1- Basic genetics

The laws of inheritance are investigated by genetics. The different nucleic acids (DNA and RNA) in the living organism play a central role in the inheritance of the different features. The information in the DNA molecule is inherited from one generation to the next generation through reproduction. It means that the hereditary material is the DNA (in some viruses the RNA), more exactly the genes which are the functional units which determine the nature of the features. Gene definition: Genes are the units of inheritance. Genes are pieces of DNA that contain information for synthesis of ribonucleic acids (RNAs) or polypeptides. Earlier only those units were regarded genes, which coded proteins. Nowadays, genes are also those, which code functional RNAs, which are not transcribed to proteins. These are called non-coding RNAs. In the so-called RNA-viruses (e.g. influenza, HIV1) genes are coded only in the form of RNA. The appearance of an organism which results from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two is called **phenotype.** The genetic background of an organism is called **genotype**. The majority of the DNA content of the cells is packaged in chromosomes and DNA can be also found in mitochondria. In diploid cells a couple of homologous chromosomes are a set of one maternal chromosome and one paternal chromosome that pair up with each other inside a cell during meiosis. These copies have the same genes in the same **locations, or loci.** In the nature a given gene can have different variations, these are called **alleles**. In a given population the most frequent allele of a gene is called wild type. If in a diploid cell the same alleles occur in a given locus of the homologous chromosomes then the organism is homozygous, if the alleles are different, it is heterozygous at this locus.

1-2 Basics of molecular biology

The central dogma in molecular biology can be described as "DNA makes RNA and RNA makes protein," a positive statement which was originally termed the sequence hypothesis by Crick (Figure 1.2). However, this simplification does not make it clear that the central dogma as stated by Crick does not preclude the reverse flow of information from RNA to DNA, only ruling out the flow from protein to RNA or DNA.

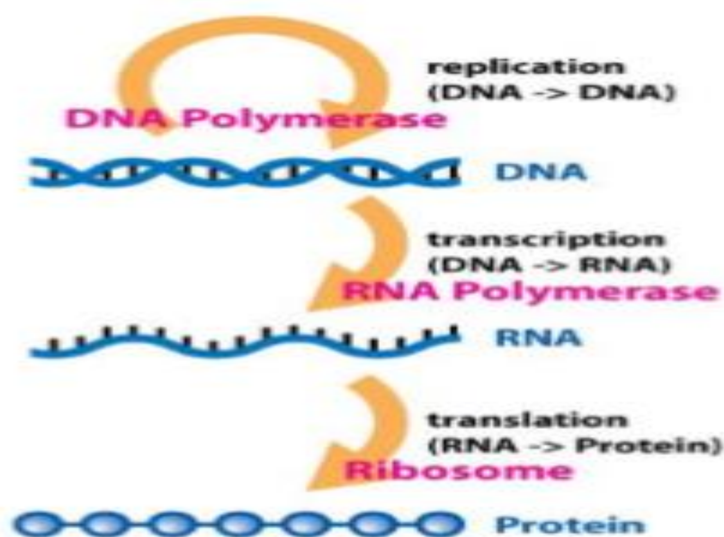


Figure 1.2. (The central dogma of molecular biology). https://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology#/media/File:Central_Dogma_of_Molecular_Biochemistry_with_Enzym es.jpg 26/02/2016.

1-3 Some characteristics of the human DNA

The proteins coded by the DNA in our cells determine the structures and functions of the cells. If there is a mutation in the DNA, it can change the structure

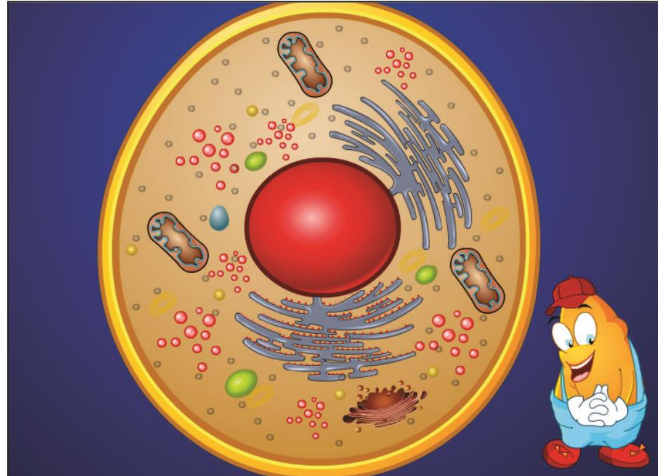
and function of the protein, which can have consequences on the function of the cell and can lead to diseases. Let's see the structure of the DNA in our cells. The backbone of the DNA strand is made from alternating phosphate and sugar residues (Figure 1.3). The sugar in DNA is 2-deoxyribose, which is a pentose (five-carbon) sugar. The sugars are joined together by phosphate groups that form phosphodiester bonds between the third and fifth carbon atoms of adjacent sugar rings. These asymmetric bonds mean a strand of DNA has a direction. In a double helix the direction of the nucleotides in one strand is opposite to their direction in the other strand: the strands are antiparallel. The asymmetric ends of DNA strands are called the 5′ (five prime) and 3′ (three prime) ends, with the 5′ end having a terminal phosphate group and the 3′ end a terminal hydroxyl group. One major difference between DNA and RNA is the sugar, with the 2-deoxyribose in DNA being replaced by the alternative pentose sugar ribose in RNA. The four bases found in DNA are adenine (abbreviated A), cytosine(C), guanine (G) and thymine (T). These four bases are attached to the sugar/phosphate to form the complete nucleotide, as shown for adenosine monophosphate. The nucleobases are classified into two types: the purines, A and G, being fused five- and six-membered heterocyclic compounds, and the pyrimidines, the sixmembered rings C and T. A fifth pyrimidine nucleobase, uracil (U), usually takes the place of thymine in RNA and differs from thymine by lacking a methyl group on its ring. Uracil is not usually found in DNA, occurring only as a breakdown product of cytosine.

In a DNA double helix, each type of nucleobase on one strand bonds with just one type of nucleobase on the other strand. This is called complementary base pairing. Here, purines form hydrogen bonds to pyrimidines, with adenine bonding only to thymine in two hydrogen bonds, and cytosine bonding only to guanine in three hydrogen bonds. This arrangement of two nucleotides binding together across the double helix is called a base pair. As hydrogen bonds are not covalent, they can be broken and rejoined relatively easily. The two strands of DNA in a double helix can therefore be pulled apart like a zipper, either by a mechanical force or high temperature. As a result of this complementarity, all the information in the double-stranded sequence of a DNA helix is duplicated on each strand, which is vital in

DNA replication. Indeed, this reversible and specific interaction between complementary base pairs is critical for all the functions of DNA in living

organisms. A DNA sequence is called "sense" if its sequence is the same as that of a messenger RNA copy that is translated into protein. The sequence on the opposite strand is called the "antisense" sequence. Both sense and antisense sequences can exist on different parts of the same strand of DNA (i.e. both strands can contain both sense and antisense sequences). In human cells DNA is in two compartments. Nuclear DNA, or nuclear deoxyribonucleic acid (nDNA), is DNA contained within a nucleus of the cell. Nuclear DNA encodes for the majority of the genome, with DNA located in mitochondria coding for the rest. Nuclear DNA adheres to Mendelian inheritance, with information coming from two parents, one male and one female. The other DNA containing compartment is the mitochondria. Mitochondria are cellular organelles within eukaryotic cells that convert chemical energy from food into a form that cells can use, adenosine triphosphate (ATP). In most multicellular organisms, including humans the mitochondrial DNA (mtDNA) is inherited from the mother (maternally inherited). Nuclear DNA and mitochondrial DNA differ in many ways. The structure of nuclear DNA chromosomes is linear with open ends and includes 46 chromosomes containing more than 3 billion nucleotides (3.38 *109). Mitochondrial DNA chromosomes have closed, circular structures, and contain 16,569 nucleotides. Nuclear DNA is located within the nucleus of eukaryote cells and usually has two copies per cell while mitochondrial DNA is located in the mitochondria and contains 100-1,000 copies per cell. Nuclear DNA contains more than 20 thousands protein coding and more than 23 thousands non-coding genes. The mitochondrial DNA contains 37 genes. Of the 37 genes 13 are protein coding, 2 rRNA and 22 tRNA coding genes. The mutation rate for nuclear DNA is less than 0.3% while that of mitochondrial DNA is generally higher. As mitochondria is the “powerhouse of the cell”, mutations of its DNA will effect on the power production processes of the cell, and will have serious consequences especially in tissues with large power need, like liver, neurons and muscle. As the mutation rate in the mitochondrial DNA higher, the mitochondrial diseases usually deteriorate with age, and can play also a role in the aging processes.

2- CELL CYCLE AND CELL DIVISION



Are you aware that all organisms, even the largest, start their life from a single cell? You may wonder how a single cell then goes on to form such large organisms. Growth and reproduction are characteristics of cells, indeed of all living organisms. All cells reproduce by dividing into two, with each parental cell giving rise to two daughter cells each time they divide. These newly formed daughter cells can themselves grow and divide, giving rise to a new cell population that is formed by the growth and division of a single parental cell and its progeny. In other words, such cycles of growth and division allow a single cell to form a structure consisting of millions of cells.

2.1Cell cycle and regulation of cell cycle

In a given organism the genetic information (DNA) is transferred from cell to cell during the cell cycle. In the cell cycle, the cellular content is duplicated then it is halved. However, a distinction must be drawn between the nuclear and cytoplasmic events. DNA duplication (in chromatin form of DNA) and halving (in chromosome form of DNA) are very precisely regulated processes, resulting two genetically identical cells. At the same time the growing of the cytoplasm followed by division in two are less strictly regulated events of cell cycle .

The duplication of cellular ingredients occurs in interphase, that is divided into G1 (preduplication or preceding DNA duplication), S (DNA synthesis) and G2 (postduplication) phases. In M-phase the previously duplicated cellular content is separated, in mitosis the chromosomes, followed by cytokinesis, the division of cytoplasm .Cell proliferation rate in an adult multicellular organism is variable. Moreover most of the cells are in so-called G0 phase, where there is no cell division, sometimes not even growth. The cells need extracellular stimuli, e.g. growth factors and / or adhesion to other cells or extracellular matrix in order to reenter G1 phase .In the cell cycle a very sophisticated control system (cell cycle control system) functions, whose essential components are the cyclin-dependent

protein kinases, the Cdk-s. Cdk-s are activated by another protein family, by cyclins, the amount of which cyclically varies during the cell cycle. Beside cyclins, the activity of cyclin-dependent kinases is regulated by other factors, too. These factors include activating and inhibiting Cdk kinases which phosphorylate Cdk-s, resulting Cdk activation and inhibition respectively. Phosphate residues are removed by phosphatases, modifying Cdk activity. According to their names, cyclin-dependent kinase inhibitors inhibit Cdk activity. The amount of all the proteins mentioned before may be regulated via transcriptional and translational level and by proteasomal degradation, followed by ubiquitination. All these together allow a highly organized, complex but gentle control of the cell cycle. The cyclindependent kinases, the main actors of cell cycle control system, operate the cell cycle through phosphorylation of many different target proteins. Recently in addition to cyclindependent kinases the role of some other kinases (e.g. Polo, Aurora etc.) was found. The phases of cell cycle are not interchangeable, they have to follow each other in a strict order. Operation of checkpoints in the cell cycle ensures to give rise to genetically identical cells by cell cycle (Figure 2-1)

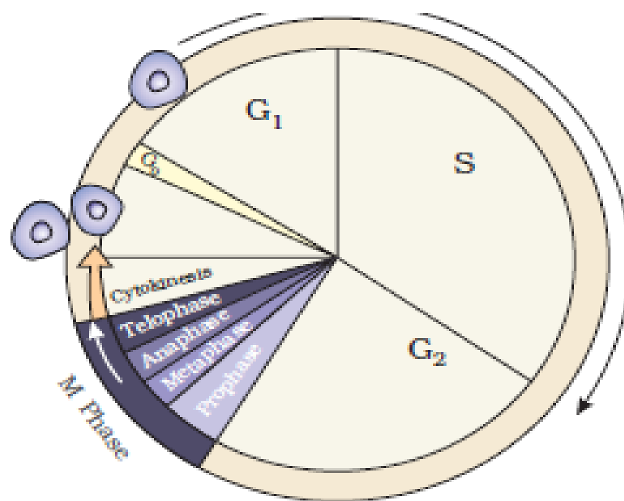


Figure 2-1A diagrammatic view of cell cycle indicating formation of two cells from one cell

cycle control system allows to overstep checkpoints if the conditions are suitable for the cell to proceed to the next phase .The main checkpoints are the following: G1 checkpoint (in higher eukaryotes it is referred to as restriction point), where first of all the integrity of DNA is checked, operates at the end of the G1 phase. The second checkpoint is at the end of G2 phase, it is the G2 checkpoint, where the accuracy and integrity of DNA is monitored. Finally, the function of M checkpoint,

in the metaphase of mitosis is to ensure the appropriate attachment of all chromosomes to the microtubules of the mitotic spindle before the duplicated chromosomes are separated. And now let us see a brief summary of multicellular (mammalian) cell cycle and the regulation.

2-2 M-phase

The M-phase is a complex process of successive steps, a series of events, used to be divided into mitosis and cytokinesis. In the first half of the M-phase, in mitosis the doubled DNA divides in two, followed by the separation of cytoplasm, by the phase of cytokinesis .

In mitosis the following phases are distinguished:

1-Prophase.

In the nucleus the nuclear chromatin gradually changes to chromosomes by the maximal condensation of DNA. Since before the M-phase the DNA has been replicated, each chromosome comprises two chromatids (sister chromatids). In the cytoplasm the centrosome, which also has been doubled in interphase, splits into two and move to opposite poles of the cell, and organize the mitotic spindle composed of microtubules.

2-Prometaphase.

Nucleolus disappears, the chromosome development continues. The nuclear membrane disintegrates, too. Kinetochore microtubules binding kinetochore protein complex associate to the centromere region of each chromatid. Metaphase. The chromosomes are arranged in the equatorial plane of the cell by the help of kinetochore microtubules. Kinetochore regions face the two poles of the cell and the kinetochore microtubules bind to sister chromatids of a chromosome from opposite direction.

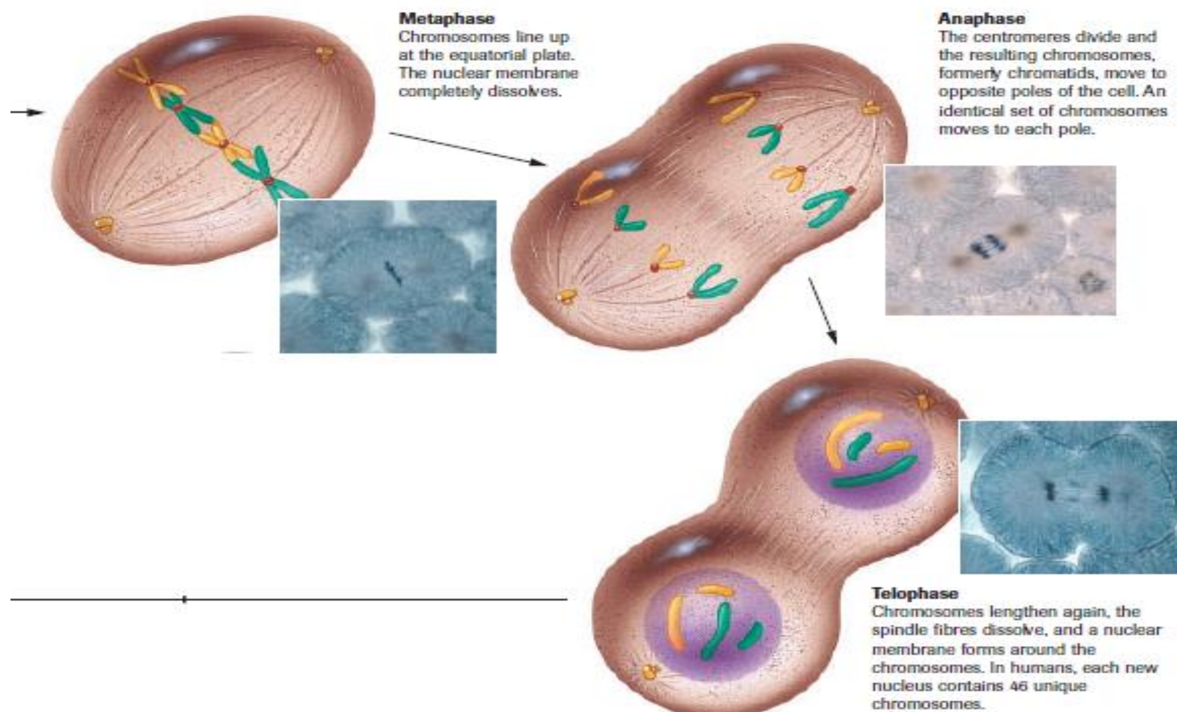
3-Anaphase.

Sister chromatids of chromosomes split and move toward the poles of the cell. In the first half of anaphase (anaphase A) the kinetochore, later in the second half of

anaphase (anaphase B) the polar microtubules operate. It is the shortest phase of mitosis.

4-Telophase.

Kinetochore microtubules disappear, nuclear membrane is reorganized around the chromatids at the cell poles. Chromosomes decondense, they become chromatin. Nucleoli are reformed. Polar microtubules lengthen further the cell. The mitosis, the division of nuclear content is followed by **Cytokinesis**. The separation of the cytoplasm begins in the late anaphase and is completed after the telophase. In the middle of the cell, perpendicular to the axis of the mitotic spindle cleavage furrow appears which gradually deepens and thus the connection between the two half cells narrows. The overlapping region of polar microtubules makes so-called midbody. Finally, the cytoplasm completely splits.



The Stages of Mitosis

2.3. Meiosis

There are two forms of genetic information transmission from one generation to the next one. Firstly asexual reproduction, which is typical for the lower organisms evolved. It is a simple process, the offspring develop from the somatic cells of a single parent, thus they are genetically identical to the parent organism. In sexual reproduction the offspring have mixed genome of two parents, so they are genetically different from both parents and from each other. Sexual reproduction has a great evolutionary advantage for the species, because the individuals gain high genetic variability allowing the adaptation to the unexpected circumstances. Sexual reproduction is crucial for the survival of species. In sexually reproducing organisms, there are two successive generations of cells: the diploid somatic cells give rise to haploid cell by meiosis and the haploid cells, which are reduced to gametes in animals. The speciesspecific chromosome number is restored by the fusion of gametes resulting diploid zygote, and the life of a new individual starts.

3.1. Phases of meiosis

In meiosis there are two successive divisions: meiosis I and meiosis II. Similarly to mitosis, in meiosis I prophase the chromosomes are condensed, the nucleolus and the nuclear envelope disappear. The main event of this phase is the homologous recombination, the exchange of sequences of paired homologous chromosomes (maternal and paternal chromosomes of the same size and shape, and having the same genes). It is the longest phase of meiosis which is divided **into five substages: leptotene, zygotene, pachytene, diplotene and diakinesis.** In leptotene the two sister chromatids (having identical DNA due to duplication in S-phase) containing chromosomes are very thin fiberlike structures, which randomly bind by their both ends to the nuclear envelope. Later they move to a distinct point of nuclear envelope, close to centrosome, forming a bouquet-like structure. Thus the homologous chromosomes are close to each other, which is necessary for the next stage process. In zygotene the pairing, also known as synapse, of homologous chromosomes begins. Recent studies have shown that even before the pairing, probably in early leptotene the double stranded DNA-s break at several hundred sites. The pairing of homologous chromosomes is helped by a ladder-like protein structure, by the synaptonemal complex. It has lateral, transversal filaments, the overlapping transversal filaments form the central region of the structure .

Due to DNA condensation, chromosomes become thicker and more visible and the synapses are completed in **pachytene.** After pairing they form structures composed of two chromosomes, maternal and paternal one (bivalent), both having two **sister chromatids (tetrad).** The tight binding between the homologous chromosomes leads to apparent decrease in number of chromosomes (pseudoreduction). The majority of double-stranded DNA breaks are repaired, but at some of them homologous recombination **(crossing-over),** exchange of corresponding chromatids occur. This process is mediated by the recombination nodules, large 100 nm sized multi-enzyme complexes, which appear on the synaptonemal complex. The detailed molecular mechanism of crossing over is not discussed here. The crossing over may occur between any chromatids, but it results new combination of genes if it happens between non-sisters. The number of crossing overs between non-sister chromatids of a chromosome pair is 1-3. There is compulsory recombination even between the basically not homologous X and Y chromosomes at their pseudoautosomal regions (PAR). Checkpoint machinery controls the appearance and the process of crossing over, underlining the significance of homologous recombination. **In diplotene** stage the **synaptonemal** complex largely detaches, thus the members of homologous pairs may slightly move away from each other, so the chromosomes are linked only at the sites of crossing overs, referred as **chiazmata.** Finally, in diakinesis the homologous separation continues, but the bivalents are still connected at chiazmata, found between sister chromatids of homologous chromosomes, and also by aploids which held together sister chromatids of a chromosome. Later the aploids dissociate from the arms and keep the chromatids together only at centromeric regions. During the prophase kinetochore region develops on chromosomes, but in contrast to mitosis, both kinetochores of a chromosome face one pole, while the kinetochore of the homologous face opposite poles (Figure 3).

