MINISTRY OF HEALTH OF UKRAINE

ODESSA NATIONAL MEDICAL UNIVERSITY

Department of clinical immunology, genetics and medical biology

APPROVED

Vice-rector for research and educational work,

_____ Eduard BURYACHKOVSKY

01 September 2023

METHODICAL DEVELOPMENTS FOR PRACTICAL LESSONS IN MEDICAL GENETICS

Faculty of Medicine Course IV

Educational discipline MEDICAL GENETICS

Level of higher education: second (master's)

branch of knowledge: 22 «Health Care»

specialty: 222 «Medicine»

educational and professional program: Medicine
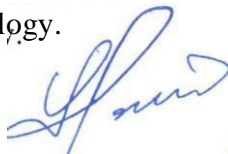
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Developments are discussed and approved at a methodic meeting of the Department of Clinical Immunology, Genetics and Medical Biology.

Minutes № 1,   28.08.2023.

Head of the department, professor.   Sergiy GONCHARUK

Reviewed and approved at a meeting of the Department

Minutes №_____ 202___

Reviewed and approved at a meeting of the Department of Medical Biology and Chemistry
Minutes № _1_ __7.09_____ 202_3_ .

Head of the department
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Reviewed and approved at a meeting of the Department of Medical Biology and Chemistry

Reviewed and approved at a meeting of the Department of Medical Biology and Chemistry
Minutes № ___ _____ 202____.

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PRACTICAL TRAINING

Practical lesson No. 1
Topic: Classification and etiology of hereditary diseases
The goal: To integrate the knowledge of the students about mutations like etiology of hereditary disorders.

Student should know :
- – frequency of congenital and hereditary pathology in different periods of ontogenesis
- – classification of mutations and mutagenic factors;
- – concept, effects of genomic imprinting,
- – lethal effects of mutations (significance in perinatal, early childhood and infant mortality, association with infertility, spontaneous abortions),
- – classification of hereditary pathology,

Student has to be able:
- • to analyze the karyotypes of the patients with the most common chromosomal diseases and determine the type of structural or numerical chromosomal aberration;
- • to detect types of single gene mutations

Control questions

1. Subject and tasks of medical genetics.
2. Definition, general characteristics and classification of hereditary disorders
3. Mutations as etiological factors of hereditary pathology. Definition and classification of mutations
4. Types of gene mutations.
5. Chromosomal mutations (structural chromosomal aberrations). Balanced and imbalanced chromosomal aberrations.
6. Numerical chromosomal mutations (genome mutations).
7. Standard nomenclature for chromosome karyotypes
8. Spontaneous and induced mutations. General characteristics and classification of mutagenic factors.
9. Somatic and germ mutations. Disorders of somatic cells.
10. Epigenetic mutations. Chromosomal and genomic imprinting (the content, notion and effects).
11. Main protective mechanisms which can decrease the pathological effects of mutations.
12. Heredity and the outcome of diseases. Lethal effects of mutations (importance in the perinatal and infancy mortality, role in infertility, spontaneous miscarriage).

PRACTICAL WORK
1. Analyze karyotypes of the patients with chromosomal pathology and determine the types of structural and numerical chromosomal mutations
2. Analyze situational tasks and determine the types of mutations

Main literature
1. Methodical recommendations on medical genetics

Additional literature:
1. Genetics in medicine. - 7th edition/Robert L/Nussbaum, Roderick R. McInnes, Huntington F. Willard. – 2007 – 585 p.
2. Emery's Elements of medical genetics. 15th ed. / Peter Turnpenny, Sian Ellard. – Elsevier, 2017. – 400 pp.

3. Lynn B. Jorde, John C. Carey, Michael J. Bamshad. Medical genetics. 5th ed. Elsevier, 2016. 356 pp.
4. Vogel and Motulsky's human genetics. Problems and approaches / M. R. Speicher, S. E. Antonarakis, F. G. Motulsky. 4th addition. – Springer, 2010. – 981 pp.
5. Young Ian.D. Medical genetics. -2nd ed. -Oxford university press, 2010. - 304 p.
6. Diseases of the fetus and newborn. Pathology, radiology and genetics. G.B.Reed, A.E. Claireaux and A.D.Bain., - Great Britain, - 1989, 812 p.
7. Human molecular genetics. Tom Strachan, Andrew P.Read. – 4th edition - Bios Scientific Publisher, 2010, 680 p.
8. Smith recognizable patterns of human malformation. Seventh edition. John M. Graham, - USA, - 2013, 976 p.
9. R. Wiedemann, K.-R. Gross, H.Dibberin ,Atlas of characteristic syndromes. A Visual Aid to Diagnosis. Second edition. -London, 1986,412 p.

13. Information resources:

https://ghr.nlm.nih.gov National librery of medicine, genetics
https://www.orpha.net The portal for rare diseases and orphan drugs
https://rarediseases.org National Organization for Rare Disorders
http://omim.org/OMIM (Online Mendelian Inheritance in Man) – An Online Catalog of Human Genes andGenetic Disorder

Genetics (Greek genesis-origin production) — science concerning inheritance and variations of organisms.

The basic principles of inheritance and variations is applicable to all living organisms as they are applicable to men. The branch of genetic which deals with inheritance and variations in men is termed as human genetics.

The medical genetics deals with the inheritance of human diseases, works out the methods of diagnosis, treatment and preventive treatment of hereditary pathology.

Clinical genetics is the branch of medical genetics solving practical problems, as to establish a diagnosis, to calculate the probability of affected child, to prevent the genetic disease.

Nowadays it is known more then 4000 mendelian monogenic or single gene diseases, more then 1000 chromosomal and a large group of multifactorial disorders. Each individual has from 5 to 30 (on the average 10) recessive gene of single gene disorders. Besides new mutation can occur in population. It has been estimated that new mutations occur in about 10% individuals in population.

Each hereditary disease is rather rare in a population. But taking into account the enormous number of nosological forms, it is possible definitely to say that hereditary diseases belong to the most wide spread group of disorders. Besides on G. Fancony expression rear disorders are really rear up to this time while they are known for us a little.

In the whole each family has the genetic risks 5 % to have children with hereditary diseases or abnormalities.

Not less then 10 % of population have multifactorial disorders (hypertension, shizophrenia, ishaemic heart diseases, tumors and others). Risk of its development can be predicted before the childbirth. The early prophylaxis permits to prevent the development of this disorders or to delay the onset of its manifestation. It is necessary to note that the further progress in the treatment and prophylaxis those diseases is closely connected to development of medical genetics and biotechnology.

All described was a ground of introduction of a new course of medical genetics in educational program of medical High School. The main purpose of this subject is systematization and integration of the students' knowledge about basic laws of heredity and variation in humans and on the basis of it to study the common principles of diagnostic, treatment and prophylaxis of

hereditary diseases, medico-social rehabilitation of the patient.

Definition, general characteristics and classification of hereditary disorders

Hereditary or genetic disorders are the disorders caused by changes in genotype (by mutations). Students often define hereditary disorder as a disease, which is transmitted in the family from generation to generation, a disease that should be obligatory inherited by children from the parents. But this is not the exact definition. Hereditary disorder often considerably reduces viability or fertility of the patient and cannot be transmitted to offspring. They always result from new mutations. For example, as a rule, a child with Down syndrome has healthy parents. On other side, certain endemic diseases are observed in parents and children and gives impression of inheritance, but they are not hereditary (endemic goiter).

Terms "hereditary" and "congenital" diseases are also different. As congenital are known all conditions which are present at birth. Most hereditary disorders are congenital, but some of them are not congenital in term of age of onset. Onset of the disease may be after several months or even years after birth (mucopolysaccharidosis, hepatolenticular dystrophy, Huntington's disease and others). On other side, not all congenital abnormalities are genetic in origin. Some of them are because of teratogenic environmental effect and are nonhereditary (e.g. alcohol syndrome).

"Family disorders" are met among members of one family. It may be either hereditary (usually autosomal-recessive) or nonhereditary (caused by the same environmental factor like harmful habits, profession, peculiarities of nourishment and others).

Classification of hereditary disorders is based on classification of mutations and character of interaction with the environment. Under modern classification all hereditary disorders may be divided into following groups.

1. Single gene (mendelian or monogenic) disorders are the disorders caused by mutations of single gene. They are usually transmitted in simple patterns as originally described by Gregor Mendel (achondroplasy, phenylketonuria, haemophilia and others).

2. Chromosomal disorders are caused by numerical or structural chromosome mutations. Examples include Down syndrome, cat cry syndrome.

3. Multifactorial disorders (disorders with hereditary predisposition) are the result of the combined effect of multiple genetic and provoking environmental factors (cleft lip and palate, clubfoot, arterial hypertension, schizophrenia and others).

4. Genetic disorders of somatic cells are caused by somatic mutations. Accumulating somatic mutations are now known to play a major role in causing cancer and they probably also explain certain sporadic cases of congenital defects as well as the ageing process itself.

5. Disorders because of mother-fetus genetic incompatibility (hemolytic anemia because of Rh-conflict or because of incompatibility on other antigens).

Clinical classification is based on organic principles: psychic, neuromuscular, skeletal, facial, cutaneous, respiratory, immunity system disorders and others.

DEFINITION AND CLASSIFICATION OF MUTATIONS

Mutation is defined as a heritable alteration or change in genetic material (in genotype). Mutations can be classified in several different ways.

1. *Somatic versus germ-line mutations.* Mutations can occur in the germ cells-gametes. They can be passed on to the progeny. Such mutations are called *germ-line, or germinal, mutations*. On the other hand, mutations can arise in the somatic sells. They may change the phenotype of the individual that suffers the mutations, but since mutation does not affect the gametes, it cannot be transmitted to offspring. Such types of mutations are called *somatic mutations*.

2. *Spontaneous and induced mutations.* Naturally occurring mutations are referred to as *spontaneous mutations*. Only from 1/10 to 1/4 of spontaneous mutations can

be explained by the effect of natural radiation, influence of free radicals and peroxides, which are constantly formed inside the cells during metabolic processes. Greater part of spontaneous mutations arise through chance errors in chromosomal division, DNA replication and DNA reparation. *Induced mutations* are caused by environmental agents. These agents are known as *mutagens*.

3. ***Gene mutations and chromosomal mutations.*** *Gene mutation* is an alteration of a DNA sequence in a single gene. C*hromosomal mutations* include rearrangements in the structure of the chromosomes (structural chromosomal mutations or chromosomal aberrations) or in the number of chromosomes (numerical chromosomal mutations or genome mutations).

TYPES OF GENE MUTATIONS

Gene mutation is an alteration of a DNA sequence in a single gene. They can occur in coding and noncoding sequences. If they occur in coding sequences they can lead to hereditary disorders. Gene mutations occur in two forms: point mutations which involve a change in the base present at any position in a gene and cross mutations which involve alterations of longer stretches of DNA sequence. Gene mutations may be considered in two main classes according to how they are transmitted (Table 1). They may be fixed (or stable) and dynamic (unstable).

Table 1. The main classes and types of mutations and effects on protein products.

Classes	Types	Effect on protein product
Stable (fixed)	Substitution	Missense – replacement of one amino acid by another Silent-same amino acid Nonsense – premature termination of translation with loss-of-function
	Insertion Deletion	Multiple of 3 (codon) - deletion or insertion of one or more amino acids- alteration of function Not multiple of 3 – altered reading frame- altered amino acid sequence, loss-of-function
	Duplication	altered reading frame- altered amino acid sequence, loss-of-function
	Inversion	Replacement of one or more amino acids
Dynamic (unstable)	Expansion of triplet repeats	Alteration of gene expression- alteration of protein structure and function

Stable mutations can be transmitted unaltered from generation to generation. They can be classified according to the specific molecular changes or according to the effects on protein structure. Main types of stable gene mutations are:

1. ***A substitution*** is the replacement of a single nucleotide by another. It is the most common type of mutations. Substitutions may have different effects on protein structure. They usually lead to replacement of one amino acid. Such kind of mutations is called *missense mutation*.

For example, sickle cell anemia is the result of a single missense mutation that effects a substitution of valine for glutamic acid in the six position of the β-globin polypeptide chain.

Normal DNA   DNA after mutation

DNA   CTC   **DNA   CAC**
mRNA GAG   mRNA GUG
amino acid Glu   amino acid Val

Due to degeneracy of the genetic code substitutions may not cause the alteration of amino acid sequence. Such substitutions usually occur in the third base position of the codon. They are called *silent mutations*. This class of mutations is the most frequently observed in coding DNA, because they are neutral mutations and not subject to selection pressure. Silent mutations tend to

accumulate in the DNA of organisms where they are known as *DNA-polymorphisms*. Sometimes a substitution can lead to replacement of a codon specifying an amino acid by a termination codon. Such type of mutations is called *nonsense mutation*. The protein will be shorter than a normal protein, or protein is not synthesized at all.

2. *An insertion* is the addition of one or more nucleotides into a gene, usually from another part of a chromosome. Several types of DNA sequences are capable of propagating copies of themselves; this copies are then inserted in other location on chromosomes (examples include mobile elements - the SINEs and *Alu* repeats, discussed in Chapter 1). Such insertions can cause frameshift mutations.

Normal DNA	CGG-TAT-TCG-ATG-AAG
Normal mRNA	GCC-AUA-AGC-UAC-UUC
Normal protein	Ala – Ile – Ser – Tyr – Phe

Insertion of **TG** →frameshift mutation

Mutant DNA	CGG-TA**T-G**TT-CGA-TGA-AG
mRNA	GCC-AUA-CAA-GCU-ACU-UC
protein	Ala – Ile – Gln – Ala – Thr

Insertions of mobile elements can cause isolated cases of type I neurofibromatosis, Duchenne muscular dystrophy, β-thalassemia, familial breast cancer, familial polyposis colon cancer and hemophilia A and B.

3. *A deletion* is the loss of one or more nucleotides.

4. *A duplication* – nucleotide dabbling.

4. *An inversion* - a portion of the DNA sequence is excised then re-inserted at the same position but in the opposite orientation. (rotation of the gene sequence by 180^0).

Mechanisms 2 – 4 cause the changes in general number of nucleotides and leads to the *frameshift mutations*. Frameshift mutation - this is result from the insertion of extra bases or the deletion of existing bases from the DNA sequence of a gene. If the number of bases inserted or deleted is not a multiple of three the reading frame will be altered and the ribosome will read a different set of codons downstream of the mutation. Frameshift mutations usually have a serious effect on the encoded protein.

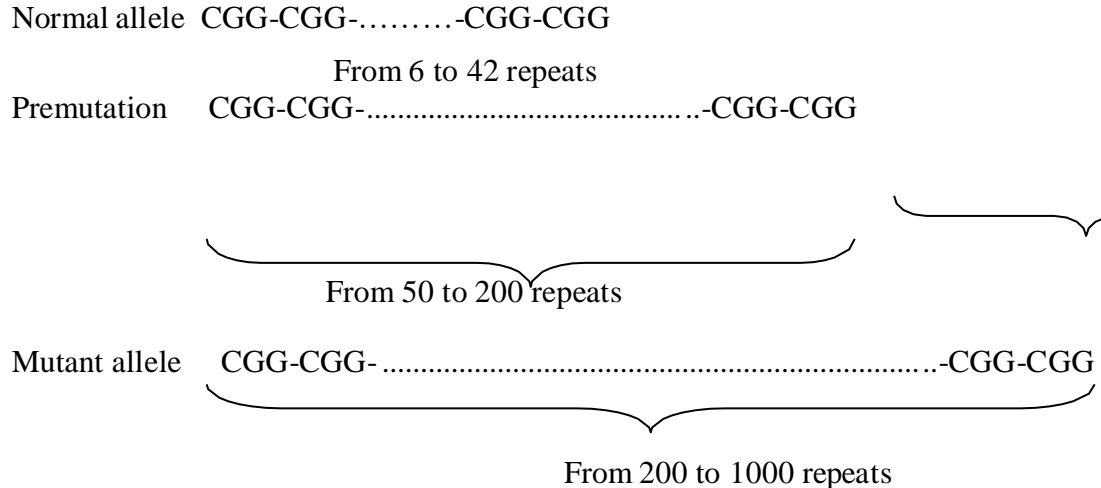|	
Normal DNA	GGG-C**C**A-TCG-GGG-A
mRNA	CCC-GGU-AGC-CCC-U
protein	Pro – Gly – Ser – Pro –
	deletion of **C**
Mutant DNA	GGG- CAT-CGG-GGA-
mRNA	CCC–GUA-GCC- CCU-
protein	Pro - Val - Ala – Pro

Dynamic mutations or expansions of trinucleotide repeats have been identified as the mutational bases for a number of different single gene disorders including Huntington's disease, Fragile X mental retardation and myotonic dystrophy.

Tandem trinucleotide repeats are very frequent in the human genome. They may be located within or near disease genes. Normal allele contains a certain number of tandem triplet repeats (usually 20 to 40), and the length of this sequence is usually stable in mitosis and meioses. Sometimes the number of repeats increases during meiosis or possibly early fetal development so that a newborn can have hundreds or thousands of repeats. It causes genetic disease.

For example, Fragile X mental retardation is inherited as recessive X-linked disorder. The gene is located on the long arm of chromosome X (Xq27.3). Normal allele has 6 to 42 tandem triplets CGG at 5'–non-translating end of the gene. Sometimes the repeats become extremely unstable and the number of repeats may increase. Increasing of triplets is called expansion of trinucleotide repeats. The number of repeats may reach up 50 to 200 (permutation). Premutation

does not have any medical consequences, but makes allele to be instable. In people with permutation the number of repeats usually increases during meiosis. Children of these parents will become allele containing more than 200 repeats (mutant allele) that leads to the disease features.

Normal allele CGG-CGG-………-CGG-CGG

From 6 to 42 repeats

Premutation   CGG-CGG-.......................................-CGG-CGG

From 50 to 200 repeats

Mutant allele   CGG-CGG- ....-CGG-CGG

From 200 to 1000 repeats

The exact mechanism of tandem repeats expansion is not known. The number of repeats may increase in descendent generations producing the phenomenon of *anticipation*. This is the phenomenon of earlier onset of a disease in succeeding generations.

Functional effects of mutations on the protein

Mutations in non-coding DNA, in general, do not have phenotypic effect unless they occur in the DNA sequences involved in gene regulation (promoter, enhancer, silencer, mutations of transcription factors genes). These mutations can affect the level of gene expression.

Mutations in the coding DNA usually cause the changes in the protein syntheses.

Gene mutations   →   mRNA changes   →   abnormal protein syntheses

Mutations can produce either a *loss-of-function* or a *gain-of-function* of the protein product. Loss-of-function mutations can result either reduced activity or complete loss of the gene product. Loss-of-function mutations in the heterozygous state are usually associated with half normal levels of the protein product. Loss-of-function mutations involving enzymes will be inherited in an autosomal or X-linked recessive manner, because the 50% catalytic activity that remains in heterozygotes is sufficient for normal function.

Gain-of-function mutations occasionally result in a completely novel protein product. More commonly, they result in overexpression of the product or inappropriate expression (i.e., in the wrong tissue or in the wrong state of development). Gain-of-function mutations produce dominant disorders (Huntington's disease).

The rate of spontaneous gene mutations

The spontaneous mutation rate is quite variable for different genes, ranging from 1×10^{-4} to 1×10^{-6} per locus per gamete, with an average of about 1×10^{-5} . There are at least two reasons for this large range of variation.

1. Certain nucleotide sequences are especially susceptible to mutation. They are termed mutation hot spots. The best known is the two-base (dinucleotide) sequence CG. In mammals, about 80% of CG dinucleotides are methylated: a methyl group is attached to the cytosine base. The methylated cytosine, 5-methylcitosine, easily looses an amino group, converting into thymine. The mutation rate at CG dinucleotides is about 12 times higher than at other dinucleotide sequences. Mutation hot spots, in the form of CG dinucleotides, have been identified in a number of important human disease genes.

2. Genes vary in size. Large genes, because of their size, are generally more likely to experience mutations than are small genes. For example, the genes responsible for Duchenne muscular dystrophy (DMD), as well as genes for hemophilia A and type I neurofibromatosis, are all very large and have high mutation rate (Table 2.)

Table 2. The spontaneous mutation rates

Disease	Pattern of inheritance	Mutation rate

Achondroplasia	АД	От 1×10^{-5} до $6\text{-}9 \times 10^{-6}$
Aniridia	АД	$2{,}9\text{-}5 \times 10^{-6}$
Apert syndrome	АД	$3\text{-}4 \times 10^{-6}$
Marfan syndrome	АД	$4.2\text{-}5.8 \times 10^{-6}$
Huntington's disease	АД	1×10^{-6}
Retinoblastoma	АД	1×10^{-5}
Neurofibromatosis	АД	От $4{,}4\text{-}4{,}9 \times 10^{-5}$ до 1×10^{-4}
Osteogenesis imperfecta	АД	$0{,}7\text{-}1{,}3 \times 10^{-5}$
Hemophilia A	ХР	$3{,}2 - 5{,}7 \times 10^{-5}$
Duchenne muscular dystrophy	ХР	$4.3\text{--}10{,}5 \times 10^{-5}$
Incontinentia pygmenti	ХД	$0.6 - 2{,}0 \times 10^{-5}$

Single gene mutations can increase with paternal age. This increase is seen in several single gene disorders, including Marfan syndrome, achondroplasia, neurofibromatosis, Apert syndrome. For example, the risk of producing a child with Marfan syndrome is approximately five times higher for a male older than 40 years of age than for a male in his 20s. The parental age affect is usually explained by the fact that the stem cells giving rise to sperm cells continue to divide throughout life, allowing a progressive buildup of DNA replication errors.

CHROMOSOMAL MUTATIONS

They include structural chromosomal mutations (chromosomal aberrations), numerical chromosomal mutations (genome mutations) and mixoploidy.

Structural chromosomal mutations

Structural chromosomal mutations include rearrangements in the structure of the chromosomes. Abnormalities of chromosome structure are usually caused by failure of meiosis (unequal crossing over). In addition, chromosome breakage can occur during mitosis and meiosis. Mechanisms exist to repair these breaks, and usually break is repaired perfectly with no damage to the daughter cells. Sometimes, however, the breaks remain, or they heal in a fashion that alters the structure of chromosome.

Structural chromosomal mutations can be balanced (the rearrangement does not produce a loss or gain of chromosome material) or unbalanced (the rearrangement causes a loss or gain of chromosome material). Carries of balanced chromosomal mutations have usually normal phenotype, but they are often at risk of chronic miscarriages or producing children with unbalanced chromosomal rearrangement. They often have low fertility. This can be explained by abnormal pairing and/or abnormal crossing over between normal chromosome and chromosome with balanced mutation.

When chromosomal mutation is unbalanced, clinical effects are usually very serious.

Structural abnormalities include duplications, deletions, ring chromosomes, inversions, translocations, marker chromosomes.

Structural chromosomal mutations

Unbalanced

Balanced

Produce a loss or gain of

Do not cause a loss or gain
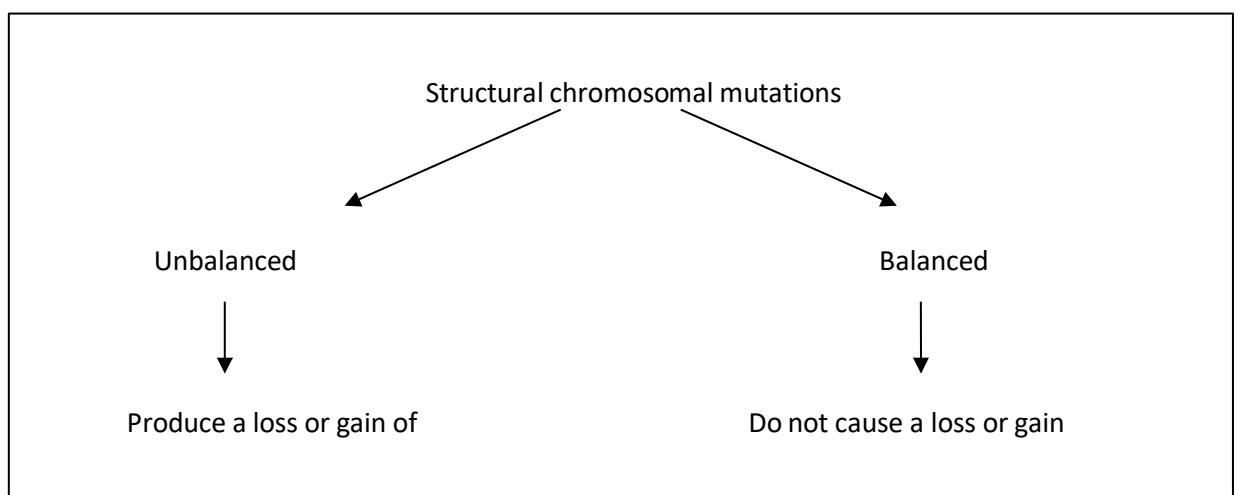
1.   ***Deletions*** involve loss of part of chromosome and result in monosomy for that segment of the chromosome. They can be terminal (loss the chromosome's tip, see below) or interstitial (an internal part of the chromosome is loosed). Any deletion resulting in loss of more than 2% of the total haploid genome will have a lethal outcome. Small deletions are compatible with survival to term but have usually serious medical consequences.

A B C Д E F **G H** → Deletion of **G H**   A B C Д E F

2.   ***Duplications*** represent a gain (doubling) of chromosome segment and result in trisomy for that segment of the chromosome. Duplications tend to produce less serious consequences than deletions, illustrating the principle that a loss of genetic material is more serious than an excess of genetic material.

A **B C** Д E F G H Duplication of **BC**   A **B C B C** Д E F G H
Deletions and duplications are usually the result of unequal crossing over

3.   ***Ring chromosomes*** arise from deletions at both tips of a chromosome and fusion of the remaining chromosome ends. The two distal chromosomal fragments are lost, so individuals with ring chromosomes have deletions of the short and long arms of the chromosome The effects are usually very serious. Ring chromosomes are often unstable in mitoses. They are often lost, resulting in monosomy for the chromosome in at list some cells (mosaicism for the ring chromosome may be seen). Sometimes this chromosomal mutation may be balanced, but carries produce unbalanced gametes.

4.   ***Inversions*** — a segment of chromosome is reversed in position, i.e. inverted. If the inverted segment involves the centromere it is termed a pericentric inversion (Fig. 2.3), if it does not involve the centromere it is called a paracentric inversion. Inversions are usually balanced rearrangements and rarely cause problems in carries. However they can lead to chromosome imbalance in offspring. The offspring of individuals who carry inversions often have chromosome deletions or duplications.

5.   ***Isochromosomes*** are usually formed due to division of the centromere transversally rather than longitudinally. As a result a metacentric chromosome forms, consisting of two same arms (deletion of one arm and duplication of the other one). Isochromosomes of most autosomes are lethal, because the genetic material is substantially altered. The most commonly observed isochromosome is that which consists of two long arms of the X chromosome. The babies with isochromosome Xq usually have features of Turner syndrome. — forms due to the reset of horizontal unparalleled division chromosomes.

6.   ***Translocations*** refer to the transfer of a chromosomal segment from one chromosome to another. Translocations can be balanced and unbalanced. Balanced translocations are the most common chromosomal aberrations in human populations, occurring in 1 of every 500 to 1000 individuals. There are three basic types of translocations – ***reciprocal, nonreciprocal*** and ***robertsonian***.

a)   *Reciprocal translocation* — in this kind of translocation there is a two-way exchange of material between homologous or non-homologous chromosomes. Autosomes or sex chromosomes may be affected. The carrier of a reciprocal translocation is usually

unaffected because he or she has normal chromosomal complement of genetic material. Carries of balance reciprocal translocation usually have reduced fertility. The carrier's offspring can be normal, can carry the same balanced translocation, or can have duplications or deletions of genetic material.

b) *Nonreciprocal translocation (transposition) involves* the one-way movement of a chromosome segment into the other position of the same chromosome or into other chromosome.

c) *Robertsonian translocation* or centric fusion is one of the common types of chromosomal mutations in human populations. Robertsonian translocations are observed between two acrocentric chromosomes of the group D (numbers 13,14,15) or G (numbers 21 and 22).

The fragments formed by the fusion of two short arms of two chromosomes are lost. The short arms of these chromosomes are very small and contain only genes for ribosomal RNA. Any two of these chromosomes lose short arms and one centromere, and then long arms fuse to form a single chromosome. This is also referred to as *centric fusion*. Loosing of rRNA genes does not have serious consequences, because these genes belong to the repeatable. Each cell has multiple copies of rRNA genes (to 10^5 copies). Loosing some of these genes is compensated with the same genes of other 8 acrocentric chromosomes. Because the carries of Robertsonian translocations lose no essential genetic material, they are phenotypically normal but have only 45 chromosomes. Their offspring, however, may inherit a missing or extra long arm of an acrocentic chromosome.

A common Robertsonian translocation involves fusion of the long arms of chromosomes 14 and 21.

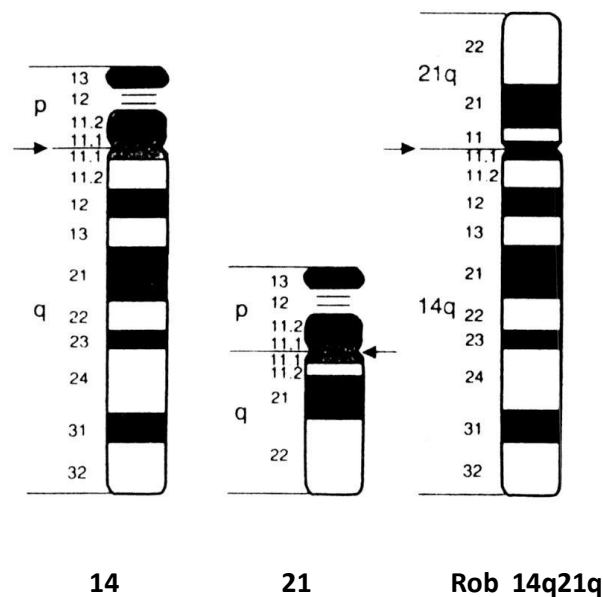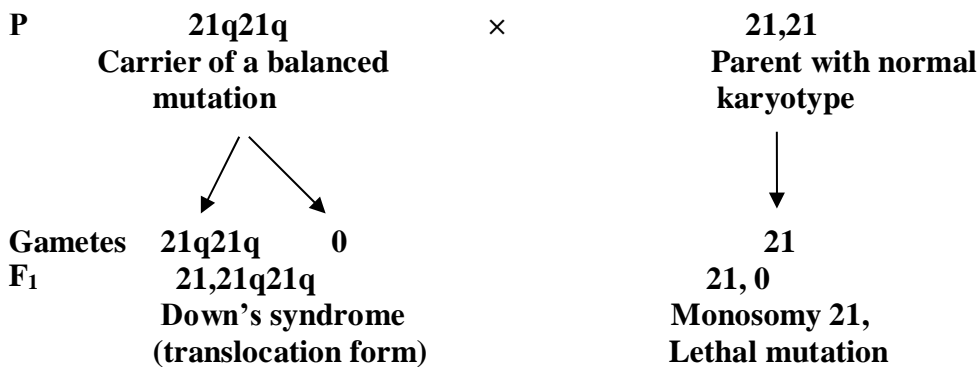**14**   **21**   **Rob 14q21q**

Fig. A balanced Robertsonian translocation between chromosomes 14 and 21. A carrier of this translocation would have single normal 14 and 21 chromosomes and a chromosome derived from a Robertsonian translocation of the entire long arms of chromosomes 14 and 21 (14q21q).

During meiosis a carrier of a 14q21q translocation can produce six types of gametes (Table 2).The third combination will result in the fertilized embryo having Down's syndrome. The last three combinations will result in zygotes with monosomy 21, monosomy 12 and trisomy 14 respectively. All of these combinations are incompatible with survival beyond early pregnancy.

Table 2. Possible gametes and zygotes which can be produced in a carrier of a 14q21q translocation.

Possible gametes in a carrier of a 14q21q translocation. The carrier has single normal 14 and 21 chromosomes and a chromosome derived from a Robertsonian translocation of the entire long arms of chromosomes 14 and 21 (14q21q). This karyotype may designed as 14,14q21q,21q	Possible zygotes which can be produced after fertilization by a normal gamete, containing single chromosomes number 14 and 21
14,21 –a normal chromosome complement	14,14,21,21 – a normal zygote
14q21q – a balanced chromosome complement (a translocation chromosome)	14,14q21q,21 – a zygote with balanced Robertsonian translocation, normal phenotype
14q21q, 21 - an unbalanced chromosome complement, possessing both the translocation chromosome and a normal 21	14,14q21q,21,21 – translocation form of Down's syndrome
14 - an unbalanced chromosome complement, with a normal 14 and a missing chromosome 21	14,14,21 – monosomy 21, lethal mutation
21 – an unbalanced chromosome complement, with a normal 21 and a missing chromosome 14	14,21,21 – monosomy 14, lethal mutation
14, 14q21q – an unbalanced chromosome complement, possessing both the translocation chromosome and a normal 14	14,14,14q21q,21 – trysomy 14, lethal mutations

Sometimes a parent may carry a Robersonian translocation, which involves long arms of two homologous chromosomes, for example 21q21q. All gametes will be either nullisomic or disomic for chromosome 21. Consequently all pregnancies will end either in spontaneous miscarriage or in the birth of a child with Down's syndrome.

P   21q21q   ×   21,21
      Carrier of a balanced   Parent with normal
           mutation   karyotype

Gametes   21q21q   0   21
F_1 21,21q21q   21, 0
        Down's syndrome   Monosomy 21,
        (translocation form)   Lethal mutation

7. *Marker chromosomes.* Occasionally in metaphase plate (in a slide of metaphase chromosomes) very small chromosomes are seen. They are called supernumerary chromosomes or marker chromosomes. Marker chromosomes may have different shape: metacentic, submetacentric, ring. They also represent the structural rearrangement because they are derivates of any other chromosome and contain a genetic material of other chromosomes. Clinical effects depend on the structure of the marker chromosome. If it consists of euchromatine it can cause chromosomal disorder. If it contains nonactive heterochromatine it may be compatible with normal phenotype.
NUMERICAL CHROMOSOMAL MUTATIONS (GENOME MUTATIONS).
Numerical chromosomal mutations are classified into two groups: *aneuploidy* and *polyploidy*.

Aneuploidy involves the loss or gain of one or more chromosomes. There are several types of aneuploidy:

    1. ***Nullisomy*** – loss of a pair of chromosomes (karyotype is 2n-2). Nullisomies are not observed in livebirths or in early spontaneous abortions because they are lethal to the conceptus.

    2. ***Monosomy*** - loss of a single chromosome (2n-1). Autosomal monosomies are lethal to the conceptus. Monosomy for the X chromosome (45,X) is common in chromosomally abnormal, spontaneously aborted embryos and also causes the condition known as Turner's syndrome.

    3. ***Trisomy*** – the presence of an extra chromosome (2n+1). Autosomal trisomies which are compatible with survival to term are Down's syndrome (trisomy 21), Patau's syndrome (trisomy 13), Edward's syndrome (trisomy 18). Most other autosomal trisomies result in early pregnancy loss. Trisomies for sex chromosomes (X or Y) have only mild phenotypic effects.

    4. ***Tetrasomy*** – the presence of two extra chromosomes (2n+2).

    5. ***Pentasomy*** – the presence of three extra chromosomes (2n+3). The tetrasomy and pentasomy for autosomes are not known in livebirths. However, tetrasomy and pentasomy for sex chromosomes are sometimes observed.
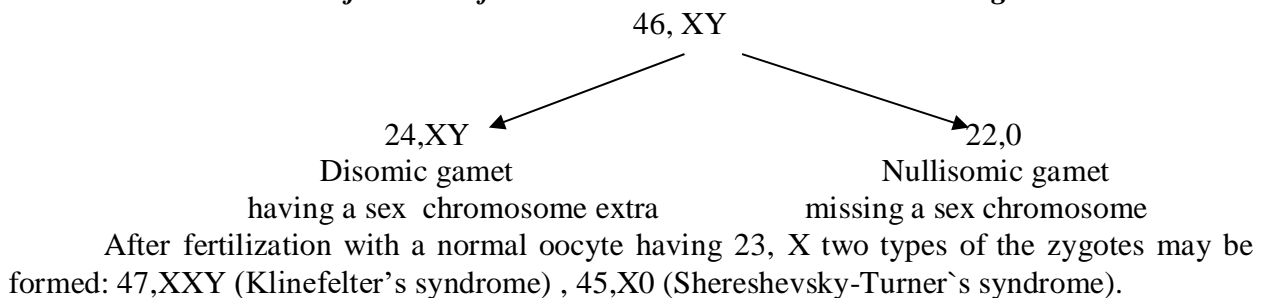
What are the causes of aneuploidy?

Aneuploidy is usually caused by failure of separation of one of the pairs of homologous chromosomes during anaphase of meiosis I or, less often, during meiosis II. This failure is called non-disjunction.

This is where either two homologous chromosomes fail to separate at meiosis I or a centromere fails to split at metaphase of meiosis II. After fertilization zygote and all embryonic cells will have abnormal number of chromosomes.
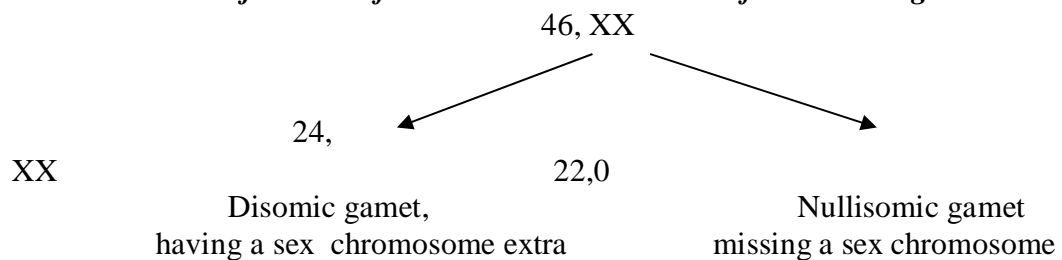
Scheme of normal meiosis

In male   In female
46,XY   46,XX

23, X   23,Y   23,X   23, X

Non-disjunction of the sex chromosomes in a male during meiosis

46, XY

24,XY   22,0
Disomic gamet   Nullisomic gamet
having a sex chromosome extra   missing a sex chromosome

After fertilization with a normal oocyte having 23, X two types of the zygotes may be formed: 47,XXY (Klinefelter's syndrome) , 45,X0 (Shereshevsky-Turner`s syndrome).

Non-disjunction of the sex chromosomes in a female during meiosis

46, XX

24,
XX   22,0
Disomic gamet,   Nullisomic gamet
having a sex chromosome extra   missing a sex chromosome

After fertilization by a normal sperm with chromosomal complement 23,X two types of zygotes may be formed: 47,XXX (triple X, or superfemale syndrome) or 45,X0 (Shereshevsky-

Turner`s syndrome). After fusion with normal sperm (23,Y) two other zygotes may be formed: 47,XXY (Klinefelter's syndrome), or 45,Y – monosomy for Y chromosome (lethal mutation).

An example bellow shows a result of non-disjunction chromosomes 21 in a woman, resulting in Down's syndrome.

P   ♀ 21,21   ×   ♂21,21

Gametes   21,21   0   21

F_1 21,21,21   21,0
           Down`s syndrome   Monosomy for chromosome 21,
                                           lethal mutation
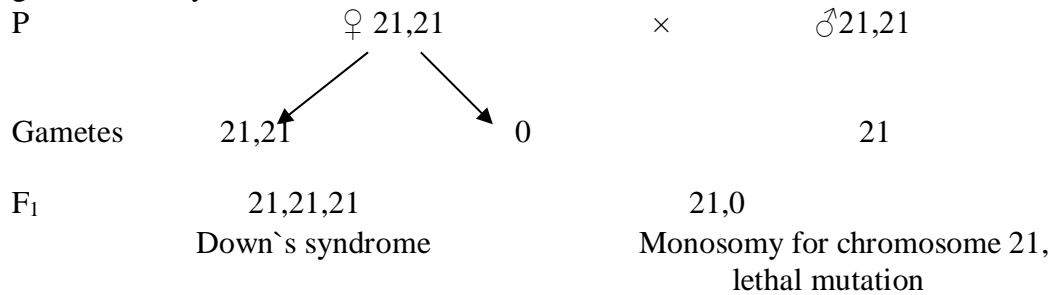
Studies using DNA markers have shown that most children with autosomal trisomy have inherited their additional chromosome as a result of non-disjunction occurring during one of the maternal meiotic divisions (Table 3). The occurrence of trisomies 13, 18 and 21 in livebirths and in spontaneous abortions increases with the age of the mother.

Table 3. Parental origin of meiotic error leading to aneuploidy

Chromosome abnormality	Paternal (%)	Maternal (%)
Trisomy 13	15	85
Trisomy 18	10	90
Trisomy 21	5	95
45,X	80	20
47,XXX	5	95
47,XXY	45	55
47,XYY	100	0

A maternal origin of aneuploidy and maternal age effect may be explained by the process of oogenesis. All of a female's oocytes are formed during her embryonic development. The meiosis I begins in primary oocytes before birth. At the birth all primary oocytes pass through prophase I and enter the phase of maturation arrest, known as dictyotene. In this stage oocytes remain suspended until meiosis I is completed at the time of ovulation when a single secondary oocyte is formed. Meiosis II begins if only the secondary oocyte is fertilized by a sperm cell. Thus the older is mother the longer is period between the beginning of meiosis and its end. This may predispose to non-disjunction because of abnormality in spindle formation.

Non-disjunction may also be under genetic control, because the recurrence risk of aneuploidies is much more higher than in general population.

Non-disjunction can also occur during an early mitotic division in the developing zygote. These results in the presence of two or more different cell lines, a phenomenon known as mosaicism (see below).
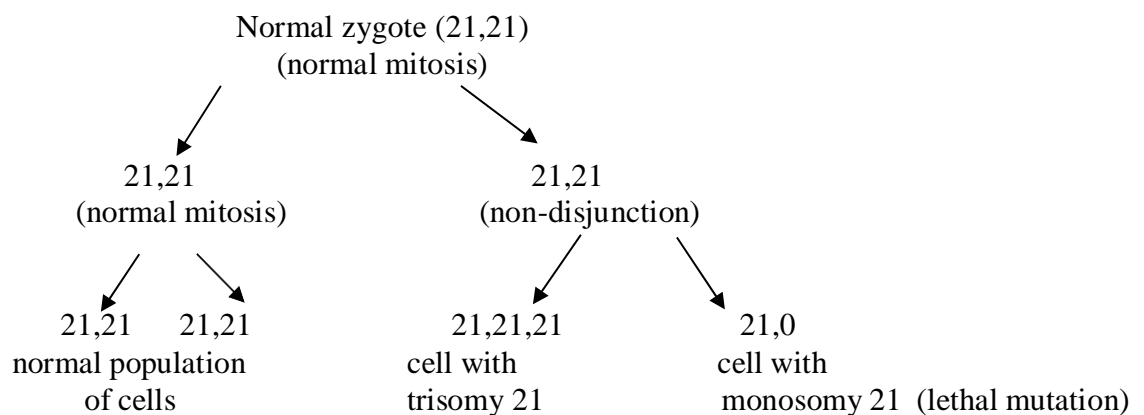
Polyploidy is the addition of one or more complete haploid sets. The base haploid number of chromosomes is **n**. Normal diploids have **2n** chromosomes. Polyploids may have **3n** chromosomes (triploidy) or **4n** (tetraploidy). Mechanism of triploidy is non-disjunction of all chromosomes in meiosis. This leads to the formation of a diploid sperm or ovum. After fertilization a triploid zygote arises. Triploidy can be also caused by fertilization of an ovum by two sperm (dispermy) or by the fusion of an ovum and a polar body with subsequent fertilization by a sperm. Tetraploidy is a consequence of non-disjunction of all chromosomes during the first cleavage division, which results in a doubling of the chromosome number immediately after fertilization.

Mixoploidy

Mixoploidy can be defined as the presence in an individual of two or more cell lines with different karyotype and genotype. Mixoploidies include mosaicism and chimerism.

Mosaicism is the presence of two or more cell lines which differ in their genetic constitution (with different karyotypes or genotypes) but are derived from a single zygote, i.e. they have the same genetic origin. Mosaicism is caused by somatic mutation. Chromosome mosaicism results from non-disjunction occurring in an early embryonic mitotic division.

If, for example, the two chromatids of a number 21 chromosomes failed to separate in the process of mitosis this would result in the embryo having three types of cells:1) normal cells with 46 chromosomes, 2) cell with 47 chromosomes (trisomy 21), 3) a cell with 45 chromosomes (monosomy 21). The scheme below shows generation of somatic mosaicism for chromosome 21 caused by mitotic non-disjunction.

Normal zygote (21,21)
(normal mitosis)

21,21   21,21
(normal mitosis)   (non-disjunction)

21,21   21,21   21,21,21   21,0
normal population   cell with   cell with
of cells   trisomy 21   monosomy 21 (lethal mutation)

Monosomy 21 is lethal mutation for cells. So the resulting embryo would have two cell lines – normal cells and the cells with trisomy. Mosaicism accounts for 1-2% of all clinically recognized cases of Down's syndrome. The number of affected cells depends on the period of embryonic development when this mutation occurs. The earlier mutation occurs the more is proportion of the affected cells and tissues and the more serious is the clinical effect of mosaicism.

Chimerism can be defined as the presence in an individual of two or more genetically distinct cell lines derived from more than one zygote. The word chimera is derived from the mythological Greek monster which had the head of a lion, the body of a goat and the tail of a dragon. Main causes of chimerism are:

- Fusion of two non-identical twins during early embryonic development. (two ova are fertilized by two sperms and the resulting two embryos are fused to form one embryo). If the two zygotes are of different sex, the chimeric embryo can develop into an individual with true hermaphroditism. The true hermaphrodites have two cell lines with karyotypes XX and XY respectively and abnormal sexual differentiation.
- Blood chimeras occur due to exchange of steam cells, via placenta, between non-identical twins in utero. The cells of non-identical twins may have erythrocytes with antigens AB0 for different blood groups.
- In leukemic patients, repopulation of bone marrow from a donor.

STANDARD NOMENCLATURE FOR CHROMOSOME KARYOTYPES

Nomenclature for normal and abnormal karyotypes is based on the International System for Human Cytogenetic Nomenclature (ISCN), which was proposed in 1995. In describing of karyotype, the first item is the number of chromosomes; second is the sex chromosomes constitution. A normal female karyotype is designated 46,XX; a normal male karyotype is 46,XY.

Autosomes are specified only if there is an abnormality. The nomenclature for various chromosome abnormalities is summarized in Table 4.

