

**MINISTRY OF SCIENCE AND HIGHER EDUCATION
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**FEDERAL STATE AUTONOMOUS EDUCATIONAL INSTITUTION OF
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NORTH-CAUCASUS FEDERAL UNIVERSITY**

Methodological Guidelines
for Students on arranging and performing laboratory works
in the Discipline of Medical Genetics
for Students majoring (Specialty) in 31.05.01 General Medicine

Laboratory work №1

Introduction to Medical Genetics. Methods of Medical Genetics.

Aim of the Session

To consolidate theoretical knowledge on the basic concepts, history, and methods of medical genetics, and to develop practical skills in analyzing and interpreting clinical and genetic data using genealogical, population-statistical, twin, and cytogenetic methods.

Learning Objectives

By the end of this session, students should be able to:

- Define key terms and concepts in medical genetics.
- Describe the historical stages of medical genetics and its foundational axioms.
- Explain the relationship between genomics and clinical medicine.
- Apply genealogical, twin, and population-statistical methods to solve genetic problems.
- Interpret karyotypes and identify chromosomal abnormalities.
- Understand the role of medical genetics in the structure of morbidity and disability.

Part 1. Theoretical Background (for Self-Study)

1.1. Basic Concepts of Medical Genetics

Medical genetics is a branch of genetics that studies the role of hereditary factors in human health and disease. It encompasses:

- Hereditary diseases (monogenic, chromosomal, multifactorial).
- Genetic predisposition to common diseases.
- The interaction between genotype and environment.

- Genetic counseling, prenatal diagnosis, and preventive measures.

1.2. History of Medical Genetics

Period	Key Event
1865	Mendel's laws of inheritance
1900	Rediscovery of Mendel's work
1902	Garrod: "inborn errors of metabolism"
1944	Avery, MacLeod, McCarty: DNA as genetic material
1953	Watson and Crick: DNA double helix
1960s–1970s	Chromosome banding techniques; prenatal diagnosis
1990–2003	Human Genome Project
2000s–present	Genomic medicine, NGS, CRISPR

1.3. Axioms (Fundamental Principles) of Medical Genetics

1. Hereditary material is organized in the form of genes located on chromosomes.
2. Genes are transmitted from parents to offspring according to Mendelian laws (with exceptions: imprinting, mitochondrial inheritance, etc.).
3. Mutations in genes or chromosomes can lead to pathological phenotypes.
4. Phenotype is the result of genotype interacting with environmental factors.
5. Genetic heterogeneity and clinical polymorphism are common features of hereditary diseases.

1.4. Genomics and Clinical Medicine

Genomics is the study of the complete set of DNA (genome) of an organism. In clinical medicine, genomics enables:

- Diagnosis of monogenic and complex diseases.

- Pharmacogenetics (individualized drug therapy).
- Gene therapy and genome editing.
- Cancer genomics and personalized oncology.

1.5. Subject and Objectives of Medical Genetics

Subject: Heredity and variation in humans, with emphasis on pathological traits.

Objectives:

- Identification of the genetic basis of diseases.
- Development of diagnostic, preventive, and therapeutic strategies.
- Genetic counseling and risk assessment.
- Public health genetics and screening programs.

1.6. The Increasing Proportion of Hereditary Pathology

Hereditary and congenital diseases account for a growing share of morbidity, disability, and mortality worldwide. The contribution of genetic factors is significant in:

- Pediatric diseases (congenital malformations, metabolic disorders).
- Multifactorial adult diseases (hypertension, diabetes, cancer).
- Rare diseases (orphan diseases) that impose a heavy burden on patients and healthcare systems.

Part 2. Methods of Medical Genetics

2.1. Clinical-Genealogical (Pedigree) Method

Purpose: To determine the mode of inheritance of a trait or disease within a family.

Practice Task 1. Construct a Pedigree

A proband is a woman with phenylketonuria (PKU), an autosomal recessive disease. Her healthy parents have two healthy brothers and one healthy sister. The

proband's husband is healthy but has a sister with PKU. Their two children are healthy.

- Construct a pedigree using standard symbols.
- Determine the genotypes of all family members.
- Calculate the recurrence risk for the next child.

2.2. Twin Method

Purpose: To estimate the relative contribution of genetic and environmental factors to a trait using concordance rates.

Formula (Holzinger's index):

$$H = (C_{MZ} - C_{DZ}) / (1 - C_{DZ})$$

where C_{MZ} = concordance in monozygotic twins, C_{DZ} = concordance in dizygotic twins.

Practice Task 2. Calculate Heritability

In a study of 50 MZ twin pairs and 100 DZ twin pairs, at least one twin had type 2 diabetes. Concordance was observed in 25 MZ pairs and 8 DZ pairs. Calculate the heritability (H) of type 2 diabetes. Interpret the result.

2.3. Population-Statistical Method (Hardy–Weinberg Principle)

Purpose: To calculate allele and genotype frequencies in populations.

Formula:

$$p + q = 1 \quad p^2 + 2pq + q^2 = 1$$

where p = frequency of dominant allele, q = frequency of recessive allele, p^2 = frequency of homozygous dominant, $2pq$ = frequency of heterozygotes, q^2 = frequency of homozygous recessive.

Practice Task 3. Calculate Carrier Frequency

Cystic fibrosis (CF) is an autosomal recessive disease with an incidence of 1 in 2,500 newborns in a European population.

- Calculate the frequency of the mutant allele (q) and the carrier frequency ($2pq$).
- How many carriers are expected in a city of 1,000,000 inhabitants?

2.4. Cytogenetic Methods (Karyotyping)

Practice Task 4. Karyotype Analysis

You are given a karyotype image (or description): 47, XX+21.

- How many chromosomes are present?
- What is the sex of the individual?
- Which chromosome shows an abnormality?
- What is the diagnosis? Describe the clinical features.

Additional Karyotype Exercises

Karyotype	Diagnosis	Clinical Features
45, X	Turner syndrome	Short stature, webbed neck, gonadal dysgenesis
47, XXY	Klinefelter syndrome	Tall stature, gynecomastia, infertility
47, XY+18	Edwards syndrome	Micrognathia, rocker-bottom feet, heart defects
47, XY+13	Patau syndrome	Cleft lip/palate, holoprosencephaly, polydactyly

2.5. Molecular-Genetic Methods (Overview)

Method	Application
PCR	Amplification of DNA for further analysis

Method	Application
Sanger sequencing	Detection of point mutations, small deletions
NGS	Whole-genome/exome sequencing; multi-gene panels
FISH	Detection of chromosomal rearrangements, microdeletions
MLPA	Detection of copy number variations
Southern blot	Detection of large DNA fragments (e.g., trinucleotide repeats)

Part 3. Questions for Self-Assessment

1. What is the object and subject of medical genetics?
2. List the main historical milestones in the development of medical genetics.
3. Explain the axioms of medical genetics and give examples.
4. How does genomics contribute to modern clinical medicine?
5. What are the objectives of medical genetics?
6. Why is the proportion of hereditary pathology increasing in the population?
7. Describe the genealogical method and its applications.
8. What is the twin method? How is heritability calculated?
9. How is the Hardy–Weinberg law used in medical genetics?
10. What cytogenetic and molecular-genetic methods are used to diagnose hereditary diseases?

Part 4. Case Studies

Case 1. Family with Huntington's Disease

A 45-year-old man presents with choreiform movements, cognitive decline, and behavioral changes. His father died at age 55 with similar symptoms. His grandfather was also affected. The patient has two children, aged 10 and 14.

- What is the most likely mode of inheritance?
- What is the genetic basis of the disease?
- What is the risk for each child to develop the disease?
- What molecular test would you recommend?

Case 2. Population Screening for Phenylketonuria (PKU)

PKU has a frequency of 1 in 10,000 newborns in a certain population.

- Calculate the frequency of the mutant allele (q).
- Calculate the carrier frequency ($2pq$).
- How many carriers are there per 100,000 people?

Case 3. Twin Study on Schizophrenia

A study reports concordance rates for schizophrenia: MZ twins – 48%, DZ twins – 17%.

- Calculate the heritability (H).
- What is the relative contribution of genetic and environmental factors?

Part 5. Assignment for Submission

1. Complete all Practice Tasks (1–4) and write the answers in your notebook.
2. Prepare a short report (2-3 pages) on the topic: "**The Role of Medical Genetics in Modern Healthcare.**"
3. Be prepared to present your pedigree analysis (Task 1) and karyotype interpretation (Task 4) during the session.

Laboratory work №2

Morphogenetic Variants of Development. Congenital Malformations.

Aim of the Session

To study the role of genetic and environmental factors in the development of congenital malformations, to analyze morphogenetic variants and their diagnostic significance, and to master the classification and mechanisms of developmental defects, while also acquiring practical skills in DNA extraction from patient samples for genetic analysis.

Learning Objectives

By the end of this session, students should be able to:

- Define and classify congenital malformations and morphogenetic variants.
- Explain the interaction between heredity and environment in pathology.
- Distinguish between primary, secondary, isolated, systemic, and multiple defects.
- Understand the etiological heterogeneity of congenital malformations.
- Identify critical periods of development and their significance.
- Classify mutations and describe their role in congenital anomalies.
- Explain the mutational process and the effects of environmental factors, including radiation.
- Perform basic DNA extraction from patient samples for molecular genetic analysis.

Part 1. Theoretical Background (for Self-Study)

1.1. The Role of Heredity and Environment in the Development of Pathology

The development of any phenotypic trait, normal or pathological, is the result of the interaction between the genotype and environmental factors.

Factor Type	Examples
Genetic	Gene mutations, chromosomal aberrations, polymorphisms
Environmental	Teratogens (drugs, alcohol, infections), nutrition, radiation
Interaction	Gene–environment interactions (G×E), epigenetic modifications

Key concept: The same genotype can produce different phenotypes under different environmental conditions (phenocopies), and the same phenotype can arise from different genetic causes (genocopies).

1.2. Morphogenetic Variants of Development

Morphogenetic variants are deviations from the typical developmental pathway that lead to structural or functional changes in the organism.

Genesis:

- Genetic: mutations in developmental genes (e.g., homeobox, growth factors).
- Environmental: exposure to teratogens during critical periods.
- Multifactorial: combination of genetic predisposition and environmental triggers.

Postnatal modification: Some morphogenetic variants can be modified after birth through surgery, therapy, or hormonal treatment.

1.3. General and Specific Morphogenetic Variants

Type	Description	Examples
General	Minor anomalies that are relatively common and non-specific	Epicanthus, clinodactyly, low-set ears
Specific	Distinctive features characteristic of a particular syndrome	Flat facial profile in Down syndrome, arachnodactyly in Marfan syndrome

Clinical significance: Morphogenetic variants, especially specific ones, are important diagnostic markers for hereditary syndromes and congenital conditions.

1.4. Classification of Hereditary Pathology

Category	Description	Examples
Monogenic (Mendelian)	Caused by mutations in a single gene	Cystic fibrosis, Huntington's disease, PKU

Category	Description	Examples
Chromosomal	Caused by numerical or structural chromosomal abnormalities	Down, Turner, Klinefelter syndromes
Multifactorial (polygenic)	Caused by multiple genes interacting with environmental factors	Diabetes, hypertension, neural tube defects
Mitochondrial	Caused by mutations in mtDNA	MELAS, MERRF, LHON
Epigenetic	Caused by changes in gene expression without DNA sequence alteration	Prader-Willi, Angelman syndromes

1.5. Congenital Malformations

Primary malformations: Result directly from a developmental defect (e.g., cleft lip, spina bifida).

Secondary malformations: Result from a primary defect or mechanical disruption (e.g., clubfoot due to oligohydramnios).

Isolated defects: Affect a single organ or system.

Systemic defects: Affect multiple structures within one organ system.

Multiple defects: Affect two or more organ systems (often syndromic).

1.6. Etiological Heterogeneity of Congenital Malformations

A single phenotype can be caused by different etiologies:

- **Locus heterogeneity:** Mutations in different genes cause the same phenotype (e.g., retinitis pigmentosa).
- **Allelic heterogeneity:** Different mutations in the same gene cause different phenotypes (e.g., FGFR3 mutations).
- **Genetic vs. environmental:** Phenocopies can mimic genetic syndromes (e.g., fetal alcohol syndrome vs. genetic causes of microcephaly).

1.7. The Teratogenic Termination Period (Critical Periods of Development)

Period	Gestational Age	Events	Teratogenic Effects
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Period	Gestational Age	Events	Teratogenic Effects
Pre-implantation	Weeks 0–2	Cell division, implantation	"All-or-nothing" effect (death or repair)
Embryonic	Weeks 3–8	Organogenesis	Major malformations (e.g., neural tube, heart, limbs)
Fetal	Weeks 9–38	Growth, maturation	Functional defects, minor anomalies, growth restriction

1.8. Mutations and Their Classification

Classification Criteria	Types
By cell type	Somatic, germline
By molecular nature	Point (missense, nonsense, silent), frameshift (indels), splice-site, regulatory
By effect on function	Loss-of-function (recessive), gain-of-function (dominant), dominant-negative
By scale	Gene (point), chromosomal (structural), genomic (aneuploidy, polyploidy)

1.9. The Mutational Process and Environmental Factors

Spontaneous mutations: Arise due to replication errors, DNA polymerase slippage, or spontaneous chemical changes (deamination, depurination).

Induced mutations: Caused by external mutagens:

- **Chemical mutagens:** Alkylating agents, base analogs, intercalating agents.
- **Physical mutagens:** Ionizing radiation (X-rays, gamma rays), non-ionizing radiation (UV light).
- **Biological mutagens:** Viruses, transposons.

Radiation mutagenesis:

- Ionizing radiation → DNA double-strand breaks → chromosomal aberrations.
- UV radiation → pyrimidine dimers → replication blocks and mutations (if unrepaired).

Part 2. Laboratory Procedures

2.1. Morphogenetic Variant Assessment (Dysmorphology Screening)

Objective: To recognize general and specific morphogenetic variants and use them as diagnostic clues.

Procedure:

1. Examine photographs or clinical descriptions of patients with known syndromes.
2. Identify and list morphogenetic variants (e.g., craniofacial, limb, skin anomalies).
3. Classify each variant as general or specific.
4. Correlate the variants with possible syndromes.