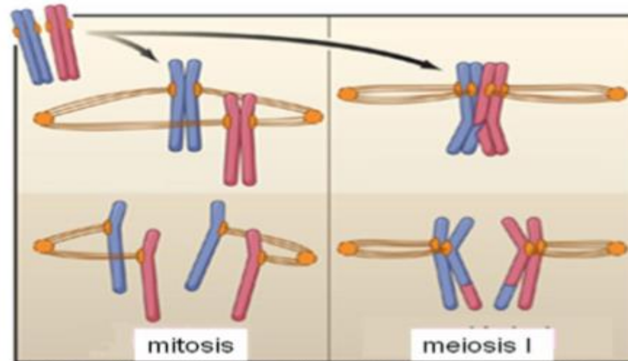


Figure 3 Kinetochor orientation in mitosis and meiosis I Source : http ://www.sciencedirect.com/science/article/pii/S0092867406011524 ; 20/02/2013.

- **In first division** metaphase not the single chromosomes, but the chromosome pairs are arranged in the equatorial plane, whereas the chiazmata still connect the homologs. Chiazmata only disappear at the end of metaphase. - **In the anaphase** the kinetochore microtubules pull the homologous chromosomes and not the chromatids toward the poles, since the kinetochores of a chromosome face the same pole. Thus the synapses not only allow the cross-over, but also needed to halve the number of chromosomes. The separation of homologous, which member of a pair is pulled to a given pole is a random process. It increases further the genetic variation. In human it is 2^{23}. **In telophase** the nuclear membrane is reorganized, and the cytoplasm splits. Arising cells are haploid, that is why the first division of meiosis is called reduction division. The chromosomes are still composed of two sister chromatids, which will separate in the following part, in meiosis II. The first division is followed by a short interphase, in which there is **no DNA replication**.

Second division of meiosis is also divided into pro-, meta-, ana- and telophase, but these phases are essentially very similar to the phases of mitosis. Thus, in metaphase the single chromosomes are arranged in the equatorial plane, and in the anaphase the sister chromatids of the chromosomes are separated. The orientation of kinetochores is also similar to mitosis.

In the telophase the nuclear envelopes are reorganized, two nuclei are formed and then the cytoplasms are also halved .

Finally the meiosis results from a diploid cell four haploid cells, the gametes. After the fusion of two haploid cells, in the zygote chromosome number of the species is reconstituted. At the same time the genetic information of the gametes is different caused by the homologous recombination in meiosis I prophase and the random assortment of homologous in meiosis I anaphase. These processes provide high genetic variability needed for the survival of the species .

The most frequent abnormality of meiosis is the **non-disjunction** (Figure 4) either in meiosis I or II. Obviously non-disjunction of both the homologous chromosomes (in the first division), and the sister chromatids (in the second division) alters the chromosome number of resulting gametes. Involvement of such gametes in fertilization may lead to so called **aneuploid genome mutation** .

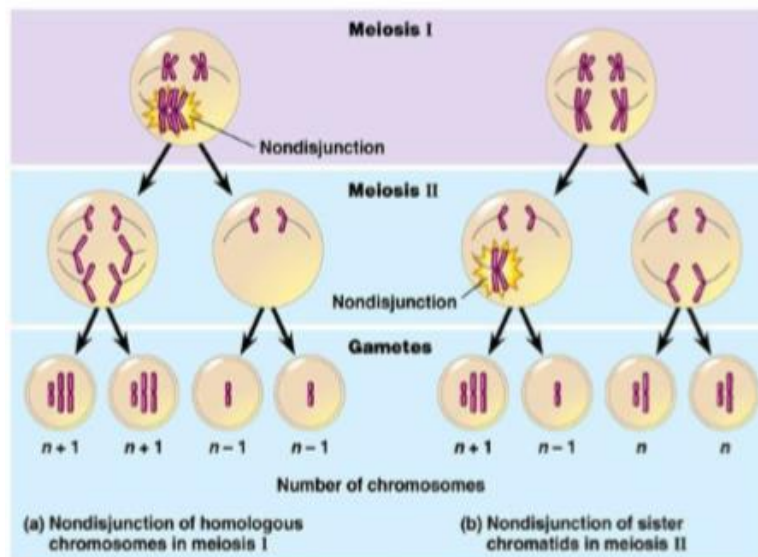


Figure 3Meiotic non-disjunction Source : http ://drugline.org/img/term/meiotic-nondisjunction-9351_1.jpg ; .20/02/2013

In vertebrates the gamete formation is a complex process, the meiosis is only a part of it. At the very beginning of ontogeny primordial germ cells migrate to the developing gonads. Several mitotic divisions are followed by meiosis, and finally in male gametogenesis a differentiation step gives rise to mature gametes.

1. What is the average cell cycle span for a mammalian cell?
2. Can there be mitosis without DNA replication in ‘S’ phase?

3. Can there be DNA replication without cell division?

4- Analyze the events during every stage of cell cycle and notice how the following two parameters change
(i) number of chromosomes (N) per cell
(ii) amount of DNA content (C) per cell
5 Name the stage of cell cycle at which one of the following events occur:
(i) Chromosomes are moved to spindle equator.
(ii) Centromere splits and chromatids separate.
(iii) Pairing between homologous chromosomes takes place.
(iv) Crossing over between homologous chromosomes takes place.

4-The chromosomes
History -structure number karyotyping

The German scientists Schleiden,[4] Virchow and Bütschli were among the first scientists who first recognized the structures now familiar as chromosomes.

In a series of experiments beginning in the mid-1880s, Theodor Boveri gave the definitive demonstration that chromosomes are the vectors of heredity; his two principles or postulates were the *continuity* of chromosomes and the *individuality* of chromosomes. It is the second of these principles that was so original.] Wilhelm Roux suggested that each chromosome carries a different genetic configuration , and Boveri was able to test and confirm this hypothesis. Aided by the rediscovery at the start of the 1900s of Gregor Mendel's earlier work, Boveri was able to point out the connection between the rules of inheritance and the behaviour of the chromosomes. Boveri influenced two generations of American cytologists: Edmund Beecher Wilson, Nettie Stevens, Walter Sutton and Theophilus Painter were all influenced by Boveri (Wilson, Stevens, and Painter actually worked with him). In his famous textbook *The Cell in Development and Heredity*, Wilson linked together the independent work of Boveri and Sutton (both around 1902) by naming the chromosome theory of inheritance the Boveri–Sutton chromosome theory (the names are sometimes reversed). Ernst Mayr remarks that the theory was hotly contested by some famous geneticists: William Bateson, Wilhelm Johannsen, Richard Goldschmidt and T.H. Morgan, all of a rather dogmatic turn of mind. Eventually, complete proof came from chromosome maps in Morgan's own lab. The number of human chromosomes was published in 1923 by Theophilus Painter. By inspection through the microscope, he counted 24 pairs, which would mean 48 chromosomes. His error was copied by others and it was not until 1956 that the true number, 46, was determined by Indonesia-born cytogeneticist Joe Hin Tjio.

4-1 Chromosome structure

In M-phase the long eukaryotic DNA molecules have to be packed in small chromosomes to be able to accurately halve without breaks. Meanwhile, the original length of the DNA (several cm) is reduced by ten thousands fold (few μm). The molecular mechanism of this packaging is still not known in detail. The major points of a widely accepted model are described below (Figure4).

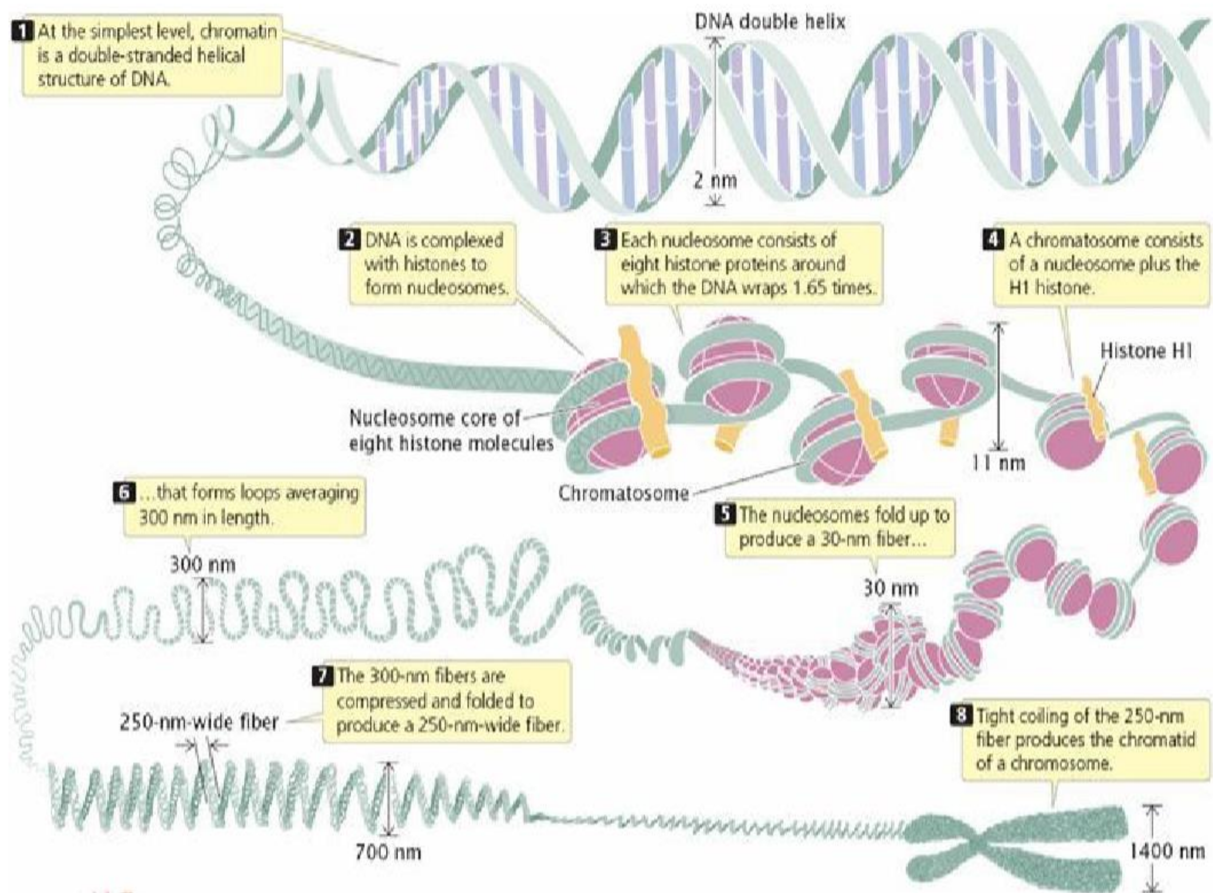


Figure 4. From the DNA to the chromosome Source: http://www.nature.com/scitable/topicpage/eukaryotic-genome-complexity437 ; 20/02/2013.

Two nm wide DNA double helix wraps the octamers of histones (2 of each H2A, H2B, H3 and H4 histone molecules) forming nucleosomes, disc-like structures connected by the continuous DNA molecule. It is called nucleosomal structure having a diameter of 11 nm. H1 histone folds six nucleosomes in one plane to give

a diameter of 30 nm fiber called chromatin or solenoid. The chromatin fiber is attached to a protein scaffold and forms loops. These loops are the basic unit of replication and transcription, and this structure is 300 nm wide. Finally, it is further compressed and folded to produce the chromatids of 1400 nm wide metaphase chromosome (Figure 4). The final step of chromosome condensation is induced by the MPF activated condensins. There are two protein complexes of similar structure influencing different DNA functions: the condensins and the cohesins. They are composed of different SMC (structural maintenance of chromosomes) proteins having ATPase activity and regulatory functions, all associate in a ring-like structure (Figure 5)

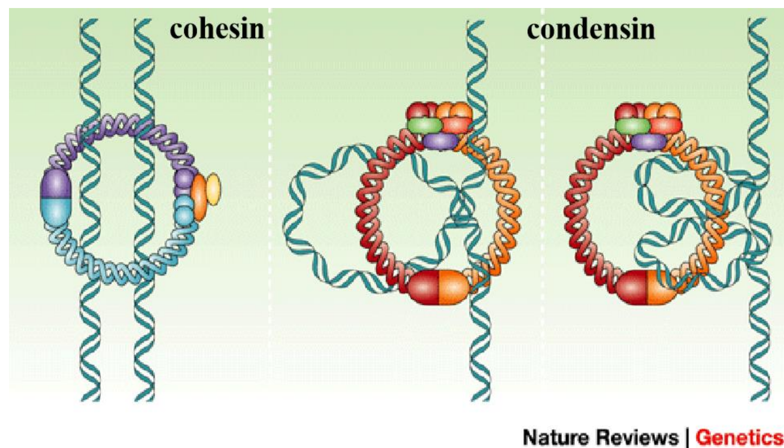


Figure 5 Structure of cohesin and condensin Source: http://www.nature.com/nrg/journal/v4/n7/box/nrg1110_BX3.html ; 19/02/2013..

Metaphase chromosome has very characteristic morphological structure. As the DNA is doubled in the S phase, chromosome comprises two sister chromatids. After DNA synthesis, the DNA molecules are held together by the ring-like cohesin complexes. Much of this cohesins detaches during the prophase, and at the end of metaphase it is found only at the primary constriction of chromosomes specified as centromere region. This pericentromeric cohesin is cleaved in early anaphase allowing the separation of chromatids. Chromosomes are usually classified according to the location of the centromere region. During prophase and prometaphase a special three-layer plate of protein structure called kinetochore associates to the centromeres of chromosomes. Beside many other proteins kinetochore contains both dynein and kinesintype motor proteins, and the role of it is to bind kinetochore microtubules (about 30– 40/sister chromatids). In

scleroderma which is an autoimmune disease, patients produce antibodies against some of the kinetochore proteins. The centromere divides the sister chromatids into two arms, the ends of the arms are called telomeres. Loss of telomeres makes the chromosomes instable (Figure 6).

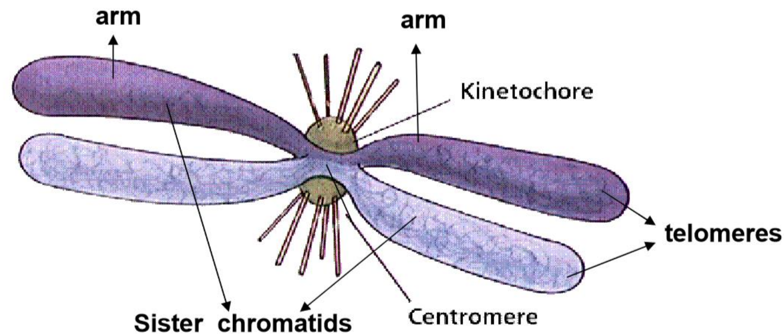


Figure 6. Eukaryotic chromosome Source: http://www.emc.maricopa.edu/faculty/farabee/biobk/biobookmito.html; 20/02/2013

6- Chromosomal abnormal

Cytogenetics is a field of genetics dealing with species or cell specific number of chromosomes, and their structure and characteristic segments, their functional roles, and all the differences - namely the chromosomal mutations - related to them. Chromosome mutations are changes in the structure or in the number of chromosomes, and since they are relatively rare in this respect they differ from normally occurring common, harmless chromosome polymorphisms. Since both types of chromosome aberrations affecting many genes, and since the size of chromosomes or their affected segments are within the limits of microscopic resolution therefore they can be examined by light microscope, as opposed to gene mutations only be identified by molecular biological techniques. However, the application of modern hybridization based (FISH and CGH) techniques allow the identification of small structural changes (e.g. microdeletions or CNVs) previously unrecognized by light microscope.

Two aspects of the chromosomal abnormalities are regarded crucial: when and where they happen. While chromosome mutations may be formed during both mitosis and meiosis, those may occur in meiosis, lead to defective gamete formation, and to the birth of affected offspring. Thus their medical significance is greater than that of mitotic chromosome aberrations. From the point of mitotic chromosomal abnormalities it is also important when during development and in what kind of cell they are formed. Mutations occurred during the early cleavage divisions may have serious consequences for the entire organism, while aberrations occurred in a continuously proliferating cell type (e.g. epithelial cells) in adulthood may have negligible role. However, certain chromosomal mutations may have a role in the formation and subsequent rapid proliferation of tumor cells.

Two chromosomal regions have special importance in the formation of chromosome aberrations: the **centromeres** and the **telomeres**

Other classification of chromosomal abnormalities depends on fact which type of chromosome is affected – autosomes (down syndrome, digeorge syndrome) or sex chromosomes (klinefelter syndrome, turner syndrome).

61. Structural chromosome aberrations

The prerequisite of structural chromosome aberrations is breakage of chromosome/s
which can be spontaneous or induced. The classification of structural aberrations is based on the number and the location of breaks within chromosomes (Figure 7).

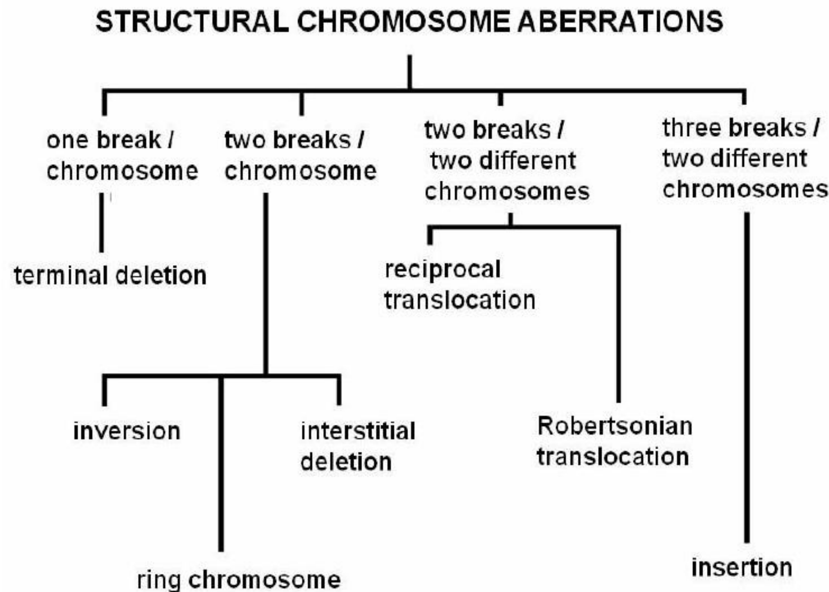


Figure 7 The classification of structural chromosome aberrations

6-1. Deletions

If a chromosome is broken, and the broken piece lost, we are talking about deletion. Then the genetic information carried by the broken piece will be absent from the cell involved, whereupon the cell does not function normally or die. Since the deletions eliminate certain functions therefore certain proteins for example enzymes are not produced. By the help of deletions the location of the gene eliminated can be mapped - it was one of the earliest methods of gene mapping, the ***deletion mapping***

If the break is close to the end of the chromosome, a ***terminal deletion*** is generated. In this case, in addition to other genes telomere is lost, too and this also contributes to the severity of symptoms, to early lethality. The best known example of a terminal deletion is the ***cat cry (cri du chat) syndrome***: the short arm of chromosome 5 is deleted (5p-). The disease is named after the affected newborns characteristic mewing cry.

There are two breaks within one chromosome in the case of ***interstitial deletion***, and the intermediate piece is lost. Such lesions usually may cause severe physical and mental disabilities, spontaneous abortion, premature death depending on the chromosome involved. The best known interstitial deletion affects the long arm of chromosome 15: del15 (q11-13). This is one of the causes of Prader-Willi or Angelman syndrome (see Chapter 5, Epigenetics and genomic imprinting). In the former case paternal deletion, in the latter one maternal deletion is found. Also interstitial, but small, so-called ***microdeletion***s are in background of Williams and DiGeorge syndromes (del7q11.23 and del22q11.2) as well.

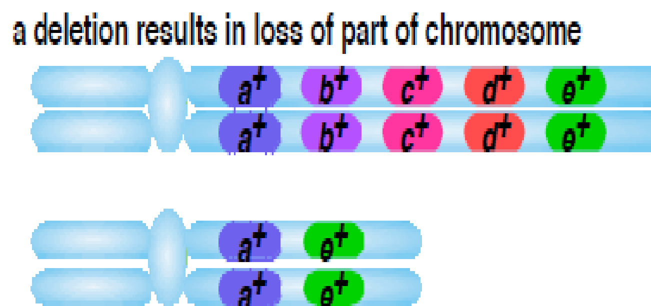


Figure deletion chromosome.

6.2. Duplications

During duplication a chromosomal segment is duplicated. It's either a replication error or due to meiotic unequal crossing over **Example - Fragile X**: the most common form of mental retardation. The X chromosome of some people is unusually fragile at one tip - seen "hanging by a thread" under a microscope. Most people have 29 "repeats" at this end of their X-chromosome, those with Fragile X have over **700** repeats due to duplications. Affects 1:1500 males, 1:2500 females.Like deletions, duplications are also used to identify the chromosomal location of a gene or group of genes, so to map a gene.

