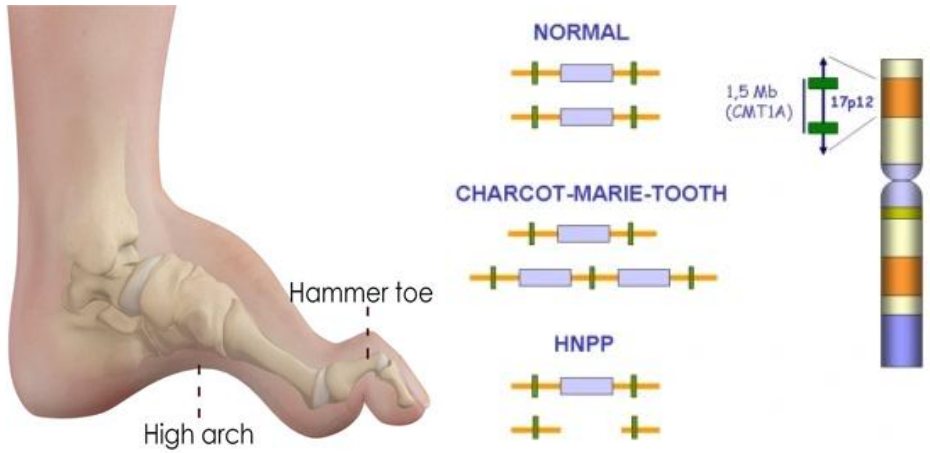
- **Hunter Syndrome (MPS II)–** chromosomal inversion
  - X-linked recessive disorder caused by the deficiency of iduronate 2-sulfatase (IDS)
  - Major rearrangements of the IDS gene.
  - Manifests almost exclusively in males – also observed in females

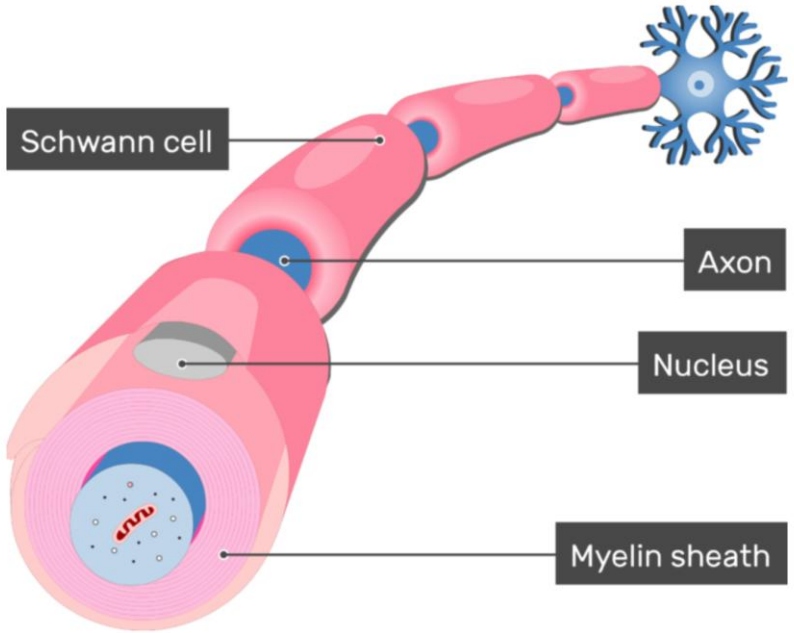




Chromosomal mutations - clinical examples

- **Charcot-Marie-Tooth disease type 1 –** chromosomal duplication
  - A subtype of CMT1 called CMT1A (caused by a duplication or, less commonly, a point mutation in the PMP22 gene on chromosome 17) accounts for around 70% to 80% of CMT1 cases, making it the most common subtype of CMT1.
  - Duplication of PMP22 leads to accumulation of the peripheral myelin protein 22 (PMP22) protein, and point mutations alter its distribution.
  - PMP22 is vital for the normal creation and maintenance of the myelin sheath.
  - CMT1 patients usually present with typical CMT onset within adolescence but remain ambulatory with no reduced life expectancy

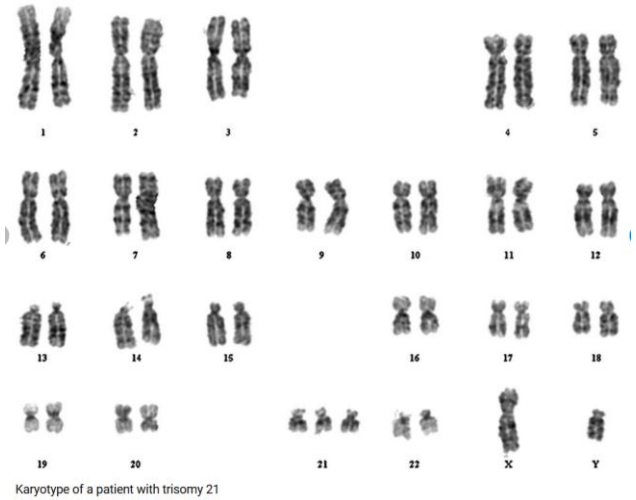




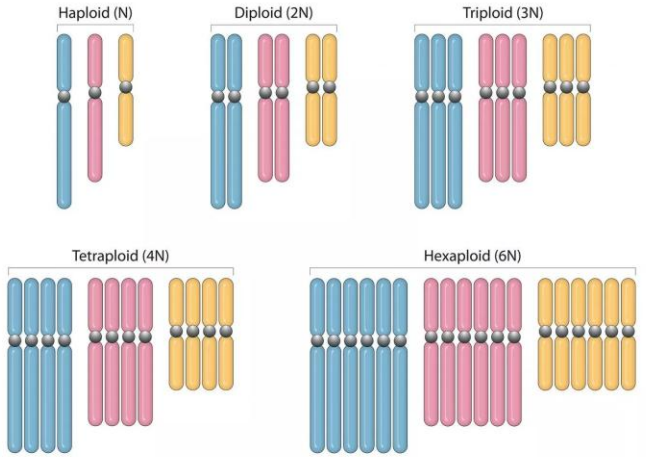
Chromosomal mutations – aberations

Numerical chromosomal aberrations

- Numerical chromosomal aberrations are defined as changes in the number of chromosomes or the entire set of chromosomes
- Structure of chromosomes is intact, but there can be pathological change in number of total genes
- Result from disturbances in the mitotic spindle apparatus during cell division. In the process called nondisjunction, chromosomes of a given pair fail to separate in the normal way during anaphase


