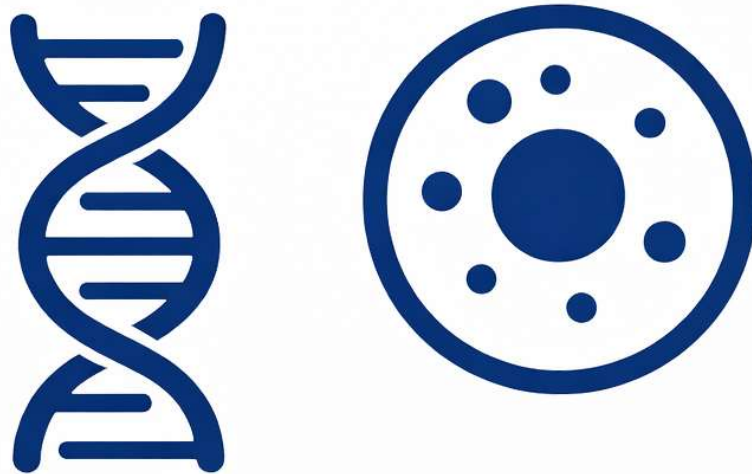


**Ľubica Milošovičová
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And colleagues**



Medical Biology and Genetics 2

Repetitorium and Model Questions

University Textbook for Undergraduate Medical Education

2026

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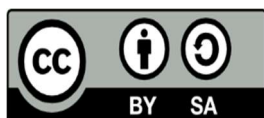
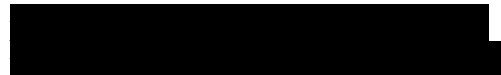
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Rewievers



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Preface

Dear students,

The presented material is intended as a guide to the study of the second semester of medical biology and genetics. This time, it focuses on more complex topics that build on and expand basic knowledge about the cell. These are areas that have a direct impact on clinical thinking and modern medicine.

The text includes key thematic areas of the second semester and is supplemented by a set of model questions. These are divided into 14 chapters according to the content of lectures and practical exercises. Their aim is to help you better sort and consolidate your knowledge, but not to replace the recommended study literature. Each chapter is also accompanied by a key of correct answers, which serves for quick feedback and self-control.

Our ambition was to create material that will support you in preparing for exams, but also contribute to a deeper understanding of biological and genetic principles important for further study and future practice. We believe that it will help you learn more systematically, more clearly and with greater confidence.

Wishing you much success in your studies,

Lubica Milošovičová, Štefan Harsányi and colleagues

Dear Students,

If you come across any kind of error, please contact MUDr. Štefan Harsányi, PhD. (stefan.harsanyi@fmed.uniba.sk). Your suggestions will be incorporated into the text. (version: May 18, 2026)

Thank you

1. The human genome, its organization. Types of DNA (nuclear and mitochondrial genomes).

The human genome represents the complete genetic information of a human, stored in the form of DNA, and consists of the nuclear genome and the mitochondrial genome. Their mutual cooperation is essential for the proper functioning of the cell, especially in the area of energy metabolism.

The nuclear genome is located in the cell nucleus and is organized into 23 pairs of chromosomes (22 pairs of autosomes and 1 pair of gonosomes). The DNA of the nuclear genome has a linear structure and is highly condensed in the form of chromatin, which is formed by DNA winding around histone proteins. The nuclear genome contains approximately 20,500 genes. About 30% consists of genes and their associated sequences (10% coding DNA and 90% non-coding DNA), while the remaining 70% is extragenomic DNA. **Coding DNA** represents only a small part of the genome (1–2%) and includes exons, which directly determine the amino acid sequence of proteins. **Non-coding DNA** represent the majority of the genome and plays an important role in the regulation of gene expression, the structural organization of chromosomes, and evolutionary processes. Non-coding DNA includes pseudogenes, gene fragments, introns, and untranslated regions. **Extragenomic DNA** consists of about 60% unique sequences or sequences with a low copy number, while 40% is made up of moderately or highly repetitive sequences (tandem, clustered, and interspersed repeats).

In addition to nuclear DNA, the human genome also includes **mitochondrial DNA (mtDNA)** located in the mitochondria, which is present in many copies in each cell. It consists of a circular DNA molecule that is present in a triple-stranded form in the region of the D-loop. The mitochondrial genome contains 37 genes, including 22 genes for tRNA, 2 genes for rRNA, and 13 genes encoding polypeptides. The mtDNA of plants and animals is rich in G and C nucleotides. The strand rich in G is called the heavy strand (H), while the strand rich in C is called the light strand (L). Unlike the nuclear genome, mtDNA does not contain introns, is characterized by a high gene density, and shows an increased rate of mutational variability. mtDNA is **inherited matrilineally** (from the mother) and is important for the study of evolution as well as for the diagnosis of certain hereditary diseases.

The variability of the human genome is relatively low. Genetic differences between individuals are mainly caused by **single nucleotide polymorphisms (SNPs)** or **copy number variations (CNVs)**, which may influence an individual's traits and susceptibility to diseases.

Genes themselves are composed of exons, introns, and regulatory regions. **Exons** encode the information for protein synthesis, while **introns** are non-coding sequences that are removed during RNA processing. In addition to genes, the genome also contains **regulatory sequences** such as promoters, enhancers, and silencers, which control when and to what extent individual genes are expressed. The genome also includes **repetitive sequences**, such as short tandem repeats (STRs), long repetitive regions, or transposons (LINEs and SINEs). Some genes exist as pseudogenes, which are nonfunctional remnants of genes created by mutations. Although repetitive sequences do not directly code for proteins, they are crucial for chromosome structure, gene expression regulation, and the evolutionary dynamics of the genome. **Tandem repeats** are sequences that occur consecutively in direct succession. Several types of these repeats are recognized, including satellite DNA, minisatellite repeats, and dispersed repeats. **Microsatellites** are mainly located in centromeric and pericentromeric regions. They form an essential part of the structural architecture of chromosomes and ensure stability and proper

chromosome segregation during cell division. **Minisatellite** repeats consist of short sequences (10–60 bp) repeated hundreds to thousands of times, with copy numbers highly variable between individuals. Microsatellites (**STRs** – short tandem repeats) are highly polymorphic and are composed of 2–5 bp repeating nucleotide units, most commonly dinucleotide CA repeats.

Dispersed repeats are spread throughout the genome and are not arranged in direct succession. These include **LINEs** (Long Interspersed Nuclear Elements), long retrotransposons of approximately 6–7 kb, which can independently copy themselves and insert into the genome. They encode proteins necessary for their own retrotransposition, particularly reverse transcriptase and endonuclease. Their presence can lead to mutations and genomic instability.

The most significant group in humans is the **LINE-1** (L1) elements, which make up approximately 17% of the genome. Another type is **SINEs** (Short Interspersed Nuclear Elements), shorter retrotransposons (~100–300 bp) that participate in gene expression regulation, alternative splicing, and genome evolution. In the human genome, the best-known example is the **Alu sequence** (approximately 300 bp), which occurs in more than one million copies. The amplification of Alu sequences has significantly contributed to the structural diversity of the genome, but it is also associated with recombination and certain genetic disorders. **Transposons** are mobile genetic elements that can move within the genome. Their activity can cause insertions, deletions, or DNA rearrangements, potentially leading to genetic diseases or evolutionary changes.

Table: Molecular organization of the genome and types of genetic elements

Structure	Characteristics	Function
Exons	Coding sequences of genes; remain in mRNA after splicing	Carry information for protein synthesis
RNA genes (rRNA, tRNA)	Do not code for protein but produce functional RNA; organized in clusters	Participate in translation
Introns	Non-coding parts of genes; not part of mature mRNA	Removed during RNA splicing
Regulatory sequences	DNA regions controlling gene activity; promoters, enhancers, silencers	Regulate gene expression
Pseudogenes	Nonfunctional copies of genes	Evolutionary significance
miRNA genes	Encode small non-coding RNAs; bind to mRNA and inhibit translation	Regulation of gene expression
Tandem repeats	Sequences repeated in direct succession; satellite DNA, minisatellites, microsatellites	Chromosome structure
Satellite DNA	Repetitive DNA	Chromosome stability
Minisatellites	10–60 bp repeats, hundreds to thousands of copies	High variability
Microsatellites	2–5 bp repeats, mainly CA repeats	Highly polymorphic
Disperse repeats	Spread throughout the genome; LINEs, SINEs	Genome evolution and structure
LINE (L1)	~6–7 kb retrotransposons; make up ~17% of the genome	Can cause mutations
SINE (Alu)	~100–300 bp retrotransposons; >1 million copies	Regulate gene expression and alternative splicing
Transposons	Mobile genetic elements	Can cause insertions, deletions, and DNA rearrangements; associated with diseases

In addition to protein-coding genes, there is a large number of **RNA genes** (approximately 60,000) that encode rRNA and tRNA and are organized into large gene clusters. Small, single-stranded non-coding RNA molecules, 21–23 nucleotides long, known as **microRNAs** (miRNAs), are involved in the regulation of gene expression. They partially bind to mRNA (usually in the 3' UTR), thereby inhibiting its translation. miRNAs are produced through transcription but are not translated into proteins. They are found in various organisms, including humans, plants, and animals, and their sequences are located in non-coding regions of the genome.

The Human Genome Project

The Human Genome Project (HGP) was an international scientific project focused on **mapping and sequencing the entire human genome**. It provided invaluable insights into genetics and genomics, while also highlighting the importance of addressing the ethical and social issues associated with the use of genetic information. The central goal of the HGP was to determine the complete sequence of the human genome. This included identifying and mapping all genes, non-coding sequences, and regulatory elements that make up human DNA. The project aimed to identify all genes in the human genome and determine their functions. The initial draft of the human genome was referred to as “**Day G**,” and **the project was successfully completed on April 15, 2003**, two years ahead of schedule. It produced a **reference human genome**, which became the foundation for genetic research, medicine, and biotechnology. HGP had several key objectives: **determining the complete sequence of human DNA, identifying all human genes, mapping genetic variations** (creating genetic and physical maps), **archiving the obtained information** (developing computer databases), and **addressing the ethical, legal, and social issues** raised by the project.

After the Human Genome Project, several new genomic projects followed. The **Human Genome Diversity Project** aimed to analyze polymorphisms in the human genome. The **1000 Genomes Project** created a catalog of common genetic variations in humans, using samples from volunteers who had declared themselves healthy – 2,500 unidentified individuals from 20 populations around the world. The **Encyclopedia of DNA Elements (ENCODE)** aimed to identify all functional elements in the genomes of humans and mice. The **Human Proteome Project** focused on a comprehensive description of proteins in the human body, including their functions, cellular localization, association with respective genes, and quantification of protein products resulting from gene expression. The **Human Microbiome Project** characterized microbial communities (bacteria, archaea, viruses, and fungi) across various parts of the human body and analyzed their impact on physiological processes, health, and disease development. The **Earth BioGenome Project** mapped the genomes of all known living plant and animal species on Earth.



Model questions

1. What were the key goals of the Human Genome Project:

- a. determining the complete sequence of human DNA
- b. identifying all human genes
- c. mapping genetic variations
- d. archiving the obtained information

2. Which other projects followed the Human Genome Project (HGP):

- a. 1000 Genomes Project
- b. Human Genome Diversity Project
- c. ECOCODE
- d. Human Microbiome Project

3. What are the possible consequences of genetic testing?

- a. it can cause frustration, especially if no treatment exists
- b. test results only apply to the tested individual and not to other family members
- c. it can reveal sensitive information, such as true paternity
- d. genetic information is already fully accessible today through mobile applications

4. Alu sequences have:

- a. high GC content
- b. high AT content
- c. a length of 3000 bp
- d. a length of 300 bp

5. Alu sequences belong to:

- a. retropseudogenes
- b. retrotransposons
- c. retroviruses
- d. endogenous retroviruses

6. Alu sequences:

- a. are retropseudogenes
- b. make up 23% of the human genome
- c. have a length of 300 bp
- d. belong to SINE sequences

7. Alu sequences:

- a. contains many guanines
- b. contains many cytosines
- c. make up 3% of the human genome
- d. make up 1% of the human genome

8. The goal of the Human Genome Project was:

- a. creating physical maps of human DNA
- b. identifying all human genes
- c. determining the complete sequence of human DNA
- d. creating a genetic map of human DNA

9. What does the nuclear genome consist of?

- a. coding DNA
- b. non-coding DNA
- c. mitochondrial DNA
- d. extragenomic DNA

10. The D-loop of mitochondrial DNA:

- a. does not contain coding sequences
- b. contains coding sequences
- c. is important for repair
- d. is important for replication

11. Gene families can be:

- a. classical, with the same sequence throughout the gene
- b. superfamilies, encoding functionally different products
- c. encoding products with short amino acid motifs, having only a short DNA motif in common while differing in other regions of the gene
- d. superfamilies, encoding functionally related products

12. Genes in mitochondrial DNA are:

- a. regulatory genes
- b. structural genes
- c. genes for rRNA
- d. genes for tRNA