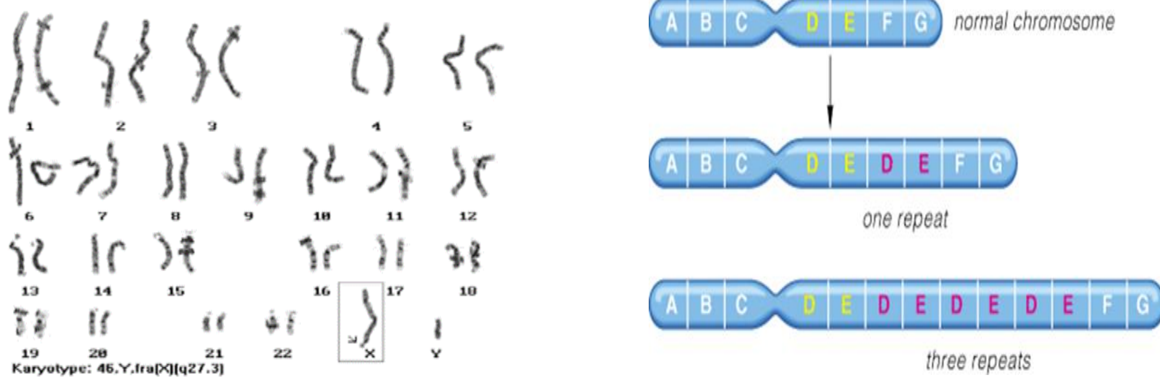


Figure. duplication chromosome

6.3. Translocations

During translocation, a part of one chromosome is transferred to another chromosome. It is very important whether the translocation is **balanced** or **unbalanced**. Balanced means that two chromosomes just exchange their parts but the number of chromosomes (46 chromosomes) as well as no loss of genetic material stays the same2,3. Fortunately – typical place of break is near the centromere, usually only small arms of the acrocentric chromosomes are lost. There are no crucially important genes coded by these chromosomal segments. So, a carrier of such robertsonian translocation can normally survive this cytogenetic change. Unfortunately problems occur during the fertilization with a gamete of a carrier of a balanced translocation. Carrier of the translocation may produce unbalanced zygotes, because the process s of homologous chromosomes pairing during meiosis is interrupted. This is very important because unbalanced gametes lead to abnormalities in offspring. The reason is that the offspring receives altered chromosome from the carrier which may lack several important genes. Therefore the only clinical symptom found in the carriers of balanced translocations may be the reproduction failure.

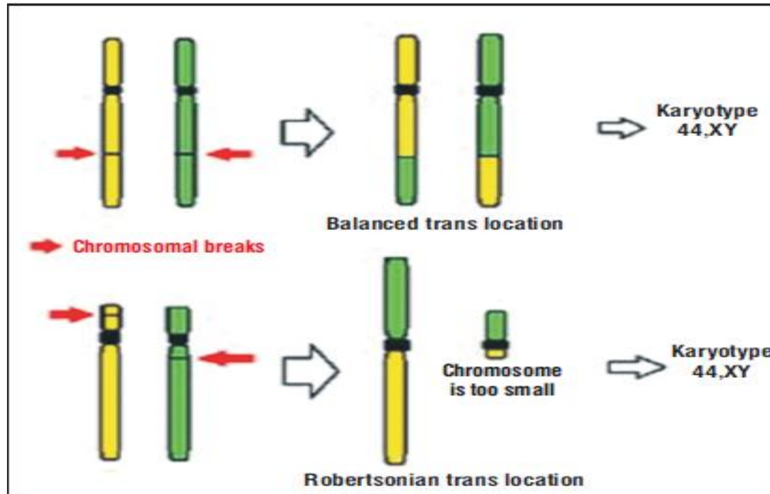


Figure. translocation chromosome

Types of translocation

A-Reciprocal – translocation between two chromosomes ("a segment" goes to "b chromosome" and "b segment" goes to "a chromosome")

B-Robertsonian – translocation (or fusion) of two acrocentric chromosomes

The best example of reciprocal translocations leading to the formation of the ***Philadelphia chromosome (Ph1)***, is between 9 and 22 chromosomes, its cytogenetic abbreviation is t(9;22)(q34;11). This translocation occurs in ***chronic myeloid (CML)*** or ***acute lymphocytic leukemia (ALL)***. The breakpoint in chromosome 22 is in the BCR (breakpoint cluster region) gene, while the breakpoint of chromosome 9 affects in the ABL (Abelson murine leukemia) proto-oncogene. Since the ABL gene encodes a tyrosine kinase as the result of the translocation a bcr / abl fusion protein is produced which not only has a greater molecular weight than the original enzyme, but also a higher activity. In fact, during this translocation the well-regulated promoter of ABL gene is lost, and the gene permanently overexpressed. Finally this leads to uncontrolled cell proliferation, i.e. the development of the tumor.

Another medically important example is the Burkitt's lymphoma caused mostly by Epstein-Barr virus. In this disease the c-myc proto-oncogene coded by chromosome 8 is translocated to chromosome 14 or 2 or 22. These two cases are examples of the relationship between translocations and proto-oncogenes, where the overexpression of a normal protein (Burkitt's lymphoma), or a fusion protein -

although of normal function - regulation of independent production (CML) is responsible for tumor formation.

6.4. Inversions

For inversion are typical two breakages in the different part of the chromosome. The newly created segments then replace each other. Inversion was discovered in 1921. Although we still don´t know why inversion exists, we know that it is the most important mechanism of reorganizing of the genome.

There are 2 types of inversion:

A-Pericentric – the chromosome breakages are on both arms, that is on both sides of the centromere. The pericentric inversion of chromosome 9 is relatively common and found frequently in couples with recurrent abortions.

B- Paracentric – breakpoints are on the same arm of the chromosome, thus in the turn of the fragment the centromere is not involved more common type, it is less harmful for its carrier. Inversion suppresses the recombination process.

6.5. Ring (ring) chromosome

In this case, there are breaks on both arms of the chromosome - usually near the telomeres - then broken ends fold and a ring chromosome is formed. The fragments broken are lost during successive divisions,

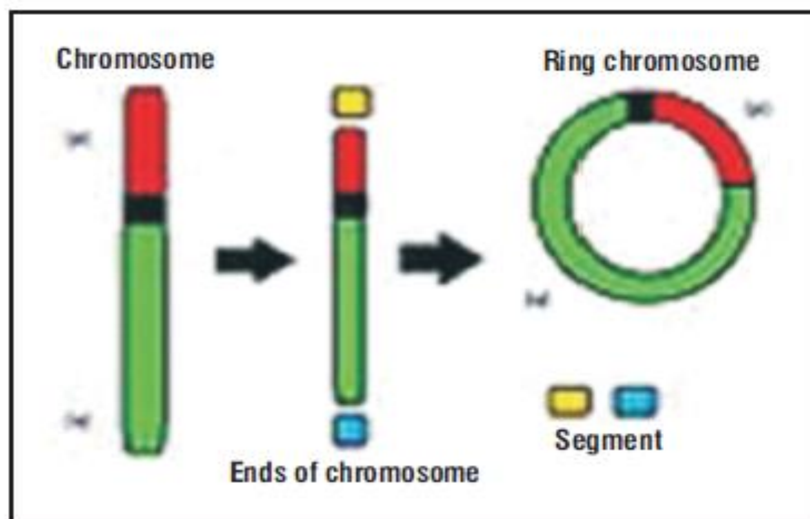


Figure ring chromosome

6.6. Isochromosome

Isochromosomes are created by the incorrect division of centromere. Normally centromere divides vertically. In this case it divides horizontally. The result is usually the loss of one arm. It means that newly created chromosome has just two long arms or two short arms which are normally connected by centromere. It occurs relatively frequently in X chromosome. It is a huge problem during the fertilization. Because fetus then becomes trisomic for one arm and monosomic for the second arm. Thus aberrant chromosomes ultimately cells containing them are formed which contain either the short arm or the long arm specific information only on both arms and the information of the other arm is lost.

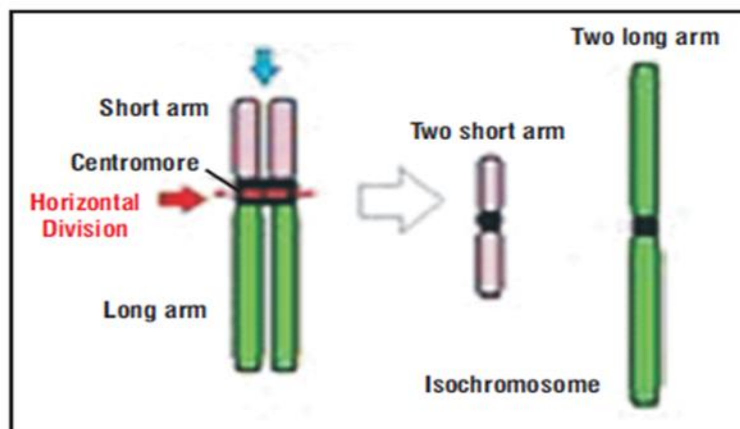


Figure 6.6 Isochromosome chromosome

6.7. Acentric fragment

More rarely broken fragment(s) without a centromere remain in the cytoplasm as small fragments. Due to the absence of centromere such pieces cannot migrate to cell poles and either a so called micronucleus is formed or they are during the subsequent cell divisions, and finally only the deleted chromosomes has retained within the cell. Since these acentric fragments are most commonly induced by some chromosome breakage causing mutagenic agents such as radiation, therefore thy can be used for testing the mutagenic effects (micronucleus test).

7- Numerical chromosome aberrations

are defined as a gain or loss of one or more whole chromosome(s) (whether an autosome or a sex chromosome) or a whole set of chromosomes. The normal

chromosome count is 46 (i.e. 2n = 46) as it is arranged in two sets of chromosomes. The numerical abnomalies, when one or more chromosomes are in excess or missing, ultimately modify the entire genome size, so they can be considered genome mutations as well.

There are three types of numerical chromosome aberrations:

1 / euploid

2 / aneuploid

3 / mixoploid mutations

Nondisjunction - Mistake in cell division where chromosomes do not separate properly in anaphase. Usually in meiosis, although in mitosis occasionally. In meiosis, can occur in anaphase I or II. Polyploidy – complete extra sets (3n, etc.) – fatal in humans, most animals. Aneuploidy – missing one copy or have an extra copy of a single chromosome. Three copies of a chromosome in your somatic cells: TRISOMY. One copy of a chromosome in your somatic cells: Monosomy Most trisomies and monosomies are lethal well before birth in humans. Generally, autosomal aneuploids tend to be spontaneously aborted. Over 1/5 of human pregnancies are lost spontaneously after implantation. Chromosomal abnormalities are the leading known cause of pregnancy loss. Data indicate that minimum 10-15% of conceptions have a chromosomal abnormality. At least 95% of these conceptions spontaneously abort (often without being noticed

The most common numerical chromosomal abnormalities

7-1 chromosome disorder autosomes :- the majority of human chromosomal abnormalities occur in autosomes .There are three trisomies that results in baby which can survive for time after birth ; the other are too devastating and baby usually dies in utero :-

A-Down syndrome (Trisomy 21)

Trisomy 21 is the cause of ***Down syndrome***. Although the non-disjunction of chromosome 21 is not the only cause of Down syndrome -a smaller proportion of the cases is due to either centric fusion or translocation - it is the most common

type. Despite the fact that trisomy 21 fetuses die in utero the average population frequency of Down syndrome is 1:650, but this value increases dramatically with maternal age, at 45 years of age it is more than 1:100!

B-*Patau syndrome*(*Trisomy 13)*

Trisomy 13 is the ***Patau syndrome***. Similar to Down syndrome it is most commonly derived from maternal non-disjunction. 65% of such non-disjunctions derived from the first meiotic division. Frequency of birth is 1:12 500 - 1:21 700. Only <5% of these infants survive the first year of life.

c-*Edwards syndrome*.(*Trisomy 18)*

18 is the ***Edwards syndrome***. It is primarily due to maternal non-disjunction. 95%! of the cases are due to non-disjunction in the first meiotic division. The frequency is 1:6000 -1:10000 live-born but the frequency at the time of conception can be much higher, since approx. 95% of the fetuses die within the womb. 30% of the Edwards syndromic abnormal newborns die within one month, > 95% of them die within a year.

7-2 chromosome disorder of sex chromosome

A- ***Turner syndrome***

Short stature; sterile (immature sex organs); often reduced mental abilities. About 1 in 2500 human female births .The ***Turner syndrome*** is characterized by ***45,X0 karyotype***. ***This is the only viable monosomy.*** The explanation for this lies in the fact that while both homologues of the autosomes are necessary to the normal phenotype - so their monosomy is lethal – by contrast in females only one X chromosome is active (see the dose compensation in X inactivation), so a functional monosomy and Barr body negativity can be maintained. However, for the normal development of female sex characteristics both X chromosome is needed, as indicated by the symptoms of Turner syndrome.

While it is easy to understand the sexual development related characteristics of the syndrome, the low height is still not fully explained. It is assumed that a gene coding a protein of the small ribosome subunit (*RPS4X*) may also play a role. Because this gene has a Y chromosomal counterpart (*RPS4Y*) as well, both in normal females and males double dose of this ribosomal protein is produced. In

Turner syndromic individuals less than sufficient 86 amount is produced, and if the ribosome number is less than normal it will largely influence the production of other proteins, and thus indirectly the body height, too. Although Turner syndrome is often characterized by normal intelligence there is a difference in verbal skills, social integration between patients inherited their X chromosome from father or the mother. Maternal X carriers, according to surveys are weaker in these features than the patients inherited paternal X. The phenomenon is explained by the different methylation of the two types X chromosome and the genomic imprinting.

B- XXY male (Klinefelter syndrome)

Often not detected until puberty, when female body characteristics develop.
Sterile; sometimes reduced mental abilities; testosterone shots can be used as a partial treatment; About 1 in 500human male births.The frequency is 1:1000.
Nearly it is derived with the same probability from maternal (56%) and paternal (44%) non-disjunction. 36% of the maternal non-disjunctions take place in the first meiotic division. Since there are two X chromosomes, thus they are Barr body positive. Their sterility can also be attributed to presence of 2 X chromosomes, since certain X chromosome a gene products are in a higher dose than in normal fertile males.

c- XXY male (XYY syndrome) ***"superman" or Jacobs syndrome***

In this case ***normal, slightly taller than the average males have 47,XYY karyotype***. The birth rate is 1:1000. They derived only from paternal second meiotic non-disjunction. In contrast to all meiotic non-disjunctions, ***the formation is not affected by age***, as paternal gametogenesis is continuous from puberty, there are no aged sperms. Usually tall, with heavy acne; some correlation with mild mental retardation and with aggressiveness; usually still fertile. About 1 in 1000 human male births.

Knowing the characteristics of meiotic division we could ask that the two aneuploidies (47,XXX and 47,XYY) with normal fertility are characterized by greater prevalence of similar disorders among offspring or not. For example, in the case of double Y syndrome the following karyotypes offspring are expected in the offspring: 2 XXY, 2 XY, 1 XX and 1 XYY. In contrast, birth of only normal offspring was reported so far, however its exact explanation is still not known.

D- XXX female (triple X syndrome)

Feminine phenotype and 47, XXX karyotype are present. 89% is of maternal, 8% is of paternal origin, and the remaining 3% is due to post-fertilization mitotic non-disjunction. Neonatal frequency is 1:1000. Two Barr bodies are typical. HOWEVER, more likely to be sterile, and if fertile, more likely to have XXY and XXX children. About 1 in 1000 human female births. Aneuploidy in human autosomes.

7-3 Mixoploid mutations

In mixoploidy or in mutations associated with mixed ploidity usually two (sometimes more) cell lines with different chromosome numbers are found within an organism. There are two forms: ***mosaicism and chimerism***

In genetics a mosaic is a living creature, where two cell lines of different chromosome numbers, but of the same origin are present in the body. They are either ***aneuploid or polyploid mosaics***.

In the case of ***gonadal mosaicism*** only the cells in the germ line have abnormal chromosome number, thus the risk of numerical aberrations in the offspring is high. Unfortunately, the detection of such defects is still not possible routinely, but the birth of an abnormal offspring of the patient can indicate this. Mosaicism in a broader sense is a somatic mutation, when different mutants (alleles) of a given gene are located in different organs or in different cells of the same organ (for example eyes with different colors: one is blue and the other is brown or a blue eye with brown spots).

After the lion-headed, bird-legged, snake-tailed monster of Greek mythology the creature that has two cell lines of different origin - derived from different zygotes - is called ***chimera***. A chimera is derived either from fusion of fraternal twins, or from double fertilization of an egg and a polar body (polocyte), or from transplacental haematopoietic stem cells exchange between fraternal twins (blood group chimerism). Recently, the chimera referred to as transgenic animals / plants, which contain cells of different origin, derived from either the fusion of few-cell-embryos, or via the microinjection of foreign genes into fertilized oocytes.

7-4. Uniparental disomy (UPD)

This abnormality which is not or hardly identifiable by cytogenetic methods were recognized - due to molecular biological techniques - in the past decades. The UPD means that the person concerned has a normal chromosome number, but the homologues of a certain chromosome – in contrast to normal - are from the same parent, either from the father or from the mother. As for the formation two consecutive numerical aberrations are in the background: a meiotic non-disjunction and an anaphase lag occurring during the early cleavage divisions. So in fact a trisomic zygote is formed first, and subsequently the 3 homologue is lost. Depending on whether first or second meiotic non-disjunction occurred, uniparental heterodisomy or uniparental isodisomy is present. The first case is when the child inherits two different homologues from the parent (one grandmaternal and one grandpaternal), that is non-disjunction occurred in the first meiosis. The latter is when the two homologues inherited are the same (either both are grandmaternal or grandpaternal) suggesting a second meiotic non-disjunction. In UPD depending on the parental origin of the homologues, and due to genomic imprinting, different symptoms may be seen. The different symptoms in some of the Prader-Willi and Angelman syndrome cases are not due to the 15q deletion, but the UDP.

Questions

1. What are the causes of aneuploidy and polyploidy?

2. What are the main regions of chromosomes?

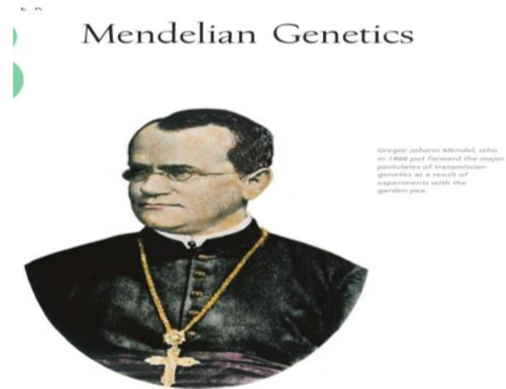
4. In what diseases has UPD an etiologic role?

5. What are the different positions of chromosomal breakpoints?

6. What are chimerism and mosaicism?

-7What techniques are used for the detection of chromosomal aberrations?

8- Mendelian inheritance



The founder of the modern science of genetics was Johann Gregor Mendel. Mendel's pea plant experiments established many of the rules of heredity, now referred to as the laws of Mendelian inheritance. Below Mendel's Laws are summarized .

Law of Segregation (the "First Law") states that the two alleles for a heritable character segregate (separate from each other) during gamete formation and end up in different gametes.

Law of Independent Assortment (the "Second Law"), also known as "Inheritance Law", states that separate genes for separate traits are passed independently of one another from parents to offspring.

Law of Dominance (the "Third Law") states that recessive alleles will always be masked by dominant alleles. Therefore, a cross between a homozygous dominant and a homozygous recessive will always express the dominant phenotype, while still having a heterozygous genotype.

A Mendelian trait is one that is controlled by a single locus in an inheritance pattern. In such cases, a mutation in a single gene can cause a disease that is inherited according to Mendel's laws. These diseases are called monogenic diseases.

Mendelian inheritance is the basis of classical genetics. Although our knowledge about classical genetics has significantly expanded lately, the understanding of the heredity of the human diseases / traits can still be related to Mendelian inheritance. Those patterns of inheritance are considered Mendelian in a simplified way, which fulfill two criteria: on the one hand Mendel's principles can be applied, on the other hand the environment has no influence on them. The classical classification of the hereditary patterns is the following: autosomal dominant and recessive, codominant, X-linked dominant and recessive, and Y-linked.

The genetics of human traits is difficult to study, as it is not possible to make backcrosses, and the time lag between the generations is much longer than in the case of classical models, such as bacteria, yeast cells, drosophila, mice and rats. Additionally, the size of the families is significantly smaller than in classical models.

Interpretation of some basic genetic terms

Gene: a DNA sequence coding for one or more functional product(s). These can be proteins via mRNA or can be any other kind of RNA, like t tRNA, rRNA, snRNA, miRNA, siRNA, etc. In the so-called RNA-viruses (e.g. influenza, HIV1) genes are coded only in the form of RNA.