Karyotype of a patient with trisomy 21

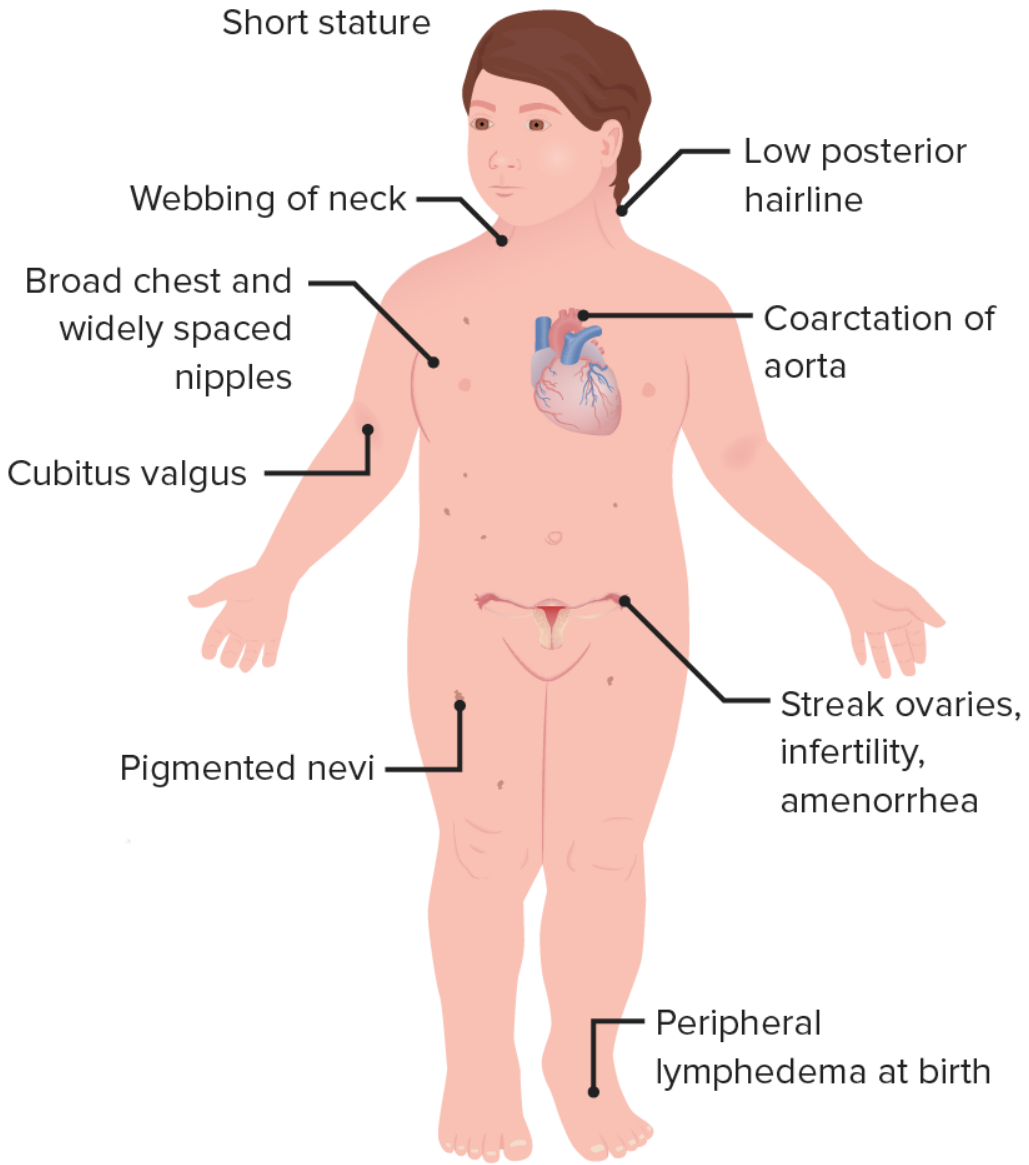
- **aneuploidy** - numerical aberation of one or more concrete chromosome – not whole set of chromosome! Chromosome can be
- Added - **trisomy** (3x – three copies of chromosome), **tetrasomy** (4x …),
- Lost - **monosomy** (1x – one copy of chromosome), **nulisomy** (0x …)



- **polyploidy** - *„heritable"!* condition of possessing more than two complete sets of chromosomes.
- Polyploidy occurs in humans in the form of triploidy, with 69 chromosomes (sometimes called 69, XXX), and tetraploidy with 92 chromosomes (sometimes called 92, XXXX)

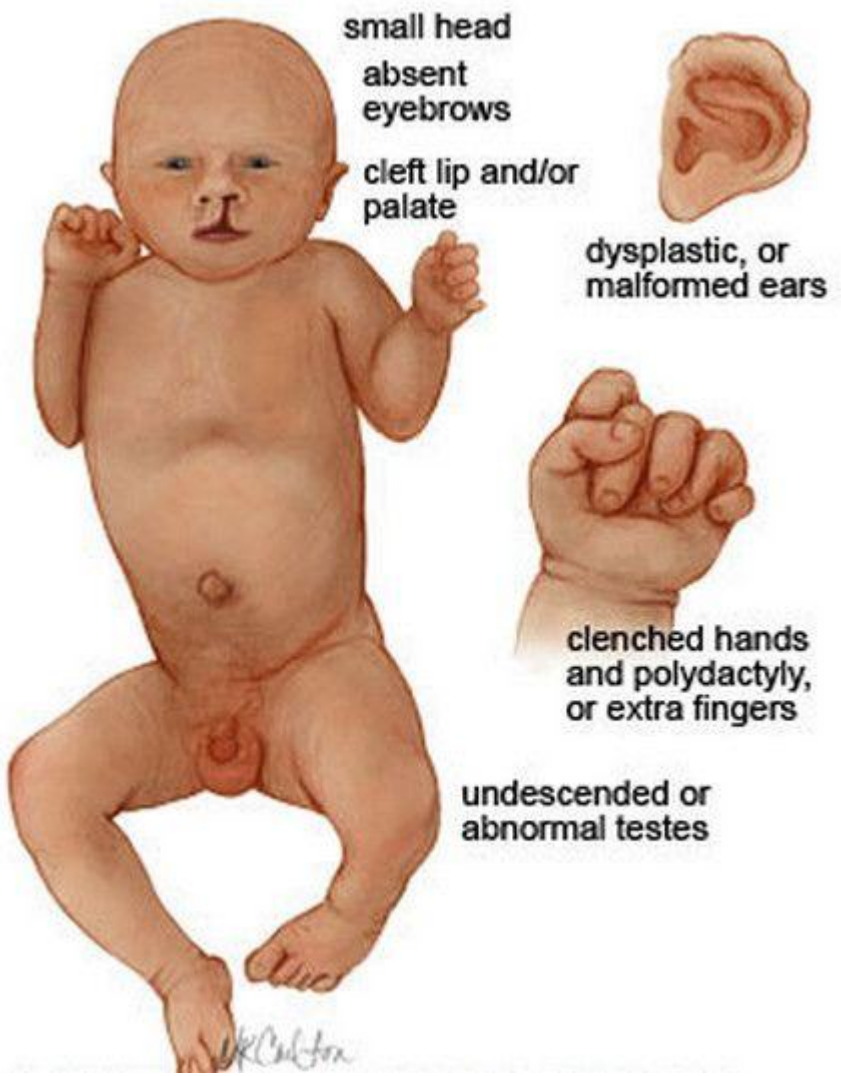
Chromosomal mutations - clinical examples