Practice Task 1. Dysmorphology Analysis

Patient	Findings	Suggested Syndrome
A	Flat facial profile, epicanthus, short neck, single palmar crease, hypotonia	
B	Long limbs, arachnodactyly, lens dislocation, aortic dilation	
C	Cleft lip, microphthalmia, polydactyly, holoprosencephaly	
D	Short stature, webbed neck, shield chest, cubitus valgus, ovarian dysgenesis	

Instructions: Fill in the suggested syndrome and list the morphogenetic variants. Explain which are general and which are specific.

2.2. Critical Periods of Development – Case Analysis

Practice Task 2. Teratogen Exposure Timing

A pregnant woman took a drug (thalidomide) during her 6th week of gestation and another drug during her 24th week. What malformations could result from each exposure, and why?

- Use the critical periods table to justify your answer.
- Describe the mechanisms of teratogenesis.

2.3. Classification of Congenital Malformations

Practice Task 3. Classify the Following Malformations

Malformation	Primary / Secondary	Isolated / Systemic / Multiple	Possible Etiology
Cleft lip with cleft palate			
Clubfoot due to oligohydramnios			
Ventricular septal defect (VSD)			
Holoprosencephaly with cyclopia			
Polydactyly as part of Patau syndrome			

2.4. Mutation Analysis (Practical Simulation)

Practice Task 4. Mutation Identification

You are given a DNA sequence from a patient with a suspected genetic disorder. The wild-type sequence is:

ATG GGC CTA GAA TTC GGG TAA

The patient's sequence is:

ATG GGC CTA GAA TTT CGG GTA A

- Identify the type of mutation (point, frameshift, etc.).
- Determine the effect on the protein (missense, nonsense, silent).

- Predict the clinical consequences.

2.5. DNA Extraction from Patient Samples (Laboratory Procedure)

Objective: To isolate high-molecular-weight DNA from buccal epithelial cells (or peripheral blood) for subsequent genetic analysis.

Materials:

- Buccal swab or blood sample (EDTA tube)
- Lysis buffer (SDS, Tris-HCl, EDTA, NaCl)
- Proteinase K
- Phenol:chloroform:isoamyl alcohol (25:24:1)
- 100% ethanol (ice-cold)
- 70% ethanol
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Microcentrifuge tubes, pipettes, water bath, centrifuge

Procedure:

Step	Procedure
1	Collect buccal cells by rotating a swab against the inner cheek (or use 200 μ L of blood). Place the swab (or blood) into a 1.5 mL microcentrifuge tube.
2	Add 400 μ L of lysis buffer and 20 μ L of Proteinase K (20 mg/mL). Vortex briefly.
3	Incubate at 56°C for 1 hour (or overnight) with occasional mixing to completely lyse cells and digest proteins.
4	Add 400 μ L of phenol:chloroform:isoamyl alcohol (25:24:1). Vortex vigorously for 30 seconds.
5	Centrifuge at 12,000 rpm for 10 minutes at 4°C. Carefully transfer the upper (aqueous) phase to a new tube.
6	Add 400 μ L of chloroform to the aqueous phase. Vortex and centrifuge again. Transfer the aqueous phase to a new tube.
7	Add 800 μ L of ice-cold 100% ethanol and 40 μ L of 3 M sodium acetate (pH 5.2). Invert gently to precipitate DNA.

Step	Procedure
8	Centrifuge at 12,000 rpm for 10 minutes. A white pellet of DNA should be visible. Discard the supernatant.
9	Wash the pellet with 500 μ L of 70% ethanol. Centrifuge for 5 minutes and discard the supernatant.
10	Air-dry the pellet for 10–15 minutes (do not over-dry). Resuspend the DNA in 50–100 μ L of TE buffer.
11	Store at -20°C for long-term storage, or at $+4^{\circ}\text{C}$ for short-term use.

Quality Control:

- Measure DNA concentration using a spectrophotometer (A_{260}/A_{280} ratio should be 1.8–2.0).
- Assess integrity by agarose gel electrophoresis (1% agarose).

Practice Task 5. DNA Extraction Report

Write a short report (1 page) describing:

- The principle of DNA extraction.
- Steps of the procedure you performed.
- Quality control results (concentration, purity, integrity).
- Potential applications of the extracted DNA in medical genetics.

Part 3. Questions for Self-Assessment

1. What is a morphogenetic variant? How does it differ from a malformation?
2. How do hereditary and environmental factors interact in the development of pathology?
3. What are general and specific morphogenetic variants? Give examples of each.
4. What is the significance of morphogenetic variants in diagnosing hereditary syndromes?
5. Classify hereditary pathology (monogenic, chromosomal, multifactorial, mitochondrial, epigenetic).
6. Differentiate between primary and secondary congenital malformations.
7. What are isolated, systemic, and multiple defects? Provide examples.
8. Explain the concept of etiological heterogeneity of congenital malformations.
9. What are the critical periods of development? Why are they important for teratogenesis?

10. List the main types of mutations and their effects on protein function.
11. How do environmental factors (chemical, physical, biological) cause mutations?
12. What are the mechanisms of radiation mutagenesis?
13. What is the purpose of DNA extraction in medical genetics?
14. What safety precautions should be observed when handling patient samples and chemicals?

Part 4. Case Studies

Case 1. Newborn with Multiple Anomalies

A newborn presents with:

- Cleft lip and palate
- Microphthalmia
- Polydactyly (six fingers on each hand)
- Holoprosencephaly on MRI
- Congenital heart defect
- What is the most likely diagnosis?
- What karyotype would you expect?
- What is the mode of inheritance?
- What critical period was likely affected?

Case 2. Family History of Neural Tube Defects

A woman who had a child with spina bifida is pregnant again. She has been taking folic acid (4 mg/day) since the first trimester. Her family history is unremarkable.

- What is the recurrence risk?
- How does folic acid act as a preventive measure?
- What environmental factors could increase the risk?

Case 3. Phenocopy or Genetic Syndrome?

A child has microcephaly, facial dysmorphism, growth restriction, and developmental delay. The mother admits to heavy alcohol consumption during early pregnancy. There is no family history of similar conditions.

- Is this condition more likely a phenocopy or a genetic syndrome?
- How would you differentiate it from a genetic cause (e.g., chromosomal microdeletion)?
- What diagnostic tests would you recommend?

Part 5. Assignment for Submission

1. Complete Practice Tasks 1–4 and submit your answers in a written report.
2. Write a laboratory report on the DNA extraction procedure (Practice Task 5), including:
 - Objective
 - Principle
 - Materials and methods
 - Results (concentration, purity, gel image)
 - Discussion (applications and troubleshooting)
3. Prepare a short presentation (5–7 slides) on one of the following topics:
 - "The Role of Morphogenetic Variants in the Diagnosis of Hereditary Syndromes"
 - "Critical Periods of Development and Teratogenesis"
 - "Radiation Mutagenesis and Its Clinical Implications"

Laboratory work №3

Gene Disorders (Monogenic Diseases). General Principles of Pathogenesis. Etiology, Classification, and Clinical Presentation.

Aim of the Session

To study the concept, classification, and pathogenesis of monogenic diseases, to analyze the clinical and genetic characteristics of autosomal dominant and recessive disorders, and to develop practical skills in PCR-based molecular diagnosis of a monogenic disease.

Learning Objectives

By the end of this session, students should be able to:

- Define monogenic disease and classify gene mutations.
- Describe the classification of monogenic disorders.
- Apply nomenclature rules for recording mutations in human genes.
- Explain genetic heterogeneity and clinical polymorphism.
- Understand the pathogenesis of monogenic diseases.
- Describe the clinical and genetic features of autosomal dominant and recessive disorders.
- Analyze hereditary motor-sensory neuropathy type 1A (CMT1A), channelopathies, cystic fibrosis, and SMA.
- Perform PCR for molecular diagnosis of cystic fibrosis or another monogenic disease.

Part 1. Theoretical Background (for Self-Study)

1.1. The Concept of Monogenic Disease

A **monogenic disease** (Mendelian disorder) is a disease caused by a mutation in a single gene. It follows classical Mendelian inheritance patterns: autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive, or Y-linked.

Types of gene mutations:

Mutation Type	Description	Example
Missense	Single nucleotide change resulting in substitution of one amino acid	Sickle cell anemia (HbS)
Nonsense	Single nucleotide change creating a premature stop codon	Duchenne muscular dystrophy (some cases)
Frameshift (insertion/deletion)	Addition or loss of nucleotides not multiple of 3 → altered reading frame	Cystic fibrosis (F508del is an in-frame deletion)
Splice-site	Mutation affecting mRNA splicing	β-Thalassemia (some cases)
Regulatory	Mutation in promoter or enhancer regions	Some forms of thalassemia

1.2. Classification of Monogenic Disorders

Inheritance Pattern	Features	Examples
Autosomal dominant	Affected individuals have one mutated allele; every generation affected; male and female equally affected; male-to-male transmission possible	Huntington's disease, Marfan syndrome, CMT1A
Autosomal recessive	Affected individuals are homozygous for the mutant allele; parents are usually carriers; often skips generations	Cystic fibrosis, PKU, SMA
X-linked recessive	Males affected more frequently; no male-to-male transmission; carrier females	Hemophilia A, Duchenne MD
X-linked dominant	Both sexes affected; affected males pass to all daughters; no male-to-male	Rett syndrome (lethal in males),

Inheritance Pattern	Features	Examples
	transmission	some forms of rickets
Y-linked (holandric)	Transmitted from father to all sons	Y-linked infertility (rare)
Mitochondrial	Maternally inherited; variable expressivity	MELAS, MERRF, LHON

1.3. Nomenclature for Recording Mutations in Human Genes

Mutations are recorded according to the **HGVS (Human Genome Variation Society)** guidelines.

Format: Gene symbol, transcript reference, description at DNA/RNA/protein level.

Examples:

- CFTR c.1521_1523delCTT (3-bp deletion leading to loss of phenylalanine at position 508 – F508del)
- HBB c.20A>T (point mutation in β -globin gene $\rightarrow$ HbS)
- HTT c.52CAG>(36 39) (trinucleotide repeat expansion)

1.4. Genetic Heterogeneity and Clinical Polymorphism

Concept	Definition	Example
Locus heterogeneity	Mutations in different genes produce the same phenotype	Retinitis pigmentosa (multiple genes)
Allelic heterogeneity	Different mutations in the same gene produce different phenotypes	FGFR3: achondroplasia, hypochondroplasia, thanatophoric dysplasia

Concept	Definition	Example
Clinical polymorphism	Same mutation → different severity or symptoms	Cystic fibrosis (mild vs. severe presentation), Huntington's disease (variable age of onset)

1.5. Pathogenesis of Monogenic Disorders

Mechanism	Description	Example
Loss-of-function	Reduced or absent protein function	Cystic fibrosis (CFTR channel defect)
Gain-of-function	Increased activity or new function of protein	Huntington's disease (toxic gain-of-function)
Dominant-negative	Mutant protein interferes with wild-type protein	Osteogenesis imperfecta (collagen defect)
Enzyme deficiency	Block in metabolic pathway	PKU (phenylalanine hydroxylase deficiency)
Structural protein defect	Abnormal protein formation	Epidermolysis bullosa (keratin defects)

1.6. Clinical and Genetic Characteristics of Monogenic Diseases

Autosomal dominant:

- Every generation affected
- 50% risk to offspring
- Late onset possible (e.g., Huntington's)

Autosomal recessive:

- Affected siblings may appear "skipped"
- Parents usually asymptomatic carriers
- 25% recurrence risk

1.7. Hereditary Motor-Sensory Neuropathy Type 1A (CMT1A)

- **Gene:** PMP22 (duplication on 17p11.2)
- **Inheritance:** Autosomal dominant
- **Clinical features:** Distal muscle weakness and wasting, foot drop, pes cavus, sensory loss, areflexia
- **Pathogenesis:** Overexpression of PMP22 disrupts peripheral nerve myelin
- **Diagnosis:** PCR-based dosage analysis, MLPA, or FISH

1.8. Channelopathies (Disorders of Ion Channels)

Disease	Affected Channel	Inheritance	Clinical Features
Cystic fibrosis	CFTR (Cl ⁻ channel)	AR	Pulmonary, pancreatic, reproductive defects
Congenital myotonia	Cl ⁻ channel (CLCN1)	AD/AR	Muscle stiffness
Long QT syndrome	K ⁺ or Na ⁺ channels	AD	Cardiac arrhythmias, syncope
Familial hemiplegic migraine	Ca ²⁺ channel (CACNA1A)	AD	Hemiplegia, migraine, ataxia

1.9. Cystic Fibrosis (Mucoviscidosis)

- **Gene:** CFTR (7q31.2)
- **Inheritance:** Autosomal recessive
- **Mutation:** Most common F508del (70% of alleles in Caucasian populations)
- **Pathogenesis:** Defective chloride transport → thick secretions in lungs, pancreas, liver, reproductive tract
- **Clinical triad:** Chronic lung disease, pancreatic insufficiency, elevated sweat chloride (>60 mmol/L)
- **Diagnosis:** Newborn screening (IRT), sweat test, CFTR sequencing

1.10. Proximal Spinal Muscular Atrophy (SMA)

- **Gene:** SMN1 (5q13.2)
- **Inheritance:** Autosomal recessive
- **Mutation:** Homozygous deletion of SMN1 (>95% of cases)
- **Pathogenesis:** Loss of SMN1 → reduced survival motor neuron protein → degeneration of anterior horn cells

- **Types:** SMA 0–4 (based on age of onset and motor milestones)
- **Diagnosis:** MLPA or qPCR (detection of SMN1 deletion)

Part 2. Laboratory Procedures

2.1. Pedigree Analysis – Monogenic Disorders

Practice Task 1. Pedigree Interpretation

Case 1 (Autosomal Dominant): A family with Huntington's disease. The proband is a 45-year-old man with chorea and cognitive decline. His father and paternal grandfather were affected.

Case 2 (Autosomal Recessive): A family with cystic fibrosis. Two healthy parents have a child with CF. The mother's sister also has CF.

Instructions:

- Construct a pedigree using standard symbols.
- Determine the mode of inheritance.
- Calculate the recurrence risk for future children.
- List the possible genotypes of family members.