Table 4. Standard nomenclature for chromosome karyotypes

Karyotype	Description
46,XY	Normal female karyotype
46,XY	Normal male karyotype
69,XXX; 69,XXY 92,XXXX; 92,XXXY	Triploidy Tetraploidy
45,X	Female with monosomy X, Sherishevsky-Turner's syndrome
47,XX,+13 or 47,XY,+13 47,XX.+18 or 47,XY,+18 47,XX,+21 or 47,XY,+21	Female or male with trisomy 13, Patau's syndrome Female or male with trisomy 18, Edward's syndrome Female or male with trisomy 21, Down's syndrome
47,XXX 48,XXXX 49,XXXXX	Female with trisomy X syndrome Tetrasomy X Pentasomy X
47,XYY 48,XYYY 49,XYYYY	Male with 47,XYY syndrome Tetrasomy Y Pentasomy Y
47,XXY 48,XXXY 49,XXXXY	Male with Klinefelter's syndrome Klinefelter's syndrome, tetrasomy Klinefelter's syndrome, pentasomy
46,XX/47,XX,+21	Mosaic form of Down's syndrome in a female (she has two populations of cells- normal cells and cells with trisomy 21)
46,XX,del (5p)	Female with a deletion of the short arm of chromosome 5, Cat-cry syndrome
46,XY,dup(11) (q12)	Male with a duplication of the ling arm of chromosome 11 band designated 12
46,X,i (Xq)	Female with one normal X chromosome and an isochromosome of the long arm of the X chromosome, cytogenetic variant of the Sherishevsky-Turner's syndrome
46,XX, inv(3)(p21;q13)	An inversion on chromosome 3that extends from p21 to q13;because it includes the centromere, this is a pericentric inversion
46,XY,r(18)	Male karyotype with ring chromosome 18
45,XX,rob (14q21q)	Female with a balanced Robertsonian translocation of chromosomes 14 and 21. Karyotype shows that one normal 14 and one normal 21 chromosomes are missing and replaced with a chromosome containing long arms of the chromosomes 14 and 21
46,XX,-14,+rob(14q21q)	Female with non balanced Robertsonian translocation of chromosomes 14 and 21. Karyotype shows that one chromosome one is missing and replaced wit chromosome containing long arms of the chromosome 14 and 21. Really one chromosome 21 is extra. Cytogenetic variant of the Down's syndrome
46,XX,t(2;4)(q21;q21)	Female with a balanced reciprocal translocation between chromosomes 2 and 4. The breakpoints are at chromosome 2 segment 2q21 and at chromosome 4 segment 4q21
46,XY,fra(X)(q27.3)	Male with a fragile X chromosome, fragile site at q27.3; fralile X syndrome

GENERAL CHARACTERISTICS AND CLASSIFICATION OF MUTAGENIC FACTORS.

Induced mutations occur under the effects of known mutagens. Mutagens are factors which can cause mutations. Mutagens just as in spontaneous mutations affect replication, reparation of DNA, recombination of genes and disjunction of the chromosomes during mitosis or meiosis. Mutagens may be physical, chemical and biological ones.

Physical mutagens

Physical mutagens include all types of ionizing radiation (X-rays, alpha, beta and gamma rays), nonionizing radiation in the form of ultraviolet light (UV). Heat is also a significant environmental mutagen.

Powerful physical mutagens are X-rays, alpha, beta and gamma rays that belong to the class of ionizing radiation. Sources of ionizing radiations fall into two classes: natural (background) and artificial. Natural ionizing radiation includes cosmic rays, external radiation from radioactive materials in earth's surface (such elements as thorium, uranium, radium and an isotope of potassium K^{40} and other) and internal radiation from radioactive materials in our tissues (they are constituents of the air we breathe, the food we eat and the water we drink). Artificial ionizing radiation includes medical radiology and occupational exposure. Ionising radiation can be produced from radioactive materials like radium, cobalt, etc. These can induce transformation of the stable atoms into reactive ions if passed through biological material. A large number of ions produced this way initiate a number of chemical reactions, which destroy the molecules – carriers of hereditary information. Ionizing radiation can also break the double-stranded DNA.

X-rays and gamma-rays can reach all cells of the body, including the germ-line cells.

Ultraviolet light (UV) is the form of nonionizing radiation. UV radiation causes the formation of covalent bonds between adjacent pyrimidine bases (i.e., cytosine or thymine). These pyrimidine dimeras are unable to pair properly with purines during DNA replication; this results in base pair substitution. Because UV radiation is absorbed by the epidermis, it does not reach the germ cells but can cause skin cancer.

Chemical mutagens.

A variety of chemicals can induce mutations, sometimes because of their chemical similarity to DNA bases. Some **chemical mutagens** are nitrogen base analogues with molecular structure very similar to a natural base and can be incorporated by DNA polymerases into the DNA during replication (5-bromouracil, 2-aminopurine, coffin and others). 5BU, for example, a base analogue derived from thymine. If it incorporates into the DNA in place of thymine at the next replication BU pairs with guanine and then guanine pairs with cytosine in next replication. Ultimately an AT pair is replaced by a GC pair.

Other mutagens are intercalating agents that disrupt DNA replication by slipping between adjacent base pairs of the double helix causing the frameshift mutation in the gene (ethidium bromide). Many mutagens act by chemically modifying bases (alkylating agents, deaminating agents). An example is nitrous acid, which removes an amino group from cytosine (deamination), converting it to uracil and causing replication errors.

Therefore, most chemical mutagens destroy DNA replication, DNA reparation and cause gene mutations.

Some chemical substances arrest the cell division and cause genome mutations, destroying the spindle fibers (colchicine, some cytostatics).

Hundreds of chemicals are now known to be mutagenic in laboratory animals. Among these are mustard gas, vinyl chloride, formaldehyde, benzene, sodium nitrite, and even saccharin and caffeine. Many of chemical mutagenic substances are present in various agricultural, industrial and pharmaceutical chemicals in common use today.

Biological mutagens — these are, first of all, viruses. Aberrations of chromosomes in somatic cells and gene mutations can be caused by the viruses of small pox, measles, chicken pox, epidemic parotitis, influenza, hepatitis and others. The toxins of certain bacteria (haemolytic streptococcus, agent of bacterial dysentery and others), fungi (aspergillum) are also mutagens.

The special attention is requited mutagenesis under the influence of vaccination because they capture many people in population.

Spreading of the mutagens in the environment leads to the increasing of mutation rate, genetic load of human being, development of hereditary diseases. One of the branches of genetics is genetic toxicology, which studies the mutagenic and carcinogenic activity of the anthropogenic environmental factors. It works out the methods and ways of genetic activity of chemical compounds estimating. Aim of the genetic toxicology is to reduce genetic danger in all fields of human activities.

MAIN PROTECTIVE MECHANISMS WHICH CAN DECREASE THE PATHOLOGICAL EFFECTS OF MUTATIONS.

Main protective mechanisms, which can decrease the pathological effects of mutations, are as follows.

1. Many mutations are lethal for zygote, early embryo, and fetus or can cause stillbirth. Lethal mutations cannot be inherited (except recessive ones) and disappear from population with death of mutant organism. The significance of this protective mechanism proves following facts. About 50-60% embryos are lost before implantation and clinically tested pregnancy. From the tested pregnancies, 15% end in spontaneous abortion and 1% in stillbirth. Chromosome abnormalities are seen in 50% of first-trimester, 20% of second trimester spontaneous abortions and in 6% of stillbirth. Lethal single gene mutations are responsible for pregnancy lost too. But there contribution has not been tested yet. From 1000 newborns not less then 5 dies during the first year of life because of hereditary pathology.

2. A characteristic of mostly all hereditary diseases is reduced fertility, caused by the failure of reproductive function.

3. Diploid set of the chromosomes allows the recessive mutant genes not to be manifested in heterozygous organism.

4. Due to degeneracy of genetic code substitutions of the third nucleotide in triplet may not cause the substitution of an amino acid.

5. Living organisms have evolved biological processes for dealing with mutations—DNA repair mechanisms. They take place in all normal cells of higher organisms. Several dozen enzymes are involved in the repair of damaged DNA. They collectively recognize an altered base, excise it by cutting the DNA strand, replace it with the correct base, and reseal the DNA. It is estimated that these repair mechanisms correct 99,9% of initial errors. DNA repair is under genetic control. Defects in DNA repair system can lead to many types of diseases (some types of cancers, xeroderma pigmentosum).

6. Mutant DNA can be repaired in meiosis. About 6 single strand and 0,1 double strand destructions of the DNA occur daily in human cells (i.e. one double strand destruction occur every ten days). Double strand injury repair happens only in meiosis during the conjugation. Normal DNA of the homologous chromosome is used as a template for reparation of the damaged DNA. Repairs of the single strand mistakes, which have not been corrected before, are possible in meiosis also. All these repair mechanisms are the result of biological adaptation during the evolutionary process. They allow controlling the mutation load and support it on the definitive level.

7. Special attention in the last years is given to anti-mutagens – the substances, which decrease the level of spontaneous and induced mutations. As anti-mutagens are termed the chemical compounds that are capable for: a) inactivation of the mutagens; b) modification of mutagens metabolism; c) decreasing of mistakes of DNA replication and repair, etc. Because of there action anti-mutagens are often called gene protectors. Gene protectors are the group of natural substances, containing in vegetable, fruits, greens, spices used in meal. Vitamins C, E, K, Folic acid, B_2, A, β-carotin (provit-A), thymine etc. belong to this group. They are widely used for prophylaxis of hereditary disorders during pregnancy and in certain professional groups of people.

EXAMPLES OF NON-MENDELIAN INHERITANCE IN HUMANS

According to mendelian lows each trait of an organism depends on a pair of allelic genes, which are inherited from both parents. Origin of these genes (maternal or paternal) is not essential. Both maternal and paternal genes are equally expressed. But inheritance of some traits may differ from classical (mendelian) one. Examples of non-mendelian inheritance are as follows:

- Uniparental diploidy, uniparental disomy, uniparental isodisomy;
- Genomic imprinting;
- X-chromosome inactivation (Barr bodies formation);
- Germ-line mosaicism;
- Mitochondrial inheritance.

Uniparental diploidy, uniparental disomy, uniparental isodisomy.

Occasionally, cells may have diploid number of chromosomes, but all chromosomes are derived from a single parent. This phenomenon is called uniparental diploidy. More commonly, cases have been reported of individuals in whom two copies of a specific chromosome are inherited from a single parent, either both homologs from that parent (uniparental disomy) or two identical copies (two chromatides) of a single homolog (uniparental isodisomy).

Uniparental diploidy results in failure of embryonic development in humans and uniparental disomy and isodisomy often contributes to disease. This is due to inherent differences in maternaly and paternaly inherited chromosomes. In all cases cells or individuals show a normal diploid set of chromosomes, but really they are abnormal due to phenomenon of genomic imprinting (different activity of some maternal and paternal genes).

An embryo with uniparental diploidy contains diploid number of chromosomes (46,XX), but all chromosomes are of maternal or paternal in origin. In case of paternal origin of chromosomes a complete hydatidiform mole is developed; in case of maternal origin ovarian teratoma may be produced.

What is a complete hydatidiform mole? In a normal pregnancy, the embryoblast of the blastocyst gives rise to the embryo, and the trophoblast gives rise to the placenta and chorion. In approximately 0.1 to 0.5 percent of all pregnancies, however, the embryo is entirely missing and the conceptus consists only of the extra-embryonic membranes. A conceptus of this type is called a complete hydatidiform mole. The chorionic villy of a complete mole are swollen and vesicular, resembling bunches of grapes ("hydatid' is from the Greek *hydatidos*, drop of water). No evidence of an embryo can be found. Complete hydatidiform moles often abort early in pregnancy. If they do not abort, they may be discovered by the physician because they cause characteristic symptoms of hypertension, edema, and vaginal bleeding in a pregnant woman. Moles and mole remnants are readily diagnosed on the basis of an abnormally high level of plasma human chorionic gonadotropin (hCG). Molar pregnancies are somewhat more common in younger than in older woman. The complete mole can undergo malignant change to become choriocarcinoma. A complete mole arises by fertilization of an empty ovum by either two sperms or a single sperm undergoing endoreduplication.

Ovarian teratomas by contrast have two maternal genomes, with a 46,XX karyotype. They consist of disorganized embryonic tissues, but without any of the extra-embryonic membranes of a normal conceptus.

Uniparental disomy is often thought to arise by loss of an extra chromosome copy from a zygote with an inviable trisomy, thereby restoring the normal number of chromosomes. If each of the three copies has an equal chance of being lost, there will be a two in three chance of a single chromosome loss leading to the normal chromosome constitution and a one in three chance of uniparental disomy (either maternal or paternal). Individuals with these abnormalities may show symptoms of hereditary disorder due to genomic imprinting.

Genomic imprinting.

For the most of human genes, the expression of an allele does not depend on whether that allele has been inherited from the mother or from the father. However, the expression of a few genes depends on its parental origin: for some genes, the maternal allele but not the paternal allele is expressed in certain cell; for others it is always paternal gene which is expressed. This different

activity of some maternal and paternal genes in the developing organism is called genomic imprinting, or "parent of origin" effect. The transcriptionally inactive gene are said to be "imprinted". Inactivation takes place during gametogenesis. Only a small proportion of the human genome is imprinted. Some genes are inactivated during oogenesis, others- during spermatogenesis. In the genes known to be imprinted, there is a strong association between methylation and transcriptional inactivation. The attachment of methyl groups to DNA at specific region of the chromosome may inhibit the binding of proteins that promote transcription. An alteration of chromatin structure may also be involved. Imprinting of affected genes occurs soon after conception and, once established, is usually transmitted to all the descendants of an imprinted cell. A gene's imprint is reversed or removed when a cell passes through gemetogenesis again. Genomic imprinting is an example of epigenetic (without altering of the gene, e.g. without altering of the DNA sequence).mechanisms which may affect the expression of genes.

What are clinical consequences of genomic imprinting?

1.   Firstly, patients with the same mutation may exhibit different phenotypes depending on whether this mutation is inherited from mother or from father. For example, a deletion in a specific region of human chromosome 11 causes Prader-Willi syndrome when the chromosome is inherited from the father but causes a phenotypically distinct condition, Angelman syndrome, when the chromosome with deletion is inherited from the mother. The Prader-Willi syndrome is characterized by short stature, obesity, hypogonadism and learning difficulty. Children with Angelman syndrome have severe mental retardation, ataxia, seizures, lack of speech and happy appearance. The difference between these two phenotypes is due to the lack of expression of maternally imprinted genes in Prader-Willi syndrome and the lack of expression of different paternally imprinted genes in the same chromosome region in Angelman syndrome. In both instances the deletion affects the chromosome that normally expresses these genes, so there is no active copy in the cell. The portion of the chromosome 15 that is deleted in both syndromes is known as the "critical region".

2.   Secondly, the phenotypic features associated with uniparental diploidy, disomy and isodysomy can be explained by phenomenon of genomic imprinting too. Human embryos that contain two maternal or two paternal genomes fail to develop, despite having normal diploid chromosome numbers. Human triploid abortuses are phenotypically different depending on whether the extra genome is maternal or paternal. Uniparental disomy can cause Angelman and Prader-Willi syndromes in some patients. When too copies of the maternal chromosome 15 are inherited, Prader-Willi syndrome results because no active paternal genes are present in the critical region. Conversely, disomy of the paternal chromosome 15 produces Angelman syndrome.

The second example is Beckwith-Wiedemann syndrome, a disorder that involves large size for gestational age, large tongue, omphalocele, and a predisposition to Wilms tumor, a kidney cancer. As with Angelman syndrome, some cases of this syndrome are caused by the inheritance of two copies of a chromosome 11 from the mother (uniparental disomy). Some genes on chromosome 11 are imprinted, including insulin-like growth factor 2 (*IGF2*). This gene is imprinted on the maternally derived chromosome and active only on the paternal chromosome. Normally, then, an individual has only one active copy of this gene. When two copies of the paternal chromosome are inherited, the active *IGF2* is present in double dose. So, Beckwith-Wiedemann syndrome is caused by overexpression of the gene. Two active copies can also result from a loss of the maternal imprint, activating the maternally inherited gene.

3.   Thirdly, certain human characters are autosomal dominant but manifest only when inherited from one parent. In some families glomus tumors are inherited as autosomal dominant character, but expressed only in people who inherit the gene from there father. Beckwith- Wiedemann syndrome (see above) is sometimes dominant but expressed only by people who inherit it from mother.

4. Allele loss in many cancers preferentially involves the paternal allele.

X-chromosome inactivation (Barr body formation).

X-chromosome inactivation or lionization is a process that occurs in all mammals, resulting in selective inactivation of alleles on one of the two X chromosome in females. It provides a mechanism of dosage compensation. This overcomes sex differences in the expected ratio of autosomal gene dosage to X chromosome gene dosage (2:1 in males and 1:1 in females): males with a single X-chromosome constitutionally hemizygous for X chromosome genes, bur females become functionally hemizygous by inactivating one of the parental X chromosome alleles. X inactivation occurs in female mammals at early stage of embryonic development (at the late blastula stage). The process is completed at different times in different embryonic tissues. In each cell of the female fetus, one of the two parental X chromosomes is randomly inactivated. Inactivated once, the inactive chromosome usually remains inactive in all progeny cells. Due to random process of inactivation female mammals are mosaics, comprising mixtures of cell lines in which the paternal X is inactivated and cell lines where the maternally inherited X is inactivated. The inactivated X chromosome in the somatic cell is called Barr body. X chromosome inactivation is another example of epigenetic regulation of gene expression.

Clinical consequences of X-chromosome inactivation:

1. A female who carries an X-linked recessive mutation on one of her two X chromosomes may express the mutant phenotype if most of her cells happen to have inactivated the X chromosome carrying the normal gene (see also Chapter 4).
2. Although monosomy for any autosome is lethal early in embryogenesis, monosomy for the X chromosome is rather common in liveborn infants and produces a relatively mild phenotype (Sherishevsky-Turner syndrome).
3. Trisomy of the sex chromosomes produces less severe phenotype than trisomy for any of the autosomes.

Germline mosaicism (germinal mosaicism, gonadal mosaicism)

Mosaicism is the presence of two or more cell lines with different genotypes in an individual (all cells arise from a single zygote). Sometimes mutation can occur at early stages of embryonic development of an organism, when germ line cells (precursor cells of the gametes) are formed. Such mutation produces a normal organism (because somatic cells are not affected) who has a clone of mutant germ line cells and who is able to produce mutant gametes repeatedly. Due to germ line mosaicism the normal parents, with no previous family history, can produce more than one affected child. Germline mosaicism is most likely to be seen in autosomal dominant or X-linked disorders because autosomal-recessive genes may not express in heterozygous offspring.

Germ-line mosaicism has been studied extensively in the lethal form of osteogenesis imperfecta. This disorder is caused by dominant mutations in the type I procollagen genes. The type I collagen is a major component of bone. When type I collagen is improperly formed, the bone loses much of its strength and fractures easily. Fetuses with this type of disorder may have hundreds of bone fractures; long bone deformities. The disorder is lethal in the perinatal period. Sometimes healthy parents produce multiple offspring affected with this disorder. First time this fact led to the conclusion, that type II osteogenesis imperfecta was an autosomal recessive trait. But later DNA was extracted from fibroblasts and sperm cells of a father of two affected children. DNA was studied with help of polymerase chain reaction. Although procollagen mutations were not detected in the fibroblast DNA, they were found in approximately 12,5 % of sperm cells. This was a direct demonstration of germ line mosaicicm in this individual. Due to germ line mosaicism the recurrence risk of type II osteogenesis imperfecta for normal parents is 6%. Other autosomal-dominant diseases in which germ-line mosaicism has been observed include achondroplasia and neurofibromatosis type I.

Mitochondrial inheritance

Mitochondrial mutations are significant cause of human genetic disorders. Mitochondrially encoded diseases have matrilineal inheritance. Inheritance is matrilineal because fathers do not pass on mitochondria to their children. Thus, a typical motochondrially inherited condition can affect both sexes, but is passed on only by affected mothers to all children.

Another unusual feature of mitochondrial inheritance is frequent heteroplasmy. Cells typically contain thousands of mtDNA molecules. If individual contains identical mtDNA in all mitochondria (either normal or mutant), this condition is called homoplasmy. However, if a new mutation arises and spreads in the mtDNA population, there will be two types of the mtDNA molecules in the cell (heteroplasmy). In some cases, some patients are heteroplasmic while others appear homoplasmic for the same mutation. The phenotype may depend on the proportion of abnormal mtDNA in some critical tissue. Mitochondrial mutations appear to evolve within individual. The proportion of normal and mutant mtDNA may change with time. Variants tend to be more frequent in older people, and an accumulation of mitochondria deficient in energy production may be a factor in aging. Some mitochondrial diseases appear to be of a quantitative nature: small mutational changes accumulate which reduce the energy-generating capacity of the mitochondrion and, at some threshold deficit, clinical symptoms appear.

External agents may precipitate disease in a susceptible individual – for example, a mitochondrial 12S RNA sequence vatiant to make individuals susceptible to hearing loss after treatment with aminoglycoside antibiotics. The same variant can interact with an autosomal locus to produce congenital hearing loss even in people who have never been given aminoglycoside antibiotics.

MCQs for self-control

1. Genetics is the science about
A. Heredity and variation
B. Hereditary disorders.
C. Hereditary characters of humans and other organisms
D. Hereditary and non-hereditary variation
E. Ontogenesis from birth to death

2. As hereditary disorders are termed the disorders
A. Caused by the gene mutations
B. Which manifests on the first year of life
C. Caused by the changing of the genotype
D. Which are inherited from the parents
E. Which are diagnosed at the moment of birth

3. Hereditary disorders are characterized by all of this **except for**
A. Are caused by gene mutations
B. Are caused by structural chromosome aberrations
C. Are caused by numerical chromosome aberrations
D. Are always inherited

E. Manifests at any age from the birth to senility.

4. Hereditary pathology is characterized by all of this **except for**
A. Early clinical manifestation
B. Participating of many organs and system of organs in pathologic process
C. Progressive course of the disease
D. Most acute onset of the disease
E. Resistance to therapy

5. Gene is the
A. Part of the DNA molecule which specifies amino acid
B. Part of the DNA molecule, which specifies primary protein structure
C. mRNA molecule, which specifies primary protein structure
D. Triplet of the tRNA, which is complementary to mRNA codon
E. All of the above

6. Gene is the
A. Part of the DNA molecule which specifies primary protein structure
B. Part of the DNA molecule, which specifies tRNA structure
C. Part of the DNA molecule, which specifies rRNA structure

D. Part of the DNA - repressor

E. All of the above

7. Human genetic code is

A. Triplet

B. Specific

C. Universal

D. Degenerative

E. All of the above

8. How many amino acids are specified by the following part of DNA molecule ATT GTC GGC AAT CGG?

A. 15

B. 4

C. 5

D. 3

E. 30

9. What is the chemical composition of the chromosomes?

A. Histone proteins

B. Non-histone proteins

C. DNA

D. DNA + histone + non-histone proteins

E. DNA + RNA

10. Aborted embryo has the karyotype 69, XXY. Which mutation is it?

A. Triploidy

B. Trisom.

C. Tetraploidy

D. Duplication

E. Deletion

11. Pregnant woman has been made placentocentesis. Cytogenetic studying of placenta samples showed karyotype 45,X. Which mutation is it?

A. Nullisomy

B. Trisom.

C. Monosomy

D. Duplication

E. Deletion

12. Women is consulted a geneticist for two spontaneous abortions in early term. Karyotyping revealed one long chromosome, which joins the hereditary material (long arms) of two chromosomes 13. Which mutation is it?

A. Duplication

B. Robertsonian translocation

C. Reciprocal translocation

D. Inversion

E. Dicentric chromosome

13. Deletion is:

A. Gene mutation – replacement of the nucleotide by complementary one

B. Chromosomal mutation – lost of the chromosome part

C. Chromosomal mutation – reverse of the chromosome part on 180^0

D. Genome mutation (numerical chromosome aberration) – diploid set of the chromosomes possesses one extra chromosome

E. Genome mutation (numerical chromosome aberration) – absence of one chromosome in diploid set

14. Inversion is

A. Gene mutation – replacement of the nucleotide by complementary one

B. Chromosomal mutation – lost of the chromosome part

C. Chromosomal mutation – reverse of the chromosome part on 180^0

D. Genome mutation (numerical chromosome aberration) – diploid set of the chromosomes possesses one extra chromosome

E. Genome mutation (numerical chromosome aberration) – absence of one chromosome in diploid set

15. Duplication is:

A. One extra chromosome in diploid set**.**

B. Doubling of the part of the chromosome

C. Additional haploid chromosomal sets

D. Replacement of the nucleotide by the complementary one

E. Transfer of the chromosome part on the non-homologous one

16. Trisomy is

A. Gene mutation – replacement of the nucleotide by the complementary one

B. Chromosomal mutation – lost of the chromosome part

C. Chromosomal mutation – reverse of the chromosome part on 180^0

D. Genome mutation (numerical chromosome aberration) – diploid set of the chromosomes possesses one extra chromosome

E. Genome mutation (numerical chromosome aberration) – absence of one chromosome in diploid set

17. Human karyotype possesses al types of the chromosomes **except** for:

A. Metacentric

B. Submetacentric

C. Acrocentric

+ D. Telocentric

E. Satellite

18. Example of monosomy in human pathology is:

A. Down syndrome

B. Patau syndrome

C. Edwards syndrome

D. Klinefelter syndrome

+ E. Shereshevsky-Turner syndrome

19. Some mutations in humans are balanced i.e. don't cause changing in phenotype, but result in infertility. Which mutation could be balanced?

A. Deletion

B. Duplication

C. Trisomy

D. Inversion

E. Monosomy

20. Aborted embryo has the karyotype 47, XX, + 21. Which mutation is it?

A. Polyploidy

B. Duplication

C. Deletion

D. Trisomy

E. Monosomy

21. Chromosomal mutations in humans may results in all of the below **except** for

A. Lethal effect

B. Tumor formation

C. Apert syndrome

D. Patau syndrome

E. Delayed psychomotor development

Answers for self-control
1-A
2-C
3-D
4-D
5-B
6-E
7-E
8-C
9-D
10-A
11-C
12-B
13-B
14-C
15-B
16-D
17-D
18-E
19-D
20-D
21-C

Practical lesson No. 2
Topic: Semiotics of hereditary diseases
<u>**The purpose of the lesson:**</u> to get the practical skills of examination the patient with the aim of diagnostics of hereditary disorder. To became acquainted with the structure of medico genetical center.

<u>**Student should know**</u>:
1. What is hereditary disorder.
2. Classification of hereditary disorders.
3. History data pointing out hereditary pathology.
4. Main congenital anomalies.
5. Tasks of medico-genetic counseling.
6. Organization of medico-genetic aid in Ukraine.

<u>**Student should be able to**</u>:
1. Reveal most common congenital micro anomalies while examining the patient or studying the photos of the patients.
2. Analyze the history of the diseases and reveal the symptoms indicating hereditary pathology.

CONTROL QUESTIONS
1. Aim of the medico-genetic counseling. Principles of organization of medico-genetic aid to the population.
2. Classification of hereditary disorders.
3. Syndromologic diagnostics in practice.
4. Describing of the phenotype of the patient with hereditary disorder.
5. Definition of congenital micro anomalies and defects and its role in diagnostics of hereditary disorders.
6. Main micro anomalies and defects of head face trunk, limbs, skin and its derivatives.
7. Peculiarities of the clinical features of hereditary disorders.
8. General principles of diagnostics of hereditary disorder.
9. Manifestation of hereditary disorder in different age groups.
10. Main genetic terms: gene, genotype, phenotype, allele genes, homozygote, heterozygote, dominant gene, recessive gene, recessive character, proband, siblings.

PRACTICAL WORK
1. To analyze phenotype of the patient, name congenial micro anomalies and defects.
2. To analyze the case history and name the characters, indicating hereditary disorder.
3. To study phenotypes of the patients with hereditary disorders, using photos of the patients with different hereditary diseases. Name the micro anomalies and congenital defects.

Main literature
Methodical recommendations on medical genetics
Additional literature:
1. Genetics in medicine. - 7th edition/Robert L/Nussbaum, Roderick R. McInnes, Huntington F. Willard. – 2007 – 585 p.
2. Emery's Elements of medical genetics. 15th ed. / Peter Turnpenny, Sian Ellard. – Elsevier, 2017. – 400 pp.
3. Lynn B. Jorde, John C. Carey, Michael J. Bamshad. Medical genetics. 5th ed. Elsevier, 2016. 356 pp.
4. Vogel and Motulsky's human genetics. Problems and approaches / M. R. Speicher, S. E. Antonarakis, F. G. Motulsky. 4th addition. – Springer, 2010. – 981 pp.
5. Young Ian.D. Medical genetics. -2nd ed. -Oxford university press, 2010. - 304 p.

6. Diseases of the fetus and newborn. Pathology, radiology and genetics. G.B.Reed, A.E. Claireaux and A.D.Bain., - Great Britain, - 1989, 812 p.

7. Human molecular genetics. Tom Strachan, Andrew P.Read. – 4th edition - Bios Scientific Publisher, 2010, 680 p.

8. Smith recognizable patterns of human malformation. Seventh edition. John M. Graham, - USA, - 2013, 976 p.

9. R. Wiedemann, K.-R. Gross, H.Dibberin ,Atlas of characteristic syndromes. A Visual Aid to Diagnosis. Second edition. -London, 1986,412 p.

13. Information resources:

https://ghr.nlm.nih.gov National librery of medicine, genetics

https://www.orpha.net The portal for rare diseases and orphan drugs

https://rarediseases.org National Organization for Rare Disorders

http://omim.org/OMIM (Online Mendelian Inheritance in Man) – An Online Catalog of Human Genes andGenetic Disorder

MCQs for self -control

1. Minor congenital anomaly is characterized by all of this except:
A. It is a morphological change
B. It doesn't influence the function.
C. It is not cosmetic defect.
D. It doesn't require medical correction.
E. It is absent in healthy person .

2. Chromosomal disorders are characterized by skin fold in the internal angle of the eye. This minor anomaly is termed as:
A. Coloboma.
B. Epicanthus.
C. Criptophtalmos.
D. Synophris.
E. Microblepharon.

3. Blue sclera and gothic palate are features typical for metabolic defects of:
A. Carbohydrates.
B. Lipids.
C. Amino acids.
D. Connective tissue.
E. Hemoglobin.

4. Coarse face with abundant hair, thick lips is typical for:
A. Cystic fibrosis.
B. Glycogeosis.
C. Phenylketonuria.
D. Mucopolysaccharidosis.
E. Marfan's disease.

5. Underdeveloped chin is typical for many chromosomal disorders. This feature is termed as:
A. Microgenia.
B. Progenia.
C. Prognatia.
D. Microstomia.
E. Microcoria.

6. Heiloschisis – is:
A. Eyebrows growing together.
B. Short palpebrae.
C. Cleft palate .
D. Cleft lip.
E. Small mouth.

7. New born girl has claw-like right hand. This defect is termed as:
A. Phocomely.
B. Arachnodactyly.
C. Syndactyly.
D. Camptodactyly.
E. Ectrodactyly.

8. A 5-months-old girl with Down's syndrome has lateral deviation of small finger. This minor anomaly is termed as:
A. Clinodactyly
B. Arachnodactyly.
C. Syndactyly.
D. Camptodactyly.
E. Ectrodactyly.

9.. Boy with Marfan's syndrome has extremely long slender fingers. This minor anomaly is termed as:

A. Phocomely.
B. Arachnodactyly.
C. Syndactyly.
D. Camptodactyly.
E. Ectrodactyly.

10. Mongolian eye slant is:
A. Large distance between inner angles of the eyes.
B. Low situated external angle of the eye.
C. Short eye slant.
D. Low situated internal angle of the eye.
E. Skin fold in the internal angle of the eye.

11. 12-years-old girl with Turner syndrome has widened neck because of wing-like skin folds. This feature is termed as:
A. Hypertelorism.
B. Hypotelorism.
C. Pterigium coli.
D. Synophris.
E. Paltoschisis

12. Boy with cri-de-chat syndrome has widely spaced eyes. This minor anomaly is termed as:
A. Hypertelorism.
B. Hypotelorism.
C. Pterigium coli.
D. Synophris.
E. Paltoschisis

13. Newborn child with Apert's syndrome is characterized by egg-shaped skull. Such shape of the head is termed as:
A. Brachicephaly.
B. Dolychocephaly.
C. Acrocephaly.
D. Scaphocephaly.
E. Trigonocephaly.

14. 8-years-old girl with mucopolysaccharidosis have flexor contracted fingers. This feature is termed as :
A. Phocomely.
B. Arachnodactyly.

C. Syndactyly.
D. Camptodactyly.
E. Ectrodactyly.

15. Excessive development of mammary gland in man is termed as:
A. Hupertelorism.
B. Polytelia.
C. Polymastia
D. Gynecomastia.
E. Hyperplasia.

16. Mental deterioration is the typica feature of:
A. Chromosomal disorders with autosomes abnormalities.
B.Chromosomal disorders of sex chromosomes.
C. Chromosomal aberrations.
D. Glycogenosis.
E. Sphingolipidosis.

17. Combination of microcephaly, microgenia and low situated abnormally shaped auricles is typical for
A. Chromosomal disorders.
B. Single gene disorders.
C. Metabolic defects of glycosaminoglycans.
D. Amino aciduria.
E. Metabolic disorders of connective tissue.

18. Slit-like defect of iris is termed as:
A. Coloboma.
B. Hypertelorism.
C. Hypotelorism.
D. Epicanthus.
E. Atresia.

19. How many minor anomalia can be normally present?
A. None.
B. 0 to 3.
C. 0 to 6.
D. 0 to 8.
E. 0 to 10.

MAIN THEORETICAL INFORMATION
Tasks of the medico genetic counseling.

Medico-genetic counseling it is one of the types of specialized medical aid to the population, directed on the prevention of birth the children with hereditary pathology. Basic tasks of the medico-genetic counseling are.

1. Exact diagnostics of hereditary disorder.

2. Determining of the type (mode of inheritance) of hereditary disorder in that particular family.

3. Calculating of the recurrent risk in that particular family.

4. Determining of the most effective way for prophylaxis.

5. Explanation of collected and analyzed information and prognosis to the family.

Medico-genetic aid for the population of Ukraine is provided by specialists of inter-district medico-genetic consulting rooms, regional medico-genetic centers, inter-regional medico-genetic centers, Ukrainian scientific center of medical genetic, scientific research center of hereditary pathology in Lvov, and clinical institutes of the Health department of Ukraine, medical institutes.

<u>Inter-district medico-genetic centers</u>: Are organized on the territory with population of 300.00 and more. They are placed in the district and regional hospital. Pediatricians or obstetricians after special training on medical genetics work in it. They carry out primary diagnostics of hereditary disorders, consultations of the families and patients with hereditary disorders, registration and monitoring of the families with hereditary disorders and congenital defects, control of the mass screening programs.

<u>Regional medico–genetic consulting centers</u> is organized on the basis of regional or city hospital, its stuff includes specialists in medical genetics (pediatricians, obstetricians). Center is equipped with cytogenetic and biochemical laboratory. Apart from medico-genetic counseling of families with hereditary disorders and co-coordination of inter-district medical consulting centers work, management of registries, controlling of mass screening programs specialists of the center actively reveal the patients with hereditary disorders, carry out prenatal ultra sound diagnostics, cytogenetic, DNA and biochemical diagnostics of hereditary diseases.

<u>Inter-regional medico genetic-centers</u> provide the specialized aid for population in diagnosis, prophylaxis and treatment of patients with hereditary disorders. Apart from specialists in medical genetic (pediatricians and obstetricians) stuff of the center includes endocrinologist and neurologists after specialization in field of medical genetics. In center is well equipped cytogenetic and biochemical laboratory, ultra sound. Center has high possibilities in diagnostics, treatment and prophylaxis of hereditary disorders. Specialists of the center organize prenatal diagnostics and carrying out of mass screening programs in the region.

All specialists of medico-genetic service carry out propaganda of medico-genetical knowledge to the population.

Specialized centers are organized for diagnostics and treatment of mostly common hereditary disorders like phenylketonuria, cystic fibrosis, hemophilia, muscular dystrophy and others.

Classification of hereditary disorders.

What is a hereditary disorder?

Hereditary disorder is a disease caused by changes in genotype (mutation). Students often define hereditary disorder as a disease, which is transmitted in the family from generation to generations, a disease that should be obligatory inherited by children from the parents.

As many hereditary diseases may not inherit, this is not the exact definition. Hereditary disorder often considerably reduces viability or fertility of the patient or is the result of fresh dominant mutation. For example, as a rule, child with Down syndrome has healthy parents. On other side, certain endemic diseases are observed in parents and children and gives impression of inheritance, but they are not hereditary (endemic goiter).

Terms "hereditary" and "congenital" diseases are also different. As congenital are known all diseases and defects, manifesting at birth. Hereditary disorders are not always congenital. Onset of the disease may be after several months or even a year (mucopolysaccharidosis, hepatolenticular dystrophy, Huntington's disease and others). Some congenital defects are because of teratogenic environmental effect and are nonhereditary.

 "Family disorders" are met among members of one family. It may be either hereditary (usually autosomal-recessive) or nonhereditary (caused by the same environmental factor like

harmful habits, profession, peculiarities of nourishment and others).

Classification of hereditary disorders is based on classification of mutation and character of interaction with the environment. Under modern classification all hereditary disorders may be divided into following groups.

1. Single gene disorders (monogenic disease) – caused by single gene mutation (achondroplasy, phenylketonuria, haemophilia and others).

2. Chromosomal disorders caused by numerical or structural chromosome mutations (Down syndrome, cat cry syndrome and others).

3. Multifactorial disorders (disorders with hereditary predisposition) are the result of interaction abnormal genotype of the individual and provoking environmental factors (cleft lip and palate, clubfoot, arterial hypertension, schizophrenia and others).

4. Genetic disorders of somatic cells – result of somatic mutation (retinoblastoma, Wilms tumor and some other tumors, certain sporadic case of congenital defects).

5. Disorders because of mother-fetus genetic incompatibility (hemolytic anemia because of Rh-conflict or because of incompatibility on other antigens).

Clinical classification is based on the systems and organs principles: psychic, neuromuscular, skeletal, facial, cutaneous, respiratory, immunity system disorders and others.

Syndromologic diagnose in clinical genetics.

Hereditary disorders are caused by changing of the genotype, i.e. mutations. Mutations result in either failure of embryonic development, or disturbance in certain link of metabolism. In different people certain hereditary disorder usually manifests with the same clinical features. As a rule it manifests not only as one symptom, but a definite complex of symptoms. This pattern of symptoms, caused by the same pathogenesis is known as a syndrome. Knowing the pattern of the symptoms for certain hereditary disease one may diagnoses the disorder on the basis of phenotype analysis. So, diagnostics of majority of hereditary disorders is syndromologic. Many of the hereditary syndromes are diagnosed only on basic characteristic external features. In many cases portrait diagnostics is carried out. In portrait diagnostics special atlases of characteristic syndromes (a visual aid to diagnosis), and computer diagnostic programs are used.

Description of patient's phenotype is not enough, exact diagnose is very important in prognosis of viability, choosing of treatment and calculating of recurrent risk. For example, at patients with Patau syndrome there is no sense to perform surgical correction of cleft lip, because this syndrome is lethal. Artesia of esophagus may be isolated congenital defect or symptoms of lethal Di George syndrome. In first case surgical treatment is indicated, in the second case it is out of sence. Apart from that, exact diagnose allows to determine mode of inheritance and this is extremely necessary for calculating of recurrent risk and choosing of prenatal diagnostics methods.

Term "syndrome" in clinical genetics often uses as a synonym for word "disease". One may say "Down disease", "Patau disease" and "Down syndrome", "Patau syndrome". For many hereditary diseases term "syndrome" traditionally is used more often: Klinefelter syndrome, Shereshevsky – Turner syndrome, Russell – Silver syndrome, Cornelia de Lange syndrome and others.

Description of the phenotype of the patient with hereditary disorder

Phenotype is the totality of external and internal signs of the organism. While describing phenotype of the patient one should pays attention on congenital defect and minor anomalies.

Congenital defect (or congenital abnormality) is a structural defect of prenatal origin, present at birth, seriously interfering function, health or viability. As synonyms of "congenital defect" terms "defects of development", "malformations" "birth defects" are used.

Minor anomalies (stigmas of disembryogenesis) are unusual morphologic features that are of no serious medical or cosmetic consequence. So, these are morphologic changes of an organ, which don't require any medical correction.

Number of minor anomalies in healthy child is from 0-6. Following are met more often: epicanthal folds, high palate, flat nasal bridge, deformation of ear auricles and others. In hereditary

diseases their number increases (in single gene disorders 8 – 15). Presence of multiple minor anomalies suggests a generalized disorder of early embryogenesis under hereditary or teratogenic factors. The more minor anomalies are present the more likely is the hereditary disorder. Specific pattern of minor anomalies forms unique phenotype of the patient. Its recognition is very important in clinical diagnoses of hereditary disorder.

Examination of the patient starts with the measurement of height, weight and head circumference. Obtained data is compared with normal age parameters.

Height: - hereditary disorder often accompanies by growth deficiency starting in embryonic or postembryonic period. Smallness of body is termed as microsomia or dwarfism. Abnormally large size of the body is observed rarely and termed as macrosomia or gigantism (Beckwith-Wiedemann syndrome).

Sometimes facial or cranial asymmetry (hemifacial hypertrophy) or asymmetry of the body (Russell–Silver syndrome) is observed.

Weight: hereditary disease often manifests in embryonic period that results in hypotrophy and hypoplasia of newborn. Hypoplasia is inappropriate for gestation age weight and height of the newborn. Hypotrophy is small weight of the child with normal height. Decrease in body weight observes in chromosomal disorders, inborn errors of metabolism, congenital defects of digestive tract and others. Rarely obesity may be observed (Prader–Willi syndrome and some others).

List of main congenital defects and minor anomalies

1. HEAD, FACE, NECK.

a) abnormal size of the head with more than of 10% deviation comparatively with age norm: microcephalia (-5cm) or macrocephalia (+5cm).

Hydrocephalus (excessive accumulation of fluid in cerebral ventricles) differs from macrocephalia with disproportional enlargement of cerebral parts of the cranium – face is relatively small, large prominent forehead, dilatation of subcutaneous veins, possible divergence of cranial sutures, enlargement of fontanels.

b) unusual cranial configuration: brachycephalia (disproportionate shortness of head, flat face), dolichocephalia (disproportionately long head), scaphocephalia (prominent forehead and occiput), trigonocephalia (triangular configuration of the skull due to premature synostosis of the cranial bones), acrocephaly or oxycephalia or turricephaly (tower skull – abnormally high, conically shaped) Premature synostosis of cranial bones (craniosynostosis) leads to the different deformations of the skull, like cloverleaf skull.

c) Low (or high) anterior and posterior hairlines.

d) unusual face: Bird-like face (Marfan syndrome), doll face (glycogenosis), coarse face (mucopolysaccharidosis – abundant thick hair of the eyebrows, thick lips), triangular face (Russell – Silver syndrome).

e) Small or very high forehead, bulging or sloping forehead.

f) Defect of eyes.

Anophthalmos – absence of one or both eyes; cryptopthalmus – congenital absence of the eyelids with skin passing continuously from forehead onto cheek over a rudimentary eye; buphthalmos – enlargement of the eyeball; microphthalmos – small size of the eyeball.

Anomaly of eyelids: distichiasis or tristichiasis – the presence of two or three rows of eyelashes on the eyelids; coloboma – slit-like defect, epicanthus - fold of skin extending from the root of the nose to the inner termination of the eyebrow; microblepharon – small vertical size of the palpebra, leading to lagophthalmos (inability to close the eyes), blepharophimosis – shortening of the eye lids and eye slit; ptosis – drooping of the eyelid, synophrys – the growing together of the eyebrows.

Blow sclerae; micro- and macrocornea – abnormal size of cornea; coloboma of cornea; aniridia – absence of iris; heterochromia – irregular distribution of pigment in one eye or in two eyes, cataract – a loss of transparency of the lens of the eyes; leukoma – albugo, a dense, opaque, white opacity of the cornea; enophthalmos – deep position of the in orbital area; exophthalmos –

protrusion of the eyeballs.

Mongoloid (external angle over the internal), or anti-mongoloid (external angle below the internal) slant of palpebral fissures.

Hypotelorism – narrow spaced eyes; hypertelorism – widely spaced eyes. Distance between internal angle of the eye normally is equal to the length of the eye slit.

In hereditary disease also is met glaucoma, divergent or convergent strabismus, short or long sight, blindness.

g) Anomaly in structure of nose and jaws;

Nasal bridge: depressed, flat, broad, narrow, bulging of the nasal root (Greek warrior helmet);

Nose: saddle-like, beak-like, potato, small nose with turned-up nostrils. Hemihypoplasia of the nose, hypoplasia of nasal porch. Distortion of the nasal septum, hypoplasia and notching of alai nasi, colobomus of nasal wings.

Upper jaw may be underdeveloped (micrognathia) – or vice versa, large, with abnormal forward projection (prognathism), abnormally small lower jaw is termed as microgenia, and its overgrowth with forward position of the chin – basilar prognatism.

Long or short philtrum (distance between the nose and upper lip).

i)   Mouth and oral cavity.

Macrostomia and microstomia – large or small size of the mouth; abnormally thick or thin lips are also the symptoms of hereditary pathology. Cheilo- or palatoschisis – middle, one or double sided cleft lip or palate.

Changes in number of the teeth: adentia – absence of teeth, oligodentia – less number of teeth), abnormal shape (conical teeth). Macro- and microdentia –change in size of teeth, diastema – a space between two adjacent teeth;   amelogenesis imperfecta – enamel dysplasia. Multiple caries. High (gothic) palate. Macroglossia and microglossia - large tongue.

j)   Auricle.

 Process of formation of external ear in embryonic development is very sensitive to the changes of genotype and action of teratogenic factors.

 Due to hereditary and congenital pathologies often observed large (macrotia) or small (macrotia) auricles, high or low position of auricles, large everted auricules. Normally lower wall of auditory canal is situated on the line between wing of the nostril and mastoid process of the temporal bone. Often are met: attached ear lobule, thick ear lobule, preauricular tags or fistulas, stenosis or atresia of auditory canal, hypo-and hyperplasia of different auricular structures.

Hearing impairment or deafness may also be observed.

k) Neck: - short, wing-like skin fold - pterygium, central or lateral cervical cysts.

2. TRUNK:

a)   deformation of chest and vertebrae column: scoliosis – lateral curvature of the spine; kyphosis – abnormal curvature of the spine with the convexity backward(usually in the thoracic region); lordosis – anterioposterior curvature of the spine, generally lumber, with the convexity looking anteriorly; kyphoscoliosis; flat spine – absence of physiological curvatures; cobbler's chest, funnel chest, keeled (chicken) chest.

b)   changes in nipples or mammary gland: absence of nipples – atelia; extra nipples – polytelia; widely spaced nipples – hypertelorism of nipples; gynecomastia – excessive development of the male mammary glands.

c) hernias of linea alba, umbilical, inguinal, inguinoscrotal hernias.

d) abnormalities of sexual organ:

in males epispadia and hypospadia – a malformation in which the urethra opens on the dorsum or on the under surface of the penis; macro- or microphalos- Crypthorchism – the failure of descent of a testis; in females – hypertrophy of clitoris; hypo- or hyperplasia of large and small pudendal lips; atresia of vagina.

3. LIMBS

Phocomelia - absence of a proximal parts of the limbs or of the whole limbs; brachymelia - shortness of limbs; brachydactylia - shortness of fingers; isodactylia - equal length of fingers; arachnodactylia - long slender fingers; syndactylia (symphalangism) any degree of webbing or fusion of fingers or toes; clinodactyly – deflection of one or more fingers; large distance between first and second toes; flat foot - arches of the foot are flattened; rockerbottom foot - flat foot with prominent heel; polydactylia – more then five digits; ectrodactylia – congenital absence of one or more fingers and toes with hand or foot like lobster's claw;

4. SKIN AND ITS DERIVATIVES (hair, nails, skin glands):

hyperkeratosis – hypertrophy of the horny layer of the epidemis; ichthyosis – congenital disorder of keratinisation characterized by dryness and fishskin-like scaling of the skin; albinism - absence or decrease amount of the melanin in skin and hair; partial hyperpigmentation of skin – nevi (birthmarks); partial hypopigmentation of skin – leukoderma; angidrosis – dry skin because of sweat glands absence; hypohydrosis – insufficient function of sweat glands; hyperhydrosis – excessive function of sweat glands; hypertrichosis - growth of the hair in excess of the normal; hypotrichosis – a deficiency of hair on the head and body; hirsutism – presence of excessive bodily and facial hair in women; alopecia – complete or partial absence of hair on the scalp; hypoplasia of nails; anonychia – absence of nails.