- **Turner syndrome (45, X0)** *(gonosomal monosomy)* – chromosomal condition that affects development in females.
  - Turner syndrome results when one normal X chromosome is present in a female's cells and the other sex chromosome is missing or structurally altered. The missing genetic material affects development before and after birth
  - Females with Turner syndrome have extra folds of skin on the neck (webbed neck), a low hairline at the back of the neck, puffiness or swelling (lymphedema) of the hands and feet, skeletal abnormalities, or kidney problems. One third to one half of individuals with Turner syndrome are born with a heart defect
  - Mosaic Turner syndrome – in an affected individual, it occurs as a random event during cell division in early fetal development. As a result, some of an affected person's cells have the usual two sex chromosomes, and other cells have only one copy of the X chromosome.



Chromosomal mutations - clinical examples

- **Patau syndrome (47, XX/XY +13)** *(autosomal trisomy)* – chromosomal condition associated with severe intellectual disability and physical abnormalities in many parts of the body.
  - The cause of Patau syndrome is the presence of three copies of chromosome 13; this is due most commonly to nondisjunction in meiosis, occurring more frequently in mothers of advanced age (age greater than 35).
  - Individuals with trisomy 13 often have heart defects, brain or spinal cord abnormalities, very small or poorly developed eyes (microphthalmia), extra fingers or toes, an opening in the lip (a cleft lip) with or without an opening in the roof of the mouth (a cleft palate), and weak muscle tone (hypotonia).
  - In some people, only a portion of cells contains the extra chromosome 13 (mosaic trisomy 13)

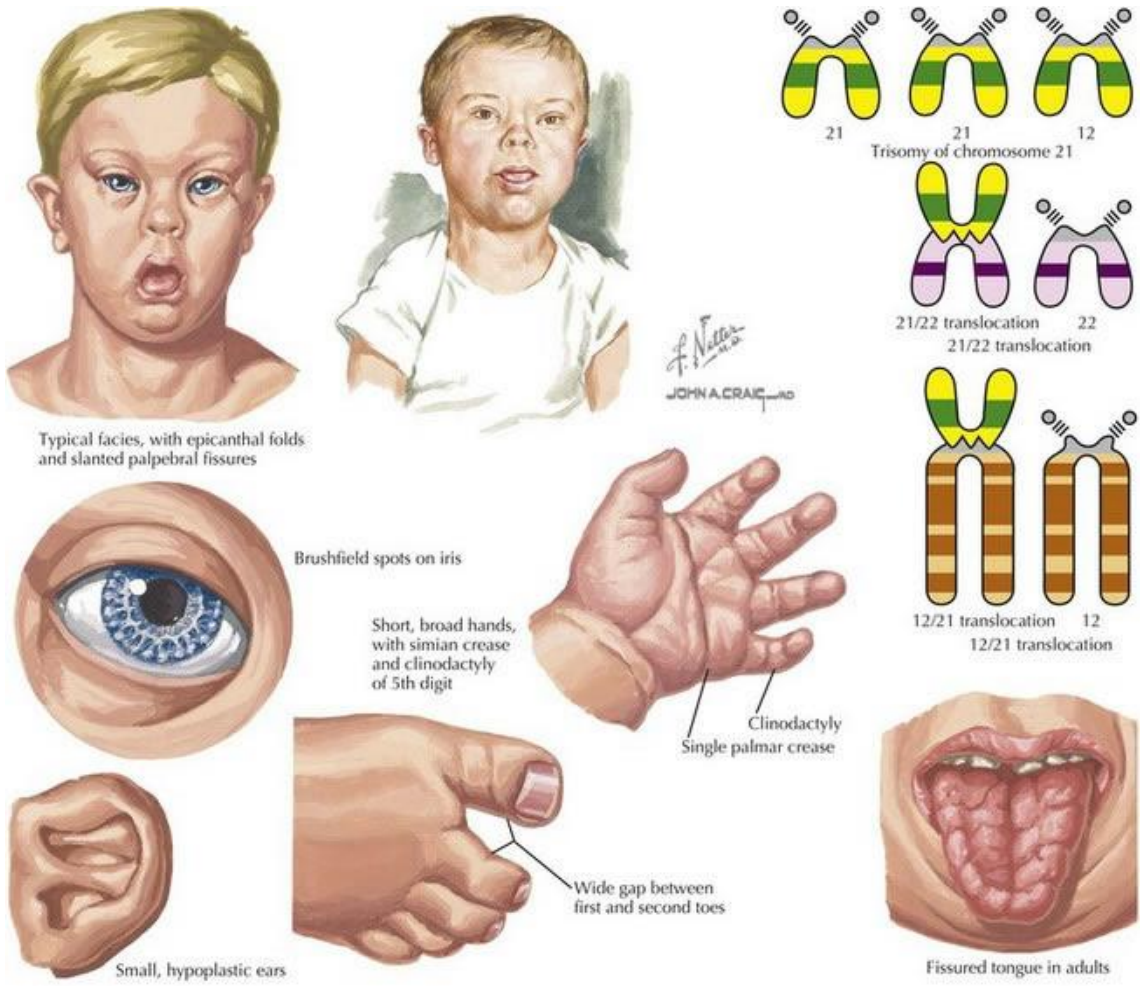


Chromosomal mutations - clinical examples

- **Edwards syndrome (47, XX/XY +18)** *(autosomal trisomy)* – chromosomal condition associated with abnormalities in many parts of the body.
  - The cause of Edwards syndrome is the presence of three copies of chromosome 18; is due to nondisjunction, mostly during meiosis II, occurring more frequently in mothers of advanced age (age greater than 35).
  - Individuals with trisomy 18 often have slow growth before birth (intrauterine growth retardation) and a low birth weight. Affected individuals may have heart defects and abnormalities of other organs that develop before birth. Other features of trisomy 18 include a small, abnormally shaped head; a small jaw and mouth; and clenched fists with overlapping fingers.