13. Genes in human DNA are divided into:

- a. huge
- b. dystrophin
- c. medium
- d. small

14. Nuclear DNA is:

- a. double-stranded
- b. linear
- c. circular
- d. chromosomal DNA

15. The smallest gene in human DNA is the gene for:

- a. pre-mRNA
- b. mRNA
- c. t-RNA
- d. dystrophin

16. Highly repetitive DNA sequences include:

- a. tandem repetitions
- b. unique repetitions
- c. dispersed repetitions
- d. transposable elements

17. When was the HUGO project successfully completed:

- a. 1999
- b. 2003
- c. 2010
- d. 2025

18. How many genes are encoded by mitochondrial DNA?

- a. 36
- b. 35
- c. 37
- d. 38

19. Which statements about microRNA (miRNA) are correct?

- a. they are 21–23 nucleotides long and regulate gene expression
- b. they are produced by translation from non-coding regions of DNA
- c. they bind to mRNA and typically inhibit translation
- d. they encode small regulatory proteins

20. Which statements about microsatellite DNA are correct?

- a. it contains repeats 9–65 nucleotides long
- b. it is highly polymorphic and often used as a genetic marker
- c. it most commonly contains trinucleotide CA repeats
- d. it can be easily analyzed using PCR

21. The light strand in mitochondrial DNA contains many:

- a. adenines
- b. thymine
- c. guanines
- d. cytosines

22. LINE1 (long interspersed nuclear elements) belong to:

- a. retroviruses
- b. endogenous retroviruses
- c. retropseudogenes
- d. retrotransposons

23. LINE1 (long interspersed nuclear elements):

- a. does not have LTR sequences
- b. have LTR sequences
- c. encodes reverse transcriptase
- d. do not encode reverse transcriptase

24. LTR retrotransposons are:

- a. endogenous retroviruses
- b. HERV (human endogenous retroviruses)
- c. SINE (short interspersed nuclear elements)
- d. retrovirus-like elements

25. Endogenous retroviruses include:

- a. HERV (human endogenous retroviruses)
- b. Alu sequences
- c. RTLV (retrovirus-like elements)
- d. LINE1 (long interspersed nuclear elements)

26. Mitochondrial DNA in the D-loop region is:

- a. linear
- b. triple-stranded
- c. double-stranded
- d. single-stranded

27. Mitochondrial DNA is:

- a. double-stranded
- b. linear
- c. circular
- d. non-coding

28. Which statement about gene families is generally true?

- a. an example is the hemoglobin gene
- b. an example is the insulin gene
- c. all have a locus control region
- d. all members shared one common promoter

29. The last completely sequenced chromosome is:

- a. X
- b. Y
- c. 21
- d. 1

30. Strands in mitochondrial DNA are:

- a. ultra-light
- b. heavy
- c. light
- d. medium-heavy

31. The heavy strand in mitochondrial DNA contains many:

- a. adenines
- b. thymine
- c. guanines
- d. cytosines

32. Transposable elements:

- a. have introns
- b. do not have introns
- c. are terminated by a polyA sequence
- d. are terminated by a cap

33. Transposable elements:

- a. are not important for evolution
- b. can jump to another location on the same chromosome
- c. can move to another chromosome
- d. cannot move to another chromosome

34. Animal mitochondrial DNA contains many:

- a. adenines
- b. guanines
- c. thymines
- d. cytosines

35. The heavy strand of mitochondrial DNA contains information for the structure of:

- a. 12 proteins
- b. 2 rRNAs
- c. 14 tRNAs
- d. 13 proteins

36. Which other genomic projects followed the Human Genome Project?

- a. Human Microbiome Project
- b. Human Genome Diversity Project
- c. Human Proteome Project
- d. a project addressing ethical issues

37. What was the goal of the 1000 Genomes Project?

- a. solving ethical issues
- b. the project analyzed the genomes of more than 2,500 individuals from dozens of populations
- c. it created a catalog of common genetic variations in humans
- d. to identify both common and rare genetic variants

38. What are the results of the 1000 Genomes Project used for today?

- a. provided a foundation for research on genetic diseases
- b. provided a foundation for research on personalized medicine
- c. provided a foundation for research on the human microbiome
- d. provided a tool for understanding human population evolution and migration

39. What was the focus of the ENCODE (Encyclopedia of DNA Elements) project?

- a. created a basis for the study of hereditary diseases
- b. identification through evolution and migration of human populations
- c. identification of all functional elements in the human and mouse genomes
- d. analysis of introns

40. What did the Human Proteome Project study?

- a. the complete set of all proteins in the human body
- b. analyzed polymorphisms in the human genome
- c. linked genes to specific proteins and their functions
- d. the structure, function, quantity, and interactions of proteins

41. What was the focus of the Human Microbiome Project?

- a. studied human proteins and their functions
- b. focused on the microbiome of the gut, oral cavity, skin, nose, vagina, and urinary tract
- c. the quantity of proteins produced by gene expression
- d. characterized microbial communities in various parts of the human body

42. What did the Earth BioGenome Project map?

- a. microbiome
- b. the genetic diversity of life on Earth
- c. mapped the genomes of all known living plant and animal species on Earth
- d. the genome of only prokaryotic organisms on Earth

43. Which types of genomic sequence variation directly contribute to genetic variability?

- a. single nucleotide polymorphisms
- b. mitochondrial DNA
- c. copy number variations
- d. non-coding RNA

44. What are genes composed of?

- a. introns
- b. exons
- c. coding sequences
- d. non-coding sequences

45. What are microRNAs (miRNAs) involved in?

- a. the production of nuclear RNA
- b. the production of mitochondrial DNA
- c. affecting variations in gene copy number
- d. the regulation of gene expression

46. What is the human genome?

- a. the complete set of DNA in a human cell
- b. the complete set of proteins in the body
- c. the complete set of RNA molecules in the body
- d. the set of mitochondrial DNA

47. What proportion of the genome is made up of coding DNA (genes)?

- a. less than 1%
- b. about 1–2%
- c. about 10–15%
- d. more than 30%

48. What is the significance of the Human Genome Project for medicine?

- a. helps develop new technologies
- b. reduces the number of genetic diseases
- c. allows a better understanding of hereditary diseases
- d. contributes to personalized medicine

49. What are single nucleotide polymorphisms (SNPs)?

- a. variations in a single DNA base between individuals
- b. special types of proteins
- c. variations in a single base in mtDNA
- d. variations in a single RNA base between individuals

50. Approximately how many genes does the human genome have?

- a. less than 1,000
- b. 1,000–5,000
- c. 20,000–25,000
- d. 100,000–200,000

51. Genes are not found in the cell:

- a. in mitochondria
- b. in nuclear ribosomes
- c. in the nucleus
- d. in the nuclear envelope

52. Which structures are part of the coding regions of the genome?

- a. introns
- b. exons
- c. RNA genes (rRNA, tRNA)
- d. satellite DNA

53. Regulatory sequences:

- a. includes promoters
- b. influence the level of gene expression
- c. includes enhancers and silencers
- d. encode amino acids

54. Which statements about miRNA are true:

- a. encodes proteins
- b. inhibits translation
- c. binds to mRNA (most often in the 3' UTR)
- d. encodes reverse transcriptase

55. Transposons:

- a. are mobile genetic elements
- b. can cause insertions or deletions
- c. are always inactive in the human genome
- d. participate in evolutionary changes

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. TTTT | 20. FTFT | 39. FFTF |
| 2. TTFT | 21. FFFT | 40. TFTT |
| 3. TFTF | 22. FFFT | 41. FTFT |
| 4. TFFT | 23. TFTF | 42. FTTF |
| 5. FTFF | 24. TTFT | 43. TFTF |
| 6. FFFT | 25. TFTF | 44. TTTT |
| 7. TTFF | 26. FTFF | 45. FFFT |
| 8. TTTT | 27. TFTF | 46. TFFF |
| 9. TTFT | 28. TFFF | 47. FTFF |
| 10. TFFT | 29. FTFF | 48. FFFT |
| 11. TFTT | 30. FTTF | 49. TFFF |
| 12. FTTT | 31. FFTF | 50. FFTF |
| 13. TFTT | 32. FTTF | 51. FTFT |
| 14. TTFT | 33. FTTF | 52. FTTF |
| 15. FFTF | 34. FTFT | 53. TTTF |
| 16. TFTT | 35. FTTT | 54. FTTF |
| 17. FTFF | 36. TTTF | 55. TTFT |
| 18. FFTF | 37. FTTT | |
| 19. TFTF | 38. TTFT | |

2. Basic Methods of Human Genetics.

Human genetics has several specific features that arise from ethical and practical limitations. In humans, it is not possible to use experimental crosses as in the genetics of plants and animals. Nevertheless, the goal is the same – to determine to what extent a trait or disease is determined by heredity and to what extent by the environment, and at the same time to apply the results in medicine for diagnosis, prevention, and genetic prognosis. Therefore, **clinical genetics** relies on methods that work with naturally occurring families and populations, especially the **twin method** (analysis of twins) and the **pedigree method** (analysis of pedigrees), supplemented by a population-genetic approach. These approaches are practical: they allow estimation of the risk of disease recurrence in a family, identification of possible carrier status for pathogenic alleles, and, in many cases, a targeted indication for further genetic testing.

The **twin method** compares monozygotic and dizygotic twins. **Monozygotic** (identical) twins arise by division of a single zygote in the early stages of development; therefore, under usual circumstances, they have an identical genotype. If phenotypic differences appear between them, these are attributed mainly to non-genetic influences, differences in the intrauterine environment, random developmental variation, or later environmental factors. **Dizygotic** (fraternal) twins arise when two different ova are fertilized by two sperm. Genetically, they are similar to ordinary siblings; on average, they share about half of their genetic material. The essence of the method is that, for a given trait or disease, we evaluate agreement of occurrence within a twin pair (**concordance**) and disagreement (**discordance**) separately in monozygotic and dizygotic twins. If a trait is strongly genetically determined, markedly higher concordance is expected in monozygotic than in dizygotic twins. If the environment is decisive, the difference between these groups becomes smaller.

To quantify the contribution of heredity, **heritability**, denoted H , is often used. In a simple approach, it is estimated by comparing the percent concordance in monozygotic twins (C_{MZ}) and dizygotic twins (C_{DZ}) using the relationship $H = (C_{MZ} - C_{DZ}) / (1 - C_{DZ})$. The value of H ranges from 0 to 1. If C_{MZ} is very high and markedly exceeds C_{DZ} , H approaches 1, and the trait has a strong genetic component (typically, traits with a simple genetic basis). If C_{MZ} approaches C_{DZ} , H approaches 0, suggesting predominance of non-genetic influences. In practice, it is important to understand that **heritability does not describe the “geneticness” of an individual**, but rather the proportion of genetic variation contributing to trait variation within a specific population under specific environmental conditions. In teaching and practical exercises, this principle is also used to compare the heritability of different diseases based on known C_{MZ} and C_{DZ} values.

The **pedigree method** (pedigree analysis) consists of constructing a standard pedigree and evaluating it. A pedigree is a clear representation of kinship relationships in a family, with the studied trait (for example, a disease) indicated using agreed symbols. The first examined family member through whom the pedigree is “opened” is referred to as the **proband**. This may be a patient with the disease or a relative who is examined first.

In clinical practice, data are obtained mainly from the medical history and documentation, with the aim of covering **at least three generations**, ideally including more extended relatives. In the neonatal period (the first 28 days), many congenital developmental anomalies become apparent. If findings suggest a genetic disorder, the child is referred to a medical genetics outpatient clinic, where one of the first steps is usually a thorough pedigree analysis, supplemented by a clinical examination and review of available specialist consultation findings.

Based on the pedigree and phenotype, the clinical geneticist then plans targeted further investigations. Pedigree analysis enables the determination of the most likely mode of inheritance. The key step is to determine whether the gene for the trait is located on the autosomes or on the sex chromosomes, and whether the trait is dominant or recessive. This results in **four basic “classical” pedigree patterns**: autosomal dominant, autosomal recessive, X-linked dominant, and X-linked recessive.

In **autosomal dominant inheritance**, the trait typically appears in every generation. Affected individuals often have an affected parent, and transmission can occur from father to son as well as from mother to son or daughter. Because a dominant pathogenic allele is usually rare in the population, an affected individual is often assumed to be heterozygous. If they have children with an unaffected partner, the risk that a child will be affected is approximately 50%.

In **autosomal recessive inheritance**, by contrast, the parents of an affected individual are usually phenotypically unaffected but are carriers (heterozygotes). The condition may “appear” among siblings without an obvious occurrence in the previous generation, and when both parents are carriers, the risk of an affected child is typically 25%.

An important practical point is that in rare recessive disorders, **consanguinity** (marriage between relatives) is more common, especially in isolated populations. This increases the probability that two identical recessive pathogenic alleles meet in the zygote. In X-linked inheritance, pedigrees show characteristic features because males are **hemizygous** for the X chromosome.