Allele: it is an alternative form of a gene (one member of a pair) that is located at a specific position (locus) on a specific chromosome. A diploid cell contains two alleles at a time. Their relationship determines whether the inheritance of a gene-coded trait is dominant or recessive.

- Allelic heterogeneity: different mutant alleles of the same gene result in similar symptoms /diseases. (e. g. mutations of FGFR3 gene)

Dominance / recessivity: the expression „dominance / recessivity" refers to phenotype, not to genotype. A trait is said to be dominant when it is visible even in heterozygous form. In case it is not, it is said to be recessive. These expressions show the relationship between the two alleles of the same gene. If the expression of one of the alleles represses the expression (the phenotypic form) of the other one, the inheritance pattern is dominant.

Modifier genes": genes that influence the expression of another gene. These are interactions between two or more genes of different loci.

Co-dominance: both alleles are manifested phenotypically in heterozygotes. AB0 blood group system is the classical example. Earlier incomplete dominance was also called codominance. Incomplete dominance occurs when the phenotype of the heterozygous phenotype is distinct from and often intermediate to the phenotypes of the homozygous phenotypes. E.g. LDLR mutations in familial hypercholesterolemia

1-Dominant-recessive inheritance

In dominantly inherited diseases only one faulty gene is enough for the manifestation of the disease. Such disease is e.g. familial hypercholesterolemia or Huntington disease. In cases of recessive diseases the faulty gene product is compensated by the normal variant. In this case two mutated homologous genes are required for the manifestation of the disease. Such diseases are e.g. cystic fibrosis or albinism. The codominant inheritance is a variation of the dominant-recessive inheritance. In case of codominant inheritance two different alleles of a gene can be expressed, and each version makes a slightly different protein. Both alleles influence the genetic trait or determine the characteristics of the genetic condition. E.g. blood type AB is inherited in a codominant pattern. Here the A and B blood group is dominant over 0 blood group and show codominant inheritance to each other. It means that if a person has one gene for A blood group, one for 0 blood group then his/her blood group will be A, in the case of one A and one B, the blood group will be AB .

.2.**Polygenic inheritance** In most cases, however, a trait or feature is determined more than one allele pair. Often the genes are large in quantity but small in effect. Examples of human polygenic inheritance are height, skin color, eye color, weight and diseases like diabetes mellitus, high blood pressure, asthma, allergy or atherosclerosis .

3-Genetic pleiotropy

It can also occur that an allele pair is responsible for more than one trait. Here, the product of the gene participates in several metabolic pathways, which have effects on different organs or tissues. In this case mutations in this gene can have different consequences. It is called genetic pleiotropy. A classic example of pleiotropy is the human disease phenylketonuria (PKU). This disease can cause mental retardation and reduced hair and skin pigmentation, and can be caused by any of a large number of mutations in a single gene that codes for the enzyme phenylalanine hydroxylase, which converts the amino acid phenylalanine to tyrosine, another amino acid .

4- **Sex-linked inheritance**

Humans have altogether 46 chromosomes, which consist of 22 pairs of autosomes in both females and males and two sex chromosomes. There are two copies of the X-chromosome in females (homogametic), but males have a single X-chromosome and a Y-chromosome (heterogametic). Genes on the X or Y chromosome are called sex-linked. Since humans have many more genes on the X than the Y, there are many more X-linked traits than Y-linked traits. The gender of the offspring is determined by the sperm. **Cytogenetics** is a field of genetics dealing with species or cell specific number of chromosomes, and their structure and characteristic segments, their functional roles, and all the differences - namely the chromosomal mutations - related to them. With cytogenetic methods (e.g. with chromosome staining) the chromosome X and Y can be easily differentiated. Chromosome X is significantly larger than the Y.

Both chromosomes contain homologous and non-homologous regions. The non-homologous regions contain genes which do not have pairs in the other chromosome. In males these genes are in hemizygotic state. Females possessing one X-linked recessive mutation are considered carriers and will generally not manifest clinical symptoms of the disorder. All males possessing an X-linked recessive mutation will be affected, since males have only a single X-chromosome and therefore have only one copy of X-linked genes. All offspring of a carrier female have a 50% chance of inheriting the mutation if the father does not carry the recessive allele. All female

The Patterns of Genetic Inheritance

<u>1- Mendelian</u>

- Autosomal Dominant ex (Huntington's disease)
- Autosomal Recessive ex :-(Phenylketonuria PKU, congenital adrenal hyperplasia, Cystic fibrosis, Haemoglobinopathies and Xeroderma pigmentosum)
- X-linked Recessive
- X-linked Dominant
- Y-linked

2- **Non-Mendelian**

- Imprinting
- Mitochondrial
- Multifactorial
- Sporadic
- Contiguous gene syndromes

Questions

1- Describe Mendel's principles!
2. Define the following terms! --- gene, allele, , , locus heterogeneity, allele heterogeneity, dominance, recessivity, codominance.

The genetics of sex

A branch of developmental genetics is dealing with the sex determination and sex differentiation, and with all the genetic process by which the male and female gender-specific phenotype develops.

Genomic Structure of the Human X and Y Chromosomes

The human X and Y chromosomes have evolved from a pair of ancestral chromosomes during the past 300 million years . While the X chromosome retained many properties of an autosome, the Y chromosome lost most fits genes and became greatly reduced in size. Its genetic function is now limited to inducing male development during embryonic development and to maintaining spermatogenesis in adult males. The two chromosomes undergo pairing and recombination at the distal ends of their short arms in the pseudo autosomal region (PAR1),whereas all other regions are exempted from recombination.

A. Genomic structure of the human X chromosome

Functional genes are distributed along the X chromosome as shown by the blue squares (each representing one gene). The approximate locations of nine selected landmark genes and their directions of transcription (arrows) are shown. Most of the short arm (Xp) consists of a region that resulted from the translocation of an ancestral autosome into Xp about 105 MYA (million years ago) (X-added region,

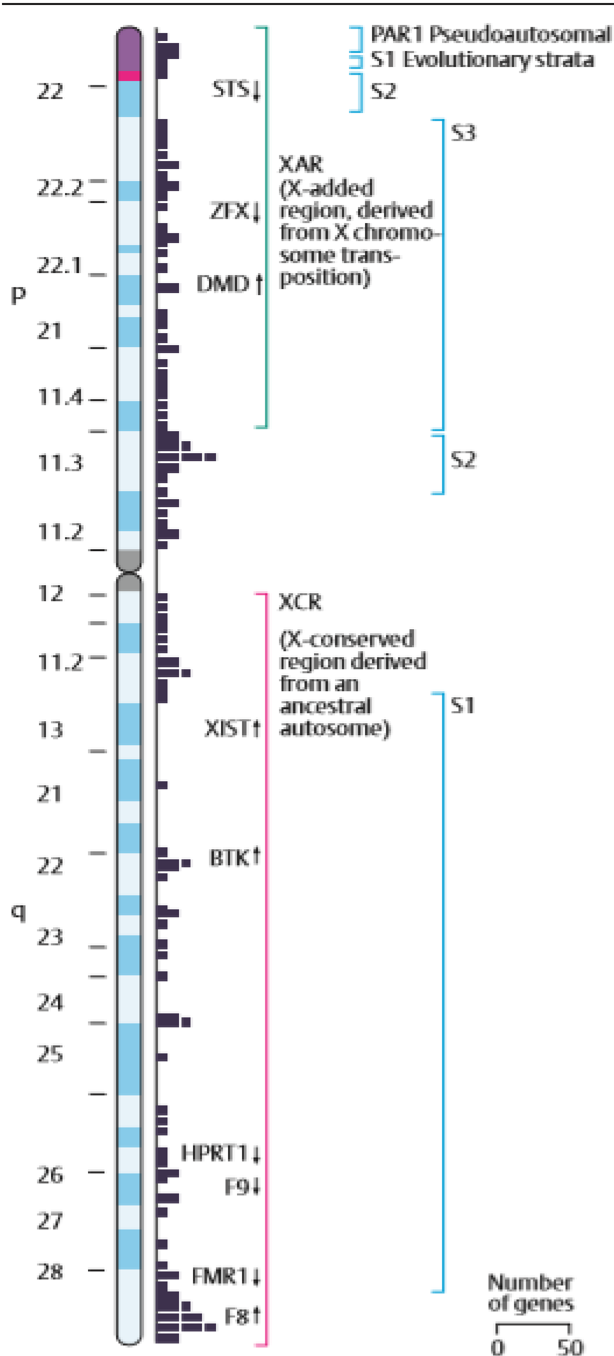
XAR). The long arm (Xq) is composed of a region that has been conserved during evolution in mammals (XCR, X-conserved region). Five regions of evolutionary conservation, called evolutionary strata S1-S5, have been identified along the X chromosomes. The human X chromosome contains 1098 genes. The X chromosome has 7.1 genes per million base pairs, one of the lowest gene densities in the human genome (average 10–13).

B- Genomic structure of the human Y chromosome

The human Y chromosome has a distinct genomic structure comprising five different regions in the euchromatic part: **(i)** two pseudo utosomal regions at the distal ends of the short (PAR1) and long arms (PAR2), **(ii)** the Y-specific male determinant (MSY) region of about 35 kb, **(iii)** about 8.6 Mb (38% of the euchromatic portion) called X-degenerate, derived from the ancestral autosome, **(iv)** 3.4 Mb derived from former X-linked genes by transposition (Xtransposed), which occurred about 3–4 MYA, and (v) 10.2 Mb amplified (amplionic) Y specific sequences, designated as P1-P8, derived from three different processes. These are thought to be derived from former X-and Y linked genes and to have acquired autosomal male fertility factors by transposition and retroposition. They are termed amplionic because they consist of amplified palindromic sequences (amplicons) of various sizes with a marked sequence similarity of 99.9% over long stretches of DNA(ten to hundreds of kilobases). They contain both coding and noncoding genes. Most genes in the amplionic segments are expressed exclusively in testes ,presumably being required for spermatogenesis. Since the male-specific sequences on the Y chromosome do not participate in crossing over, they are deprived of one mechanism for replacing mutations or structural rearrangements with normal sequences. Gene conversion between these palindromic sequences (Y-Y conversion) presumably serves as a mechanism for restoring normal sequences that have been rendered nonfunctional in one arm of a palindrome.

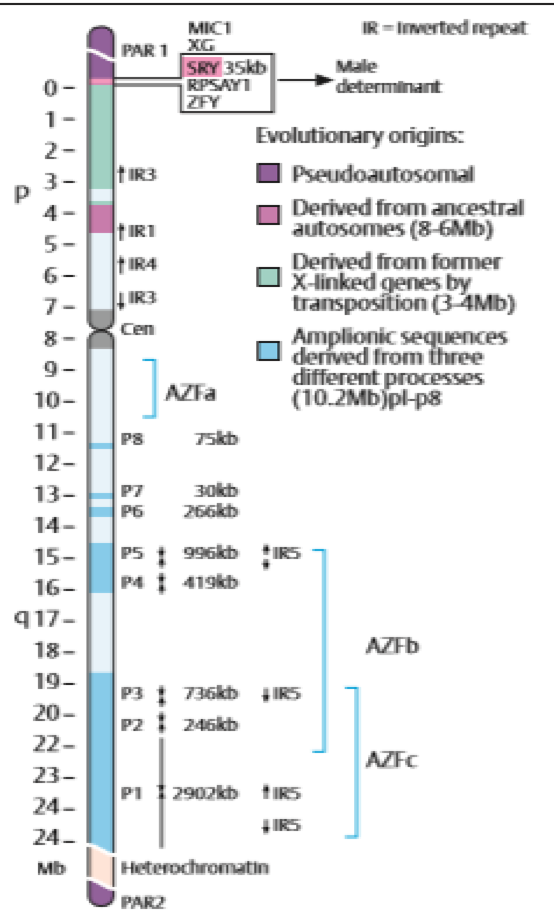
C. Homologies between and the X and Y chromosomes

The X and Y chromosomes share regions of homology due to their common evolutionary origin. Medical relevance Three regions, AZFa, AZFb, and AZFc ,in the long arm of the Y chromosome are associated with male infertility when deleted, due to failure to produce viable sperm cells (azoospermia).

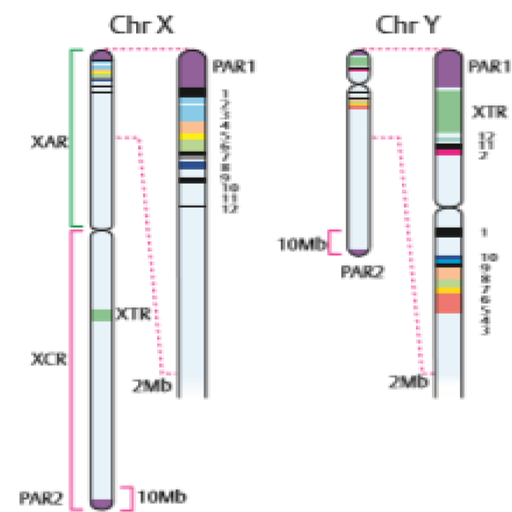


Selected landmark genes:
STS: steroid sulfatase, ZFX: Zinc finger protein, XIST: X-inactivation-specific transcript, BTK: Bruton agammaglobulinemia tyrosine kinase, HPRT1: Hypoxanthine-phosphoribosyltransferase 1, F9 and F8: Hemophilia A and B, FMR1: Familial Mental Retardation type 1.

A. Genomic structure of the X chromosome



B. Genomic structure of the Y chromosome



C. Homologies of the X and Y chromosome

Sex-determining region SRY

Sex-determining region SRY Experiments in animals and clinical observations of human males with various sized deletions of the Y chromosome indicate that only a small region of the distal short arm of the Y chromosome is required to induce male development. This region is named SRY(sex-related Y). SRY is a small region on the short arm of the human Y chromosome. Within this region, the gene SRY (sex-determining region Y) was identified. It is located just proximal to the pseudo autosomal region 1 (PAR1). The PAR1 is homologous to the distal segment of the short arm of the X chromosome. Homologous pairing occurs here with crossing over during male meiosis.

SRY gene The SRY gene, located atYp11.32, consists of a single exon. It has a TATAAA motif for binding transcription factor TFIID**.** SRY is a member of the SOX family of transcription factors. It contains conserved high-mobility group (HMG)motif, which binds to DNA and causes reversible bending **(2).** The bending opens the double helix and permits access of transcription factors. HMG proteins are no histone DNA-binding proteins.

There are also sex revertants, when female phenotype is formed because of a mutated SRY. In these cases, the HMG (high mobility group) part, the DNA binding domain of the protein is wrong, and in the absence of DNA binding the differentiation cascade cannot start. Although the SRY alone is sufficient for male sex determination, i.e. to induce the differentiation, however, many other autosomal (e.g. chromosome 17 localized SOX9 [SRY HMG box related genes] a transcription factor encoding gene), and X chromosome localized genes are necessary to switch on SRY and to the whole process of sexual differentiation. For the normal sexual differentiation not only the sufficient quality and quantity of the inductors, but their adequate receptors are necessary, too. Their mutations also cause disturbed sexual development.

Sex Differentiation

Sex differentiation is a series of consecutive developmental processes during early embryogenesis resulting in either the female or the male gender. Initially all anatomical structures involved are undifferentiated. Under the influence of various genes they develop into either sex.

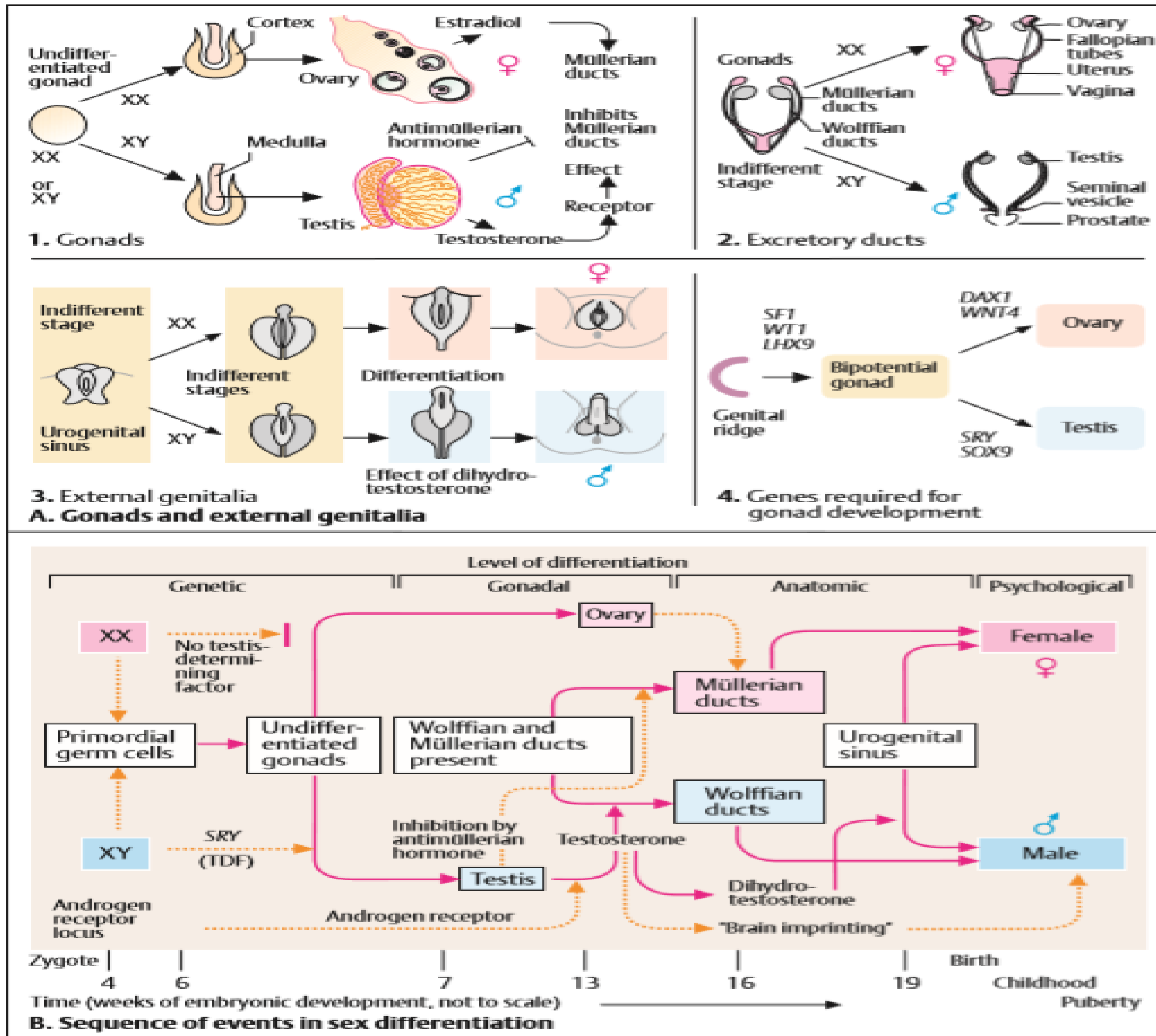
A. Gonads and external genitalia

The gonads **(1),** the efferent (mesonephric and paramesonephric) ducts **(2),** and the external genitalia **(3)** all develop from an indifferent anlage. At about the end of the sixth week of pregnancy in humans, after the primordial germ cells of the embryo have migrated into the initially undifferentiated gonads, an inner portion (medulla) and an outer portion (cortex) of the gonads can be distinguished **(1).**In XY embryos, early embryonic testes develop at about the 10thweek ofpregnancy under the influence of a testis-determining factor(TDF),the SRY gene. If this is not present, ovaries develop. The early embryonic testis produces two hormones, testosterone, with a male differentiating effect, and the Müllerian inhibition factor MIF (anti Müllerian hormone). MIF inhibits the development of female anatomical structures. The excretory ducts differentiate under the influence of the hormones produced by the early gonads **(2).** The Müllerian ducts, precursors of the Fallopian tubes, the uterus, and the upper vagina, develop when a male differentiating influence is absent. The Wolffian ducts, precursors of the male efferent ducts (vas deferens, seminal vesicles, and prostate), develop under the influence of testosterone, a male steroid hormone formed in the fetal testis. If testosterone is absent or ineffective, the Wolffian ducts degenerate. The external genitalia develop after the gonads have differentiated into testes or ovaries. In humans this occurs relatively late, in the 15th to 16th week **(3).** Full development of male external genitalia depends on a derivative of male-inducing testosterone, 5-dihydrotestosterone, a metabolite of testosterone producedbytheenzymaticactionof5α-reductase. The differentiation of the gonadal ridge in to the bipotential gonad, and this into ovary or testis, requires several genes .see figure (Male sex determination)