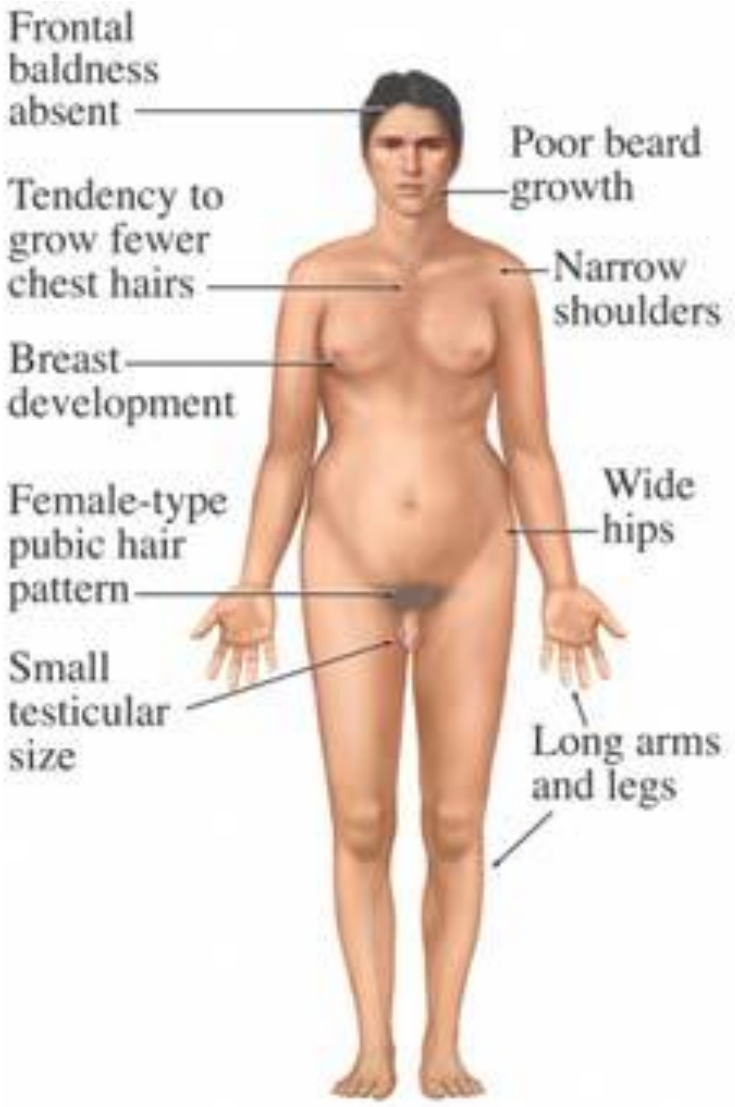
Chromosomal mutations - clinical examples

- **Down syndrome (47, XX/XY +21)** *(autosomal trisomy)* – chromosomal condition that is associated with intellectual disability, a characteristic facial appearance, and weak muscle tone (hypotonia) in infancy.
- The majority of full trisomy 21 is caused by chromosomal nondisjunction occurring during maternal meiotic division (~90%). Errors occur more frequently in the first maternal meiotic division than the second (73% vs. 25%)
- Individuals with Down syndrome often have a characteristic facial appearance that includes a flattened appearance to the face, outside corners of the eyes that point upward (upslanting palpebral fissures), small ears, a short neck, and a tongue that tends to stick out of the mouth. Affected individuals may have a variety of birth defects.



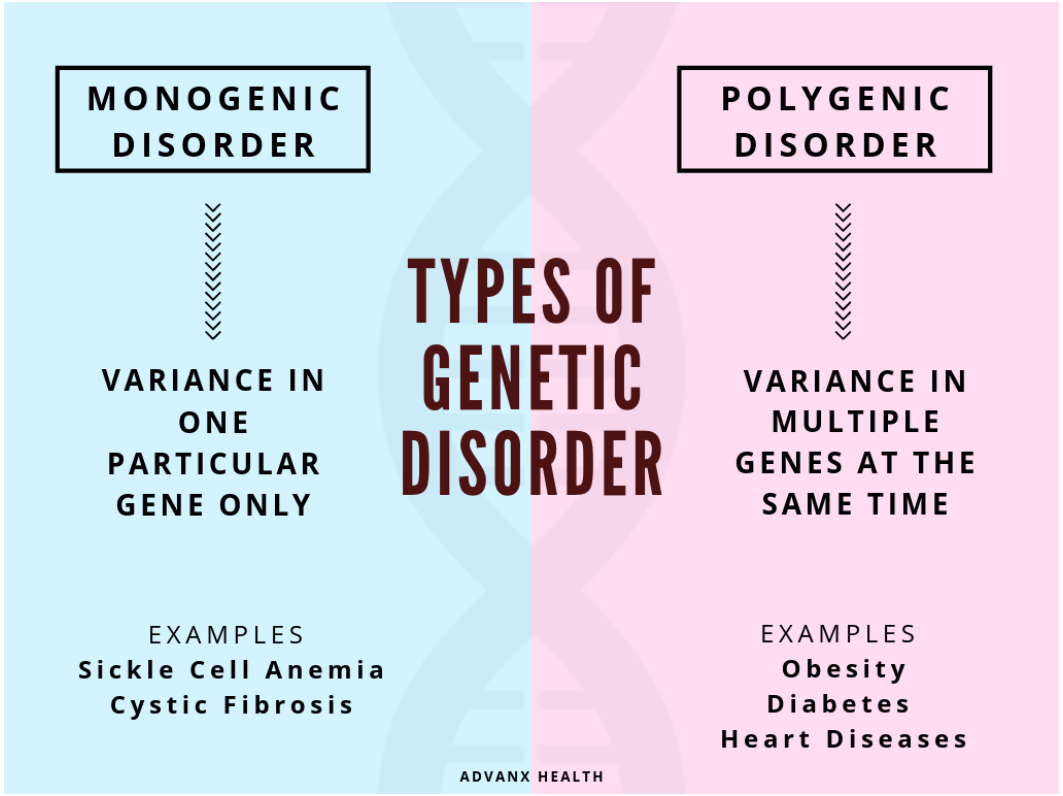
Chromosomal mutations - clinical examples

- **Klinefelter (47 XXY)** *(gonosomal trisomy)* – the most common chromosomal disorder in men and is associated with hypergonadotropic hypogonadism and infertility
- **Triple X syndrome (47 XXX)** *(gonosomal trisomy)* – characterized by the presence of an additional X chromosome in each of a female's cells. Although females with this condition may be taller than average, this chromosomal change typically causes no unusual physical features. Most females with triple X syndrome have normal sexual development and are able to conceive children.
- **47, XYY syndrome (47 XYY)** *(gonosomal trisomy)* – characterized by an extra copy of the Y chromosome in each of a male's cells. Although many males with this condition are taller than average, the chromosomal change sometimes causes no unusual physical features. Most males with 47,XYY syndrome have normal production of the male sex hormone testosterone and normal sexual development, and they are usually able to father children.