2.2. Mutation Nomenclature

Practice Task 2. Interpret the Following Mutations

Mutation	Gene	Interpretation	Disease
CFTR c.1521_1523delCTT			
HBB c.20A>T			
HTT c.52CAG>(36-39)			

Instructions:

- Write the correct HGVS-style nomenclature.
- Identify the disease.
- Explain the effect on the protein.

2.3. Genetic Heterogeneity – Case Analysis

Practice Task 3. Heterogeneity in Retinitis Pigmentosa

A family has multiple members with retinitis pigmentosa (RP). Different affected individuals have been found to have mutations in different genes (e.g., RHO, RP1, USH2A).

- What type of heterogeneity is this?
- How does this affect genetic counseling and diagnostic testing?
- What molecular test would you recommend?

2.4. Pathogenesis and Clinical Presentation

Practice Task 4. Match the Disease to Its Pathogenic Mechanism

Disease	Pathogenic Mechanism
Cystic fibrosis	
Huntington's disease	
CMT1A	
PKU	
Duchenne MD	

Options: Loss-of-function, Gain-of-function, Dominant-negative, Enzyme deficiency, Structural protein defect, Toxic gain-of-function.

2.5. PCR Analysis of a Monogenic Disease (Cystic Fibrosis – F508del Detection)

Objective: To perform a PCR-based screening for the F508del mutation in the CFTR gene.

Principle: PCR amplifies the target region of the CFTR gene. The F508del mutation (3-bp deletion) is detected by fragment length analysis (electrophoresis) or allele-specific amplification.

Materials:

- DNA samples (positive control for F508del, negative control, patient samples)

- PCR primers specific for the CFTR exon 10 region (F508del)
- dNTPs, Taq polymerase, reaction buffer, MgCl₂
- Thermal cycler
- Agarose gel electrophoresis equipment

Primer sequences :

- Forward: 5' GTT TTC CTG GAT TAT GCC TGG CAC 3'
- Reverse: 5' GTT GGC ATG CTT TGA TGA CGC TTC 3'

PCR Protocol:

Step	Temperature	Time	Cycles
Initial denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	35
Annealing	60°C	30 sec	
Extension	72°C	45 sec	
Final extension	72°C	5 min	1

Expected fragment sizes:

- Normal allele: ~98 bp (including 3-bp sequence)
- Mutant allele (F508del): ~95 bp

Results interpretation:

- Two bands (98 bp and 95 bp) → heterozygous carrier
- One 95 bp band → homozygous affected
- One 98 bp band → normal homozygous

Practice Task 5. PCR Analysis Report

Write a report including:

- Objective
- Principle of F508del detection
- PCR conditions and primer sequences
- Gel electrophoresis results (draw or attach image)
- Interpretation and conclusion
- Clinical significance of the result

2.6. Channelopathies – Clinical Case Discussion

Case Study: Long QT Syndrome

A 16-year-old girl experiences syncope during exercise. Her ECG shows a prolonged QTc interval (480 ms). Her mother had a similar episode and was diagnosed with long QT syndrome. Genetic testing reveals a mutation in KCNQ1.

- What is the mode of inheritance?
- What is the pathophysiological mechanism?
- What treatment options are available?
- What is the risk to her future children?

Part 3. Questions for Self-Assessment

1. What is a monogenic disease?
2. List and describe the types of gene mutations.
3. Classify monogenic disorders by inheritance pattern.
4. How are mutations recorded according to HGVS?
5. What is genetic heterogeneity? Provide examples.
6. What is clinical polymorphism? Give an example.
7. Describe the pathogenesis of monogenic diseases.
8. What are the clinical features of CMT1A?
9. What are channelopathies? Provide three examples.
10. Describe the etiology and pathogenesis of cystic fibrosis.
11. What is the role of SMN1 in spinal muscular atrophy?
12. How is cystic fibrosis diagnosed molecularly?
13. What are the ethical considerations in genetic testing for monogenic disorders?

Part 4. Case Studies

Case 1. Family with CMT1A

A 30-year-old man presents with progressive weakness in his feet, high-arched feet (pes cavus), and difficulty walking. His father and paternal grandmother had similar symptoms.

- What is the most likely diagnosis?
- What is the mode of inheritance?
- What genetic test would you recommend?
- What is the recurrence risk for his children?

Case 2. Newborn with SMA Type 1

A newborn presents with severe hypotonia, weak cry, tongue fasciculations, and areflexia. Genetic testing reveals a homozygous deletion of SMN1 exon 7.

- What is the diagnosis?
- What is the prognosis?
- How is SMA inherited?
- What is the role of SMN2 in modifying the phenotype?

Case 3. Prenatal Diagnosis for Cystic Fibrosis

A couple, both carriers of the F508del mutation, is planning a pregnancy.

- What is the risk of having an affected child?
- What prenatal diagnostic options are available?
- What are the benefits and limitations of each option?
- What are the ethical considerations?

Part 5. Assignment for Submission

1. Complete Practice Tasks 1–5 and submit your written answers.
2. Write a laboratory report on the PCR analysis for F508del (Practice Task 5), including:
 - Objective
 - Principle
 - Materials and methods
 - Results (gel image and interpretation)
 - Discussion (clinical significance, limitations)
3. Prepare a short presentation (5–7 slides) on one of the following topics:
 - "Molecular Diagnosis of Cystic Fibrosis"
 - "The Spectrum of Channelopathies"
 - "Genetic Counseling in Spinal Muscular Atrophy"

Laboratory work №4

Chromosomal Disorders (Chromosomal Diseases)

Aim of the Session

To study the general characteristics, etiology, pathogenesis, classification, and clinical features of chromosomal disorders, to master cytogenetic diagnostic methods, and to develop practical skills in microscopic analysis of human chromosomes in normal and pathological karyotypes.

Learning Objectives

By the end of this session, students should be able to:

- Describe the general characteristics and etiology of chromosomal disorders.
- Understand the cytogenetic basis and pathogenesis of chromosomal abnormalities.
- Classify chromosomal abnormalities (numerical and structural).
- Recognize the general clinical features of chromosomal disorders.
- Apply diagnostic methods for chromosomal disorders (karyotyping, FISH, molecular techniques).
- Evaluate therapeutic and rehabilitation options for patients.
- Analyze normal and pathological chromosome preparations under a microscope.

Part 1. Theoretical Background (for Self-Study)

1.1. General Characteristics and Etiology of Chromosomal Disorders

Chromosomal disorders are conditions caused by abnormalities in chromosome number or structure. They are a major cause of congenital malformations, intellectual disability, and reproductive failure.

Etiology:

- **Numerical abnormalities:** Nondisjunction during meiosis or mitosis (e.g., trisomy, monosomy)
- **Structural abnormalities:** Chromosome breakage and rearrangement (e.g., deletions, duplications, inversions, translocations)
- **Parental factors:** Advanced maternal age (nondisjunction), balanced translocations in parents

- **Environmental factors:** Radiation, chemical mutagens, viral infections (less common)

1.2. Cytogenetics of Chromosomal Disorders

Cytogenetics is the study of chromosomes and their structure, function, and abnormalities.

Key techniques:

- **Karyotyping:** Arrangement of metaphase chromosomes by size, centromere position, and banding pattern.
- **Banding methods:**
 - **G-banding (GTG):** Most common; reveals dark and light bands (G-positive and G-negative).
 - **Q-banding:** Fluorescent bands (Q-positive and Q-negative).
 - **R-banding:** Reverse pattern of G-banding.
 - **C-banding:** Stains constitutive heterochromatin (centromeric regions).
- **FISH (Fluorescence In Situ Hybridization):** Detects specific DNA sequences using fluorescent probes.
- **SKY (Spectral Karyotyping):** Uses multiple fluorescent labels to identify all chromosomes.

1.3. Pathogenesis of Chromosomal Disorders

Mechanisms:

- **Gene dosage imbalance:** Extra or missing chromosomal material leads to altered expression of hundreds of genes.
- **Critical regions:** Specific regions are more sensitive to dosage changes (e.g., 22q11.2 in DiGeorge syndrome).
- **Disruption of developmental pathways:** Leads to complex pleiotropic phenotypes (e.g., congenital heart defects, craniofacial anomalies, intellectual disability).
- **Mosaicism:** Presence of two or more cell lines with different karyotypes; severity depends on the proportion of abnormal cells.

1.4. Classification of Chromosomal Abnormalities

Numerical abnormalities:

Type	Description	Examples
Aneuploidy	Loss or gain of one or more	Trisomy 21, 18, 13; Monosomy

Type	Description	Examples
	chromosomes	X (Turner)
Polyploidy	Extra set(s) of chromosomes (3n, 4n)	Triploidy (lethal)

Structural abnormalities:

Type	Description	Examples
Deletion	Loss of a chromosome segment	22q11.2 deletion (DiGeorge), 5p- (Cri-du-chat)
Duplication	Gain of a chromosome segment	Charcot-Marie-Tooth type 1A (PMP22 duplication)
Inversion	Reversal of a chromosome segment	Pericentric inversion 9 (often benign)
Translocation	Exchange of segments between chromosomes	Robertsonian translocation (e.g., 13;14), balanced reciprocal translocation
Ring chromosome	Chromosome forms a ring after breaks	Ring chromosome 13, 18
Isochromosome	Loss of one arm and duplication of the other	Isochromosome Xq in Turner syndrome

1.5. General Clinical Characteristics of Chromosomal Disorders

Feature	Description
Growth	Prenatal and postnatal growth retardation
Craniofacial	Dysmorphic features (microcephaly, epicanthus, low-set ears, hypertelorism)

Feature	Description
Neurological	Intellectual disability, developmental delay, hypotonia, seizures
Cardiovascular	Congenital heart defects (VSD, ASD, PDA)
Other	Skeletal anomalies, genitourinary malformations, organomegaly
Variable expressivity	Same abnormality may present with different severity (e.g., mosaic vs. non-mosaic)

1.6. Diagnostic Methods for Chromosomal Disorders

Method	Application
Karyotyping (G-banding)	Detection of aneuploidies and large structural abnormalities; resolution ~5–10 Mb
FISH	Detection of specific microdeletions, duplications, or gene rearrangements
Array CGH (aCGH)	Detection of submicroscopic deletions/duplications; resolution ~50–100 kb
MLPA	Detection of copy number variations in specific regions
NIPT (Non-Invasive Prenatal Testing)	Screening for fetal aneuploidies using cell-free fetal DNA from maternal blood
Prenatal invasive methods	Amniocentesis, CVS for fetal karyotyping

1.7. Therapeutic and Rehabilitation Options for Patients

- **Symptomatic treatment:** Management of congenital heart defects, feeding difficulties, seizures, infections.

- **Early intervention:** Physical, occupational, and speech therapy; educational support.
- **Growth hormone therapy:** For Turner syndrome and some other conditions.
- **Hormonal replacement:** Estrogen therapy in Turner syndrome; testosterone in Klinefelter syndrome.
- **Surgical correction:** Repair of cleft lip/palate, cardiac defects, gastrointestinal anomalies.
- **Genetic counseling:** Recurrence risk assessment, family planning, and psychosocial support.
- **Multidisciplinary care:** Regular follow-up with specialists (pediatricians, cardiologists, endocrinologists, neurologists).

Part 2. Laboratory Procedures

2.1. Microscopic Analysis of Chromosome Preparations (Normal and Abnormal Karyotypes)

Objective: To identify and analyze normal and abnormal human chromosomes using microscopic examination of metaphase chromosome spreads.

Materials:

- Prepared microscope slides of metaphase chromosome preparations (G-banded)
- Light microscope with 100× oil immersion objective
- Karyotype reference chart (Denver classification)
- Patient karyotype images (normal and pathological)

Procedure:

Step	Procedure
1	Place the slide on the microscope stage and focus under low power (10×) to locate metaphase spreads.
2	Switch to high power (40×) and then to oil immersion (100×) for detailed observation.
3	Identify chromosomes based on size, centromere position (metacentric, submetacentric, acrocentric, telocentric), and banding pattern.
4	Count the total number of chromosomes.

Step	Procedure
5	Identify the sex chromosomes (XX or XY).
6	Compare the observed karyotype with a standard karyotype chart.
7	Detect any numerical or structural abnormalities.

Practice Task 1. Karyotype Analysis

You are given five different karyotype images (or slides). For each, complete the following table:

Karyotype	Total Chromosomes	Sex	Abnormality	Diagnosis
A				
B				
C				
D				
E				

Instructions:

- Draw or describe the karyotype.
- List the clinical features associated with each syndrome.
- Explain the mechanism of formation (nondisjunction, translocation, deletion).

2.2. Cytogenetic Banding Pattern Recognition

Practice Task 2. Identify Chromosomes by Banding Patterns

Using a standard G-banding ideogram, match the following band designations to the correct chromosome:

Band Designation	Chromosome	Region/Feature
18q21		

Band Designation	Chromosome	Region/Feature
7q11.23		
5p15.2		
Xq28		
22q11.2		

Instructions:

- Identify the chromosome.
- Name a disease or syndrome associated with a deletion or duplication in that region.

2.3. FISH Analysis – Microdeletion Detection

Practice Task 3. FISH Interpretation

A FISH probe specific for the 22q11.2 region (DiGeorge syndrome) was used on a patient's metaphase spread. The result shows two red signals on one chromosome 22 and one red signal on the other.

- What does this indicate?
- What is the most likely diagnosis?
- What clinical features would you expect?
- What is the mode of inheritance?
- What other diagnostic tests would you recommend?

2.4. Classification of Chromosomal Abnormalities

Practice Task 4. Classify the Following Abnormalities

Abnormality	Type (Numerical/Structural)	Specific Type	Associated Syndrome
47, XY+18			
46, XX, del(5p)			

Abnormality	Type (Numerical/Structural)	Specific Type	Associated Syndrome
47, XXX			
46, XY, t(11;22)			
45, X/46, XX			

2.5. Pathogenesis and Clinical Correlations

Practice Task 5. Mechanism of Nondisjunction

A child is born with Down syndrome. The mother is 42 years old.

- What is the most likely mechanism causing the trisomy?
- At what stage of meiosis did the error occur?
- What is the recurrence risk for a subsequent pregnancy?
- How does maternal age affect the risk?

Practice Task 6. Mosaicism

A patient has a mild form of Turner syndrome. Karyotyping reveals 45, X/46, XX mosaicism (20% 45, X; 80% 46, XX).