In **X-linked dominant inheritance**, an affected father passes his X chromosome to all daughters, so all daughters will be affected. However, a father passes a Y chromosome to his sons, and therefore (if the mother is unaffected) the sons are unaffected.

In **X-linked recessive inheritance**, affected individuals are mainly boys. An affected male passes the mutated X chromosome to all daughters, who then become carriers. A typical pedigree feature can be “skipping” of generations (for example, grandfather → grandson through a carrier mother).

The pedigree method is used not only to determine the mode of inheritance but also to **estimate the risk** of recurrence of a disorder within a family, **identify** likely carriers in recessive conditions, and **plan genetic testing**. When interpreting pedigrees, it is important to consider phenomena that can “distort” the pattern. These include **incomplete penetrance** (an individual with a risk genotype may not express the phenotype), **variable expressivity** (differences in the severity of manifestations), and late-onset disease (when a trait appears only in adulthood). These concepts are often addressed in model tasks and in the interpretation of family data.

Today, issues of **biological parentage** or unclear kinship relationships can be professionally resolved through DNA analysis, significantly increasing the reliability of pedigree conclusions.

Population genetics shifts the perspective from the family level to the population level. It examines how often particular alleles and genotypes occur in a population, how the gene pool changes, and which factors cause these changes. A population is understood as a group of individuals of one species living in a reproductive community with a shared gene pool. The **gene pool** is the sum of all alleles present in a population and can also be described using the “gamete pool” (alleles in gametes) or the “zygote pool” (alleles in zygotes), which together

represent the population genetic make-up. From a practical medical perspective, population genetics is important, especially for understanding the occurrence of inherited diseases, carrier status, and risk in different population groups, as well as for preventive programs.

The gene pool changes due to random and non-random factors. In small populations, chance plays a major role – **genetic drift**, where allele frequencies change between generations simply because the next generation receives a random sample of gametes. In large populations, non-random factors have a greater effect, especially mutation, selection, and migration. **Mutations** are a source of new alleles for population genetics and medicine. Particularly relevant are heritable mutations and allelic variants that affect protein function. **Selection** (natural selection) influences whether a carrier of a particular allele leaves offspring: negative selection removes strongly harmful alleles from the gene pool, whereas positive selection can, under specific conditions, favour carriers (a classic example is the advantage of sickle-cell heterozygotes in regions with malaria). **Migration** mixes gene pools between populations and changes local mutation frequencies. Modern genetic methods also allow historical population movements to be traced retrospectively based on the distribution of DNA variants.

A key foundation of population genetics is the **Hardy–Weinberg principle**. In a **panmictic population** (random mating) and under ideal conditions, allele and genotype frequencies reach equilibrium after one generation. If the frequency of the dominant allele is “p” and the recessive allele is “q”, the equilibrium relationship is “ $p + q = 1$ ”. Genotype frequencies are “ p^2 ” (dominant homozygotes), “ $2pq$ ” (heterozygotes), and “ q^2 ” (recessive homozygotes), i.e., “ $(p + q)^2 = 1$ ”. In practice, this model is used to estimate carrier frequency for recessive disorders and to assess whether observed genotype frequencies match expectations in a given population. For equilibrium to hold, the population must be panmictic, sufficiently large, and not influenced by selection, migration, or mutation. In the real world, these conditions are never met perfectly, but the principle is very useful as a reference framework.

If **inbreeding** (non-random mating between genetically related individuals) predominates in a population, genotype frequencies shift. The proportion of homozygotes increases, and the proportion of heterozygotes decreases (inbreeding effect; often expressed using Wright’s inbreeding coefficient, F). This is medically important mainly because it increases the probability of homozygosity for rare recessive pathogenic alleles, and thus the occurrence of recessive disorders. Other mating patterns include **homogamy** (assortative mating, i.e., a preference for partners with a similar phenotype, which can increase the frequency of specific phenotypes in offspring) and **panmixia**, the ideal model of random mating. Population-genetic reasoning, therefore, connects with clinical genetics. It helps explain why some disorders have different frequencies in different regions, ethnic groups, or isolates, and why the risk of recessive disorders is higher in some families.

Alongside these methods, yet very important in practice, is **forensic genetics**. This is a separate field that mainly uses molecular genetic methods to identify individuals and biological traces in legal practice, mass disasters, or disputes over biological relatedness (for example, paternity testing). Kinship analyses in forensic practice overlap conceptually with pedigree reasoning, and DNA evidence can confirm or exclude biological relationships with high reliability. Approaches such as genetic genealogy and genogeography are also being developed; these link genetic data to family history and historical population context. In medicine, this may expand the possibilities for estimating the risk of inherited disorders within families across generations, while always with careful attention to ethical and legal rules governing the handling of genetic data.



Model questions

1. What determines the value of H (heritability)?

- a. the proportion of phenotypic variance explained by genetic variance in a population
- b. the proportion of phenotypic variation caused by environmental factors
- c. genetic variation within a population
- d. the degree of genetic similarity between relatives

2. What does it mean when $H = 0.3$?

- a. about 30% of the phenotypic variance in the population is attributable to genetic differences
- b. about 30% of the phenotype is due to the environment
- c. about 3% of the phenotype is due to genotype
- d. about 70% of the phenotypic variance in the population is attributable to non-genetic factors (including environment)

3. What does it mean when $H = 0.95$?

- a. about 5% of the phenotype is due to genotype
- b. about 5% of the phenotypic variance in the population is attributable to non-genetic factors (including environment)
- c. about 0.95% of the phenotype is due to genotype
- d. about 0.5% of the phenotype is due to the environment

4. What is the pedigree (genealogical) method?

- a. analysis of twin studies
- b. pedigree analysis
- c. a method used in human genetics
- d. population analysis

5. The mathematical expression of the Hardy–Weinberg principle is:

- a. $(p + q)^2 = 1$
- b. $p - q = 1$
- c. $p^2 + 2pq + q^2 = 1$
- d. $2pq = 1$

6. What does the variable “p” represent in the Hardy–Weinberg principle?

- a. the frequency of heterozygotes
- b. the frequency of the dominant allele
- c. the frequency of the recessive allele
- d. the frequency of unaffected individuals

7. What does the variable “q” represent in the Hardy–Weinberg principle?

- a. the frequency of heterozygotes
- b. the frequency of the dominant allele
- c. the frequency of the recessive allele
- d. the frequency of unaffected individuals

8. What does “ $2pq$ ” represent in the Hardy–Weinberg principle?

- a. the frequency of heterozygotes
- b. the frequency of the dominant allele
- c. the frequency of the recessive allele
- d. the frequency of unaffected homozygotes

9. What does “ p ” represent in the Hardy–Weinberg principle?

- a. the frequency of heterozygotes
- b. the frequency of the dominant allele
- c. the frequency of the recessive allele
- d. the frequency of dominant homozygotes

10. What does “ q^2 ” represent in the Hardy–Weinberg principle?

- a. the frequency of heterozygotes
- b. the frequency of the dominant allele
- c. the frequency of the recessive allele
- d. the frequency of recessive homozygotes

11. If a population is in Hardy–Weinberg equilibrium, it holds that:

- a. the population is panmictic, and there is no selection
- b. the population is panmictic, and there is no mutation
- c. the population is panmictic, and there is no migration
- d. the population is not panmictic

12. Why does human genetics have specific features compared with genetic research in plants and animals?

- a. human genetics does not focus on the inheritance of pathological traits
- b. experimental crosses cannot be performed in humans
- c. humans have a different DNA structure than plants and animals
- d. genetic studies in humans must respect ethical restrictions

13. What is the aim of clinical genetics?

- a. to perform experimental crosses in humans
- b. to diagnose inherited disorders and determine the probability of their occurrence
- c. to eliminate the need for prenatal genetic testing
- d. to assess genetic risk (genetic prognosis) and understand the basis of genetically determined disorders

14. Methods used in human genetics include:

- a. the twin method (gemellological) – twin research
- b. the pedigree method (genealogical) – pedigree analysis
- c. population-based methods
- d. cytogenetic methods

15. What does the pedigree method in human genetics allow?

- a. determining the mode of inheritance of a trait based on pedigree analysis
- b. determining the probability of a mutation in mitochondrial DNA
- c. identifying carriers of a pathogenic allele in recessive disorders
- d. determining gene order on a chromosome using recombination frequencies

16. Which statements about constructing a pedigree are true?

- a. a pedigree should include at least three generations, ideally four
- b. family members within a generation are labeled with Roman numerals from left to right
- c. a legend (key) is part of the pedigree and includes additional information
- d. identifying the biological father is not important from a genetic point of view

17. Which statements about the twin method are correct?

- a. it is used to estimate the contribution of inherited and non-inherited factors to a trait
- b. it is based on comparing monozygotic and dizygotic twins
- c. it is based on experimental crosses of selected individuals
- d. it does not use concordance and discordance assessment

18. How do monozygotic (identical) twins arise?

- a. by fertilization of two different eggs by two sperm
- b. by splitting of the early embryo (e.g., at the morula stage) during early embryogenesis
- c. they always have different genotypes
- d. they originate from a single zygote

19. Which statements about monozygotic twins are correct?

- a. under usual circumstances, they have the same genotype
- b. their coefficient of relatedness is ~100% ($r \approx 1.0$)
- c. phenotypic differences between them are mainly due to non-genetic factors, although some genetic/epigenetic differences can arise over time
- d. their coefficient of relatedness is 50%

20. How do dizygotic (fraternal) twins arise?

- a. by the splitting of a single zygote into two parts
- b. by fertilization of two different eggs by two different sperm
- c. they develop in the uterus at the same time
- d. their coefficient of relatedness is 100%

21. Which statements about dizygotic twins are correct?

- a. their coefficient of relatedness is about 50%
- b. they have a genetic make-up similar to other siblings
- c. they have exactly the same genotype
- d. all phenotypic differences are exclusively genetic

22. What is compared between MZ and DZ twins in the twin method?

- a. concordance (agreement) of the occurrence of the same trait form
- b. discordance (disagreement) of the occurrence of the same trait form
- c. gene order on a chromosome
- d. mutation pressure in the population

23. What does H (heritability) express?

- a. the proportion of phenotypic variance attributable to genetic factors (in a given population and environment)
- b. the coefficient of relatedness between siblings
- c. the occurrence of alleles in the population gene pool
- d. the type of reproductive population

24. Which variables are used in the formula to calculate H?

- a. C_{MZ} – percentage concordance of a trait in monozygotic twins
- b. C_{DZ} – percentage concordance of a trait in dizygotic twins
- c. p – frequency of the dominant allele
- d. q – frequency of the recessive allele

25. When is a trait completely genetically determined?

- a. when $C_{MZ} = 100\%$
- b. when $H = 1$
- c. when $C_{MZ} = C_{DZ}$
- d. when $H = 0$

26. Which example represents a completely genetically determined trait?

- a. ABO blood group
- b. spontaneous abortions
- c. population migration
- d. stillbirths

27. When is H equal to zero?

- a. when C_{MZ} equals C_{DZ}
- b. when the trait is entirely determined by non-genetic factors (within the given population and environment)
- c. when $C_{MZ} = 100\%$
- d. when $C_{DZ} = 0\%$

28. What is the essence of the pedigree method?

- a. constructing and analyzing a standard pedigree
- b. direct analysis of DNA polymorphisms
- c. twin research
- d. assessing the gene pool of the whole population

29. Which data are recorded in pedigree analysis besides pathological conditions and anomalies?

- a. spontaneous abortions
- b. stillbirths
- c. fertility disorders
- d. drug prices

30. What is the family member called from whom pedigree analysis begins?

- a. proband
- b. mutant
- c. zygote
- d. gamete

31. Which statements about collecting pedigree data are correct?

- a. data should, if possible, be obtained from multiple sources
- b. data should be verified (e.g., medical records, registries)
- c. an oral statement without verification is sufficient
- d. the data are not used to construct the diagram

32. Which rules for constructing a pedigree are correct?

- a. a pedigree should include at least three generations
- b. generations are labelled with Roman numerals from oldest to youngest
- c. individuals within a generation are labelled with Arabic numerals from left to right
- d. the pedigree should avoid including more distant relatives

33. Which statements about the content of a pedigree are correct?

- a. the pedigree should be developed both “horizontally” (breadth) and “vertically” (depth)
- b. a legend (key) with important information is part of the pedigree
- c. the pedigree is labelled only with letters without numbers
- d. deviations in symbols are not included in the legend

34. How can each family member be identified in a pedigree?

- a. using coordinates (generation number / individual number within the generation)
- b. only by first and last name
- c. only by date of birth
- d. using the value of H

35. From a genetic perspective, what is most important in pedigree analysis, even though it may be difficult?

- a. establishing the true biological parents, especially the father
- b. determining the size of the gamete pool
- c. determining only the geographic location of the family
- d. estimating mutation pressure without DNA