Classification of genetic causes of diseases

- practically every diseases (i.e. onset, progression and outcome) is, to some extent, modified by genetic make-up subject; however, under the different mode
  - with except of trauma, serious intoxications and highly virulent infections
  - **chromosomal aberrations -** inborn but nor inherited!
  - **monogenic diseases**
    - single critical "error" (allele) of a single gene is almost entirely responsible for the development of disease (phenotype)
    - characteristic pedigree (segregation of phenotype) due to the mode of inheritance (recessive x dominant)
  - **complex (polygenic) diseases**
    - genetic dispositions + effect of non-genetic factors
    - combination of several alleles in several loci



Monogenic diseases

- The development of molecular-biological methods has enabled a detailed analysis of the genetic basis of many Mendelian inherited **monogenic diseases**.
- In these diseases, the hereditary basis is a **large factor**, meaning it is present in practically all patients and it is provably a causal factor (e.g. defects in the dystrophin gene in muscular dystrophies), to which are added *additional* genetic factors and external environmental factor. The cause of these diseases are mainly rare alleles.
- Determined by alleles in one locus
- Characteristic way of inheritance in families

Monogenic diseases

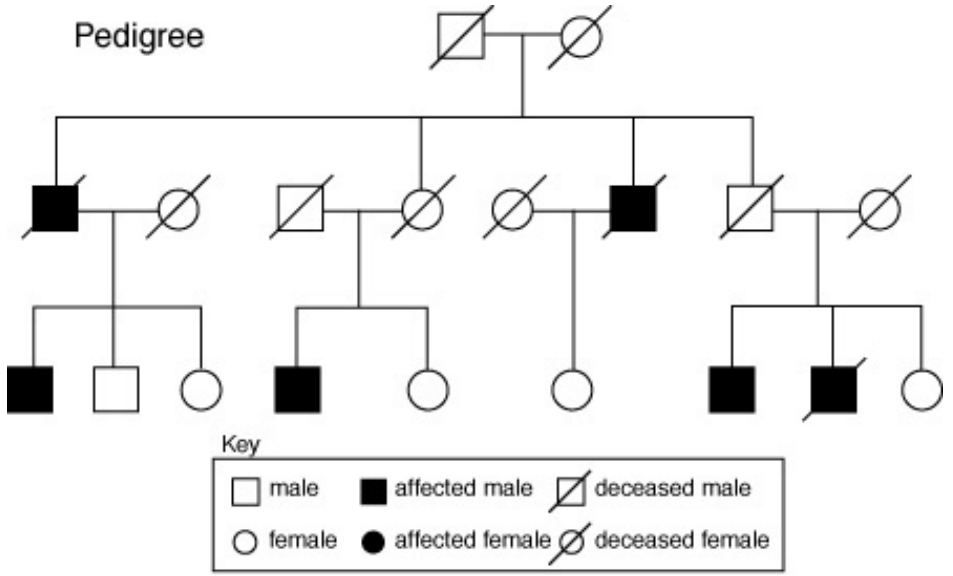
- Childhood diseases
- Less than 10% of them appear after puberty and only 1% appear after reproductive age
- often significantly pathological
- In a population study of 1 million infants, the incidence of serious monogenic diseases was estimated at 0.36%, with 6-8% of hospitalized children considering monogenic diseases.
- so far known diseases comprised in OMIM (On-line Mendelian Inheritance in Man) ~6000 clinically significant phenotypes
- Four basic types of inheritance:

	Dominant	Recessive
Autosomal	Autosomal dominant	Autosomal recessive
X-linked	X-dominant (XD)	X-recessive (XR)

Monogenic diseases

Types of inheritance

- **autosomal**
  - genes on both autosomes are normally active
- **gonosomal (X-chromosom linked)**
  - men are normally hemizygote
  - women have 1 X-chromosom inactivated
- **(imprinting, mosaicism)**



According to the expression of genotype in the phenotype

- **recessive**
  - diseases develops only in mutated homozygote
- **dominant**
  - disease in both heterozygote and mutated homozygote
- **incomplete dominance**
  - gradual increase of severity of the disease in heterozygote and mutated homozygote
- **co-dominant**
  - both normal and pathological allele expressed in phenotype

Monogenic diseases – AD x AR

Autosomal dominant AD

- Result of both mutations transmitted between generations and newly arise
- Disease is manifested in every generation - the affected individual has a affected parents (and grandparents), a mother or father
- Risk 0,5; if both parent are affected then 0,75, but this is extremely rare

- familiar hypercholesterolemia (1/500),
- myotonic dystrophy (1/1000)
- Huntington disease (1/3000)