Terms, used for description of the phenotype

Agenesis - absence, failure of formation or imperfect development of any part;

aplasia - congenital absence of an organ or tissue;

hypoplasia - a) underdevelopment of tissue or an organ, usually due to a decrease in the number of cells, b) mass and growth of newborn, of corresponding with the period of gestation;

hypertrophy - an increase in number of cells in tissue or organ, excluding tumor formation whereby the bulk of the part or organ is increased;

macrosomia — abnormally large size of the body;

pachy – prefix is used to mark the thickness of definitive part of the organ For example: pachygyria - unusually thick convolutions of the cerebral cortex related to defective development; pachydactyly - enlargement of fingers or toes;

heterotopy - a displacement of parts of the organs, tissues;

ectopy - congenital displacement of any organ or part of the body;

duplicitas - doubling of part;

multiplicity of definitive organs is marked with Greek prefix - **poly-** or Latin - **multi-**. For example: polydactyly —the presence of more then five digits an either hand or foot, polygyria — a condition in which the brain has an excessive number of convolutions;

atresia - congenital absence of a normal opening or normally patent lumen;

stenosis - a narrowing of any canal;

-pagus - suffix is used to mark conjoined twins. For examples craniopagus conjoined twins with fused sculls, thoracopagus — conjoined twins with fusion in the thoracic region;

syn- prefix is used to mark fusion or adhesion of the organs. For example: syndactility - any degree of webbing or fusion of fingers or toes, syncheilia —a more or less complete adhesion of the lips;

persistence - obstate continuation of existence the embryonic structures that normally should vanish;

hydro- prefix is used to mark excessive accumulation of fluid dilating the organ. For example: hydrocephalus - excessive accumulation of cerebrospinal fluid in cerebral ventricles;

hydronephrosis - dilation of the pelvis and calices of one or both kidneys resulting from obstruction to the flow of urine;

inversion - mirror disposition of internal organs.

Main peculiarities of clinical manifestations of hereditary disorders

Despite a great variety of clinical symptoms all hereditary disorders have common specific features, which are explained by the action and interaction of genes.

1. Familial character of disease, as a rule, indicates the hereditary disorder.

At the same time presence of the disease only in one person in the family, does not exclude hereditary character of the disease, as parents of the patient may be heterozygous carriers of recessive pathological gene. Dominant single gene disorders may be the result of fresh mutation. Chromosomal disorders usually are also caused by mutations.

2. Chronic, progressive, recurrent course.

Pathologic genotype effects constantly. This explains chronic progressive course (with age of the patient severity of the disease increases). Especially this is characteristic for storage disorders with reduced catabolism of macromolecules.

3. Congenital character of disease is typical, but not for all of the hereditary diseases. All chromosomal diseases and nearly 25% of single gene disorders manifests from the moment of birth. Other starts at different age, from few days to senile period. On the other side, congenital disease not always is hereditary (for example, fetal alcohol syndrome, diabetic embryopathy and others).

4. Multiple affection - participating in pathological process of many organs or even of system of organs makes to think about hereditary pathology. Multiple affection is explained with pleyotropic action of genes (influence of one gene on the formation of many signs).

5. Pattern of specific symptoms does not characterizes all hereditary diseases. But if they are present, it recognition permits to diagnose hereditary pathology in time. Examples of such specific symptoms are: mice odor of urine and sweat in phenylketonuria, mewing-like crying of newborns in chromosomal "cat cry" syndrome and others.

6. Hereditary disorders are resistant for the most traditional methods of therapy. At present times methods of treatment for some hereditary diseases are proposed, but they are very much differentiated from methods of treatment for common non-hereditary diseases.

General principles of clinical diagnostics for hereditary diseases.

Peculiarity of clinical genetics is that object for examination is not a particular patient, but whole family. Gathering information about a family starts with proband. Proband is the patient or member of the family that brings the family under study. It may be child, adult person or couple. Children of same parents are termed as sibs (siblings). Family in narrow meaning is a couple and their children, but sometimes may include other relatives.

At present time for medico-genetic counseling often come families having already sick child. In this case medico genetic counseling is known as retrospective.

Diagnostics of hereditary diseases, as a rule has two stages:

1) general clinic examination of the patient, genealogic and laboratory, when required. (ultra-sound, x-rays examination, endocrinologic, immunologic and other assays).

2) In suspicion on certain hereditary disorder - special genetic studies like (cytogenetic, DNA, special biochemical and other assays).

Examination of the patient is carried out in accordance with ordinary scheme. While taking family history one should pay attention on following moments.

1. Surname, name, and paternal name of the parents. Maiden surname of mother should also be ascertained. Coinciding of surnames in paternal and maternal family may indicate consanguineous marriage. In such marriages risk to have a child with recessive disorder increases.

2. Age of proband (including date and month of birth). Hereditary disorders may manifest in different age. Age of the parents at the moment of proband birth is also important. With paternal age increases probability of fresh dominant mutations (for example – achondroplasy and neurofibromatosis). With maternal age increases risk of chromosomal abnormalities.

3. Nationality. Some rare hereditary disorders are relatively common in certain nationalities. Ethnic backgrounds of the parents may be an indication for specific carrier testing or genetic screening.

For example are common:

a) in Ashkenazi Jews and Canadians of French origin - Tay-Sachs disease;

b) in Greeks and other inhabitants of Mediterranean region - Beta-thalassaemia.

c) in Afro-Americans – cycle-cell anemia.

d) in inhabitants of south – eastern Asia - alpha-thalassaemia.

e) in inhabitants of northern Europe and of south of Ukraine – cystic fibroses.

4. Residence of the family to exclude endemic diseases and influence of the environmental factors.

5. Residence of maternal and paternal families. If in few generations all members of the family live in the same small village there is a high probability of consanguineous marriages.

6. Work conditions. Possible contact with mutagens and teratogens factors should be taken into account.

7. Kind of military service of the father, possible contacts with mutagen factors during this period.

8. Chronic diseases of mother. Disorders of cardiovascular and respiratory systems often leads to fetal hypoxia, which is one of teratogenic factors; diabetes mellitus causes the risk of diabetic embryofetopathy; treatment for epilepsy includes many teratogenic anticonvulsants; maternal compensated phenylketonuria if woman do not keep a diet during the pregnancy, cause phenylpyruvic embryofetopathy.

9. Pathologic obstetric history.

Spontaneous abortions or stillbirth in anamnesis may indicate balanced chromosomal mutation in either paternal or maternal organisms. Both parents also may be carriers of the same recessive pathological gene (each person has 4-5 recessive lethal genes in average). Sometimes dominant lethal mutations form de novo.

10. Loaded family anamnesis. Presence in the consulted family or family of close relatives children with heredity disorders or congenital defects, and neonatal death because of unknown reasons indicates hereditary pathology. In all cases of stillbirth and neonatal deaths is necessary to get the conclusion of pathologist if possible.

11. Pathologic obstetric symptoms in pregnancy, which ends by giving birth to proband (patient).

a) Risk of spontaneous abortion is characteristic for chromosomal and many single gene disorders;

b) Intrauterine growth retardation may be revealed with ultrasonographic measurements of head and abdomen circumference. Possible reasons of growth retardation are: chromosomal syndromes; single gene disorders; intrauterine fetal infection (cytomegalovirus, rubella, syphilis); ionizing or non-ionizing radiation; multiple pregnancy; aplasia of pancreas. Growth delay also may be due to the influence of some maternal factors (smoking, alcohol and others).

Intrauterine growth retardation needs to be distinguished from syndromes hereditary dwarfism.

c) oligohydramnios is the symptom of different renal disorders with inadequate fetal urine output and may be teratogenic mechanical factor by itself.

d) polyhydramnios is observed in different defects of gastrointestinal tracts with swallowing failure.

e) decreased motility of fetus is characteristic for arthrogryposis (limitation of range of joint motion and contractures present at birth).

Symptoms of hereditary disorder, which can be revealed during the examination of the patient

While examining the patient it is necessary to pay attention on following symptoms, indicating hereditary disorder and congenital abnormality.

IN NEWBORNS

1. Congenital malformations may be hereditary (monogenic, chromosomal, multifactorial) or because of different teratogenic factors.

2. Prematurity of the newborn is typical for mane chromosomal disorders.

3. Hypotrophy or hypoplasia at birth is the symptoms of many chromosomal and single gene disorders.

4. Macrosomia is observed in Beckwith-Wiedemann syndrome, diabetic embryofetopathy and others.

5. Minor congenital anomalies (more than 6).

6. Muscular hypotony, and hyporeflexia are the symptoms of neuromuscular single gene disorders, Down syndrome and others.

7. Convulsions are the symptom of inborn errors of metabolism, defects CNS.

8. Microgenitalism or malformation of genitalia are symptoms of congenital adrenal hyperplasy, many single gene and chromosomal syndromes.

9. Acid – alkali misbalance (alkalosis or acidosis) are the symptoms of inborn errors of metabolism.

IN INFANTS

1. Growth retardation is observed in inborn errors of metabolism, chromosomal disorders, may be a symptom of hereditary dwarfism. Influence of different teratogenic factors like alcohol and others may cause as prenatal as postnatal growth delay.

2. Psychomotor retardation. It is a sign of chromosomal disorder if combined with congenital defects and minor anomalies. Is common in amino acidurias (inborn errors of amino acid metabolism). May be the symptom of neuromuscular congenital disease.

3. Mental deterioration is the symptom of storage disease (sphingolipidosis, leukodistrophy and others).

4. Microcephaly may inherits like recessive disorder or is a symptom of many single gene and chromosomal syndromes. Sometimes is caused teratogenic factors (intrauterine infection, hypoxia and others).

5. Macrocephaly may be familial feature or the result of hereditary pathology (fragile X syndrome, mucopolysaccharidosis, achondroplasia and others).

6. Deviation in physical development.
   a) Hypertrophy and asymmetry of face and cranium (syndrome of hemifacial hypertrophy and others).
   b) Hypertrophy and asymmetry of limbs (Russel-Silvers syndrome and others).
   c) Promoted physical growth (Beckwith – Wiedemann syndrome and others).
   d) Disproportion of trunk and limbs (in Marfan syndrome and homocystinuria – long slender limbs, in achondroplasy - shortening of proximal parts of limbs and others).
   e) Shortening of trunk in bone anomalies of vertebrae column.

7. Diffuse or local pigmentation defects. Stains on skin of coffee with milk color is characteristic for neurofibromatosis, hypopigmented stains for tuberose sclerosis, skin like geographic maps for, plural pigmentation of_____in syndrome Basal celled_____ For albinism, ectoderm displasia, phenylketonuria general hypopigmentation is typical.

8. Unusual smell of sweat and urine is characteristic for many inborn errors of metabolism (maple syrup disease or FKU with mousy odor).

IN CHILDREN OF PRESCHOOL AGE

1. Mental retardation exhibits very clear in children of school age. Usually it is preceded by psychomotor delay.

Basic reasons for mental retardation

ETIOLOGY	EXAMPLES
Autosomal dominant disorder	Tuberose sclerosis, myotonic dystrophy
Autosomal recessive disease	Phenylketonuria, mucopolysaccharidose
X-linked disorder	Fragile X syndrome, stenosis of
Multifactorial	Non-specific mental retardation, hydrocephlia
Chromosomal disease	Down syndrome, Prader – Willi and other's
Terratogenic influence	Fetal alcohol syndrome, congenital rubella
Sporadic case	Prenatal hypoxia, intra-cranial hemorrhage
Syndromes with unknown etiology	Cornelia de Lange syndrome

2. In this age at first may manifest some congenital errors of metabolism (mucopolysaccharidosis) or neuromuscular disorders (muscular dystrophy) e.t.c.

3. Chronic anemia may be a result of hemoglobinopathies (thalessaemia) or disturbances in metabolism of erythrocytes (glucose–6-phosphate dehydrogenase deficiency).

IN ADOLESCENT AND ADULT AGE.

1. Hypogenitalism and hypogonadism. Primary amenorrhea may be observed in syndrome of testicular feminization, primary amengrrhea and underdevelopment of secondary sexual characters are observed in Shereshevsky-Turner syndrome (45 XO), underdevelopment of secondary sexual characters in boys with Klinefelter syndrome (47, XXY).

2. Early pubescence may be observed in Beckwith-Wiedemann, Poland, Albright syndromes and others. This also may be a symptom of the tumor of pituitary, adrenal gland or ovary.

3. Some hereditary disease of nervous system manifests at this age: Friedreikch's disease, Huntington's disease and others. Epilepsy may start in adolescent period.

4. Hereditary nephritis and polykistosis of kidneys may manifest. One of the first clinical signs may be arterial hypertension.

5. In people with genetic predisposition in this or in more early age may be malignant tumors.

6. Early onset of middle-age disorders (ischemic heart disease or arterial hypertension) may be the result of single gene disorders (familial hypercholesterinemia).

7. Infertility may the result of balanced structural chromosomal mutation, presence of lethal gene or chromosomal disorder (Shereshevsky-Turner syndrome, or congenital defect of the uterus in women). Male infertility because of oligospermia often observed in Kleinefelter syndrome, cystic fibrosis, deletion of the Y chromosome.

8. Chronic miscarriage is observed in balanced chromosomal abnormalities of in both parents.

Practical lesson 3.
Topic: General characteristics of chromosomal diseases. Cytogenetic methods of diagnosis.
The purpose of the lesson: to study etiology, clinics and diagnosis, treatment of patients with Down syndrome. To be able to reveal the symptoms, characteristic for chromosomal diseases while history taking and examination of the patient and to work out plan of the clinical examination of a patient.
Student should know:
1. Characteristic of normal human karyotype.
2. Chromosomal aberrations and genome mutations.
3. General clinical symptoms of chromosomal disorders.
4. Key symptoms of Down syndrome.

Student should be able:
1. To select patients for cytogenetic analisis.
2. Formulate a possible diagnosis of Down syndrome

- Characteristics of the normal human karyotype .
- Classification of genomic and chromosomal mutations.
- What is uniparental disomy?
- What is chromosomal polymorphism?
- The value of chromosome and genome mutations in ontogenesis.
- What is a chromosomal disease? The frequency in the population.
- Classification of chromosomal diseases: a complete and mosaic forms, sporadic, hereditary.
- Pathogenesis of chromosomal diseases.
- Common symptoms of chromosomal diseases.
- Down syndrome: karyotype, the frequency in the population, the correlation with maternal age. Clinical characteristic of this syndrome in different age periods, the calculation of genetic risk.
- Diagnosis of chromosomal disease. Cytogenetic methods.
- Principles of medical genetic counseling. Prenatal diagnostics of chromosomal diseases.

Practical work
1. To examine the patient with Down syndrome, to reveal the typical symptoms, and formulate diagnose, to compose the plan of clinical and laboratory examination.
2. To study karyotypes of the patients with Down syndrome.

Main literature
Methodical recommendations on medical genetics
Additional literature:

1. Genetics in medicine. - 7th edition/Robert L/Nussbaum, Roderick R. McInnes, Huntington F. Willard. – 2007 – 585 p.
2. Emery's Elements of medical genetics. 15th ed. / Peter Turnpenny, Sian Ellard. – Elsevier, 2017. – 400 pp.
3. Lynn B. Jorde, John C. Carey, Michael J. Bamshad. Medical genetics. 5th ed. Elsevier, 2016. 356 pp.
4. Vogel and Motulsky's human genetics. Problems and approaches / M. R. Speicher, S. E. Antonarakis, F. G. Motulsky. 4th addition. – Springer, 2010. – 981 pp.
5. Young Ian.D. Medical genetics. -2nd ed. -Oxford university press, 2010. - 304 p.

6. Diseases of the fetus and newborn. Pathology, radiology and genetics. G.B.Reed, A.E. Claireaux and A.D.Bain., - Great Britain, - 1989, 812 p.
7. Human molecular genetics. Tom Strachan, Andrew P.Read. – 4th edition - Bios Scientific Publisher, 2010, 680 p.
8. Smith recognizable patterns of human malformation. Seventh edition. John M. Graham, - USA, - 2013, 976 p.
9. R. Wiedemann, K.-R. Gross, H.Dibberin ,Atlas of characteristic syndromes. A Visual Aid to Diagnosis. Second edition. -London, 1986,412 p.

13. Information resources:
https://ghr.nlm.nih.gov National librery of medicine, genetics
https://www.orpha.net The portal for rare diseases and orphan drugs
https://rarediseases.org National Organization for Rare Disorders
http://omim.org/OMIM (Online Mendelian Inheritance in Man) – An Online Catalog of Human Genes andGenetic Disorder

Chromosomal disorders is a group of the diseases because of structural and numerical chromosome changes. Genetics that deal with the studying of chromosomes is termed as cytogenetics. Its development starts at the beginning of the XX century. In 1903 Satton and Bovery were the first to suggest that genes are situated in chromosomes. In 1903 Satton introduced the term "cytogenetics", in 1911 T. Morgan formulated the main postulates of chromosomal theory of heredity.

Though the main discovering in cytogenetics of animals and plants have been made at the beginning of the century, development of human cytogenetics started only in fiftieth. Tijo and Levan in 1956 used a new methodic for studying of human chromosomes and revealed that there are 46 chromosomes in human karyotype. In 1959 was studied the karyotype of patients with Down, Turner and Klinfelter syndromes.

## Normal human karyotype (chromosomal set). International classification of chromosomes.

Karyotype is a diploid chromosomal set of organism, specific for the species, which is characterized by constant number, size and shape of chromosomes. There are 46 chromosomes or 23 pairs in human's karyotype. Twin chromosomes are called homologous, they have the same length and shape and contain allele genes. The material of the chromosomes is called chromatin. Chemical composition of chromatin is:
DNA – 40%, histone proteins – 50%, acid (non-histone) proteins - 8,5%, RNA - 1,5%,
traces of lipids, Ca^{2+}, Mg^{2+}.

Gene is a segment of DNA that contains biological information and codes one RNA and/or one polypeptide molecule.

Chromosomes in cells may present in two structural and functional states: condensed and uncondensed. In interfase they are in condensed state, in mitoses chromosomes are maximally condensed during metaphase. In metaphase a chromosome is found to possess two similar threads called chromatides, attached to each other at centromere or primary constriction area. The centromere divides the chromosome into two arms. The upper arm is often shorter than the lower one, being designated p and q respectively. Some chromosomes has the secondary constrictions and sattelite.

Structure of the metaphase chromosome.

arms   centromere   satellites

telomere
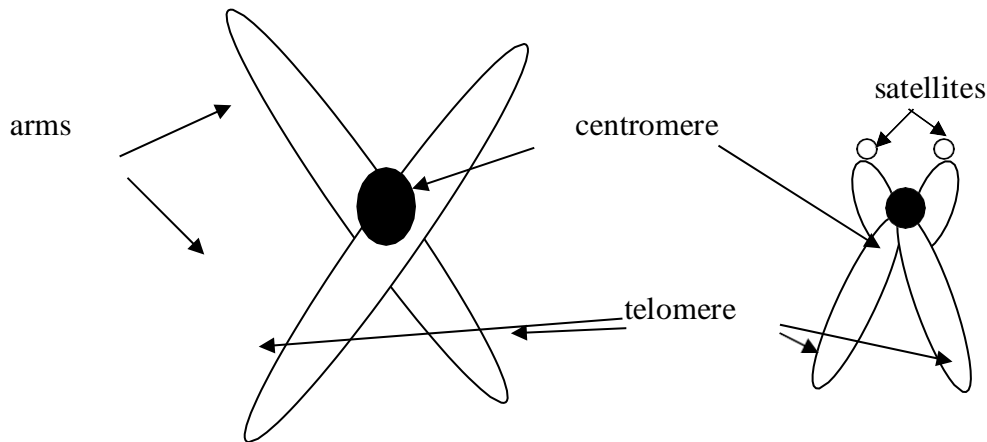
The position of centromere is fixed for a chromosome. In 1960 Patau proposed to classify chromosomes in accordance of comparative length of the arms and position of centromere (centromere index). Depending on it there are metacentric chromosomes (centromere is right in the center), submetacentric chromosomes (difference between chromosome arms is insignificant) and acrocentric chromosomes (one arm is much more shorter than another).

metacentric   submetacentric   acrocentric
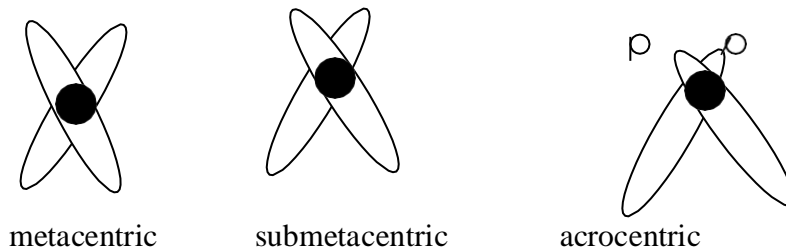
International (Denver) classification of chromosomes was adopted in 1960 on the international genetic conference in Denver. The main principles of classification were worked out by Patau, who proposed to take into account size and shape of the chromosomes. Under this classification, pairs of chromosomes from 1 to 23 are numbered with Arabian digits, 1 - 22 chromosome pairs are called autosomes, 23rd pair - sex chromosomes. Female karyotype is 46 XX, male - 46 XY.

According to centromere index and size 22 pairs of autosomes are divided into 7 groups from "A" to "G", 23rd pair (sex chromosomes) form separate group.

Group "A" (1-3). Large, metacentric and submetacentric chromosomes. Chromosome 1 - is the largest metacentric chromosome. Centromere is located in the middle, centromere index 48-49. The largest submetacentric chromosome is chromosome 2 with centromere index 38-40. Chromosome 3 with centromere index 45-46 is 20% shorter than chromosome 1 and, consequently, easily identified. Length is from 11 (1 pair) to 8.3 (2 pair).

Group "B" (4 -5). Large submetacentric chromosomes (centromere index 24-30) are not distinguished between each other without radio autography and differential staining.Length is 7.7 mcm.

Group "C"(6-12) Chromosomes of medium size, submetacentric. At routine staining X-chromosome can't be differed from other chromosomes of this group. X- chromosome is metacentric, by size it's similar with chromosomes of 8 and 7 pairs. Chromosomes 6, 7, 8, 11 and 12 are relatively submetacentric, their centromere index is 27-55. Length is 7.7 (6 pair) to 5.8 mcm.

Group "D" (13-15) Chromosomes are acrocentric, there is a great difference between them and other human chromosomes by shape. All three pairs have satellites on the short arm. Centromere index is about 15 - is the smallest in human karyotype.

Short arm of these chromosomes exhibits wide interchromosome diversity. Length of proximal parts of short arms varies, satellites may be absent or very large in size, may also give bright fluorescence or not.

Group "E" (16 - 18) Relatively short metacentric and submetacentric chromosomes. Length is 2.9 mcm.

Group "F" (19 - 20) – small metacentric. At routine staining they look similar, but at differential staining they are considerably different.
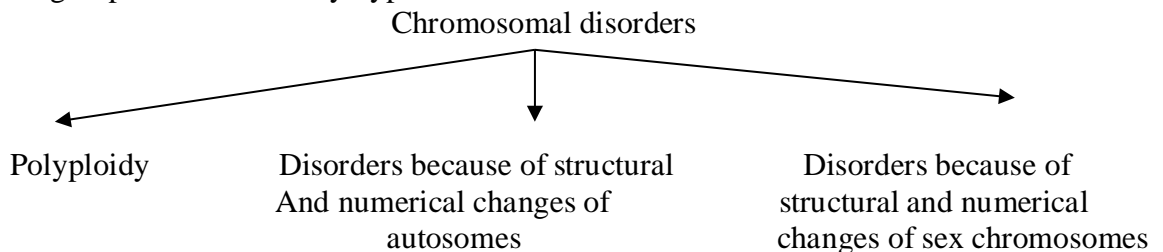
Group "G" (21-22). The smallest acrocentric chromosome/ Short arm bares satellite. Variability of its short arms is also considerable, as in chromosomes of group "D".

Y- chromosome is small acrocentric with absolute length 2,8 mcm. Usually (but not always) larger than chromosomes of group "G", and chromatids of its long arm, as usual, lay parallel to each other. That is the difference between Y-chromosomes and chromosomes of group "G", which exhibits wide angle between chromatids of long arms.

X - chromosome at routine staining is similar with chromosomes of group "C", but distinguishes while using of differential staining.

Classification of chromosomal disorders.

Chromosomal disorders is the group of the disorders because of numerical and structural chromosome anomalies. More then 1000 chromosomal disorders are described. About 100 of them have a clear clinical picture and are termed as syndromes. All chromosomal disorders are divided into three groups because of karyotype abnormalities.

Chromosomal disorders

| Polyploidy | Disorders because of structural And numerical changes of autosomes | Disorders because of structural and numerical changes of sex chromosomes |

Because of percentage of the cells with abnormal karyotypes complete and mosaic types of the chromosomal disorders are distinguished. Complete type of the disease is the result of germ mutation in parent's germ cell. All embryonic cells have abnormal karyotype. Mosaic type of the disease is the result of somatic mutation in embryo cells in the period of embryonic development. Because of this some embryonic cells have normal and some abnormal karyotype.

Sporadic chromosomal disorders are because of fresh mutation, hereditary ones are because of balanced chromosomal rearrangement or chromosomal disorder in parents. For example, persons with Klinefelter syndrome, polysomy X in females and polysomy Y in males, women with Downs syndrome are fertile.
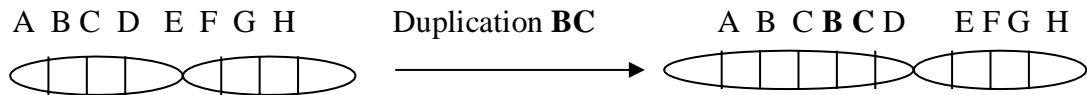
Etiology of chromosome diseases.

In etiology of chromosome diseases structural and numerical abnormalities are distinguished. Numerical anomalies can result either in aneuploidy and polyploidy. Change of normal chromosome number in haploid or diploid cell, which is not equal to haploid is termed as aneuploidy. Addition of complete haploid chromosome sets (more than 2) in somatic cell is termed as polyploidy. In human cells triploidy (3n=69) and tetraploidy (4n=92) are described. The group of aneuplidy monosomy (2n-1), trisomy (2n+1), tetrasomy (2n+2), pentasomy (2n+3) are included. Tetra- and pentasomy in human are observed only in sex chromosomes. Nyllesomy - absence of one chromosome pair (2n-2) is lethal mutation.

Chromosomal mutations or structural chromosome aberrations occur as a result of chromosomes reorganisation under meiosis. There are 5 main types of chromosomal aberrations.
1. Deletion — loss of chromosomes part.

A B C D E F **G H**   Deletion **GH**   A B C D E F
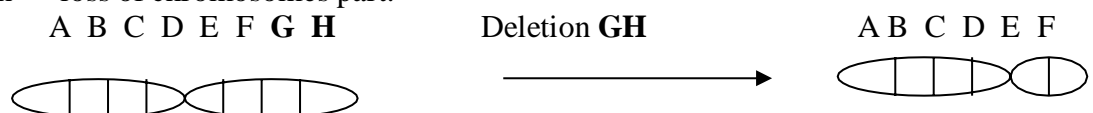
2. Duplication — doubling of chromosomes sectors.

A B C D E F G H   Duplication **BC**   A B C **B C** D   E F G H

3. Inversion — a segment of chromosome is reversed in position or inverted. If the inverted segment involves the centromere if is termed a pericentric inversion, if it does not involve the centromere it is called a paracentric inversion.

A B **C D E F** G H   pericentric inversion   A B **F E   D C** G H

A B C D   E **F G** H   paracentric inversion   A B F E   D **G F** H
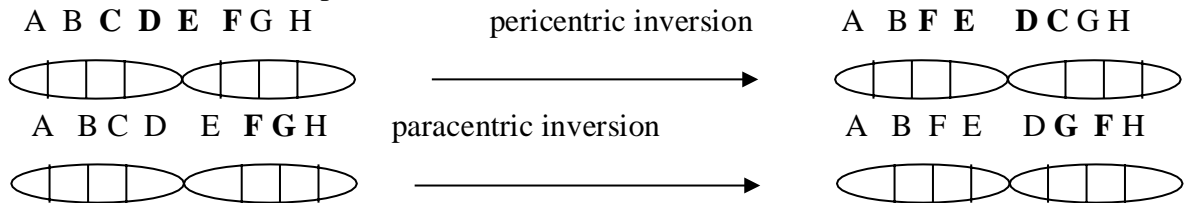
As an individual with an inversion usually has correct total amount of chromosome material, there will be no physical changes with the chromosome mutations (balanced mutation) but there may be important when such an individual comes to have children. Persons who carry an inversion can produce unbalanced gametes.

3. Ring chromosomes — occur as a result loosing of two telomer ends. The deleted ends, which are more "sticky" have adhered to each other to form a ring.

are lost
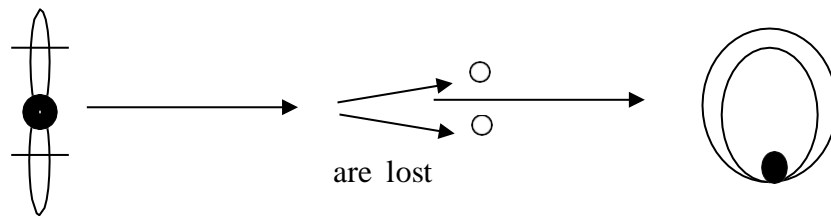
Persons with ring chromosomes also can produce unbalanced gametes.

5. Isochromosome — forms due to the reset of horizontal unparalleled division chromosomes. As a result metacentric chromosome forms, consisting of two same arms.
For example, isochromosome has duplication of the long arm and no short arm.

p are lost   Isochromosome
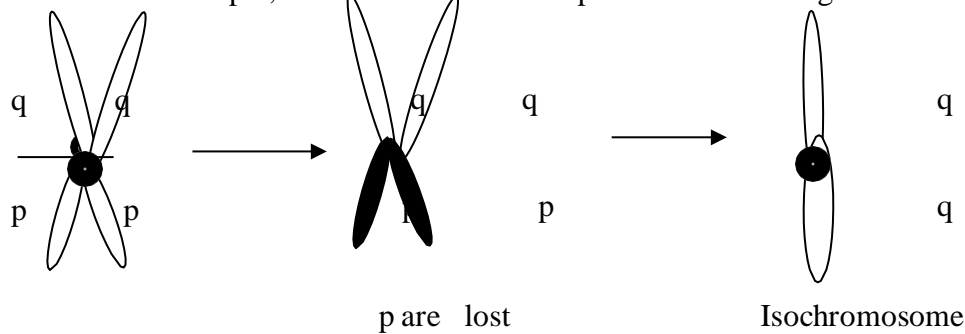
6. Translocation — transfer of chromosomes sector to another chromosome.
6.a. Robertsonian translocation or centric fusion is one of the common types of chromosomal mutations in human population. Observes between acrocentric chromosomes of D and G groups. The chromosomes of those groups have a small arm, containing nucleoli organizer (r RNA genes). Two chromosomes of these groups lose short arms and one centromere, and then long arms fuse. The fragments formed by the fusion of two short arms of two chromosomes are lost.
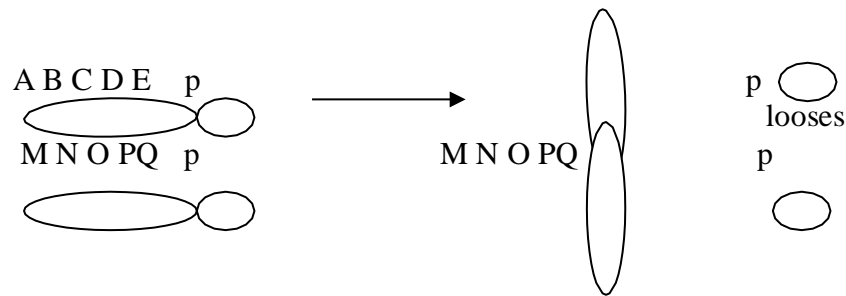
Loosing of r RNA genes does not occur change in phenotype, i.e. these genes belong to the repeatable. Each cell has a lot of copies of these genes (to 10^5 copies). Loosing of the genes is compensated with the same genes of other 8 acrocentric chromosomes. So, the carriers of such anomalies are healthy but produce abnormal gametes and are of high risk to have spontaneous abortions and children with chromosomal disorders.

Sometimes a parent may carry a translocation, which involves homologous chromosomes, for example 21/21. This person produces gametes of two types:

(1)with two joined chromosomes – 21/12, and (2) without chromosome 21. The risk of having affected offspring will be 100%.

P ♀ 21/21 x ♂ 21,21
G 21/21, 0 21
F_1 21/21 21; 21 0

6.b. Reciprocal translocation — in this kind of translocation there is a two-way exchange of material between homologous or non-homologous chromosomes. Autosomes or sex chromosomes may be affected, and no chromosomal material is lost. Carriers of reciprocal translocations are phenotypically normal, and as in Robertsonian translocations, the medical significance is for future generations.


7. Centrical division — aberration that is opposite to centrical fusion. One chromosome divides into two. Under this must form a new centromere. In opposite case chromosome without centromere looses under cellular division.

8. Transposition – part of the chromosome can change its position and without reciprocal exchange leaves in the same chromosome or in some other.
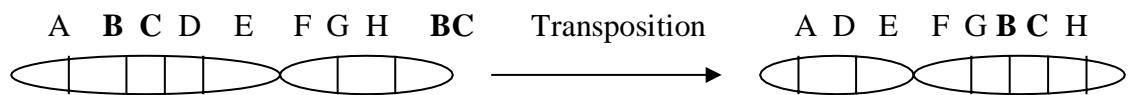
9. Dicentric chromosome consists of two centromeres. Two daughter chromatids lose telomer engs and form a one chromosome.

Fragments are lost   Dicentric chromosome
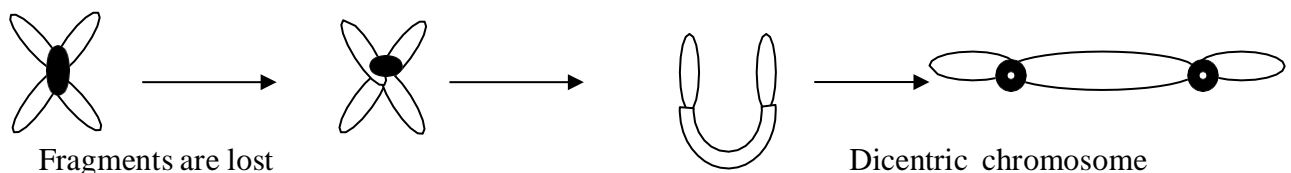
Genome Mutations (numerical mutations).

Genome mutation – is the change in number of chromosomes (numerical mutations).
There are 2 types of genome mutation:
1) Poliploidy.
2) Heteroploidy (aneuploidy).
Poliploidy is the numerical chromosome mutation with complete additional chromosome set 2n 3n,4n, ... In humans triploids (3n) contain 69 chromosomes and tetraploids (4n) - 92 chromosomes. →

Aneuploidy (heteroploidy) is increase or decrease in the number of chromosomes unequal to the haploid set. There are three kinds of aneuploidy —nullesomy, monosomy, polisomy (tri-, tetra-, pentasomy).

Normal diploid (2n) XX   XX   XX
                         1   2   3

a)Monosomy: absence of one chromosome (2n-1)
                  XX   XX   X-
                  1   2   3

b) Nullesomy — absence of two homologous chromosomes (2n-2)
                  XX   XX   —
                  1   2   3

c) Trisomy — one extra chromosome (2n+1)
                  XXX   XX   XX
                  1   2   3

d) Tetrasomy — two extra chromosome (2n+2)
                  XXXX   XX   XX
                  1   2   3

e) Pentasomy — (3n+3)
                  XXXXX   XX   XX
                  1   2   3

Basic mechanisms of chromosome numerical changes are:
1) Nondisjunction of chromosomes under mitosis or meiosis.
This is where either two homologous chromosomes fail to separate at meiosis I or a centromere fails to split at metaphase of meiosis II. After fertilization zygote and all embryonic cells will have abnormal number of chromosomes.
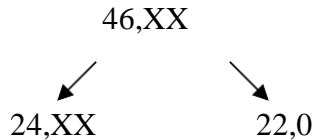
Scheme of Normal meiosis.

       Male   Female
      46,XY   46,XX

      23,X 23,Y   23,X   23,X

Meiosis with nondisjunction of the sexual chromosomes in male:
                  46, XY

        24,XY   22,0
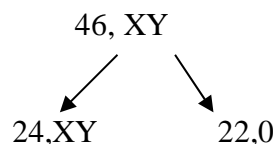
After fusion with normal ova (23,X) forms two types of the zygote: 47,XXY (Klinefelter's syndrome) , 45,XO (Shereshevsky-Turner`s syndrome).

Meiosis with nondisjunction of the sexual chromosomes in female.
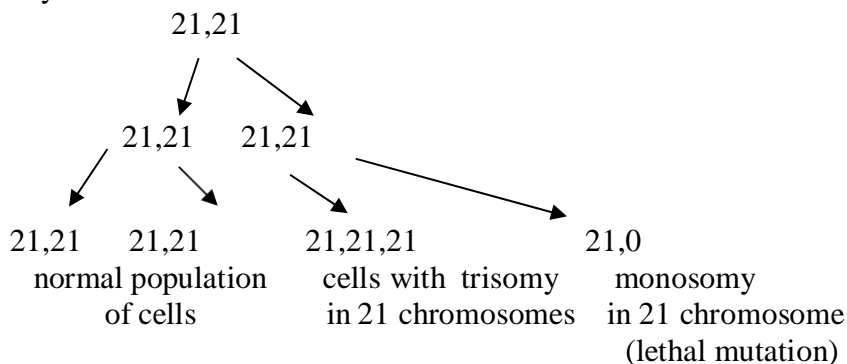
46,XX

24,XX   22,0

Fusion with normal sperm (23,X) forms two types of zygote: 47,XXX triple X (super female) syndrome or 45,XO Shereshevsky -Turner`s syndrome. After fusion with normal sperm (23,Y) forms zygote 47,XXY (Klinefelter's syndrome), or 45,YO (lethal mutation).

Nondisjunction of 21 chromosome under meiosis in mother resulting in Down's syndrome.

P   ♀ 21,12   X   ♂21,21

Gamete   21,21   0   21
F$_1$ 21,21,21   21,0
          Down`s syndrome   lethal mutation
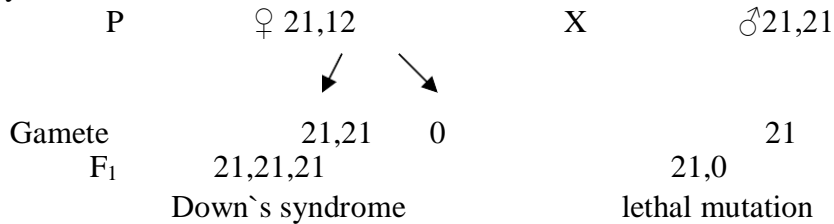
If chromosomes are not separated in mitosis in the process of cleavage or other stages of embryonic development, mosaic phenotype is formed. Scheme of mosaic formation is following in Down`s syndrome.

21,21

21,21   21,21

21,21   21,21   21,21,21   21,0
normal population   cells with trisomy   monosomy
of cells   in 21 chromosomes   in 21 chromosome
                                        (lethal mutation)

The latter is the mutation the less is number of abnormal cells.

2)   Chromosome lost in mitotic anaphase in embryonic or post-embryonic period. These chromosomes don't enter the nucleus and are destroyed with the cytoplasm enzymes. Lost of the chromosome cause the mosaic karyotype with normal and monosomic cell populations.

Risk of further chromosome anomalies as chromosome lost is higher in cells with aneuploidy.

3)   Children birth in parents with chromosomal disorders. Equal numbers of normal or abnormal gametes received from parents with trisomy or monosomy determine the theoretical probability of children birth with 50% gene mutation. This mechanism occurs rarely, that is aneuploidy characterises by the lowered vital activity and fertility. Normal and abnormal human karyotype.

In the year 1971 in Paris there was a conference which approved special nomenclature representation in man.

Normal karyotype in man:
          46,XX — female, 46,XY — male
Female and male poliploid karyotype
          69,XXX: 69,XXY   —   triploidy.
          92,XXXX: 92,XXXY —tetraploidy.
Female monosomic karyotype
          45,XO — the only one monosomy that is possible in living beings (Turner's syndrome)

Trisomic autosomal karyotype in female and male

47,XX,+21 or 47,XY,+21 — trisomy in 21 chromosome (Down`s syndrome)

47,XX,+13 or 47,XY,+13 — trisomy in 13 chromosome (Pateau's syndrome)

47,XX,+18 or 47,XY,+18 — trisomy in 18 chromosome (Edward`s syndrome).

Karyotype for trisomy in sex chromosomes:

47,XXX — Trisomy X in female

47,XXY — Trisomy Y in male

Tetrasomy and Pentasomy in sex chromosomes:

48,XXXX — Tetrasomy X.

48,XXXXX — Pentasomy X.

48,XXXY: 49,XXXXY:—modification of Klinefelter syndrome

48,XYYY: 49,XYYYY:—modification of Y polysomy in male.

Karyotype for chromosomal aberration:

46,XX, del 5p. — deletion of the short arm of the 5th chromosome in female.

46,XY, del 4p. — deletion of the short arm of the 4th chromosome in male.

46,X, i (X_q) — isochromosome X in the long arm in female.

46,XY, i (18) —radical 18 chromosome in male.

45,XX,-D,-Y,+T (D_q,Y_q) —.balanced Robertsonian translocation, forming a link between the long arm of single `D` and single Y-chromosome in female

Karyotype for mosaics:

45,X/46,X — part of cell which have normal karyotyre.

(46,XX) and part of it with monosomy X (45,X) we are concerned with mosaic form of Turner syndrome.

47,XX,+21/46,XX — mosaic form of Down`s syndrome.

Pathogenesis of chromosomal disorders.

In chromosomal disorders as a rule a misbalance in great number of genes take place. Abnormal genotype manifests in early stages of embryonic development, when more then 1000 genes controlling different stages of ontogenesis are expressed. These genes are located in all chromosomes. Numerical and structural chromosome anomalies cause misbalance in all genes including ones that regulate embryonic development It leads to failure in hystogenesis and organogenesis and formation of congenital defects. More often these defects are fatal and cause intrauterine death, in other cases it results in birth of the children with different malformations.

About 35 – 50% (on modern data up to 70%) of human embryos die on blastocyst stage before implantation. In majority of such cases embryo is chromosomally abnormal. Chromosomal anomalies cause near 45% of all cases of intrauterine death after implantation. The earlier is spontaneous abortion the more is the possibility of chromosomal misbalance. If abortion is at first 2 – 4 weeks of gestation, chromosomal anomalies exhibit 60% - 70% embryos, at first trimester of gestation - 50%, second trimester – 30%, at 2- -27 week of gestation – 7% and about 6% of stillbirth is because of different chromosomal anomalies.

If embryo survive, child birth with malformation, developmental delay, poor physical growth and mental retardation.

About 1% of live births are with different chromosomal disorders. All clinical symptoms in this cases manifest from the moment of birth as congenital defects, excluding the cases of sex chromosome involving. Latter manifests in adolescents and adults as reproductive failure.

As in chromosomal disorders early stages of embryonic development are destroyed, affection is usually multi system. It makes clinical symptoms of different chromosomal disorders similar. The more is chromosomal misbalance the more is similarity. Despite this polymorphism is characteristic for each chromosomal disorder as individual genotype effects gene expression.

COMMON FEATURES OF CHROMOSOMAL DISORDERS

A chromosome abnormality may be suspected in the following clinical situations:

During the pregnancy:

- Intrauterine growth retardation;
- Toxemia of pregnancy;
- Olygohydramnios or excessive amount of amniotic fluid;
- Risk of miscarriage.

General features of chromosomal abnormality in a newborn:
- Hypoplasy;
- Psychomotor delay in infancy;
- Mental retardation at older age;
- Poor physical growth;
- Facial dysmorphias like micro anomalies of eyes (mongoloid or antimongoloid slant of the palpebral fissures, epicanthic folds and other), dysplastic and low situated external ears, microgenia and others;
- Anomalies of limbs like polydactyly, partial syndactyly, rockerbottom feet, radial deviation of the little fingers, widely spaced big and second toes, simian crease on the palms and other dermatoglific changes;
- Multiple congenital defects, especially anomalies of nervous, circulatory, digestive and urogenital systems.

GENETIC AND CLINICAL CHARACTERISTICS OF DOWN SYNDROME

Down's syndrome (trisomy 21)

Down's syndrome was described by English physician Dr Langdon Down in 1866, and termed as «mongoloid idiocy». In 1959 F. Lejenne found extra 21 chromosome in such patients.

Minimal diagnostic signs are*: mental retardation, muscular hypotonia, brachicephaly, mocrocephaly, flat face, mongoloid slant of palpebral fissures, congenital cardiac abnormalities and congenital defects of other organs, immunodeficiency, and extra chromosome 21.*

Incidence. Approximately 1 in 700-800 newborns has Down's syndrome. The rate increases with the age of the mother. Among mothers younger than 30 years of age, the risk is less than 1:1000. It increases to approximately 1:300 at age 35 years, 1:100 at age 40, and approximately 1:25 after age 45.

Table 2. The prevalence of Down's syndrome among livebirth in relation to age of the mother.

Maternal age at birthe	Risk of having a child with Down's syndrome
Before 18	1 : 45
20	1 : 1800
25	1 : 1300
30	1 : 1000
35	1 : 300
40	1 : 100
45	1:30
49	1 : 12

Karyotypes. In 94% of all cases Down's syndrome is caused be the presence of an extra copy of chromosome 21 (trisomy 21). Karyotype is 47,XY,+21 or 47,XX,+21. The additional chromosome is maternal in origin in over 90% of cases. Robertsonian translocations account for approximately 4% of all cases, and mosaicism is seen in 2%. The most common cause of mosaicism in trisomy is a trisomic conception followed by loss of the extra chromosome in some cells during mitosis in the embryo. Children with mosaicism are often less severely affected than in the full syndrome.

Clinical features. The most common finding in the newborn period is severe muscular hypotonia. This symptom is helpful in making of diagnoses. The newborn has a characteristic appearance: brachycephaly with relatively flat occiput; flat face with a flat nasal bridge, mongoloid slant of palpebral fissures; epicanthic folds; Brushfield's spots (small white porcelain spots in the light iris of the young infant); protruding tongue (macroglossia), small, dysplastic and low situated ears; high hard palate; dysodontiasis; short-appearing neck wit loose skin (more apparent in young children), wide palms with single palmar crease, clinodactyly of the fifth fingers; wide gap between first and second toes. Rather often there is hypoplasia of external genitalia (cryptorchism, small penis and scrotum), umbilical and inguinal hernias.

Congenital cardiac abnormalities are present in 40-45% of children with Down's syndrome and this is the most significant medical problem. The most common structural heart defects are atrial and ventricular septal defects, common atrioventricular canal, patent ductus arteriosus. About 4% of patients develop an obstruction of the duodenum or atresia (absence) of the esophagus, duodenum, or anus.

Dermatoglyphic findings at Down's syndrome are: single palmar creases (simian creases) are found in 50% of Down's syndrome children in contrast to 2-3% in the general population, single creases on the fifth fingers due to small middle phalanx, distal position of palmar axial triradius, ulnar loops dermal ridge pattern on all digits.