B-Sequence of events in sex differentiation

Four levels can be schematically defined: **(i)** genetic,**(ii)** gonadal, **(iii) anatomical**, as prenatal stages, and **(iv)** from early childhood on, psychological .A fifth, the legal gender, recorded as “female” or “male” in all legal documents, can be added .Each level is reached in a series of temporally regulated successive steps. First the primordial germ cells differentiate into early embryonic testes under the influence of the testis-determining factors (TDF), mainly the SRY gene (in humans) or the Sry gene in other mammals and other genes. Male differentiation includes suppression of the Müllerian ducts by the Müllerian Inhibitor Factor. In the absence of SRY no testes develop and no subsequent male differentiation stages

occur. In the absence of testes, ovaries develop, the Wolffian ducts degenerate, and the Müllerian ducts differentiate in to Fallopian tubes, uterus, and the upper vagina. The male differentiating effect of testosterone depends on the function of an intracellular androgen receptor. Testosterone also has an effect on the central nervous system by influencing the psychosexual orientation apparent later in life ("brain imprinting"). When testosterone is absent or ineffective due to a receptor defect, gender orientation is female.



the sex differentiation abnormalities can be primarily caused by the following inherited disorders:

a. mutations of SRY, rarely of RSPO1 or structural abnormalities affecting these genes

b. disorders of steroid (androgen / estrogen) biosynthesis

c. mutations of the androgen receptor

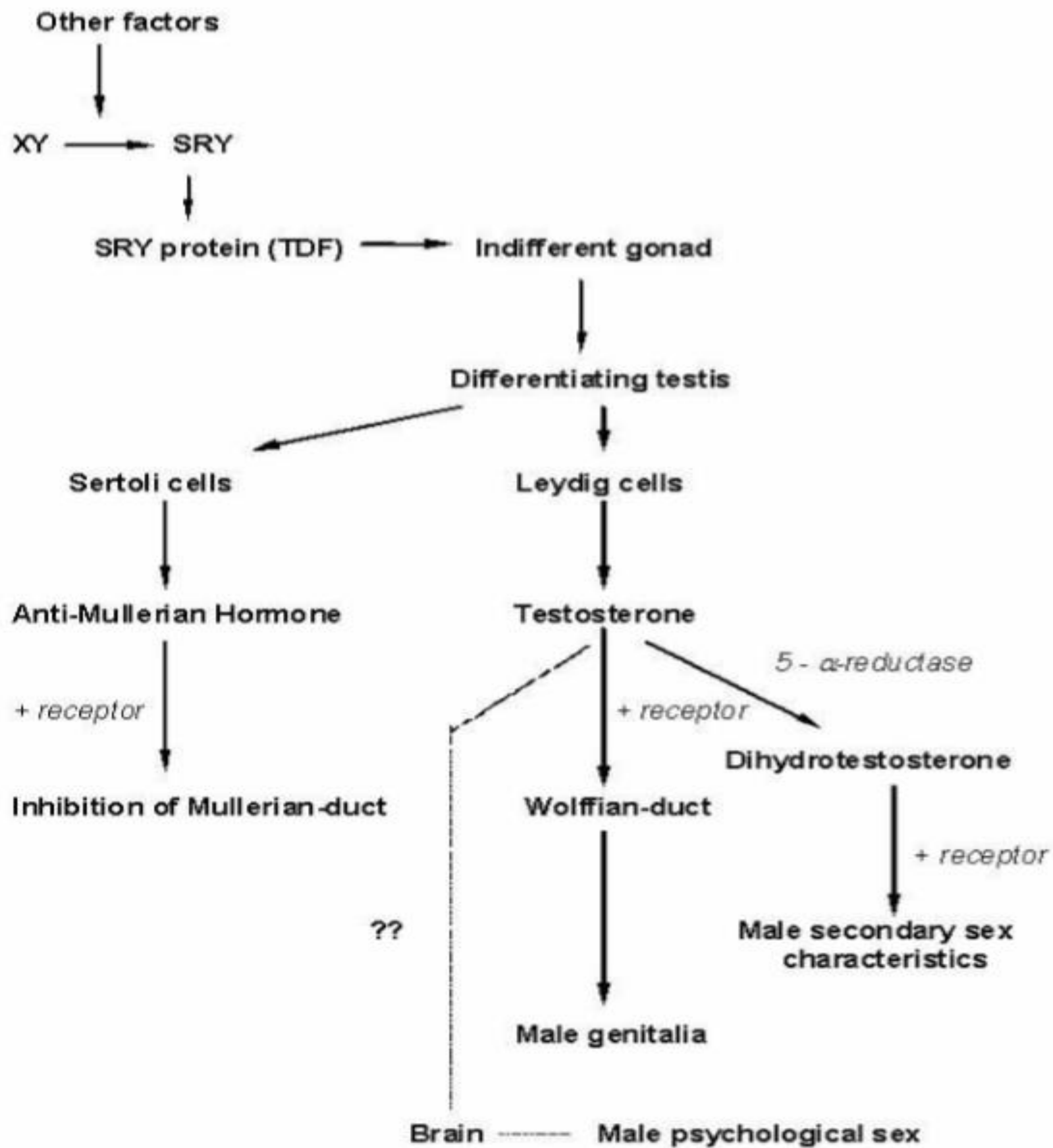
d. defects of the AMH gene

e. X0/XY mosaicism

f. mutations in genes involved in the differentiation of mesoderm or the nephrogonotome (for example SF1, WT-1).

The X chromosome inactivation

the extra-embryonic membranes (placenta) have imprinted parental origin dependent X chromosome inactivation. The placenta always has the paternal X in inactive form. The inactive X chromosome can be detected in interphase. Adhering to the nuclear membrane, a heavily stained sex chromatin, the so-called Barr body is seen in the epithelial cell nuclei Adrumstick-shaped appendix of the segmented nucleus of neutrophils is a particular manifestation of the inactive X.



(figure Male sex determination)

6- Mutation

Most biological molecules have a limited lifetime. Many proteins, lipids and RNAs are degraded when they are no longer needed or damaged, and smaller molecules such as sugars are metabolized to compounds to make or store energy. In contrast, DNA is the most stable biological molecule known, befitting its role in storage of genetic information. according to the classic definition mutations are sudden heritable changes in the DNA. The process (change) itself is still called mutation, but due to the fast development of genetics and genomics, two terms related to the variations in the sequence had to be modified. Next to the above mentioned definition the term **mutatio**n is also used to indicate a disease-causing change or sometimes rare change. Similarly, **the term polymorphism** is used both to indicate a non disease-causing change or a change found at a frequency of 1% or higher in the population. In the era of advanced DNA sequencing tools and personal genomics, these earlier definitions of mutation and polymorphism are antiquated. a mutation would be a “DNA variant” acquired over the lifetime of an organism, i.e. a **somatic mutation**. In this sense, mutations are the principal causes of many diseases like cancer but are typically not inherited by their offspring. Alterations in the DNA of germ cells – sperms and eggs – can be inherited by offspring and are currently called **germline mutations**. In this case, the term mutation should be used only if the germline “variant” has been detected using as a reference the germline DNA of the same individual.

6-1 Mutagens and type

Mutations that are caused by agents that damage the DNA are known as **induced mutations**. Agents that mutate DNA are called **mutagens** and are of three main types: mutagenic chemicals, radiation and heat. Even if there are no dangerous chemicals or radiation around, mutations still occur, though less frequently. These are **spontaneous mutations**. Some of these are due to errors in DNA replication.The enzymes of DNA replication are not perfect and sometimes make mistakes. In addition, DNA undergoes certain spontaneous chemical reactions (alterations) at a low but detectable rate and this rate goes up with increasing temperature.

A- The most common mutagens are toxic chemicals that react with DNA and alter the chemical structure of the bases. For example, EMS (ethyl methane sulfonate) is widely used by molecular biologists to mutagenize growing cells. It adds an ethyl group to bases in DNA and so changes their shape and their base-pairing properties. Nitrite converts amino groups to hydroxyl groups and so converts the base cytosine to uracil . Nitrite is used experimentally to mutate purified DNA, such as a cloned gene carried on a plasmid, while the plasmid is in the test tube.The mutagenized DNA is then transferred back into a cell to identify the mutations that were generated. During DNA replication, the DNA polymerase misidentifies these altered bases and puts in the wrong bases in the new complementary strand of DNA it is making .

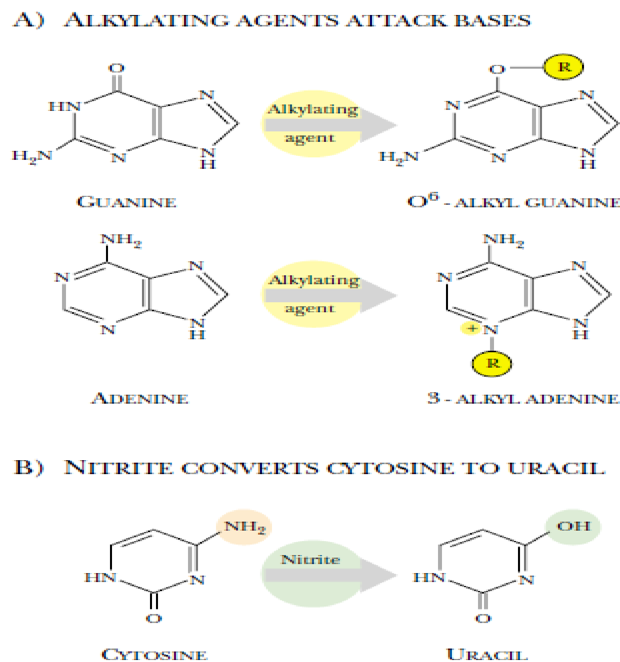


FIGURE 13.13 ***Base Alteration by Chemical Mutagens***
A) Alkylating agents alter the structure of bases by adding alkyl groups. B) Nitrite will convert cytosine to uracil (which pairs with
adenine).

Base analogs are chemical mutagens that mimic the bases found in natural DNA. For example, bromouracil resembles thymine in shape. It is converted by the cell to the DNA precursor, bromouridine triphosphate, which DNA polymerase inserts where thymine should go. Unfortunately, bromouracil can flip-flop between two alternative shapes . In its alternate form, bromouracil resembles cytosine and

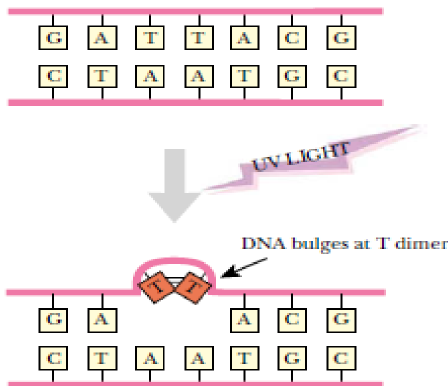
pairs with guanine. If bromouracil is in its misleading form when DNA polymerase arrives, a G will be put into the new strand opposite the bromouracil instead of A. Some mutagens imitate the structure of a base pair rather than a single base. For example, **acridine orange** has three rings and is about the size and shape of a base pair. Acridine orange is not chemically incorporated into the DNA. Instead, it squeezes in between the base pairs in the DNA a process referred to as **intercalation**. During DNA replication, the DNA polymerase mistakes the intercalating agent for a base pair and puts in an extra base when making the new strand. As discussed above, insertion of an extra base will change the reading frame of the protein encoded by agene. Since this will completely destroy the function of the protein, intercalating agents are highly hazardous mutagens.

A **teratogen** is an agent that causes abnormal development of the embryo, which results in gross structural defects. Teratogens may or may not cause mutations. The most famous example is thalidomide, which resulted in the birth of malformed children with missing limbs. Thalidomide interferes with the development of embryos as opposed to causing mutations. Although the mechanism responsible for the malformations remains uncertain, it is known that thalidomide prevents blood vessels from forming (i.e. it is anti-angiogenic), which may partly explain the drug's ability to cause birth defects.

B- **Radiation Causes Mutations**

Some types of radiation cause mutations. High frequency electromagnetic radiation, ultraviolet radiation (UV light), X-rays and gamma rays (g-rays), directly damage DNA. X-rays and g-rays are **ionizing radiation**; that is, they react with water and other molecules to generate ions and free radicals, notably hydroxyl radicals. Ionizing radiation is responsible for about 70 percent of the radiation damage to DNA. The other 30 percent of the radiation damage is due to direct interaction of X-rays and g-rays with DNA itself. In the early days of molecular biology, X-rays were often used to generate mutations in the laboratory. X-rays tend to produce multiple mutations and often yield rearrangements of the DNA, such as deletions, inversions and translocations. Ultraviolet radiation is electromagnetic radiation with wavelengths from 100 to 400 nm. It is nonionizing and acts directly on the DNA. The bases of DNA show an
absorption peak at around 254 nm and UV close to this wavelength is absorbed very efficiently by DNA. In particular, UV causes two neighboring pyrimidine bases to cross-react with each other to give dimers. Thymine dimers are especially frequent . Although DNA polymerase can proceed by skipping over thymine dimers, this leaves a single-stranded region that needs repairing. The repair process

in turn causes the insertion of incorrect bases in the newly synthesized strand . Ultraviolet radiation is emitted by the sun. Most of it is absorbed by the ozone layer in the upper atmosphere, so it does not reach the surface of the earth. Damage to the ozone layer by the chlorinated hydrocarbons used in aerosol sprays and refrigerants has allowed more UV radiation to reach the surface of this planet, especially in certain areas. This has probably contributed to the increased frequency of skin cancer noted in recent years. **In addition to electromagnetic radiation**, there are other forms of radiation, such as the a-particles and b-particles emitted by radioactive materials along with g-rays. Most a-particles are too weak even to penetrate skin but b-particles may cause significant damage to DNA and other biological molecules. However, a-emitters can be mutagenic if they have entered the body, for example by being breathed in or swallowed.



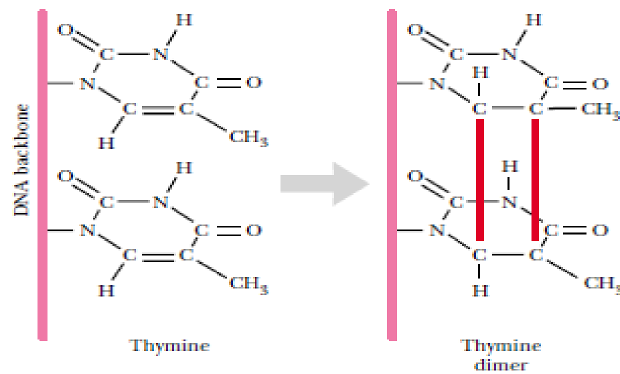


FIGURE ***Thymine Dimers***
A) Ultraviolet light (UV) sometimes results in the formation of a thymine dimer (red). B) The detailed chemical structure of the thymine dimer is shown.

C- Biological mutagens:- such as virus and transposons that can isert themselves with in a gene and destroy its function

6-2 Phenotypical classification of mutation

Although all mutations represent biochemical changes ,they have various manifestations such as

1- **Colour mutation ;-** these change in the colour e.g. red eyes to white eyes in *Drosophila* , red flowers to whit flowers in peas plant
2- **Morphological mutation**;- these change the morphology of the mutant e.g normal wings to curly wings in *Drosophila* , normal coloniesto fluffy colonies in the fungus *Aspergillus* .
3- **Resistant mutation** ;- these make mutant capable of growth on chemical or antibiotic that are toxic to the wild type e.g drug resistant mutations in microorganisms .
4- **Auxtrophic (nutritional) mutations** ;- these make mutant unable to grow on the minimal medium (MM) unless a certain nutrient is added to the medium .the wild type can grow on MM and is called prototrophic .

	MM	MM+ Lysine
Lys + (w.t)		
Lys – (auxo)		

6-3 The Major Types of Mutation

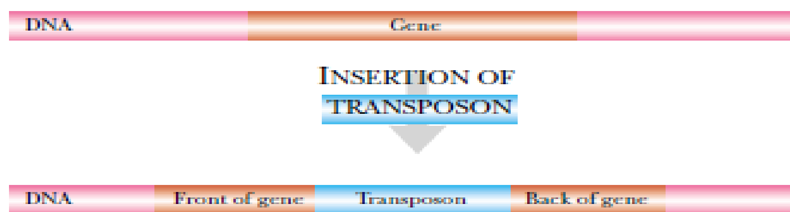
A single mutation is a single event and a multiple mutation is the result of several events. A single mutational event, however large or complex its effect, is regarded as a single mutation. A mutation that involves only a single base is known as a **point mutation**.A **null mutation** totally inactivates a gene; the expression "null mutation" is a genotypic term. Complete absence of a gene product may or may not cause a detectable phenotype. A **tight mutation** is one whose phenotype is clear-cut.The complete loss of a particular enzyme may result in no product in a particular biochemical pathway. For example, the complete inability of a bacterium to grow if provided with a certain sugar is an example of a tight mutation.A **leaky mutation** is one where partial activity remains. For example, 10 percent residual enzyme activity might allow a bacterium to still grow, albeit very slowly.
The major types of sequence alteration are as follows, and will be discussed separately below:

1- Base substitution: one base is replaced by another base.

If one base is replaced by another, a base substitution mutation has occurred. These may be subdivided into **transitions** and **transversions**. In a transition a pyrimidine is replaced by another pyrimidine (i.e., T is replaced by C or vice versa) or a purine is replaced by another purine (i.e., A is replaced by G or vice versa). A transversion occurs when one base is replaced by another of a different type; for example, a pyrimidine is replaced by a purine or vice versa.

2- **Insertion:** one or more bases are inserted into the DNA sequence. Genes may also be inactivated by insertions of DNA. If a foreign segment of DNA is inserted into the coding region, then the gene is said to be disrupted. (Fig. 6-2).The cause of insertion mutations may be divided into two distinct categories. Some of these mutations are the result of **mobile genetic elements**, usually thousands of bases long, inserting themselves into a gene. Other insertion mutations, usually only one or a few bases long, are caused by mutagenic chemicals or by mistakes made by DNA polymerase.

A) DNA INSERTION OF TRANSPOSON



B) POLAR EFFECT IN BACTERIA

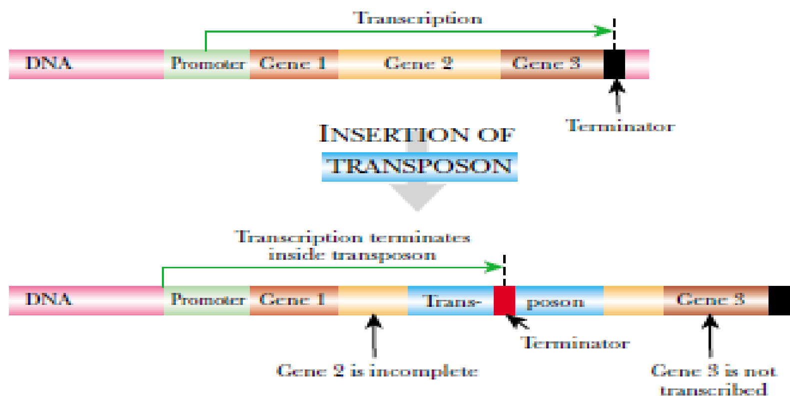


FIGURE 6-2 ***Effects of Insertion Mutations***
A) Insertion of a transposon into the middle of a gene interrupts the coding sequence. B) Insertion of a transposon into the second gene of a bacterial operon with three genes. Gene 1 is the only gene correctly transcribed since the transposon disrupts gene 2 and causes premature termination. Gene 3 will not be transcribed, although its coding sequence is still intact

Occasionally, insertions may activate genes. If an insertion occurs in the recognitionsite for a repressor, binding of the repressor will be prevented and activation of the gene may result.

3- Deletion: one or more bases are deleted from the DNA sequence . In particular, we should distinguish between point mutations where one (or a very few) bases are affected, and gross deletions and insertions that affect long segments of DNA. Point deletions and insertions may have major effects due to disruption of the reading frame—see below. Here we will consider the effects of larger deletions. Large deletions may remove part of a gene, an entire gene or several genes. Deletions may also remove part or all of the regulatory region for a gene. Depending on the precise region removed, gene expression may be decreased or increased. For example, a deletion that removes the binding site for a repressor may result in a large increase in activity of the gene in question. Thus loss of DNA may result in elevated activity.

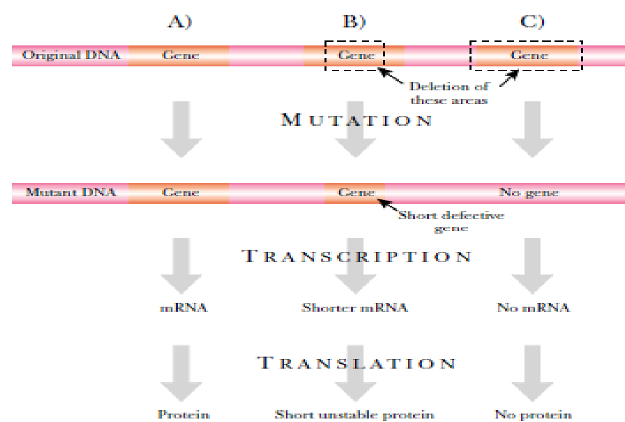


FIGURE 6- ***Effects of Deletion Mutations***
A) The wild-type gene produces a normal mRNA and a normal protein. B) A large deletion causes a shorter mRNA and a short unstable protein. C) Deletion of an entire gene results in no mRNA and no protein.