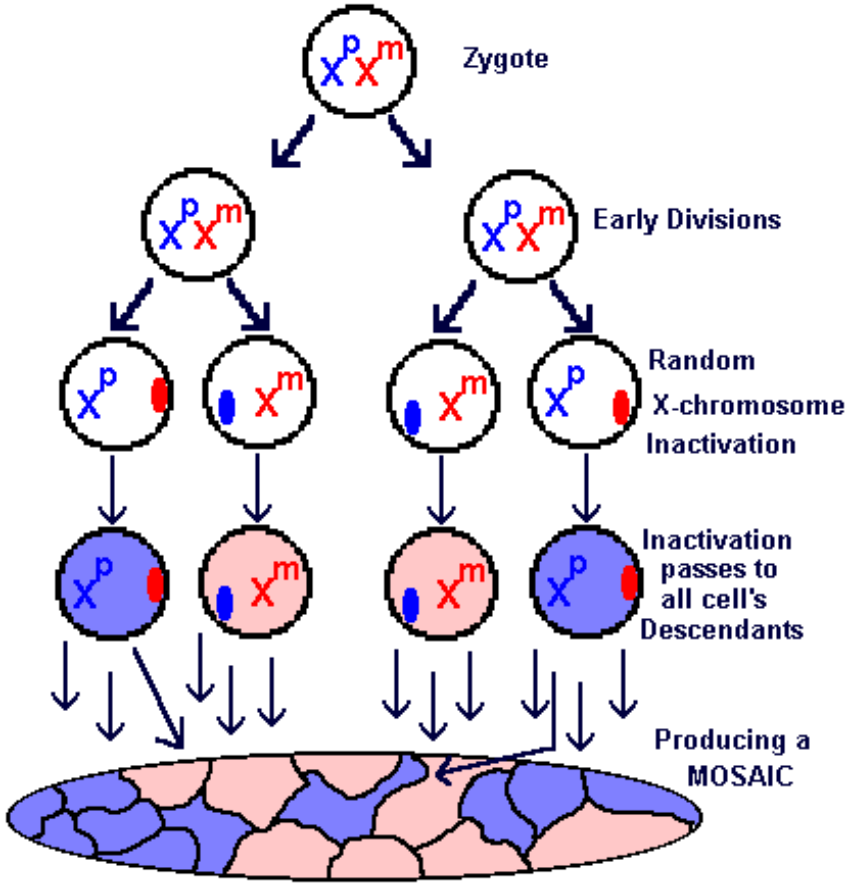
Autosomal recessive AR

- very often enzyme defects
- affected mutated homozygote, heterozygous parents are carriers (asymptomatic)
  - risk $0.50 \times 0.50 = 0.25$
- both genders affected equally
- frequency of carriers in population → frequency of disease
- most common AR diseases in whites is cystic fibrosis
  - f disease 1/2000, f carriers 1/22 !!!
- consanguinity and inbreeding significantly increase the risk
  - arranged marriages
  - genetically isolated populations (e.g. Ashkenazi Jews – Tay-Sachs disease)

Monogenic disease - X-linked

- women 3 genotypes, men only 2
- X-linked diseases manifest in all men who have inherited the mutation and only in homozygous women
- Hemophilia A
- Duchenne muscular dystrophy
- Wiskott-Aldrich syndrome (immunodeficiency)

- Inactivation of X-chromosome in women
  - Dose compensation and expression of X-linked genes
  - Lyon hypothesis ("lyonization")
  - in somatic cells one X chromosome is inactivated and appears in the interphase as a "Barr" body
  - the process is random, it can involve both paternal and maternal X.
    - the result is variable expression of X-linked genes in heterozygotes ("manifesting carrier")
    - functional mosaicism

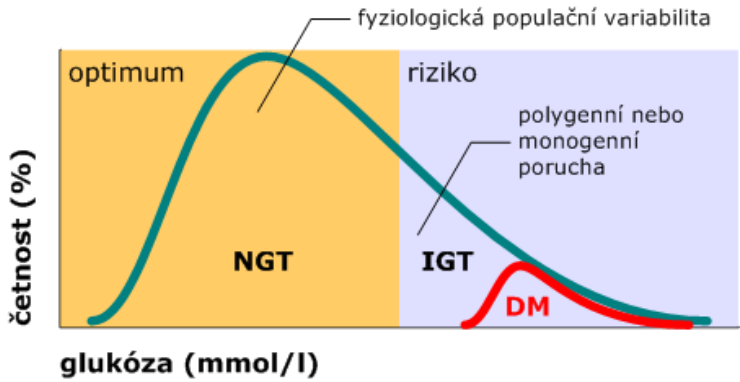


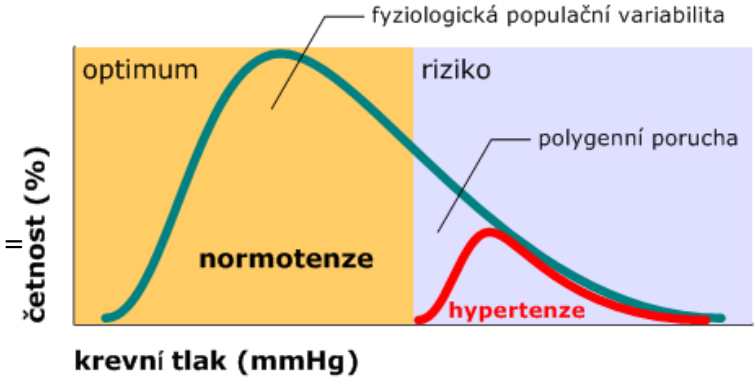
Complex diseases

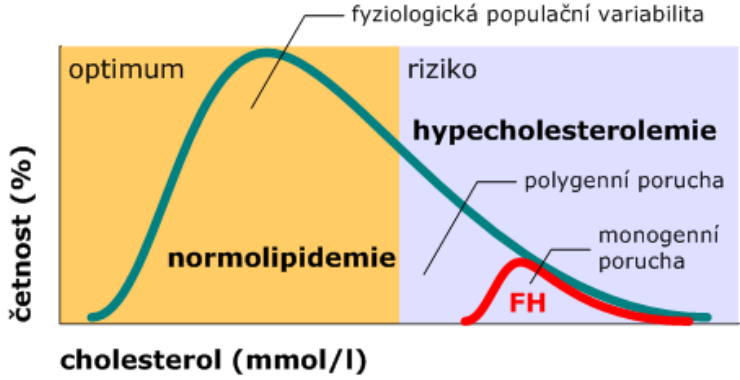
- Multifactorial, polygenic.
- Combination of both certain genes and certain environmental respectively is important.
- Unlike the genetics of Mendelian diseases, most of the general principles of multifactorial diseases are still unclear.
- In clinical practice, the results of genetic studies that seek to reveal the genetic basis of complex diseases often vary, from unreasonable expectations about the genes found to have a large effect to great skepticism about the existence of genetic evidence in a population of numerous diseases (over 1%) - for example essential hypertension in cardiology.
- if the disease has a provably familial occurrence, we must expect the genetic basis to contribute to its manifestation, even if it is not yet well defined or we do not consider the current knowledge to be convincing.
- Even relatively remote proximal phenotypes, such as the quality of life in patients with chronic cardiovascular disease, have a genetic background.
- **Each disease has a genetic background, the role of which in the manifestation of the disease is different.**