All affected children have moderate to severe mental retardation (IQ ranging from 25 to 60).Several other medical problems occur in infants and children with Down syndrome. Respiratory infections are quite common in patients with Down syndrome and the risk of developing leukemia is 15 to 20 times higher than in the general population. Other features include hypothyroidism, conductive or sometimes neural hearing loss, and various eye abnormalities. Short stature is also specific for patients with Down's syndrome. Adult height is usually around 150cm.

Because of medical problems seen in children with Down syndrome, their survival rates are significantly decreased. The life span depends on presence of congenital defects of inner organs, infections of respiratory tract, and leukemia. Congenital heart defects are the most important single cause of decreased survival, they lead to early death in 15-20% of cases. Nowadays due to improvements in corrective surgery, antibiotic treatment, and management of leukemia, approximately 80% of children with Down syndrome may survive to age 10 years, and about half survive to 50 and even 60 years of age. All males are infertile; females have reduced fertility and 50% risk of having a trisomy 21 pregnancy. However, because approximately 75% of trisomy 21 conceptions are spontaneously aborted, the real (empiric) occurring genetic risk is about 10%.

Most affected adults develop Alzheimer's disease in later life (a type of presenile dementia).

Plan for routine medical care infants and children with Down's syndrome is as follows:

2. Evaluation of heart defects. An echocardiogram have to be performed during the newborn period.

3. Because Down's syndrome patients often have strabismus (deviation of the eye from its normal visual axis) and other eye problems, an ophthalmological examination before the age of 4 years is recommended to evaluate visual acuity.

4. Hypothyroidism is common especially in adolescence. Therefore, thyroid hormone levels should be measured annually.

5. Hiaring loss is often seen in children with this syndrome. The routine following-up should include a hearing test at 6 to 8 month of age and as needed afterward.

6. Instability of the first and second vertebrae has led to spinal cord injuries in some older Down syndrome patients. Imaging studies have to be

carries out in children with neurological syndromes and in those planning to participate in athletic activities.

7. It is appropriate to refer children with Down's syndrome to preschool programs to provide intervention for developmental disabilities.

Molecular approaches, such as cloning and DNA sequencing, are being used to identify specific genes responsible for this disorder. A candidate for mental retardation in Down's syndrome is *DYRK* , a kinase gene located on 21q. Overexpresion of this gene in the mouse model causes learning and memory defects. Another gene located in the critical region, *APP*, encodes the amyloid β precursor protein. A triple copy of *APP* is likely to accent for the occurrence of symptoms of Alzheimer's disease in nearly all Down's syndrome patients by 40 years of age.

Diagnosis of Down's syndrome is made on the basis of specific features and karyotype examination. Karyotype examination is important as for establishing of diagnoses so for genetic counseling.

Recurrence risk. The recurrence risk depends on the karyotype of the patient with Down's syndrome and his or her parents, and the age of the mother.

The recurrence risk for Down's syndrome (full trisomy 21) among mothers younger than 30 years age is about 1% (i.e., ten times higher than the population risk for this age group), and it increases with maternal age. After 35 it considered to be high and required prenatal invasive diagnostics.

If one of the parents is a carrier of balanced translocation, recurrence risk depends on type of translocation and maternal or paternal origin of chromosomal anomaly and next pregnancy requires invasive prenatal diagnostics. In familial translocation cases (Robertsonian translocation involving chromosome 21 and any other from nonhomologous chromosomes number 13,14,15,22), the recurrence risks vary from around 1-3% for male carriers up to 10-15% for female carriers. For very rare carries of a 21q21q translocation the recurrence risk is 100%.

In parents' mosaicism recurrence risk is considered to be high and is about 30%.

DIAGNOSIS OF CHROMOSOMAL DISORDERS

For diagnosis of chromosomal disorders different cytogenetic methods are used. These methods include karyotyping (or karyotype preparation method), sex chromatin (Barr bodies) studying, and molecular-cytogenetic methods.

Indications for chromosome analysis:
10. Patients with symptoms of definite chromosomal disorder;
11. Multiple congenital abnormalities;
12. Unexplained mental retardation;
13. Sexual ambiguity or abnormality in sexual development;
14. Infertility;
15. Recurrent miscarriage;
16. Unexplained stillbirth;
17. Malignancy and chromosome breakage syndrome.

Karyotyping

Karyotyping is based on the examination of karyotype. Karyotype can be studied in metaphase of mitosis when chromosomes are maximally condensed and therefore most easily visible. The slide of metaphase chromosomes of a cell is called metaphase spread (metaphase plate). Diagnostic cytogenetic analysis can also be performed with prometaphase and even prophase chromosomes. A variety of tissues have been used in studying human chromosomes but most commonly specimens of skin, red bone marrow, peripheral blood, and cells from amniotic fluid, placenta, and chorion.

Chromosomes from cultured lymphocytes of peripheral blood provide the shortest and most convenient method for routine cytogenetic analysis. This method includes the following stages:

- A small amount of venous blood is taken and added to a small volume of nutritive medium containing phytohaemagglutinin (PHA), which stimulates T lymphocytes to division by mitosis. PHA is extracted from the French beans (Phaseolus vulgaris). The cells are cultured under sterile conditions at body temperature (37ºC) for 72 hours.
- During this time cells divide and 3 hours before the end of incubation colchicine is added. Colchicine is a complex organic compound which is extracted from the autumn crocus. Colchicine has the unique property of preventing the formation of the spindle fibres and stops mitosis at the stage of metaphase.
- After 3 hours or so a hypotonic saline solution is added (of KCl), which causes swelling of cells and better separation of individual chromosomes. It is done to avoid the overlapping of the chromosomes.
- Cells are then fixed, mounted on a glass, and stained.

In karyotyping, several different staining methods can be utilized to identify individual chromosome:

- **G (Gimsa) banding.** This is the most commonly used method. The chromosomes are treated with trypsin (proteolytic enzyme) and then stained with a DNA binding dye known as Romanovsky-Gimsa. The banding patterns of each chromosome are specific and make it possible to identify each individual chromosome and to identify different types if chromosomal aberrations. It gives each chromosome a characteristic pattern of light and dark bands. The dark bands are called G bands; the light bands are R bands. In an average metaphase preparation, approximately 400 dark and light bands can be resolved in a haploid set of chromosomes.
- **High-resolution banding.** This method captures chromosomes in prometaphase, when they are less condensed than in metaphase. In high-resolution preparations, each band seen in metaphase chromosomes can be resolved into sub-bands, which allows for resolution of 800 or more bands. This method is more convenient for diagnosis of chromosomal aberrations.
- **Q (Quinacrine) banding** uses quinacrine to stain chromosome, which are viewed with ultraviolet fluorescence microscopy. Alternating bands of bright and dull fluorescence correspond to those seen by G banding for most areas of chromosomes. Bright Q bans are equivalent to dark-staining G bands. Areas lacking this similarity are called variable bands.
- **R (reverse) banding** uses Romanovsky-Gimsa dyes under elevated temperatures to produce the reverse of the pattern seen in G banding or Q banding.
- **C (Centromeric heterochromatin) banding** requires heating in an alkali solution and staining with Romanovsky-Gimsa. The centromeres of all chromosomes and the long arm of the Y chromosome (Yq) are preferentially stained. These are areas of constitutive heterochromatin containing highly repetitive DNA sequences.

- Karyotype analysis. The cytogeneticist analyses the slide either while looking down the immersion microscope or, less commonly, on a photograph of the metaphase spread which can now be produced electronically. Firstly, the cytogeneticist counts the number of chromosomes. Usually the total chromosome count is determined in 10-15 cells, but if mosaicism is suspected then 30 or more cell counts will be undertaken. Next step is detailed analysis the banding pattern of the individual chromosomes. For this, each pair of homologous chromosomes is analysed in approximately 3-5 metaphase spreads, which show high quality banding. Modern cytogenetic laboratories usually use computer scanners and special computer programmes to analyse and classify the karyotype of an individual on computer. The chromosomes of a single cell are arranged in pairs under International System of Cytogenetic Nomenclature (1995).

Sex chromatin studying

Sex chromatin (Barr body) is an inactive X chromosome. The number of Barr bodies is one less than number of X-chromosomes (Table 5._). So normal male has only one X chromosome and lack Barr bodies, normal female has two X chromosomes and one Barr body.

For Barr bodies examination epithelial cells of buccal mucosa should be taken. The scraping from the cheek is evenly spread on the slide, fixed in alcohol, stained with aceto-orsein or any other basic dyes, and then studied under the microscope. X-chromatin can be found within the nucleus and looks like a small darkly stained particle under nuclear membrane.

The studying of sex chromatin is used as express method for:
- Diagnosis of chromosomal disorders with abnormal number of X-chromosomes;
- In forensic medicine for definition of sex of victim or criminal;
- Definition of the chromosomal sex in hermaphrodites.

Table 5._ The number of Barr bodies in normal males and females and in patients with chromosomal disorders (Always one less than the number of X-chromosomes)

Number of sex chromosomes	Sex	Number of Barr bodies
XY	Male	0
XX	Female	1
X0	Female	0
XYY	Male	0
XXY	Male	1
XXXY	Male	2
XXX	Female	2
XXXX	Female	3

Genes of Y chromosome are not subjected to the dosage compensation. Y-chromosome never forms Barr body even if there are more than one Y-chromosomes in the cell. A large portion of Y chromosome is heterochromatic and is a strongly fluorescent body, which is called Y-chromatin. To demonstrate it during the interphase a fluorescence method is used. The number of Y-chromatin bodies is equal to the number of Y chromosomes.

Molecular-cytogenetic methods (fluorescent in situ hybridization – FISH)

These methods combine conventional cytogenetic methods with molecular genetic technology. In situ hybridization method specially labeled DNA probes are hybridized to metaphase chromosomes or to interphase chromatin. Method is based on the ability of a portion of single stranded DNA (DNA probe) to anneal with its complementary sequence on a metaphase spread or in interphase chromatin. The DNA probes are labeled with reported molecules, such as biotin, dygoxigenin, and dinitrophenyl, which can be visualized under the microscope by coupling of reporter molecules to a fluorescent signal or other dye. Fluorescent dyes are most widely used, giving rise to the term fluorescent in situ hybridization.

Different DNA probes are available to clinical cytogenetic laboratories now:
- ***Centromeric probes*** are complementary to the centromeres of specific chromosomes (13, 18, 21, X, Y). They are used for rapid diagnosis of common chromosome disorders (trysomy 13,18,21,X,Y) in interphase cells obtained from a prenatal diagnostic sample of chorionic villi.
- ***Probes for single-copy genes***. They are useful for identifying tiny submicroscopic deletions and duplications (Prader-Willi's and Angelman's syndromes).
- ***Probes for individual whole*** chromosome (whole chromosome paint probes). These consist of a set of probes for different parts of a particular chromosome. These probes are used for diagnosis of complex rearrangements such as

translocations and for identifying the origin of small marker chromosomes that cannot be fully characterized by banding.

Molecular-cytogenetic methods can be used for diagnosis of all types of chromosomal disorders and especially for the detection of submicroscopic chromosomal rearrangements (deletions, duplications, translocations). Different modifications of FISH technology are available now for cytogenetic studying of cancer.

Prenatal diagnosis for chromosome diseases.

Two types of prenatal tests are used to detect chromosomal disease in a fetus: screening tests and diagnostic tests. Screening tests estimate the risk that a fetus has DS; diagnostic tests can tell whether the fetus actually has the condition.

Screening methods

There are two methods of screening for chromosomal syndromes: serum screening and ultrasound screening. These can be used in combination.

Serum markers for Down's syndrome include:

- Pregnancy Associated Plasma Protein A (PAPP-A): produced by placental syncytiotrophoblasts; Low levels of PAPP-A as measured in maternal serum during the first trimester may be associated with fetal chromosomal anomalies including trisomies 13, 18, and 21. In addition, low PAPP-A levels in the first trimester may predict an adverse pregnancy outcome, including a small for gestational age (SGA) baby or stillbirth.
- Beta- human chorionic gonadotrophin (β-HCG): produced by placental syncytiotrophoblasts. An elevated beta-HCG coupled with a decreased AFP suggests Down syndrome.
- Alfa-fetoprotein (AFP): produced by fetal yolk sac and liver; reduced levels in pregnancies affected by Down's syndrome.. The AFP has the greatest sensitivity between 16 and 18 weeks gestation, but can still be useful between 15 and 22 weeks gestation.
- Unconjugated oestriol (uE$_3$): produced by placenta and fetal adrenals. reduced levels in pregnancies affected by Down's syndrome. Estriol tends to be lower when Down syndrome is present and when there is adrenal hypoplasia with anencephaly.
- Inhibin-A: produced by placenta; raised levels in pregnancies affected by Down's syndrome.

Factors that can affect these serum markers include: Weight: marker levels are higher in lighter women and lower in heavy women. Ethnic group: Afro-Caribbeans have higher levels of AFP and HCG compared to Caucasians. Twins: increased levels if serum markers are present. Assisted conception: if a donor egg is used, Down's risk should be calculated using age of donor; HCG levels can be increased in assisted conception. Previous pregnancy history. Gestational age.

Ultrasound examination.

Nuchal translucency scanning. Fetal nuchal translucency (NT or FNT) screening uses ultrasound to measure the size of the nuchal pad at the nape of the fetal neck. It should be performed between 11 weeks and 13 weeks. Increased nuchal translucency reflects fetal heart failure, and is typically seen in any serious anomaly of the heart and great arteries, and strongly associated with a chromosomal abnormality. In one study, 84% of karyotypically proven trisomy 21 fetuses had a nuchal translucency >3mm at 10-13 weeks' gestation (as did 4.5% of chromosomally normal fetuses). The greater the extent of FNT, the greater the risk of abnormality. It is a straightforward test but will have a 20% false positive rate (FPR) if the thresholds are set to detect 85% (if used alone and maternal age adjusted).

Adding nasal bone screening during the same examination may increase sensitivity further and reduce the FPR. One study concluded that an absent nasal bone should be considered as a highly predictive marker of Down's syndrome.

The 'combined test' (nuchal translucency, beta-human chorionic gonadotrophin (β-HCG) and pregnancy-associated plasma protein-A) should be the screening test offered to women between 11 weeks 0 days and 13 weeks 6 days. For women who book later, triple or quadruple test) should be offered between 15 weeks 0 days and 20 weeks 0 days.

Chromosomal diseases screening tests currently available
- Maternal age alone (30% detection rate; 5% false positive rate)
- 1st trimester tests (11-14 weeks)
  o Nuchal translucency alone
  o Combined test: nuchal translucency scanning plus serum measurement of free β-HCG and PAPP-A
- 2nd trimester tests (15-20 weeks)
  o Double test: measures uE_3 and β-HCG
  o Triple test: measures AFP, HCG and uE_3
  o Quadruple test: measures free β-HCG, AFP, inhibin A and uE_3

"Triple" or "Quadruple" test

Combining the maternal serum assays may aid in increasing the sensitivity and specificity of detection for fetal abnormalities. The classic test is the Ttriple screenϒ for frsalpha-fetoprotein (MSAFP), beta-HCG, and estriol (uE3). The "quadruple screen" adds inhibin-A.

Condition	AFP	uE3	HCG
Neural tube defect	Increased	Normal	Normal
Trisomy 21	Low	Low	Increased
Trisomy 18	Low	Low	Low
Molar pregnancy	Low	Low	Very High
Multiple gestation	Increased	Normal	Increased
Fetal death (stillbirth)	Increased	Low	Low

After an abnormal result, the procedure includes counseling, directed ultrasound, and invasive methods with fetal karyotyping (if the patient so chooses).

Practical lesson 4
Topic: Chromosomal diseases associated with changes in the number or structure of autosomes.
Practical lesson 5
Topic: Chromosomal diseases associated with changes in the number of sex chromosomes.

The purpose of the lesson: to study etiology, clinics and diagnosis, treatment of patients with the most widespread chromosomal diseases. To be able to reveal the symptoms, characteristic for chromosomal diseases while history taking and examination of the patient and to work out plan of the clinical examination of a patient.

Student should know:
1. General clinical symptoms of chromosomal disorders.
4. Key symptoms and karyotypes of most spread chromosomal disorders.
5. Principles of prenatal diagnostics of chromosomal disorders.

Student should be able:
3. To select patients for cytogenetic analisis.
4. Formulate a possible diagnosis of chromosomal aberrations and determine the need for additional testing, including specific genetic methods.

Control questions.

1. Classification of chromosomal diseases.
2. Poliploidy: karyotype, clinical features, prognosis, medical and genetic counseling.
3. General symptoms of chromosomal disease due to changes in the number and structure of the autosomes.
4. Clinical -cytogenetic characteristics of chromosomal diseases associated with changes in the number and structure of autosomes: Patau syndrome, Edwards, "cat cry".
5. Peculiarities of clinical picture of chromosomal syndromes due to changes in the number of sex chromosomes.
6. Clinical-cytogenetic characteristics of Shereshevsky - Turner syndrome, polysomy X - syndrome, polysomy Y, Klinefelter's syndrome.
7. What is mikrocytogenetic syndromes? The peculiarity of the clinical picture, examples of syndromes.
8. Syndroms of Angelman and Prader - Willy. What is genomic imprinting.
9. Diagnostic of chromosomal diseases. Cytogenetic methods.
10. Principles of medical-genetic counseling for chromosomal diseases.

Practical work
1. To examine the patient with chromosomal disorder, to reveal the typical symptoms, and formulate diagnose, to compose the plan of clinical and laboratory examination.
2. To analyze the phenotype of the patients with different chromosomal disorders, using photos of the patients.
3. To study karyotypes of the patients with chromosomal disorders.

Main literature
    Methodical recommendations on medical genetics
Additional literature:

1. Genetics in medicine. - 7th edition/Robert L/Nussbaum, Roderick R. McInnes, Huntington F. Willard. – 2007 – 585 p.
2. Emery's Elements of medical genetics. 15th ed. / Peter Turnpenny, Sian Ellard. – Elsevier, 2017. – 400 pp.
3. Lynn B. Jorde, John C. Carey, Michael J. Bamshad. Medical genetics. 5th ed. Elsevier, 2016. 356 pp.

4. Vogel and Motulsky's human genetics. Problems and approaches / M. R. Speicher, S. E. Antonarakis, F. G. Motulsky. 4th addition. – Springer, 2010. – 981 pp.
5. Young Ian.D. Medical genetics. -2nd ed. -Oxford university press, 2010. - 304 p.
6. Diseases of the fetus and newborn. Pathology, radiology and genetics. G.B.Reed, A.E. Claireaux and A.D.Bain., - Great Britain, - 1989, 812 p.
7. Human molecular genetics. Tom Strachan, Andrew P.Read. – 4th edition - Bios Scientific Publisher, 2010, 680 p.
8. Smith recognizable patterns of human malformation. Seventh edition. John M. Graham, - USA, - 2013, 976 p.
9. R. Wiedemann, K.-R. Gross, H.Dibberin ,Atlas of characteristic syndromes. A Visual Aid to Diagnosis. Second edition. -London, 1986,412 p.

13. Information resources:
https://ghr.nlm.nih.gov National librery of medicine, genetics
https://www.orpha.net The portal for rare diseases and orphan drugs
https://rarediseases.org National Organization for Rare Disorders
http://omim.org/OMIM (Online Mendelian Inheritance in Man) – An Online Catalog of Human Genes andGenetic Disorder

GENETIC AND CLINICAL CHARACTERISTICS OF THE MOST COMMON CHROMOSOMAL SYNDROMES
Polyploidy

The polyploid conditions that have been observed in humans are triploidy (69 chromosomes in the nucleus of each cell) and tetraploidy (92 chromosomes). The karyotypes for these two conditions would be designated 69,XXX; 69,XXY; 69,XYY and 92,XXXX or 92,XXYY respectively.

Triploidy is a relatively common finding in material cultured from spontaneous abortions (tryploidy is seen in 15% of chromosomally abnormal abortuses), but is seen only rarely in a liveborn infant (about 1 in 10000 livebirths are triploids).

Triploid pregnancies may be accompanied by varying degree of toxemia. The placenta has focal trophoblastic hyperplasia and hydatidiform changes to the chorionic villi (partial hydatidiform mole). Unlike the true molar pregnancy, a small embryo is usually present. On ultrasound, growth retardation and progressive olygohydramnios are recognized. The fetus has relatively large head, congenital heart lesions, and syndactyly. Premature labor is typical on the 36th weak of gestation. Weight of the newborn child is 1300 g in average, maximally up to 2000 g. Life expectancy is about 9 days (rare cases of surviving for 4 – 7 months were reported). Clinical symptoms are nonspecific as great chromosomal misbalance is present. Craniofacial abnormalities includes dysplastic calvaria with large posterior fontanel, ocular hypertelorism with eye defects ranging from colobomata to microphthalmia, low nasal bridge, low-set, malformed ears, micrognathia, microstomia, cleft lip and palatum. Syndactyly of third and fourth fingers, simian crease, talipes equinovarus, camptodactyly and clinodactyly are common. Brain anomalies, including hydrocepalus and holoprosencephaly, cyclopy, renal anomalies like cystic dysplasia and hydreoneprosis, heart valvulae defects, adrenal hypoplasia, hypogenitalia and other congenital defects are observed.

Most tetraploid fetuses are lost in the first trimester. In the rare instance of ongoing pregnancy fetus has marked growth retardation, microcephaly and multiple malformations.

Recurrence risks: neither triploidy nor tetraploidy has an increased risk of recurrence.

Edward's syndrome (trisomy 18)
This syndrome was described by J.Edwards in 1960.

Minimal diagnostic signs are: prenatal growth deficiency, single umbilical artery, small placenta, multiple congenital defects of inner organs, trisomy 18.

Incidence. The newborn incidence is approximately 1 in 5000-7000. More than 70% of patients with Edward's syndrome are females, probably, because of their higher viability. As in trisomy 21, there is a significant maternal age affect.

Karyotypes. More than 95% of patients with Edward's syndrome have full trisomy 18. Karyotype is 47,XY,+18 or 47,XX,+18. Seldom cases of Edwards' syndrome are mosaic forms and as exclusion there may be translocation types of the syndrome. Clinically all these types are not distinguished.

Clinical features. The newborn with trisomy 18 has hypoplasia due to intrauterine growth retardation, a small cranium (microcephaly) with prominent occiput (dolihocephaly), microgenia, multiple micro anomalies like ant-mongoloid slant of palpebral fissures, a small mouth that is often hard to open, small and low-set malformed auricles, typical overlapping of finger (index finger over the third, fifth finger over the fourth), rocker-bottom heels, and short toes.. Almost all of these newborns have cardiac as well as other internal malformations (omphalocele, diaphragmatic hernia, and, occasionally, spina bifida.

About 50% of infants with trisomy 18 die within the first several weeks of life, and only aboyt 5% may survive more than 1 year. Mental retardation in survivors is profound.

Patau's syndrome (trisomy 13)

Patau's's syndrome was described by K. Patau in 1960.

Minimal diagnostic signs are: microcephaly, polydactyly, cleft lip and palate, multiple congenital defects of inner organs, extra chromosome 13.

Incidence. The newborn incidence is approximately 1 in 5000-7000. The rate increases with the age of the mother

Karyotypes. 80-85% of all patients with Patau's syndrome have full trisomy 13. Karyotype is 47,XY,+13 or 47,XX,+13. Robertsonian's translocation, mosaicism, reciprocal translocation, inversions, circle chromosome 13, etc. may also occur.

Clinical features. The newborn has hypoplasia due to intrauterine growth retardation, midline brain and facial abnormalities (scalp cutis aplasia, holoprosencephaly, hypotelorism, central or unilateral facial clefts), the anomalies of the CNS (microcephaly, trigonocephaly, arhinencephaly), anomalies of genitalia, omphalocele, malformations of limbs (a flexor position of the hands, polydactyly, rocker-bottom feet). Cardiac abnormalities occur in at least 90% of all cases.

Almost all affected newborns have lethal cardiac and CNS anomalies and die during the firs few weeks of life. In the unusual event of long-term survival there is severe mental retardation.

Wolf-Hirschhorn syndrome (4 p⁻)

A deletion of the short arm of the fourth chromosome in a human is accompanied by the following *minimal diagnostic signs*: microcephaly, specific face, psychomotor delay, severe mental retardation, deletion of the short arm of 4th chromosome.

Incidence. The newborn incidence is approximately 1 in 45000-50000.

Karyotypes. The disorder is caused by deletion of the short arm chromosome 5. Karyotypes are 46,XX, del 4p⁻ or 46,XY,del 4p⁻ .Sometimes deletion is very small and unvisible under light microscope. The presence of a very subtle deletion can be demonstrated by FISH method using locus-specific probes for 5p.

Clinical features. The infants have a prominent forehead and a broad nasal root ("Greek warrior helmet"). The philtrum is short, and mouth is down-turned. The cleft lip and palate, small facial skin hemangiomae are often observed. These infants are severely mentally retarded. They commonly have cardiac and kidney defects. Other features include muscle hypotony, flexor position of fingers, eye abnormalities like strabismus, exophthalmia, coloboma of iris, cataract, microphthalmia; hypospady and cryptorchism in affected boys.

Cri du chat (cat cry) syndrome (5 p⁻)

This syndrome was the first autosomal deletion to be described by F. Lejenne in 1963.

The minimal diagnostic signs: *unusual cat-like cry, microcephaly, round face, anti-mongoloid slant of palpebral fissures, mental retardation, a deletion of the short arm of the 5th chromosome.*

Incidence. The newborn incidence is approximately 1 in 45000-50000.

Karyotypes. 46,XX, del 5p⁻ or 46,XY,del 5p⁻

Clinical features. The infants have growth deficiency of prenatal onset, a cat-like cry, which disappears with time, and cardiac defects. They usually have a characteristic round face, facial asymmetry, a broad nasal bridge, micrognathia, low situated auricles, anti-mongoloid slant of palpebral fissures, hypertelorism, epicanthic folds. Cat-like cry results from underdevelopment of the larynx. Predisposition to the infectious diseases of the respiratory tracts is observed. A round face, cat's cry, muscle hypotony in a majority of the cases' disappears with age. Infants with cat cry syndrome are severely mentally retarded. Survival to adulthood has been observed but is not common.

Disorders of the sex chromosomes
Klinefelter's syndrome

The syndrome was described by Harry Klinefelter in 1942. The chromosomal basis of Klinefelter's 's syndrome was established in 1959.

Minimal diagnostic signs are: *hypogenitalism, hypogonadism, infertility in males, karyotype 47,XXY.*

Incidence. It is relatively common condition with an incidence of 1in 800-1000 male live births.

Karyotypes. In most cases patients with Klinefelter's syndrome have one X chromosome extra (karyotype 47,XXY). Individuals with the 48,XXXY and 49,XXXXY karyotypes have also been reported. Because they have a Y chromosome, they have a male phenotype, but the severity of the disorder increases with each additional X chromosome. X-chromatin test is positive. Persons with karyotype 47,XXY demonstrate one Barr body.

Clinical features. Males with Klinefelter's syndrome tend to be taller than average, with the disproportionately long arms and legs, eunuchoid habitus, and poor musculature. Gynecomastia (breast development) is present in approximately one third of affected males and leads to an increased risk of breast cancer. The risk can be reduced by mastectomy. Most patients have small soft testes, and are infertile due to atrophy of the seminiferous tubules and absence of sperm in their semen (azoospermia). Although fertility has been achived for a small number of affected males using the technique known as ICSI (intra-cytoplasmic sperm injection – injection of a single sperm cell into oocyte). Testosterone levels in adolescents and adults are low, and there is an increased incidence of osteoporosis due to testosterone deficiency. Males with Klinefelter's syndrome usually are not mentally retarded, but the ICQ is on average 10-15 points lower than that of the affected individual's siblings. Sometimes there is predisposition for learning disabilities.

Diagnosis during childhood is helpful in allowing for prospective testosterone replacement therapy beginning at the age of 11 to 12 years. This will bring about a more usual adolescent development and may prevent many of the features of adult Klinefelter's syndrome that are due to testosterone insufficiency.

Polysomy Y syndrome

Minimal diagnostic signs are: *Clinical signs vary from normal tall men with normal fertility to men with mild social problems due to emotional immaturity, hyperactivity, and impulsive behavior*

Incidence. The incidence of this syndrome is 1in 1000 in males in newborn surveys and 1 in 10 in males taller than 2 m. This syndrome is found in 2-3% of males who are in institutions because if mental retardation or antisocial criminal behavior.

Karyotypes. In most cases patients with polysomy Y syndrome have one Y chromosome extra (karyotype 47,XYY). Individuals with the 48,XYYY and 49,XYYYY karyotypes have also been reported.

Clinical features. Clinical features include tall stature and mild social problems, but the majority of affected males are thought to be normal with normal fertility. The average height of these patients is 186 cm, and each Y chromosome adds 15 cm. Males with karyotype 47,XYY may have 10- to 15-point reduction in average IQ. As soon as this syndrome was discovered in 1960, it was found, that its incidence in the male prison population was 1in 30, compared with 1 in 1000 in the general male population. This led to the suggestion that this karyotype might confer a predisposition to violent, criminal behavior. A number of studies have shown that XYY males are not really inclined to commit violent crimes. But they can show emotional immaturity, hyperactivity, attention deficit disorder, learning disabilities, and impulsive behavior.

Shereshevsky-Turner's syndrome

The syndrome was described by M.Shereshevsky in 1925 and by Henry Turner in 1938. The chromosomal basis of this syndrome was established in 1959.

Minimal diagnostic signs are: *in a newborn girl congenital lymphedema of the hands and feet, hypotony, loose skin in the posterior neck. In older children sexual infantilism, primary amenorrhea, short stature, "webbed neck", congenital defects of the cardiovascular, excretory and other systems, monosomy X.*

Incidence. This syndrome relates to the one of the most widely spread chromosomal anomalies. The population frequency of the syndrome is 1:3000-3500 of the newborn girls. It is necessary to mark a high frequency of the spontaneous abortions of X monosomic fetuses before 6 week of gestation, which is ¼ -1/5 among the all cases of pregnancy lost in this term of gestation.

Karyotypes. 50% of all patients with Shereshevsky-Turner's syndrome have the full form of monosomy X (karyotype 45,X0 or 45,X). Other karyotypes are possible too (Table 5. _). The absence of a Barr body is other typical cytogenetic finding.

Table 5.Chromosome findings in Shereshevsky-Turner's syndrome

Karyotype	*Mutation*	*Frequency (%)*
45,X	Monosomy X	50
45,X/46,XX	Mosaicism	20
46,X,i(Xq)	Isochromosome	15
46,X,r(X)	Ring X chromosome	5
46,X,del(Xp)	Deletion of the short arm of the chromosome X	5
Other		5

Clinical features. Individuals with Shereshevsky-Turner's syndrome are female and have a characteristic phenotype. Infants with this syndrome may be normal, or they may have features such as residual neck webbing from cystic hygroma, shield chest, and edema of the hands and feet more often as puffiness of the fingers and toes.

Other findings can include a low posterior hair-line, a triangle-shaped face, anti-mongoloid slant of palpebral fissures, epicanthic folds, posteriorly rotated ears, low posterior hairline, webbed posterior neck, broad chest with widely spaced nipples, short fourth metacarpals, excessive pigmented nevi.

Intelligence in Shereshevsky-Turner's syndrome is normal. The three medical problems are:

1. congenital defects,
2. short stature,
3. sexual infantilism and ovarian dysgenesis.

Typical congenital abnormalities are cardiac defects (bicuspid aortic valve in 50% of patients, coarctation of aorta 15% to 30%, valvular aortic stenosis, septal defects). About 50% of patients have renal anomalies (horseshoe kidney, renal hypoplasy, double or cleft renal pelvis, hydronephrosis), etc. Severe cardiac and renal abnormalities should be surgically repaired.

The short stature becomes apparent by midchildhood, and the average adult height is 145 cm. Growth hormone administration produces increased height in these girls. Gonadal dysgenesis is commonly seen in Shereshevsky-Turner's syndrome. Instead of ovaries, most females with this syndrome have streaks of connective tissue. Lacking normal ovaries, they do not usually develop secondary sexual characteristics, and most women with this condition are infertile. Estrogen replacement therapy should be initiated at adolescence for development of secondary sexual characteristics and long-term prevention of osteoporosis. In vitro fertilization using donor eggs offers the prospect of pregnancy for women with Shereshevsky-Turner's syndrome.

The multiple endocrine dysfunctions (an obesity, diabetes mellitus) can be observed. A life prognosis depends on presence of severe congenital defects.

Molecular studies have shown that approximately 60% to 80% of all patients with Shereshevsky-Turner's syndrome receive an X chromosome only from the mother, and a paternally derived sex chromosome is absent. It has been reported that affected females who receive the X chromosome from their father have higher verbal IQ scores and better social cognition than those who receive the X chromosome from mother. This difference implies the presence of a genomic imprinting effect on a specific region of the X chromosome.

Polysomy X syndrome (trisomy X, tetrasomy X, and pentsomy X)

Trisomy X (47, XXX) initially detected in 1959.

Minimal diagnostic signs are: *Clinical signs vary from healthy fertile women to patients with expressed hypogonadism with overproduction of gonadotropin, infertility and mental deficiency, one or more X chromosomes are extra.*

Incidence. Population rate is 1 : 1000 – 1 : 1200 girls. Polysomy X syndrome is diagnosed among mentally retarded women in 1 %.

Karyotypes. Most patients have karyotype 47,XXX (trisomy X). Tetrasomy (48,XXXX) and pentasomy (49,XXXXX). Patients with this syndrome can have 2 Barr bodies (in case of karyotype 47,XXX), 3 Barr bodies (karyotype 48,XXXX) and 4 Barr bodies (karyotype 49,XXXXX).

Clinical features. The majority of 47,XXX females have no clinical manifestations and have normal fertility and produce normal offspring. A small number of these women sometimes suffer from sterility, menstrual irregularity, premature menopause, mild mental retardation. Normal phenotype is explained with inactivation of extra X chromosome in embryonic period and Barr bodies formation. Due to this mechanism only one chromosome is active.

There is an increased risk of psychiatric problems, specifically schizophrenia, in these women.

Women with more than three X chromosomes show a high incidence of mental retardation, the severity of which increases with each additional X chromosome.

Microdeletion syndromes

These syndromes are caused by submicroscopic deletions or microdeletions. Some of these microdeletions involve loss of only a few genes at closely adjacent loci. The presence of a very subtle deletion can be demonstrated by FISH method using locus-specific probes. Examples of microdeletion syndromes are given in Table 5

Table 5.___. Mycrodeletion syndromes.

Syndrome	Chromosomal deletion	Clinical features
Prader-Willi	15q11-13 (paternal origin)	Mental retardation, short stature, obesity, hypotonia, characteristic facies, small feet and hands
Angelman syndrome	15q11-13 (maternal origin)	Mental retardation, ataxia, uncontrolled laughter, seizures
Williams	7q1	Developmental disability, characteristic facies, supravalvular aortic stenosis

Deletion 1p36	1p36	Mental retardation, seizures, hearing loss, heart defects, growth failure
Aniridia/ Wilms tumor	11p13	Mental retardation, aniridia, predisposition to Wilms tumor (a kidney tumor), genital defects

Angelman's syndrome

The syndrome was described by H.Angelman in 1965 г.

Minimal diagnostic signs are: mental retardation, ataxia, uncontrolled laughter, seizures, lack of speech and happy appearance.

Incidence. It is relatively common condition with an incidence of 1in 15.000 live births.

Karyotypes. About 70% of patients with Angelman's syndrome have microdeletion in a specific region of chromosome 15 (46,XX, del5q- or 46,XY,del 15q-). Angelman syndrome develops when the chromosome 15 with deletion is inherited from the mother (genomic imprinting, see chapter 2.2.6.2). Molecular analysis has identified several specific genes in the critical region of chromosome 15, which are responsible for Angelman's syndrome. The Angelman's syndrome gene encodes a protein involved in ubiquitin-mediated protein degradation during brain development (consistent with the mental retardation and ataxia observed in this disorder. In brain tissue, this gene is active only on the chromosome inherited from the mother. A maternally inherited deletion removes the single active copy of this gene.

Several mechanisms in addition to chromosome deletions can cause Angelman's syndrome. One of these is uniparental disomy, a condition in which the individual inherits two copies of a chromosome from one parent and none from the other. When two copies of paternal chromosome 15 are inherited, Angelman's syndrome results because no active maternal genes are present in the critical region. Point mutations in the identified gene can also produce disease.

Clinical features. Children with Angelman syndrome have microcephaly, severe mental retardation, ataxia, seizures, and lack of speech. Other symptoms include uncontrolled laughter and happy appearance that gives the other name of this disorder – "happy pappy syndrome".

Prader-Willi's syndrome

Disorder was described by A.Prader and H.Willi in 1956.

Minimal diagnostic signs are:

Karyotypes. Disorder may be caused by the microdeletion in a specific region of chromosome 15 (46,XX, del5q- or 46,XY,del 15q-). Karyotype is the same as in patients with Angelman syndrome, but microdeletion mast be inherited from the father (genomic imprinting). The difference between these two phenotypes is due to the lack of expression of maternally imprinted genes in Prader-Willi's syndrome and the lack of expression of different paternally imprinted genes in the same chromosome region in Angelman's syndrome. The portion of the chromosome 15 that is deleted in both syndromes is known as the "critical region". Several genes at the critical region are involved in Prader-Willi syndrome, and they are active only on the chromosome inherited from the father. One of these genes, *SNRPN*, encodes a small nuclear riboprotein that is expressed in the brain. Uniparental disomy (two copies chromosomes 15 are inherited from the mother) and gene mutations can also cause this syndrome.

Clinical features. The Prader-Willi syndrome is characterized by muscular hypotonia, short stature, obesity, hypogonadism. Other symptoms include mental retardation, learning difficulty, characteristic facie, small feet and hands.

Table 1.

**Cytogenetic and clinical characteristics of
the most common chromosomal disorders**

Syndromes	Karyotype	Incidence in the newborns	Clinical features
Tryploidy	69,XXX or 69,XXY	1:10.000	During pregnancy severe growth retardation and progressive oligohydramnios, the newborn has hypoplasy, relatively large head, congenital defects of the heart, eyes, CNS. Meningomyelocel and syndactyly of the third and fourth fingers are common findings. Life expectancy is about 9 days (rare cases of surviving for 4 – 7 months have been reported)
Down's syndrome	47,XY,+21 or 47,XX,+21	1 in 700-800	Mental retardation, muscular hypotonia, brachicephaly, microcephaly, flat face, mongoloid slant of palpebral fissures, congenital cardiac abnormalities in 50% of the patients and congenital defects of other organs, immunodeficiency, severe cause of infectious disorders, the risk of leukemia. Life span depends on the severity of congenital defects. Some patients may live to 50-60 years
Edwards syndrome	47,XY,+18 or 47,XX,+18	1 in 5000-7000	Prenatal growth deficiency, single umbilical artery, small placenta, multiple congenital defects of inner organs. Other features include a small cranium with prominent occiput (dolihocephaly), microgenia, typical overlapping of finger (index finger over the third, fifth finger over the fourth), rocker-bottom heels, and short toes. About 50% of infants with trisomy 18 die within the first several weeks of life, and only about 5% may survive more than 1 year. Mental retardation in survivors is profound.
Patau's syndrome	47,XY,+13 or 47,XX,+13	1 in 5000-7000	Microcephaly, polydactyly, cleft lip and palate, multiple congenital defects of inner organs. Almost all affected newborns have lethal cardiac and CNS anomalies and die during the firs few weeks of life. In the unusual event of long-term survival there is severe mental retardation.
Wolf-Hirschhorn's syndrome (4 p⁻)	46,XX, del 4p⁻ or 46,XY,del 4p⁻	1 in 45000-50000	Microcephaly, psychomotor delay, severe mental retardation. Patients have specific face with prominent forehead and a broad nasal root ("Greek warrior helmet"), the cleft lip and palate, small facial skin hemangiomae. They commonly have cardiac and kidney defects, eye abnormalities like strabismus, exophthalmia, coloboma of iris, cataract, microphthalmia
Cri du chat (cat cry) syndrome (5 p⁻)	46,XX, del 5p⁻ or 46,XY,del 5p⁻	1 in 45.000-50.000	Unusual cat-like cry, microcephaly, round face, anti-mongoloid slant of palpebral fissures. Infants with cat cry syndrome are severely mentally retarded. Survival to adulthood has been observed but is not common. mental retardation
Angelman's syndrome	46,XX, del 15q- or 46,XY,del 15q- of	1:50.000	Microcephaly, severe mental retardation, ataxia, seizures, and lack of speech. Other symptoms include uncontrolled laughter and happy appearance that gives the other name of this disorder – "happy pappy syndrome"

	maternal origin		
Prader-Willi's syndrome	46,XX, del 15q- or 46,XY,del 15q- of paternal origin	1:25.000 – 1:50.000	Muscular hypotonia, short stature, obesity, hypogonadism. Other symptoms include mental retardation, learning difficulty, characteristic facie, small feet and hands
Klinefelter's syndrome	47,XXY 48,XXXY 49,XXXXY	1 in 800-1000 male live births	Males with the disproportionately long arms and legs, eunuchoid habitus, poor musculature, hypogenitalism, hypogonadism, infertility. Gynecomastia (breast development) is present in approximately one third of affected males. Intelligence is usually normal. Persons with karyotype 47,XXY demonstrate one Barr body.
Polysomy Y syndrome	In most cases 47, XYY, in the rare cases 48,XYYY or 49,XYYYY	1 in 1000 male newborns	Clinical signs vary from normal tall men with normal fertility to men with mild social problems due to emotional immaturity, hyperactivity, and impulsive behavior. Patient usually show, attention deficit disorder and learning disabilities
Shereshevsky-Turner's syndrome	45,X in 50% of all patients	1:3000-3500 in the newborn females	In a newborn girl congenital lymphedema of the hands and feet, muscular hypotony, loose skin in the posterior neck. At adult patients sexual infantilism, primary amenorrhea, infertility, short stature, "webbed neck", congenital defects of the cardiovascular, excretory and other systems
Polysomy X syndrome (trisomy X, tetrasomy X, and pentsomy X)	47,XXX, in the rare cases 48,XXXX or 49,XXXXX	1 : 1.000 – 1 : 1.200 in the female	Clinical signs vary from healthy fertile women to patients with expressed hypogonadism with overproduction of gonadotropin, infertility and mental deficiency and 2 Barr bodies (in case of trisomy) or 3 Barr bodies (tetrasomy) and even 4 Barr bodies (pentasomy)

Practical lesson 6
Topic: General characteristics and classification of monogenic diseases. Genealogical method

The goal: to collect anamnesis and pedigree information, make a pedigree, analyze a type of inheritance of disease or a character in a family.

Student should know :
      1. Genetic terminology such as : proband , sibs , genealogy.
      2. Symbols for pedigree charting.
      3. Rules of pedigree construction.
      4. Sense of genealogical method.
      5. Peculiarities of pedigrees with different patterns of inheritance.

Student has to be able to :
      1. Compose the pedigree
      2. Analyse pedigree and identify the pattern of inheritance.

–   **Control questions**
    1. Medical importance of clinical genealogical method
    2. Stages of pedigree method
    3. What is genealogy, proband (proposites), sibs?
    4. Peculiarity of collection of genealogic anamnesis. Symbols for pedigree charting. .Rules of pedigree charting.
    5. Peculiarities of pedigree for autosomal-dominant, autosomal-recessive, X-linked (dominant and recessive), mitochondrial types of inheritance.
    6. Peculiarities of pedigree in mitochondrial inheritance.

Main literature
    Methodical recommendations on medical genetics

Additional literature:

    1. Genetics in medicine. - 7th edition/Robert L/Nussbaum, Roderick R. McInnes, Huntington F. Willard. – 2007 – 585 p.
  2. Emery's Elements of medical genetics. 15th ed. / Peter Turnpenny, Sian Ellard. – Elsevier, 2017. – 400 pp.
  3. Lynn B. Jorde, John C. Carey, Michael J. Bamshad. Medical genetics. 5th ed. Elsevier, 2016. 356 pp.
  4. Vogel and Motulsky's human genetics. Problems and approaches / M. R. Speicher, S. E. Antonarakis, F. G. Motulsky. 4th addition. – Springer, 2010. – 981 pp.
  5. Young Ian.D. Medical genetics. -2nd ed. -Oxford university press, 2010. - 304 p.
  6. Diseases of the fetus and newborn. Pathology, radiology and genetics. G.B.Reed, A.E. Claireaux and A.D.Bain., - Great Britain, - 1989, 812 p.
  7. Human molecular genetics. Tom Strachan, Andrew P.Read. – 4th edition - Bios Scientific Publisher, 2010, 680 p.
  8. Smith recognizable patterns of human malformation. Seventh edition. John M. Graham, - USA, - 2013, 976 p.
  9. R. Wiedemann, K.-R. Gross, H.Dibberin ,Atlas of characteristic syndromes. A Visual Aid to Diagnosis. Second edition. -London, 1986,412 p.

13. Information resources:
https://ghr.nlm.nih.gov National librery of medicine, genetics
https://www.orpha.net The portal for rare diseases and orphan drugs
https://rarediseases.org National Organization for Rare Disorders
http://omim.org/OMIM (Online Mendelian Inheritance in Man) – An Online Catalog of Human Genes andGenetic Disorder

The main theoretical information

Clinico-genealogical method is based on composing and analyzing of pedigree. It's compulsory stage in examination the patient with hereditary pathology. Synonym of the word «pedigree» is «genealogy». Here comes the name of the method.

The method allows to reveal the following:
1. Is this trait single in the family or there are some cases of that pathology (family character);
2. To identify the pattern of inheritance.
3. To identify the risk of birthing the child with genetic disorder
4. To detect the persons, which need medico-genetic counselling, to calculate clinical prognosis for proband and his family subject to   peculiarity of the disease and its genetic characteristic;
5. To estimate the expressivity and penetrance of the gene.

This method doesn't required special equipment, long laboratory analysis, it's simple and is obtainable for each doctor. It's consist of the following stages:
1. Recording the family history (genealogical anamnesis)
2. Pedigree charting
3. Analysis of pedigree
So this method combines clinical examination of the patient and analysis of pedigree.

I stage . Collection of the information begins with proband. Proband - is a person which pedigree should be composed. Frequently proband is a sick person with hereditary disease, rarely - healthy person which has affected relatives. Proband is also named propositus if male or proposita if female. As a rule we start with anamnesis of proband disorder. Important is information about pregnancy terminations. We pay attention at abortions, stillbirths, infant death, as it can promote to decide the pattern of inheritance. Information about sibs (or another relatives) is gathered in order of birth.

Next stage is to get the information about proband's family.

We ask about proband's sibs (sibs are brothers and sisters of proband), nearest relatives (parents, than relatives on mother's line and on father's line). We have to find out if in the family consanguineous mating takes place. Each person has from 10 to 30 pathological recessive genes in it's genotype. Closed relatives have more chances to turned out to be heterozygous on the same pathological gene. By consanguineous mating the risk of birthing child with recessive disease is increased.

Than in chronological order we gather information about mother's relatives and than about father's. We should point the age of deaths and the cause of it.

So question list for gathering the genealogical anamnesis should be as follows:

1. Which on a count a child was proband?
2. How many pregnancies did his mother have?
3. What was the outcome of her first, second and others pregnancies?
4. What disease did sibses have ?
5. What was the cause of death, it they are dead.
6. What was the time and cause of abortion, if the pregnancy interrupt in ?
Questions about mother's and father's family:
1. Which on a count a child was mother /father/ in the family?
2. Do her /his/ sisters and brothers have children?
3. Number of the children in order of their birth and condition of their health.
4. In what age did they die, it they are dead.
Than we have to gather the information about grandmother, grandfather and their relatives on mother's and father's side. We should pay attention on the causes and the age of their death also.