- How does mosaicism arise?
- Why is the phenotype milder than in non-mosaic Turner syndrome?
- How does mosaicism affect diagnosis and genetic counseling?

2.6. Diagnostic Methods – Case Scenarios

Case 1. Prenatal Diagnosis

A 35-year-old pregnant woman undergoes amniocentesis due to advanced maternal age. The fetal karyotype is 47, XY+18.

- What is the diagnosis?
- What clinical features are associated with this condition?
- What is the prognosis?
- What are the ethical considerations in managing this pregnancy?

Case 2. Adult with Infertility

A 28-year-old man presents with azoospermia and small testes. Karyotyping reveals 47, XXY.

- What is the diagnosis?
- What are the clinical features?
- How is this condition treated?
- What is the risk of passing the abnormality to offspring?

Case 3. Child with Developmental Delay

A 5-year-old child has microcephaly, dysmorphic facies, cleft palate, and a heart defect. FISH reveals a 22q11.2 deletion.

- What is the diagnosis?
- What is the underlying genetic mechanism?
- What other clinical features may be present?
- What is the recurrence risk for the parents?

Part 3. Questions for Self-Assessment

1. What are the general characteristics of chromosomal disorders?
2. What are the main etiological factors leading to chromosomal abnormalities?
3. Describe the role of cytogenetics in diagnosing chromosomal disorders.
4. What is the pathogenesis of chromosomal disorders?
5. Classify chromosomal abnormalities with examples.
6. What are the general clinical features of chromosomal disorders?
7. What diagnostic methods are used for chromosomal disorders?
8. What are the therapeutic and rehabilitation options for patients with chromosomal disorders?
9. What is the difference between G-banding and FISH?
10. How does mosaicism arise and how does it affect phenotype?
11. What is the clinical significance of a balanced translocation carrier?
12. How does maternal age influence the risk of trisomy?

Part 4. Case Studies

Case 1. Newborn with Multiple Anomalies

A newborn presents with microcephaly, bilateral cleft lip and palate, microphthalmia, polydactyly, and a heart defect. Karyotyping reveals 47, XY+13.

- What is the diagnosis?
- What is the prognosis?
- What is the mode of inheritance?

- What are the management options?

Case 2. Adolescent with Short Stature

A 15-year-old girl is evaluated for short stature. She has a webbed neck, low posterior hairline, broad chest with widely spaced nipples, and delayed puberty. Karyotyping reveals 45, X.

- What is the diagnosis?
- What are the treatment options?
- What is the risk of infertility?
- What other health issues should be monitored?

Case 3. Couple with Recurrent Miscarriages

A couple has had three spontaneous miscarriages in the first trimester. The female partner is phenotypically normal. Karyotyping reveals she carries a balanced Robertsonian translocation (45, XX, t(13;14)).

- What is a balanced translocation?
- How does it lead to recurrent miscarriages?
- What prenatal diagnostic options are available?

Part 5. Assignment for Submission

1. Complete Practice Tasks 1–6 and submit your written answers.
2. Write a laboratory report on the microscopic analysis of chromosome preparations (Practice Task 1), including:
 - Objective
 - Materials and methods
 - Observations (normal and abnormal karyotypes)
 - Interpretation (diagnosis and clinical correlation)
 - Discussion (limitations of karyotyping, role of molecular cytogenetics)
3. Prepare a short presentation (5–7 slides) on one of the following topics:
 - "Microscopic Analysis of Human Chromosomes in Normal and Pathological Karyotypes"
 - "The Role of FISH and Array CGH in Modern Cytogenetics"
 - "Genetic Counseling in Families with Chromosomal Disorders"

Laboratory work №5

Clinical and Genetic Characteristics of Syndromes Associated with Autosomal Numerical Abnormalities

Aim of the Session

To study the classification, etiology, pathogenesis, clinical features, diagnosis, and management of the most common autosomal numerical abnormalities, and to develop practical skills in karyotype interpretation and clinical case analysis.

Learning Objectives

By the end of this session, students should be able to:

- Classify chromosomal disorders.
- Describe the etiology, pathogenesis, clinical features, and management of Down, Edwards, Patau, and Turner syndromes.
- Interpret karyotypes associated with these syndromes.
- Recognize the clinical features from case descriptions and photographs.
- Understand the principles of diagnosis and genetic counseling.

Part 1. Theoretical Background (for Self-Study)

1.1. Classification of Chromosomal Disorders

Category	Subtype	Examples
Numerical abnormalities	Aneuploidy (trisomy, monosomy)	Down, Edwards, Patau, Turner
	Polyploidy (triploidy, tetraploidy)	Lethal in most cases
Structural abnormalities	Deletions, duplications, inversions, translocations, ring chromosomes, isochromosomes	Cri-du-chat, DiGeorge, CMT1A

Aneuploidies are caused by nondisjunction during meiosis or mitosis.

1.2. Down Syndrome (Trisomy 21)

Etiology:

- 95%: Nondisjunction during maternal meiosis I (most common) or II.
- 3–4%: Robertsonian translocation (t(14;21), t(21;21)).
- 1–2%: Mosaicism (47,XX+21 / 46,XX or 47,XY+21 / 46,XY).

Pathogenesis:

- Overexpression of genes on chromosome 21 (e.g., *DYRK1A*, *APP*, *SOD1*).
- Leads to altered neurodevelopment, cardiac and gastrointestinal malformations, and accelerated aging.

Clinical features:

- **Facial:** Brachycephaly, epicanthus, flat nasal bridge, small ears, protruding tongue.
- **Musculoskeletal:** Hypotonia, short stature, hyperflexibility.
- **Cardiovascular:** Congenital heart defects (AVSD, VSD, ASD) – 40–50% of cases.
- **Gastrointestinal:** Duodenal atresia, Hirschsprung disease.
- **Neurological:** Intellectual disability (mild to moderate), early-onset Alzheimer's disease.
- **Other:** Increased risk of leukemia, hypothyroidism, hearing loss.

Management:

- Multidisciplinary care: cardiology, gastroenterology, endocrinology, audiology, speech therapy.
- Early intervention programs (physical, occupational, speech therapy).
- Regular screening for thyroid dysfunction, hearing loss, and leukemia.
- Lifelong medical follow-up.

1.3. Edwards Syndrome (Trisomy 18)

Etiology:

- Complete trisomy 18 (most common): Nondisjunction during maternal meiosis.
- Mosaicism and translocations are rarer.

Pathogenesis:

- Overexpression of genes on chromosome 18 disrupts fetal development, causing severe growth retardation and organ dysmorphogenesis.

Clinical features:

- **Growth:** Intrauterine growth restriction (IUGR), microcephaly.
- **Facial:** Prominent occiput, micrognathia, low-set ears, small mouth.
- **Limb:** Clenched hands with overlapping fingers (index over middle, fifth over fourth), rocker-bottom feet.
- **Cardiovascular:** VSD, PDA.
- **Other:** Omphalocele, severe intellectual disability.

Prognosis:

- Median life expectancy: 5–15 days; <10% survive to 1 year.

Management:

- Palliative care, nutritional support, seizure control.
- Surgical interventions considered only if compatible with quality of life.

1.4. Patau Syndrome (Trisomy 13)

Etiology:

- Complete trisomy 13 due to maternal meiotic nondisjunction.
- Mosaic and translocation forms are less common.

Pathogenesis:

- Overexpression of genes on chromosome 13 → severe midline defects and neural tube anomalies.

Clinical features:

- **Craniofacial:** Microcephaly, holoprosencephaly (midline brain fusion defect), cleft lip/palate, microphthalmia.
- **Limb:** Polydactyly (extra digits).
- **Cardiovascular:** VSD, ASD, PDA.
- **Other:** Omphalocele, severe intellectual disability.

Prognosis:

- Most infants die within the first weeks of life; survival beyond 1 year is extremely rare.

Management:

- Palliative care, nutritional support, anticonvulsants.
- Surgical decisions are individualized.

1.5. Turner Syndrome (Shereshevsky–Turner Syndrome; 45,X)

Etiology:

- 45,X (monosomy X) – most common (paternal nondisjunction in ~80% of cases).
- Mosaicism (45,X/46,XX; 45,X/46,XY).
- Structural abnormalities: isochromosome Xq, ring X, partial deletions.

Pathogenesis:

- Haploinsufficiency of genes that escape X-inactivation (e.g., *SHOX*, *RPS4X*) → short stature, skeletal anomalies, ovarian failure, cardiovascular/renal malformations.

Clinical features:

- **Growth:** Short stature (adult height ~140–145 cm), lymphedema of hands and feet (in newborns).
- **Craniofacial:** Webbed neck, low posterior hairline, broad chest with widely spaced nipples (shield chest).
- **Skeletal:** Cubitus valgus.
- **Reproductive:** Ovarian dysgenesis (streak gonads) → infertility, delayed puberty.
- **Cardiovascular:** Bicuspid aortic valve, coarctation of the aorta.
- **Intelligence:** Normal, but some have difficulties with spatial perception and executive function.

Management:

- Growth hormone therapy (GH) – improves final height.
- Estrogen replacement therapy at puberty – for secondary sexual characteristics and bone health.
- Cardiac monitoring (echocardiography), hearing aids.
- Reproductive assistance (oocyte donation, IVF).
- Multidisciplinary long-term care.

Part 2. Laboratory Procedures

2.1. Karyotype Interpretation – Numerical Abnormalities

Practice Task 1. Karyotype Analysis

You are given five karyotype images (or descriptions). For each, complete the following table:

Karyotype	Total Chromosomes	Sex	Abnormality	Diagnosis
47, XX+21				
47, XY+18				
47, XX+13				
45, X				
47, XXY				

Instructions:

- Identify the syndrome.
- List three clinical features for each syndrome.
- Describe the mechanism of formation (nondisjunction, translocation, mosaicism).

2.2. Clinical Case Matching

Practice Task 2. Match the Case Description to the Syndrome

Case	Description	Syndrome
A	Newborn with microcephaly, cleft lip/palate, polydactyly, and holoprosencephaly	
B	Infant with prominent occiput, micrognathia, clenched hands, and rocker-bottom feet	
C	Child with brachycephaly, epicanthus, hypotonia, AVSD, and intellectual disability	
D	Adolescent girl with short stature, webbed neck, shield chest, and ovarian dysgenesis	
E	Male with tall stature, gynecomastia, small testes, and infertility	

Instructions:

- Identify the syndrome.
- State the karyotype.
- Describe the pathogenesis.
- List management strategies.

2.3. Nondisjunction and Recurrence Risk

Practice Task 3. Mechanism of Nondisjunction

A couple has a child with Down syndrome. The mother is 38 years old.

- What is the most likely mechanism causing the trisomy?
- At what stage of meiosis did the error likely occur?
- What is the recurrence risk for a subsequent pregnancy?
- How does maternal age affect the risk?

Practice Task 4. Translocation Down Syndrome

A child has Down syndrome. Karyotyping reveals 46, XX, t(14;21). The mother has a balanced translocation (45, XX, t(14;21)).

- How does this differ from the typical nondisjunction case?
- What is the recurrence risk for the parents?
- What prenatal diagnostic options are available?

2.4. Mosaicism – Clinical Significance

Practice Task 5. Mosaic Trisomy 21

A child has features of Down syndrome but is less severely affected. Karyotyping reveals 47,XX+21 / 46,XX (30% / 70%).

- How does mosaicism arise?
- Why is the phenotype milder?
- What is the recurrence risk?
- How is the diagnosis confirmed?

2.5. Clinical Management and Prognosis

Practice Task 6. Management Plan

For each syndrome, prepare a brief management plan including:

Syndrome	Multidisciplinary Team	Key Interventions	Prognosis
Down syndrome			
Edwards syndrome			
Patau syndrome			
Turner syndrome			

2.6. Prenatal Diagnosis – Case Scenarios

Case 1. Advanced Maternal Age

A 40-year-old pregnant woman undergoes first-trimester screening. The results show an increased risk for trisomy 21.

- What non-invasive and invasive prenatal diagnostic options are available?
- What are the advantages and limitations of each?
- What are the ethical considerations?

Case 2. Ultrasound Findings

A fetal ultrasound at 20 weeks reveals a nuchal translucency, hydrops fetalis, and a heart defect. The mother is 35 years old.

- What chromosomal abnormality should be suspected?
- What diagnostic test would you recommend?
- What is the prognosis?

Part 3. Questions for Self-Assessment

1. Classify chromosomal disorders.
2. What is the etiology of Down syndrome?
3. Describe the pathogenesis of trisomy 21.
4. List the clinical features of Down syndrome.
5. What are the management strategies for Down syndrome?
6. What is the etiology and pathogenesis of Edwards syndrome?
7. What are the clinical features of trisomy 18?
8. What is the prognosis for Edwards syndrome?
9. Describe the etiology, pathogenesis, and clinical features of Patau syndrome.

10. What is Turner syndrome? How does it arise?
11. What are the clinical features and management options for Turner syndrome?
12. How does mosaicism affect the phenotype of chromosomal disorders?
13. What is the role of genetic counseling in families with autosomal numerical abnormalities?
14. What are the ethical issues in prenatal diagnosis?

Part 4. Case Studies

Case 1. Newborn with Multiple Anomalies

A newborn presents with microcephaly, bilateral cleft lip and palate, microphthalmia, polydactyly, and a heart defect. Karyotyping reveals 47, XY+13.

- What is the diagnosis?
- What is the prognosis?
- What management options are available?
- What is the recurrence risk for the parents?

Case 2. Adolescent with Short Stature

A 15-year-old girl is evaluated for short stature. She has a webbed neck, low posterior hairline, broad chest with widely spaced nipples, and delayed puberty. Karyotyping reveals 45, X.

- What is the diagnosis?
- What treatment options are available?
- What is the risk of infertility?
- What other health issues should be monitored?

Case 3. Child with Global Developmental Delay

A 3-year-old child has delayed milestones, flat facial profile, epicanthus, and hypotonia. A heart murmur is detected. Karyotyping reveals 47, XX+21.

- What is the diagnosis?
- What additional health problems should be monitored?
- What is the role of early intervention?

Case 4. Couple with Recurrent Pregnancy Loss

A couple has had two miscarriages. Cytogenetic analysis of fetal tissue reveals trisomy 18. The parents are phenotypically normal.