36. How can the professional aspect of establishing biological relatedness be addressed today?

- a. identification of individuals using DNA analysis methods
- b. exclusively by family memories
- c. by experimental crosses
- d. only using the Hardy–Weinberg principle

37. What does population genetics study?

- a. genetic phenomena at the level of a whole population or part of it
- b. the occurrence of alleles in the gene pool and genotypes in the population
- c. experimental human crosses
- d. exclusively the diagnosis of a single patient without family data

38. Which statements about a population are correct?

- a. a population is a group of individuals of one species living in a reproductive community
- b. population members are potentially able to interbreed
- c. a population has a shared gene pool
- d. a population is always composed only of related individuals

39. What is the gene pool?

- a. the sum of all genes (alleles) of the members of a given reproductive population
- b. the set of alleles only in zygotes
- c. the set of alleles only in gametes
- d. a list of all chromosomal aberrations in a family

40. Which statements about the gamete pool and zygote pool are true?

- a. the gamete pool is the set of all alleles in the gametes of individuals in a population
- b. the zygote pool is the set of alleles in zygotes formed in the population
- c. the gene pool can be viewed as the sum of the gamete pool and zygote pool of a population
- d. the gamete pool includes only recessive alleles

41. Which factors cause changes in the gene pool?

- a. genetic drift
- b. mutation
- c. selection
- d. migration

42. In which populations do random changes in the gene pool play a major role?

- a. in small populations
- b. in large populations, where their effect is not masked by random changes
- c. in panmictic populations regardless of size
- d. in populations where only positive selection occurs

43. What is genetic drift?

- a. a random change in allele frequencies between generations in small populations
- b. a predictable consequence of migration in large populations
- c. a process that monitors allele occurrence in gametes
- d. an equilibrium state of genotype frequencies after one generation

44. Which statements about selection are true?

- a. selection affects whether a carrier of a mutation can contribute to reproduction
- b. negative selection is more common
- c. positive selection is rarer
- d. selection has no effect on the gene pool

45. Which example represents positive selection?

- a. heterozygote advantage for the sickle-cell allele in malaria-endemic regions
- b. formation of a morula during embryogenesis
- c. complete genotype identity in MZ twins
- d. Hardy–Weinberg equilibrium

46. What is migration in population genetics?

- a. movement of large population groups
- b. a factor that affects the distribution of mutations in local populations
- c. a process with no influence on mutation occurrence
- d. a form of homogamy

47. What areas does human population genetics study?

- a. the distribution of genetic differences, mutations, and polymorphisms in a population
- b. how genetic differences change due to selection, migration, and genetic drift
- c. exclusively pedigree analysis of families
- d. only diagnosis of newborns in the first 28 days

48. Which types of reproductive populations (mating patterns) are recognized?

- a. panmixia
- b. homogamy
- c. inbreeding
- d. gamete pool

49. Which statements about panmixia are correct?

- a. it's a sufficiently large population with random mating of genetically unrelated individuals
- b. it is an open population without major barriers (e.g., geographic)
- c. in such a population, random processes fully determine allele combinations and genotype occurrence
- d. it is a population with non-random mating of related individuals

50. Which statements about inbreeding are true?

- a. it is non-random mating between genetically related individuals
- b. in humans, it occurs mainly in isolates (geographic, ethnic, religious, economic)
- c. it markedly increases the probability of offspring being homozygous for a mutant allele
- d. it is typical only of panmictic populations

51. Inherited diseases are caused by changes in genetic material of different types, for example:

- a. monogenic
- b. multifactorial
- c. mitochondrial
- d. infectious

52. Which of the following is another type of genetic change associated with inherited diseases?

- a. chromosomal aberrations
- b. changes in blood pressure
- c. disturbances of the glycaemic curve
- d. vitamin deficiencies

53. Practical application of genetic knowledge in medicine makes it possible to:

- a. estimate the risk of a genetically determined condition in a family (genetic prognosis)
- b. in some cases, confirm or exclude a genetic condition prenatally
- c. replace all clinical examinations with pedigree information alone
- d. understand the basis of various genetically determined pathological conditions

54. Population genetics:

- a. uses modelling and compares a model population with a real population
- b. has a strong preventive and medically practical focus
- c. is based on experimental crosses in humans
- d. tracks allele frequencies in the gene pool and genotype frequencies in the population

55. By methodological approach, population genetics has two basic branches:

- a. theoretical population genetics, based on mathematical models
- b. empirical population genetics
- c. cytogenetic population genetics
- d. forensic population genetics

56. Which statements about homogamy are correct?

- a. it is preference for marriage and parenthood between phenotypically similar individuals
- b. it can increase the probability of the same form of pathological phenotypes in offspring
- c. it is random mating of genetically unrelated individuals
- d. it decreases the probability of the same phenotypes in offspring

57. According to Wright's inbreeding coefficient (F), compared with a panmictic population, there is:

- a. an increase in the frequency of both types of homozygotes at the expense of heterozygotes
- b. a decrease in the frequency of heterozygotes
- c. an increase in heterozygotes and a decrease in homozygotes
- d. maintenance of genotype frequencies according to the Hardy–Weinberg equilibrium

58. Which of the following statements is true?

- a. in the neonatal period, most congenital developmental anomalies become apparent
- b. if a genetic disorder is suspected, the child is referred to a medical genetics outpatient clinic
- c. the first step is always DNA analysis without the need for pedigree analysis
- d. a pedigree covering at least three generations is part of the first-step genealogical analysis

59. The main branches of forensic genetics are:

- a. forensic casework genetics (criminalistics / crime-scene genetics)
- b. identification genetics
- c. investigation of biological relatedness (e.g., paternity testing)
- d. the twin method

60. In practice, forensic genetics is mainly used for:

- a. identification of persons and biological traces in legal practice
- b. treatment of monogenic diseases using gene therapy
- c. determining only the ABO blood group for transfusions
- d. confirming or excluding biological relatedness (e.g., maternity testing)

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. TFFF | 21. TTFF | 41. TTTT |
| 2. TFFT | 22. TTFF | 42. TFFF |
| 3. FTFF | 23. TFFF | 43. TFFF |
| 4. FTTF | 24. TTFF | 44. TTTF |
| 5. TFTF | 25. TTFF | 45. TFFF |
| 6. FTFF | 26. TFFF | 46. TTFF |
| 7. FFTF | 27. TTFF | 47. TTFF |
| 8. TFFF | 28. TFFF | 48. TTTF |
| 9. FFFT | 29. TTTF | 49. TTTF |
| 10. FFFT | 30. TFFF | 50. TTTF |
| 11. TTTF | 31. TTFF | 51. TTTF |
| 12. FTFT | 32. TTTF | 52. TFFF |
| 13. FTFT | 33. TTFF | 53. TTFT |
| 14. TTTT | 34. TFFF | 54. TTFT |
| 15. TFTF | 35. TFFF | 55. TTFF |
| 16. TFTF | 36. TFFF | 56. TTFF |
| 17. TTFF | 37. TTFF | 57. TTFF |
| 18. FTFT | 38. TTTF | 58. TTFT |
| 19. TTTF | 39. TFFF | 59. TTTF |
| 20. FTTF | 40. TTTF | 60. TFFF |

3. Monogenic inheritance.

Monogenic inheritance refers to the transmission of traits and diseases in which a specific trait form is determined by a mutation or variant in a **single gene at a single locus**. Such traits may be **normal** (for example, blood groups) or **pathological** (for example, genetic diseases). Depending on whether the locus is located on an autosome or on a sex chromosome, we distinguish **autosomal** and **gonosomal (sex-linked)** inheritance. With respect to allele relationships, monogenic traits may be inherited in a **dominant** or **recessive** manner, and dominance may be **complete**, **incomplete** (intermediate), or **codominant**.

The basic biological basis of monogenic pathological conditions is most often a mutation at the gene level (a **“point mutation” in the broader sense**), which leads to a change in the synthesis or function of the gene product, usually a protein. The **effect of a mutation** depends not only on its type and extent, but especially on its location. If a mutation affects the promoter, transcription may not occur, and the protein will not be produced. If it affects a region encoding the active site of a protein, the protein may be produced but will be non-functional.

An **allele** is a particular version of a gene at a given locus. A **genotype** is the set of alleles of an individual (and also a specific allele combination for a given trait), while a **phenotype** is the set of an individual's traits or the specific form of one trait. A **homozygote** has two identical alleles for a given gene, whereas a **heterozygote** has two different alleles. A compound heterozygote has two different mutant alleles of the same gene; the mutations are at different sites and lead to the production of different **protein isoforms**. A **hemizygote** is an individual with one active allele for a given gene, typically a male for genes located in the non-homologous region of the X chromosome. A **locus** is the position of a gene on a chromosome, and gene loci are arranged linearly. In some systems, **multiallelism** (multiple alleles - more than two alleles in the population) and different dominance patterns are important. In **complete dominance**, one dominant allele is sufficient for trait expression, and we cannot distinguish a heterozygote phenotypically from a dominant homozygote. In **incomplete dominance**, both alleles contribute to the phenotype, and the result may be intermediate. In codominance, both alleles are expressed independently, and two phenotypic expressions occur simultaneously (classically, the AB blood group).

In **clinical practice**, however, the relationship between genotype and phenotype is often not “one mutation = one disease”. The same clinical picture may arise from different mutations and sometimes even from mutations in different genes. We distinguish allelic and locus heterogeneity. **Allelic heterogeneity** refers to the situation in which different mutations in the same gene can lead to the same disease or to phenotypically related diseases (for example, different mutations in the DMD gene can lead to Duchenne or Becker muscular dystrophy). The difference often relates to whether the mutation preserves the reading frame. **Locus heterogeneity** refers to cases where the same phenotype can be caused by mutations in different genes (for example, congenital deafness or retinitis pigmentosa, where a similar clinical picture may result from defects in many different genes).

For **clinical practice**, the concepts of penetrance and expressivity are crucial. **Penetrance** is the proportion of carriers of the same genotype that express the phenotype (reported as a percentage). A fully penetrant gene is expressed in all carriers, while an incompletely penetrant gene is expressed only in some. **Expressivity** refers to the degree of phenotypic expression (for example, mild, moderate, severe), and variable expressivity means that carriers of the same mutation may have different severity. These two phenomena help explain why some monogenic

diseases look “atypical” in pedigrees or why the same disease can present differently, including a different age at onset.

Autosomal dominant inheritance means that the locus is on an autosome and one dominant allele is sufficient for expression of the trait. The condition typically occurs with similar frequency in males and females and often appears in every generation, producing so-called **vertical transmission**. Father-to-son transmission is possible. If both parents are heterozygous, in theory, 75% of offspring will show the dominant trait. If an individual does not show the dominant trait, they are a recessive homozygote. If both parents are carriers of the recessive form, a dominant trait in the offspring would arise only as a *de novo* mutation.

In clinical genetics, it is also useful to understand how an allele “becomes” autosomal dominant. Common mechanisms include **haploinsufficiency** (one functional copy is not enough), **gain of function** (acquisition of a new or increased function), or a **dominant-negative effect** (the mutant product interferes with the function of the normal product).

Among the best-known normal traits with a simple inheritance pattern are **blood groups**. In the **ABO system**, phenotypes are determined by the presence of **agglutinogens** on the surface of erythrocytes, and the serum contains naturally occurring **antibodies - agglutinins**. This system is a typical example of multiple alleles (A, B, O), in which A and B are codominant with each other and both are dominant over O. Therefore, there are six genotype combinations but only four phenotypes (A, B, AB, O). Clinically, it is important that in phenotype A or B, we cannot determine the genotype directly from the phenotype, because it may be AA or AO, or BB or BO, respectively, and genetic risk calculations must consider both possibilities. In a basic approach, **the Rh system** behaves as an autosomal dominant: an Rh-positive individual has the antigen on erythrocytes, whereas an Rh-negative individual does not, with Rh-negative being a recessive homozygote. This is explained using Fisher’s theory: at the locus, there may be alleles **C, D, E**, where the decisive factor is the dominant D allele, and an antigenic product of allele **d is not detected**.

Autosomal dominant diseases have several typical features. In pedigrees, they usually appear across multiple generations; an affected individual typically has an affected parent, and occurrence is not linked to sex. From a union of a heterozygously affected individual and an unaffected individual, the theoretical ratio of affected to unaffected children is 1:1. In practice, selection also plays a role. In life-threatening conditions, negative selection operates, and such diseases are then often observed as **de novo mutations**. Exceptions include diseases with late onset or with a course that does not prevent reproduction. A typical example is **Huntington’s disease**, a degenerative disorder of the basal ganglia with progressive choreiform movements and dementia, with an average onset in adulthood. The molecular basis is the expansion of a CAG triplet repeat at the relevant locus. The larger the expansion, the earlier the onset, and PCR is used as a practical detection method.

Other examples of autosomal dominant diseases include **neurofibromatosis types 1 and 2**, with variable expressivity, and **achondroplasia**, where it is emphasized that dominant homozygotes are not viable and that, in heterozygotes, the phenotype may be less severe due to incomplete dominance.