It's desirable to exam patients personally. If it's impossible to do in any cases we should to analysis their phenotype by their family photography. In some cases we need to get the results of autopsy or learn medical documents. All these information is necessary to decide the pattern of inheritance in the family.

II stage: Pedigree construction.

Symbol	Meaning
□	Male
○	Female
◇	Sex undesignated
(□)	Adopted
◇P	Pregnancy
⊠ ⊘	Deceased
■ ●	Affected with trait
◧ ◐	Carrier for trait
⊙	Carrier for X-linked trait
□—○	Mating
□=○	Consanguineous Mating
○□□	Siblings
③ ②	Number of children
□╫○	Divorced or Separated
◇↓	Miscarriage, SAB
○○ ○□	Dyzigotic Twins (Fraternal Twins)
○○	Monozigotic Twins (Identical Twins)
□↓○ ♀ ♂	No offspring
↗○ ↗□	Patient initiating genetic work-up (Proband, index case consultant)
□╫○—□	Two mating

There are following rules of pedigree charting:
1. We have to start pedigree charting from the middle of the list.
2. Sibls are placed from the left to the right in order of birth.
3. All members of one generation are placed on one line.
4. Pedigree charting is convenient to begin from proband's mother and her sibses, than her relatives.
5. Mother and her relatives are situated at the right. Father and his relatives are situated at the left.
6. Proband and his sibs are situated in the middle between families of mother and father.
7. Generations are numbered at the left by roman numerals from up to down. Members of one generation are numbered at the left to the right by arabic numerals. Each representative has its own code (I - 5, II – 7 and so on)
8. We have to point age of each member of the family by numerals near symbol (it's better to point the date of birth)
9. To exam the patient privately (!)

10. Wives of proband's relatives aren't pictured in the family tree if there are healthy and do not influent at the disease expression.

11. Pedigree should not include more than 3-4 generations.

12. We have to point the date of pedigree construction

III stage. Genealogical analysis.

The first question which we should answer by analyzing the pedigree is whether the trait inherits or it is consequence of a new mutation. Appearance of congenital defects is possible under activation of teratogenic factor in period of pregnancy.

The second question is the pattern of disease inheritance in the family .

The third one is parent's genotype and birthing the affected child posibility.

For monogenous diseases are known autosomal-dominant, autosomal-recessive and linked with X-chromosome dominant and recessive patterns of inheritans. Normal traits can also be inherited linked with Y-chromosome .

Autosomal-dominant pattern of inheritance .
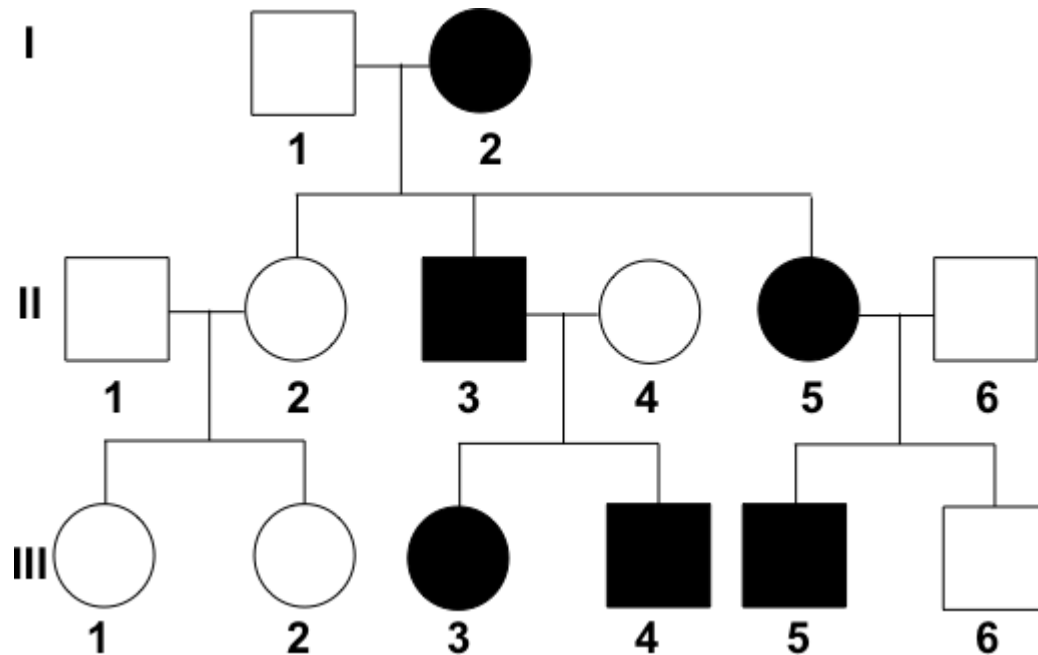
1.A disease is specified by gene A , norm by – a. Affected person have genotype Aa and AA rarely. A disease in homozygotes shows a severe form. For many diseases dominant gene in homozygous condition has lethal effect.

2. Both males and females are equally affected.

3.   The trait is inherited "on vertical". i. e . appears in each generation.

4.   Affected person has one affected parent as a rule .

5.   If one of the parents is affected so the probability of affected child birth is 50%

P   ♀Aa   X   ♂aa

gametes   A,a   a

F1   Aa   aa
+   sick 50%   healthy 50%

Sometimes pedigree of autosomal-dominant disordes is different from classical ones. In 60-80% dominant trait appears because of a new mutation in one of parents gametes (often father's).
Risk of a new dominant mutation is increased with the father's age. Risk of second mutation is insignificant as a rule.

Sometimes few children of healthy parents can have dominant disorder. In that case we can think of gonadal mosaicism in one of the parents. Mutation happened in predecessors of sexual cells by forming gonads. Then a lot of sexual cells can have mutant gene, and risk of birthing the second affected child can be very high. For example the risk of child with ectrodactily birth is 14%,though the parents are healthy.

The second reason of atypical pedigree is that the disease can manifests not from the moment of birth but later (Huntington's chorea in 40-50age). Incomplete penetrance and variable clinical expressivity is typical for some dominant genes.

Autosomal-recessive type of inheritance.
The example of pedigree.
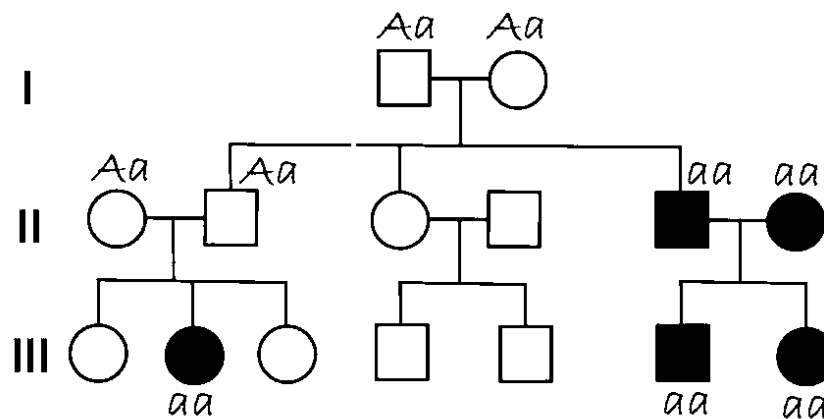
For this type is typical the following :
1. A disease is specified by recessive gene a, norm by – A. Sick man has genotype aa, healthy one –AA or Aa. For genes complete penetrance is typical. Variable expressivity is met rarely.
2.Male and female are equally affected.

3.The trait is inherited "on horizontal", i. e. is met at sibs of one generation.

4.Patient's parents are usually healthy heterozygous (Aa).

5.Risk of sick child birth at two heterozygous parents is 25%.

P   ♀ Aa   X   ♂ Aa

Gametes   A   a   A   a

F1   AA, Aa,   Aa, aa

                          25%

 b) If both parents are ill the trait is inherited in 100%

P   ♀ **aa**   **X**   ♂**aa**

gametes   a   a

      F1   **aa**

              100%

7.The recessive traits are often met in consanguineous marriages. If the consanguinity is repeated, pedigree looks like antosomal – dominant one. Unusual pedigree with estimated recessive pattern of inheritance may be explained by mutation in maternal or paternal gene or by uniparental isodisomia.

1.Mutation in one of the parents – homozygote on dominant gene is not concealed:

P Aa   x   AA

          a   a

            aa
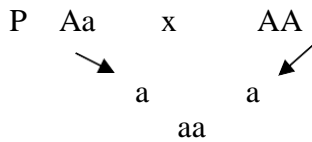
2.   Uniparental isodisomia is possible :

Aa   AA

a
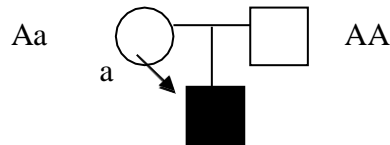
By violation of the second meiotic division gamete can get two chromatids

(†a † a) Zygote will get two chromosomes †a † a from one gamete and † from another one. Chromosome † is lost in first mitotic division of zygote. Uniparental origin of two chromosomes is proved by the results of examinations of chromosomes with help of banding staining method.

The cases of birthing healthy children from two sick parents with albinism or congenital deafness are known. It's explain by genetic heterogenity of diseases.

A mother's disease is specified with one mutant gene (aaBB) and father's (Aabb) with another one.

P   **♀ aaBB**   **X**   **♂Aabb**

Gametes   **aB**   **Ab**

F1   **AaBb**

                   100%   **are**   **healthy**
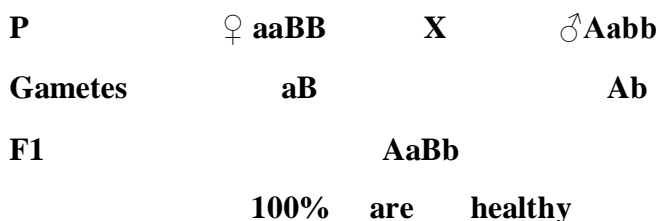
Sex-linked inheritance
There is no description about human diseases linked with Y-chromosome (association of – "skin of porcupine "- with Y-chromosome is contested by a lot of geneticists). That's why speaking about sex – linked inheritance we mean X-chromosome linked inheritance. For recessive X-chromosome linked inheritance is typical:

1.Mainly men are affected
2.We can't see transmission of the diseases from man to man because son never inherits X-chromosome from his father:

P   **♀ *XX***   ***X***   **♂ *XY***
Gametes   X   X   X   Y

F1	XX	XX	XY	XY
	Girls		*boys*	

3. The gene is inherited from sick father to his daughters in 100% and they become healthy heterozygous carriers:

P   ♀ $X^a X^a$ X   ♂ $X^a Y$

gametes X^a X^a ,Y

F1 $X^A X^a$ $X^A Y$

     100% of daughters are heterozygous carriers

4. From heterozygous mother a trait is inherited in 50% of daughter (which become carriers) and 50% of sons (are affected)

P   ♀$X^A X^a$ X   ♂ $X^a X^A$
gametes X^A , X^a X^a ,Y
F1 $X^A X^A$, $X^A X^a$, $X^A Y$, $X^a Y$
     Daughters - carriers   50%   sons - ill 50%

5. Affected son more often inherits disease from healthy heterozygous mother - carrier of pathological gene. Affected boys can have ill brother and uncles on mother's line. Son's disease can be caused in some cases by new mutation in mother's X-chromosome.

6. Sometimes X trait can be expressed phenotypically as a consequence of selective inactivation of X chromosome at heterozygous carriers.

An example of pedigree with recessive X-linked trait:

Dominant X-linked inheritance.
1.   Women are in twice more affected than men
2.   If mother is heterozygous carrier of pathological gene ($X^A X^a$) the trait is inherited by 50% of daughters and sons .
mother is heterozygous on dominant X-linked gene:

P   ♀$X^A X^a$ X   ♂$X^a Y$
Gametes $X^A X^a$ $X^a Y$
F1 $X^A X^a$,$X^a X^a$, $X^A Y$,$X^a Y$

    Affected are 50%

3.A trait from affected man is inherited by 100% of daughter and never by sons because son inherits Y-chromosome .

Father is affected, mother is healthy:

P   ♀ $X^a X^a$ X   ♂ X^A Y

Gametes X^a X^A Y

F1 $X^A X^a$, X^a

100% of affected daughter   100% of healthy sons

4.Disease at women are seen to be in more mild form because they are more frequently heterozygous ($X^A X^a$). Disease at homozygous men (X Y) is expressed in more severe form.

Pedigree of dominant X-linked pattern of inheritans
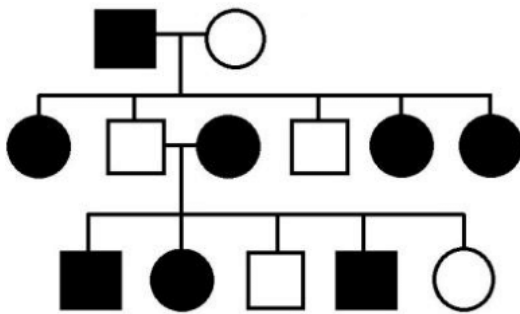
Some problems can appear in interpretation of pedigree .Sometimes man hasn't offspring because they die without becoming sexual mature (for example: muscular degeneration of Dushen) or men can be sterile .

Sometimes X-linked dominant diseases are characterized by lethal effect for mail embryo. In that case only girls are affected (Bloch-Sulzberg's syndrome)

If mother is heterozygous 2/3 daughters and 1/3 sons will be born. Sons and half of daughters will be healthy.

X- linked disease

P   ♀$X^A X^a$ X   ♂X^a Y

Gametes X^A X^a X^a Y

F1 $X^A X^a$ $X^a X^a$ X^a Y X^a Y

                affected   healthy   healthy doys   intrauterine death

Y-linked pattern of inheritance

There are genes in Y-chromosome which control body, extremities and teeth growth, , hairy pinna. Trait is inherited from father to son (holandric inheritance).

P   ♀ XX   X   ♂ X Y^g

gametes   X   X Y^g

F1   XX   X Y^g

                Trait at 100% of sons

Y-linked
Inheritance
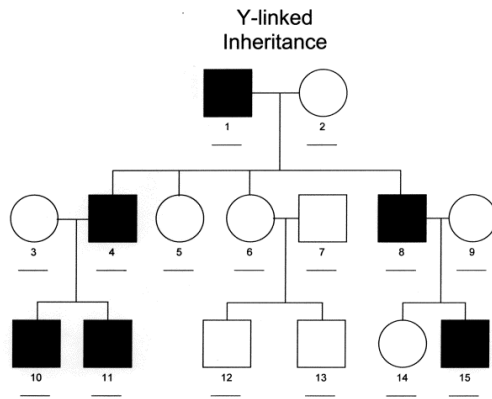
MCQs for self -control

1. For pedigree with autosomal-dominant pattern of inheritance is typical:

A. "Vertical" inheritance of the trait, affected child as a rule has one of the parents affected
B. Males and females have the same risk to be affected, for heterozygous parents risk to have an affected child is 25%.
C. Trait is inherited by all of the daughters and never by the sons of affected father
Д. Mainly men are affected. If mother is a heterozygous carrier, 50 % of sons may be affected.
E. Mainly men are affected. Trait is inherited by 100 % of sons.

2. For pedigree with autosomal-recessive pattern of inheritance is typical:

A. "Vertical" inheritance of the trait, affected child as a rule has one of the parents affected
B. Males and females have the same risk to be affected, for heterozygous parents risk to have an affected child is 25%.
C. Trait is inherited by all of the daughters and never by the sons of affected father
Д. Mainly men are affected. If mother is a heterozygous carrier, 50 % of sons may be affected.
E. Mainly men are affected. Trait is inherited by 100 % of sons.

3. Parents of the affected child are healthy, but same disease has sibs of both sexes. It is typical for:

A. Autosomal-dominant pattern of inheritance.
B. Autosomal-recessive pattern of inheritance.
C. Recessive X-linked pattern of inheritance.
D. Dominant X-linked pattern of inheritance.
E. Polygenic pattern of inheritance.

4. For autosomal-recessive pattern of inheritance is typical all except:

A. Both sexes are equally affected.
B. 'Vertical" transmission of the disease in the pedigree.
C. Parents of the sick person may be healthy.
D. Parents are relatives.
E. The risk to have an affected child is 25 % if both parents are heterozygotes.

5. For X-linked-recessive pattern of inheritance is typical all except:

A. Healthy parents have healthy children.
B. Mainly men are affected.
C. Sons of the affected father are also affected.
Д. If woman is a carrier a risk to be affected for her sons is 50%.
E. Daughters of affected father are the carriers.

6. Genotype of a boy with phenylketonuria is

A. AA
B. Aa
C. aa
D. $X^A y$
E. $X^a y$

7. Genotype of a boy with Duchenne muscular dystrophy is:

A. AA
B. Aa
C. aa
D. XᴬУ
E. XᵃУ

8. Genotype of a man with phosphate diabetes is:

A. AA
B. Aa
C. aa
D. XᴬУ
E. XᵃУ

9. The most possible genotype of a woman with Marfan's syndrome is:

A. AAA.
B. Aa
C. aa
Д. XᴬУ
E. XᵃУ

10. Healthy parents have a child with cystic fibrosis. What is the most possible genotype of the parents and child?

A. Mother Aa, father aa, child Aa.
B. Mother Aa, father Aa, child aa.
C. Mother XᵃXᵃ, father XᴬУ, child XᴬУ.
D. Mother XᴬXᵃ, father XᴬУ, child XᵃУ.
E. Mother aa, father aa, child Aa.

11. Healthy parents have a child with hemophilia. What is the most possible genotype of the parents and child?

A. Mother Aa, father aa, child Aa.
B. Mother Aa, father Aa, child aa.
C. Mother XᵃXᵃ, father XᴬУ, child XᴬУ.
D. Mother XᴬXᵃ, father XᴬУ, child XᵃУ.
E. Mother aa, father aa, child Aa.

12. Healthy parents have a child with ectrodactyly (autosomal-dominant character). Fresh mutation is supposed. What is the recurrence risk?

A. About 0%.
B. 25%
C. 50%
D. 75%
E. 100%.

13. Healthy woman has brother and son with fragile-X syndrome. Risk to have one more affected child is

A. Almost 0%
B. 50% of all children.
C. 50% of the sons.
D. 25% of the sons.
E. 25% of all children.

There can be one or several correct answers.

14. .Sibs are:

A. proband's parents;
B. proband's children;
C. proband's brothers;
D. proband's sister;
E. all proband's relatives.

15. .For autosomal-dominant pattern is typical the following :

a) Patient's parents is more often healthy heterozygotes ;

b) If one of the parents is affected the risk of sick child birth is 50%;
c) Risk of sick child birth 25%;
d) Men and women are affected equally;
e) The trait is inherited on vertical.

16. Parents are heterozygous carriers of pathological gene. Risk of sick child birth in the family is

A. 0%
B. 25%
C. 50%
D. 75%
E. 100%

17. .Both parents have phenylketonuria (have already passed through the reatment) .Phenylketonuria is recessive disease. Risk of affected child birth in the family is :

a)0%

b)25%
c)50%
d)75%
e)100%

a)autosomal-dominant disease
b)autosomal-recessive disease
c)recessive X-linked disease
d)dominant X-linked disease

18. .For autosomal –recessive pattern of inheritance is typical the following :

a)high penetrance of genes ;
b)men and women are affected equally;
c) risk of the sick child birth is higher in consanguineous marriage;
d) sick child has one sick parent as a rule;
e) parents of sick child are phenotypically healthy .

19. In which pattern of inheritance men are mainly affected

autosomal-dominant
autosomal-recessive
recessive X-linked
dominant X-linked

20. .In which pattern of inheritance women are affected more often

autosomal-dominant ;
autosomal-recessive;
recessive X-linked
dominant X-linked

21. Information about couple and their parents origin from one place is important for diagnostic of:

22. In what cases two healthy parents can have sick child:

a)if both are heterozygous carriers of recessive pathological gene ;
b)if one of them is with happened mutation;
c)if mother is carrier of recessive X-linked gene

23 .For recessive X-linked inheritance is typical

a)men are mainly ill;
b)sons of mother-carrier will be affected with probability 50%;
c)from sick father gene is inherited by 100% of daughters and never by sons .
d)The trait is inherited on vertical in pedigree

24. For dominant X-linked pattern of inheritance is typical all except:

a) men and women are affected often;
b) from father gene is inherited by daughters and never by sons;
c) from sick heterozygous mother trait is inherited by 50% of sons '
d) gene can cause death of mail embryo ;
e) disease at women are seen to be in more mild form

Practical lesson 7-8
Topic: Monogenic diseases with autosomal dominant and X-linked types of inheritance

CONTROL QUESTIONS
1. Etiology and pathogenesis of single gene disorders. Classification of gene mutations.
2. Classification of single gene disorders.
3. Single gene disorders and syndromes with autosomal dominant pattern of inheritence: achondroplasy, Marphan's syndrome, acrocephalosyndactilia. Clinics, diagnostics, treatment and monitoring of the patients, prenatal diagnostics, genetic counseling, calculation of genetic risk.

The purpose of the lesson: To know the etiology, pathogenesis, classification, diagnosis and principles of treatment of single gene disorders with autosomal-dominant and X-linked inheritance; to be able to reveal the symptoms of inborn errors of metabolism while gathering anamnesis and examining the patient; to analyze the results of a biochemical investigation of the patient with cystic fibrosis and phenylketonuria.

Student should know:
1. Classification of the single gene disorders;
2. General characteristics of single gene disorders with different patterns of inheritance;
3. The examples of single gene disorders with different patterns of inheritance;
4. The principles of genetic risk calculation;

Student has to be able to:
1. To calculate the genetic risk for the most common of single gene disorders with with autosomal-dominant and X-linked inheritance;
2. To reveal the pattern of anomalies, which characterize the frequent single gene disorders with autosomal-dominant and X-linked inheritance.

CONTROL QUESTIONS
4. Etiology and pathogenesis of single gene disorders. Classification of gene mutations.
5. Classification of single gene disorders.
6. Single gene disorders and syndromes with autosomal dominant pattern of inheritance: achondroplasy, Marphan's syndrome. Clinics, diagnostics, treatment and monitoring of the patients, prenatal diagnostics, genetic counseling, calculation of genetic risk.
7. Single gene disorders with X-linked pattern of inheritance: Duchenne muscular dystrophy, fragile-X syndrome.
8. Methods of laboratory diagnosis of single gene disorders
9. Prenatal diagnostics of single gene disorders.

PRACTICAL WORK:
1. To examine the patient with single gene disorder, to mark the typical symptoms, to compose pedigree and identify the pattern of inheritance.
2. To work out plan of examining the patient with single gene disorder.
3. To analyze the phenotype of the patients with single gene disorders, using the photos of the patients.

Main literature
Methodical recommendations on medical genetics
Additional literature:

1. Genetics in medicine. - 7th edition/Robert L/Nussbaum, Roderick R. McInnes, Huntington F. Willard. – 2007 – 585 p.

2. Emery's Elements of medical genetics. 15th ed. / Peter Turnpenny, Sian Ellard. – Elsevier, 2017. – 400 pp.
3. Lynn B. Jorde, John C. Carey, Michael J. Bamshad. Medical genetics. 5th ed. Elsevier, 2016. 356 pp.
4. Vogel and Motulsky's human genetics. Problems and approaches / M. R. Speicher, S. E. Antonarakis, F. G. Motulsky. 4th addition. – Springer, 2010. – 981 pp.
5. Young Ian.D. Medical genetics. -2nd ed. -Oxford university press, 2010. - 304 p.
6. Diseases of the fetus and newborn. Pathology, radiology and genetics. G.B.Reed, A.E. Claireaux and A.D.Bain., - Great Britain, - 1989, 812 p.
7. Human molecular genetics. Tom Strachan, Andrew P.Read. – 4th edition - Bios Scientific Publisher, 2010, 680 p.
8. Smith recognizable patterns of human malformation. Seventh edition. John M. Graham, - USA, - 2013, 976 p.
9. R. Wiedemann, K.-R. Gross, H.Dibberin ,Atlas of characteristic syndromes. A Visual Aid to Diagnosis. Second edition. -London, 1986,412 p.

13. Information resources:
https://ghr.nlm.nih.gov National librery of medicine, genetics
https://www.orpha.net The portal for rare diseases and orphan drugs
https://rarediseases.org National Organization for Rare Disorders
http://omim.org/OMIM (Online Mendelian Inheritance in Man) – An Online Catalog of Human Genes andGenetic Disorder

MAIN THERETICAL INFORMATION

Single gene disorders are the disorders, caused by single gene mutation. There are about 100000 (10^{-5}) genes in human genotype. Single gene disorders are the result of about 30.000 – 5.00 genes mutations. Mutations of other genes lead to the death of the cell and can not results in hereditary disorder. In nowadays more than 4000 single gene disorders have been described.

Gene is the part (nucleotide sequence) of a DNA molecule, which contain the information about single protein structure. There are different types of gene mutations:

1. A point mutation is the replacement of one nucleotide by another. For instance sickle cell anemia is the result of mutation in gene, encoding the *B*-chain of hemoglobin molecule. There is a CAC triplet instead of CTC, which leads to the substitution of Gly by Val.

Normal DNA	DNA after mutation
DNA CTC	DNA CAC
mRNA GAG	mRNA GUG
amino acid Glu	amino acid Val

2. An insertion is the addition of anything from one base pair up to quite extensive pieces of DNA, usually from another part of a chromosome.

3. A deletion is the loss of a portion of the DNA sequence. Deletions can be as small as a single base or much large.

4. A duplication – nucleotide dubbling.

4. An inversion - a portion of the DNA sequence is excised then re-inserted at the same position but in the opposite orientation. (rotation of the gene sequence by 180).

Mechanisms 2 – 4 cause the changes in general number of nucleotides and leads to the frameshift mutations Frameshift mutation - this is result from the insertion of extra bases or the deletion of existing bases from the DNA sequence of a gene. If the number of bases inserted or deleted is not a multiple of three the reading frame will be altered and the ribosome will read a different set of codons downstream of the mutation. Frameshift mutations usually have a serious effect on the encoded protein.

Norma DNA:
DNA GGG-CC**C**A-TCG-GGG-A
mRNA CCC-GGU-AGC-CCC-U
protein Pro – Gly – Ser – Pro –
Mutant DNA after **C** deletion
DNA GGG- CAT-CGG-GGA-
mRNA CCC–GTU-GCC- CCU-
protein Pro - Val - Ala -

Some times point mutation changes a codon specifying an amino acid into termination codon (nonsense mutation). The protein will be shorter than a normal protein, or proteins are not synthesized at all.

So gene mutations lead to the changes of protein structure or stoppage of its synthesis.

DNA changes ⟶ mRNA changes ⟶ abnormal protein or its absence

Mutations may be dominant and recessive. Dominant mutations manifests immediately, recessive ones – are kept in population in heterozygous condition and then manifests in homozygous offspring of heterozygous couple. Mutations may involve as genes of autosomes as genes of sex chromosomes. It may change structural, transport, enzymes, and embryonic proteins that determine phenotypic effect and clinical symptoms.
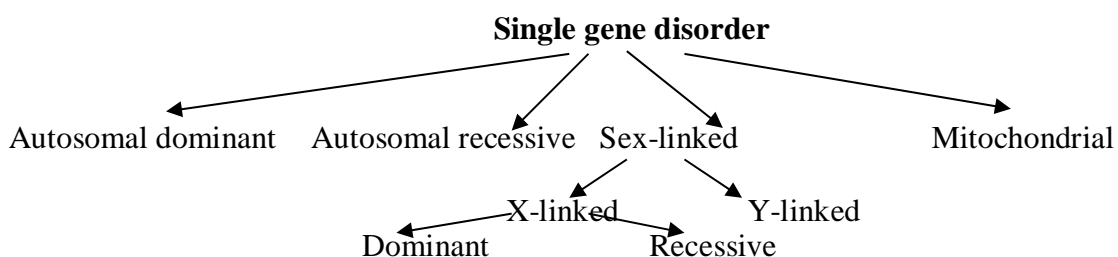
Results of pathological mutation may be:

1. Death of the embryo in early stages of the embryonic development or later, before birth. Some time lethal effect of gene manifests before implantation as infertility in women with normal sexual activity.

2. Single gene disorder.

If development of embryo is not terminated, disease may manifest with multiple congenital abnormalities (mutation of embryonic development gene) or inborn errors of metabolism (mutation of the enzyme) or combined states.
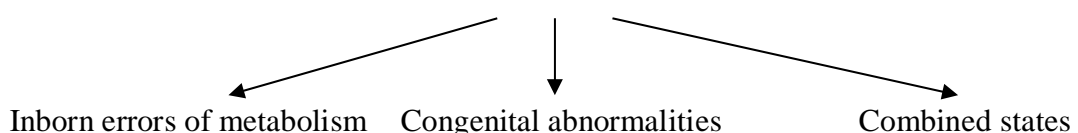
Classification of single gene disorders

Classification is based on the three different principles: genetic, clinical and pathogenetic.

Genetic classification is based on the pattern of inheritance.

Single gene disorder

Autosomal dominant   Autosomal recessive   Sex-linked   Mitochondrial

X-linked   Y-linked

Dominant   Recessive

Clinical classification is based on the primary affection of certain organ or system of organs: hereditary disorders of nervous system, neuro-muscular disorders, gene dermatoses, ocular, endocrine, blood, circulatory system, urogenital system, gastro-intestinal system disorders and so on.

Pathogenetic classification is based on the main link of pathogenesis.

Pathogenetic classification

Inborn errors of metabolism   Congenital abnormalities   Combined states

In nowadays more complicate clinical and pathogenetical classifications are proposed. According this classification single gene disorders may be divided into following groups.

1. Congenital abnormalities.
2. Enzymopathias – disturbance in enzyme synthesis, which are manifests as metabolic disorders or endocrinopathias.
3. Storage disorders – a variant of enzymopathias.
4. Disorders because of transmembrane conduction disturbance (cystic fibrosis)
5. Disturbance in function and structure of blood proteins – hemoglobin, factors of blood clotting, transport proteins.
6. Hereditary immune deficient states.
7. Disorders of DNA reparation (xeroderma pigmentosum, Blum's syndrome)
8. Mitochondrial and peroxysome disorders
9. Disorders because of uniparental isodisomy (inheritance of both homologous chromosome from one parent – Silver-Russell syndrome, Beckwith-Wiedeman syndrome)
10. Syndromes with unknown etiology (Cornelia de Lange syndrome)

Pattern of inheritance is very important for the genetic counseling. That why the material above is in accordance with genetic classification.

General characteristic of single gene disorders

1). Variable age of the onset from intrauterine to old. The majority of pathologic mutations 25% of disorders manifest in newborn babies, 45% in infants till 3 years old and 20% in adolescent period. In sum it will be 90%. Other 10% manifests after 20.

2). Genetic heterogeneity. Some clinical form of the hereditary disease may be caused with mutation in different locus or different mutation in one locus. Such cases are termed as genocopies. For example phenylketonuria may be caused by the mutation of two different genes. There are 11 types of mucopolysaccharidosis, caused by the mutations of 11 different genes. Deafness and albinism in different families are caused with the mutation of two different genes. Some times there may be different types of the mutation in the same gene. The longer is the gene the more frequent take place a mutation. There are about 200 mutations in gene of phenylketonuria, more then 300 mutations in gene of cystic fibrosis. Protein structure can be damaged in different ways in case of different mutations. That is why in different families the same hereditary disorder may vary in the time of the onset, severity of the course and other peculiarities. That means clinical polymorphism except genetic one.

3) Clinical polymorphism also may be the result of specific genes regulators, which modify the activity of pathologic gene.

4) Prolonged chronic course with increased severity is typical for storage diseases and enzymopathias. Progredient course is not characterized for all genetic disorders. In the development of certain diseases (ectrodactily, achondroplasy) formation of terminal phenotype takes place in definitive age.

Single gene disorders with autosomal-dominant pattern of inheritance.

Pathologic character is encoded with dominant gene A, normal - with recessive allele a. Affected persons have genotype AA or Aa, healthy persons - aa, probability of birth of affected child depends upon genotypes of parents.

```
P            ♀ Aa   x   ♂ aa
Gametes    A , a              a
F₁         Aa(50%)        aa(50%)
```

Risk of affected child birth is 50%.

Exclusions of this rule may be follows:

1) Ratio between affected males and females are not equal 1 because of lethality for definite sex.

2) Affection of the child, having the healthy parents may be explained with fresh mutations in gametogenesis.

3) Penetrance of the gene may be low and a person with pathologic dominant gene phenotypicaly is healthy.

For dominant genes incomplete penetrance and variable expressivity are common. Penetrance is the percentage of persons in which disease is manifested (pathological gene is expressed in phenotype) for the all carriers of this gene.

Penetrance of dominant gene can be calculated using the following formulae P= number of patients / number of people with genotypes AA or Aa x 100%

In analysis of pedigree penetrance can be calculated in formulae P= number of patient / number of expected patient x 100%

Expressivity is individual variability of trait expression in different patients. For instance, polycystosis of kidneys (adult type with autosomal dominant pattern of inheritance) in some patients may manifest with renal insufficiency and in others as renal hypertension with normal function of kidneys. This is explained with different regulatory genes and different mutation of the same gene in different families.

Pleyotropy is also quite common and clinically manifests as affection of several systems of organs.

Achondroplasia.

Population rate is In 80 – 90% is a fresh mutation. The frequency is
1/ 50000 – 1/ 100 000 in adult.

Typical features are short stature (height of newborn is 46 – 48 cm., in adults is about 120-130 cm), macrocephaly, flattened nose, prognatia, shortness of proximal part of extremities. The palms are wide and short, finger is tregent, usually take place isodactily, lumber lordose is expressed very well. Motor development is delayed, intelligence is normal. Diagnosis is portrait (based on the specific appearance). Treatment is symptomatic, orthopedic correction is proposed. Genetic risk for offspring is 50%. Prenatal diagnosis is made with the help of ultrasound examination on the 24 – 26 week of gestation – measuring of the femoral and humoral bone length.

Marfan's syndrome.

Population rate is 1:1000 – 1:5000, sex ratio is M1: F1; 75% patients with Marfan's syndrome have affected parents, 25% of cases is the result of fresh mutation.

Disease is caused by the mutation of gene coding fibrillin – one of the proteins of connective tissue. Gene is localized in 15q21. In disturbance of fibrillin synthesis increasing of connective tissue extension happens. The disorder in acid glycosaminoglycans metabolism take place as in fibrils as in intercellular matter. That leads to its storage and excessive excretion with the urine. The hydroxyproline metabolism (collagen component) is also abnormal, so it can be revealed in urine.

Hyperextension of connective tissue leads to the affection of skeleton, circulatory system, eyes and other organs.

Patients are characterized by high height (about 10 – 15 cm more than normal height). Dolichocephaly, narrow bird–like face, gothic palatum, and blue sclera are common.

The skeletal changes are arachnodactyly (excessive length of the long bones). Especially distal part of vertebra get deformed, scoliasis, thoracic lordosis, kiphosis, deformation of thorax; funnel like sternum, laxness of joints.

Cardiovascular system: vessels and bi- and trycuspidal valves abnormalities. Arises prolaps of mitral valves, aneurysm of the aorta, dissecting or diffuse.

Eyes disorders: lens dislocation, myopia, glaucoma and other.

Lung affection includes emphysema and spontaneus pneumothorax as a result of bullous emphysema.

Femoral and inguinal hernias, muscular hypoplasia and hypotonia are common.

Diagnosis - In urine of patient is increased amount of proline and oxyproline and GAG. Treatment includes thorough control of blood pressure, avoidance of marked physical excretion,

diet reached with collagen and vitamins, especially vit. C, angioprotectors, Vit. B_1, B_6, B_{12}, and immune modulators.

Risk for the offspring of heterozygous parent is 30%

Prenatal DNA diagnosis is possible in future.

Apert syndrome
(acrocephalosyndactily of first type)

Population rate is 1 : 160000, sex ratio is M1 : F1, belongs to the group of acrocephalosyndactilies, few types of which are described.

Apert syndrome (acrocephalosyndactily of first type) is characterized with

1) craniofacial abnormalities – acrocephaly (short anteroposterior diameter of cranium with high, full forehead and flat occipiut), irregular craniosynostosis, especially of coronal suture. Facial signs include flat face, shallow orbits, hypertelorism, strabismus, antimongoloid eye slanting, palpebral fissures, small nose, maxillary hypoplasia.

2) Osseous and/or cutaneous syndactily, varying from total fusion to partial fusion, most commonly with complete fusion of second, third and fourth fingers.

3) Sever mental retardation with impossible social adaptation.

Other abnormalities are synostosis of radius and humerus, pyloric stenosis, ectopic anus, ventricular septal defect.

Etiology is autosomal dominant with the vast majority of cases representing a fresh mutation. One factor in the sporadic cases has been older parental age. The recurrence risk for the unaffected parent of a child with Apert syndrome is negligible. Prenatal US diagnosis is possible on the 24-26 week of gestation.

Single gene disorders with autosomal-dominant pattern of inheritance

Syndrome or diseases	Population rate	Location of gene	Minimal diagnostic signs
Apert syndrome	1:160000		Acrocephaly (peaked head), the second, third and fourth fingers are fused into a mass with the common nail, sever mental retardation.
Achondroplasy	1 : 100000		Dwarfism because of shortness of proximal parts of extremities
Marfan's syndrome	1 : 10000 – 1 : 15000	15q	High stature, excessive length of extremities, arachnodactyly, laxness of joints, deformation of sternum, vertebrae column, bilateral ectopia lentis, and vascular defects.
Neurofibromatosis (Recklinghausen's disease)	1 : 4000 – 1 : 3300 (in 50%-70% cases fresh mutation)	17q	Pigmented skin lesions (cafe-au-lait spot) in infancy or early childhood, followed by development of multiple benign subcutaneus neurofibromas of periphery nervous trunks, acoustic and optic neurinomas.
Syndactyly	1 : 2500 – 1 : 3000		Any degree of webbing or fusion of fingers or toes, involving soft parts only or including bone structure.
Pollydactyly	1 : 3300 – 1 : 630		The presence of more than five digits on either hand or foot.
Ectrodactyly			Congenital absence of one or more fingers or toes (claw-like hand)

Sex linked single gene disorders

If gene is localized in X or Y chromosome it is termed as sex linked inheritance.

X-linked characters may be dominant and recessive. Recessive X-linked genes are marked as X^h. Males are affected more often (X^aY) as they have only one X chromosome. Genes of X chromosome don't have allele pair and manifests phenotypically in all cases.

Females become heterozygous carriers of the pathological gene (X^AX^a) and quite rare are affected homozygotes ($X^a X^a$). Sometimes affected females are "manifesting heterozygote" because of random inactivation ("lyonisation") of X-chromosome. Normally all female embryos on the stage of 16 – 32 blastomeres in all cells (except sex cells precursors) one of the X-chromosomes undergoes inactivation. Inactive chromosome is termed as X-chromatin or Barr body. Another X-chromosome is active. If by chance in most of cells of the heterozygous women the X-chromosome with normal dominant gene is inactive she will be ill. In case of clinically manifested X-linked disorder in girl X monosomy (Terner syndrome, 45 X) also should be excluded.

If mother is a heterozygous carrier of recessive character, she will transmit pathologic gene to 50% of sons and 50% of daughters. Sons will be ill and daughters become heterozygous carriers. If the father is affected (X^aY) he transmits the pathologic gene for all daughters, which become heterozygous carriers. All sons inherit Y chromosome. Examples: hemophilia, color blindness, Duchenne muscular dystrophy, fragile X syndrome, anhidrotic ectodermal dysplasia.

Dominant X linked disorders are expressed either in women or in men. Sick mother transmit the character to 50% daughters and sons, which are affected. Affected father gives the disorder to 100% daughters and never to the sons. Example: phosphate diabetes.

Duchenne-Becker muscular dystrophy

Disease is the result of mutation in gene responsible for specific protein dystrophin synthesis. This protein is a structural component of muscular cell's sarcolemma which maintain its stability. Structural change in sarcolemma results in metabolic disorders, degeneration of cytoplasm, increased entrance of K^+, which cause the cells destruction and replacement of skeletal muscles with connective and fat tissue. Gene is recessive and localized in the short arm of the X-chromosome (xp-21.2). It is the largest gene among studied, it consist of 2 x 10 nucleotyde pairs, m-RNA molecule is 16000 nucleotyde pairs in length. Fresh mutations form about 30% of all cases of the disease. Two types of the disease are distinguished: Duchenne muscular dystrophy because of stoppage of protein synthesis and Becker muscular dystrophy because of decreased amount of the protein or its abnormal structure.

Duchenne muscular dystrophy

Population rate is 1 : 3000 among males (X^aY), sex ratio is M1: F0.

The onset of the disease, as a rule, is in first three ears of life. After learning to walk, with delay and difficulty and frequent falls, the child develops a rocking, weaving, or waddling gait. It is difficult for children to jump, to go upstairs, to get up from the horizontal position, in the final process "climbing on himself" is present.

The disorder is characterized with constantly progressive course, unrelenting progression of the disease leads to the invalidism (wheelchair) usually about the end of the first decade of life. The main clinical sign is starting with the musculature of the pelvic girdle and thigh, ascending degeneration of the musculature, clinically involving that of the remaining trunk, then that of the shoulder girdle, upper arm, and other regions. Forms hyperlordosis with protruding abdomen, protruding "loose" shoulders. Pseudohypertrophy (fatty infiltration) especially of the calves, also the thigh and buttock musculature, less frequently of other muscle regions develops. Tendency for contractures to develop, especially in the lower extremities: relatively early tendency to develop talipes equinus and toe-walking. Mental retardation is in about 30% of cases.

Life expectancy is about 20 – 35 years. Lethal outcome is because of respiratory infection or heart failure, as on the final stage facial and pharyngeal muscles, respiratory muscles, cardiac muscle are involved.

Diagnosis is based on the specific clinical symptoms, increasing of serum creatine phosphokinase activity (10 – 100 times), especially in the initial stages of the process. EMG

demonstrates signs of myopathy, and ECG degeneration of myocardium and conduction disturbances.

In 70 % cases recognition of heterozygous females can be achieved by the combined use of enzyme creatine phosphokinase activity determinations, EMG, and muscular biopsy. In sonography of hips in heterozygous carriers rarefaction of femur contour is revealed.

Becker Muscular Dystrophy is a benign form of dystrophy. Frequently is 1:20000 in male. The disease manifests not earlier then 10-15 year of life. At 20-30 year they conserve the working ability, cardiomyopathy, mental retardation are absent. Activity of creatin phosphokinase is elevated insignificantly.

Either both types of muscular dystrophy or heterozygous carriers may be revealed with the molecular-genetic methods (PCR).

Haemophylia A

Population rate is 1 : 2500, sex ratio is M1 : F0.

Usually the symptoms arise at 2-3 years of life after the child starts to walk. In severe cases at the time of birth kephalohematomes, umbilical cord bleeding etc. are present. Later clinical features are abnormal bleeding following trauma, or without any significant reasons, recurrent hemarthrosis and internal hemorrhages. Hematomas are usually intramuscular or cutaneus, localize in the joints region. Bleeding after trauma may occur later (delayed bleeding), be prolonged and recurrent. Hemarthrosis leads to the limited join movements. Internal hemorrhages are quite rare.

Laboratory diagnosis consists of prolonged clotting time and partial thromboplastin time (PTT), confirmed by assay for factor VIII. In mild cases an activity of VIII factor is decreased up to 30%, in sever cases it is up to 1 %.

It is X-linked recessive disorder. The gene is localized at Xq 28. Prenatal DNA diagnostics is worked out.

Anhidrotic ectodermal dysplasia
(Crist-Siemens-Touraine syndrome)

Population rate is unknown, sex ratio M1 : F0.

Hypoplasia of sweat glands (hypohidrosis) in patients leads to the heat intolerance. Rise in body temperature sometimes with minimal physical excretion (especially in the young) or increased environmental temperature is typical. Hyperthermia may case convulsions or even lethal outcome, be the cause of mental retardation. Typical facial features includes bulging forehead, prominent suprorbital ridges, hypertelorism, deep-set nasal root, short nose with hypoplastic alae, hypoplasia of the upper jaw, full protruding lips, protruding chin, and possible prominent ears coming to a point at the top ("satyr ears"). Other external features are sparse, light scalp hair: short, fine and dry; absent or merely suggested eyebrows and eyelashes, little or no body hairs, premature baldness. Patients have fine wrinkling of the periocular and sometimes perioral skin, often heavily pigmented, hyperkeratosis of palms and soles is possible. Very typical feature is hypoplasia of alveolar ridges with adontia or hypodontia and abnormal leaf-like shape of the incisive. Supplementary findings are dry, irritated mucous membranes with the tendency to atrophy, deficiency of tears, conjunctivitis, tendency to chronic atrophic rhinitis, hypo- or aplasia of the mammary gland or nipples, hearing impairment, respiratory infections.

Female carriers may show mild dental dysplasia, decreased ability to sweat (regional aplasia of the sweat glands), little breast development.

Fragile-X syndrome
(Martin-Bell syndrome)

It is the second cause of mental retardation in population after Down's syndrome. Population rate is 1 : 2000 – 1 : 2500. Males are affected 2 – 3 times more then females and have more severe course.

Clinical presentation of the fragile X syndrome includes slow mental development with I.Q. of 30 to 55, but some times extending into the mildly retarded border-line-normal range. The

patient's growth rate is slightly increased in the early years, with delayed motor milestones but no evidence of deterioration. Testicular size may be increased before puberty, but this increase became more obvious postpubertally. A characteristic speech pattern, referred to as "cluttering", is observed in higher functioning individuals. Psychological profile is characterized by hyperkinetic behavior, emotional instability, hand biting, and other autistic features. All of these tend to improve at puberty.

The main facial abnormalities includes prominent jaw, thickening of nasal bridge extending down to the nasal tip, large ears with soft cartilage, pale blue irides.

It is unique in the sense that is caused by a combination of a mutant gene with an associated cytogenetic abnormality. The marker on the X chromosome is a fragile site at X q 27-28, so there is a site, resembling secondary constriction in long arm of the X chromosome. Pathologic FMR-1 gene has a large number of trinucleotyde repeats (CGG) in the 5' non-translating region of this gene. Normally there are 6 – 42 repeat, 50 – 200 repeats is considered to be a pre-mutation. In next generation number of repeats may reach 1000 which leads to the clinical manifestation of the disease. Severity of the disease depends on the number of the repeats. If woman inherits increased number of repeats she also may be affected.

Diagnosis is based on the typical clinical features confirmed with molecular-genetic assay (PCR) or cytogenetic method. Cytogenetic study needs a little modification in procedure of karyotyping. The culture medium used to grow cells is deficient in folic acid and thymidine. Affected persons show fragile site in long arm of the X chromosome, that resemble secondary constriction and satellite. Prenatal diagnosis has been successfully accomplished using cultured amniocytes and fetal blood samples obtained at invasive methods.

Syndrome of hereditary vitamin D resistant rickets
(phosphate diabetes syndrome: familial hypophosphataemic rickets)

Disease is X-linked dominant with correspondingly milder manifestations of the disorder in girls. Gene is localized in Xp 22.2 – 22.1. Frequency is 1:100000 in newborn.

It is a hereditary metabolic syndrome with hypophosphataemia, growth deficiency and rachitic bone changes because of decreased reabsorption of phosphates in kidney. Hypophosphataemia and hyperphosphatemia may be revealed at birth or in the course of the first half year of life. Clinically disorder manifests mostly in the 2nd and 3rd half-years of life and

thereafter, when children start to walk. Main signs are moderate grown deficiency with accentuated changes in lower extremities, pronounced bow-legs, less frequently knick-knees, wadding gait, coxae varae, dysplasia of nails. During childhood, the other rachitic bony changes, including rachitic rosary, swelling of the wrist and ankle joints develop. Dental changes, such as enamel defects, delayed eruption and premature loss may present. Joint and back pain and complaints of stiffness during adulthood are frequent. Adult height is between about 1.30 and 1.60.