- What is the risk of recurrence?

- What chromosomal abnormality is most likely in the parents?
- What prenatal testing options are available?

Part 5. Assignment for Submission

1. Complete Practice Tasks 1–6 and submit your written answers.
2. Write a laboratory report on karyotype analysis, including:
 - Objective
 - Materials and methods
 - Observations (normal and abnormal karyotypes)
 - Interpretation (diagnosis and clinical correlation)
 - Discussion (limitations of karyotyping, role of molecular cytogenetics)
3. Prepare a short presentation (5–7 slides) on one of the following topics:
 - "Down Syndrome: From Prenatal Diagnosis to Lifelong Care"
 - "Turner Syndrome: Diagnosis, Management, and Quality of Life"
 - "Ethical Issues in Prenatal Diagnosis of Chromosomal Abnormalities"

Laboratory work №6

Diseases with Non-Traditional Inheritance Patterns

Aim of the Session

To study the mechanisms, inheritance patterns, pathogenesis, clinical features, and diagnosis of diseases with non-Mendelian inheritance, including X-linked, Y-linked, mitochondrial, imprinting, trinucleotide repeat, prion, and multifactorial disorders.

Learning Objectives

By the end of this session, students should be able to:

- Describe sex-linked inheritance patterns (X-linked recessive, X-linked dominant, Y-linked).
- Explain the etiology, pathogenesis, and clinical features of Duchenne/Becker muscular dystrophy.
- Characterize mitochondrial diseases and their maternal inheritance.
- Understand the mechanisms of genomic imprinting and associated disorders.
- Explain trinucleotide repeat expansion diseases and anticipation.
- Describe prion diseases and their unique pathogenesis.
- Discuss diseases with hereditary predisposition (multifactorial diseases).

Part 1. Theoretical Background (for Self-Study)

1.1. X-Linked Recessive Inheritance. Duchenne and Becker Muscular Dystrophy

Duchenne Muscular Dystrophy (DMD)

- **Gene:** *DMD* (Xp21.2)
- **Protein:** Dystrophin
- **Inheritance:** X-linked recessive
- **Mutation:** Frameshift or nonsense mutations → absence of dystrophin
- **Onset:** 2–5 years
- **Clinical features:** Progressive proximal muscle weakness, calf pseudohypertrophy, Gower's sign, elevated CK, wheelchair by age 12, death from cardiac/respiratory failure by 20–30 years

Becker Muscular Dystrophy (BMD)

- Milder form; in-frame deletions → partially functional dystrophin
- Later onset, slower progression

Diagnosis: DNA analysis (MLPA, NGS), muscle biopsy (immunohistochemistry)

1.3. Holandric (Y-Linked) Inheritance

- Genes on Y chromosome transmitted exclusively from father to sons.
- No female-to-male transmission.
- Examples: Y-linked infertility (AZF deletions), hairy ears (rare).

1.4. Mitochondrial Diseases

- Caused by mutations in mtDNA or nuclear genes affecting mitochondrial function.
- **Maternal inheritance:** mtDNA transmitted through the egg.
- **Pathogenesis:** Oxidative phosphorylation defects → reduced ATP, increased ROS.
- **Examples:** MELAS, MERRF, LHON, Kearns–Sayre syndrome.
- **Diagnosis:** Muscle biopsy (ragged red fibers), enzyme assays, mtDNA sequencing.

1.5. Diseases of Genomic Imprinting

- **Imprinting:** Epigenetic phenomenon – genes expressed in a parent-of-origin-specific manner.
- **Mechanisms:** Deletions, uniparental disomy, epimutations.

Syndrome	Defect	Clinical Features
Prader-Willi	Paternal 15q11.2-q13 deletion	Hypotonia, hyperphagia, obesity, hypogonadism, mild ID
Angelman	Maternal 15q11.2-q13 deletion (UBE3A)	Severe ID, ataxia, absence of speech, seizures, happy demeanor

1.6. Trinucleotide Repeat Expansion Diseases

- Caused by expanded repeats (e.g., CAG, CGG) beyond a threshold.
- **Anticipation:** Increasing severity and earlier onset in successive generations.

Huntington's Disease (HD)

- **Gene:** *HTT* (4p16.3)
- **Mutation:** CAG repeat ≥ 36
- **Inheritance:** Autosomal dominant (with anticipation)

- **Pathogenesis:** Toxic gain-of-function of mutant huntingtin → degeneration of GABAergic neurons in striatum.
- **Clinical triad:** Choreic movements, psychiatric symptoms, cognitive decline.
- **Diagnosis:** PCR fragment analysis (repeat sizing).

1.7. Prion Diseases

- Caused by misfolded prion protein (PrP^{Sc}) converting normal PrP^C.
- **Types:** Sporadic, hereditary (*PRNP* mutations), acquired (infectious).
- **Examples:** CJD, vCJD, Kuru, FFI, GSS.
- **Clinical features:** Rapid cognitive decline, myoclonus, cerebellar ataxia.
- **Diagnosis:** RT-QuIC, CSF 14-3-3 protein, MRI, *PRNP* sequencing.

1.8. Diseases with Hereditary Predisposition (Multifactorial / Polygenic)

- Caused by interaction of multiple susceptibility genes (polygenic) and environmental factors.
- **Examples:** Type 2 diabetes, hypertension, coronary artery disease, schizophrenia, neural tube defects.
- **Risk assessment:** Family history, polygenic risk scores (PRS), gene-environment interactions.
- **Prevention:** Lifestyle modification, screening, pharmacogenetics.

Part 2. Laboratory Procedures

2.1. Pedigree Analysis – Non-Traditional Inheritance Patterns

Practice Task 1. Pedigree Interpretation

Case	Pedigree Description	Inheritance Pattern	Affected Gene / Mechanism
A	Affected males only; transmitted from father to all sons		
B	Affected males and females; all children of affected mothers affected		
C	Males affected more frequently; no male-to-male transmission; carrier females		

Case	Pedigree Description	Inheritance Pattern	Affected Gene / Mechanism
D	Increasing severity and earlier onset in successive generations		

Instructions:

- Identify the inheritance pattern (X-linked recessive, X-linked dominant, Y-linked, mitochondrial, trinucleotide repeat).
- Name a disease for each pattern.
- Explain the molecular mechanism.

2.2. Duchenne Muscular Dystrophy – Case Analysis

Practice Task 2. DMD Family

A family has a boy with Duchenne muscular dystrophy. His mother is a carrier. His younger brother is healthy.

- What is the recurrence risk for future male children?
- What is the risk for future female children?
- What genetic test would you recommend for prenatal diagnosis?
- What is the role of muscle biopsy in diagnosis?

2.3. Mitochondrial Inheritance – Pedigree and Mutation Analysis

Case Study: MELAS Syndrome

A 25-year-old woman presents with stroke-like episodes, seizures, and elevated lactate. Her mother, maternal aunt, and maternal grandmother have similar but milder symptoms. Her brother is healthy.

- What is the inheritance pattern?
- What is the molecular basis of MELAS?
- What is the recurrence risk for her children?
- What prenatal diagnostic options are available?

Practice Task 3. mtDNA Heteroplasmy

A patient has a mitochondrial disease with 80% mutant mtDNA in muscle tissue and 20% in blood.

- What is heteroplasmy?
- How does it affect phenotype and diagnosis?

- What are the implications for genetic counseling?

2.4. Genomic Imprinting – Prader-Willi and Angelman Syndromes

Practice Task 4. Imprinting Disorders

Feature	Prader-Willi Syndrome	Angelman Syndrome
Chromosomal region		
Mechanism		
Clinical features		
Inheritance pattern		

Instructions:

- Fill in the table.
- Explain how uniparental disomy can cause these syndromes.

Case Study:

A child is diagnosed with Prader-Willi syndrome. Molecular testing reveals a paternal deletion of 15q11.2-q13.

- What is the recurrence risk for the parents?
- What is the role of methylation analysis in diagnosis?

2.5. Trinucleotide Repeat Expansion – Huntington's Disease

Practice Task 5. HD Repeat Sizing

A patient with chorea and cognitive decline undergoes genetic testing. The HTT gene shows 42 CAG repeats.

- What is the diagnosis?
- What is the risk of inheritance for his children?
- What is anticipation? How does it affect this family?
- What are the ethical issues in predictive testing for HD?

Molecular Simulation (PCR Fragment Analysis):

You are given gel electrophoresis results showing the following fragment sizes:

Sample	Fragment Size (CAG repeats)	Interpretation
Normal	18, 22	
Carrier	18, 40	
Affected	42, 45	
Child	18, 55	

- Interpret each result.
- Explain anticipation in the child.

2.6. Prion Diseases – Diagnostic Approach

Case Study: Suspected CJD

A 60-year-old patient presents with rapidly progressive dementia, myoclonus, and ataxia. MRI shows cortical ribboning and pulvinar sign.

- What is the most likely diagnosis?
- What is the etiology (sporadic, hereditary, acquired)?
- What diagnostic tests would you recommend (RT-QuIC, CSF 14-3-3, PRNP sequencing)?
- What are the infection control precautions?

2.7. Multifactorial Diseases – Risk Assessment

Practice Task 6. Polygenic Risk Assessment

A 45-year-old man has a family history of type 2 diabetes (mother, maternal uncle). His BMI is 32, and he has hypertension.

- What is his risk for developing type 2 diabetes?
- What environmental factors contribute to the risk?
- What lifestyle modifications can reduce the risk?
- What is the role of pharmacogenetics in management?

2.8. Genetic Counseling – Non-Traditional Inheritance

Practice Task 7. Counseling Scenarios

Scenario	Inheritance Pattern	Recurrence Risk	Prenatal Options	Ethical Issues
Father with Huntington's disease (CAG 42)				
Mother with MELAS (80% mutant mtDNA)				
Couple with a child with Angelman syndrome (maternal deletion)				
Father with Y-linked infertility				
Couple with a family history of schizophrenia				

Part 3. Questions for Self-Assessment

1. What is X-linked recessive inheritance? Give an example.
2. Describe the clinical features and genetic basis of Duchenne muscular dystrophy.
3. What is the difference between Duchenne and Becker muscular dystrophy?
4. What are the characteristics of X-linked dominant inheritance?
5. What is holandric (Y-linked) inheritance? Give an example.
6. What are mitochondrial diseases? How are they inherited?
7. Explain the etiology and pathogenesis of mitochondrial disorders.
8. What is genomic imprinting? Provide examples of imprinting disorders.
9. Describe the clinical features of Prader-Willi and Angelman syndromes.
10. What are trinucleotide repeat expansion diseases? Give an example.
11. What is anticipation? How does it relate to Huntington's disease?
12. What are prion diseases? How do they arise?
13. Describe the clinical features and diagnosis of Creutzfeldt-Jakob disease.
14. What are diseases with hereditary predisposition (multifactorial diseases)?
15. How are polygenic risk scores used in clinical practice?
16. What are the ethical considerations in genetic testing for Huntington's disease?

Part 4. Case Studies

Case 1. Boy with Progressive Weakness

A 4-year-old boy presents with difficulty climbing stairs, frequent falls, and enlarged calves. CK levels are elevated (20,000 U/L). His mother has a brother who died at age 22 from a muscle disease.

- What is the most likely diagnosis?
- What is the inheritance pattern?
- What genetic test would you recommend?
- What is the risk for his sisters being carriers?

Case 2. Woman with Stroke-Like Episodes

A 32-year-old woman with a history of migraines and exercise intolerance presents with sudden onset of hemiparesis and visual disturbances. MRI shows stroke-like lesions. Her mother has a history of hearing loss and diabetes.

- What is the most likely diagnosis?
- What is the inheritance pattern?
- What molecular test would you recommend?
- What is the recurrence risk for her children?

Case 3. Child with Obesity and Hypogonadism

An 8-year-old child presents with severe obesity, small hands and feet, hypotonia, and hypogonadism. He has a history of feeding difficulties as an infant. Methylation analysis reveals a paternal deletion of 15q11.2-q13.

- What is the diagnosis?
- What is the molecular mechanism?
- What is the recurrence risk for the parents?
- What management strategies are available?

Case 4. Family with Early-Onset Dementia

A 45-year-old man presents with chorea, depression, and cognitive decline. His father died at age 55 with similar symptoms. His mother is healthy. He has two children, ages 10 and 12.

- What is the most likely diagnosis?
- What is the inheritance pattern?
- What genetic test would you recommend?
- What is the risk for his children?

- What are the ethical issues in predictive testing?

Part 5. Assignment for Submission

1. Complete Practice Tasks 1–7 and submit your written answers.
2. Write a laboratory report on one of the following topics:
 - "Pedigree Analysis in Non-Traditional Inheritance Patterns"
 - "Molecular Diagnosis of Trinucleotide Repeat Disorders"
 - "Genetic Counseling in Mitochondrial Diseases"
3. Prepare a short presentation (5–7 slides) on one of the following topics:
 - "From Genotype to Phenotype: Huntington's Disease as a Model of Anticipation"
 - "Mitochondrial Inheritance: Challenges in Diagnosis and Counseling"
 - "Prion Diseases: A Unique Paradigm of Inheritance and Transmission"

Laboratory work №7

Prevention of Hereditary and Congenital Pathology

Aim of the Session

To study the genetic foundations of prevention, the principles of medical genetic counseling, and modern methods of prenatal and neonatal diagnosis for hereditary and congenital disorders.

Learning Objectives

By the end of this session, students should be able to:

- Explain the genetic foundations of primary, secondary, and tertiary prevention.
- Describe the types and stages of medical genetic counseling.
- Calculate genetic risk for monogenic and multifactorial diseases.
- Distinguish between non-invasive and invasive prenatal diagnostic methods.
- List indications for invasive prenatal diagnosis.
- Understand the principles and goals of neonatal screening programs.

Part 1. Theoretical Background (for Self-Study)

1.1. Genetic Foundations of Prevention

Prevention of hereditary and congenital pathology is based on understanding the genetic and environmental causes of disease. It is divided into three levels:

Level	Goal	Examples
Primary prevention	Prevent the occurrence of genetic disorders	Carrier screening, preconception counseling, folic acid supplementation, vaccination, avoidance of teratogens
Secondary prevention	Early detection and intervention to reduce morbidity	Prenatal diagnosis, neonatal screening, early treatment of PKU, congenital hypothyroidism
Tertiary	Rehabilitation and	Physical therapy, speech therapy,

Level	Goal	Examples
prevention	management of established disease	surgical correction, genetic counseling for recurrence

1.2. Medical Genetic Counseling

Definition: A communication process that helps individuals or families understand and adapt to the medical, psychological, and familial implications of genetic disorders.