Autosomal recessive inheritance of pathological traits means that disease expression requires identical pathogenic alleles at both loci. Therefore, the affected individual is usually a recessive homozygote. The disorder typically does not appear in every generation and often occurs among

the siblings of an affected individual (a so-called **horizontal pattern of inheritance**). The parents are usually clinically unaffected heterozygotes (**carriers**), and when both parents are carriers, the theoretical risk of an affected child is 25%. At the same time, it should be noted that, in humans, the actual distribution of genotypes in small families can be due to chance. In autosomal recessive disorders, parents are more often related, and a typical consequence of a pathogenic gene is an enzyme defect (**enzymopathy**), often presenting as an inherited metabolic disorder.

Examples of autosomal recessive disorders include alkaptonuria, cystic fibrosis, phenylketonuria, and others. **Alkaptonuria** is a disorder of phenylalanine and tyrosine metabolism characterized by the accumulation of homogentisic acid, darkening of urine, deposition of ochronotic pigment in connective tissues, and later joint complications. A markedly higher prevalence has also been reported in Slovakia, along with multiple mutation types in the responsible gene. PCR is mentioned in diagnostics, with subsequent confirmation by sequencing.

Cystic fibrosis is caused by impaired transport of chloride ions and water in exocrine glands, leading to the production of viscous mucus. It is common among people of European ancestry, and a specific mutation in the CFTR gene is often cited: F508del. **Phenylketonuria** is a congenital disorder of amino acid metabolism, often detected by newborn screening. It involves a deficiency of phenylalanine hydroxylase, with typical clinical manifestations if untreated. A large number of known mutations are also mentioned, along with the possibility of prenatal diagnosis using DNA analysis.

Gonosomal inheritance is linked to the sex chromosomes X and Y. An important difference from autosomes is that a male has only one X chromosome and is therefore hemizygous for genes located in the non-homologous region of X. As a result, X-linked recessive alleles in males often manifest with a single pathogenic allele. The X chromosome is one of the larger chromosomes and contains many genes, whereas the Y chromosome is small. Y-linked (holandric) inheritance is considered of limited practical importance in human clinical genetics.

In **X-linked dominant inheritance**, the pathogenic gene is on the X chromosome, and females typically predominate among affected individuals, while father-to-son transmission is not possible. If the mother is heterozygous, the risk for both sons and daughters is 50%. An affected male has no affected sons, but all his daughters will be affected. In males, the course is often more severe or even lethal, and in heterozygous females, the normal allele may mitigate the effect of the pathogenic allele. Such conditions are also rarer because negative selection often operates. Examples include X-linked hypophosphatemic rickets (**vitamin D-resistant hypophosphatemic rickets**) and **Rett syndrome**. In hypophosphatemic rickets, phosphate deficiency due to decreased reabsorption in the proximal renal tubule is described, with a clinical picture of rickets and the note that physiological doses of vitamin D are not therapeutically effective. In Rett syndrome, the occurrence almost exclusively in girls is emphasized, with apparently normal early development followed by regression with loss of speech and purposeful hand use. Inheritance is described as X-linked dominant with incomplete penetrance and variable expressivity.

In **X-linked recessive inheritance**, affected individuals are predominantly males, and father-to-son transmission is not possible. If the father is unaffected and the mother is a carrier, the probability that sons will be affected is 50%. Daughters are usually unaffected, but about 50% of them may be carriers. An affected father passes the pathogenic allele to all daughters, who

will be carriers (if they receive a normal allele from the mother). In carrier females, milder manifestations may occur depending on **X-chromosome inactivation according to the Lyon hypothesis**, which is related to cellular mosaicism and to which X chromosome is active in a given cell. **X-inactivation** is usually a **random process** occurring in early embryonic development (in each cell, either the maternal or the paternal X chromosome is randomly “switched off”), creating a mosaic of cells with different active X chromosomes. With markedly skewed inactivation, clinical manifestations can be more pronounced even in carriers.

Examples of X-linked recessive disorders include **haemophilia A** (deficiency of coagulation factor VIII) and **haemophilia B** (deficiency of factor IX), **Duchenne muscular dystrophy and Becker muscular dystrophy** (both related to the dystrophin gene, with different mutations in the same gene), **daltonism** (red–green color blindness), **adrenoleukodystrophy**, and **Fabry disease**. In hemophilia, typical bleeding is emphasized, and the fact that available factor replacement therapies significantly improve the clinical course. In Duchenne muscular dystrophy, a severe progressive course with death at a young age is described, as well as the large DMD gene with frequent exon deletions. Becker muscular dystrophy has a later onset and a milder course.

Y-linked (holandric) inheritance is determined by genes located on the Y chromosome. It affects males only, because only males have a Y chromosome (karyotype 46, XY). The typical pattern is transmission from an affected father to all sons, whereas daughters cannot be affected or be carriers because they do not inherit the Y chromosome. Compared with the X chromosome, the Y chromosome contains far fewer genes. Most of them are related to spermatogenesis and male fertility. Clinically, Y-linked disorders are rare and often involve male infertility (for example, microdeletions in the AZF region). The pedigree pattern is therefore simple: only males are affected and the trait is transmitted strictly through the male line without skipping generations (if the affected father has sons).

Monogenic inheritance links the molecular basis (mutation → altered protein or altered expression) with typical pedigree transmission patterns. Dominant disorders more often show “vertical” transmission across generations, while recessive disorders show “horizontal” occurrence among siblings. In gonosomal inheritance, the key factors are male hemizygosity for the X chromosome and female mosaicism due to X-inactivation, which strongly influence both the frequency of affected individuals and clinical variability.

Inheritance type	Gene location	Typical pattern	Father -to-son	Key risks
Autosomal dominant (AD)	autosome	often in every generation; males = females	+	heterozygote × unaffected: 1:1; heterozygote × heterozygote: 3:1 phenotype ratio (75% dominant phenotype)
Autosomal recessive (AR)	autosome	often among siblings; not every generation; males = females	+	two heterozygotes: 25% affected; heterozygote × affected: 1:1
X-linked dominant (XD)	X	more females; males often more severe/lethal	-	heterozygous mother: 50% sons and 50% daughters affected; affected father: all daughters affected, sons not
X-linked recessive (XR)	X	more males; females are often carriers (sometimes symptomatic Lyonization)	-	carrier mother + unaffected father: 50% sons affected and 50% daughters carriers; affected father: all daughters carriers (if mother provides a normal X)
Y-linked (holandric)	Y	affected males only; in every generation through the male line	always	affected father: 100% sons affected; daughters never affected



Model questions

1. Explain the inheritance of the Rh factor:

- a. The Rh factor is inherited in an autosomal dominant manner, meaning that one copy of the Rh-positive allele is sufficient for an Rh-positive phenotype.
- b. The Rh factor is inherited in an autosomal recessive manner, and two copies of the Rh-negative allele are required to express the Rh-negative phenotype.
- c. The Rh factor is influenced by both genetic and environmental factors, and the gene determines the blood group only under certain conditions.
- d. Rh inheritance is recessive and sex-linked.

2. An individual who has only one allele for a particular gene is called a:

- a. hemizygote
- b. heterozygote
- c. dominant homozygote
- d. recessive homozygote

3. The frequency with which a trait is actually expressed in carriers of a genotype is:

- a. complete dominance
- b. codominance
- c. variable expressivity
- d. penetrance

4. An allele is:

- a. a specific form of a gene located at a specific position on a chromosome
- b. a version of genetic information
- c. the frequency of traits in a given population
- d. a structural or functional trait of an organism

5. A locus is:

- a. the position of a gene on a chromosome
- b. an alternative form of a gene
- c. a gene region on the centrosome
- d. the frequency of a trait in a population

6. Expressivity is:

- a. the frequency of a trait
- b. the degree of expression of an allele (e.g., severity of clinical manifestations)
- c. a phenomenon where a trait occurs in several family members in the same form
- d. usually expressed as a percentage of occurrence in a population

7. Penetrance is:

- a. the frequency of a trait among all carriers of a given genotype
- b. the degree of expression of an allele (e.g., severity of clinical manifestations)
- c. a phenomenon where a trait occurs in several family members in the same form
- d. usually expressed as a percentage of trait expression among carriers of the same genotype

8. Codominance means that:

- a. a trait is determined by environmental influences and has the same phenotype as a genetically determined trait
- b. both alleles contribute to the phenotype independently of each other
- c. a trait occurs in several family members in the same form
- d. two phenotypic expressions encoded by one allele pair occur at the same time

9. Intermediacy means that:

- a. a trait is determined by environmental influences and has the same phenotype as a genetically determined trait
- b. both alleles contribute independently of each other
- c. both alleles are expressed to the same degree
- d. two different phenotypic expressions arise

10. Incomplete dominance is:

- a. a trait determined by environmental influences with the same phenotype as a genetic trait
- b. a phenomenon where both alleles contribute independently
- c. both alleles are expressed to the same degree
- d. the formation of two different phenotypic expressions

11. In a heterozygote with intermediacy, trait formation involves:

- a. only the dominant allele
- b. only one allele
- c. alleles depending on distance from the centromere
- d. both alleles

12. Minor (small-effect) genes:

- a. each individual gene has a small effect on the phenotype
- b. each individual gene has a small effect on trait formation
- c. each individual gene has a large effect on the phenotype
- d. a mutation in a single gene usually does not change the trait form

13. Gonosomal inheritance:

- a. has no clinical significance
- b. does not exist
- c. involves the transmission of genes located on the X and Y chromosomes
- d. involves the transmission of genes located on the autosomes X and Y

14. Monogenically inherited traits are traits:

- a. whose phenotypes are determined by genes at a single locus
- b. determined by a single allele pair
- c. always determined by a single allele
- d. determined by the environment

15. Based on locus location, monogenic inheritance can be:

- a. dominant
- b. recessive
- c. autosomal
- d. gonosomal (sex-linked)

16. Variable expressivity means that:

- a. carriers of the same pathogenic allele can show different phenotype severity
- b. the gene is expressed in all carriers
- c. the trait is inherited only through males
- d. the trait always has a continuous distribution

17. The mother has blood group O and the child has blood group A. Determine the possible father's blood group:

- a. BO
- b. AO
- c. AB
- d. AA

18. Diseases with an autosomal dominant inheritance pattern include:

- a. achondroplasia
- b. Huntington disease
- c. alkaptonuria
- d. cystic fibrosis

19. Huntington's disease is:

- a. autosomal dominant
- b. autosomal recessive
- c. X-linked dominant
- d. X-linked recessive

20. Achondroplasia is:

- a. autosomal dominant
- b. autosomal recessive
- c. gonosomal recessive
- d. gonosomal dominant

21. In autosomal dominant inheritance:

- a. the gene is located on an autosome
- b. unaffected family members have only affected children
- c. unaffected family members can pass the disease to their children
- d. the gene is located on a gonosome

22. A disease allele is autosomal dominant. One parent is heterozygous, and the other is a recessive homozygote. The probability of disease in offspring is:

- a. 75%
- b. 25%
- c. 50%
- d. all will be affected

23. In autosomal dominant inheritance:

- a. the pathogenic gene is located on an autosome
- b. unaffected family members do not pass the disease to their children
- c. unaffected family members can pass the disease to their children
- d. the gene is located on an autosome or a gonosome

24. In an autosomal dominant disorder:

- a. an affected individual can be a dominant homozygote
- b. an affected individual can be a recessive homozygote
- c. an affected individual cannot be a dominant homozygote
- d. an affected individual can be a heterozygote

25. A man with neurofibromatosis type 2 has an unaffected son and an affected daughter with a healthy woman. The following statement is true:

- a. the man has a mutation in the NF1 gene on chromosome 22
- b. the man has a mutation in the NF2 gene on chromosome 17
- c. the man has a mutation in the NF2 gene on chromosome 22
- d. the affected daughter is a dominant homozygote

26. In autosomal recessive inheritance:

- a. the affected individual usually has affected parents
- b. parents of affected individuals are never related
- c. offspring of two healthy individuals never include affected children
- d. offspring of two healthy individuals may include affected children

27. Autosomal recessive diseases include:

- a. haemophilia A
- b. red–green color blindness
- c. alkaptonuria
- d. cystic fibrosis

28. Phenylketonuria is:

- a. autosomal dominant
- b. autosomal recessive
- c. X-linked dominant
- d. X-linked recessive

29. Cystic fibrosis is:

- a. autosomal dominant
- b. autosomal recessive
- c. X-linked dominant
- d. X-linked recessive

30. In autosomal recessive inheritance:

- a. parents are usually affected
- b. the pathogenic gene product is most often a defective enzyme
- c. the sex ratio among affected individuals is 3:1
- d. the sex ratio among affected individuals is 1:3

31. A disease allele is autosomal recessive. One parent is a dominant homozygote, and the other is heterozygous. The probability of affected offspring is:

- a. no child will be affected
- b. 50%
- c. 25%
- d. all children are affected

32. Cystic fibrosis:

- a. is a rare non-inherited metabolic disorder
- b. is inherited autosomally dominantly
- c. is inherited autosomally recessively
- d. is a common non-inherited metabolic disorder

33. Fabry disease is:

- a. autosomal dominant
- b. autosomal recessive
- c. X-linked dominant
- d. X-linked recessive

34. In X-linked recessive inheritance, what is the probability of affected children if the mother is a carrier and the father is affected?