Diagnosis is biochemical: hypophosphataemia with normal level of Ca, elevation of serum alkaline phosphatase.

X linked hereditary disorders

Syndrome or diseases	Population rate	Location of gene	Minimal diagnostic signs
X-linked recessive pattern of inheritance			
Hemophilia A – disturbance in blood clotting factor VIII synthesis.	1:2500 males	Xq 28	Prolonged bleeding following trauma, recurrent hemarthrosis (elbow, knee, ankle joints) and internal hemorrhages. Activity of VIII factor is decreased. Prolonged clotting time and partial thromboplastin time (PTT).
Daltonism			Green – red color blindness

Lesh- Nichan syndrome			Disturbance in purine metabolism. Is described in previous part.
Duchenne-Becker muscular distrophy Disturbance in dystrophin of skeleton muscle sarcolemma synthesis, Muscular cells destruction and replacement with connective and fat tissue.	Duchenne – 1: 3000 males; Becker – 1: 30000 males.	Xp 21.2.	Muscular weakness in proximal muscles groups, pseudohypertrophy (fatty infiltration) of musculature (calves, thigh and buttock, less frequently of other muscle regions). At 10 – 15 age patients are confined to bed, at 20 –30 dies. Becker muscular dystrophy is more benign.
Anhidrotic ectodermal dysplasia (Crist-Siemens-Touraine syndrome)	Unknown	X chromosome	Hypohidrosis of sweat glands (hypoplasia), heat intolerance. Hypodontia, hypotrichosis, abnormal teeth. Dry skin and mucous membranes.
X linked dominant pattern of inheritance			
Phosphate diabetes syndrome (Vit D resistant rickets)- decreased reabsorption of phosphates in kidney		X p22.2 – p 21.2.	Rickets, resistant for therapy with Vit D. Rachitic bone changes on the 1 – 2 year of life. Varus deformation of lower limbs. Hypophosphataemia, normal level of Ca, elevation of serum alkaline phosphatase.

Diagnosis of the single gene disorders.

Following methods are used:
1) portrait diagnostics – for congenital abnormalities;
2) biochemical diagnostics – for inborn errors of metaboplism;
3) DNA diagnostics;
4) cytogenetic method - for fragile X syndrome;

For the clinical diagnosis of single gene disorders, that manifests as congenital abnormalities, portrait diagnosis is used. Portrait diagnosis is the comparing of the patient's phenotype with the phenotype of the patients in special albums. Pathologic gene causes the formation of abnormal phenotype. As the mechanism of disorder formation is the same in all patients with the same single gene disorders, the patients are more similar with each other, than with there parents.

Biochemical methods are multi step ones. Objects for biochemical diagnostics may be urine, sweat, blood plasma and serum, blood enzymes, cell cultures.

Biochemical diagnosis is carried out in two stages.

1). Screening stage.

Screening is the identification of persons from a population with a particular disorder with the help of rapid reliable methods. It can be divided into mass neonatal screening and selective one.

Selective screening is the laboratory investigation of a person with the clinical signs of metabolic disorder with broad, generally routine eliminating procedures. In one sample of blood or urine about 30 different biochemical assays are carried out (detection of proteins, amino acids, mono- and disaccharides, GAG, chlorides, copper, bilirubin and others). Selective screening permits to reveal the abnormal link of metabolic chain, and proceeds by stages to sophisticated technology for definitive diagnosis.

Mass screening is the total biochemical investigation of newborns for the early revealing of affected children.

Program of neonatal mass screening includes following stages: 1) getting the samples from all newborns and its transportation to diagnostic laboratory;

2) screening biochemical analysis;

3) confirmatory tests on a second specimen in case of positive screening result;

4) treatment and long- term monitoring of the patients;

5) genetic counseling of the family.

Introduction of mass neonatal screening in practice is a social task. List of disorders, recommended for neonatal screening includes phenylketonuria, congenital hypothyroidism, galactosaemia, cystic fibrosis and congenital adrenal hyperplasia.

Screening for PKU : Before to be discharged from the hospital few drops of blood are taken from the newborn's heel on the filter paper. Samples are dried and send to the special laboratory. Two methods are used as the screening tests.

1) Guthrie test - comparing the amount of growth, induced by the sample with standards in a strain of the bacteria *Bacillus subtilis*, which requires phenylalanine for growth. Intensity of growth depends on PU amount in blood.

2) Fluorometric method: filter paper with blood sample is situated in the test tube with eluating solution and ninhydrin is added. In case of positive result lilac color appears. The intensity of coloration depends on the phenylalanine level in blood. It can be measured with the help of special instrument "Fluoroscan".

A useful but less reliable later screening test depends on detecting elevated urinary levels of phenylpyruvate with ferric chloride (Feling's reactive - 10%
$FeCl_3$). To 3-ml urine is added 1 drop of 1M solution of HCl, then solution is shacked and few drops of Fe Cl_3 are added. In case of positive result green color appears.

Screening for congenital hypothyroidism, and congenital adrenal hyperplasia is based on the measuring of hormones in blood.

Screening for galactosaemia is microbiologic test revealing galactose contains in blood.

Screening for cystic fibrosis is based on the measuring of tripsin activity in blood and albumin contains in meconium.

The main criteria for screening programs are:

1) Rate in population not less then 1 : 10000 (for galactosemia 1 : 40000)

2) Disorder leads to the early invalidism without treatment;

3) There are the measures of preventive treatment;

4) Test should be sufficiently sensitive without the false negative results;

Prenatal diagnosis of single gene disorders.

For prenatal diagnostics following methods can be used:

1) Ultrasonography if the disorders manifest as the congenital abnormalities. Optimal for the first examination is 18 –18 week of gestation, for the second one – 24 – 26 week. Because of medical indications examination can be carried out in other terms (on 6 – 8 week abnormal place of implantation and delayed intrauterine development can be revieled; on 13 – 14 week – reduction of limbs, at 23 week – heart abnormalities).

System or organ	Abnormalities
CNS	Anencephaly, spina bifida, holoprosencephly, cerebral hernia
Limbs	Reduction of limbs, achondroplasia (measuring of femur length on 24 – 26 week of gestarion), osteogenesis imperfect.
Heart	Sever heart abnormalities
Kidneys	Agenesis of kidneys, cyst of kidneys
Digestive system	Atresia of duodenum

2) invasive methods (amniocentesis, chorionic villus biopsy, placentocentesis, kordocentesis) with further biochemical assay or DNA studies. Unifactorial disorders, that can be diagnoses with direct DNA analysis in Ukraine are: cystic fibrosis, Duchenne and Becker muscular

dystrophy, haemophilia A and B, phenylketonuria, fragile-X mental retardation syndrome, Lesch-Nyhan syndrome, Huntington's chorea, Wilson's disease, some types of mucopolysaccharidosis, familial hypercholesterolemia. For X-linked disorders diagnostics determination of sex with cytogenetic methods can be used, if there is no methods of DNA diagnostics.

Practical lesson 9.
Topic: Monogenic diseases with autosomal recessive type of inheritance. Hereditary metabolic diseases

The purpose of the lesson: To know the etiology, pathogenesis, classification, diagnosis and principles of treatment of single gene disorders with autosomal-recessive mode of inheritance; to be able to reveal the symptoms of inborn errors of metabolism while gathering anamnesis and examining the patient; to analyze the results of a biochemical investigation of the patient with cystic fibrosis and phenylketonuria.

Student should know:

5. General characteristics of single gene disorders with autosomal-recessive inheritance;
6. The examples of single gene disorders with autosomal-recessive inheritance;
7. The principles of genetic risk calculation;
8. The general symptoms of enzymopathias, principles of its diagnostics.

Student has to be able to:

1. To calculate the genetic risk for the most common single gene disorders with autosomal-recessive inheritance;
2. To reveal the pattern of anomalies, which characterize the frequent single gene disorders.

CONTROL QUESTIONS

1. General characteristics of autosomal-recessive pattern of inheritance
2. Single gene disorders with autosomal recessive pattern of inheritance: adrenal hyperplasia syndrome, phenylketonuria, cystic fibrosis, hypothyrosis.
3. Inborn errors of metabolism, classification. Symptoms, which are typical for inborn errors of metabolism.
4. What is lysosomal storage disease? Examples.
5. Biochemical methods of diagnosis of single gene disorders. Screening of the newborns.
6. Methods of DNA diagnostics.
7. Prenatal diagnostics of single gene disorders.

PRACTICAL WORK:

1. To examine the patient with single gene disorder, to mark the typical symptoms, to compose pedigree and identify the pattern of inheritance.
2. To work out plan of examining the patient with single gene disorder.
3. To analyze data of laboratory investigation of the patient with phenylketonuria and cystic fibrosis.
4. To analyze the phenotype of the patients with single gene disorders, using the photos of the patients.

MAIN LITERATURE:

Main literature
    Methodical recommendations on medical genetics
Additional literature:

1. Genetics in medicine. - 7th edition/Robert L/Nussbaum, Roderick R. McInnes, Huntington F. Willard. – 2007 – 585 p.

2. Emery's Elements of medical genetics. 15th ed. / Peter Turnpenny, Sian Ellard. – Elsevier, 2017. – 400 pp.
3. Lynn B. Jorde, John C. Carey, Michael J. Bamshad. Medical genetics. 5th ed. Elsevier, 2016. 356 pp.
4. Vogel and Motulsky's human genetics. Problems and approaches / M. R. Speicher, S. E. Antonarakis, F. G. Motulsky. 4th addition. – Springer, 2010. – 981 pp.
5. Young Ian.D. Medical genetics. -2nd ed. -Oxford university press, 2010. - 304 p.
6. Diseases of the fetus and newborn. Pathology, radiology and genetics. G.B.Reed, A.E. Claireaux and A.D.Bain., - Great Britain, - 1989, 812 p.
7. Human molecular genetics. Tom Strachan, Andrew P.Read. – 4th edition - Bios Scientific Publisher, 2010, 680 p.
8. Smith recognizable patterns of human malformation. Seventh edition. John M. Graham, - USA, - 2013, 976 p.
9. R. Wiedemann, K.-R. Gross, H.Dibberin ,Atlas of characteristic syndromes. A Visual Aid to Diagnosis. Second edition. -London, 1986,412 p.

13. Information resources:

https://ghr.nlm.nih.gov National librery of medicine, genetics
https://www.orpha.net The portal for rare diseases and orphan drugs
https://rarediseases.org National Organization for Rare Disorders
http://omim.org/OMIM (Online Mendelian Inheritance in Man) – An Online Catalog of Human Genes andGenetic Disorder

MAIN THEORETICAL INFORMATION

Single gene disorders with autosomal – recessive pattern of inheritance.

The disease is encoded by autosomal-recessive gene - **a**, normal – by dominant gene – **A**. The patient have genotype – **aa**, healthy person – **AA** or **Aa**.

This pattern of inheritance is characterized by equal affection of males and females. If patients are heterozygotes, the probability of affected child birth is 25%.

P ♀ Aa x ♂ Aa
G A,a A,a
F_1 AA, 2Aa, **aa** (25%)

If one of the parents is healthy (AA), and another one – is affected, all children are healthy but heterozygous carriers of pathologic gene.

Diseases with autosomal – recessive pattern of inheritances include congenital abnormalities and large group of enzymopathies or inborn errors of metabolism.

Examples of congenital abnormalities with autosomal-recessive pattern of inheritance.

Syndrome	Minimal diagnostic signs
Anophthalmia	Congenital absence of one or both eyes
Holoprosencephaly	Failure of the forebrain to divide into hemispheres or lobes, clift of the lip and palatum.
Microcephaly (primary)	Abnormal smallness of the head with hypoplasy of brain and mental retardation. Secondary microcephaly is a symptom of some single gene disorders, chromosomal disorders and non-hereditary congenital abnormalities.

Inborn errors of metabolism.

Inborn errors of metabolism form the most numerous group of autosomal recessive disorders. Inborn errors of metabolism or enzymopathias are the result of mutation of genes, which coded enzymes synthesis.

Most of inborn errors of the metabolism are inherited like autosomal recessive disorders, some of them like X linked recessive ones.

Classification and examples of enzymopathias

1) Disturbance in amino acids metabolism (phenylketonuria, albinism)

2) Disturbance in carbohydrates metabolism (galactosemia, fructosuria, hereditary disaccharides intolerance, glycogenosis, mucopolysaccharidoses)

3) Disturbance in lipids metabolism (familial hypercholesterolemia, Tay-Sachs disease).

4) Disturbance in purines metabolism (Lesch - Nyhan's disease)

5) Disturbance in urea cycle (ornithine transcarbamylase deficiency)

6) Disturbance in mineral metabolism (hepatolenticular degeneration (Wilson's - Konovalov disease)

7) Disturbance in organic acids metabolism (methylmalonic, propionic, isovaleric acidaemia)

8) Disturbance in hormones synthesis (congenital hypothyrosis)

9) Disturbance in transport of chlorides (cystic fibrosis)

10) Disturbance in bilirubin metabolism (Crigler-Najjar disease)

General symptoms of inborn errors of metabolism.

A) Data of anamnesis

1. Cases of infant death in the family

2. Normal course of the pregnancy. At the moment of birth children are healthy.

B) Clinical signs

1. Vomiting, diarrhea, intolerance of definite products and medicines. Vomiting and acidosis after feeding on milk may show the disturbance of amino acid or carbohydrate metabolism (galactosemia).

2. Unusual color and smell of urine. This symptom is typical for amino acids disturbance: "mousy" odor (phenylketonuria) odor of cabbage (methionine malabsorption syndrome), odor of maple syrup (maple syrup urine disease -disturbance of leucine, isoleucine, valine metabolism), "cabbagelike" odor or odor of rotten fish (thyrosinemia), odor of sweaty feet (isovaleric acidemia).

3. Hepatomegaly and splenomegaly. It is typical for storage diseases when metabolites accumulates in cells of the liver and spleen (glycogenosis).

4. Delayed psychomotoric development of infants, mental retardation in older children, delayed growth. It present in many disorders like group of amino aciduria (phenylketonuria), storage disorders (mucopolysaccharidosis), disturbances in carbohydrates metabolism.

5. Neurological disorders (convulsions, hypotonicity or hypertonicity, spastic paresis).

6. Faint and coma. Neurological disorders are typical for amino acidurias and errors of organic acids metabolism.

7. Cataract, disturbance in vision and hearing. Cataract is one of the symptoms of galactosemia and mucopolysaccharidosis.

8. Ineffective standard treatment, progressive course of the disease without a proof of infection or affection of nervous system.

Other clinical symptoms include dermatitis abnormal skin pigmentation, deformation of the skeleton and others.

C) Laboratory data

1. Acidosis

2. Hyperammoniemia

3. Steady change in other biochemical exponents like hypoglykemia, hyperbilirubinemia, ketonuria.

All clinical symptoms as a rule manifests after a normal period of different continuity. Normal course of the pregnancy and delayed onset of the disease are explained by the compensation of enzyme deficiency with maternal enzymes.

Syndrome or diseases	Population rate	Location of gene	Minimal diagnostic signs
Disturbance in amino acid metabolism			
Phenylketonuria	1 : 8000 – 1 : 6000	12q	Disturbance in hydroxylation of PA into tyrosine, accumulation of PA and its metabolites in blood and excretion with urine. Hyperreflexy, hypertonicity, "mousy odor", later microcephaly, depigmentation of skin, hair and cornea, mental deterioration.
Albinism	1 : 39000		Skin and hair are depigmented, retina is pink or light-blue. Typical clinical features are nystagmus, strabism, photophobia, retinities, cataract, and decreased visual acuity.
Disturbance in carbohydrates metabolism			
a) defects of mono- and disaccharide metabolism			
Galactosemia – disturbance of monosaccharide galactose (component of milk sugar - lactose)	1 : 35000 – 1 : 150000	9q	Galactosemia manifests after the newborn starts to feed on milk. Delayed psychomotoric development, hepatomegaly, jaundice, cataract. Galactosuria. In case of early administration of galactose-free diet – normal development.
b) defects of polysaccharide metabolism			
Mucopolysaccharidoses – disturbance in glycosamino-glycans (GAG) catabolism, storage diseases. 14 types (with subtypes) are described.		Hurler's syndrome 4p, 7,22,Xq and others	Affection connective tissue: limitation of motion at the joints, joint contractures, deformation of vertebrae column, short stature. Accumulation of GAG in liver, spleen, heart. Affection of CNS (mental retardation), eyes (cataract), ears (hearing impairment), circulatory system (cardiomegaly, valvulae defects)
Glycogenoses - storage diseases, result of glycogen catabolism failure. There are XI types of the disease depending of the enzyme deficiency. Type 1 – von Gierke's disease.			Glycogen accumulates in excessive amounts in skeletal muscle, cardiac muscle or liver. Growth retardation, hepatomegaly, hypoglycaemia, muscular hypotonicity, cardiomegaly, "doll face".
Disturbance in lipids metabolism			
Familial hypercholesterolemia - hyperbetalipoproteinemia (LDL) due to defective LDL receptors. Autosomal	Aa rate 1 : 200 – 1 : 500		Increased incidence of atherosclerosis and coronary heart diseases in early age (in 30 – 40), heart and brain infarction, xanthomas and atheromas. Heterozygous persons are affected less severe then homozygous.

dominant pattern of inheritance			
GM$_2$ gangliosidosis or Tay-Sachs disease (amavrotic familial idiocy), defect of gangliosids catabolism. Storage disease.	1 : 500 among Jews.	15q	Psychomotor deterioration from the 4-5th month of life, cherry-red spot in the eye. Blindness, deafness, idiocy. Death on the 3-4th year of life. Hexoaminidase A deficiency in blood serum and tissues.
Disturbance in purine metabolism			
Lesch - Nyhan's disease . X-linked pattern of inheritance.		Xq	Uncontrolled movements, hyperreflexia, spasticity, mental retardation and compulsive self-mutilation. Elevated level of uric acid in blood and urine.
Disturbance in copper metabolism			
Hepatolenticular degeneration (Wilson's - Konovalov disease)		13q	Decreased plasma levels of ceruloplasmin, accumulation of copper in the liver, brain, kidneys. Hepatitis, resembling chronic active one, hepatosplenomegaly, affection of central nervous system (hyperkinesis, dysphagia, dysarthria). Kaiser-Fleisher ring in cornea (deposition of copper). Treatment is successful.
Disturbance in chloride transport			
Mucoviscidosis (cystic fibrosis) – disturbance of chloride transport, excessively viscid mucous.	1 : 1600	7q	Recurrent pneumonitis, disturbance in excretion of pancreatic enzymes and absorption in small intestine. Fruitlessness in men. Elevated level of sodium and chloride in sweat.
Disturbance in hormone synthesis			
Congenital hypothyrosis –group of the diseases with different etiology.	1 : 3500 – 1 : 4000		Psychomotor retardation, typical appearance (short neck, saddle-like nose, edema of eyelids, large tongue, dry hairs, general myxoedema) Prolonged jaundice, rough throaty cry, bradycardia, hypothermia. Falling of T$_4$ level. Starting in time, treatment is sucsessful.
Congenital adrenal hyperplasia – disturbance in synthesis of steroid hormones. 5 types are described, in 95% of cases - deficiency of 21-hydroxylase,	1 ; 5000	6p	Salt-losing forms of CAH is becuse of mineral corticods deficiency: vomiting, diarrhea, weakness, dehydration and hypotension, hyposodiumaemia and hyperpotassiumaemia, acidosis. In simple virilizing form virilisation and ambiguous genitalia in newborn female, postnatal virirlization at 5 – 6 year of life in males. Replacement hormone therapy is effective.

Phenylketonuria:

Population rate is 1 : 8000 – 1 : 6000. Rate of the disease in Odessa is 1 : 6793. Sex ratio is M1 : F1.

Disease is the result of abnormal phenylalanine metabolism (mutation of gene, coding the enzyme phenylalanine–4–hydroxilase, which converts phenylalanine into tyrosine). Phenylalanine

accumulates in blood, turn into phenylpyruvic, phenylacetic, pnenyllactic acid. Much of the phenylacetate is conjugated in the liver with glutamine and excreted in the urine as the conjugate, phenylacetylglutamine. The presence in urine of the keto acid phenylpyruvate gives the disease its name – phenylketonuria. Phenylalanine and its metabolites, which accumulate, are toxic for CNS and lead to the failure of axon's myelin sheath formation. Decrease in content of tyrosine, disturbance in melanin synthesis cause depigmentation in eye and hair. High concentration of phenylalanine inhibits metabolism of other amino acids. Synthesis of serotonin and GABA are inhibited.

Children with FKU born as healthy, i.e. processe is compensated due to mother's organism. In first 2 – 3 months, clinical symptoms are not expressed significantly, phenomena of "mousy" odor of urine is present, it caused by phenylpyruvic acid. From 2 – 6 clinical picture manifests: child fades – hair and eye grow lighter (light – colored hair, blue or gray eye). Reaction towards the environment decreases, loss of early gained abilities (mental deterioration), and progressive delay of psychomotor development, in 50% - convulsions, hypertonus are present.

The major consequence of untreated classic PKU is the mental retardation that results in IQ below 70 in late childhood (old name of the disease phenylpyruvic idiocy). Additional clinical signs include seizures, psychoses, eczema.

Diagnosis is based on the typical clinical symptoms and detection of PA level in urine and blood.

Screening test with ferric chloride (Feling's reactive - 10% $FeCl_3$) became positive from the second month of life. In case of positive result green color appears.

Methods of mass biochemical screening of the newborns are elaborated. Before to be discharged from the hospital from the newborn's heel few drops of blood are taken on the filter paper. In special screening laboratory the piece of paper with blood is situated in the test tube with eluating solution and ninhydrin is added. In case of positive result lilac color appears. The intensity of coloration depends on the phenylalanine level in blood. It can be measured with the help of special instrument "Fluoroscan".

Early treatment prevents manifestation of the disease. Treatment for phenylketonuria is keeping the diet with phenylalanin-free protein hydrolysates for a prolonged period (up to 19) with constant control of PA on level 2,5 – 3,5 mg %.

Gene of classic phenylketonuria (phenylalanine–4–hydroxylase) is situated in the long arm of 12th chromosome (12q22 – 24). Gene is sequenced. Prenatal diagnosis of classical PKU in both the homozygote and heterozygote state is now feasible, using cloning and gene mapping techniques.

Cystic fibrosis (mucoviscidosis)

Population rate is 1 : 1600. Frequency of heterozygous carriers is 1 : 20. Sex ratio is 1 M : 1 F. Disorder is caused by the mutation of genes that encodes transport protein for chlorides (transmembrane conductance regulator). The mutation of gene leads to the failure in the transport of chlorine as a result of which excessively viscid mucous of exocrine glands is secreted pancreatic, bronchial glands and glands of glands of intestinal mucous membrane). It caused obstruction of passageways, the drainage tubes of pancreas are blocked by the thick mucous so cysts are formed (from here is derived the name of the disease – cystic fibrosis). Enzymes of the pancreas do not come into intestinal lumen. Overproduction of mucous in the bronchial tree leads to the blockage of small bronchi and joining up of non-specific respiratory infection. Same processes take place in the seminal vesicles and accessory nasal sinuses. Sodium and chloride content of sweat are increased throughout the patient's life that is used for the diagnosis of disease. The main types of the disease because of definite organ affection are: combined bronchial and intestinal type, bronchial type, intestinal type, meconium ileus.

Affection of respiratory system manifests at the 1 – 3 year of life with chronic bronchitis pneumonia. Emphysema develops in all patients.

Intestinal symptoms are the result of the pancreatic ducts blockage and disturbance of the pancreatic enzyme passage into the small intestine. The main clinical features are poor growth

despite good appetite, foul bulky stool. In 10 – 20% of patients is present prolapse of rectum, in 9 – 22% liver cirrhosis, and fat infiltration of liver. Hypotrophy is very common. Meconium ileus is in about 10% of the newborns with cystic fibrosis. It exhibits the clinical picture of intestinal obstruction. Perforation of the distended gut may lead to the meconium peritonitis in the intrauterine period or shortly after birth.

Diagnosis is biochemical: measuring of sodium and chloride level in sweat, concentration of sodium in nails and saliva, proteolytic enzyme activity of duodenum contents and stool, coprologic test. Sweat test is positive if Na^+ and Cl^- level in sweat is more than 60 mmol/litre. (40 - 60 is the border level, test should be repeated). For the measuring of proteolytic (pancreatic) enzymes activity Shvahman's test with X-ray film is used.

In diet therapy administration of protein-reached diet with restricted amount of fats and increased amount of salt is very important. Dosage of replacement digestive enzymes is chose on the base of mass dynamics and amount of fats in stool. Antibiotics, mucolytics for inhalation, massage, and medical physical training are used. Prognosis is very serious.

Gene is localized in 7th chromosomes (7q 31 - 32). Nearly 300 different mutation takes place in this gene, that may lead to mucoviscidosis. The most common mutations (70%) is deletion of phenylalanine in 508 position (P 508).

Congenital (infantile) hypothyrosis.

Population rate is 1 : 3500 – 1 : 4000; in Ukraine – 1 : 4200. Sex ratio is 1M : 1F. The main causes of congenital hypothyrosis are: hypoplasia (agenesis or ectoplasia) of thyroid gland, usually sporadic, sex ratio is 2F : 1M, result of embryonic defect or auto immune process; disorder in the synthesis of thyroid gland hormones (10% - 15% of all cases); deficiency of pituitary gland TS hormone (rare disorder, rate is 1 : 110000); non susceptibility of thyroid gland towards TSH; using of radioactive iodine, iodides etc. during gestation; iodine deficiency (endemic cretinism) – inadequate maternal intake iodine during gestation; idiopathic congenital hypothyrosis.

Newborns are of normal weight and height. Enlargement of fontanels at first days of life is present. Some time first clinical symptom is prolonged jaundice with elevated level of direct bilirubin. During 1 - 2 months of life lost of appetite, constipation, hypotonicity, hypothermy, disorder of peripheral microcirculation develop. After the 6 month – rough throaty cry, large tongue, general myxoedema, dry hairs, umbilical hernia, specific face: edema of eyelids, saddle-like nose, macroglossy. Later it results in stunting of bodily growth and of mental development. If the treatment does not start before 3 month of life, hypothyrosis leads to the decrease in intelligence (cretinism).

Diagnosis is based on the elevation of plasma TSH level and falling of thyroxine level.

Treatment is immediate replacement therapy with the thyroxyne in daily dosage 0,01 mg/kg. Adequately treated are most likely to be intellectually and physically normal.

Early revealing of the disease is provided with newborn screening. It is based on measuring of TSH and T_4 levels. Disorder may be revealed in concentration of T_4 less then 0,006 mg/ml and TSH more then 20 microunits/ml on the 2 – 5 day in examination of blood drop on the filter paper.

What are the storage diseases?

It is the group of disorders in which a deficiency in one of the enzymes leads to the disturbance of the macromolecule catabolism. Non-depredated molecules accumulate in cells and intercellular matter of different organs and tissues.

To this group mucopolysaccharidosis (disturbance in GAG metabolism), glycogenosis (disturbance in glycogen metabolism) and sphingolipidosis (disturbance in lipids metabolism) are included. As the majority of disorders are caused with lysosomal enzymes deficiency, they were termed as lysosomal diseases. Children born with these disorders are normal at birth but with the passage of time commence a downhill course of differing duration.

Storage diseases are characterized by progrediente (progressive) and severe course, early invalidism and death.

Mucopolysaccharidoses are the disturbance in glycosaminoglycans (GAG) (or mucopolysaccharides) metabolism with its accumulation in different tissues.

It is described 14 types (with subtypes) of the disease now, caused by the mutations of different genes in different chromosomes. Most of them are autosmal recessive disorders, Hunter syndrome is X-linked recessive one. Each of thenm is caused with the defect of specific lysosomal hydrolase, participating in consequent splitting of GAG. GAGs accumulate in lysosomes of different cells and intercellular matter of tissues and organs - liver, spleen, kidneys, neurons, cartilage, bones, cornea and others. Derivatives of GAG are excreted with urine.

Progressive course is typical for all mucopolysaccharidoses, but severity of the disease varies greatly. As GAG is one of the main components of the connective tissue, affection of skeleton is typical. Main signs are small stature after a period of normal growth in infancy, very short neck, macrocephaly, deformation of pectus (pectus carinatum), ribs and vertebrae column, swelling and limitation of motion at the joints, joint contractures, claw hands (camptodactily). Hands and feet are short and stubby.

Typical facial dismorphism includes depressed flat nasal root, broad tip of the nose and large nostrils, hypertelorism, exophtalmos, thick pouting lips, large tongue, widely spaced teeth, hypertrophy of the alveolar processes and gums, multiple caries. Hair of the head is abundant, thick and strong. Thick, bushy eyebrows, sometimes synophyrs are present.

Other clinical findings, depending on the type of the disease, are inguinal and umbilical hernias, hepatosplenomegaly, corneal clouding, glaucome, hearing impairment, heart valvulae defects, cardiomegaly, and more or less severe mental retardation. Lethal outcome of the disease is because of the respiratory and cardiac insufficiency on the first (Hurler syndrome) or second decade of life.

All types of the disease except one (Morquio syndrome) caused by the defect of enzyme, splitting dermatan sulfate and heparan sulfate (separately or both). In patients with Morquio syndrome catabolism of keratin sulphate is abnormal.

Diagnosis is confirmed by the physical appearance, elevated (5 –10 times) level of GAG, decreased level of oxyproline in urine. Type of the disease can be cleared after measuring the activity of specific enzymes in cultured fibroblasts, leukocytes, and blood serum. Treatment is symptomatic. Orthopaedic measures and hearing aids are used when required. In some cases life expectancy may be up to 60 years.

Diagnosis of the single gene disorders.

Following methods are used:
1) portrait diagnostics – for congenital abnormalities;
2) biochemical diagnostics – for inborn errors of metaboplism;
3) DNA diagnostics;
4) cytogenetic method - for fragile X syndrome;

For the clinical diagnosis of single gene disorders, that manifests as congenital abnormalities, portrait diagnosis is used. Portrait diagnosis is the comparing of the patient's phenotype with the phenotype of the patients in special albums. Pathologic gene causes the formation of abnormal phenotype. As the mechanism of disorder formation is the same in all patients with the same single gene disorders, the patients are more similar with each other, than with there parents.

Biochemical methods are multi step ones. Objects for biochemical diagnostics may be urine, sweat, blood plasma and serum, blood enzymes, cell cultures.

Biochemical diagnosis is carried out in two stages.

1). Screening stage.

Screening is the identification of persons from a population with a particular disorder with the help of rapid reliable methods. It can be divided into mass neonatal screening and selective one.

Selective screening is the laboratory investigation of a person with the clinical signs of metabolic disorder with broad, generally routine eliminating procedures. In one sample of blood or urine about 30 different biochemical assays are carried out (detection of proteins, amino acids, mono- and disaccharides, GAG, chlorides, copper, bilirubin and others). Selective screening permits to reveal the abnormal link of metabolic chain, and proceeds by stages to sophisticated technology for definitive diagnosis.

Mass screening is the total biochemical investigation of newborns for the early revealing of affected children.

Program of neonatal mass screening includes following stages: 1) getting the samples from all newborns and its transportation to diagnostic laboratory;

2) screening biochemical analysis;

3) confirmatory tests on a second specimen in case of positive screening result;

4) treatment and long- term monitoring of the patients;

5) genetic counseling of the family.

Introduction of mass neonatal screening in practice is a social task. List of disorders, recommended for neonatal screening includes phenylketonuria, congenital hypothyroidism, galactosaemia, cystic fibrosis and congenital adrenal hyperplasia.

Screening for PKU : Before to be discharged from the hospital few drops of blood are taken from the newborn's heel on the filter paper. Samples are dried and send to the special laboratory. Two methods are used as the screening tests.

1) Guthrie test - comparing the amount of growth, induced by the sample with standards in a strain of the bacteria *Bacillus subtilis*, which requires phenylalanine for growth. Intensity of growth depends on PU amount in blood.

2) Fluorometric method: filter paper with blood sample is situated in the test tube with eluating solution and ninhydrin is added. In case of positive result lilac color appears. The intensity of coloration depends on the phenylalanine level in blood. It can be measured with the help of special instrument "Fluoroscan".

A useful but less reliable later screening test depends on detecting elevated urinary levels of phenylpyruvate with ferric chloride (Feling's reactive - 10% $FeCl_3$). To 3-ml urine is added 1 drop of 1M solution of HCl, then solution is shacked and few drops of Fe Cl_3 are added. In case of positive result green color appears.

Screening for congenital hypothyroidism, and congenital adrenal hyperplasia is based on the measuring of hormones in blood.

Screening for galactosaemia is microbiologic test revealing galactose contains in blood.

Screening for cystic fibrosis is based on the measuring of tripsin activity in blood and albumin contains in meconium.

The main criteria for screening programs are:

1) Rate in population not less then 1 : 10000 (for galactosemia 1 : 40000)

2) Disorder leads to the early invalidism without treatment;

3) There are the measures of preventive treatment;

4) Test should be sufficiently sensitive without the false negative results;

Prenatal diagnosis of single gene disorders.

For prenatal diagnostics following methods can be used:

1) Ultrasonography if the disorders manifest as the congenital abnormalities. Optimal for the first examination is 18 –18 week of gestation, for the second one – 24 – 26 week. Because of medical indications examination can be carried out in other terms (on 6 – 8 week abnormal place of implantation and delayed intrauterine development can be revieled; on 13 – 14 week – reduction of limbs, at 23 week – heart abnormalities).

System or organ	Abnormalities
CNS	Anencephaly, spina bifida, holoprosencephly, cerebral hernia
Limbs	Reduction of limbs, achondroplasia (measuring of femur length on 24 – 26 week of gestatrion), osteogenesis imperfect.

Heart	Sever heart abnormalities
Kidneys	Agenesis of kidneys, cyst of kidneys
Digestive system	Atresia of duodenum

2) invasive methods (amniocentesis, chorionic villus biopsy, placentocentesis, kordocentesis) with further biochemical assay or DNA studies. Unifactorial disorders, that can be diagnoses with direct DNA analysis in Ukraine are: cystic fibrosis, Duchenne and Becker muscular dystrophy, haemophilia A and B, phenylketonuria, fragile-X mental retardation syndrome, Lesch-Nyhan syndrome, Huntington's chorea, Wilson's disease, some types of mucopolysaccharidosis, familial hypercholesterolemia.

For X-linked disorders diagnostics determination of sex with cytogenetic methods can be used, if there is no methods of DNA diagnostics.

Practical lesson 10.

Topic: General characteristics and classification of multifactorial diseases.

The purpose of the lesson: To study a general characteristic of the disorders with hereditary predisposition; to formulate the concepts of ecogenetics, pharmacogenetics and oncogenetics.
 Control questions
 1. What are the multifactorial disorders?
 2. Classification of multifactorial disorders.
 3. Genetic characteristics of multifactorial pathology and principles of genetic risk calculation.
 4. What is ecogenetics? Examples of ecogenetic reactions.
 5. Pharmacogenetics: definition, examples of genetic pharmacological reactions.
 6. History of oncogenes identification.
 7. Subject of oncogenetics. Basis of pathogenetic mechanisms. Oncological disorders from the genetic point of view.

Phenotype of the human being including his normal and pathological attributes is formed on the basis of a genotype and under the influence of the environmental factors.

Environment ⟶ Phenotype ⟵ Genotype

All human disorders depending on the interaction of a genotype and the factors of an environment is possible to divide into three groups.

The first group - are hereditary disorders, on 100 % caused by the influence of a genotype. The second group - disorders with hereditary predisposition. The genotype creates hereditary predisposition, but for the development of the disease the change of a genotype does not suffice. The influence of the provoking factors of an environment is necessary also. The third group - nonhereditary disorders, in an origin of which genotype does not play any role (trauma, bums, and infections). Genotype in this group determines the recovering processes, reparation velocity, complications etc.

It is true to say that uncommon for heredity or environment to be entirely responsible for

any particular trait or disease. In most cases both factors are responsible though some times one may appear more important than other.

At one extreme we have diseases such as Duchenne muscular dystrophy, which are exclusively genetic in origin and where the environment seems to play no direct part in etiology. At the other extreme we have infectious diseases which are almost entirely the result of environmental factors. Between these extremes are such conditions as diabetes mellitus, hypertension, ischaemic heart disease and some others.

Traits that results from combination of factors, genetic as well as non-genetic, are defined as multifactorial traits.

Multifactorial inheritance is responsible for most normal phenotypic differences among individuals as well as for many congenital anomalies and certain common diseases of adulthood, which also some times are termed as diseases of "modern society". Normal traits, which are inherited in this way, include intelligence, stature, skin color, total dermal ridge count, certain components of ocular refraction and blood pressure.

Abnormal traits are termed as multifactorial disorders and can be divided into following groups.

1) Congenital abnormalities. Examples are isolated cleft lip and palate, anencephalia with spina bifida, hydroencephalia, meningomyelocele, congenital heart disease, pyloric stenosis, congenital dislocation of hips, congenital club foot, hypospady.

2) Common psychiatric disorders includes schizophrenia, manic depressive psychosis, epilepsy.

3) Common disorders of adulthood. To this group belong such disorders as hypertension, ischeamic heart disease, peptic ulcer, diabetes mellitus, rheumatoid arthritis, ankylosing spodylitis, psoriasis, and bronchial asthma.

Though the mode of inheritance is not Mendelian, multifactorial mode of inheritance is an extension of Mendelian concepts concerning unifactorial inheritance.

Genetic component of multifactiorial traits is usual polygenic or quantitative. As a rule it possess following features

- characters can be measured;

- characters exhibit continuos variability. This means that they can assume an unlimited number of intermediate values between extremes;

- a child phenotypic value tends to resemble the average of the parent's values. This average is called the midparent value.

There are two main mechanisms of development of abnormal multifactorial traits.

1. Inheritance of some multifactorial disorders can be thought of in terms of extreme manifestations of a multifactoral quantitative trait. Such multifactorial diseases are defined clinically in terms of quantitative traits. The difference between "normal" and "disease" in such cases is arbitrary For example, hypertension is diagnosed on the basis of a patient's blood pressure being higher than a certain number. Blood pressure is a continuous variable, and the pathologic effect of having "high" blood pressures of 140/110, 120/85, or 125/81 compared with "normal" blood pressures of 120/80 or 110/75 is only one of degree.

2. Other multifactorial diseases differ qualitatively from the normal state. Cleft palate is one example. In such cases, a multifactoiial predisposition to the disease is inherited, and the disease either occurs or does not occur, depending on the aggregate strength of the predisposing factors. The multifactorial-threshold model is a formal explanation of how a multifactorial predisposition can produce a trait that is qualitatively distinct from normal. According to this model, all of an individual's genetic and environmental disease-predisposing factors when considered together constitute liability. Liability follows a normal distribution in the population. If an individual's liability exceeds a certain threshold value, he or she will have the disease. If the liability does not exceed that threshold, the disease will not occur.

Other models can also be used to explain genetic predispositions to disease and often fit available data as well as or better than the multifactorial-threshold model.

Mixed models postulate a major gene of large effect (i.e., a mendelian factor with relatively high penetrance) operating on a multifactorial background.

Oligogenic (i.e., few gene) models postulate the presence of a small number of genes operating in combination.

Genetic heterogeneity may occur, in which some cases have a mendelian cause and others have a nongenetic or multifactorial cause.

In attempting to understand the genetics of a particular condition, different genetic methods are used. One can study the incidence of the disease among relatives, compare the incidence in identical and non-identical twins, determine the effect of environmental changes (e.g. adoption), compare incidences in various racial groups, study the association of the disease with various other characteristics such as the blood groups, study pathological components of the disease in relatives, e.g. serum lipids among the relatives of patients with ischaemic heart disease. The study of animal analogues (diseases in animals which are analogous to diseases which occur in humans) may also be helpful.

Linkage studies and the isolation of contributory genes by molecular methods also should help to clarify the underlying basis of most multifactorial diseases.

Clinico-genetic haracteristics of multifactorial diseases include the following:

1) meet more often than single gene disorders. For single gene disorders population rate considered to be high if it exceeds 1: 10000 1 incidence in 10 000). Frequency of majority of single gene disorders is much higher.

2) the diseases tends to be familial but does not follow any monogenic pattern of inheritance. For familial accumulation of multifactorial disorders is typical similar clinical picture of the disease in close relatives and effect of anticipation (earlier and more severe disease with vertical transmission).

3) multifactorial disorders tend to occur more frequently in one sex than in the other. For example defects of nervous tube, systemic lupus erythematosus are more common in females; cleft lip and palate, pyloric stenosis, peptic ulcer are more common in males.

4) the clinical picture at the different patients gives variation row from mild to severe forms (diabetes mellitus, ischeamic heart disease, hypertension and others);

5) association with certain genetic markers like antigens of erythrocytes and antigens of HLA system. For example I (0) blood group is associated with increased risk of peptic ulcer, II(A) blood group is associated with malignant tumors of stomach, large intestine, ovaries, cervix. B_{27} of HLA system is associated with ankylosing spodylitis, DR_7 with psoriasis and so on.

Calculation of genetic recurrence risk is quite serious problem. For multifactorial disaeses it is necessary combination of specific genetic and environmental factors. The risk of the disease development will be the more expressed hereditary predisposition and the more intensive factors of an environment act.

Let's for example trace the formation of congenital dislocation of the hip. The condition is predisposed to by a shallow acetabulum, the shape of which appears to be under polygenic control. The condition is also predisposed to by joint laxity, which in the families of affected individuals appears to be inherited as an autosomal dominant trait. Further, experiments in animals have shown that joint laxity is influenced by oestrogen levels and this might therefore account for why congenital dislocation of the hip is commoner in females.

So, recurrence risk is calculated with the help of special empiric tables. It is influenced with following factors:

1) the recurrence risk is lower in populations exhibiting a lower incidence of a condition. The recurrence, risk in first-degree relatives of an affected individual is approximately equal to the square root of the frequency of the condition in the population.

For multifactorial congenital anomalies that have population incidences of about 1/1000, the recurrence risk in siblings or children of an affected individual is usually 2%-4%.

For multifactorial diseases of adulthood that have population prevalences in the range of

1 %, the recurrence risk in siblings and children is usually in the range of 5%-10%.

2) relationship to the affected person; The recurrence risk is the same for all relatives who share the same proportion of genes. For example, each sibling, child, and parent shares half of his or her genes with a proband, on the average, so the recurrence risk in all such first-degree relatives is expected to be about the same.

The recurrence risk in a family drops off quickly as the relationship to an affected individual becomes more remote. For example, the recurrence risk for cleft lip with or without cleft palate is about 4% for the sibling but only about 0.5% for the first cousin of an affected child. It is explained with proportion of common with affected individual genes.

Degree of relationship	Part of common genes
Identical twins	100%
I degree (parents-children; sibs)	50% (1/2)
II degree (uncle, aunt – nephew, niece; grandparents - grandchildren)	25% (1/4)
III degree (cousins)	12,5% (1/4)
IV degree (second cousins)	3,125% (1/32)

So recurrence risk is estimated as high in relatives of I and II degrees of relationship, moderate in relatives of III degree and low in IV degree of relationship.

3) Number of affected relatives. The recurrence risk is higher if more than one close relative is affected. For example, the risk for cleft lip with or without cleft palate in a child who has one affected sibling is approximately 4%. If the child has two affected sibs, the risk is approximately 10%. Risk for the child to have diabetes mellitus is about 1,8% if only one parent is affected and 12% if both parents are affected. This reflects the fact that a couple who have already had more than one affected child are more likely to have more predisposing factors (i.e., more liability) than a couple with just one affected child. This is quite different from monogenic inheritance, in which the risk of recurrence depends on the parental genotypes and is independent of the number of previously affected children.

Multifactorial diseases are more common in consanguineous marriages. This is because parents who are related are more likely to share similar disease-predisposing genes.

4) The recurrence risk is higher in the relatives of an affected individual of the *less* frequently involved sex. Individuals of the less frequently involved **sex** must have more predisposing factors to manifest the disease so their relatives are at greater risk of being similarly affected. For example, pyloric stenosis occurs five times as often in males as in females. The recurrence risk in the brother of an affected child is 3.8% if the affected child is male, but 9.2% if the affected child is female.

5) The recurrence risk is higher in relatives of more severely affected probands. Such individuals must have more predisposing factors to manifest the disease severely; therefore, their relatives are at greater risk.

For example, the risk of recurrence of cleft lip with or without cleft palate is 2.5% in the sibling of a child with unilateral cleft lip but almost f>% in the sibling of a child with bilateral cleft lip and palate. That this is very different from the situation with monogenic and chromosome disorders, in which the severity of disease in the proband does not influence the recurrence risk.

The empiric risk in some multifactorial disorders is following

Disorder	Risk for sibs %	Risk for posterity %
Anencephalia	2 - 5	-
Cleft lip and palate	4	4
Cleft palate	2	6 – 7
Club foot	2	
Epilepsy	3 – 12	

Schisophrenia		
if one parent is sick		10
if both parents are sick		40

The recurrence risks used for multifactorial conditions do not apply to situations in which the condition is not multifactorial. The clinical features of a multifactorial disorder rarely distinguish it from similar lesions that have another cause. For example, cleft lip and cleft palate, alone or in combination, usually exhibit multifactorial inheritance. There are, however, about 200 other conditions that have cleft lip or cleft palate as a feature. Some of these conditions are due to chromosome abnormalities, others to monogenic disorders, and still others to teratogenic environmental effects. Multifactorial congenital anomalies typically occur in patients who do not have embryologically unrelated birth defects. For example, a common congenital anomaly, such as spina bifida, in a child who is otherwise completely normal is likely to be multifactorial. Spina bifida in a child who also has congenital heart disease, kidney malformations, and cleft lip is not likely to be multifactorial. The recurrence risk for the sibling of a child with congenital heart disease, omphalocele, and clubfeet cannot be calculated from the recurrence risks estimated for these conditions when they occur as isolated anomalies.

Because multifactorial diseases are caused by combinations of genetic and environmental predisposition, environmental triggers have the greatest chance of causing disease in individuals with existing genetic predispositions. This means that identification of environmental factors that predispose to a given disease should be easiest among genetically predisposed individuals. Identification of individuals with genetic predispositions to multifactorial disease may lead to disease prevention through manipulation of nongenetic factors. For instance under the high risk of diabetes mellitus level of glucose, weight should be constantly controlled, diet with restricted amount of carbohydrates is recommended. This administrations permit to decrease the risk of the disorder and its severity.

Ecogenetics

Ecogenetics is the branch of genetics deals with influence of surrounding factors on the inheritance.

Ecogenetics study two types of environmental effects:

1) changes in manifestation of certain alleles under the action of specific environmental factors.

2) changes in hereditary material structure (induced mutagenesis). It plays very important role in etiology of hereditary disorders.

First group of effects is termed as ecogenetic reactions or ecogenetic disorders. Humans evolution during the long period of time takes place in environment without any artificial pollution. The problem of artificial pollution appears recently because of intensive industrialization, especially because of chemical and atomic industry development. Many human genes were not under the natural selection during the evolutionary process. In conditions of total artificial pollution some genes, which were "silent" earlier, determine different pathologic conditions under the action of different artificial factors. On population level its action manifests as the lower adaptation ability of the population.

Genetically determined pathologic reactions of humans on the action of ecological factors are termed as ecogenetic reactions or ecogenetic disorders.