Types:

- **Prospective (preconception/pre-pregnancy):** Assesses recurrence risk before conception; aimed at couples at risk.
- **Retrospective (postnatal):** After the birth of an affected child; addresses recurrence risk, diagnosis, and management.

Stages of Genetic Counseling:

1. Taking family history and constructing a three-generation pedigree.
2. Establishing the diagnosis and determining the mode of inheritance.
3. Estimating the recurrence risk (genetic risk).
4. Discussing reproductive options (prenatal diagnosis, PGD, adoption, etc.).
5. Providing psychological support and follow-up.

1.3. Genetic Risk – Concept and Calculation Methods

Genetic risk: The probability that a couple will have a child with a genetic disorder.

Calculation methods:

- **Mendelian segregation:** For monogenic disorders (e.g., 25% for AR, 50% for AD).
- **Bayesian analysis:** For complex pedigrees with variable penetrance.
- **Empirical risk tables:** For multifactorial diseases (based on population incidence and family history).
- **Polygenic risk scores (PRS):** For complex traits (based on SNP profiles).

Risk categories:

- **High risk (>10%)** – indication for prenatal diagnosis.
- **Moderate risk (1–10%)** – genetic counseling and/or screening.
- **Low risk (<1%)** – routine management.

1.4. Non-Invasive and Invasive Prenatal Diagnostic Methods

Method	Type	Gestational Age	Indications	Advantages	Disadvantages
Ultrasound	Non-invasive	First and second trimesters	Fetal anomalies, nuchal translucency, growth	Safe, widely available	Limited sensitivity for genetic disorders
Maternal serum screening	Non-invasive	10–20 weeks	Aneuploidy risk assessment (PAPP-A, β -hCG, AFP, uE3, inhibin A)	Low risk, non-invasive	Screening, not diagnostic
NIPT (cell-free fetal DNA)	Non-invasive	≥ 10 weeks	Aneuploidy screening (Trisomy 21, 18, 13)	High sensitivity and specificity (>99%)	Only for common aneuploidies; cost
CVS (Chorionic Villus Sampling)	Invasive	10–13 weeks	Karyotyping, molecular testing	Early diagnosis	Miscarriage risk (1–2%); fetal limb defects (rare)
Amniocentesis	Invasive	15–20 weeks	Karyotyping, FISH, aCGH,	High accuracy	Miscarriage risk (0.5–1%); later

Method	Type	Gestational Age	Indications	Advantages	Disadvantages
			molecular testing		diagnosis
Cordocentesis (PUBS)	Invasive	>18 weeks	Rapid karyotyping, fetal blood analysis	Rapid results	High miscarriage risk (>2%); limited use

1.5. Indications for Invasive Prenatal Diagnosis

1. Maternal age ≥ 35 years at delivery.
2. Abnormal maternal serum screening or NIPT result.
3. Previous child with a chromosomal abnormality or monogenic disorder.
4. Parental balanced chromosomal translocation or mosaicism.
5. Family history of a Mendelian disorder (e.g., cystic fibrosis, muscular dystrophy, hemophilia).
6. Ultrasound findings suggestive of fetal anomalies (e.g., increased nuchal translucency, cystic hygroma, structural defects).
7. High risk of X-linked disorders.
8. Parental consanguinity (increased risk of autosomal recessive disorders).
9. Parental request (after counseling).

1.6. Neonatal Screening

Purpose: Early detection of treatable disorders in newborns to prevent irreversible damage.

Specimen: Dried blood spots (Guthrie test) collected from a heel prick within 24–72 hours of life.

Common disorders screened:

- Phenylketonuria (PKU)
- Congenital hypothyroidism
- Cystic fibrosis (CF)
- Sickle cell disease (SCD) and other hemoglobinopathies
- Galactosemia
- Maple syrup urine disease (MSUD)
- Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)

- Biotinidase deficiency
- Congenital adrenal hyperplasia (CAH)
- Severe combined immunodeficiency (SCID) (in some programs)
- Spinal muscular atrophy (SMA) (in some programs)

Follow-up:

- Positive screening → confirmatory diagnostic testing → early intervention and long-term management.

Part 2. Laboratory Procedures

2.1. Genetic Risk Calculation – Monogenic Disorders

Practice Task 1. Recurrence Risk Calculation

Case	Inheritance Pattern	Parental Genotype	Recurrence Risk	Explanation
A	Autosomal recessive	Both carriers ($Aa \times Aa$)		
B	Autosomal dominant	One affected parent ($Aa \times aa$)		
C	X-linked recessive	Carrier mother ($X^H X^h \times X^H Y$)	For sons:	
			For daughters:	

Instructions:

- Calculate the recurrence risk for each case.
- Explain the basis of the calculation.
- What is the risk if the couple already has an affected child?

2.2. Pedigree Analysis and Genetic Risk

Practice Task 2. Pedigree with Cystic Fibrosis (AR)

A couple, both healthy, has a child with cystic fibrosis. The mother's sister also has CF.

- Construct a pedigree.
- Determine the genotypes of all family members.
- What is the recurrence risk for future children?
- What is the carrier risk for a healthy sibling?

Practice Task 3. Pedigree with Huntington's Disease (AD)

A 45-year-old man has Huntington's disease. His father died of the disease. He has two children, aged 10 and 12.

- What is the risk for each child?
- What is the role of predictive genetic testing?
- What are the ethical issues?

2.3. Calculation of Empirical Risk (Multifactorial Diseases)

Practice Task 4. Neural Tube Defects (NTD)

The population incidence of neural tube defects is 0.1%. The recurrence risk after one affected child is 3–5%; after two affected children, it is 10–12%.

A couple has one child with spina bifida and is planning another pregnancy.

- What is the recurrence risk?
- What preventive measures can reduce the risk?
- What prenatal diagnostic methods are available?
- What is the role of folic acid supplementation?

2.4. Prenatal Diagnostic Methods – Matching and Decision-Making

Practice Task 5. Match the Method to the Indication

Indication	Most Appropriate Method	Rationale
Maternal age 39 years at 16 weeks		
Family history of Duchenne muscular dystrophy (10 weeks)		
Abnormal serum screening at 12 weeks		
Fetal hydrops on ultrasound at 22 weeks		

Indication	Most Appropriate Method	Rationale
Parental balanced translocation (18 weeks)		

Instructions:

- Choose a method (ultrasound, serum screening, NIPT, CVS, amniocentesis, cordocentesis).
- Explain your choice.
- Discuss the advantages and limitations of each.

2.5. Indications for Invasive Prenatal Diagnosis – Case Scenarios

Practice Task 6. Indications Assessment

Case	Invasive Procedure Indicated?	Reason	Recommended Method
A woman, age 32, with normal screening and no family history			
A couple with a previous child with Down syndrome			
A woman, age 40, with a positive NIPT for trisomy 21			
A woman with an ultrasound showing increased nuchal translucency			
A couple with a family history of spinal muscular atrophy			

Instructions:

- Justify each answer.

- Explain the benefits and risks of each procedure.

2.6. Neonatal Screening – Interpretation and Follow-Up

Practice Task 7. Newborn Screening Results

Case	Screening Result	Confirmatory Test	Diagnosis	Management
Newborn with elevated phenylalanine				
Newborn with positive CFTR screening				
Newborn with low T4 and elevated TSH				
Newborn with positive sickle cell test				

Instructions:

- Propose confirmatory tests.
- Suggest management and long-term follow-up.

Part 3. Questions for Self-Assessment

1. What are the genetic foundations of prevention of hereditary pathology?
2. What are the types and stages of medical genetic counseling?
3. What is genetic risk? How is it calculated?
4. What are non-invasive prenatal diagnostic methods?
5. What are invasive prenatal diagnostic methods?
6. What are the indications for invasive prenatal diagnosis?
7. What is neonatal screening? Which disorders are screened?
8. What is the role of folic acid in prevention?
9. What is the difference between primary, secondary, and tertiary prevention?

10. What are the ethical issues in prenatal diagnosis and neonatal screening?

Part 4. Case Studies

Case 1. Couple with Cystic Fibrosis Carrier Status

A couple, both of whom are carriers of the F508del mutation (CFTR), is planning a pregnancy.

- What is the recurrence risk?
- What preconception and prenatal options are available?
- What are the benefits and limitations of each?
- What are the ethical considerations?

Case 2. Advanced Maternal Age and NIPT

A 40-year-old woman has a positive NIPT for trisomy 21.

- What is the next step?
- What invasive diagnostic test would you recommend?
- What is the risk of miscarriage with the procedure?
- What should be included in genetic counseling?

Case 3. Newborn Screening for PKU

A newborn has a positive screening test for phenylketonuria (PKU).

- What confirmatory test should be done?
- What is the treatment?
- What are the consequences of untreated PKU?
- What is the role of newborn screening?

Case 4. Family History of Neural Tube Defects

A woman who had a child with spina bifida is pregnant again. She has been taking folic acid (4 mg/day) since the first trimester.

- What is the recurrence risk?
- What prenatal diagnostic methods are available?
- How does folic acid reduce the risk?
- What is the prognosis for the fetus?

Part 5. Assignment for Submission

1. Complete Practice Tasks 1–7 and submit your written answers.
2. Write a laboratory report on one of the following topics:
 - "Genetic Risk Calculation and Counseling in Monogenic Disorders"
 - "Prenatal Diagnosis: Indications and Methods"
 - "Newborn Screening: Principles and Practice"
3. Prepare a short presentation (5–7 slides) on one of the following topics:
 - "Primary Prevention of Hereditary Diseases: Carrier Screening and Preconception Counseling"
 - "Non-Invasive Prenatal Testing (NIPT): Advantages and Limitations"
 - "Neonatal Screening: Successes, Challenges, and Future Directions"

Laboratory work №8

Hereditary Metabolic Disorders. Genetic Aspects of Carcinogenesis.

Aim of the Session

To study the general characteristics, pathogenesis, clinical features, and diagnostic approaches of inherited metabolic disorders, and to understand the genetic basis of carcinogenesis.

Learning Objectives

By the end of this session, students should be able to:

- Describe the general characteristics of monogenic metabolic disorders.
- Identify and explain disorders of amino acid, carbohydrate, and lipid metabolism.
- Understand inherited red blood cell disorders (spherocytic and non-spherocytic anemias).
- Characterize lysosomal storage diseases, mucopolysaccharidoses, and peroxisomal disorders.
- Explain the multistep genetic concept of carcinogenesis.
- Apply diagnostic approaches to metabolic and cancer genetics.

Part 1. Theoretical Background (for Self-Study)

1.1. Metabolic Disorders. General Characteristics of Monogenic Pathology

Inborn errors of metabolism (IEM) are genetically determined enzyme deficiencies or transport protein defects that disrupt metabolic pathways. Most are autosomal recessive (or X-linked). Clinical manifestations are age-dependent (neonatal, infantile, late-onset), often triggered by fasting, infections, or dietary intake.

Diagnosis: Biochemical assays (blood, urine, CSF), enzyme activity analysis, DNA testing.

Management: Dietary restriction, enzyme replacement therapy (ERT), pharmacological treatment, and gene therapy (emerging).

1.2. Inherited Disorders of Amino Acid Metabolism (Aminoacidopathies)

Disorder	Enzyme Defect	Clinical Features	Treatment
Phenylketonuria (PKU)	Phenylalanine hydroxylase	Intellectual disability, seizures, hypopigmentation, eczema	Phenylalanine-restricted diet
Tyrosinemia type I	Fumarylacetoacetate hydrolase	Liver/kidney failure, neurological crises	Nitisinone (NTBC), liver transplantation
Albinism (OCA, OA)	Tyrosinase or other melanin synthesis enzymes	Hypopigmentation, nystagmus, photophobia, reduced visual acuity, increased skin cancer risk	Lifelong sun protection, low-vision aids, skin surveillance

1.3. Inherited Disorders of Carbohydrate Metabolism

Disorder	Enzyme Defect	Clinical Features	Treatment
Galactosemia	Galactose-1-phosphate uridylyltransferase (GALT)	Vomiting, jaundice, hepatomegaly, cataracts, intellectual disability	Lactose/galactose-free diet
Glycogen storage diseases (GSDs)	Various enzymes (e.g., glucose-6-phosphatase in GSD I)	Hypoglycemia, hepatomegaly, lactic acidosis, hyperlipidemia	Frequent carbohydrate feedings (cornstarch), dietary modification

1.4. Inherited Disorders of Lipid Metabolism

Disorder	Genetic Basis	Clinical Features	Treatment
Familial hypercholesterolemia (FH)	<i>LDLR, APOB, PCSK9</i> mutations (AD)	Elevated LDL, xanthomas, premature atherosclerosis, early MI	Statins, PCSK9 inhibitors, lifestyle modification
Tangier disease	ABCA1 deficiency (AR)	Low HDL, orange tonsils, peripheral neuropathy	Symptomatic

1.5. Inherited Disorders of Erythrocytes (Red Blood Cell Disorders)

Disorder	Mechanism	Clinical Features	Treatment
Hereditary spherocytosis (HS)	Mutations in spectrin, ankyrin, band 3 (AD/AR)	Hemolysis, jaundice, splenomegaly, gallstones	Folate, splenectomy
G6PD deficiency	X-linked enzyme deficiency	Hemolytic anemia triggered by oxidants (fava beans, drugs, infections)	Avoid triggers, supportive care
Pyruvate kinase deficiency	AR enzyme deficiency	Chronic hemolysis, anemia	Blood transfusions, splenectomy

1.6. Lysosomal Storage Diseases (LSDs)

Disorder	Enzyme Defect	Clinical Features	Treatment
Gaucher disease	Glucocerebrosidase	Hepatosplenomegaly, bone lesions, pancytopenia	ERT, SRT
Tay–Sachs disease	Hexosaminidase A	Neurodegeneration, cherry-red macula, early death	Supportive
Niemann–Pick disease	Sphingomyelinase	Hepatosplenomegaly, neurodegeneration	Supportive, ERT (some types)

1.7. Mucopolysaccharidoses (MPS)

Type	Enzyme Defect	Clinical Features	Treatment
MPS I (Hurler/Scheie)	α -L-iduronidase	Coarse facies, corneal clouding, organomegaly, skeletal dysplasia, intellectual disability	ERT, HSCT
MPS II (Hunter)	Iduronate-2-sulfatase (X-linked)	Similar to MPS I, but no corneal clouding	ERT
MPS III (Sanfilippo)	Heparan sulfate accumulation (various enzymes)	Severe neurodegeneration, behavioral problems	Supportive

1.8. Peroxisomal Disorders

Disorder	Defect	Clinical Features	Diagnosis
Zellweger syndrome	Peroxisomal biogenesis	Craniofacial dysmorphism, hypotonia, liver dysfunction, seizures, early death	VLCFA levels, molecular testing
X-linked adrenoleukodystrophy (X-ALD)	ABCD1 (VLCFA accumulation)	Adrenal insufficiency, progressive neurodegeneration	VLCFA, Lorenzo's oil, HSCT

1.9. Stages of the Genetic Concept of Carcinogenesis

Knudson's two-hit hypothesis:

- **First hit:** Germline mutation in a tumor suppressor gene (inherited).
- **Second hit:** Somatic mutation in the same gene → loss of heterozygosity (LOH) → tumor development.