Complex diseases

- diseases developing due to the ethiopathogenic "complex" of genetic, epigenetic and environmental factors
  - phenotype does not follows Mendel rules (dominant or recessive mode of inheritance)
- **"predisposing genes/alleles"** increase probability to become affected, however, do not determine unequivocally its development
  - effect of non-genetic factors is a necessary modifier
    - diet, physical activity, smoking, ….
  - genes interact between themselves
- typical features of complex diseases
  - incomplete penetrance of pathological phenotype
    - some subjects inherited predisposing alelles never become ill
  - existence of phenocopies
    - pathological phenotype can develop in subjects not predisposed, entirely due to the non-genetic factors
  - genetic heterogeneity (locus and allelic)
    - manifestation (clinical) is not specific but the same syndrom can develop as a consequence of various loci (= locus heterogeneity) in which there could be several variants (= allelic heterogeneity)
  - polygenic inheritance
    - predisposition to disease is significantly increased only in the presence of the set of several risk alleles (polymorphisms), hence their high population frequency
      - in isolated occurrence the effect is mild
  - other modes of transmission
    - mitochondrial,
- examples of complex diseases: essential hypertension, diabetes (type 1 and 2), dyslipidemia, obesity, allergy, Alzheimer disease, …





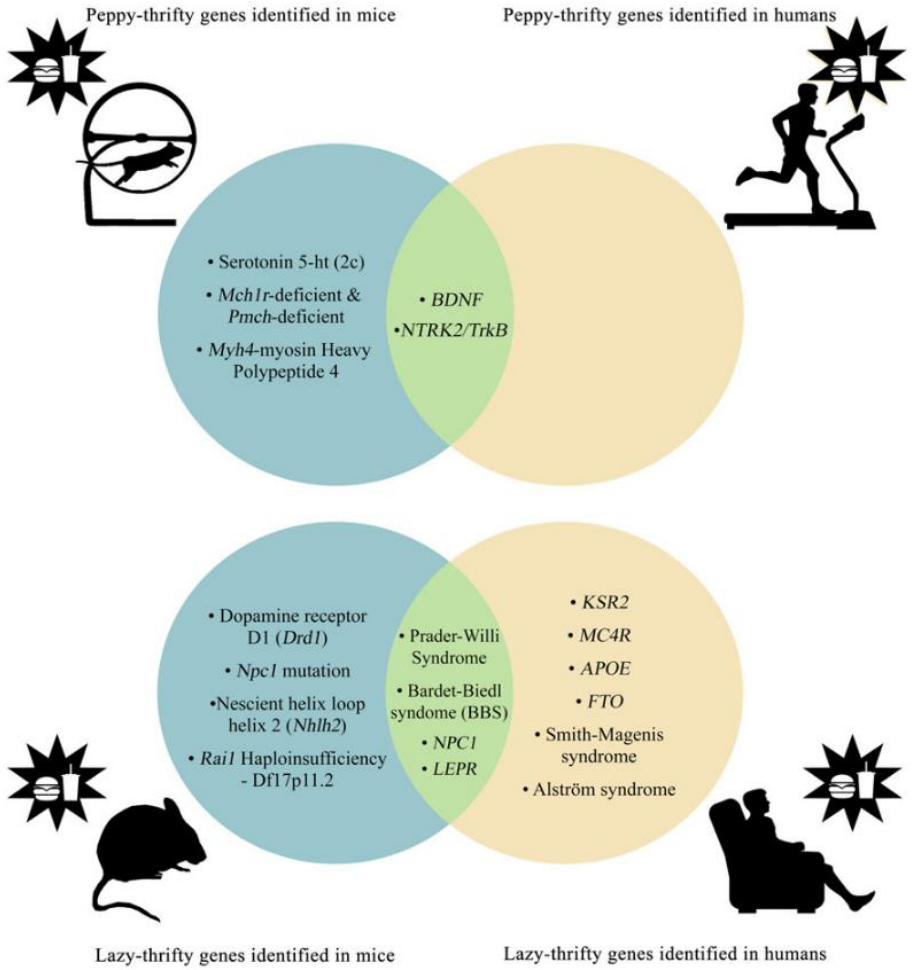


Complex diseases

- Complex diseases are characterized by:
  - incomplete penetrance of the pathological phenotype
    - in a certain group of people, even though they inherit an unfavorable genotype the pathological phenotype does not develop
  - existence of phenocopies
    - the pathological phenotype may be present in people who are not carriers of said genotype
  - genetic heterogeneity (locus and allelic)
    - the clinical picture is not specific, but it can develop due to changes in genes located at different loci (= locus heterogeneity), there may be several mutations or polymorphisms in individual genes (= allelic heterogeneity)
  - Polygenic inheritance
    - the predisposition to develop a pathological phenotype increases only when a certain set of alleles occur simultaneously
  - high population frequency of alleles responsible for the development of the pathological phenotype
    - each individual predisposing allele does not have to be significantly pathogenic
  - interaction of other transmission mechanisms
    - mitochondrial inheritance, imprinting

Complex diseases - clinical examples

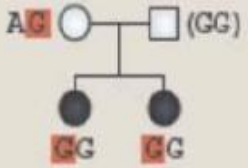
- **The Thrifty Gene Hypothesis** *(autosomal trisomy)* – suggests that particular gene variants evolved to favor efficient use of nutrients in the calorie-limited environment in which humans evolved.
- Promotes obesity and type 2 diabetes in the modern, calorie-rich environment.
- A large number of studies in animals and humans linked poor prenatal nutrition with subsequent predisposition to disease, including obesity later in life and in subsequent generations.
- Despite considerable study, there are very few concrete details on the molecular mechanisms that promote a thrifty phenotype, although, there are some indications that leptin resistance and epigenetic mechanisms are .involved

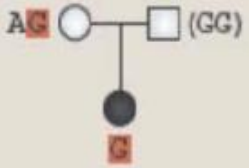


Genetic studies

- The basic debate over the genetic basis of diseases logically starts with the strategy of selecting **candidate genes.** This question is much simpler in Mendelian diseases, where the altered function of one gene is easier to identify.

- Another important approach is the selection of a statistical methodology that evaluates the strength of the association of genes with diseases. There are basically two options: **linkage analysis** and **association studies**. In principle, both methods can be used to detect specific genetic regions and genes involved in disease transmission.





Property of mapping approach	Linkage analysis	Association analysis
Data type studied	Relatives	Unrelated or related individuals
Relevant parameter	Recombination fraction	Association statistic
Range of effect detected (linkage or association)	Long (≤5 Mb)	Short (≤100 kb)
Number of markers required for genome-wide coverage	Moderate (500–1,000)	Large (>100,000)
Statistics used	Cumbersome (requires tailor-made likelihood methods)	Elegant; can use the range of classical statistical tools
Dealing with correlated markers	Pose problems in presence of ungenotyped individuals	Can be handled efficiently
Biological basis of approach	Observe (or infer) recombination in pedigree data	Exploit unobserved recombination events in past generations
Dealing with allelic heterogeneity	Not a problem	Reduces power
Detecting genotyping errors	Potentially detected as Mendelian inconsistencies	Potentially detected only in family data, but not in case–control data
Most suitable application	Rare, dominant traits	Common traits

Nature Reviews | Genetics

Genetic studies

Linkage analysis

- Tests cosegregation of gene marker and disease phenotype in the family → their marker and the disease should always occur together in a given family.