Ecogenetic disorders may be determined by rare mutant recessive genes, which are inherited like monogenic traits. For instance, persons with alfa-1-antitrypsin deficiency in dusty conditions or while smoking are of increased risk of pulmonary emphysema forming. In European populations recessive homozygotes with mutant gene are met in 0,05% (ZZ), heterozygotes (MZ) – in 4,5 – 10%. Homozygous persons develop chronic broncho-pulmonary inflammatory processes and emphysema. In heterozygous persons affection of respiratory system takes place only in unfavorable conditions (working in dusty places or smoking). Methods of revealing alfa-1-

antitrypsin deficiensy are elaborated, so it may be used in selection of person for certain works.

Specific reactions on alcohol, in which genetic factors are involved, are described. Some of this evidence is based on twin studies, which have shown high concordance rates, and family studies, which have shown a high prevalence rate among relatives of alcoholics, though clearly behavior patterns within families would artificially inflate what might appear to be genetic factors. Similarly, apparent racial differences in the incidence of alcoholism, such as the high incidence among certain American Indians and Eskimos, could well be inflated by subtle social factors. Perhaps the most convincing evidence of the role of genetic factors in alcoholism comes from the study of isozyme variants of alcohol metabolising enzymes in alcoholics and their relatives. Alcohol is metabolised in the liver by alcohol dehydrogenase (ADH) to acetaldehyde, and then further degraded by aldehyde dehydrogenase (ALDH). Human liver ADH has three loci but only one of these (ADH;) is active in adult life, an 'atypical' allele of which (ADH^) has higher enzymatic activity than the normal allele (ADH^). The atypical allele therefore leads to an increased formation of acetaldehyde and therefore to more unpleasant symptoms after drinking alcohol. The frequency of ADH^ is greater in Orientals, who also tolerate alcohol less well and among whom the incidence of alcoholism is lower than in Europeans. However at the present moment the story is far from complete because isozyme variants of ALDH exist as well and may also be involved in determining the reaction, and possible addiction, to alcohol.

Pharmacogenetics is the branch of genetic, which deals wit genetically determined variations that became evident with a altered response to drugs. The term pharmacogenetics was introduced by Vogel in 1959.

Phrmacogenetics refers to the influence of genes on the response to drug therapy. These responses may take the form of an exaggerated physiologic response to a drug, resistance to drug effects, or an increased frequency of side effects. Pharmacologic agents can also trigger the effects of certain genetic diseases.

The sequence of events which is involved when a drug is metabolized is usually as follows: intake – absorption - distribution - drug-cell interaction – breakdown - excretion. Transformation of drugs involves some important processes:

1) conjugation wit the carbohydrate glucuronic acid. It occurs chiefly in the liver. In the case of morphine and its derivatives, such as codeine, their elimination is almost entirely depend on this process.

2) Acetylation. Occurring once again in the liver it involves an addition of acetyl group to the original molecule. Isoniazid follows this manner of inactivation. Other drugs which are often acetylated before they are excreted include the sulphonamides.

Drug metabolism is determined in large part by genetic factors. There is considerable variation in the way different individuals respond to certain drugs and the variability in response may be continuous or discontinuous. If test is carried out on a large number of subjects and their responses are plotted, in continuous variation the results form a bell-shaped or unimodal distribution, but in discontinuous variation the curve is bimodal or sometimes trimodal. A unimodal distribution implies that the metabolism of the drug in question is under the control of many genes and the analysis of genetic factors in such cases is extremely complicated. In the cases of discontinuous response the metabolism of drug is under monogenic control. If the normal metabolism of the drug is controlled by a dominant gene **A** and if some people are unable to metabolize the drug because they are homozygous for a recessive gene **a**, there will be three classes of individuals: **AA, Aa, aa**. If AA and Aa are distinguishable then a trimodal distribution will result, each peak or mode representing a different phenotype.

Among the best known examples of drugs which have been responsible **for** revealing genetic variability are hydrogen peroxide, isoniazid, succinylcholine, primaquine, certain anricoagulant drugs, anaesthetic agents, the thiopurincs, phenylbutazone, debrisoquine, organophosphates and alcohol.

Acatalasia. In 1946, Takahara, a Japanese otorhinolaryngologist, treated an 11-year-old girl for a gangrenous lesion of her mouth. The infected tissue was excised and hydrogen peroxide was poured on the wound for sterilization. Normally with this treatment the blood oozing from the wound remains bright red and there is frothing. But Takahara observed that the blood which came in contact with the peroxide turned brownish-black and no bubbles formed. Takahara suggested that the patient's red cells might be deficient in the enzyme catalase which breaks down hydrogen peroxide into water and oxygen. He argued that if this enzyme were absent then hydrogen peroxide would not be broken down, there would therefore be no frothing and the hemoglobin would oxidized into methemoglobin, which is brownish-black in color.

Subsequent studies showed that this condition is in fact due to lack of catalase and it has therefore been called acatalasia. Investigations of this girl's family and other families have shown that acatalasia is a rare recessive trait. Measurements of blood catalase activity have distinguished three classes of persons: those homozygous (AA) for the normal gene with normal levels of enzyme; those homozygous (aa) for the acatalasia gene with no enzyme in their blood, and heterozygous individuals (Aa) with intermediate levels of enzyme. It prooves thst this is Mendelian character with incomplete dominance. Acatalasia is not limited to Japan but has since been described in other parts of the world. Only about half of those with acatalasia have oral sepsis, many have no symptoms at all and are perfectly healthy.

Isoniazid is one of the most important drugs used in the treatment of tuberculosis. It has been shown that isoniazid is rapidly absorbed from the gut, resulting in an initial high blood level which is slowly reduced as the drug is inactivated and excreted. With regard to the metabolism of isoniazid two classes of persons can be clearly distinguished: rapid and slow inactivators. In the former, blood levels of the drug fall rapidly after an oral dose; in the latter, blood levels remain high for some time. Family studies have shown that slow inactivators of isoniazid are homozygous for an autosomal recessive gene for the liver enzyme N-acetyl-transferase.

The implication in these studies is that the rapid inactivator produces an enzyme which inactivates isoniazid but that this enzyme is absent in slow inactivators. In the United States and Europe about 50% of the population are slow inactivators.

In slow inactivators, isoniazid may cause toxic symptoms such as polyneuritis or a systemic lupus erythematosus-like disorder. It might be predicted that blood levels of isoniazid would remain higher for longer periods in slow inactivators. Slow inactivators have a significant risk of developing toxic symptoms on doses which rapid inactivators would require to ensure adequate blood levels for successful treatment. Conversely, it appears that rapid inactivators have an increased risk of liver damage due to isoniazid. One would also expect that patients with tuberculosis who are treated with isoniazid might do better if they are slow rather than rapid inactivators. Isoniazid inactivation status does not seem to influence the response to treatment.

Phenelzine, a drug used in the treatment of depressive illness, has a molecular configuration similar to isoniazid. Not all patients respond to phenelzine and interestingly it may be possible to predict which patients will respond by their ability to inactivate isoniazid. Studies suggest that *slow* inactivators of isoniazid respond better to phenelzine than fast inactivators. Similarly with hydralazine, an antihypertensive, and sulphasalazine, a sulphonamide derivative used to treat Crohn's disease, toxic side effects are more likely in slow inactivators of isoniazid.

Succinylcholine sensitivity is inherited like autosomal recessive trait. Succinylcholine *(syn:* suxamethonium) is curare - like drug which also produces muscular relaxation, though by a different mechanism from curare. This drug has an advantage over curare in that its action is only short-lived. The muscles of respiration are paralyzed as well as the rest of the skeletal musculature, and consequently breathing stops for a short period, usually 2 or 3 minutes, after an injection of succinylcholine has been given. During this period of respiratory arrest (apnoea) the anaesthetist maintains respirations by artificial means. However, in about 1 patient in every 2000 the period of apnoea is very much prolonged and may last an hour or more. In such cases the apnoea can be corrected by a transfusion of blood taken from a normal person, otherwise the anaesthetist has to maintain the respirations until the effects of the drug have worn off.

Succinylcholine is normally destroyed in the body by the enzyme pseudocholinesterase which is present in the blood plasma. In patients who are highly sensitive to succinylcholine the plasma pseudocholinesterase in their blood is abnormal and does not destroy the drug at a normal rate. In some very rare cases there is no enzyme.

Now it is possible to study plasma pseudocholinesterase in the blood by using the local anesthetic dibucaine *(.syn:* cinchocaine). The result is termed the *dibucaine number.* The frequency distribution of dibucaine number values in families with succinylcholine sensitive individuals, gives a trimodal curve. The three modes represent the normal homozygotes, the heterozygotes and the affected homozygotes. Experimental evidences clearly indicates that there are at least two different forms of plasma pseudocholinesterase: the one in the normal homozygote and the one in the affected homozygote. The two enzymes differ not only in the way in which they are inhibited by dibucaine (the enzyme in the affected homozygote being less ihibited) but also in their enzyme kinetics, the normal enzyme being more efficient than the abnormal enzyme in destroying acetylcholine and other choline esters.

Glucose-6-phosphate dehydrogenase deficiency is inherited as an X-linked recessive disorder. Patients who are deficient in this enzyme may develop rapid hemolysis as an adverse reaction to treatment with certain commonly used drugs, including antimalarial agents and sulfonamides.

This disorder was described after the introducing of primaquine (antimlarial drug) as a drug of choice. The drug could be taken for a few days with no apparent ill effects, and then suddenly the patient would begin to pass very dark, often black, urine. Jaundice developed and the red cell count and haemoglobin concentration gradually fell as the red blood cells were destroyed. The patient usually recovered from such a hemolytic episode but occasionally the destruction of the red cells was so massive as to be fatal. The cause of primaquine sensitivity was subsequently shown to be due to a deficiency in the red cells of the enzyme glucose-6-phosphate dehydrogenase (G6PD). However, the precise mechanism whereby G6PD deficiency brings about haemolysis in the presence of primaquine is still not clearly understood. Persons with G6PD deficiency are sensitive not only to primaquine but also to many other compounds as well, such as phenacetin, furadantin, certain sulphonamides and perhaps acetylsalicyclic acid (aspirin). The accidental ingestion of moth balls (naphthalene) may also lead to a hemolytic crisis in sensitive individuals and a deficiency of G6PD has been demonstrated in persons who developed hemolytic crises after eating fava beans (favism). In fact the main medical risks of G6PD deficiency are favism and, for reasons, which are not entirely, clear, neonatal jaundice. Drug-induced hemolysis is uncommon. Red-cell G6PD deficiency is much more common in Negroes than Caucasians but in affected Negroes the enzyme activity in their *white cells* is normal whereas it is greatly reduced in most affected males of Mediterranean extraction.

The **coumarin** anticoagulant drugs are used in the treatment of myocardial infarction to prevent the blood from clotting. Observations have suggested that there is discontinuous variation in the response of patients taking these drugs. In this case there is not an increased sensitivity but an increased *resistance* to the effects of the drug. For example, a patient has been described who required 20 times the usual dose in order to maintain adequate anticoagulation. This resistance appears to be transmitted as an autosomal dominant trait.

Recently, the phenomenon of **"malignant hyperpyrexia"**, a rare complication of anesthesia, has been recognized to be genetic. Susceptible individuals develop hyperthermia, with temperatures as high as 42.3°C (108"F), and often with muscle rigidity, during anesthesia usually when halothane is used as the anaesthetic agent and succinylcholine for intubation. If not recognized early and treated with vigorous cooling, the patient often dies. It appears to be inherited as a rare (roughly 1 in 10000) autosomal dominant trait. Individuals who are genetically predisposed to developing malignant hyperpyrexia often have a raised serum level of creatine kinase and in families of affected individuals those at risk may be screened for in this way. However, a more accurate prediction of an individual's susceptibility to hyperpyrexia is possible by demonstrating an increased sensitivity to halothane, succinylcholine and caffeine of muscle biopsy specimens in

vitro. The basic defect in this disorder appears to be a reduced uptake and binding of calcium ions to the sarcoplasmic reticulum. A patient known or suspected of having malignant hyperpyrexia can undergo surgery provided that precipitating anaesthetic agents are avoided. Should hyperpyrexia develop during surgery it can be treated by intravenous procaine or procainamide but most effectively by dantrolene. A non-invasive test for family members at risk may be available in the near future by use of polymorphic DNA markers on chromosome 19, which have been linked to the malignant hyperthermia locus in some families.

A group of substances known as **thiopurines**, which include 6-mercaptopurine, 6-thioguanine and azathioprine, are extensively used in the treatment of patients with various neoplasms as well as to prevent rejection in recipients of organ transplants. They are very effective drugs but unfortunately leucopenia and severe liver damage are serious side effects. It has not been possible to predict either those who arc likely to develop such side effects or the possible therapeutic response in the individual patient because the response to these drugs varies widely. An important step in the metabolism of these compounds is a process of methylation catalyzed by the enzyme thiopurine methyltransferase (TPMT). It appears that erythrocyte TPMT activity is a polymorphism, with roughly 0.3% of the population having undetectable activity, and it may be these individuals who not only respond less well to these drugs but who also may be in danger of developing serious side effects. Those with high TPMT activity may be treated more aggressively with these drugs, resulting in a better therapeutic response. This has been shown to be the case in childhood leukemia (Lennard et al 1990).

Phenylbutazone is used in the treatment of the more severe forms of arthritis. The metabolism of this drug differs from the examples discussed so far in that it appears to be under polygenic control, though it is very likely that it is not unique in this regard. Individuals who are relatively slow in metabolizing phenylbutazone are most likely to develop drug-associated toxic side effects such as hypoplastic anaemia.

Debrisoquine is a drug frequently used in the treatment of hypertension. However, there is essentially a bimodal distribution in response to the drug in the general population. Poor metabolizers (homozygotes for a recessive gene) are more prone to overreact to the drug and develop hypotension during therapy. They are also more susceptible to adverse reactions to several other drugs and at least some individuals with defective hydroxylation of debrisoquine may be predisposed to Parkinsonism (Barbeau et al 1985) and possibly some forms of cancer. Routine phenotyping has therefore been recommended which might be facilitated by recombinant DNA methods (Heim & Meyer 1990). Recent molecular studies have now revealed that at least four different mutations give rise to the poor metabolizer phenotype. All these mutations occur not within the coding exons of the responsible gene but in the introns, producing a series of incorrectly spliced messenger-RNAs.

Paraoxonase is an enzyme which catalyses the breakdown of organophosphates. Some individuals have high serum enzyme activity and others low activity which results from a two-allele polymorphic system. It could be that those who are homozygous for the low-activity allele may be predisposed to particular sensitivity to organophosphates, which are widely employed in agriculture and industry (LaDu 1988).

HEREDITARY DISORDERS WITH ALTERED DRUG RESPONSE

It was pointed out at the beginning that not all investigators include hereditary disorders with altered drug response within the sphere of pharmaco-genetics. However, since it is a subject of immense importance in medical practice and touches on both genetics and pharmacology it is perhaps best discussed at this point.

Porphyria variegata, one of the several types of porphyria, is inherited as an autosomal dominant trait. Some affected individuals have skin lesions -particularly on exposed surfaces - others have attacks of severe abdominal pain, muscular paralysis and even mental disturbances. During an acute attack the patient may die. It has become recognised during the last few years that

in persons with porphyria an acute attack may be precipitated by barbiturates. In pans of South Africa, where as many as 1% of the population have porphyria, the possibility that any particular patient may have the disease and therefore be liable to an acute exacerbation if given barbiturates is a very real problem. Fortunately, in Europe and the United States porphyria is much less common than in South Africa. Similar precautions have to be borne in mind in several other hereditary disorders in which severe exacerbations may be provoked by a particular drug-In G6PD deficiency, as we have seen already, the administration of sulphon-amides to an affected person may result in severe haemolysis. Since G6PD deficiency is rare in most Caucasians but affects about 10% of Negro males, sulphonamides, primaquine and other sensitising drugs have to be administered with caution to Negroes. Of course in a person *known* to be G6PD deficient such drugs would be absolutely contraindicated.

In the treatment of congestive heart failure a number of drugs are commonly used which have a diuretic effect. These drugs increase the excretion of water and consequently reduce the amount of edema fluid. The most important of these diuretics is chlorothiaride which has been widely used because of its effectiveness and because it can be taken by mouth. However, not long after its discovery in 1957, it was realized that in persons with gout, the drug could cause serious side effects. Gout is a hereditary disorder associated with a raised serum level of uric acid. In genetically predisposed individuals attacks often follow dietary excesses, which lead to an elevation in the serum uric acid. Chlorothiazide has a similar effect and this is particularly unfortunate because persons with gout often have hypertension and may develop congestive heart failure later in life. The genetics of gout is still not clearly understood. Some believe that the disease is inherited as a sex-influenced autosomal dominant trait, but recent work suggests it may be multifactorial. In any event, it now seems clear that the disorder is heterogeneous. Since chlorothiazide raises the serum level of uric acid in genetically predisposed individuals the drug has been used in order to detect preclinical cases of the disease: persons genetically predisposed to the disease but who do not have symptoms. Unfortunately the results so far have not been very encouraging.

In the Crigler-Najjar syndrome the abnormal response to a drug has been used to detect carriers of this disease. This syndrome is characterised by severe non-haemolytic jaundice often associated with cerebral disturbances. The jaundice appears on the first or second day after birth and persists throughout life. Affected children often die in infancy. The condition is inherited as an autosomal recessive trait, the heterozygote being perfectly healthy. The basic defect appears to be the inability of the liver to conjugate bilirubin with glucuronides due to a deficiency of the enzyme glucuronyl transferase. When drugs, which normally undergo glucuronide conjugation (such as salicylates) are given to affected patients it is possible to show that they are unable to conjugate these substances. Defective salicylate conjugation has also been demonstrated in some carriers of this disease and the test has been used to identify such carriers.

In non-insulin dependent diabetes inherited as an autosomal dominant trait, it has been suggested that those at risk of developing the disease may develop facial flushing after alcohol if they are first given the oral hypoglycaemic drug chlorpropamide. Although it was hoped that this might prove a useful test for familial susceptibility to diabetes, this has not been the case.

ONCOGENETICS. GENERAL NATURE OF NEOPLAS1A

A neoplasm is abnormal tissue that grows beyond normal cellular control mechanisms. Neoplasms may involve almost any tissue in the body and may be either benign or malignant, depending on whether they possess the potential to spread to other parts of the body (i.e., to metastasize).

It appears to be a multistep process that varies for patients with histologically similar tumors, as well as for patients with different kinds of tumors.

Most neoplasms are of multifactorial etiology in the sense that both inherited and non-inherited factors are involved in their pathogenesis. Many of the noninherited factors are genetic, however; somatic mutation is a key component of the neoplastic process. In some instances, strong environmental predispositions to neoplasma exist; often, these act through somatic genetic mechanisms.

A small percentage (5% or less) of patients with malignant neoplasms have a strong predisposition that is inherited as a simple mendelian trait.

Patients with monogenic predispositions to neoplasia are characterized by the following features: The neoplasms have an earlier age of onset; the neoplasms tend to be bilateral and/or multifocal; only one or a few specific types of neoplasms occur in each mendelian condition; in some conditions, there are associated phenotypic abnormalities that are characteristic of the specific mendelian trait.

In individuals with mendelian conditions, the risk of developing a malignancy may be very high (75%-100%), but most cells of the affected types do not develop into tumors. Thus, the predisposition to develop neoplasia, not the neoplasia itself, is inherited. Even in patients with strong genetic predispositions, other events are necessary to cause the cells to become neoplastic.

Mendelian conditions that produce strong predispositions to neoplasia are of several types:

a. Syndromes of multiple benign or malignant neoplasms. These conditions are inherited as autosomal dominant traits. Examples include: neurofibfomatosis, multiple endocrine neoplasia, familial adenomatous polyposis of the colon, Li-Fraumeni syndrome

b. Abnormalities of DNA or chromosome repair. Affected individuals exhibit abnormal DNA repair or increased frequencies of chromosome breakage. Most of these syndromes are associated with other phenotypic abnormalities and are inherited as autosomal recessive traits.. Examples include: Xeroderma pigmentosum, Fanconi pancytopenia syndrome, ataxia-telangiectasia

Some constitutional chromosome abnormalities are associated with an increased frequency of certain kinds of malignancy. Leukemia occurs in about 1% of patients with Down syndrome. Retinoblastoma develops regularly in children with constitutional deletions of band q14.1 of chromosome 13. Wilms tumor of the kidney often occurs in children with constitutional deletions of band p 13 of chromosome 11.

2. Acquired chromosome abnormalities arise in most malignant neoplasms. In these instances, the patient does not usually have a constitutional chromosome abnormality, but karyotypic changes develop during the formation of the neoplasm. Often, a neoplasm exhibits cytogenetic changes that are characteristic of a specific tumor type. For example, a t(8;14)(q24; q32) occurs in most cases of Burkitt's lymphoma, and a t(9;22)((q34; q11) is found in most cases of chronic myelogenous leukemia (CML). The der(22)t(9;22) of CML is sometimes called the Philadelphia (Ph) chromosome.

b. Some chromosome abnormalities are associated with differences in the malignant behavior of a neoplasm. In acute myelocytic leukemia (AMD, for example, the presence of t(8;21) is a good prognostic sign, whereas the presence of t(9;22) is a poor prognostic sign.

c. Frequently, neoplasms exhibit multiple chromosome abnormalities. Some of these

cytogenetic alterations arc characteristic of a specific tumor type, others may be changes that occur in many different kinds of neoplasms, and still others are more or less unique to an individual patient.

Cell lines exhibiting different karyotype abnormalities are often seen within a single neoplasm. Sometimes, almost every cell has a karyotype that varies from other cells studied from the *same* tumor. In general, however, all of the cell lines are clonally related, which means it is possible to trace the cytogenetic evolution of the lines back to a single abnormal progenitor.

As the neoplasm progresses (i.e., becomes more malignant), the karyotype tends to become more abnormal. For example, AML associated with t(8;2l) usually has a relatively benign course until other cytogenetic changes, such as trisomy 8, appear. Once the additional chromosome alterations are present, the disease usually behaves in a more malignant fashion.

Karyotypic abnormalities may precede clinical evidence of relapse or worsening of the disease. For example, the appearance of additional changes such as trisomy 8, trisomy 19, or an isochromosome 1 7q in Ph chromosome-positive CML may precede the development of a blast crisis by weeks or months.

MALIGNANCY AS A GENOTYPE.

Two main classes of genes involved in the neoplastic process are oncogenes and tumor suppressor genes. Oncogenes are normal genes that when altered, inappropriately expressed, or overex-pressed can lead to neoplasia. Oncogenes are widely conserved in evolution. More than 100 oncogenes have been identified as part of the normal genome of human cells. These normal cellular genes are called proto-oncogenes or c-onco-genes to indicate that in their normal state and under normal physiological controls, these genes do not cause tumors. Proto-oncogenes are generally thought to have important functions in cell growth and differentiation. Examples include the following: The c-$rc proto-oncogene codes for a cytoplasmic protein kinase that affects phos-phorylation of certain amino acids in target proteins and thereby influences their activity. Some proto-oncogene products appear to be growth factors and growth factor receptors expressed in certain cell types. *nt-2* is a pcoto-oocogene related to the fibroblast growth factor gene.The product of the *c-fun* proto-oncogene is a transcription factor that regulates gene expression.. The product of the *c-fas* proto-oncogene is a guanosine triphosphate (GTP)-binding protein that plays a key rote in a signal cascade that transmits messages from cell surface receptors to the nucleus. Proto-oncogenes must be activated to express their oncogenic potential by mutations.

Tumor suppressor genes are normal genes that function to prevent the development of neoplasma. Tumor suppressor genes are widely conserved in evolution.

Tumor suppressor genes are defined by the effect of their absence. Tumors tend to develop in cells in which both normal alleles of a tumor suppressor gene have been lost or inactivated. Tumor suppressor genes usually have a recessive effect at the cell level. One normal allele is sufficient to prevent the neoplastic effect, even if the second allele has been inactivated or lost. The development of neoplasma results from a loss of function. Loss or inactivation of a normal tumor suppressor gene may be constitutional (i.e., affecting every cell of the body, including the germ line) or acquired somatically in a single clone of cells. The *Kb* gene is a good example of a tumor suppressor gene. The *Rb* gene product is a phosphoprotein involved in control of the cell cycle. Normal activity of the *Rb* gene helps prevent the development of retinoblastoma and other neoplasms. Retinoblastoma, which is a malignant tumor of the retina, develops in early childhood and is usually fatal if not treated. The incidence of retinoblastoma is about 1/20,000 infants. Most retinoblastomas occur sporadically, but approximately 10% of affected children have a parent who also had retinoblastoma. The inherited form of the disease, called hereditary retinoblastoma, is transmitted as an autosomal dominant trait. Tumors in hereditary retinoblastoma are often bilateral or multifocal and exhibit an earlier age of onset than sporadic retinoblastomas. Retinoblastoma develops when *both* alleles of the *Rb* tumor suppressor gene are lost or inactivated in a retinoblast. Loss or inactivation of the *Rb* gene may be constitutional or acquired as a somatic mutation.

In hereditary retinoblastoma, a chromosome containing an allele of the *Rb* gene that has

been deleted or inactivated by mutation is transmitted in typical autosomal dominant fashion. All of the cells of the body of a person who has inherited this abnormality begin with only one normal copy of the *Rb* gene. Retinoblastoma develops in a person who has inherited this mutation only if the second *Rb* allele is also inactivated or lost somatically in a retinoblast. Although the abnormal *Rb* phenotype (i.e., loss of tumor suppression) is recessive at the cellular level, the predisposition to hereditary retinoblastoma is transmitted dominantly at the level of the whole organism.

The *Rb* gene lies in band q14.1 of chromosome 13. Children with multiple congenital anomalies due to cytogenetically apparent constitutional deletions of chromosome 13q14.1 have only one normal *Rb* allele in every cell of their bodies. Development of retinoblastoma occurs in these children if their remaining *Rb* allele is inactivated or lost somatically in a retinoblast.

In most people with sporadic unilateral retinoblastoma, loss or inactivation of both *Rb* alleles occurs somatically in the clone of cells that give rise to the tumor. C. Loss or inactivation of a normal *Rb* allele may occur by a variety of mechanisms. Mutation producing absent or altered transcripts is a common mechanism of inactivation of the *Rb* allele transmitted in hereditary retinoblastoma. This is a less common mechanism of *Rb* allele inactivation in somatic cells. Deletions of the *Rb* locus account for most other cases of hereditary retinoblastoma. Most of these deletions are submicroscopic; that is, they are not apparent on conventional cytogenetic studies. Loss of a whole chromosome 13 is a common somatic event associated with loss of a normal *Rb* allele from a cell. In some cases, duplication of the other chromosome 13. which carries a deleted or inactivated *Rb* allele, is seen with loss of the normal chromosome 13. Mitotic crossing over between a chromosome with a normal *Kb* allele and one with an inactive *Rb* allele can result in homozygosity for the inactivated state in one *of* the daughter cells, which may then go on to produce a malignant cell clone.

In general, each tumor suppressor gene locus is involved in controlling the development of several different kinds of tumors, for example, the *Rb* locus is involved in (he development of at least some cases of osteosarcoma and small cell carcinoma of the lung.

6. A general method of identifying chromosome regions containing tumor suppressor genes is by observing reduction to homozygosity of markers in these regions within tumor cells. Reduction to homozygosity implies loss of one chromosome or a part (hereof with elimination of one allele of a tumor suppressor gene. . for example, if a patient is heterozygous for 1 series of markers on chromosome 17p in his normal tissues but is found to show only one allele at each of these loci in tissue from a neoplasm, reduction to homozygosity is said to have occurred. b. Reduction to homozygosity may result from a variety of mechanisms

Other genes are also involved in (he development of neoplasia.

1. The various monogem'c conditions that predispose to tumor development suggest the nature of some of these additional genes, including those that control repair of DNA damage and those involved in immunologic function.

2. The *p53* gene produces a DNA binding protein that affects a variety of functions including the cell cycle, DNA synthesis, and programmed cell death. The p53 protein induces the activity of some genes and represses the activity of many others. The *p53* gene functions as a tumor suppressor gene in normal cells. It is lost or inactivated during the development of many different neoplasms. In some tumors, especially colon cancers, increased amounts of p53 protein occur, indicating that the action of this gene sometimes promotes tumor development. This is thought to represent a dominant negative effect

Constitutional mutations of *p53* occur in Li-Fraumeni syndrome; a dominantly inherited condition that strongly predisposes to several kinds of malignancy. Mutation or loss of the *p53* gene is the most common genetic change that occurs in neoplasma and is seen in a wide variety of cancers, including those of the colon, breast, lung and brain.

Similar tumors often have similar *p53* mutations, and different tumors usually have different *p53* mutations.

Certain specific *p53* mutations occur in tumors associated with particular environmental exposures. For example, a G - T point mutation at nucleotide 249 of the *p53* gene is seen in most

cases of hepatocellular carcinoma associated with exposure to aflatoxin, a mutagen that is common in the diet in some parts of the world.

Environmental factors that predispose to the development of neoplasma may act by promoting mutation or alteration of expression of cellular genes, thereby disrupting their normal control over division and differentiation.

Environmental factors, inherited genetic factors, acquired somatic mutations, and acquired chromosome alterations all may interact in the development of neoplasia.

Practical lesson 11.
Topic: Methods of laboratory diagnosis of hereditary diseases
The goal: Select patients for cytogenetic analysis, special biochemical and molecular genetic studies.
Student should know :
- – To know the indications for biochemical diagnosis and stages of biochemical diagnosis
- – To know indications for cytogenetic and molecular cytogenetic diagnosis
- – To know indications for molecular – genetic analysis.
- – Explain PCR as a basic method for molecular diagnostics.

Student has to be able to :

Control questions
1. Indications for cytogenetic diagnosis. Karyotyping. Methods routine and differential staining of chromosomes.
2. Molecular- cytogenetic methods. FISH-method
3. Definition of sex chromatin.
4. Indications for DNA diagnostics. Molecular - genetic methods.
5. Stages of DNA diagnostics with using of polymerase chain reaction (PCR).
6. Direct and indirect methods of DNA diagnostics.
7. The method of dermatoglyphics.
8. Biochemical methods. Indications for biochemical diagnosis.

MAIN LITERATURE:
Main literature
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Importance of correct diagnosis. Classification of genetic methods.

Correct diagnosis of the hereditary disorder is very important for prognosis, management and genetic counseling. Recognition of correct diagnosis may answer following questions:

1) Prognosis. Is the patient likely to survive; is there associated mental retardation and if so, how sever; and are there other anomalies that might not be obvious on physical examination or that might develop later in life?

2) Management. Are there early interventions that could prevent severe cause of the disorder and complications? Is corrective surgery available and how good is it (if patient has congenital malformations)? Are there measures to be taken to minimize risk for feature children (e.g, taking folate supplements for prevention of neural tube defects).

3) Genetic counseling. What is genetic risk for future children? If diagnosis is wrong, the counselor may give an incorrect genetic risk for future affected children and the wrong risk for other relatives within the family.

For diagnosis of hereditary disorders following methods are used:

1) Syndrome-oriented examination: physical examination of the patient, examination of the patient's and family history, portrait diagnosis, and using of Internet and computer diagnostic programs.
2) Laboratory investigations: cytogenetic methods, methods of DNA- analysis, and biochemical diagnostics.

Syndrome-oriented examination.

The method includes physical examination of the patient, examination of the patient's and family history, portrait diagnosis with atlas of malformation syndromes, and using of computer diagnostic programs.

1) Syndrome-oriented physical examination is essential for correct diagnosis of all groups of hereditary disorder and especially for diagnosis of congenital malformations of any etiology. It is very important to look at facial features of the patient. Patients with hereditary disorder may have characteristic appearance: "doll face" (glycogen storage disease type 1 – von Gierke's disease), "coarse face" (mucopolysaccharidose), bird-like face (Marfan's syndrome). Some disorders are characterized by cranial asymmetry or asymmetry of the limbs or the body in general. Does the patient move the limbs and head? Patients with hereditary disorders may have unusual movements (puppet-like movements in patients with Angelman's syndrome). It is important to measure the size of the head, the length of the limbs, the fingers, and the body in general.

Muscle tone should be examined. Congenital hypotonia (the floppy baby) may be caused by severe defects of the brain, chromosomal disorders (Down's syndrome, Prader-Willis's syndrome), and spinal muscle atrophy. Unusual cry may be helpful in diagnosis of some hereditary disorders. Examples include mewing kitten-like cry of the child with cri du chat syndrome, the low-pitched, almost growly, cry of the patient with hypothyroidism or Hurler's syndrome (a type of mucopolysaccharidosis).

General physical examination also includes careful systematic examination of the patients head, neck, chest, back, abdomen, limbs, genitalia and skin. Looking for specific congenital defects and microanomalies (see chapter 3.2) may be helpful for correct diagnosis because many

hereditary disorders are characterized by very specific appearance of the affected child. Any unusual feature should be examined carefully.

2) Diagnostic imaging. For the infant with a congenital defect, a total body X-ray examibation is indicated with special attention to any area that is malformed or asymmetrical. Ultrasonography is also important for the soft-tissue defects (brain, liver, hard). Computed tomographic scans, magnetic resonance imaging, and echocardiography will be valuable when indicated.

3) Syndrome-oriented examination of the patients and family history.

Pregnancy. Counselor should ask about possible exposure to teratogens during pregnancy which ended with birth of affected child (for example, maternal infections, drugs, alcohol). It is important to know about the results of any prenatal testing, such as maternal serum screening, ultrasound. Abnormal results of prenatal screening are specific for chromosomal disorders and for congenital defects of any etiology. But inborn errors of metabolism are characterized by normal pregnancy.

Development. Learning the timing of the major developmental milestones is essential for correct diagnosis. It is important to now, if the disorder is congenital or there was a period of time over which the development was definitely normal. Normal period of life is specific for inborn errors of metabolism. Chromosomal disorders are usually congenital (except for disorders which are caused by mutations of the sex chromosomes).

The family photographs. The family photograph album should be examined. It is important to compare features of the patient with those of siblings, parents, and other relatives. Presence the same features in close relatives may be helpful in determining of dominant mode of inheritance. In addition, many families document their children's development on videotape which may be also available for examination.

Family history. The family history including not less than 3-4 generations is usually sufficient (see chapter 4). Often it is essential to see and examine the parents and siblings. The physician should consider whole spectrum of manifestations, because variable expressivity may be characteristic for some genes. If possible, copies of the medical records should be obtained.

4) Portrait diagnosis. For the clinical diagnosis portrait diagnosis is widely used. Portrait diagnosis is the comparing of the patient's phenotype with the phenotype of the patients in special atlas of malformations syndromes, like "Smith's recognizable patterns of human malformation" and so on. Pathologic mutation causes the formation of abnormal phenotype. As the mechanism of disorder formation is the same in all patients with the same mutation, the patients are more similar with each other, than with there parents. A specific diagnosis is usually dependent on recognition of the overall pattern of anomalies, and the detection of minor defects may be as helpful as that of major anomalies in this regard.

Portrait diagnosis seems to be the only method for diagnosis of single gene disorders that manifests as congenital abnormalities. It is useful auxiliary method for diagnosis of chromosomal disorders, because most of them also have specific phenotype. And it is the only method for diagnoses of multifactorial congenital abnormalities.

Examples of atlases which are available now:

1. Jones KL. Smith's recognizable patterns of human malformation. 5th ed. Philadelphia:WB Saunders;1997. *Good description of a wide variety of malformation syndromes.*

2. Gorlin RJ, Cohen MM, Levin LS. Syndromes of the head and neck. 3rd ed. New York: Oxford University Press; 1990. *Good description of congenital defects and minor anomalies of the head and neck.*

3. Taybi H, Lachman R. Radiology of syndromes, metabolic disorders and skeletal dysplasias. 4th ed. St. Louis: Mosby; 1996. *The "bible" of disorders affecting the skeletal system.*

5) Using Internet and computer - assisted diagnosis programs. It is known now more than 1000 chromosomal disorders, about 3500 monogenic disorders and a lot of multifactorial and teratogenic syndromes. The amount of newly discovered diseases increases every year. A doctor handling genetic problems cannot store in his memory information about all kinds of nosologic

forms. The Internet is a source of latest information on syndromes, reviews of clinical manifestations, and new tests, but less helpful in computer-assisted diagnosis.

The best of computer-assisted diagnosis programs is POSSUM (the London Data Bases). It contains information about hereditary diseases and photographs of the patients. This program is not on-line, but is available in every genetics center. In the USA the computer program analogous to the McKusic catalogue of Mendelian inherited diseases has been created and has being filled up every year. Today, the online version of McKusck's compendium (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) is available in Internet.

To use the computer programs the geneticists are to know all terms those mean minor anomalies and congenital anomalies, because the computer searches the hereditary syndrome, using these symptoms, chooses the relative group of diseases in which these symptoms are seen.

Laboratory investigations

Since the end of the 50th years, laboratory methods of diagnosis of hereditary diseases are widely used. Some of them are discussed here:

- Cytogenetic methods;
- Methods of DNA- analysis;
- Biochemical methods.

Cytogenetic methods

These methods are based on studying of chromosomes and include karyotyping (or karyotype preparation method), sex chromatin (Barr bodies) studying, and molecular-cytogenetic methods.

Indications for chromosome analysis:
1. Patients with symptoms of definite chromosomal disorder;
2. Multiple congenital abnormalities;
3. Unexplained mental retardation;
4. Sexual ambiguity or abnormality in sexual development;
5. Infertility;
6. Recurrent miscarriage;
7. Unexplained stillbirth;
8. Malignancy and chromosome breakage syndrome.

Karyotyping

Karyotyping is based on the examination of karyotype. Karyotype can be studied in metaphase of mitosis when chromosomes are maximally condensed and therefore most easily visible. The slide of metaphase chromosomes of a cell is called metaphase spread (metaphase plate). Diagnostic cytogenetic analysis can also be performed with prometaphase and even prophase chromosomes. A variety of tissues have been used in studying human chromosomes but most commonly specimens of skin, red bone marrow, peripheral blood, and cells from amniotic fluid, placenta, and chorion.

Chromosomes from cultured lymphocytes of peripheral blood provide the shortest and most convenient method for routine cytogenetic analysis. This method includes the following stages:
1. A small amount of venous blood is taken and added to a small volume of nutritive medium containing phytohaemagglutinin (PHA), which stimulates T lymphocytes to division by mitosis. PHA is extracted from the French beans (Phaseolus vulgaris). The cells are cultured under sterile conditions at body temperature (37ºC) for 72 hours.
2. During this time cells divide and 3 hours before the end of incubation colchicine is added. Colchicine is a complex organic compound which is extracted from the autumn crocus. Colchicine has the unique property of preventing the formation of the spindle fibres and stops mitosis at the stage of metaphase.

3. After 3 hours or so a hypotonic saline solution is added (of KCl), which causes swelling of cells and better separation of individual chromosomes. It is done to avoid the overlapping of the chromosomes.
4. Cells are then fixed, mounted on a glass, and stained.

In karyotyping, several different staining methods can be utilized to identify individual chromosome:

- **G (Gimsa) banding.** This is the most commonly used method. The chromosomes are treated with trypsin (proteolytic enzyme) and then stained with a DNA binding dye known as Romanovsky-Gimsa. The banding patterns of each chromosome are specific and make it possible to identify each individual chromosome and to identify different types if chromosomal aberrations. It gives each chromosome a characteristic pattern of light and dark bands. The dark bands are called G bands; the light bands are R bands. In an average metaphase preparation, approximately 400 dark and light bands can be resolved in a haploid set of chromosomes.
- **High-resolution banding.** This method captures chromosomes in prometaphase, when they are less condensed than in metaphase. In high-resolution preparations, each band seen in metaphase chromosomes can be resolved into sub-bands, which allows for resolution of 800 or more bands. This method is more convenient for diagnosis of chromosomal aberrations.
- **Q (Quinacrine) banding** uses quinacrine to stain chromosome, which are viewed with ultraviolet fluorescence microscopy. Alternating bands of bright and dull fluorescence correspond to those seen by G banding for most areas of chromosomes. Bright Q bans are equivalent to dark-staining G bands. Areas lacking this similarity are called variable bands.
- **R (reverse) banding** uses Romanovsky-Gimsa dyes under elevated temperatures to produce the reverse of the pattern seen in G banding or Q banding.
- **C (Centromeric heterochromatin) banding** requires heating in an alkali solution and staining with Romanovsky-Gimsa. The centromeres of all chromosomes and the long arm of the Y chromosome (Yq) are preferentially stained. These are areas of constitutive heterochromatin containing highly repetitive DNA sequences.

5. Karyotype analysis. The cytogeneticist analyses the slide either while looking down the immersion microscope or, less commonly, on a photograph of the metaphase spread which can now be produced electronically. Firstly, the cytogeneticist counts the number of chromosomes. Usually the total chromosome count is determined in 10-15 cells, but if mosaicism is suspected then 30 or more cell counts will be undertaken. Next step is detailed analysis the banding pattern of the individual chromosomes. For this, each pair of homologous chromosomes is analysed in approximately 3-5 metaphase spreads, which show high quality banding. Modern cytogenetic laboratories usually use computer scanners and special computer programmes to analyse and classify the karyotype of an individual on computer. The chromosomes of a single cell are arranged in pairs under International System of Cytogenetic Nomenclature (1995).

Sex chromatin studying

Sex chromatin (Barr body) is an inactive X chromosome. The number of Barr bodies is one less than number of X-chromosomes (Table 5._). So normal male has only one X chromosome and lack Barr bodies, normal female has two X chromosomes and one Barr body.

For Barr bodies examination epithelial cells of buccal mucosa should be taken. The scraping from the cheek is evenly spread on the slide, fixed in alcohol, stained with aceto-orsein or any other basic dyes, and then studied under the microscope. X-chromatin can be found within the nucleus and looks like a small darkly stained particle under nuclear membrane.

The studying of sex chromatin is used as express method for:
- Diagnosis of chromosomal disorders with abnormal number of X-chromosomes;
- In forensic medicine for definition of sex of victim or criminal;

- Definition of the chromosomal sex in hermaphrodites.

Table 5. The number of Barr bodies in normal males and females and in patients with chromosomal disorders (Always one less than the number of X-chromosomes)

Number of sex chromosomes	Sex	Number of Barr bodies
XY	Male	0
XX	Female	1
X0	Female	0
XYY	Male	0
XXY	Male	1
XXXY	Male	2
XXX	Female	2
XXXX	Female	3

Genes of Y chromosome are not subjected to the dosage compensation. Y-chromosome never forms Barr body even if there are more than one Y-chromosomes in the cell. A large portion of Y chromosome is heterochromatic and is a strongly fluorescent body, which is called Y-chromatin. To demonstrate it during the interphase a fluorescence method is used. The number of Y-chromatin bodies is equal to the number of Y chromosomes.

Molecular-cytogenetic methods (fluorescent in situ hybridization – FISH)

These methods combine conventional cytogenetic methods with molecular genetic technology. In situ hybridization method specially labeled DNA probes are hybridized to metaphase chromosomes or to interphase chromatin. Method is based on the ability of a portion of single stranded DNA (DNA probe) to anneal with its complementary sequence on a metaphase spread or in interphase chromatin. The DNA probes are labeled with reported molecules, such as biotin, dygoxigenin, and dinitrophenyl, which can be visualized under the microscope by coupling of reporter molecules to a fluorescent signal or other dye. Fluorescent dyes are most widely used, giving rise to the term fluorescent in situ hybridization.

Different DNA probes are available to clinical cytogenetic laboratories now:
- ***Centromeric probes*** are complementary to the centromeres of specific chromosomes (13, 18, 21, X, Y). They are used for rapid diagnosis of common chromosome disorders (trysomy 13,18,21,X,Y) in interphase cells obtained from a prenatal diagnostic sample of chorionic villi.
- ***Probes for single-copy genes***. They are useful for identifying tiny submicroscopic deletions and duplications (Prader-Willi's and Angelman's syndromes).
- ***Probes for individual whole*** chromosome (whole chromosome paint probes). These consist of a set of probes for different parts of a particular chromosome. These probes are used for diagnosis of complex rearrangements such as translocations and for identifying the origin of small marker chromosomes that cannot be fully characterized by banding.

Molecular-cytogenetic methods can be used for diagnosis of all types of chromosomal disorders and especially for the detection of submicroscopic chromosomal rearrangements (deletions, duplications, translocations). Different modifications of FISH technology are available now for cytogenetic studying of cancer.

Methods of DNA-analysis

Many methods of DNA analysis are based on denaturation and renaturation of the DNA molecules; they involve the use of nucleic acid probes, restriction enzymes and the process of nucleic acid hybridization. What are the definitions of these terms?

Denaturation and renaturation. When double stranded DNA is heated or treated by alkali, the hydrogen bonds that stabilize the double helix are disrupted. The helix becomes unstable and the two strands of the DNA molecule separate. This process is called denaturation. If the temperature is then reduced the double helix reforms and the original double-stranded DNA molecule is recovered. This process is called renaturation or reannealing.

Hybridisation. In fact, any two single-stranded nucleic acid molecules are capable of forming a double-stranded molecule as long as their base sequences are mostly complementary. If DNA molecules from two sources which have been denatured by heat to make them single stranded are mixed, complementary single DNA strands may form a double stranded molecule under appropriate conditions. Such double-stranded molecule consisting of complementary DNAs from two different sources is called a hybrid and its formation is called hybridization. Hybrids can form between two strands of DNA, between DNA and RNA and between two strands of RNA. This feature of nucleic acids is used in a series of analytical techniques in which specific DNA or RNA sequences in complex mixtures are detected by using a nucleic acid of complementary sequence.

Probes. The detection of nucleic acid sequences using techniques based on hybridization requires the use of probes. Nucleic acid probes are usually short single stranded DNA sequences which are radioactively or non-radioactively labeled and can be used to detect DNA or RNA fragments with sequence homology (by hybridization). Sometimes RNA probes can be also used. Probes must be single-stranded. DNA probes can come from variety of sources: 1) they may be produced synthetically (synthesis is based on knowledge of the protein amino acid sequence); 2) they may be cloned (DNA cloning is the selective amplification of a specific DNA fragment). Synthetic oligonucleotides and RNA obtained by *in vitro* transcription of cloned DNA sequences are also used as probes. A DNA probe can be labeled by isotope ^{32}P or by fluorescent dyes (fluorescein or rhodamine). Hybridization of a radioactively labeled DNA probe with complementary DNA sequences on a nitrocellulose filter can be detected by autoradiography while DNA fragments which are fluorescently labelled can be detected by exposure to the appropriate wavelength of light (fluorescent in situ hybridization).

Restriction endonucleases (restriction enzymes) are produced by various bacterial species. They restrict (cleave, cut) the DNA at specifically recognized sequences. These sequences are referred to as restriction sites or recognition sites. For example, the intestinal bacterium *Escherichia coli* produces a restriction enzyme, called *Eco*RI, that recognizes the DNA sequence GAATTC. The enzyme cleaves (cuts) the sequence between the G and the A (this process is called a restriction digest). This produces DNA restriction fragments. Examples of restriction enzymes and their restriction sites are given bellow (table _____). Sometimes the mutation causing the disease happens to alter a recognition sequence for a specific restriction enzyme. In this case the mutation creates a restriction site polymorphism that can be detected after patients DNA digestion with this enzyme.

Table---. **Examples of restriction enzymes and there restriction sites.**

Restriction enzymes	Bacterial species producing enzymes	Restriction sites (5'- 3')
EcoR1	*Escherichia coli RY 13*	G-AATTC
BamH1	*Bacillus amyloliquefaciens H*	G-GATCC
HaeIII	*Haemophilus aegiptius*	GC-CC
HindIII	*Haemophilus influenzae Rd*	A-AGCTT

Agarose (polyacrilamide) gel electrophoresis

This method is used in molecular biology to separate DNA or RNA molecules by size. This is achieved by moving negatively charged nucleic acid molecules through an agarose matrix with an electric field (electrophoresis). Agarose gel electrophoresis is a procedure that consists of loading DNA into small wells of an agarose gel and then applying an electric current to the gel.