- a. sons will be 50% unaffected and 50% affected
- b. all sons will be affected
- c. all daughters will be affected
- d. daughters will be 50% affected and 50% unaffected carriers

35. Diseases with an X-linked recessive inheritance pattern include:

- a. haemophilia A
- b. red–green color blindness
- c. alkaptonuria
- d. cystic fibrosis

36. Diseases with an X-linked recessive inheritance pattern include:

- a. haemophilia A
- b. Duchenne muscular dystrophy
- c. Rett syndrome
- d. cystic fibrosis

37. In X-linked recessive inheritance, what is the probability that parents will have affected children if the mother is healthy and the father is affected?

- a. daughters will be 50% healthy and 50% carriers, sons will be healthy
- b. all sons will be affected
- c. all daughters will be affected
- d. daughters will be 100% healthy carriers and sons will be healthy

38. In X-linked recessive inheritance, what is the probability of affected children if both mother and father are affected?

- a. daughters will be 50% healthy and 50% affected
- b. all sons will be affected
- c. all daughters will be affected
- d. daughters will be 50% affected and 50% carriers

39. Haemophilia A is:

- a. the most common inherited bleeding (coagulation) disorder
- b. caused by a deficiency of factor IX
- c. caused by a deficiency of factor XI
- d. caused by a deficiency of factor III

40. In X-linked recessive inheritance, what is the probability of affected children if the mother is affected and the father is healthy?

- a. daughters will be 50% healthy and 50% affected
- b. all sons will be affected
- c. all daughters will be affected
- d. daughters will be 100% healthy, but they will be carriers

41. In X-linked recessive inheritance, who receives the pathogenic allele from an affected father?

- a. only sons
- b. only daughters
- c. 50% of daughters and 50% of sons
- d. all sons and all daughters

42. Haemophilia B is a disease with:

- a. autosomal dominant inheritance
- b. autosomal recessive inheritance
- c. X-linked dominant inheritance
- d. X-linked recessive inheritance

43. In X-linked recessive inheritance:

- a. only sons receive the pathogenic gene from an affected father
- b. only daughters receive the pathogenic gene from an affected father
- c. 50% of daughters and 50% of sons receive it from an affected father
- d. all sons and all daughters receive it from an affected father

44. Can a female carrier of an X-linked recessive disease show clinical signs? If yes, under what circumstances?

- a. yes, when in most cells the X chromosome carrying the dominant (normal) allele is inactivated
- b. no
- c. yes, when in most cells the X chromosome carrying the recessive allele is inactivated
- d. yes, but only if the recessive allele is inherited from the mother

45. In X-linked dominant inheritance, what is the probability that parents will have healthy children if the mother is healthy and the father is affected?

- a. all daughters will be carriers
- b. 50:50
- c. all daughters will be healthy
- d. all sons will be healthy

46. In X-linked dominant inheritance, what is the probability of affected children if the mother is healthy and the father is affected?

- a. all daughters will be carriers
- b. all sons will be affected
- c. all daughters will be healthy
- d. 50:50

47. Diseases with an X-linked dominant inheritance pattern include:

- a. hyperphosphataemic vitamin D-resistant rickets
- b. red-green color blindness
- c. hypophosphataemic vitamin D-resistant rickets
- d. Rett syndrome

48. In X-linked dominant inheritance:

- a. male patients may have a severe to lethal course
- b. male patients generally have a milder course
- c. an affected male has all daughters healthy
- d. an affected male will have affected sons

49. In X-linked dominant inheritance:

- a. disease is more severe in male patients
- b. males predominate among patients
- c. the sex distribution is equal
- d. the disease never occurs in males

50. In X-linked dominant inheritance:

- a. an affected male has all daughters as carriers
- b. an affected male has all daughters affected
- c. an affected male has no affected daughters
- d. an affected male has all children affected

51. In X-linked dominant inheritance:

- a. the disease is transmitted from father to son
- b. father-to-son transmission is not possible
- c. father-to-son transmission occurs with a 50% risk
- d. father-to-son transmission occurs with a 25% risk

52. In X-linked dominant inheritance:

- a. disease may have a milder course if a normal allele is present in the affected individual
- b. disease is more severe in males
- c. homozygously affected females have a milder course than heterozygous females
- d. heterozygously affected females have a milder course than males

53. In X-linked inheritance due to a dominant allele:

- a. males predominate among patients
- b. only females can be affected
- c. father-to-son transmission is possible
- d. father-to-son transmission is not possible

54. For X-linked dominant inheritance:

- a. daughters of an affected father must also be affected
- b. daughters of an affected father are not affected
- c. affected women occur twice as often as affected men
- d. sons of an affected father inherit the disorder from him

55. Y-linked inheritance:

- a. is holandric
- b. genes are located on any sex chromosome
- c. genes are located on the Y chromosome (in the non-recombining region)
- d. genes are located on a “Y autosome”

56. What is holandric inheritance?

- a. inheritance linked to the Y chromosome
- b. inheritance transmitted exclusively from father to son
- c. inheritance transmitted by the mother
- d. inheritance linked to an autosome

57. A homozygote is:

- a. an individual with two identical alleles for a particular gene
- b. an individual with two different alleles for a particular gene
- c. an individual with one allele for a particular gene
- d. an individual with an inactivated X chromosome

58. A heterozygote is:

- a. an individual with two different alleles for a particular gene
- b. an individual with two identical alleles
- c. an individual with one allele
- d. an individual with three alleles at a locus

59. A compound heterozygote is:

- a. an individual with two different mutant alleles of the same gene, with mutations at different sites
- b. an individual with one allele on the X chromosome
- c. an individual with two identical mutant alleles
- d. an individual with a mutation only in the promoter

60. An protein isoform is:

- a. a variant form of a protein (often an isoform) that differs due to genetic variation and may retain at least partial function
- b. a protein produced without transcription
- c. only an enzyme that is always completely non-functional
- d. a synonym for agglutinin

61. Usually, one locus contains:

- a. one structural gene encoding one protein
- b. always three structural genes
- c. only regulatory regions without a gene
- d. only minor genes

62. The Rh factor locus:

- a. contains three structural genes C, D, E
- b. contains exclusively the CFTR gene
- c. is located on the X chromosome
- d. is located on chromosome 7

63. The genome usually matches the genotype, but an exception is:

- a. X-chromosome inactivation (only one X is active in a cell)
- b. multiple alleles in the ABO system
- c. threshold effect in multifactorial traits
- d. genetic drift

64. The ABO system is characterized by:

- a. three alleles (A, B, O) – multiple alleles
- b. alleles A and B are codominant
- c. alleles A and B are dominant over allele O
- d. only two phenotypes exist

65. Which ABO phenotypes and genotypes are correct?

- a. phenotype A can be AA or AO
- b. phenotype B can be BB or BO
- c. phenotype AB is AB
- d. phenotype O is AO

66. For phenotype A or B in the ABO system:

- a. the genotype may not be determined unambiguously from phenotype alone
- b. heterozygous arrangements AO or BO must be considered
- c. phenotype A always has genotype AA
- d. phenotype B always has genotype BB

67. Agglutinins in the ABO system are:

- a. naturally occurring antibodies in serum
- b. antigens on the surface of erythrocytes
- c. also present in the Rh system
- d. always present in blood group AB

68. The Rh system:

- a. has no agglutinins
- b. Rh⁺ has an antigen on erythrocytes
- c. Rh⁻ does not have the antigen on erythrocytes
- d. is determined by a locus on the X chromosome

69. The gene for the Rh factor is located:

- a. on chromosome 1
- b. on chromosome 7
- c. on the X chromosome
- d. on chromosome 4

70. Fisher's theory of the Rh factor states that:

- a. the Rh factor is determined by three alleles (C, D, E)
- b. the decisive allele is D
- c. the antigenic product of allele d was not demonstrated
- d. the Rh system is identical to the ABO system

71. Huntington's disease:

- a. is autosomal dominant with high penetrance
- b. has an average age of onset of about 38 years
- c. was first described in 1872
- d. is autosomal recessive

72. Molecular basis of Huntington disease:

- a. CAG triplet expansion in exon 1
- b. the gene is located at 4p16.3
- c. normal repeat number is ~ 6–53
- d. it is a deletion of exons 2 and 3

73. Huntington's disease:

- a. the larger the CAG expansion, the earlier the onset
- b. diagnosis in practice uses PCR to detect the expansion
- c. it is an X-linked recessive disease
- d. an intermediate (“premutation”) range is ~ 9–35 repeats

74. Neurofibromatosis type 1:

- a. has variable expressivity
- b. is autosomal codominant
- c. is caused by the tumor suppressor gene NF1 at 17q11.2
- d. the responsible gene is on Xp28

75. In neurofibromatosis type 1, diagnostic methods include:

- a. FISH
- b. sequencing
- c. hemagglutination only
- d. Hardy–Weinberg law only

76. Neurofibromatosis type 2:

- a. is associated mainly with the acoustic nerves (often bilateral)
- b. is caused by the tumor suppressor gene NF2 (Merlin) at 22q12.2
- c. the most common cause is deletions in exons 2 and 7
- d. is autosomal recessive

77. Achondroplasia (chondrodystrophy):

- a. has a clinically significant autosomal dominant pattern
- b. homozygous achondroplasia is usually lethal (similar to thanatophoric genotype)
- c. is a disorder of the growth cartilage of the short bones
- d. is always X-linked

78. The mutated gene in achondroplasia:

- a. is FGFR3
- b. is located at 4p16.3
- c. encodes a receptor with tyrosine kinase activity
- d. is CFTR

79. Enzymopathies are:

- a. disorders caused by the absence of an enzyme or the production of a non-functional enzyme
- b. accompanied by substrate accumulation
- c. often inherited metabolic disorders
- d. always autosomal dominant

80. Alkaptonuria:

- a. is autosomal recessive
- b. is caused by a defect in homogentisate 1,2-dioxygenase (HGD)
- c. leads to dark urine
- d. is X-linked dominant

81. In alkaptonuria, the following accumulates:

- a. homogentisic acid
- b. phosphate in serum
- c. chloride ions in mucus
- d. glycosphingolipids in lysosomes

82. Cystic fibrosis:

- a. is autosomal recessive
- b. is related to impaired chloride and water transport in glandular cells
- c. leads to viscous mucus and blockage of ducts
- d. is caused by factor VIII deficiency

83. The gene associated with cystic fibrosis:

- a. is called CFTR
- b. is located at 7q31.2
- c. has 27 exons
- d. is located at Xp21.2

84. Phenylketonuria (PKU):

- a. is autosomal recessive
- b. is the most common congenital disorder of amino acid metabolism
- c. newborn screening is used for detection
- d. is caused by phosphate deficiency

85. In phenylketonuria, the affected enzyme is:

- a. phenylalanine hydroxylase (mainly in the liver, not “only in the liver”)
- b. coagulation factor IX
- c. homogentisate 1,2-dioxygenase
- d. alpha-galactosidase A

86. The most important Y-chromosome gene for male sex determination is:

- a. SRY (historically referred to as TDF)
- b. CFTR
- c. DMD
- d. MECP2

87. Y-linked (holandric) inheritance:

- a. has little practical importance for clinical genetics
- b. is the most common type of human inheritance
- c. is typical for haemophilia
- d. is typical for Rett syndrome

88. If the mother is heterozygous in X-linked dominant inheritance, the risk of being affected is:

- a. 50% for daughters
- b. 50% for sons
- c. 25% for daughters
- d. 25% for sons

89. Vitamin D-resistant hypophosphataemic rickets:

- a. is X-linked dominant
- b. is associated with decreased phosphate reabsorption in the proximal renal tubule
- c. physiological doses of vitamin D have no therapeutic effect
- d. is caused by factor VIII deficiency

90. Rett syndrome:

- a. occurs almost exclusively in boys
- b. has X-linked dominant inheritance with incomplete penetrance and variable expressivity
- c. early development appears normal up to about 6–18 months
- d. is autosomal recessive

91. Haemophilia A:

- a. is X-linked recessive
- b. is the most common inherited coagulation disorder (about 80% of haemophilia cases)
- c. is related to coagulation factor VII
- d. is related to coagulation factor IX

92. Haemophilia B:

- a. is X-linked recessive
- b. is caused by absence or reduced level of factor IX
- c. is five times more common than haemophilia A
- d. is autosomal dominant

93. Duchenne muscular dystrophy:

- a. is X-linked recessive
- b. is related to the DMD gene on Xp22.1
- c. often arises de novo (about one third of cases)
- d. has a milder course than Becker dystrophy