4- **Inversion:** a segment of DNA is inverted, but remains at the same overall location.
5- **Duplication:** a segment of DNA is duplicated; the second copy usually remains at the same location as the original.
6- **Translocation:** a segment of DNA is transferred from its original location to another position either on the same DNA molecule or on a different DNA molecule.

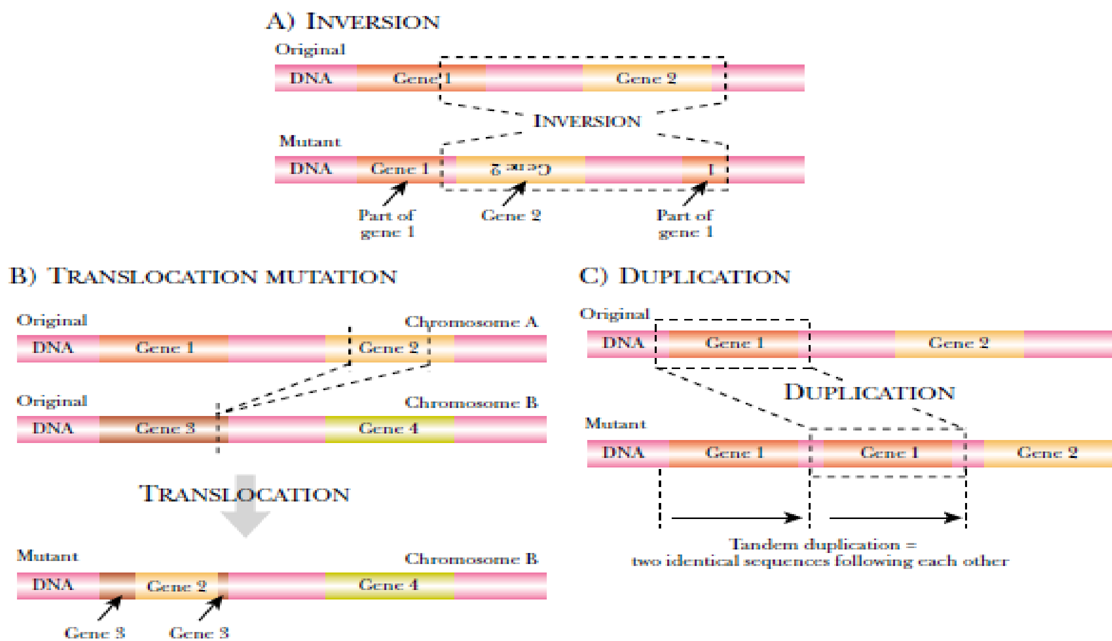


FIGURE 6-4.5.6 *Inversions, Translocations and Duplications*

Writhe type mutations effect on protein????

Questions

1. When the terms mutation and polymorphism may be used?

2. What kind of mutations do you know according to their origin?

? 3. Give examples of some physical and chemical mutagens!

4. Why could a double-stranded DNA break lead to structural chromosomal abnormality

7 - Diseases due to the mutation of structural genes

.1. Marfan syndrome

Fibrillin gene is mutated. Pleiotropy in this case is clearly understandable, since fibrillin is one of the most important extracellular proteins present in the elastic and nonelastic connective tissues. Multiple organs can be affected: lungs, skin, kidneys, skeletal system, vascular system, cornea, etc. The type of the affected organs and the severity of the symptoms may show individual differences, implicating variable expressivity. The arousal of the mutation shows strong correlation with paternal age.

.2.Osteogenesis imperfecta

The different mutations of collagen genes produce differently severe symptoms. The collagen gene family comprises 45 genes, distributed on different chromosomes, coding for proteins of somewhat different nature. Considering that collagen is the most abundant protein in the extracellular matrix, it is not surprising that the mutations of these genes have pleiotropic effect as well. Also expressivity is variable; the fragility of the bones in homozygotes can be so severe, that it causes death already during the process of birth. On the other hand it is possible that either deafness - as a result of the abnormal development of the ear bones, or only breakable bones develop. Sometimes the inheritance of osteogenesis imperfect a shows incomplete penetrance. It has not been revealed exactly yet, why some heterozygous persons do not show the symptoms in AD diseases, but it is suggested that modifier genes are involved in the process .

8 - Diseases due to mutations of receptor genes

.1.Achondroplasia

The disease is caused by the mutation of FGFR3 (fibroblast growth factor receptor type 3) gene. Depending on the locus of the mutation in the gene, three different diseases are distinguished: - achondroplasia, hypochondroplasia and tanatophoroplasia - . The mutation is a „gain of function" mutation; the receptor stays active without ligand as well. The mutation arises usually as new mutation and it shows strong correlation with the age of the father.

2-Familial hypercholesterolemia:

it is a relatively frequent disease with a prevalence of 1:500. The severity of the symptoms depends on whether the genotype is homozygous or heterozygous. More than 100 mutations have been identified in the gene of LDL receptor- this is a case

of allelic heterogeneity. But variants in other genes (e.g. APOB, PCSK9) can cause similar symptoms, i.e. In addition the disease also has recessive types, like mutations in the ARH or CYP7A1 genes.

.3. Polycystic kidney disease:

with a prevalence of 1:800, it is a quite frequent AD disease. The disease is connected to the malfunction of a receptor-ion channel complex that consists of two polycyistin proteins, regulating G-protein signalization and a membrane bound Ca++ channel. PKD1 gene codes for the protein polycystin1 and PKD2 gene for polycystin2. 80% of the mutations hit the PKD1 gene. The onset of the disease is in early or late adulthood, it shows variability even in the same family regarding both the time of the onset and the severity of the symptoms. The differences depend on environmental factors, as infections can promote the development of the symptoms. As for genetic influence, the manifestation may depend on the site of the mutation in the affected allele. Additionally, the outcome of the disease is strongly influenced by modificator genes whose variants further complicates the picture. Some variants of nitrogen-monoxide synthase gene (NOS3) induce an early and more severe development of the disease.

9- Genetic Causes of Cancer:

Background

Cancer cells (malignant cells) break two rules imposed on all cells in a multicellular organism: they and their progeny do not adhere to restrained cell division, and they invade and colonize tissues reserved for other cell types. A cancer is medically classified according to the cell type from which it originates: carcinoma from epithelial cells, sarcoma from connective tissue and muscle cells, leukemia from hematopoietic cells, and lymphoma from lymphoid cells. Genetically, cancer is either non hereditary, due to somatic mutations, or hereditary, due to a predisposing mutation in the germline.

10- Oncogenetics

Although the process and causes of carcinogenesis have already been discussed in several other subjects, this section will cover the major genetic events of the development of tumors, because at cellular level tumors may also be considered genetic disorders. The cancers affect 1 in 3 people worldwide; a man has ~ 40% chance of the cancer. Even this high frequency indicates that tumors are usually not of monogenic origin, with the exception of rare monogenic tumors such as retinoblastoma, or Li-Fraumeni syndrome. There are a number of underlying genetic susceptibility factors (mutations) and environmental effects. The cancer can be described as a group of diseases characterized by unlimited proliferation and spread of mutant cells in the body.

The following steps are the hallmarks of carcinogenesis: a. growth signal autonomy

b. unlimited replicative potential

c. evasion of growth inhibitory signals

d. evasion of apoptosis

e. angiogenesis

f. invasion and metastasis

Four basic types of genetic alteration in tumor cells

The many genetic alterations affecting growth controlling genes can be classified into four major categories: (i) change in the DNA sequence of a growth-controlling gene (somatic mutation), (ii) reciprocal chromosome translocation disrupting a gene expressed in a tissue that depends on controlled cell division (e.g., immune system, blood cell formation in bone marrow), (iii) gross alteration in chromosome number in somatic cells during tumor progression, and (iv) amplification of a growth controlling gene. Here are four examples.

(**1**) *Change in DNA sequence*. A deletion of two adenines (A) in a series of ten in the gene *TGFBR2* for receptor type 2 of the transforming growth factor beta (TGF_ R2) in a colorectal cancer cell line changes the codon AAG (lysine) to GCC (alanine). This converts the subsequent codons into TGG (tryptophan) and TGA (stop codon), resulting in a truncated protein.

(**2**) *Chromosome translocation*. A reciprocal translocation between a chromosome 1 and a chromosome 17 in a neuroblastoma (MIM 256700) cell line disrupts genes involved in neuroblastoma located on chromosomes 1 and 17.

(**3**) *Gross chromosomal change*. Loss of a chromosome 3 and a chromosome 12 (yellow arrows) occurred in a clone of a cell line (SW837) of colorectal cancer cells (CRCs). Such gross changes are frequent during tumor progression.

(**4**) *Gene amplification*. In some tumor cells in culture, small chromosomal derivatives (double minutes) or homogeneously stained regions (HSRs) are visible. HSRs, first described by Biedler & Spengler (1976), are cytological manifestations of gene amplification. Specific DNA sequences are replicated to a disproportionately higher degree than normal. Here a metaphase from a clone of the CRC cell line SW837 expanded through 25 generations is shown.

Principal types of genetic changes in tumor cells:

1. Change in DNA sequence (Mutation in *TGFB2* gene)

	Codon	125	126	127	128	129	130	
normal		Glu	Lys	Lys	Lys	Pro	Gly	
		GAA	AAA	AAA	AAG	CCT	GGT	
mutant		GAA	AAA	AAA	GCC	TGG	TGA	Deletion of two adenines
		Glu	Lys	Lys	Ala	Trp	Stop	

Cancer and genetic

A- Categories of Cancer Gene

Tumors and cancer are the result of uncontrolled cell division. Normally, cell division is regulated by a family of extracellular growth factors, proteins that cause resting cells to divide and, in some cases, differentiate. Defects in the synthesis, regulation, or recognition of growth factors can lead to cancer. Cancer is a common genetic disease that affects 1 of every 4 individuals. More than 100 genes in the human genome contribute to cancer when altered by mutations. They are classified into three basic categories according to the effects of their mutations: too much activity of a gene product (**oncogenes**) Their mutant forms, called *oncogenes*, drive a cell to divide when it normally should not (gain-of-function mutations). A single activating mutation is the first step towards cancer , insufficient activity (***tumor suppressor*** **genes**) They require two mutational events to induce tumor development (comparable to a defective brake). The initial mutation predisposes the cell to become a cancer cell. The second mutation then inactivates the other allele (loss-of-function mutation) and results in loss of cell division control. , and **disruption of *genome stability* genes,** Mutations in the third class of cancer genes, called *stability genes* or *caretakers*, affect the stability of the genome by disrupting one of the various repair processes.

B- Oncogene activation

Oncogene were originally discovered in tumor-causing viruses, then later found to be closely similar to or derived from genes in the animal host cells, protooncogenes, which encode growth-regulating proteins. During viral infections, the DNA sequence of a protooncogene is sometimes copied by the virus and incorporated into its genome.

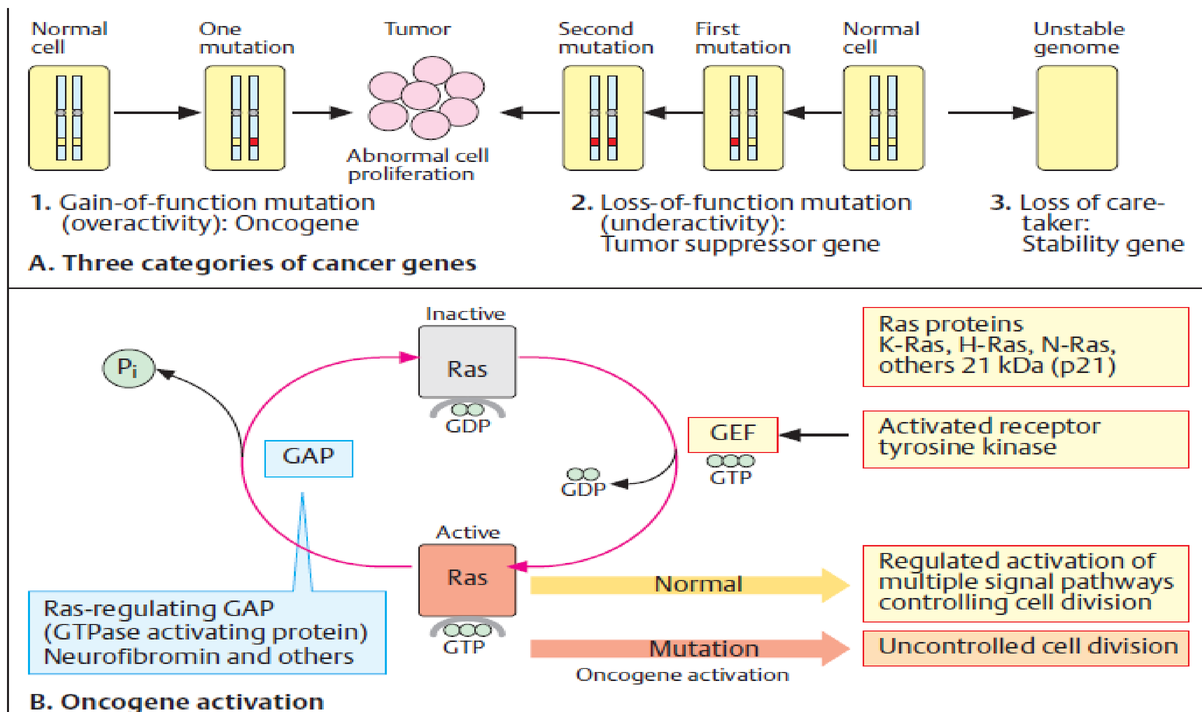
Proto-oncogenes can become oncogenes without a viral intermediary. The genetic mechanisms that activate oncogenes include point mutations, chromosome rearrangement (chromosomal translocation), and gene amplification. chemical agents, and radiation are among the factors that can cause oncogenic mutations. The mutations that produce oncogenes are genetically dominant; if either of a pair of chromosomes contains a defective gene, that gene product sends the signal "divide" and a tumor will result.

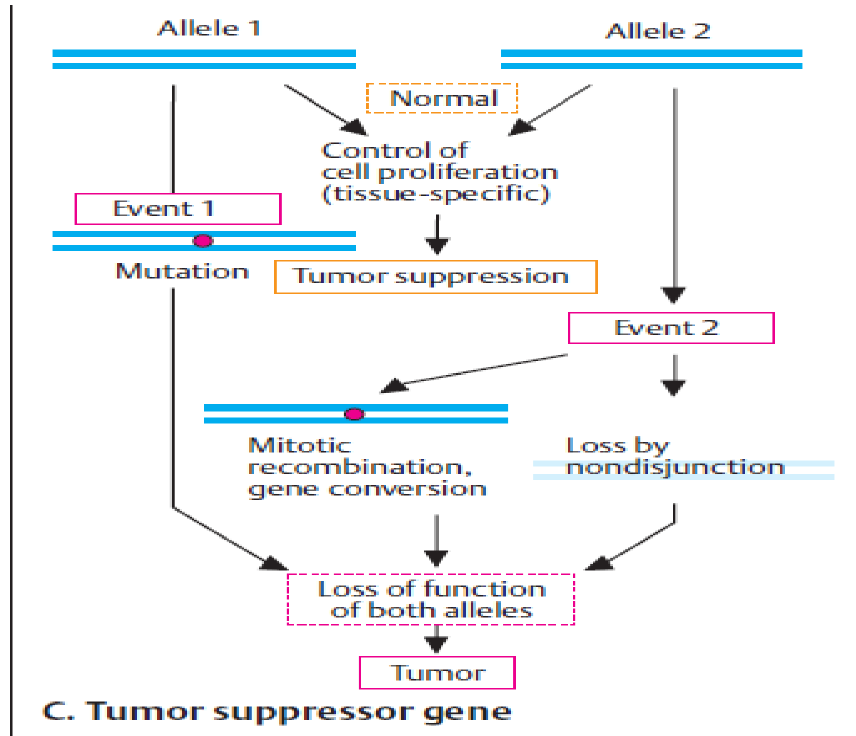
Oncogenes serve in signal pathways controlling cell division. For example the *Ras* genes encode a family of related cell growth-controlling proteins. Ras proteins are GTPase-binding proteins functioning as switches, inactive when bound to GDP (guanosyldiphosphate) and active when bound to GTP (guanosyltriphosphate). Ras is activated by a receptor tyrosine kinase, which activates a guanine nucleotide exchange factor (GEF). GTPase-activating proteins (GAPs) increase the hydrolysis of GTP bound to Ras and inactivate Ras by removing GTP. Mutant forms of Ras are hyperactive and do not respond to GAPs. Instead they remain bound to GTP, sending continuous cell division-promoting signals to the nucleus through several pathways and causing uncontrolled cell divisions.

C. Tumor suppressor genes

Two successive mutational events are required within the same cell (**1**). The first event inactivates one allele and predisposes the cell to uncontrolled divisions. If the other allele is also inactivated by a mutation, cell division control is lost and a tumor develops. One of several mechanisms may be responsible: a further mutation, chromosome loss during cell division (mitotic nondisjunction), or mitotic recombination with gene conversion. Tumor suppressor genes can be assigned to two groups, ***gatekeepers*** and ***caretakers***. ***Gatekeeper* genes *directly*** inhibit tumor growth. Inactivation **of caretaker genes** leads to genetic instability, indirectly promoting tumor growth. Loss of one allele in a somatic cell carrying a mutation in the other allele. (**2**). Whereas somatic cells heterozygous at a marker locus give two signals, tumor cells, which have lost both alleles of the gene, give one signal (loss of heterozygosity, LOH). LOH is a hallmark of a tumor suppressor gene. It occurs with variable frequency in different tumors and may be useful in detecting a

mutation indirectly. Mutations in tumor suppressor genes may be present in the zygote (by transmission or by new mutation) or occur in a somatic cell (**3**). A germline mutation predisposes all cells to develop into tumor cells. A somatic mutation predisposes a single cell. Germline mutations are the basis for hereditary forms of cancer; somatic mutations for the nonhereditary forms. A germline mutation occurring after the initial division of the fertilized egg may result in amosaic of mutated and normal cells.





-10-The *p53* Tumor Suppressor Gene

The *p53* tumor suppressor gene plays a central role in cell cycle control, apoptosis, and maintenance of genetic stability. It encodes a 53-kDa nuclear phosphoprotein translated from a 2.8- kb mRNA. The gene spans about 20 kb on the short arm of human chromosome 17 (17p13). The p53 protein binds to specific DNA sequences and controls the expression of different regulator genes involved in growth. It interacts with other proteins in response to DNA damage and mediates apoptosis (cell death) of the cell when the damage is beyond repair. Its basic function is to control entry of the cell into the S phase . Somatic mutations in the *p53* gene occur in about half of all tumors. Mutations in the gene for p53 also cause tumors; in more than 90% of human cutaneous squamous cell carcinomas (skin cancers) and about 50% of all other human cancers, *p53* is defective. Those very rare individuals who *inherit* one defective copy of *p53* commonly have the Li-Fraumeni cancer syndrome, in which multiple cancers (of the breast, brain, bone, blood, lung, and skin) occur at high frequency and at an early age. The explanation for

multiple tumors in this case is the same as that for *Rb* mutations: an individual born with one defective copy of *p53* in every somatic cell is likely to suffer a second *p53* mutation in more than one cell in his or her lifetime.

Apoptosis Is Programmed Cell Suicide

Many cells can precisely control the time of their own death by the process of **programmed cell death,** or **apoptosis** (app_-a-toe_-sis; from the Greek for "dropping

off," as in leaves dropping in the fall). In the development of an embryo, for example, some cells must die. Carving fingers from stubby limb buds requires the precisely timed death of cells between developing finger bones. During development of the nematode *Caenorhabditis elegans* from a fertilized egg, exactly 131 cells (of a total of 1,090 somatic cells in the embryo) must undergo programmed death in order to construct the adult body.

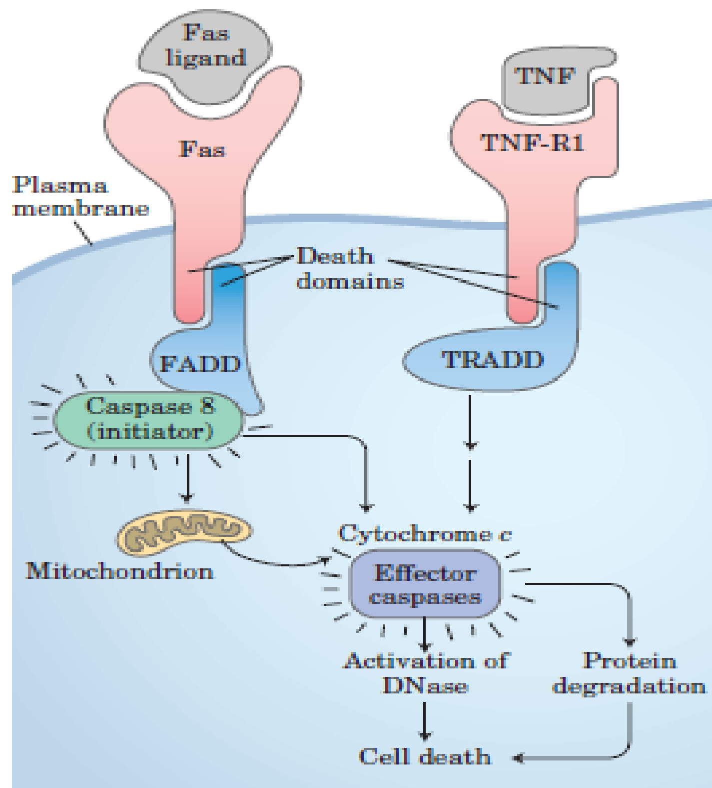
Apoptosis also has roles in processes other than development. When an antibody-producing cell begins to make antibodies against an antigen normally present in the body, that cell undergoes programmed death in the thymus gland—an essential mechanism for eliminating anti-self antibodies. The monthly sloughing of cells of the uterine wall (menstruation) is another case of apoptosis mediating normal cell death. Sometimes cell suicide is not programmed but occurs in response to biological circumstances that threaten the rest of the organism.