Association studies

- Genetic association studies test for a correlation between disease status and genetic variation to identify candidate genes or genome regions that contribute to a specific disease.
- A higher frequency of a single-nucleotide polymorphism (SNP) allele or genotype in a series of individuals affected with a disease can be interpreted as meaning that the tested variant increases the risk of a specific disease
- SNPs are the most widely tested markers in association studies, but microsatellite markers, insertion/deletions, variable-number tandem repeats (VNTRs), and copy-number variants (CNVs) are also used
- **Case-Control Study**
  - The simplest study design used to test for association is the case–control study, in which a series of cases affected with the disease of interest are collected together with a series of control individuals.

Clinical genetic

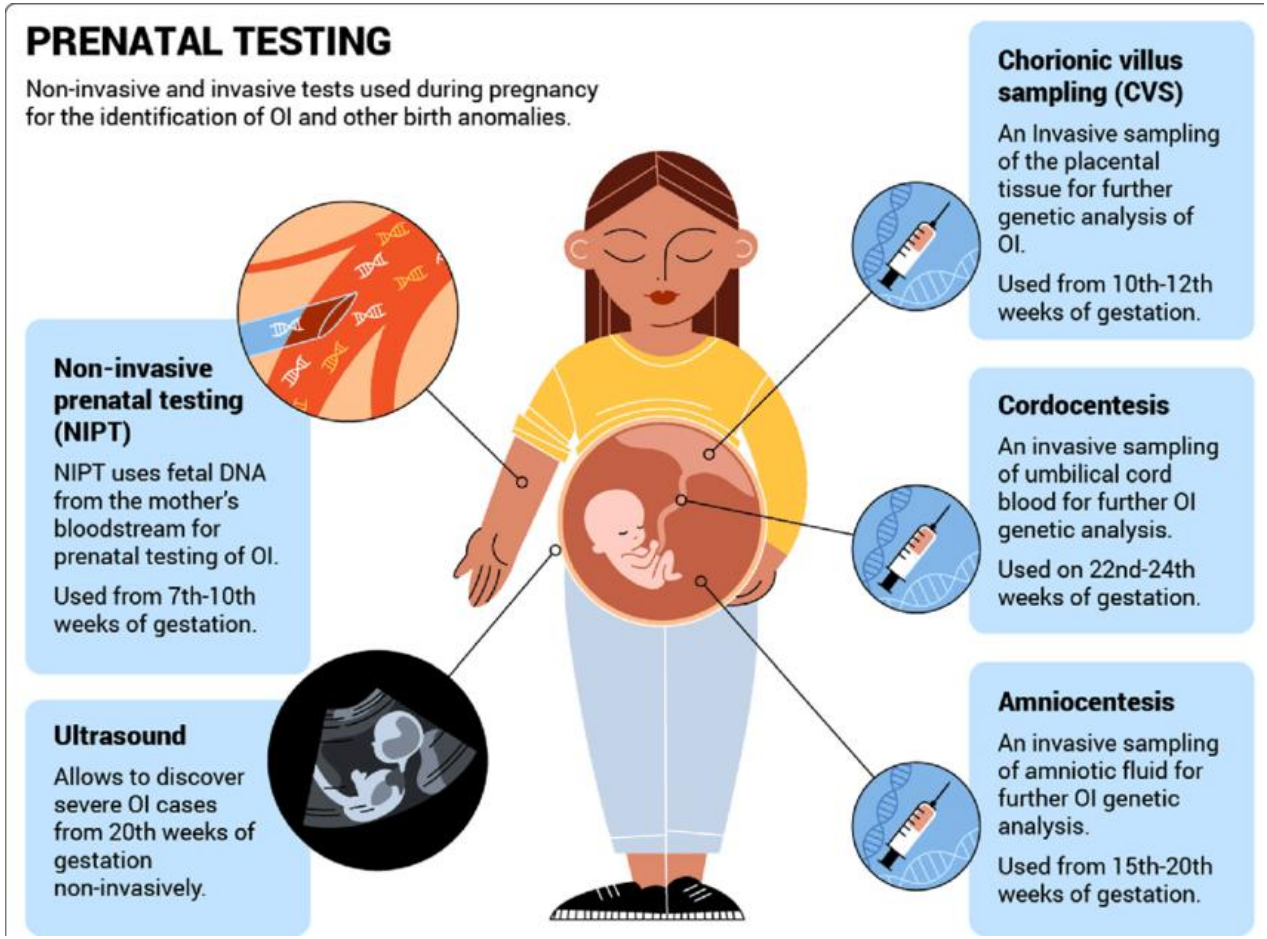
- **Deals with diagnostics, treatment and prevention of genetic diseases**

- **Genetic counselling**
- **congenital malformations - disorders of organ formation that occurred during the period of intrauterine life, as well as functional disorders (mental retardation) and disorders at the biochemical and molecular level (ongenital metabolic defects).**

Group	Cause	Repr.
Primary (genetic)	Chromosomal aberation	10 %
	Monogenic inheritance	20 %
Secondary (environment)	Drugs, infection, radiance	5 %
	Birth wounds	12 %
	After birth infrection	7 %
Unknown (multifactorial)	Gene + environment	46 %

Clinical genetics – Prenatal diagnosis

- **Karyotype test -** looks at the size, shape, and number of chromosomes. Methods of sample collection:
  - Amniocentesis
  - Chorionic villus sampling (CVS)
- **Ultrasound**
- **MRI**
- **Quantitative fluorescent PCR (QF-PCR) –** or amnioPCR is used for prenatal diagnosis of the most common aneuploidies
  - comparison of DNA markers of mother and fetus → determination of the number of individual chromosomes in the fetus.
- **Cell-free fetal DNA (cffDNA) –** *cffDNA circulating in maternal plasma* has enabled development of safer, earlier testing based on a simple maternal blood sample. A number of clinical diagnostic tests have now been implemented, including **fetal sex determination**, RHD blood group determination and the detection or exclusion of de novo or paternally inherited monogenic disorders
- **+Preimplantation diagnostics**




Thank you for your attention