As a result, the smaller DNA strands move faster than the larger strands through the gel toward the positive current. The size of the DNA fragments can be determined by comparing it with a *DNA ladder*, which contains DNA fragments of known size, also loaded onto the gel.

Sometimes the sizes of normal and mutant DNA fragments are equal, but an alteration in the DNA sequence can result in a different conformation which, under appropriate gel conditions, results in different electrophoretic mobility.

Method is used for analysis of PCR (see below) products (in molecular genetic diagnosis or genetic fingerprinting) and for separation of restricted genomic DNA in Southern blotting (or RNA in Nothern blotting).

Methods of nucleic acid hybridisation.

The most commonly used methods of nucleic acid hybridization are Southern and Northern blotting.

Southern blotting, named after Edwin Southern who developed the technique, involves digesting DNA by a restriction enzyme gel electrophoresis with the use of specific probes. The method includes following steps:

1. *Obtaining DNA, restriction, gel electrophoresis*. Usually DNA is obtained from white blood cells. Approximately 5-10 µg of DNA are treated with a restriction enzyme that makes sequence-specific cuts. Restriction digest typically produces more than million DNA fragments. The resulting DNA fragments are separated by gel electrophoresis (agarose gel is usually used). This separates the restriction fragments by size: the smaller fragments migrate faster than the larger ones.

2. *Denaturation DNA*. The DNA fragments in the gel are then denatured with alkali. DNA fragments become single stranded.

3. *Blotting of the DNA fragments*. The single stranded DNA fragments are transferred to a nitrocellulose filter which binds the single stranded DNA (so called Southern blot). The filter contains many thousands of DNA fragments arrayed according to their size. If we looked at all of this fragments at once, it would be impossible to distinguish one from another because of their large number.

4. *DNA hybridization*. A radioactively labeled DNA (or RNA) probe is added to the DNA fragments on the nitrocellulose filter. The probe recognizes and hybridizes with a complementary DNA sequence.

5. *Visualizing of the hybridized DNA probes by autoradiography*. To visualize the position of the hybridized probe, the blot is exposed to x-ray film, which darkens at the probes position due to emission of radioactive particles from the labeled probe. These darkened positions are usually referred to as bands and the film is termed an autoradiogram, and all process is called autoradiography.

Mutations causing the disease can alter a recognition sequence for a specific restriction enzyme. These variations at restriction sites produce different DNA fragment lengths after digestion with this enzyme, which are sorted by electrophoresis and visualized trough the use of labeled probes. This process of DNA mutations detection is called the detection of the *restriction fragment length polymorphisms (RFLPs).*

Southern blotting is very precise and specific method but there are several limitations. Significant amounts of DNA a needed for the Southern blotting. The technique is also labor intensive and may take 7-14 days to achieve the results. Southern blotting usually involves the use of radioactive material, which requires special training and safety precautions for laboratory personnel.

Northern blotting differs from Southern blotting by the use of mRNA as the target nucleic acid in the same procedure. mRNA is unstable molecule because of cellular ribonucleasis which cut this molecule. Use of ribonuclease inhibitors allows isolation mRNA which if run on an electrophoretic gel, can be transferred to a filter. Hybridizing the blot with a DNA probe allows

determination of the size and quantity of the mRNA transcript. In some gene disorders in which mutation has not been identified in the coding sequences, an alteration in the size of the mRNA transcriptesuggests the possibility of a mutation in a non-coding region of a gene such as the splice site. Northern blotting can also be used to demonstrate the differential pattern of expression of a gene in different tissues or at different time of development.

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a powerful molecular biology technique for selective and rapid enzymatic amplification of target DNA *in vitro*. *Amplification* means multiple replication of a DNA sequence. PCR is used to amplify specific region of a DNA strand. This can be a single gene, a part of a gene, or non-coding sequence. Most PCR methods typically amplify DNA fragments of up to 10 kilo base pares (kb). Some PCR methods can copy DNA fragments of up to 40 kb in size.

PCR was invented by Kary Mullis, an American scientist, in 1983. He shared the Nobel Prize in Chemistry with Michael Smith in 1993.

PCR requires several basic components. These components are:

- *DNA template*. This contains the region of the DNA fragment to be amplified. DNA can be obtained from any nucleated cell. A small amount of DNA is needed for this method. It is possible to start with quantities of DNA as small as that from a single cell. RNA can also be used for PCR by first making a DNA copy using the enzyme reverse transcriptase.
- *Primers*. Each PCR requires a pair of oligonucleotide primers. These are short single stranded DNA molecules (typically 20 bases) obtained by chemical synthesis. Primers are complementary to the 3'ends of opposite DNA strands on both sides of the sequence to be amplified. The primers when added to denaturated DNA will bind to the complementary DNA sequences and will flank the target DNA fragment. Primers are needed for DNA replication, because enzyme DNA polymerase can not to start DNA replication itself;
- *DNA polymerase*. A number of polymerases are used for PCR. All are thermostable and can withstand the high temperature (up to 100°C) required. The most commonly used enzyme is *Taq* DNA polymerase (with a temperature optimum at around 72°C) which was first isolated from *Thermus aquaticus,* a bacterium that lives in hot springs and hydrothermal vents. The role of the Taq polymerase in PCR is to copy (to amplify) DNA molecules. It can amplify a 1kb strand of DNA in roughly 30 seconds at 72°C. The enzyme binds to single–stranded DNA and synthesizes a new strand complementary to the original strand. DNA polymerases require a short region of double-stranded DNA to begin the DNA synthesis. In PCR this is provided by the oligonucleotide primers which create short double stranded regions by binding on either side of the DNA sequence to be amplified. In this way the primers direct the DNA polymerase to copy only the target DNA sequence.
- Deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP) from which the DNA polymerase builds the new DNA;
- Buffer solution, which provides a suitable chemical environment for optimum activity and stability of the DNA polymerase;
- Divalent cations, magnesium (Mg^{2+}) or manganase (Mn^{2+}), monovalent cation potassium ions.

PCR allows the amplification of target DNA sequences through repeated cycles of DNA synthesis. Each cycle of PCR involves three stages (denaturation, primer annealing, elongation) which take place at different temperatures and together result in the synthesis of target DNA.

1. ***Denaturation.*** The PCR reaction is heated to a temperature of 94-96°C. At this temperature the double stranded DNA is destabilized and the DNA molecules separate into single strands capable of being copied by the DNA polymerase. This step usually lasts for 20-30 second.

2. ***Primers annealing.*** The reaction is cooled to a temperature that allows binding of the primers to the single stranded DNA without permitting the double helix to reform between the template strands. This process is called annealing. The temperature used varies (typically 40-60°C) and is determined by the sequence and the number of bases in the primers. The duration of this period is usually 20-40 seconds.
3. ***Elongation (extension).*** This stage is carried out at the temperature at which the DNA polymerase is most active. For *Taq* polymerase, this is 72°C. The DNA polymerase, directed by the position of the primers, copies the intervening target sequence using the single-stranded DNA as a template. Duration of this stage is usually 45 seconds. And then these three stages are repeated. To deal with the large number of incubations needed, the PCR is carried out using a microprocessor-controlled heating block known as a ***thermal cycler***.

A total of 20-40 PCR cycles is carried out depending on the abundance of target DNA sequence in the template DNA. During the firs cycle DNA sequences which were obtained from tissues are used as templates for copying and the number of target DNA is doubled. Doubled number of target DNA act as a template for the synthesis of new target molecules in the next cycle. As a result, the amount of target DNA increases with each cycle until it becomes the dominant DNA molecule in the reaction. During the early cycles, DNA synthesis increases exponentially but in later cycles, as the amount of target DNA to be copied increases and the reaction components are used up, the increase becomes linear and then reaches a plateau.

In the first cycle, DNA molecules are synthesized which extend beyond the target sequence. This is because there is nothing to prevent the DNA polymerase continuing the copy the template beyond the end of the target sequence. However in subsequent cycles, newly synthesized DNA molecules which end with the primer act as a templates and limit synthesis to the target sequence so that the amplified DNA contains only the target sequence.

Sequences up to several thousand base pairs can be amplified.

After electrophoresis, sufficient quantities of the target DNA can be visualized in agarose gel by ultraviolet fluorescence after ethidium bromide staining, without the need to use indirect detection techniques.

Applications of polymerase chain reaction: PCR as a research tool is commonly used in medical and biological research labs as separately so in association with other techniques. For example, DNA amplified by PCR can be used for DNA sequencing, as a probe in Northern and Southern blotting. PCR is used for a variety of tasks, such as the diagnosis of hereditary disorders, prenatal diagnosis of hereditary disorders, the identification the genetic fingerprints, the diagnosis of infectious diseases, the cloning of genes, paternity testing. PCR has also applications in anthropology and archeology. Some examples will be discussed below.

DNA sequencing

Methods allow obtaining the exact DNA sequence. DNA sequencing is usually carried out by the dideoxy chain termination method (the Sanger method). The bases of this method is that the DNA molecule whose sequence is to be determined is copied in en enzyme reaction by a DNA polymerase. Modified nucleotide triphosphates (dideoxynucleotide triphosphates, ddNTP) are included in the reaction that causes termination of DNA synthesis randomly at each of the four bases where they occur in the template DNA.

To sequence the DNA molecule four separate enzyme reactions are prepared. Each contains:

- Template DNA (the DNA to be sequenced) in single stranded form;
- DNA polymerase;
- Primer (it binds to DNA forming a short double-stranded region, determining the point at which the sequencing reaction is initiated);
- Normal nucleotides for synthesis of the sequenced DNA template;

- One of the four modified nucleotides ddNTPs (ddATP, ddTTP, ddGNT, ddCTP).

Modified nucleotides (ddNTP) differ from normal nucleotides in that they lack of a hydroxyl group on the 3' carbon of the ribose sugar. They are incorporated into the growing DNA polynucleotide by the polymerase but their lack of a 3' hydroxyl group prevents formation of a phosphodiester bond with the next nucleotide to be added. This prevents further elongation of the DNA polynucleotide being synthesized. The ddNTPs thus act as specific inhibitors of DNA synthesis. Each of the four sequencing reactions contains a different ddNTP. Depending on which ddNTP is present, synthesis will terminate where that nucleotide is incorporated. At any point during DNA synthesis, the DNA polymerase may incorporate a normal dNTP into the growing polynucleotide chain in which case synthesis continues or it may incorporate a ddNTP in which case chain elongation is blocked and synthesis ends at that position. The overall effect is that in each of the four reactions a series of DNA molecules of different lengths is produced each terminating at a position corresponding to the presence of that base in the template DNA. For example, in the sequencing reaction containing ddATP a series of DNA molecules will be synthesized each of which ends in an A corresponding to the position of a T in the template.

When the sequencing reaction is completed the synthesized DNA molecules are separated according to size by electrophoresis on polyacrilamide gels with the four reactions run in adjacent lanes. By adding dNTP which contains radioactive phosphorus to the sequencing reactions, the synthesized DNA becomes radioactive allowing it to be detected by placing the polyacrilamide gel against a sheet of X-ray film (autoradiography). When the X-ray is developed a series of bands is visible in the four lanes which form a ladder. The sequence of the template DNA can be read directly from an autoradiograph of the gel by identifying the smallest band followed by successively larger bands.

Use of fluorescently labelled primers or nucleotides, which can be detected by a computerized laser detection system, allows rapid, accurate automated sequencing. Automated sequencing has been used in the sequencing of the human genome and genomes of other organisms.

DNA sequencing allows direct assessment of whether the gene of the patient has any sequence changes (mutation) when compared with a normal control. The technology is still expensive and, therefore, is still primarily used for research purposes. DNA sequencing is likely to be of major importance in the future, when the technology has improved and the cost has decreased sufficiently.

Allele-specific oligonucleotide (ASO) probe analysis

The technique uses two synthetic oligonucleotide probes: one probe specific for a normal gene and the other probe (probes) specific for a known genetic mutation (mutations). The ASO probes are used as hybridization probes to determine whether a patient has two copies of a normal gene or a mutation particular for specific disease. This method is most helpful for diagnosis genetic disorders that typically arise from a single mutation. ASO probe analysis can be used on DNA that has been selectively amplified by PCR, which allows rapid and accurate diagnosis. ASO probe analysis is possible only with precise knowledge of the structure of the normal gene and of the molecular bases of mutation. ASO probe method is used in families having specific and separate mutation.

DNA chips (DNA microarrays)

Principles of allele-specific oligonucleotide probe analysis in a modified manner are used for creating of DNA chips (DNA micriarrays) - a new DNA technology. To make a DNA microarray, oligonucleotides are robotically placed on a small glass slide. A single slide (1cm^2) can contain hundreds of thousands of different oligonucleotides. These oligonucleotides consist of normal DNA sequences as well as DNA sequences that contain known disease-causing mutations. Fluorescently labeled DNA from a subject is hybridized with the normal or with the mutation-containing oligonucleotides, and a pattern of hybridization signals is analyzed by a computer.

The technology of DNA chips can be used for direct DNA analysis, in population screening programs. Another application of DNA chips is to determine which fenes are being expressed in a

given tissue sample (e.g., from tumor). mRNA from the tissue is extracted and used as a template to form a complementary DNA sequence, which is then hybridized on the slide with oligonucleotides representing many genes. The pattern of positive hybridization signals indicates which genes are expressed in the tissue sample.

The DNA chips approach offers the extraordinary speed, miniaturization, and accuracy of computer-based mutation analysis.

Applications of methods of DNA-analysis

Methods of DNA analysis are used for diagnosis of single gene disorders, prenatal diagnosis of single gene disorders, detection of heterozygous carries of mutant recessive genes, for presymptomatic testing of single gene disorders with late onset, for diagnosis of multifactorial disorders, and for diagnosis of infective disorders. These methods are also used in forensic science for different purposes. Some of these applications are considered bellow.

8.3.2.7.1. Diagnosis of single gene disorders can be ***direct and indirect.***

1) ***Direct methods of diagnosis*** are available in case if mutational basis of single gene disorders is known. Mutations that are directly detectable include major gene rearrangements (deletions, insertions, duplications) and single-nucleotide substitutions. Major gene rearrangements are easily detected using Southern blot analysis or PCR (PCR products of normal and mutant genes move in gel with different speed). Examples of disorders that are caused by major gene rearrangements include α-talassemia, Duchenne-Becker muscular dystrophy, familial hypercholesterolemia, hemophilia.

Single gene substitutions or other small rearrangements (point mutations) represent the most common type of mutations underlying genetic diseases. Sometimes such mutations alter a recognition sequence for a restriction enzyme. In this case the mutation itself creates a restriction site polymorphism and the restriction fragments lengths polymorphisms (RFLPs) techniques can be used for direct diagnosis. In this case PCR amplification and digestion with the appropriate enzyme may reveal an altered pattern of restriction fragments between normal and affected persons. RFLPs can be also detected by the Southern blotting.

A good example of disorder caused by point mutation that alters restriction site is sickle-cell anemia. Normal individuals have glutamic acid at position 6 of the β-globin polypeptide (the DNA sequence GAG). Patients with sickle-cell anemia have valine at this position (the DNA sequence GTG). The normal codon GAG creates a restriction site for the restriction enzyme *Mst*II. Thus, *Mst*II recognizes and cleaves the DNA sequence of the normal chromosome at this site as well as at the restriction sites pn either side of it. The result is two DNA fragments, one 1100 bp long and another only 200 bp long. The mutant DNA with abnormal codon GTG is not cleaved at this site. However, the restriction sites flanking the site of interest, which does not vary, are still cleaved. The result is a single DNA fragment (1300 bp long instead of two short fragments). Because the resulting RFLP reflects the disease-causing mutation directly, RFLP analysis in this context is an example of direct diagnosis of the disease.

Most mutations do not occur at a known restriction site. After a specific region of DNA has been amplified by PCR, it can be sequenced directly with technique described previously. Mutations are then detected by comparing the DNA of a patient with that of an unaffected individual.

If mutation does not alter the restriction site, it is possible also to use allele specific oligonucleotide (ASO) probes. By using appropriate reaction conditions, the normal and mutant DNA can be distinguished by demonstrating differential hybridization of the normal and mutant oligonucleotide probes corresponding to the normal and mutant DNA sequences. For example, the most common cause of cystic fibrosis is the three-base deletion that results in the loss of a phenylalanine molecule at position 508 of the CFTR protein. This mutation is labeled ΔF508 (Δ for deletion and F for phenylalanine at position 508). ΔF508 accounts for nearly 70% of all CF mutations. This and other mutations can be revealed with help of specific oligonucleotide probes. ASO probe analysis provides a rapid and simple way to screen for common cystic fibrosis

mutations in the general populations. It is possible to detect approximately 90% of carries by this method.

DNA chips approach can be also used for direct diagnosis. It allows detection of many possible mutations at the gene of interest at once.

2) **Detection of unknown mutations: indirect DNA diagnosis**. This approach is used if mutation causing the disorder in a particular family is unknown. These disorders can be diagnosed indirectly using markers for the mutant gene. These markers are variations in DNA that do not cause the disease but have been demonstrated to be close to or linked to the gene or mutation of interest.

Indirect diagnosis includes several steps. 1) first of all, affected and normal members of a family are tested on specific polymorphic markers closely linked to the mutant gene; 2) secondly, allele of the DNA marker which is located on the same chromosome as a disease is identified; 3) once linked marker allele is established in a family, the analysis of the pedigree is performed to reveal the members of the family who inherited the marker that is segregating with the mutant gene. For example, in the pedigree for autosomal dominant breast cancer (figure 8---) several marker alleles were identified (alleles 1,2,3,4). The alleles are transmitted in generations as codominant traits. Each individual possesses two alleles (one from father and one from mother). The analysis of closely linked marker on chromosome 17 shows that the mutation is on the same chromosome as marker allele 1 in the affected mother in generation II. This indicates that the daughter in generation III has inherited the mutation-bearing chromosome from her mother and is highly likely to develop a breast cancer (the penetrance of this autosomal dominant gene is 80%).

There are several limitations of indirect DNA diagnosis:

- Numerous family members are needed to determine the form of the marker that segregates with the mutant gene in that family.
- The distance between marker and the mutant gene limits the accuracy of indirect DNA diagnosis. For each distance of 1 million bp between the marker and gene, there is a 1% chance of recombination during each meiosis, which can make the results incorrect.
- Genetic heterogeneity is other possible complication. The same single gene disorder in different families may be caused by mutations at different genes (locus heterogeneity). If a DNA marker near the gene on one chromosome is used in all instances to determine the risk of someone having the mutant gene, the results of DNA testing will likely be incorrect in families who have the causative gene on another chromosome.

DNA polymorphisms which can be used for indirect DNA analysis as markers. DNA polymorphisms include single nucleotide polymorphisms, restriction fragments length polymorphisms and tandem repeats polymorphisms.

Single nucleotide polymorphisms (SNPs). There is an enormous amount of DNA sequence variation in the human genome. Variations in the nucleotide sequences occur approximately once every 200 bp. These single base pair differences in DNA nucleotide sequence, usually only diallelic, are inherited in a Mendelian codominant manner. They can have no phenotypic effect as they usually occur in intergenic non-coding DNA. This DNA sequence variation, known as single nucleotide polymorphisms (SNPs), has the advantage that it can be detected by DNA chips. SNPs can be used as markers in indirect DNA analysis of single gene disorders or (more often) in association studies of common (multifactorial) disorders.

Some of these single base pair variants may change restriction sites for specific restriction enzymes or may create new once.

Restriction fragments length polymorphisms. If a difference in DNA sequence occurs within nucleotide recognition sequence of a restriction enzyme, the DNA fragments produced by that restriction enzyme will be of different lengths in different people. This can be recognized by the altered mobility of the restriction fragments on gel electrophoresis, so-called restriction fragment length polymorphisms, RFLPs. Some of these polymorphisms may be linked with gene

of interest and may be used for indirect diagnosis of a single gene disorder in particular family. RFLPs can be detected by the Southern blotting or by analysis of DNA fragments generated by polymerase chain reaction. So, restriction fragments length polymorphism can be used as for direct DNA analysis (if mutation in a coding DNA changes restriction site) so for indirect DNA analysis (if mutation changing restriction site occurs in the non-coding DNA sequence linked to the gene of interest).

Tandem repeats polymorphisms. Approximately 10% of the human genome is composed of tandemly repeated DNA sequences. Satellite DNA is named because of how it separates on density-gradient centrifugation. There are two categories of tandemly repeated sequences which are used as genetic markers: minisatellites and microsatellites.

- *Minisatelites* are extremely polymorphic tandem repeats between 100 bp and 30 kb in length. The repeated sequence varies from 15 to 500 bp. Minisatellites may occur at only one site in the genome (single locus minisatellites) or at many different loci (multilocus minisatellites). The number of repeats in a specific locus can vary substantially from individual to individual which gives the name of this polymorphic marker- *variable number tandem repeats,* or VNTRs. VNTRs are detected using an approach similar to that used for RFLPs. DNA is digested with a restriction enzyme, and the fragments are electrophoresed, denatured, and transferred to a filter (Southern blotting). The principal difference is that special probes are used that hybridize only to a given minisatellite region. VNTRs reveal polymorphisms because of different numbers of repeats located between two restriction sites. Minisatellites can be also identified by PCR.

- *Microsatellites* are extremely polymorphic DNA segments that are usually less than 1kb long and are composed of tandemly repeating units of 2, 3, or 4 nucleotides (from 1 to 13).An example is the $(CA)_n$ repeat, which is composed of the two nucleotides - C and A. The sequence …CACACACACACA…..may have 10 to 60 units. The variations in the number of units of microsatellites is called short tandem repeat polymorphisms (STRPs). STPRs are present in numerous copies throughout genome. For example there are approximately 50000 different $(CA)_n$ loci in humans. These short tandem repeats polymorphisms (STRPs) differ from VNTRs just discussed in terms of there size and also in that they are not defined by restriction sites flanking the repeat region. The PCR technique is used to isolate them.

Both types of tandem repeats polymorphisms (VNTRs and STRPs) are used in forensic applications, such as paternity testing and identification of criminal suspects.

Diagnosis of infective disorders. DNA technology, especially PCR, has found application in the diagnosis and managements of infective disorders. The polymerase chain reaction can be used to detect the presence of DNA sequences specific to a particular infectious organism before conventional evidence such as antibody response or the results of cultures are available. This method allows the detection of specific mutations in the DNA of bacteria or virus responsible for acute overwhelming infections, where early diagnosis prompt institution of the correct antibiotic or antiviral agent with the prospect of reducing morbidity and mortality.

Forencic uses of DNA analysis

Polymorphic DNA sequences (minisatellites and microsatellites) are used for establishing identity and paternity testing. There two approaches –DNA fingerprinting and DNA typing. DNA fingerprinting method was investigated by Alec Jeffreys in 1985.

Other polymorphisms arise due to changes in the number of tandem repeats (VNTRs - variable number of tandem repeats, STRs - short tandem repeats polymorphisms). All these markers can be used in linkage (indirect) analysis.

Practical classes 12, 13.
Topic: Levels and methods of prevention of hereditary diseases. Medical genetic counseling. Credit
The goal: *To conduct preventive measures directed on the prevention of the heredity and congenital diseases origin.*
<u>Student should know</u> :
- *To know levels, kinds, ways of conduction of medical-genetic counseling.*
- *To know indications to perform medical-genetic counseling.*
- *To know indications to perform prenatal diagnostics.*
- *To know the methods of prenatal diagnostics.*
- *To analyze the results of biochemical screening.*
- *To know the principles of selection of nosological forms that is liable to screening preclinical diagnostics.*
- *To know deontological aspects of screening programs.*

Control questions
- Medical and social aspects of the hereditary and congenital pathology.
- Genetic risk common to all population.
- Main directions of primary prevention of the hereditary pathology.
- Purpose and stages of the medico-genetic counseling.
- Organization of the medico-genetic help in Ukraine.
- Indications for the medico-genetic counseling.
- Preconceptional prevention of hereditary diseases and congenital abnormalities.
- Secondary prevention. Classification of methods of prenatal diagnostics.
- Non-invasive methods: serum markers, ultrasonography. Prenatal screening. The possibility of using fetal cells circulating in maternal blood.
- Invasive methods.
- Diagnosis of hereditary diseases in a system of modern reproductive technologies.
- What is fetology? The possibility of prenatal treatment of hereditary diseases and abnormalities.
- Mass screening of newborns. Requirements for screening programs.
- Examples of hereditary diseases, which are designed programs of newborns mass screening.

- Diagnosis of recessive genes heterozygous carriers of monogenic hereditary diseases. Determination of genetic predisposition to multifactorial diseases.
- Genetic passport rights.
- Ethical, moral and legal problems in medical genetics.

MAIN LITERATURE:

Manuals on medical genetics

ADDITIONAL REFERENCES:

1. Ian.D Young/ Medical genetics. -2nd ed. -Oxford university press. - 304 p.

2. M.R. Speicher, S.E.Antonarakis, F.G. Motulsky. Vogel and Motulsky's human genetics. Problems and approaches. 4th addition. – 2010. –981 pp.

GENETIC COUNSELING

Genetic counseling - is a process in which patients or their relatives are informed about the genetic risk, about the consequences of the disorder, its transmission and the ways by which this can be prevented or mitigated.

Indications for genetic counseling can change with time as new methods of diagnosis become available or as specific therapies are developed. The most common indications include:

1. Advanced maternal age (over 35 years) or paternal age (over 40 years)

2. Known or suspected hereditary condition in the family (chromosomal disorder, single gene disorder, a fetus or a child with birth defects, a child with mental retardation, or several relatives with a specific type of malignancy, pathologic obstetric history - recurrent spontaneous abortions or stillbirth in anamnesis)

3. Consanguinity

4. Exposure to known or suspected teratogens during 1st trimester of gestation

5. Pathologic obstetric symptoms in present pregnancy6

- Risk of spontaneous abortion is characteristic for chromosomal and many single gene disorders.
- Intrauterine growth retardation can be the clinical feature of chromosomal syndromes; single gene disorders; intrauterine fetal infection (cytomegalovirus, rubella, syphilis); ionizing or non-ionizing radiation; multiple pregnancy. Growth delay also may be due to the influence of some maternal factors (smoking, alcohol and others). Intrauterine growth retardation needs to be distinguished from syndromes hereditary dwarfism.
- oligohydramnios is the symptom of different renal disorders with inadequate fetal urine output and may be teratogenic mechanical factor by itself.
- polyhydramnios is observed in different defects of gastrointestinal tracts with swallowing failure.

e) decreased motility of fetus is characteristic for arthrogryposis (limitation of range of joint motion and contractures present at birth).

The goals of genetic counseling as defined by the American Society of Human Genetics (1975) are to help the patient or family in the following ways.

1. Comprehending the medical facts. After the most precise diagnosis is made, the diagnosis, prognosis, appropriate investigations needed, and ongoing management of the condition are discussed with the family. If a diagnosis is not possible, the counseling should present what is understood as well as uncertainties regarding prognosis or inheritance.

2. Understanding the mode of inheritance and the recurrence risks**.** The inheritance should be

clearly explained (the use of diagrams and examples may be helpful) to the parents or to other family members. Recurrence risks may involve standard mendelian genetics, calculated risks, or risks observed in the population (empiric).

The origin of the risk and whether it pertains to a specific diagnosis (e.g., cystic fibrosis) or to a nonspecific diagnosis (e.g., nonspecific mental retardation) should be made clear.

3. Understanding the options. When faced with a distinct recurrence risk, the reproductive options ior the couple may include methods of contraception, adoption, insemination by donor sperm, use of donated ova, prenatal diagnosis with or without termination of the affected fetus, and an unmonitored pregnancy.

4. Choosing a course of action. The couple is encouraged to choose the best course for themselves in light of the recurrence risk, the perceived burden (i.e., psychological, social, economic), the goals of the family, and their religious and ethical standards.

5. Adjusting to the condition. The role of the counselor is to assess the family's reaction to the diagnosis and recurrence risk. Follow-up counseling, by either the medical genetics team or a psychologist, may be indicated to help the family deal with the condition affectively.

Problems in counseling

1. Genetic heterogeneity often complicates the establishment of a precise diagnosis. Similar disorders caused by mutation at different loci or involving different alleles may have different forms of inheritance. For example, retinitis pigmentosa may be inherited as an autosomal recessive or dominant condition, as well as an X-linked condition.

2. Phenocopies may mimic genetic disorders. They are caused by environmental factors, and are, therefore, unlikely to recur. Microcephaly may have both genetic and nongenetic causes.

3. The sporadic case is common in nuclear families. Here, the pedigree is not helpful, and the counselor must depend on an accurate diagnosis. The advent of molecular methods of diagnosis has helped in some areas.

4. Nonpaternity or questioned paternity may invalidate the risks quoted in a counseling session. Interpretation of molecular analysis is dependent upon known paternity, with the use of both direct and indirect methods. The inadvertent discovery of nonpaternity during the course of such testing creates ethical concerns regarding whether to disclose the fact.

PRENATAL DIAGNOSIS

Prenatal diagnosis is a diagnosis of hereditary disorders and congenital defects during pregnancy. There are two types of prenatal techniques- non-invasive and invasive. Non-invasive methods are: ultrasound and maternal serum screening. Invasive methods are amniocentesis, placentocetesis, chorionic villus sampling, cordocentesis.

1) Non-invasive methods include ultrasound examination and maternal serum screening.

Ultrasound was first used as a method of prenatal diagnosis in 1972 for anencephaly. Since then, many fetal structural anomalies have been diagnosed using this technique. Common uses of ultrasound in pregnancy are listed in Table 1.

1. Ultrasound may be used as a screening procedure for fetal viability, growth, and detection of fetal anomalies. The best time for screening ultrasound is 10-14 weeks gestation, between 16 and 18 weeks' gestation (first time) and 24-26 weeks' gestation (second time).

2. Detailed ultrasound is used when there are risks for specific anomalies.

TABLE 1. Uses of Ultrasound in Pregnancy

Trimester	Uses
First	Dating of pregnancy Investigating bleeding Determining viability of fetus Screening for chromosomal diseases (nuchal translucency scanning and nasal bone screening during the same examination)
Second	Determining presence of twins

	Determining placenta! position Screening for fetal anomalies Aiding procedures (e.g., chorionic vittus sampling) Screening for suspected fetal anomalies Checking high-risk situations (e.g., a family history of structural abnormalities or maternal disease associated with an increased risk for fetal anomalies)
Third	Measuring interval growth Determining fetal size and positioning Screening for suspected fetal anomaly

3. The detection by ultrasound of a placental or fetal anomaly may prompt additional studies, including karyotyping. A number of subtle fetal features are associated with an increased risk *of* either chromosome abnormalities or single gene conditions (Table 2).

TABLE 2. Fetal Features Associated with an Increased Risk of Abnormalities

Feature	Abnormality
Increased nuchal translucency and absent nasal bone	Highly predictive marker of Down's syndrome. In one study, 84% of karyotypically proven trisomy 21 fetuses had a nuchal translucency >3mm at 10-13 weeks' gestation (as did 4.5% of chromosomally normal fetuses).
Increased nuchal translucency	Reflects fetal heart failure, and is typically seen in any serious anomaly of the heart and great arteries
Large placenta	Triploidy Hydrops Thahssemia fetalis
Early growth retardation	Trisomies Triploidy
Abnormality of body "Lemon sign" of brain shape Talipes	Neural tube defect Neurologic problems Trisomies
Cleft of lip and palate Nuchal skin thickening	Trisomies or triploidy Turner syndrome Down syndrome

Doppler analysis involves the generation of flow velocity waveforms with a continuous wave unit. The technique is used to evaluate blood flow in the umbilical and placental fetal circulation. Fetal cardiac pathology can be defined with Doppler analysis of fetal blood flow patterns.

1. In normal pregnancy, the circulation in the cord and placenta is high flow with low resistance.

2. In growth-retarded infants, placenta) blood *flow* may be abnormal with high resistance.

2) **Maternal serum screening** involves the use of unique fetal markers, proteins made by the fetus during early gestation that are present in predictable levels in maternal serum throughout pregnancy.

- **AFP** is the principal plasma protein in early fetal life, and is gradually replaced by albumin. Levels of AFP can be measured in fetal blood, amniotic fluid, and maternal serum.

    Maternal serum levels increase during pregnancy, whereas amniotic fluid levels fall. Levels, therefore, must be correlated with gestational age.

    Neural tube and abdominal wall defects in the fetus allow leakage of fetal serum into amniotic fluid. In the 1970s, amniotic fluid AFP measurement was introduced as a method to diagnose these conditions. Subsequently, the level of AFP was also found to be elevated in the maternal serum in most but not all cases in which there was a fetal neural tube defect.

    Low levels of maternal serum AFP are associated with an increased risk for **Down** syndrome in the fetus.

- **Unconjugated estriol (uEs) concentrations** maternal serum increase throughout pregnancy. Pregnancies with fetal Down syndrome have significantly lower uEs levels in the second trimester. If the estriol level drops, then the fetus is threatened and delivery may be necessary emergently. Estriol tends to be lower when there is adrenal hypoplasia with anencephaly.

- **Human chorionic gonadotropin** (hCC) originates from the placenta, and levels decrease sharply between 10 and 20 weeks of pregnancy. Down syndrome pregnancies have higher hCG levels, and hCG levels may be the best single marker for Down syndrome.

- **Pregnancy-associated plasma protein A (PAPP-A).** Low levels of PAPP-A as measured in maternal serum during the first trimester may be associated with fetal chromosomal anomalies including trisomies 13, 18, and 21. In addition, low PAPP-A levels in the first trimester may predict an adverse pregnancy outcome, including a small for gestational age (SGA) baby or stillbirth. A high PAPP-A level may predict a large for gestational age (LGA) baby.

- Inhibin-A. Inhibin is secreted by the placenta and the corpus luteum. Inhibin-A can be measured in maternal serum. An increased level of inhibin-A is associated with an increased risk for trisomy 21. A high inhibin-A may be associated with a risk for preterm delivery.

-
    4. Triple screening, utilizing maternal serum AFP, uE3, and hCG, is now used in many areas for risk assessment in pregnant women. Triple screening should meet the following **criteria.**

- Any woman with a positive screening test should be at sufficiently high risk to justify the use of further invasive confirmatory testing.

- **After an abnormal** result, the procedure includes counseling, directed ultrasound, and amniocentesis with measurement of amniotic fluid AFP and fetal karyotyping (if the patient so chooses).

Invasive methods

Chorionic villus sampling (CVS) is a first-trimester method of prenatal diagnosis involving either transcervical or transabdominal biopsy of the developing placenta (the chorionic villi), using a flexible catheter or needle. First developed in the 1960s, CVS was not commonly used until the availability of improved ultrasound. The chorion differentiates into the chorion frondosum, which becomes part of the placenta and is biopsied during CVS, and the chorion laeve, which becomes part of the fetal membranes. The frondosum is mitotically very active. The villi are derived from three different cell lineages: polar trophectoderm, extraembryonic mesoderm, and primitive embryonic streak. . Both the extraembryonic mesoderm and the embryo proper originate from the inner cell mass, but only a small number of inner cell mass cells (3-8) become progenitors of the embryo itself.

    CVS is routinely done between 9 and 12 weeks' gestation. Transabdominal placental biopsy is possible throughout the pregnancy and may be an alternative to amniocentesis in selected instances.

    Sample tissue can be analyzed directly or cultured. Direct analysis or short-term culture involves cells from the trophectoderm lineage. Cells in the long-term cultures of chorionic villi represent the extraembryonic and embryonic mesoderm lineages. Results of CVS chromosome analysis usually take 2 weeks. Biochemical or DNA analysis results vary and depend on whether cell culture is necessary and the complexity of the analysis. Generally, CVS tissue is preferred for

molecular studies.

Complications of CVS include the following.

a. Bleeding. Approximately 40% of women having CVS wilt experience a minor amount *of* bleeding slice the sampling is from a vascular site.

.b. Spontaneous abortion randomized Canadian trial comparing CVS with amniocentesis showed no difference in loss rates. The loss rate due to spontaneous abortion is usually quoted as 1% (the background rate for spontaneous abortion at this stage of pregnancy is 3-5%).

c. Maternal contamination. If the villi are not carefully cleaned before being analyzed, maternal cells may be present in sufficient numbers in long-term cultures to confuse the results.

d. Confined chorionic mosaicism is defined as a dichotomy between the chromosome constitution of the placental cells and the cells of the embryo (fetus). It is reported in 2% of pregnancies studied by CVS. Mosaicism may originate in earfy development through nondisjunction, anaphase lag, or structural rearrangement, resulting in both a normal diploid and an aneuploid ceil line. If the mutation occurs in trophoblast or cxtraembryonic mesoderm progenitor cells, the mosaicism may be confined to the placenta and not extend to the fetus.When diagnosed, the usual recommendation is to offer amnioccntesis to document normalcy of the fetal karyotype. If the fetal chromosomes are normal, the usual pregnancy outcome is a normal infant. Some pregnancies result in growth-retarded infants or in unexplained perinatal death. This may be due to the chromosomally mosaic placenta, or to uniparental disomy in the infant. Pregnancies diagnosed as having confined chorionic mosaicism should be followed carefully for ongoing fetal growth.

CVS may be done prior to 9 weeks' gestation, but available data indicate an increased risk of transverse limb malformations in pregnancies in which CVS has been performed this early. The basis may be vascular. The risk of transverse limb malformations in infants born after later CVS is thought to be no more than 1/1000 above the background rate.

Amniocentesis involves the aspiration of amniotic fluid with a fine-gauge spinal needle for amniotic fluid cell culture and analysis of the fluid for fetal markers, such as α-feto-protein (AFP). As with CVS, the technique should be done under ultrasound control. Amniocentesis is usually done between 15 and 17 weeks' gestation. Approximately 15-20 ml of amniotic fluid are withdrawn. Results from amniotic fluid cell culture take 2-4 weeks, depending on the culture technique used. Complications of amniocentesis include the following. Failure to aspirate fluid may be due to a uterine contraction, which obliterates the available sampling space, or tenting of the membranes. Maternal complications include spotting or amniotic fluid leakage and are usually short-lived.

The risk of fetal loss related to the procedure is 0.5% in most centers.

Fetal blood sampling [percutaneous umbilical blood sampling (PUBS)] was first developed to diagnose conditions not expressed in cells obtained by amniocentesis or CVS. As ultrasound resolution improved, the technique became more widely used as a faster method to obtain genetic information on the fetus. The technique involves percutaneous sampling from the umbilical cord near the placental insertion under ultrasound guidance. The procedure is done from 17 weeks'gestation to near term. Usually, no more than 0.5-1.0 ml of fetal blood is removed, and the sample is immediately analyzed to ensure that it is of fetal origin (maternal placental vessels are in close proximity to the sampling site).

Chromosome analysis is possible in 48 hours, so the sample is most often used for urgent fetal karyotyping. Fetal blood sampling may also be used for viral or bacterial cultures or analysis of various hematologic indices (e.g., platelet count). Complications of fetal blood sampling include the following. a. Fetal loss rates are difficult to obtain because the fetus being studied may be at increased risk for loss by virtue of the abnormalities present. However, a risk of 2% is seen with experienced centers In pregnancies with a compromised fetus (for example, with severe growth retardation), the loss risk may be approximately 5%. Because the complications of fetal blood sampling may lead to rapid delivery of the fetus after the period of fetal viability, facilities for emergency delivery should be available. Fetal bleeding from the sampling site may continue for

1-2 minutes and is usually not a major problem. Fetal bradycardia, thought to be secondary to vascular spasm, may be documented immediately after the procedure but is usually brief.

Indications for Prenatal Diagnosis

Test	Indications
Fetal karyotype	Maternal age s35 years at time of delivery Previous stillbirth or livebirth with chromosome abnormality Parental translocation or chromosome abnormality Fetal ultrasound abnormality suggestive of chromosome abnormality Low maternal serum o-fetoprotein (MSAFP)
Amniotic fluid or maternal serum α-fetoprotein	Previous pregnancy with neural tube defect Maternal diabetes mellitus Maternal use of sodium valproate Ultrasound finding suggestive of open fetal defect
Fetal blood sampling	Ultrasound abnormality suggestive of chromosome abnormality Hemoglobinopathies or immune deficiencies Diagnosis of fetal infection
Detailed ultrasound	Raised MSAFP at 16-18 weeks of gestation Past history of child with structural malformation Patient affected with, or having family history of, structural malformation Patient affected by medical disorder associated with increased risk for fetal structural malformation Environmental exposure associated with increased risk of structural malformation in fetus

GENETIC SCREENING

Definition. Genetic screening is the identification of a genetic disease, a genetic predisposition to a disease, or a genotype in an individual that increases the risk of having a child with a genetic disease.

1. **The purpose of identifying individuals with a genetic disease** is **usually to permit mangement** of the disease or the complications of the disease in a more effective manner than would otherwise be possible. Examples include:

a. Screening for phenylketonuria (PKU) in newborn infants. Recognition of affected infants within the first few weeks of life permits institution of dietary treatment that is effective in preventing the severe mental retardation that develops in untreated PKU patients.

b. Serum triple marker screening of pregnant women for Down syndrome. In this case, no effective treatment of the fetal condition is possible. Subsequent amniocentesis that shows a woman is carrying an affected fetus gives the woman the option of terminating the pregnancy or continuing it to term and preparing for the birth of an abnormal child.

2. The purpose of identifying individuals at increased risk of having children with a serious genetic disease is to permit them to take advantage of reproductive options that may prevent the birth of affected children. Examples include:

a. Detection of heterozygous carriers for Tay-Sachs disease among Ashkenazi Jewish populations
(**1**) Identification of couples in which both partners are heterozygous carriers of Tay-Sachs disease permits them to avoid having affected children.
(**2**) Such couples may choose to do so by not having children of their own, by having children via artificial insemination from an unrelated donor, or by using prenatal diagnosis with abortion of affected pregnancies.
 b. Use of DNA testing to identify women who carry the gene for DMD within the family of an affected boy provides another example Recognition of women who are carriers permits them to avoid having sons with this disease.

TABLE 3. Population Screening for Genetic Diseases and Carrier States

Individuals with Genetic Disease	Genetic Diseases
Newborns	Congenital hypothyroidism Galactosemia Phenylketonuria Sickle cell disease
Fetuses	Down syndrome Neural tube defects Trisomy 18
Heterozygous Carriers (in High-Risk Populations)	Sickle cell disease (African-Americans and Africans) Tay-Sachs disease (Ashkenazi Jews) ά-thalassemia (Chinese, Southeast Asians) β-thalassemia (Creeks, Italians)

3. The purpose of identifying individuals with a genetic predisposition to a disease is to enable them either to institute measures that will prevent or delay development of the disease or to deal with the disease more effectively if it does develop.

Criteria for screening. There are thousands of genetic diseases, but screening is used routinely for only a few. The most frequent examples are given in Table 3. Genetic screening is usually undertaken only when certain conditions relating to the disease, the screening test, and the system for implementing them are met.

1. Characteristics of the disease

a. The disease should be relatively frequent within the population screened. The more frequent the disease, the more effective will be the screening program. Some conditions are sufficiently common in the general population to warrant screening in everyone. This is called population screening. Other diseases are not common enough to justify screening the entire population but are sufficiently frequent within an easily identifiable subgroup. Examples include Tay-Sachs disease among Ashkenazi Jews, sickle-cell disease among African-Americans, and ά-thalassemia among Southeast Asians. In many uncommeon diseases,genetic screening can be justified only within families in which an affected individual has been born. DMD is such a condition.

b. The disease must produce severe impairment or death. Screening for a trivial genetic condition such as postminimal polydactyly, which produces a tiny extra digit, could not be justified because it can be safely and inexpensively treated and produces no long-term morbidity.

c. Some beneficial intervention must be possible if the condition is recognized. This intervention is usually an effective treatment or prevention strategy.

2. Characteristics of the test. An appropriate test must exist that is capable of identifying people who have the relevant condition. Appropriate tests should be highly sensitive and specific, have a reasonably high positive predictive value, and be relatively inexpensive to perform. a. Sensitivity is defined as the proportion of individuals affected by the disease or genotype in question who have a positive test. False-negative results are produced when individuals who have the disease or genotype have a negative test. A good test will have a very low frequency of false-negatives. Specificity is defined as the proportion of those who are not affected with the disease or genotype m question who have a negative test. False-positive results are produced when individuals who do not have the disease or genotype have a positive test. A good test will produce few false-positives. Positive predictive **value** is (he proportion of persons with a positive test who actually have the disease or genotype being tested for. The higher the positive predictive value, the lower the proportion of false-positives.

Expense. Tests applied to large populations are usually much less expensive to

perform if automation is possible. Economy can often be achieved by **combining more than one screening test.** For example, newborn screening for PKU and congenital hypothyroidism (which is usually not a monogenic disorder) is often done together to decrease **costs.**

3. Characteristics of the screening system. Effective genetic screening requires (he **use of** an appropriate test within the context of a **well-organized and comprehensive program for dealing with abnormal results.**

Genetic screening is usually a multistep process.

1. To avoid false-negatives, screening tests frequently produce a substantial number **of falspositive results.** a. In such circumstances, **all patients who have a positive result on** initial screening require a more definitive test to confirm that they exhibit the condition or geno-type of interest. b. A good example is provided by serum triple screening of pregnant women for Down syndrome fetuses. (1) Most women with abnormal triple screening tests are *not* carrying a fetus with Down syndrome (i.e., most positive triple screening tests are false-positives). (2) Additional testing by ultrasound and amniocentesis is required to distinguish true-positive from false-positive results.

2. Concentrating genetic testing in certain subpopulations known to have a higher fre-quency of the condition than the general population can be thought of as initial screening that precedes the test in some cases. For example, β-thatassemia is much more common among some ethnic groups (e.g., Italians, Greeks, and Africans) than among others. Performing genetic screening only in high-risk ethnic groups increases the efficiency of testing.

3. Screening is often most effective when information obtained from various sources is combined to determine the result. For example, the risk of Down syndrome in the fetus of a woman with an abnormal serum triple screen depends on the absolute values obtained in the test, the woman's ethnic origin, the gestational age, her weight,. whether she is carrying twins, and whether she has diabetes. All of these factors can be included in a single risk estimate.

Ethical and legal concerns regarding genetic screening. Although genetic screening programs are developed to benefit patients and families, they sometimes have a negative impact on screened populations. a. For example, people found to have a certain disease or genotype have sometimes been stigmatized so that they are unable to obtain employment or insurance. b. Education, both professional and public, is the most important tool in preventing stigmatization.

2. Genetic screening may be mandated by legislation, which can raise issues of personal conscience, privacy, and consent.

<div align="center">Future perspective: the genetic profile</div>

1. Recent advances in molecular genetics have increased the ability to identify individuals who have genotypes that produce disease (e.g., in Huntington disease), have genetic predispositions to disease (e.g., in hyperlipidemias), or are at high risk for having a child with a given disease (e.g., detection of heterozygous carriers for cystic fibrosis).

2. Predicting most of the diseases that each individual is likely to develop or pass on to his or her children should be possible if advances in genetic research continue. a. Such knowledge would enable patients to:

   (1) Alter their lives to reduce nongenetic risk factors of particular relevance and thereby forestall the diseases to which they are genetically predisposed

   (2) Take advantage of all available reproductive options if potential offspring carry genetic risks

b. Privacy. Appropriate safeguards will be necessary to make certain that knowledge of a person's genetic endowment is maintained with strictest confidentiality and does not result in discriminatory practices against him or her.

3. When a sufficiently large number of genotypes can be identified, every individual will be found to carry some disease-predisposing traits. Thus, genetic screening will be used to determine the individual genetic predispositions of every person rather than to identify "normal" or "abnormal" people.