For example, a virus-infected cell that dies before completion of the infection cycle prevents spread of the virus to nearby cells. Severe stresses such as heat, hyperosmolarity, UV light, and gamma irradiation also trigger cell suicide; presumably the organism is better off with aberrant cells dead. The regulatory mechanisms that trigger apoptosis involve some of the same proteins that regulate the cell cycle.

The signal for suicide often comes from outside, through a surface receptor. Tumor necrosis factor (TNF), produced by cells of the immune system, interacts with cells through specific TNF receptors. These receptors have TNF-binding sites on the outer face of the plasma membrane and a "death domain" of about 80 amino acid residues that passes the self-destruct signal through the membrane to cytosolic proteins such as TRADD (*T*NF *r*eceptor-*a*ssociated *d*eath *d*omain) . Another

receptor, Fas, has a similar death domain that allows it to interact with the cytosolic protein FADD (*F*as-*a*ssociated *d*eath *d*omain), which activates a cytosolic protease called caspase 8. This enzyme belongs to a family of proteases that participate in apoptosis; all are synthesized as inactive proenzymes, all have a critical *C*ys residue at the active site, and all hydrolyze their target proteins on the carboxyl-terminal side of specific *Asp* residues (hence the name caspase). When caspase 8, an "initiator" caspase, is activated by an apoptotic signal carried through FADD, it further self-activates by cleaving its own proenzyme form. Mitochondria are one target of active caspase 8.

The protease causes the release of certain proteins contained between the inner and outer mitochondrial membranes: cytochrome *c* and several "effector" caspases. Cytochrome *c* binds to the proenzyme form of the effector enzyme caspase 9 and stimulates its proteolytic activation. The activated caspase 9 in turn catalyzes wholesale destruction of cellular proteins—a major cause of apoptotic cell death. One specific target of caspase action is a caspase-activated deoxyribonuclease. In apoptosis, the monomeric products of protein and DNA degradation (amino acids and nucleotides) are released in a controlled process that allows them to be taken up and reused by neighboring cells. Apoptosis thus allows the organism to eliminate a cell without wasting its components.



Initial events of apoptosis.

Genomic Instability Diseases

Ataxia-telangiectasia, Fanconi anemia, and Bloom syndrome are important examples of hereditary diseases resulting from mutations in genes that contribute to genome stability. Different patterns of chromosomal breaks and rearrangements are visible by light microscopy of metaphase cells. The underlying genetic defects predispose affected individuals to different types of cancer.

A. Ataxia-telangiectasia (A-T)

A-T is a variable disease due to autosomal recessive mutations in the *ATM* gene at gene map locus 11q23. The main manifestations are immune defects, cerebellar ataxia, and characteristic telangiectasias of the conjunctivae (**1**), which develop in early childhood. Affected individuals are highly sensitive to irradiation and are prone to develop lymphomas and leukemias. The *ATM* gene has 66 exons spanning

150 kb of genomic DNA. A 3056- amino acid (350kD) protein kinase, ATM, is translated from its alternatively spliced 13-kb transcript. ATM is activated in response to double-strand DNA breaks. It has a central role in a network of proteins that regulate cellular responses to DNA damage and recombination. Mutations in a related gene result in the Nijmegen breakage syndrome.

B. Fanconi anemia (FA)

FA is a heterogeneous group of autosomal recessive and X chromosomal diseases manifest in early childhood as pancytopenia, growth deficiency (**1**), hypoplastic radius often with hypoplastic or absent thumbs (**2**), and other malformations. About eight FA genes form a complementation group (see table in appendix). The proteins encoded by these genes form the FA complex. Together with other proteins, they detect DNA damage or errors in replication. The most prevalent mutation is of *FA-A* (also called *FANCA*), in about 65% of patients. FA cells are hypersensitive to DNA-crosslinking agents, such as diepoxybutane (DEB), which induces chromosomal breaks.

C. Bloom syndrome (BLM)

BLM is a prenatal and postnatal growth deficiency disease (birth weight 2000g, birth length 40 cm, adult height ca. 150 cm) with a distinct phenotype (**1**) including a narrow face, sunlight-induced facial erythema, variable immune deficiency, and a greatly increased risk of different malignancies (about 1in 5 patients). Chemotherapy is very poorly tolerated. The hallmark is a tenfold increase in the spontaneous rate of sister chromatid exchanges. Breaks in one or both chromatids and exchanges between homologous chromosomes occur in about 1–2% of metaphase cells. BLM results from autosomal recessive mutations in the *BLM* gene at gene map locus 15q26.1, encoding a member of the RecQ family of DNA helicases. The 1417-amino acid BLM protein interacts with the FA complex and is involved in meiotic recombination. It is homologous to yeast Sgs1 (slow growth suppressor) and the human WRN protein (Werner syndrome). Mainly protein-truncating nonsense mutations are distributed fairly evenly along the gene (**4**), but some missense mutations exist. Most distinct is a founder mutation in populations of Ashkenazi Jewish origin, consisting of a 6-bp deletion/7-bp insertion at nucleotide 2281. Homozygosity for mutations in the *BLM* gene results in an increased rate of somatic mutations.

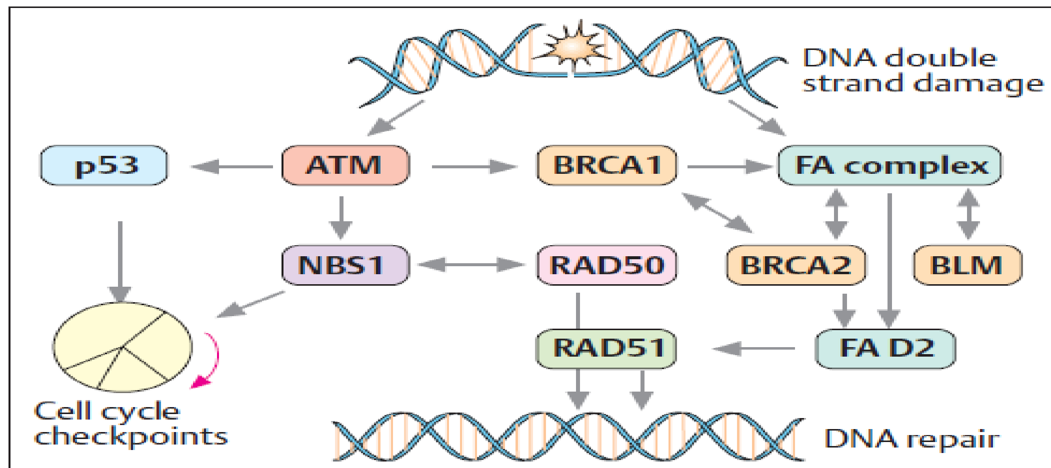


Figure: Relation of ATM to other proteins Maintaining genomic stability

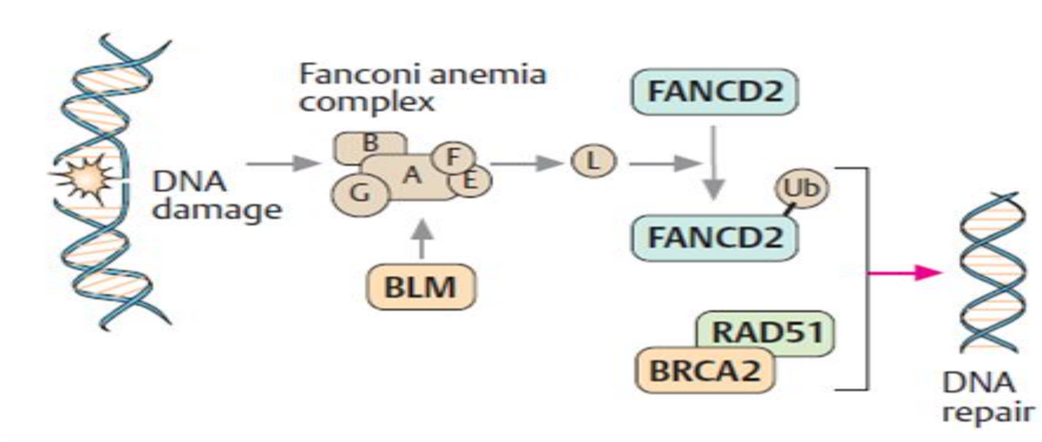


figure: Fanconi anemia-associated proteins

Chromosomes and cancer cells

Two prominent features of cancer cells are abnormal numbers of chromosomes **(aneuploidy)** and large-scale structural rearrangements of chromosomes.

These chromosome aberrations are caused by genomic instabilities inherent to most cancers. **Aneuploidy arises through chromosomal instability (CIN**) by the persistent loss and gain of whole chromosomes.

Chromosomal rearrangements occur through **chromosome structure instability (CSI**) as a consequence of improper repair of DNA damage. The mechanisms that cause CIN and CSI differ.

Both CIN and CSI are associated with advanced stage tumors with increased invasiveness and resistance to chemotherapy, indicating that targeted inhibition of these instabilities might slow tumor growth. Here, we review recent efforts that define the mechanisms and consequences of CIN and CSI.

Breast Cancer Susceptibility Genes

Breast cancer is one of the most common forms of cancer, accounting for 32%of all cancers in the Western world. Two genes confer susceptibility to breast and ovarian cancer when mutated, the breast cancer genes *BRCA1* and *BRCA2*. Both encode multifunctional proteins that play important roles in genomic stability, homologous recombination, and double-stranded and transcription- coupled DNA repair. The BRCA1 and BRCA2 proteins interact and participate in cell cycle control .

A. The breast cancer susceptibility gene *BRCA1*

The *BRCA1* gene on chromosome 17 at q21.1 accounts for 20–30% of inherited, autosomal dominant forms of breast cancer. This gene has 24 exons spanning 80 kb of genomic DNA. Somatic mutations in breast tissue and germline mutations observed in unrelated patients are evenly distributed throughout the gene. About 55% of all mutations occur in the large (3.4-kb) exon 11. A deletion of an adenine (A) and a guanine (G) in nucleotide position 185 (185delAG) and an insertion of a cytosine in position 5382 (5382insC) are the most frequent, each accounting for about 10% of mutations.

B. The breast cancer susceptibility gene *BRCA2*

Mutations in the *BRCA2* gene, at 13q12, occur throughout the gene. A deletion of thymine at nucleotide position 6174 (6174delT) is relatively frequent (1%) in the Ashkenazi Jewish population. The BRCA2 protein has distinct functional domains. A large central domain consists of eight copies of a 30–80-amino acid repeat, which are conserved in all mammalian BRCA2 proteins (BRC repeats).

Telomerase

It is known that eukaryotic DNA is shortened in somatic cells from division to division because of the characteristics of replication. This occurs in the subtelomeric and telomeric repetitive sequences of chromosomes, and following approx. 50-70 divisions it leads to cell senescence, arrest of cell division and aging.

In germ line cells the telomerase enzyme, which comprises a reverse transcriptase, and a telomeric DNA complementary RNA can restore the length of the telomere. It's crucial in the transmission of the same sized genome from generation to generation. However, telomerase activity is also linked to cancer cells. They can restore the telomeres either by up-regulating telomerase enzyme or by recombination based alternative telomere lengthening. If a cell - due to different mutations - avoids cell death caused by the extreme short telomeres, its genome becomes unstable, leading to the oncogenic transformation of the cell through the aforementioned mutations (amplifications, translocations).

pharmacogenomics

This deals with the influence of genetic variations on drug response in patients by correlating gene expression or single-nucleotide polymorphisms with a drug's efficacy or toxicity. By doing so, pharmacogenomics aims to develop rational means to optimize drug therapy, with respect to the patients' genotype, to ensure maximum efficacy with minimal adverse effects. Pharmacogenomics is the whole genome application of pharmacogenetics, which examines the single gene interactions with drugs. These types of studies are at least as significant as the discovery of new drug targets. Genomic differences between people can result in significant differences in their responses to the drugs. E.g. in 30% of people the β-blockers used against hypertension are ineffective, while the antidepressants are ineffective in 50% of the treated persons. The situation is similar in the cases of most drugs

Goals of Pharmacogenomics has two main goals. One of them is to search for new drugs and drug targets with the help of genomic methods. It has great significance, because current existing therapies only hit about 400 different drug targets compared to the 20-22 thousand protein coding genes coding for about 2 million different proteins (because of e.g. posttranslational modifications, splice variants). In addition, DNA or RNA sequences can also be regarded as potential targets, and we know from the ENCODE project (http://www.nature.com/encode/#/threads) and the amount of conserved regions that about 10% of the human genome has some functions, and the number of cell specific enhancer regions is about 400,000 . Naturally, only a fraction of these are

really drug targets, but according to estimations there are at least 10 times more drug targets than presently exists.

Genomic background of adverse effects One of the main questions of pharmacogenomics is that, what the mechanism is, with which the genetic variants influence the drug-response. **There are three main possible mechanisms** :

Pharmacokinetic: Genetic variations, which influence the mechanisms of absorption and distribution of the administered drug, the chemical changes of the substance in the body, and the effects and routes of excretion of the metabolites of the drug .

Pharmacodynamic: Genetic variants, which are in the genes of the drug targets or in their associated pathways. Pharmacodynamics is often summarized as the study of what a drug does to the body, whereas pharmacokinetics is the study of what the body does to a drug.

Idiosyncratic: Genetic variations in genes coding for proteins, which are not in the drug target or pharmacokinetic pathways, but could influence the drug response. The adverse effects could be caused by e.g. an enzymopathy, so that the triggering substance cannot be processed properly in the organism and causes symptoms by accumulating or blocking other substances to be processed.

Difficulties of the pharmacogenomic researches

1- that the main development of the high throughput genomic, bioinformatic and other methods have been carried out only in the last few years, and there was not enough time (10-15 years) for the marketing of the drugs developed by the new methods.
2- Often environmental factors can cause similar effects as the genetic variants, which is called phenocopy. From a statistical point of view it can cause great difficulties in the evaluation.
3- Another disturbing factor is gene-gene interactions

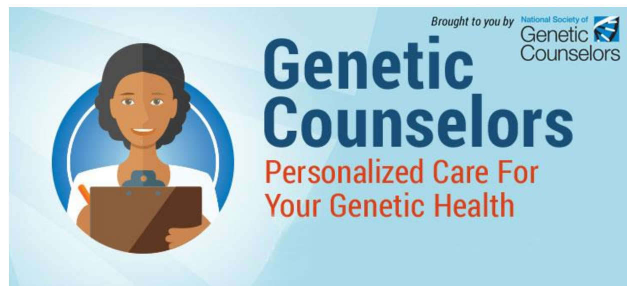
4- There is also a less ethical explanation to why the pharmacogenetics results are so few today. In some cases the pharmaceutical companies are not

interested that their drugs may be used only on people where the drugs are really effective, because it can result in less user and less profit.

Question

1- What are relationship between Ras gene and cancer ?
2- What are different between Apoptosis and Oncogene?
3- What main goals has pharmacogenomics?
4- What is the significance of pharmacogenomics?
5- With what mechanisms can genetic variations influence the drug-response?
6- What are the difficulties of pharmacogenomic researches?

Prenatal diagnosis - principles of diagnostic procedures and genetic counseling



Prenatal diagnosis is enables early diagnosis of congenital anomalies and genetic disorders in utero. Advanced imagining techniques as well as cytogenetic and molecular biology methods provide the means to diagnose prenatally numerous congenital structural malformations and genetic disorders in highrisk families. Early diagnosis in utero can prove essential to management of the pregnancy, prenatal and postnatal medical care, and treatment. It is also crucial to making informed decisions about continuing or terminating the pregnancy.

Prenatal diagnosis techniques Methods of prenatal diagnosis can be divided into non-invasive and invasive techniques.

1- Non-invasive procedures (Non-invasive prenatal testing NIPT):- Used for diagnosing congenital anomalies and risk assessment of given genetic disorders (screening).include

• ultrasound:

– routine obstetric ultrasound scan
– high-resolution ultrasound scan and Doppler studies
– fetal heart echocardiography
• magnetic resonance imaging (MRI)
• maternal serum biochemistry testing (measurement of indicative enzymes in maternal blood serum).

Routine obstetric ultrasound scanning. Performed by the obstetrician managing the pregnancy. Standards for normal pregnancies provide for four scans carried out at: 11-14 weeks, 21-26 weeks, 27-32 weeks, and 40 week of gestation (as recommended by the Ministry of Health, 10 July 2003).

High resolution ultrasound scanning. Performed in any pregnancy with an increased risk of fetal structur al abnormalities, isolated or part of a genetic syndrome (Table 1). Women are referred for high-resolution ultrasound to specialist centers managing high-risk pregnancies. According to the Ultrasound Section of Polish Gynecological Society's recommendations the first scan should take place at 11-13 (+6 days) weeks of gestation (crown rump length 45-84 mm), followed by another scan at 18-23 weeks of gestation. In recent years three-dimensional ultrasound (3D) and four-dimensional ultrasound (4D) have started to play an increasing role in prenatal diagnosis. They can be applied in assessing facial features, central nervous system abnormalities and skeletal defects .

Doppler studies. Detect abnormal blood flow in umbilical, placental, and fetal vessels that may be suggestive of a genetic syndrome (Table 1). Fetal heart echocardiography. Performed at 18-23 weeks of gestation in the presence of an increased risk of heart defect (for example: heart defect in a parent or sibling, abnormal routine ultrasound) .

Magnetic Resonance Imaging. MRI is used in combination with ultrasound, usually at or after 18 weeks' gestation. MRI provides a tool for examination of fetuses with large or complex anomalies, and visualization of the abnormality in relation to the entire body of the fetus. Apparently MRI is a risk-free method .

Table 1. Ultrasound markers of fetal congenital abnormalities or genetic syndromes found in: (A) first trimester scanning [at 11-13 (+6 days) weeks' gestation], (B) second trimester scanning [at 1824 (+6 days) weeks' gestation].

- nuchal translucency > 3 mm: trisomy 21, Turner and other syndromes; heart and great vessels defects
- absence of the fetal nasal bone: trisomy 21
- hypoplastic maxilla: about 50% of fetuses with trisomy 21
- abnormal blood flow velocity in the fetal ductus venosus: in 80% of fetuses with trisomy 21
- omphalocoele: trisomy 18
- hypoplastic bladder: trisomies 18 and 13
- single umbilical artery: trisomy 18
- intra-uterine growth trisomy 18 and triploidy, rare in trisomy 13 and Turner syndrome

(B)

- congenital heart defects, ventriculomegaly - trisomies 13, 18, 21; triploidy, Turner syndrome
- echogenic intracardiac focus (EICF) found at 16-20 weeks of gestation; right-handed, bilateral, large, isolated foci in women aged above 35 or in women with abnormal biochemistry tests results – trisomies 13, 18, 21
- holoprosencephaly - trisomies 13 and 18
- choroid plexus cysts > 3mm (found at 14-24 weeks of gestation) in women > 35 years of age, in women with abnormal biochemistry tests results – trisomies 13, 18, 21; in fetuses with other malformations – trisomies 18, 21
- agenesis/hypoplasia of corpus callosum - trisomy 18
- enlarged cisterna magna >10 mm with additional abnormalities found in ultrasound scan – trisomy 18, di George syndrome, Merkel-Gruber syndrome
- Dandy-Walker complex – trisomies 13,18, triploidy
- cleft lip - trisomies 13, 18
- micrognathia - trisomy 18, triploidy
- diaphragmatic hernia – trisomies 13, 18
- encephalocele - trisomy 18, triploidy
- omphalocoele - trisomy 18
- polydactyly/syndactyly - trisomy 13, triploidy
- thickened nuchal fold or nuchal oedema (>5mm at 16-18 weeks of gestation, >6mm at 18-24 weeks of gestation), cystic hygroma – Turner syndrome, trisomies 13, 18, 21, Noonan syndrome, neonatal bone dysplasia, heart defects
- duodenal atresia & echogenic bowel - trisomies 13, 18 , 21, cystic fibrosis, congenital infections, bowel malformations)
- kidney defects - trisomies 13, 18, 21, Turner syndrome
- mild ventriculomegaly (10-15 mm) found at 16-20 weeks of gestation – trisomy 21, central nervous system abnormalities, congenital infections
- short femur/humerus length - trisomy 21, neonatal bone dysplasia
- fifth finger clinodactyly (absence or shortening of the mid-phalanx of the fifth finger) - trisomy 21
- intra-uterine growth retardation - trisomy 18, Turner syndrome, triploidy

Biochemistry testing (maternal serum markers)

can be applied as a screening technique for every pregnant woman. Screening in the first trimester involves the measurement of PAPP-A(pregnancy associated plasma protein A) and free -HCG (-human chorionic gonadotropin) levels in maternal serum. These measurements are used in conjunction with ultrasound scanning that includes assessment of ultrasound markers such as nuchal translucency (NT) thickness and absence/presence of the nasal bone (NB). The detection rate (DR) of these combined methods is about 8590% in regard to trisomy 21 and 18, for a false positive rate of 5%. DR for nuchal translucency alone is 75% for a false positive rate of 5%. Abnormal nuchal translucency

thickness measurements can be associated with other disorders such as: heart defects, Beckwith-Wiedemann syndrome, achondroplasia, SmithLemli-Opitz syndrome, osteogenesis imperfecta, Noonan syndrome, and with pregnancies complicated by arterial hypertension or gestosis.