Multistep carcinogenesis:

- Accumulation of mutations in proto-oncogenes → oncogenes.
- Inactivation of tumor suppressor genes (e.g., TP53, RB1, APC).
- Defects in DNA repair genes (e.g., BRCA1/2, MSH2/MLH1).
- Epigenetic changes.

Examples of hereditary cancer syndromes:

Syndrome	Gene(s)	Associated Cancers
Li–Fraumeni	TP53	Breast, brain, sarcoma, leukemia
BRCA1/2	BRCA1, BRCA2	Breast, ovarian, prostate, pancreatic
Lynch syndrome	MSH2, MLH1,	Colorectal, endometrial,

Syndrome	Gene(s)	Associated Cancers
	MSH6	ovarian
Familial adenomatous polyposis (FAP)	APC	Colorectal, gastric, duodenal
Retinoblastoma	RB1	Retinoblastoma, osteosarcoma

Part 2. Laboratory Procedures

2.1. Clinical Case Analysis – Metabolic Disorders

Practice Task 1. Differential Diagnosis of Metabolic Disorders

Case	Symptoms	Laboratory Findings	Suspected Disorder	Confirmatory Test
A	Neonatal vomiting, jaundice, hepatomegaly, cataracts	Reducing substances in urine (galactose)		
B	Newborn with fair skin, blue eyes, seizures, musty odor	Elevated phenylalanine in blood		
C	Child with hepatomegaly, hypoglycemia, lactic acidosis	Deficiency of glucose-6-phosphatase		
D	Adult with xanthomas, high LDL,	LDLR mutation		

Case	Symptoms	Laboratory Findings	Suspected Disorder	Confirmatory Test
	premature MI			
E	Newborn with coarse facies, organomegaly, skeletal dysplasia	α -L-iduronidase deficiency		

Instructions:

- Identify the disorder.
- Describe the pathogenesis.
- Propose treatment options.

2.2. Aminoacidopathies – PKU and Albinism

Practice Task 2. PKU Pedigree

A couple, both carriers of PKU, has a child with PKU. They are planning another pregnancy.

- What is the recurrence risk?
- What is the role of newborn screening?
- What is the treatment for PKU?
- What are the consequences of untreated PKU?

Practice Task 3. Albinism – Genetic Counseling

A family has a child with oculocutaneous albinism (OCA). The parents are healthy.

- What is the inheritance pattern?
- What is the risk for future children?
- What preventive measures should be taken (sun protection, eye care)?
- What is the risk of skin cancer?

2.3. Carbohydrate Metabolism Disorders

Practice Task 4. Galactosemia and GSD

Case A: A newborn develops vomiting, diarrhea, and jaundice after breastfeeding.

- What is the most likely diagnosis?
- What enzyme is deficient?
- What dietary modification is needed?

Case B: A child with hepatomegaly, hypoglycemia, and lactic acidosis.

- What is the most likely diagnosis?
- What type of GSD is it (type I)?
- What is the treatment?

2.4. Lipid and Erythrocyte Disorders

Practice Task 5. Hereditary Spherocytosis

A child presents with jaundice, splenomegaly, and anemia. Family history reveals similar symptoms in the father.

- What is the most likely diagnosis?
- What is the inheritance pattern?
- What laboratory findings are expected (spherocytes on blood smear, increased osmotic fragility)?
- What treatment options are available?

Practice Task 6. G6PD Deficiency

A patient develops hemolytic anemia after taking an antimalarial drug.

- What is the most likely diagnosis?
- What is the inheritance pattern?
- What triggers should be avoided?
- How is G6PD deficiency diagnosed?

2.5. Lysosomal and Peroxisomal Disorders

Practice Task 7. LSD and MPS Diagnosis

Case	Clinical Features	Enzyme Deficiency	Diagnosis
A	Coarse facies, organomegaly, skeletal dysplasia, corneal clouding	α -L-iduronidase	
B	Neurodegeneration, cherry-red macula, early death	Hexosaminidase A	

Case	Clinical Features	Enzyme Deficiency	Diagnosis
C	Hepatosplenomegaly, bone lesions, pancytopenia	Glucocerebrosidase	
D	Craniofacial dysmorphism, hypotonia, liver dysfunction, early death	Peroxisomal biogenesis	

Instructions:

- Identify the diagnosis.
- Describe the pathogenesis.
- Outline treatment options.

2.6. Genetic Aspects of Carcinogenesis – Hereditary Cancer Syndromes

Practice Task 8. Multistep Carcinogenesis – Two-Hit Hypothesis

A patient with retinoblastoma has a germline mutation in RB1.

- What is the two-hit hypothesis?
- Why is the risk of bilateral retinoblastoma higher in hereditary cases?
- What is the risk of secondary cancers (e.g., osteosarcoma)?

Practice Task 9. Cancer Genetic Testing

Case	Personal/Family History	Suspected Syndrome	Gene	Risk Management
A	Breast cancer at 35, ovarian cancer in mother			
B	Colorectal cancer at 40, endometrial cancer in sister			
C	Multiple polyps in colon, colorectal cancer at 25			
D	Sarcoma, breast cancer, brain tumor in relatives			

Instructions:

- Identify the syndrome.
- Name the gene.
- Propose screening and preventive measures.

Part 3. Questions for Self-Assessment

1. What are the general characteristics of monogenic metabolic disorders?
2. Describe the pathogenesis, clinical features, and treatment of PKU.
3. What is albinism? How is it inherited?
4. What is galactosemia? What enzyme is deficient?
5. What are glycogen storage diseases? Give two examples.
6. Describe familial hypercholesterolemia.
7. What are spherocytic and non-spherocytic anemias?
8. What are lysosomal storage diseases? Give three examples.
9. What are mucopolysaccharidoses? How are they diagnosed?
10. Describe peroxisomal disorders.
11. What is the two-hit hypothesis of carcinogenesis?
12. What are hereditary cancer syndromes? Give three examples.
13. What is the role of proto-oncogenes and tumor suppressor genes in cancer?
14. How is genetic testing used in hereditary cancer syndromes?

Part 4. Case Studies

Case 1. Newborn with Vomiting and Jaundice

A newborn develops vomiting, diarrhea, and jaundice after breastfeeding. Reducing substances are detected in the urine.

- What is the diagnosis?
- What is the enzyme deficiency?
- What is the treatment?
- What is the role of newborn screening?

Case 2. Child with Coarse Facies and Organomegaly

A child presents with coarse facies, corneal clouding, hepatosplenomegaly, and skeletal dysplasia. Enzyme assay shows α -L-iduronidase deficiency.

- What is the diagnosis?
- What is the inheritance pattern?
- What treatment options are available (ERT, HSCT)?
- What is the prognosis?

Case 3. Woman with Breast and Ovarian Cancer

A 40-year-old woman is diagnosed with breast cancer. Her mother died from ovarian cancer at age 50. Genetic testing reveals a BRCA1 mutation.

- What is the diagnosis?
- What is the recurrence risk for her children?
- What screening and preventive measures are recommended?
- What are the ethical issues in genetic testing?

Case 4. Adult with Premature MI and Xanthomas

A 45-year-old man has tendon xanthomas, high LDL cholesterol, and a family history of early myocardial infarction.

- What is the most likely diagnosis?
- What is the inheritance pattern?
- What genetic test would you recommend?
- What treatment options are available?

Part 5. Assignment for Submission

1. Complete Practice Tasks 1–9 and submit your written answers.
2. Write a laboratory report on one of the following topics:
 - "Diagnostic Approach to Lysosomal Storage Diseases"
 - "Genetic Testing in Hereditary Cancer Syndromes"
 - "Newborn Screening for Metabolic Disorders"
3. Prepare a short presentation (5–7 slides) on one of the following topics:
 - "Inborn Errors of Metabolism: Diagnosis and Treatment"
 - "The Genetic Basis of Cancer: From Two-Hit Hypothesis to Targeted Therapy"
 - "Ethical Issues in Genetic Testing for Hereditary Cancer Syndromes"

Laboratory work №9

Prevention of Hereditary Pathology. Principles of Treatment of Hereditary Diseases.

Aim of the Session

To study the principles of primary, secondary, and tertiary prevention of hereditary diseases, to understand the role of medical genetic counseling and prenatal diagnosis, and to explore the principles and ethical issues of treating hereditary diseases, including gene therapy.

Learning Objectives

By the end of this session, students should be able to:

- Explain the role of medical genetic counseling in primary prevention.
- Describe prenatal and preimplantation genetic diagnostic methods.
- Understand biochemical screening programs for secondary prevention.
- Differentiate between symptomatic, pathogenetic, and etiological treatment.
- Explain the principles of gene therapy.
- Discuss ethical and legal issues in medical genetics and gene therapy.

Part 1. Theoretical Background (for Self-Study)

1.1. Medical Genetic Counseling as Primary Prevention

Definition: A communication process that helps individuals understand and adapt to genetic risks and make informed reproductive decisions.

Primary prevention aims to prevent the occurrence of hereditary diseases before conception by:

- Carrier screening (e.g., Tay–Sachs, cystic fibrosis, SMA).
- Preconception counseling (e.g., folic acid supplementation, avoidance of teratogens).
- Population screening and risk assessment.
- Reproductive options (PGD, adoption, gamete/embryo donation).

1.2. Prenatal and Preimplantation Genetic Diagnosis

Method	Description	Gestational Age / Timing	Indications
Non-invasive prenatal testing (NIPT)	Cell-free fetal DNA from maternal blood	≥10 weeks	Aneuploidy screening
Chorionic villus sampling (CVS)	Trophoblast biopsy	10–13 weeks	Karyotyping, molecular testing
Amniocentesis	Amniotic fluid aspiration	15–20 weeks	Karyotyping, FISH, aCGH, molecular testing
Cordocentesis (PUBS)	Fetal blood sampling	>18 weeks	Rapid karyotyping, fetal blood analysis
Preimplantation genetic diagnosis (PGD)	Embryo biopsy during IVF	Day 3 (blastomere) or Day 5–6 (trophectoderm)	Monogenic disorders, chromosomal rearrangements, HLA matching

PGD is used for couples at high risk of transmitting:

- Autosomal recessive disorders (e.g., CF, SMA)
- Autosomal dominant disorders (e.g., Huntington's disease)
- X-linked disorders (e.g., Duchenne MD)
- Chromosomal translocations

1.3. Biochemical Screening Programs (Secondary Prevention)

Secondary prevention aims to detect treatable disorders early, before clinical symptoms appear.

Neonatal screening (heel prick test):

- Dried blood spots (Guthrie test) – 24–72 hours of life.
- Target disorders:
 - Phenylketonuria (PKU)

- Congenital hypothyroidism
- Cystic fibrosis
- Sickle cell disease
- Galactosemia
- MCADD
- SCID, SMA (in some programs)

Maternal serum screening:

- First-trimester: PAPP-A, β -hCG, nuchal translucency.
- Second-trimester: AFP, uE3, β -hCG, inhibin A.

Carrier screening: For disorders common in specific populations (e.g., Tay–Sachs in Ashkenazi Jews).

1.4. Principles of Treatment of Hereditary Diseases

Level	Goal	Examples
Symptomatic	Alleviate symptoms without addressing the cause	Anticonvulsants, pain management, respiratory support, surgical correction
Pathogenetic	Target pathophysiological mechanisms	Dietary restriction (PKU), enzyme replacement therapy (Gaucher, Pompe), substrate reduction therapy, organ transplantation, HSCT
Etiological (causal)	Correct the genetic defect	Gene therapy, gene editing, cell-based therapies

1.5. Gene Therapy

Definition: Introduction, removal, or correction of genetic material to treat or prevent disease.

Types:

- **Gene addition (replacement):** Functional copy of the defective gene using viral vectors (AAV, lentivirus, retrovirus). Examples: SMA (Zolgensma), hemophilia B, Leber congenital amaurosis.
- **Gene editing:** CRISPR/Cas9, TALENs, ZFNs to correct mutations. Examples: sickle cell disease, beta-thalassemia (ex vivo editing of HSCs).

- **Gene silencing (antisense):** Oligonucleotides to modulate splicing, reduce expression of mutant proteins. Examples: nusinersen (Spinraza) for SMA, eteplirsen for Duchenne MD.
- **Cell-based gene therapy:** Genetically modified cells (e.g., CAR-T cells, modified HSCs) for cancer or immune disorders.

Challenges: Vector safety, immune response, delivery efficiency, long-term expression, cost, ethical concerns.

1.6. Ethical and Legal Issues in Medical Genetics

- **Genetic confidentiality:** Protection of genetic information; risk of discrimination in employment, insurance, education.
- **Informed consent:** Patients must be adequately informed about genetic testing, risks, implications, and limitations.
- **Genetic testing of minors:** Testing for adult-onset disorders in children when no immediate benefit exists is generally discouraged.
- **Direct-to-consumer (DTC) testing:** Concerns about test quality, interpretation, psychological impact.
- **Reproductive autonomy:** Access to prenatal and preimplantation diagnosis, termination of pregnancy, reproductive choices.
- **Non-discrimination policies:** GINA in the US; similar legislation is being developed elsewhere.