94. Which statements about the ABO system are true?

- a. there are 3 alleles (A, B, O)
- b. A and B alleles are codominant
- c. A and B alleles are recessive to O
- d. O allele is dominant over A and B

95. Which statements about Huntington's disease are correct?

- a. the average age of onset is 51 years
- b. the molecular basis is CAG triplet expansion
- c. the responsible gene is located at 17q11.2
- d. inheritance is autosomal dominant with high penetrance

96. Daltonism (red–green color blindness):

- a. is an X-linked recessive disorder
- b. is more often clinically expressed in males
- c. is transmitted autosomal dominantly
- d. an affected father passes it to all sons

97. Becker muscular dystrophy:

- a. is typically lethal in childhood
- b. usually has a milder course and later onset than Duchenne dystrophy
- c. is inherited autosomally recessively
- d. is caused by mutations in the dystrophin gene

98. Duchenne muscular dystrophy:

- a. has a mild course with adult onset
- b. is related to a large gene with frequent exon deletions
- c. is an X-linked recessive disorder
- d. affected females are more common than males

99. Daltonism:

- a. belongs to X-linked recessive disorders
- b. females are affected more often than males
- c. a male with this disorder is hemizygous for the pathogenic allele
- d. it is a codominant inheritance disorder

100. Daltonism:

- a. is transmitted from an affected father to all sons
- b. can show mild symptoms in carrier females due to X-inactivation
- c. typically involves red–green color vision deficiency
- d. the gene is located on an autosome

Answer Key to Model Questions

- | | | |
|----------|----------|-----------|
| 1. TFFF | 35. TTFF | 69. TFFF |
| 2. TFFF | 36. TTFF | 70. TTTF |
| 3. FFFT | 37. FFFT | 71. TTTF |
| 4. TTFF | 38. FTTF | 72. TTFF |
| 5. TFFF | 39. TFFF | 73. TTFF |
| 6. FTFF | 40. FTFT | 74. TFTF |
| 7. TTFT | 41. FTFF | 75. TTFF |
| 8. FTFT | 42. FFFT | 76. TTFF |
| 9. FFTF | 43. FTFF | 77. TTFF |
| 10. FFTF | 44. TFFF | 78. TTTF |
| 11. FFFT | 45. FTFT | 79. TTTF |
| 12. TTFT | 46. FFFT | 80. TTTF |
| 13. FFTF | 47. FTTT | 81. TFFF |
| 14. TTFF | 48. TFFF | 82. TTTF |
| 15. FTTT | 49. TFFF | 83. TTTF |
| 16. TFFF | 50. FTFF | 84. TTTF |
| 17. FTTT | 51. FTFF | 85. TFFF |
| 18. TTFF | 52. TTFT | 86. TFFF |
| 19. TFFF | 53. FFFT | 87. TFFF |
| 20. TFFF | 54. TFTF | 88. TTFF |
| 21. TFFF | 55. TFTF | 89. TTTF |
| 22. FFTF | 56. TTFF | 90. FTTF |
| 23. TTFF | 57. TFFF | 91. TTFF |
| 24. TTFT | 58. TFFF | 92. TTFF |
| 25. FFTF | 59. TFFF | 93. TFTF |
| 26. FFFT | 60. TFFF | 94. TTFF |
| 27. FTTT | 61. TFFF | 95. FTFT |
| 28. FTFF | 62. TFFF | 96. TTFF |
| 29. FTFF | 63. TFFF | 97. FTFT |
| 30. FTFF | 64. TTTF | 98. FTTF |
| 31. TFFF | 65. TTTF | 99. TFTF |
| 32. FFTF | 66. TTFF | 100. FTTF |
| 33. FFFT | 67. TFFF | |
| 34. TTFT | 68. TTTF | |

4. Polygenic and multifactorial inheritance.

Polygenic and multifactorial inheritance are sometimes confused in practice, but they are not identical in meaning. **Polygenic inheritance** means that a single trait is determined by more than one major gene, that is, by several genes (allele pairs) at different loci. This refers to a situation in which the final form of the trait is influenced by several “**large-effect genes**”, while the influence of the environment is usually minimal. Examples include iris color, which is described here as being influenced by two genes, and skin color, where three genes are considered.

Multifactorial inheritance (often referred to in English-language literature as complex traits) is a broader concept. Multifactorial traits arise from a combination of **genetic factors** and **environmental** (including epigenetic) factors. The genetic component is understood as a predisposition, a heritable tendency that is determined by a large number of minor (**small-effect**) genes. A typical feature of minor genes is that a single gene does not have a decisive effect on the trait form, and a mutation in one such gene usually does not, by itself, change the trait form. What matters is their shared additive effect. Alleles of many minor genes act over a long period in different combinations and over time and, together with epigenetic factors, shape the resulting phenotype.

A **multifactorial predisposition** is often suggested by **familial occurrence**, meaning that a particular pathological trait is found more frequently in certain families than in the population as a whole. However, this familial pattern does not automatically imply “simple” inheritance. It may reflect a combination of genetically determined susceptibility and shared epigenetic factors within the family (for example, similar dietary habits or lifestyle). Examples of epigenetic factors mentioned include hormonal profile, dietary components, geographic conditions, socio-economic situation, daily routine, overall lifestyle, maternal age, viral infections, exposure to harmful agents, and others.

In individual **clinical cases**, it is often difficult to decide whether a specific environmental factor plays the decisive role in causing the pathological trait form, or whether predisposition mainly reflects increased individual susceptibility to that factor. This uncertainty complicates explaining the etiology in a particular patient, estimating prognosis, and especially estimating recurrence risk within a family. Therefore, for diagnosis, prevention, and prenatal strategy in multifactorial traits, the search for **candidate genes** is important. These are major genes that are identified indirectly, for example through studies of microdeletion syndromes. If the clinical picture of a microdeletion syndrome includes features that are also typical of a particular multifactorial pathological trait, the deleted region becomes a target for searching a gene whose mutation (or a specific interaction of its gene product) may produce a similar pathological phenotype. If such a link is confirmed, possibilities for diagnosis and preventive interventions expand.

Multifactorial traits are classified as quantitative or qualitative. Quantitative traits are expressed in defined units (e.g., cm, kg, years) and include, for example, height, body dimensions, degree of pigmentation, number of dermatoglyphic ridges, age at disease onset, blood pressure, IQ, and similar traits. Most normal quantitative traits show a **continuous** (approximately normal) **distribution** in the population. Such a distribution is described by the **Gaussian** (normal) curve. Trait occurrence is characterized by the mean value (arithmetic mean) and the standard deviation, which reflects the “steepness” of the curve and the spread of values. It is stated that the interval $\text{mean} \pm \text{standard deviations}$ includes approximately 95% of the population.

Necessary fix: the usual rule is ± 2 standard deviations $\approx 95\%$, while ± 1 standard deviation $\approx 68\%$. An important idea is that the shape of the curve does not change, but the curve can shift along the X-axis depending on the population means (for example, differences in average height between geographic regions), while the distribution pattern remains the same.

At the edges of the distribution are **extremes**, that is, very low or very high trait values, which can still represent physiological variability. If a value exceeds normal variability and has a pathological character, it may more often reflect a monogenic pathological condition (for example, extremely short stature may be related to achondroplasia, whereas extreme height may result from overproduction of growth hormone by the pituitary gland). In familial studies of quantitative traits, a tendency toward average values is often observed, that is, **regression to the mean**: parents with extreme values tend to have children whose values are closer to the population mean.

A closely related concept in quantitative multifactorial traits is **heritability**. Heritability is defined as the proportion of additive **genetic variance** in the total **phenotypic variance** of a trait. It indicates whether the genetic component (predisposition) contributes a large or small share to variability in that phenotype. The higher the heritability, the larger the contribution of genetic factors to the phenotype (or predisposition to the phenotype). At the same time, it is emphasized that heritability is a population parameter tied to a specific environment and a specific population in which the variance is assessed.

Qualitative traits with multifactorial inheritance are described rather than measured. Even normal qualitative multifactorial traits can vary (e.g., upper lip shape, age-related changes). A key concept in pathological qualitative traits is the **threshold effect**. There is no gradual transition between trait forms in the population. Instead, the **distribution is discontinuous**. Individuals are classified as “unaffected” or “affected” depending on whether their combined predisposition, together with epigenetic factors, exceeds a threshold value. The threshold is the boundary beyond which the pathological trait form manifests. The more severe the pathological trait, the less frequently it occurs, because the simultaneous presence of high predisposition and strong negative epigenetic influences is rare.

An example of the threshold effect is palatal development. During normal development, the lateral palatal shelves fuse to form an intact palate, and within the normal range, there is shape variability (from typical to a high-arched palate). If the shelves fail to fuse, a cleft palate occurs, and the trait form shifts abruptly to a pathological state. Variable normal forms are considered subthreshold in this model, while pathological forms are suprathreshold.

Predisposition may manifest in two typical ways. **In the first**, predisposition is directed toward the development of a pathological trait, and epigenetic factors influence whether, how, and when the disease manifests. As an illustrative example, pyloric stenosis is described as occurring about five times more often in males than in females, with females being partly “protected” by estrogens. Therefore, sons of an affected woman have a markedly higher risk than sons of an affected man.

In the second, predisposition means increased susceptibility of cells and tissues to harmful agents, and the result may be congenital anomalies (for example, cleft lip and palate, neural tube defects, congenital heart defects) or “civilization-related” diseases (atherosclerosis, peptic ulcer disease, essential hypertension, some types of diabetes).

A specific subgroup are **congenital anomalies** and diseases with a **multifactorial mode of inheritance**. According to population frequency, three groups are listed:

- rare (population frequency < 1%; for example, cleft lip and palate, neural tube defects, congenital heart defects, developmental dysplasia of the hip),
- moderately frequent (< 5%; for example, some severe psychiatric disorders such as schizophrenia, bipolar disorder, intellectual disability - oligophrenia),
- high-frequency disorders (for example, hypertension, non-insulin-dependent diabetes mellitus, peptic ulcer disease, atopy).

In genetic counselling for complex disorders, simple Mendelian calculations cannot be used. Risk is estimated either theoretically (for example, using the Edwards formula) or practically using empirical risks derived from large pedigree datasets.

Empirical risks follow general rules. As the number of affected close relatives increases, the risk for additional relatives increases. If only distant relatives are affected, the risk is lower. If a disorder affects the sexes with different frequencies, then being affected in the less frequently affected sex increases the risk for relatives (an analogy is provided by developmental dysplasia of the hip, which is more common in girls; an affected boy suggests a higher familial predisposition). The Edwards formula is used to estimate risk for first-degree relatives and is given as: $r = \sqrt{\text{population frequency of the disease}}$. If more first-degree relatives are affected, the result is multiplied by the number of affected relatives. In a case with a population frequency of 0.09% and two affected first-degree relatives, the risk is 6%.

The **dynamics of the threshold** are also important. Adverse epigenetic factors may “shift” the threshold so that manifestation occurs at a lower level of predisposition (effectively a higher disease risk). Conversely, reducing negative influences may raise the threshold and reduce the probability of manifestation. This principle is illustrated using cardiovascular diseases: with high predisposition (when it is not a monogenic disorder), healthy lifestyle measures may delay onset or mitigate the course.

Examples include cleft lip and palate, neural tube defects, pyloric stenosis, pes equinovarus, and congenital heart defects.

Cleft lip and palate are among the most common congenital anomalies and account for a substantial proportion of all congenital anomalies. However, they are not a homogeneous entity. Two genetically independent groups are described. Cleft lip with or without cleft palate (frequency about 1:1,000), arising around day 35 of intrauterine development due to abnormal fusion of the maxillary process with the medial nasal processes.

Left-sided clefts are more common than right-sided clefts and occur more frequently in males. A median cleft of the upper lip is rare and may be associated with additional developmental defects. **Isolated cleft palate** (frequency about 1:2,500), more frequent in females. An association is mentioned with the later fusion of palatal shelves in female fetuses.

Clefts can have **multiple causes**. They may be due to mutations in major genes (with loci reported on multiple chromosomes), may be part of many syndromes, may be associated with chromosomal aberrations, may result from teratogens or mechanical influences (for example, amniotic bands), and a substantial proportion may result from multifactorial interaction between predisposition and external factors.

A group of genes involved in orofacial clefts is listed (e.g., *TGF- α* and *TGF- β 2/3* genes, genes indirectly related to orofacial development, homeotic genes *MSX1* and *MSX2*, and genes involved in xenobiotic metabolism). For recurrence risk estimation, the number of affected and unaffected children already born is emphasized, as well as the sex and severity of the defect. More severe defects and affection of the less commonly affected sex increase the estimated risk. Empirical risks may vary between populations, so data from the population of origin should be used.

Neural tube defects (NTDs) arise from failure of neural tube closure. Under normal circumstances, the neural tube closes around day 28 of embryogenesis. Closure proceeds from the mid-body region both cranially and caudally; the anterior neuropore closes around days 25–26, and the posterior neuropore about two days later. If closure fails, NTDs occur, including anencephaly, encephalocele, and spina bifida.