Second trimester maternal serum biochemistry (at 14-18 weeks of gestation) involves the "triple," "quadruple" (triple screen and inhibin A) and "integrated" screen. The "triple" screen is the measurement of alphafetoprotein (AFP), free beta human chorionic gonadotropin (β-HCG), free estriol (uE3) levels in maternal serum. The values of these parameters can be influenced by the presence of maternal diabetes type 1, smoking and pregnancy-related weight gain . The detection rate for this test is increased by determining the Ulm index (which eliminates the influence of maternal age on test results). Second trimester maternal serum biochemical testing is carried out as a screening method for Down and Edwards syndrome, open neural tube defects (anencephaly, myomeningocele, omphalocele and gastroschisis). DR for trisomy 21 and 18 is 60-70%, for a false positive rate of 6%. Abnormal second trimester maternal serum biochemical test results are an indication for high-resolution ultrasound in the second and third trimester and/or invasive prenatal diagnosis. Evaluation of the severity and the etiology of the anomaly is an important prognostic factor.

2- **Invasive procedures:-** Invasive procedures involve direct examination of fetal cells or tissues. Classical cytogenetic**, molecular and biochemical methods (performed on uncultured or cultured cells) are the most frequently used in prenatal invasive diagnosis**.

The procedures should take place in specialist centers that manage high-risk pregnancies. When considering invasive methods all indications and criteria need to be carefully evaluated as there is a considerable risk to the pregnancy . Invasive techniques include:

- **chorionic villus sampling** (trophoblast cells analysis)
- **amniocentesis** (amniotic fluid cells analysis)
- **cordocentesis** (Percutaneous Umbilical Blood Sampling)

Chorion villi sampling (CVS)-

a sample of the developing placenta is obtained transcervically or transabdominally at 8-11 weeks of gestation under ultrasound guidance.

A variety of diagnostic techniques can be employed on CVS cells:

• **karyotype analysis (classical and molecular cytogenetic methods)** - detects all numerical and many structural chromosome aberrations, including microdeletions that cause syndromes such as Prader-Willi or William's syndrome,

• **enzyme studies,** for example when there is a risk of inborn errors of metabolism (phenylketonuria, Gaucher disease, mucopolysaccharidosis, haemoglobinopathies such as thalassaemia),

• **DNA analyses in** monogenic disease (a preferred method for molecular studies).

The following problems may arise in CVS:

• placental mosaicism (confined to trophoblast tissue not fetal tissue),

• contamination by maternal tissue

Amniocentesis - can be performed at 13-15 weeks of gestation (early amniocentesis) but usually done at 1618 weeks of gestation. A sample of about 15 ml of amniotic fluid is obtained transabdominally under ultrasound guidance. **Employed methods of analysis include**:

• karyotyping (cytogenetic as well as molecular cytogentic methods) • DNA analysis (monogenic disease diagnosis, such as congenital adrenal hyperplasia, cystic fibrosis)

• biochemical studies:

– measurement of AchE and AFP levels when considering neural tube defects

– measurement of 17α-hydroprogesterone when a risk of congenital adrenal hyperplasia

– inborn errors of metabolism diagnosis (mucopolysaccharidosis, familial hypercholesterolaemia, adrenoleucodystrophy, homocysteinuria, maple syrup urine

disease) The risk of amniocentesis is around 0.5-1% and it includes miscarriage, transient amniotic fluid leakage and intrauterine infection

Cordocentesis (Percutaneous Umbilical Blood Sampling) - a sample of 0.5-1 ml fetal blood is obtained from the umbilical vein (close to the placenta) usually at 18-23 weeks of gestation under ultrasound guidance. The blood sample can be used for genetic and biochemical studies, including chromosomal analysis and monogenic disease diagnosis (phenylketonuria, cystic fibrosis, Duchenne muscular dystrophy). Furthermore it is also possible to detect haemoglobinopathies, immunological deficiency syndromes (ataxia-teleangiectasia) and intrauterine infections (toxoplasmosis, rubella, cytomegaly). The risk is estimated to be around 2% with fetal death, premature birth, bleeding (usually transient) and fetal bradycardia (usually short lasting) being the most frequent complications.

Currently in Europe there is an on-going discussion about allowing for changes to prenatal diagnosis algorithms because of the introduction of new rapid diagnostic techniques (Rapid Tests - RT), of chosen chromosomal defects. These recommended diagnostic techniques include:

1. Rapid-FISH (rapid fluorescence in situ hybridization),
2. MLPA(multiple ligation PCR amplification)
3. QF-PCR (Quantitative Fluorescent Polymerase Chain Reaction).

Additionally the use of methods such as FISH, PCR, MLPA or array-CGH (micro array comparative genomic hybridization) is suggested, the latter being especially useful in detecting genomic imbalance in the fetus (duplications/deletions)

NIPT raises a broad range of ethical issues, which might be understood in terms of the following values:

■ Choice, autonomy and consent – our ability to make free, informed choices about the medical tests and treatments we undergo is considered to be an important principle in modern healthcare.

■Avoidance of harm – the Government has a duty to eliminate or reduce harms caused by healthcare interventions such as NIPT that are available through the NHS, or to consumers in the private healthcare sector.

■ Equality, inclusion and fairness – the Government and NHS each have a duty to promote equality and ensure that all people are treated fairly. This involves developing policies that address prejudice, bias and discrimination, and ensuring that public money is spent fairly.

What Is Genetic Counseling?

The National Society of Genetic Counselors (NSGC) defines genetic counseling as the process of assisting people with understanding and adapting to the medical, psychological, and familial implications of genetic contributions to disease. **This process includes**

1- the interpretation of family
2- medical histories to assess the chance of disease occurrence or recurrence.Genetic counseling **usually involves** providing education about inheritance, testing options, disease management, and prevention. Genetic counseling also promotes informed choices and adaptation to the risk or condition.

Role of genetic counselor

- Diagnose
- Explain risks of recurrence
- prognosis and management
- Explain possible methods for prevention, including prenatal screening and diagnosis, and preimplantation diagnosis
- NOT DIRECTIVE

Ethical issues in genetic counseling

- Autonomy: the couple should take their own decision
- Informed choice: the decision is based on the information given by the counselor
- Informed consent: The individual or couple should give their informed consent for any investigation
- Confidentiality

Pedigree charts

Pedigree chart:- are diagrams that show the phenotypes and/or genotypes for a particular organism, its ancestors, and descendants. While commonly used in human families to track genetic diseases, they can be used for any species and any inherited trait. Geneticists use a standardized set of symbols to represent an individual's sex, family relationships and phenotype. These diagrams are used to determine the mode of inheritance of a particular disease or trait, and to predict the probability of its appearance among offspring.

Pedigree analysis is therefore an important tool in basic research, agriculture, and genetic counseling. Each pedigree chart represents all of the available information about the inheritance of a single trait (most often a disease) within a family.. In real pedigrees, further complications can arise due to incomplete penetrance including age of onset) and variable expressivity of disease alleles, , the phenotype accurately reflects the genotype. A pedigree may be drawn when trying to determine the nature of a newly discovered disease, or when an individual with a family history of a disease wants to know the probability of passing the disease on to their children. In either case, a tree is drawn, as shown in Figure 6-2, with circles to represent females, and squares to represent males. Matings are drawn as a line joining a male and female, while a consanguineous mating (closely related, such as siblings or first cousins) is two lines.The symbols commonly used in pedigrees are summarized in figure 1

	Male	Female	Sex unknown or unspecified
Unaffected individual			
Individual affected with trait			
Obligate carrier (carries the gene but does not have the trait)			
Asymptomatic carrier (unaffected at this time but may later exhibit trait)			
Multiple individuals (5)	5	5	5
Deceased individual			
Proband (first affected family member coming to attention of geneticist)	P	P	P
Family history of individual unknown	?	?	?
Family— parents and three children: one boy and two girls in birth order	I 1 2 II 1 2 3		
Adoption (brackets enclose adopted individuals. Dashed line denotes adoptive parents; solid line denotes biological parent)			
Twins	Identical	Nonidentical	Unknown ?

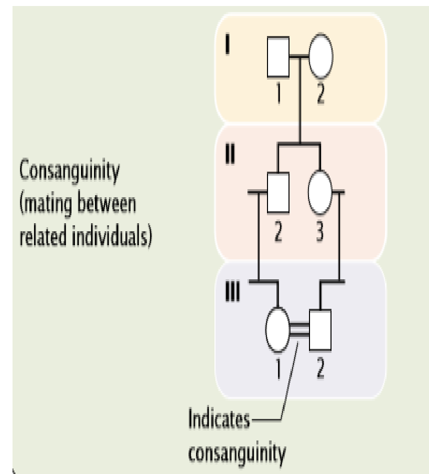


Table 6.1 Pedigree characteristics of autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant, and Y-linked traits

Autosomal recessive trait

1. Appears in both sexes with equal frequency.
2. Trait tends to skip generations.
3. Affected offspring are usually born to unaffected parents.
4. When both parents are heterozygous, approximately 1/4 of the offspring will be affected.
5. Appears more frequently among the children of consanguine marriages.

Autosomal dominant trait

1. Appears in both sexes with equal frequency.
2. Both sexes transmit the trait to their offspring.
3. Does not skip generations.
4. Affected offspring must have an affected parent, unless they possess a new mutation.
5. When one parent is affected (heterozygous) and the other parent is unaffected, approximately 1/2 of the offspring will be affected.
6. Unaffected parents do not transmit the trait.

X-linked recessive trait

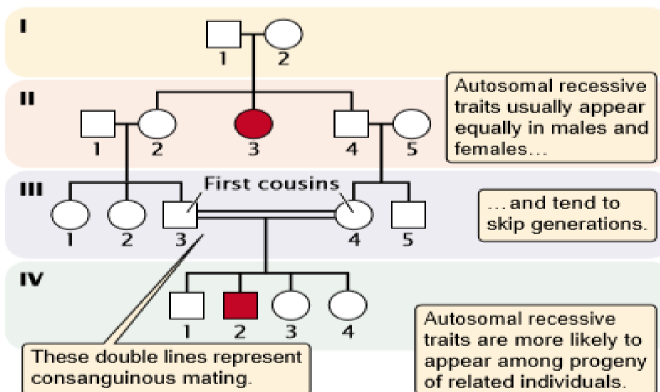
1. More males than females are affected.
2. Affected sons are usually born to unaffected mothers; thus, the trait skips generations.
3. A carrier (heterozygous) mother produces approximately 1/2 affected sons.
4. Is never passed from father to son.
5. All daughters of affected fathers are carriers.

X-linked dominant trait

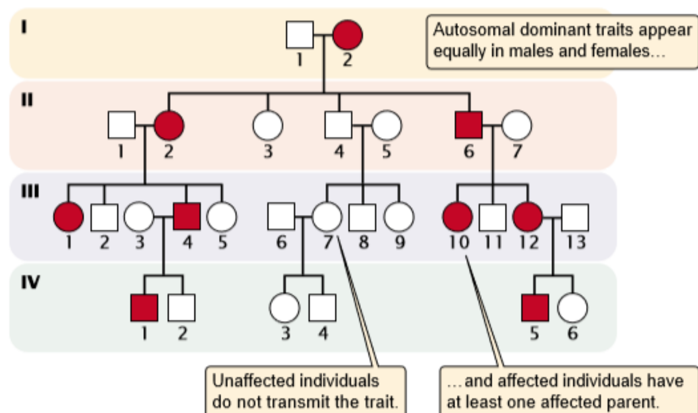
1. Both males and females are affected; often more females than males are affected.
2. Does not skip generations. Affected sons must have an affected mother; affected daughters must have either an affected mother or an affected father.
3. Affected fathers will pass the trait on to all their daughters.
4. Affected mothers (if heterozygous) will pass the trait on to 1/2 of their sons and 1/2 of their daughters.

Y-linked trait

1. Only males are affected.
2. Is passed from father to all sons.
3. Does not skip generations.

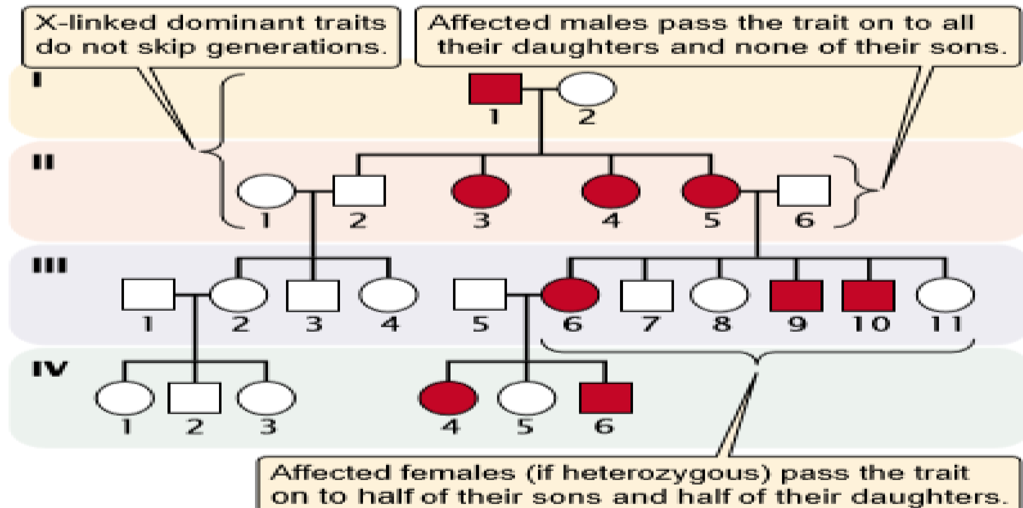


6.4 Autosomal recessive traits normally appear with equal frequency in both sexes and seem to skip generations.

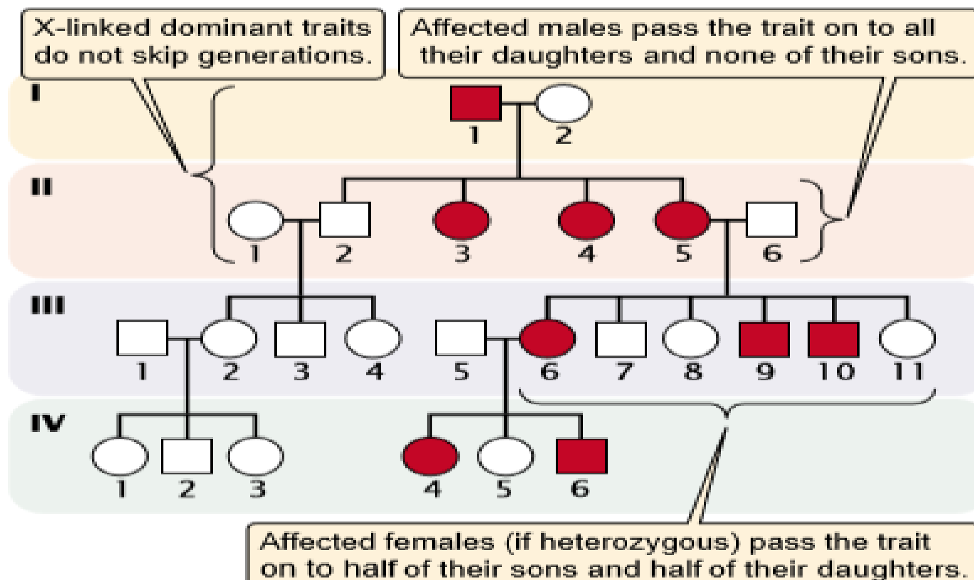


Autosomal dominant traits normally appear with equal

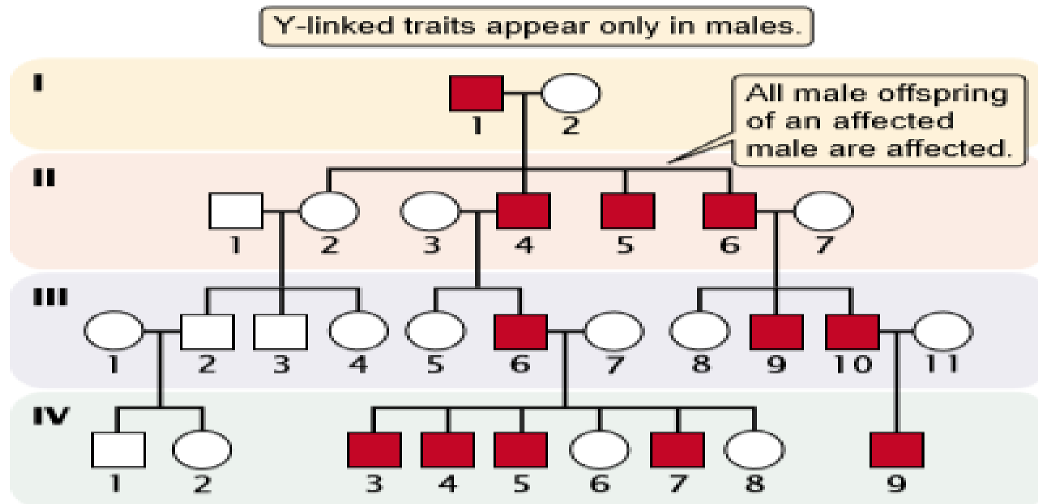
frequency in both sexes and do not skip generations



6.9 **X-linked dominant traits affect both males and females.** An affected male must have an affected mother.

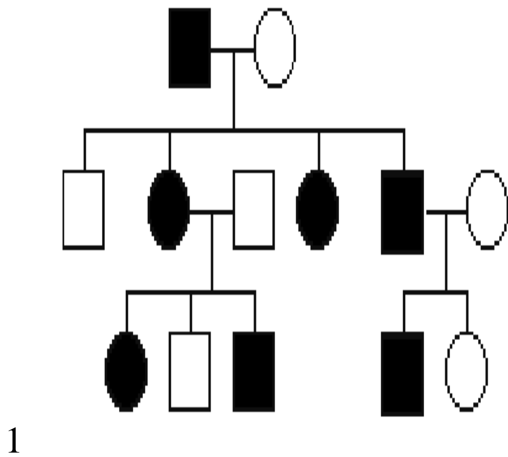


6.9 **X-linked dominant traits affect both males and females.** An affected male must have an affected mother.



6.10 **Y-linked traits appear only in males and are passed from a father to all his sons.**

Q1/What is the mode of inheritance in the following pedigrees?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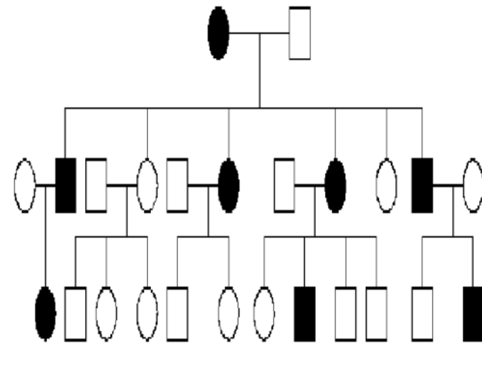
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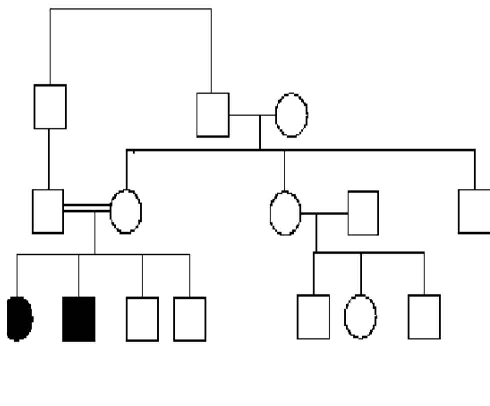
3

Q2/ Mr E is a 28-year-old man with a younger brother who died from type 2 Gaucher disease, an autosomal recessive lysosomal storage disorder that is fatal by the age of 2 years. What is the probability that Mr E is a heterozygous carrier for the Gaucher mutation? A) 0.25 B) 0.33 C) 0.50 D) 0.67 E) 0.75