1.7. Bioethical Problems of Gene Therapy

- **Somatic vs. germline gene therapy:** Somatic therapy is more accepted; germline therapy raises concerns about heritability, unforeseen effects, and genetic enhancement.
- **Informed consent in clinical trials:** Complex due to uncertain long-term risks; genetic counseling essential.
- **Equity and access:** Gene therapies are extremely expensive (e.g., Zolgensma ~\$2.1 million per patient).
- **Long-term safety:** Risks of insertional mutagenesis, immune reactions, off-target editing.
- **"Designer babies" and enhancement:** Potential misuse for non-medical traits (intelligence, appearance).
- **Public engagement and oversight:** Need for transparent regulatory frameworks, ethics committees.
- **Intellectual property and commercialization:** Patenting of gene therapy technologies may limit access.

Part 2. Laboratory Procedures

2.1. Genetic Counseling – Role-Play and Risk Assessment

Practice Task 1. Genetic Counseling Scenario

A couple, both carriers of the F508del mutation (CFTR), is planning a pregnancy. They are concerned about having a child with cystic fibrosis.

- What is the recurrence risk?
- What reproductive options are available?
- What is the role of prenatal diagnosis and PGD?
- What ethical considerations apply?

Instructions:

- Prepare a genetic counseling summary.
- Explain the risks, options, and limitations.

2.2. Prenatal Diagnosis – Case Decision-Making

Practice Task 2. Indications for Prenatal Diagnosis

Case	Indication for Prenatal Diagnosis?	Method	Rationale
A	Woman, age 39, with positive NIPT for trisomy 21		
B	Couple with a child with Down syndrome		
C	Family history of spinal muscular atrophy (SMA)		
D	Ultrasound showing increased nuchal translucency		
E	Parental balanced translocation t(14;21)		

Instructions:

- Justify the choice of method.
- Discuss risks and benefits.

2.3. Biochemical Screening – Interpretation

Practice Task 3. Newborn Screening Results Interpretation

Case	Screening Result	Confirmatory Test	Diagnosis	Management
A	Elevated phenylalanine			
B	Low T4, elevated TSH			
C	Positive CFTR screening			
D	Positive sickle cell test			
E	Positive galactose in urine			

Instructions:

- Propose confirmatory tests.
- Describe treatment and long-term follow-up.

2.4. PGD – Clinical Application

Practice Task 4. PGD for Huntington's Disease

A couple has a family history of Huntington's disease. The husband is at risk (his father was affected). He does not want to know his own carrier status, but wants to have a child free of the disease.

- What are the options (preconception, prenatal, PGD)?
- What are the ethical issues?
- How is PGD performed (embryo biopsy, genetic testing)?
- What are the advantages and limitations of PGD?

2.5. Principles of Treatment – Matching

Practice Task 5. Treatment Types and Examples

Treatment Type	Example	Disease
Symptomatic		
Pathogenetic		
Etiological (causal)		

Examples to match:

- Enzyme replacement therapy (ERT) for Gaucher disease
- Phenylalanine-restricted diet for PKU
- Zolgensma (gene therapy) for SMA
- Anticonvulsants for seizures in metabolic disorders
- HSCT for Hurler syndrome (MPS I)
- Dietary restriction of galactose for galactosemia

Instructions:

- Match each treatment to the correct type.
- Explain the mechanism.

2.6. Gene Therapy – Case-Based Analysis

Practice Task 6. Gene Therapy Clinical Scenario

A newborn is diagnosed with spinal muscular atrophy type 1 (SMA1). The parents are offered gene therapy (Zolgensma).

- What is the mechanism of action of Zolgensma (AAV9-SMN1)?
- What are the indications and contraindications?
- What are the risks (hepatotoxicity, immune response)?
- What is the cost and cost-effectiveness?
- What ethical issues arise?

2.7. Ethical and Legal Issues – Case Discussion

Practice Task 7. Bioethical Dilemmas

Scenario	Ethical Issue	Considerations
A teenager at risk for Huntington's disease requests		

Scenario	Ethical Issue	Considerations
predictive testing		
A couple requests PGD for non-medical traits (e.g., sex selection, eye color)		
A family refuses newborn screening due to religious beliefs		
A biotech company patents a gene therapy for a rare disease and charges \$2 million per patient		
A researcher proposes germline gene editing to eliminate a hereditary disorder		

Instructions:

- Identify the ethical issue(s).
- Discuss the pros and cons.
- Propose a reasoned position.

Part 3. Questions for Self-Assessment

1. What is the role of medical genetic counseling in primary prevention?
2. What are the types of prenatal diagnosis? When is each used?
3. What is preimplantation genetic diagnosis (PGD)? What are its indications?
4. What are biochemical screening programs for secondary prevention?
5. What is the difference between symptomatic, pathogenetic, and etiological treatment?
6. How does gene therapy work? Give examples.
7. What are the challenges and risks of gene therapy?
8. What are the ethical issues in genetic testing and counseling?
9. What are the bioethical problems of gene therapy?
10. What is the role of informed consent in genetic testing and gene therapy?
11. How does gene therapy differ from traditional drug therapy?
12. What are the legal protections against genetic discrimination?

Part 4. Case Studies

Case 1. Couple with Cystic Fibrosis Carrier Status

A couple, both carriers of CFTR mutations, is planning a pregnancy. They are considering PGD.

- What is the recurrence risk?
- What is the process of PGD?
- What are the advantages and limitations?
- What are the ethical issues?

Case 2. Newborn with Positive PKU Screening

A newborn has a positive PKU screening test.

- What is the confirmatory test?
- What is the treatment?
- What are the consequences of untreated PKU?
- What is the role of newborn screening?

Case 3. Family with Huntington's Disease

A 45-year-old man with Huntington's disease has two children. The parents want to know if the children are at risk.

- What is the recurrence risk?
- What are the options for prenatal testing?
- What is PGD and how does it differ from prenatal testing?
- What are the ethical issues in testing minors?

Case 4. Gene Therapy for SMA

A newborn is diagnosed with spinal muscular atrophy type 1. The parents are offered gene therapy (Zolgensma).

- What is the mechanism of action?
- What are the risks and benefits?
- What is the cost and who pays?
- What are the ethical issues?

Case 5. Ethical Dilemma: Germline Editing

A couple with a family history of a severe genetic disorder requests germline gene editing to prevent transmission to their children.

- What is the difference between somatic and germline gene therapy?
- What are the risks and potential consequences?
- What are the ethical arguments for and against?
- What are the current regulatory frameworks?

Part 5. Assignment for Submission

1. Complete Practice Tasks 1–7 and submit your written answers.
2. Write a laboratory report on one of the following topics:
 - "Genetic Counseling and Prenatal Diagnosis: A Practical Guide"
 - "Gene Therapy: Principles, Applications, and Challenges"
 - "Ethical Issues in Medical Genetics"
3. Prepare a short presentation (5–7 slides) on one of the following topics:
 - "From Prevention to Treatment: The Role of Medical Genetics in Modern Medicine"
 - "Gene Therapy: Hope, Hype, and Ethical Dilemmas"
 - "Ethical Issues in Prenatal Diagnosis and PGD"

List of Main Literature:

1. Genetics and genomics in medicine / Ronald Doron Cohn, Stephen W. Scherer, Ada Hamosh - Elsevier. – 2024. – 612 p.

List of Additional Literature:

1. Genetics: analysis & principles, Sixth edition / Robert J. Brooker - New York NY : McGraw-Hill Education. -2018. – 872 p.
2. Lewin's GENES XII / Jocelyn E. Krebs, Elliott S. Goldstein, Stephen T. Kilpatrick. - Jones & Bartlett Learning . – 2018. – 1026p.

**MINISTRY OF SCIENCE AND HIGHER EDUCATION
RUSSIAN FEDERATION**

**FEDERAL STATE AUTONOMOUS EDUCATIONAL INSTITUTION OF
HIGHER EDUCATION
NORTH-CAUCASUS FEDERAL UNIVERSITY**

Methodological Guidelines
for Students on arranging and performing independent work
in the Discipline of Medical Genetics
for Students majoring (Specialty) in 31.05.01 General Medicine

Methodological Recommendations for Studying Theoretical Material

When performing the independent study of theoretical material, the student must follow the rules as set below. The working curriculum of the respective academic discipline shall be recommended to be used as the basis for such activity. According to the schedule of classroom lessons and independent work, a different number of hours is to be allocated for studying individual topics. A significant portion of the student's independent work should be allocated to mastering the material on the covered topic, writing a summary (notes) on the specified theoretical blocks, and searching for additional literature based on the NCFU library and via the Internet system. The lecture material shall not cover all theoretical material; the greater part of it must be mastered through independent work. Given the above, working with the recommended literature is mandatory. Special attention must be paid to the content of the main provisions and conclusions, the explanation of phenomena and facts, and the understanding of the practical application of the theoretical issues under consideration. In the process of this work, the student should strive to understand and memorize the main provisions of the material being considered, the examples illustrating it, as well as to understand the illustrative material. Preparation should be completed by drawing up a plan (summary/notes) on the material (issue) being studied. This allows for the creation of a concentrated, concise representation of the issues being studied. First, studying the recommended literature is to be done. Special attention must be paid to the content of the main provisions and conclusions, the explanation of phenomena and facts, and the understanding of the practical application of the theoretical issues under consideration.

During this work, the student should strive to understand and memorize the main provisions of the material being considered, the examples illustrating it, as well as to understand the illustrative material. Preparation should be completed by drawing up a plan (summary/notes) on the material (issue) being studied. This allows for the creation of a concentrated, concise representation of the issues being studied. If necessary, one should seek (advice) consultation from the instructor. When going to a consultation, it is necessary to thoroughly think through the questions that require clarification. Keeping records contributes to turning reading into an active process, mobilizing motor memory along with visual memory. The following should be born in mind: a student who systematically keeps records creates their own individual stock of auxiliary materials for a quick review of what has been read and for the mobilization of accumulated knowledge. Records are especially important and useful when they reflect the thoughts that arose during independent work.

It is important to develop the students' capacity to compare sources and to think through the material being studied.

Improving note-taking skills among students is of great importance.

The instructor may recommend the following main forms of recording to students: a plan (simple and detailed), excerpts, and theses (abstracts).

The results of note-taking can be presented in various forms.

- A plan is an outline of the read material, a brief (or detailed) list of questions reflecting the structure and sequence of the material.
- A detailed plan fully replaces a summary.
- A summary (notes) is a systematized, logical presentation of the source material. Four types of summaries are distinguished:
 - Outline-summary (Plan-notes) – this is an expanded, detailed plan in which sufficiently detailed records are provided for those points of the plan that need explanation.
 - Textual summary – this is the reproduction of the most important provisions and facts of the source.
 - Free summary – these are clearly and briefly formulated (stated) main provisions resulting from a deep understanding of the material. It may contain excerpts, quotations, and theses; part of the material may be represented by a plan.
 - Theme-based summary – this is compiled on the basis of studying a number of sources and gives a more or less exhaustive answer according to a certain scheme (issue).

Methodological Guidelines for the Preparation for Practical Activities

When preparing for practical activities, the recommendation implies studying the control questions for the given lesson and the theoretical material from one's own notes.

The main form of work during the lessons is an interview (discussion). Each student must be ready to speak on all issues raised in the plan and demonstrate maximum activity during their consideration.

The speech must be constructed freely, convincingly, and with arguments. The instructor ensures that the speech is not reduced to a reproductive level (simple reproduction of the text); simple reading of the summary is also not allowed.

It is necessary for the speaker to show their own attitude towards what they are talking about, express their personal opinion and understanding, substantiate it, and be able to draw the correct conclusions from what has been said. In doing so, the student may refer to the entries in the summary, directly to the primary sources, use knowledge of previously studied disciplines, etc. In addition, to obtain a grade at an advanced level, students solve situational tasks based on the results of the mastered topics.

During the execution of practical works, a defense of practical skills is provided, the full list of which is specified in the fund of assessment tools.

Methodological Recommendations for the Preparation of Presentations and Reports

The report must contain elements of novelty; along with the fundamental aspect, an analysis of the current state of the problem under study must be carried out, as well as inclusion in regional issues. The report must include an introduction, a theoretical part, a conclusion, a list of literature, and a presentation that reveals the essence of the topic in graphs, pictures, diagrams, and formulas.

The report is recommended to be submitted on 1 printed sheet. The text of the work must be printed with 1.5 line spacing on one side of a standard sheet (A4) of white paper.

The text and other printed elements of the work must be black, and the contours of letters and signs must be clear, without halos and shading. The font must be Times New Roman, size 14. The titles of chapters and paragraphs are highlighted in bold. The sheet with text must have margins: left – 30 mm, right – 10 mm, top – 20 mm, bottom – 20 mm.

Page numbering of the text is done in the lower right corner of the sheet. It is necessary to number pages starting from the page where the Introduction is printed, on which the number 3 is placed. After that, all pages, including appendices, are numbered.

A space equal to two intervals is placed between the title of the chapter and the title of the paragraph of this chapter, and the title of the paragraph must not be separated from the text of this paragraph by a space. The titles of paragraphs shall be separated from the text of the previous paragraph by a space equal to two intervals. Each chapter, as well as the introduction, conclusions, appendices, and the list of used literature, begin on a new page. The word Chapter is not to be written. Chapters have ordinal numbers within the entire work, designated by Arabic numerals (for example: 1, 2, 3), followed by a period.

The word **PARAGRAPH** or the paragraph symbol (§) is not placed in the title. Paragraphs have ordinal numbers within the chapters, designated by Arabic numerals (for example: 1.1. and 1.2.).

Headings of chapters and paragraphs in the text must be centered; a period shall not be placed at the end of the title of the chapter and paragraph. It is not allowed to hyphenate (transfer) a part of a word in a heading. 4.5. The numbering of tables and figures can be running (continuous) or relate to the number of the chapter and paragraph. For example, if a table or a figure is included in the text of the first paragraph of the second chapter, the numbering is as follows: Table 2.1.1., Fig. 2.1.1.

The last digit means the ordinal number of the table (or figure) in this paragraph.

The table is to be placed as the next page after the first mention of it in the text.