A smaller proportion of cases (under 10%) has an identifiable cause, such as amniotic bands, monogenic syndromes, chromosomal aberrations, or teratogens. Anencephaly is a lethal defect with the absence of a large part of the brain and cranial vault, and survival after birth is only very brief. Encephalocele is a herniation of brain tissue and meninges through a skull defect with intact skin, and it may be combined with other abnormalities. Spina bifida is a defect of vertebral development with failure of closure of the bony covering of the spinal cord. Its frequency varies geographically and ethnically, and it may occur within a multifactorial spectrum or as part of some syndromes.

Pyloric stenosis is caused by hypertrophy of the pyloric sphincter, with impaired gastric emptying, and often presents around the 3rd–4th week of life. A multifactorial threshold model is used to explain familial occurrence. A higher frequency in boys is reported, as well as different recurrence risks depending on whether the father or mother is affected. This supports a threshold model with different “hormonal background” and different thresholds between sexes (the highest risk is reported in sons of affected mothers).

Pes equinovarus is a deformity (**clubfoot**) with an incidence of about 1.2:1,000 live births and a higher occurrence in boys. It may be unilateral or bilateral. Causes may be genetic and epigenetic, for example, predisposition related to “weaker” ligamentous structures, as well as environmental or mechanical conditions in the uterus (limited space, oligohydramnios, amniotic bands, uterine septa, or tumors). Pes equinovarus may also be associated with other congenital disorders.

Congenital heart defects are among the most common congenital anomalies. Shunt defects are described (mixing of oxygenated and deoxygenated blood, for example, patent ductus arteriosus and septal defects) and non-shunt defects (without mixing, for example, coarctation of the aorta). A specific example is tetralogy of Fallot with its four typical components, clinical manifestations (e.g., cyanosis, clubbing, fatigue), and the importance of surgical treatment. Patent ductus arteriosus is also described (physiologically closing shortly after birth), atrial septal defect (including situations where a small persistent opening may have no functional consequences), and ventricular septal defect.

Table: Main differences between polygenic and multifactorial inheritance

Area	Polygenic inheritance	Multifactorial inheritance
Core concept	A single (usually qualitative) trait is determined by more than one major gene (multiple loci, multiple allele pairs).	A trait arises from a combination of genetic predisposition (many minor/small-effect genes with an additive effect) and epigenetic factors.
Role of environment / epigenetics	Minimal influence of the environment on the final trait form.	Major influence of epigenetic factors.
Familial occurrence	A more genetically “stable” pattern of expression.	Typically shows familial clustering.
Risk estimation in genetic counselling	Focus is more on what is genetically possible based on allele combinations. In practice, it is less often addressed as “disease risk” and more as trait variability.	Key elements are empirical risks, number of affected relatives, and degree of relatedness, defect severity, sex (if prevalence differs by sex), and assessment/possible modification of epigenetic factors.
Preventive and “modifying” recommendations	Limited (because the environment has little influence).	Very important: reducing risk epigenetic factors may shift the threshold and reduce the probability of manifestation.
Diagnostic approach	Mainly, the genetic interpretation of allele combinations for the trait.	Search for predisposition, use of the threshold model, and when possible, identification of candidate genes.



Model questions

1. Polygenic inheritance means that a trait is:

- a. determined by a single gene
- b. determined by several major genes
- c. strongly influenced by the environment
- d. the result of epigenetic changes

2. Polygenic traits are typically:

- a. discontinuously distributed
- b. influenced by large-effect genes
- c. minimally influenced by the environment
- d. always pathological

3. An example of a polygenically determined trait is:

- a. iris color
- b. pyloric stenosis
- c. cleft lip
- d. blood pressure

4. Multifactorial inheritance includes:

- a. genetic factors only
- b. genetic and epigenetic factors
- c. mutations in major genes only
- d. chromosomal aberrations exclusively

5. Minor genes are characterized by the fact that:

- a. individually they have a large effect
- b. they act additively
- c. mutation of one gene fundamentally changes the phenotype
- d. they are small-effect genes

6. Familial occurrence of a multifactorial trait means:

- a. monogenic inheritance
- b. increased occurrence in a family
- c. influence of a shared environment
- d. absence of a genetic component

7. Epigenetic factors include:

- a. hormonal profile
- b. geographic conditions
- c. allelic variability
- d. lifestyle

8. In multifactorial traits, a common problem is:

- a. precise identification of one causal gene
- b. a simple Mendelian risk calculation
- c. distinguishing environmental effects from genetic predisposition
- d. absence of phenotypic variability

9. Candidate genes are searched for mainly:

- a. in monogenic diseases
- b. in microdeletion syndromes
- c. based on phenotypic similarity
- d. exclusively by pedigree analysis

10. Quantitative multifactorial traits:

- a. have a discontinuous distribution
- b. can be measured in units
- c. often have a normal distribution
- d. are always pathological

11. Multifactorial traits are:

- a. determined by a large number of minor genes
- b. determined by multiple genes (allele pairs)
- c. characterized by familial clustering
- d. determined by genetic and non-genetic factors

12. The Gaussian curve describes:

- a. a discontinuous distribution
- b. a normal distribution
- c. the spread of values around the mean
- d. the threshold effect

13. The interval mean $\pm$ standard deviation(s) includes approximately:

- a. 50% of the population
- b. 68% of the population
- c. 95% of the population
- d. 100% of the population

14. Multifactorial traits can be divided into:

- a. qualitative and quantitative
- b. qualitative (dominant) and quantitative (recessive)
- c. qualitative (recessive) and quantitative (dominant)
- d. minor and familial

15. Regression to the mean means that:

- a. extreme values tend to be less extreme in the next generation
- b. the phenotype becomes pathological
- c. children have the same values as their parents
- d. extremes accumulate

16. Heritability expresses:

- a. absolute genetic determination of a trait
- b. the proportion of genetic variance in phenotypic variance
- c. individual disease risk
- d. a population parameter

17. Heritability is:

- a. unchangeable
- b. specific to a particular population
- c. independent of the environment
- d. dependent on the environment

18. Qualitative multifactorial traits are characterized by:

- a. a threshold effect
- b. a gradual transition between forms
- c. a discontinuous distribution
- d. expression in units

19. The threshold effect means:

- a. an abrupt shift to a pathological form
- b. a gradual change in phenotype
- c. the same expression in all individuals
- d. dependence on the combination of predisposition and environment

20. More severe multifactorial disorders are:

- a. more frequent
- b. rarer
- c. without a genetic component
- d. always lethal

21. Multifactorial traits are determined by:

- a. genetic factors only
- b. non-genetic factors only
- c. genetic and non-genetic factors
- d. external factors only

22. Pyloric stenosis is characterized by:

- a. hypertrophy of the pyloric sphincter
- b. more frequent occurrence in girls
- c. more frequent occurrence in boys
- d. a threshold multifactorial model

23. Sons of a woman affected by pyloric stenosis have:

- a. lower risk
- b. the same risk
- c. higher risk
- d. zero risk

24. Congenital multifactorial defects include:

- a. cleft lip
- b. achondroplasia
- c. neural tube defect
- d. pes equinovarus (clubfoot)

25. Cleft lip and palate:

- a. form a homogeneous entity
- b. consist of two genetically independent groups
- c. are always syndromic
- d. can be multifactorial

26. Isolated cleft palate is:

- a. more common in males
- b. more common in females
- c. linked to earlier shelf fusion
- d. linked to later shelf fusion

27. Genes associated with orofacial clefts include:

- a. MSX1
- b. MSX2
- c. the insulin gene
- d. TGF- β

28. Neural tube defects arise:

- a. after day 60 of embryogenesis
- b. due to failure of neural tube closure
- c. around day 28 of development
- d. always due to teratogens

29. Anencephaly is:

- a. compatible with life
- b. a lethal disorder
- c. a form of spina bifida
- d. characterized by the absence of the brain

30. Spina bifida is:

- a. a disorder of vertebral development
- b. always part of a syndrome
- c. associated with failure of closure of the bony covering of the spinal cord
- d. without geographic differences in frequency

31. Qualitative multifactorial traits are expressed by:

- a. description
- b. metric units
- c. comparison
- d. the threshold effect

32. Quantitative multifactorial traits are expressed:

- a. by description
- b. in metric units
- c. by comparison
- d. in defined units

33. Quantitative multifactorial traits show a population distribution that is:

- a. continuous
- b. can be represented by a Gaussian curve
- c. discontinuous
- d. step-like

34. Qualitative multifactorial traits show a population distribution that is:

- a. continuous
- b. can be represented by a Gaussian curve
- c. discontinuous
- d. continuous (ongoing)

35. The Edwards formula is used for:

- a. calculation of Mendelian risk
- b. estimation of recurrence risk
- c. risk estimation for first-degree relatives
- d. calculation of heritability

36. If more first-degree relatives are affected:

- a. the risk is divided
- b. the risk is multiplied
- c. the risk decreases
- d. the risk does not change

37. A shift in the threshold value may cause:

- a. increased risk with adverse factors
- b. decreased risk with healthy lifestyle measures
- c. disappearance of genetic predisposition
- d. development of a monogenic disorder

38. Congenital heart defects are:

- a. rare congenital anomalies
- b. among the most common congenital anomalies
- c. exclusively monogenic disorders
- d. multifactorial defects

39. Shunt heart defects are characterized by:

- a. mixing of oxygenated and deoxygenated blood
- b. absence of connections between chambers
- c. septal defects
- d. coarctation of the aorta

40. Tetralogy of Fallot includes:

- a. four typical components
- b. always a patent ductus arteriosus
- c. cyanosis
- d. absence of surgical treatment

41. Patent ductus arteriosus:

- a. is always pathological
- b. physiologically closes after birth
- c. is a typical non-shunt defect
- d. leads to blood mixing

42. Atrial septal defect:

- a. is always clinically significant
- b. may be asymptomatic
- c. belongs among shunt defects
- d. is exclusively syndromic

43. In genetic counselling for multifactorial disorders:

- a. Mendelian laws are used
- b. empirical risks are used
- c. the population of origin is considered
- d. the sex of the affected individual is ignored

44. Which statements about cleft lip and/or palate are correct?

- a. left-sided clefts are more common than right-sided clefts
- b. they are always syndromic disorders
- c. they occur more often in males
- d. they arise after day 60 of embryogenesis

45. A median cleft of the upper lip:

- a. is the most common type of cleft
- b. is rare
- c. is often associated with other developmental anomalies
- d. arises due to failure of neural tube closure

46. Isolated cleft palate:

- a. arises at the same time as a cleft lip
- b. is genetically independent of cleft lip
- c. has a lower frequency than cleft lip
- d. is more common in males

47. Factors that increase the risk of cleft lip and palate include:

- a. exposure to teratogens
- b. amniotic bands
- c. monogenic inheritance in all cases
- d. multifactorial predisposition

48. Neural tube defects include:

- a. anencephaly
- b. encephalocele
- c. spina bifida
- d. cleft palate

49. Encephalocele is characterized by:

- a. herniation of brain tissue and meninges
- b. disruption of the skin covering
- c. a defect of the skull bone
- d. always a lethal course

50. Spina bifida:

- a. has the same frequency in all populations
- b. may be part of a multifactorial spectrum
- c. arises due to a defect in vertebral development
- d. is always the result of a chromosomal aberration

51. Fewer than 10% of neural tube defects have a cause:

- a. in teratogens
- b. in monogenic syndromes
- c. in amniotic bands
- d. exclusively in multifactorial predisposition

52. Clinically, pyloric stenosis presents with:

- a. impaired gastric emptying
- b. hypertrophy of the pylorus
- c. onset most commonly in the 3rd–4th week of life
- d. cyanosis

53. Which statements about pyloric stenosis are correct?

- a. it is more common in girls
- b. it follows a multifactorial threshold model
- c. hormonal differences between sexes influence risk
- d. it has no familial occurrence

54. Pes equinovarus (clubfoot):

- a. has an incidence of about 1.2 per 1,000
- b. is more common in boys
- c. is always genetically determined
- d. may be unilateral or bilateral

55. Possible epigenetic/environmental causes of pes equinovarus include:

- a. oligohydramnios
- b. limited space in the uterus
- c. mutation of MSX genes
- d. amniotic bands

56. Congenital heart defects:

- a. are among the most common congenital anomalies
- b. are always lethal
- c. can be shunt or non-shunt defects
- d. do not have a multifactorial etiology

57. Shunt congenital heart defects are typically associated with:

- a. blood mixing
- b. septal defects
- c. coarctation of the aorta
- d. patent ductus arteriosus

58. Tetralogy of Fallot:

- a. belongs among non-shunt defects
- b. causes cyanosis
- c. has typical clinical signs such as clubbing (“drumstick fingers”)
- d. is not treated surgically

59. Ventricular septal defect:

- a. is a congenital heart defect
- b. is always clinically severe
- c. may be part of multifactorial disorders
- d. arises due to failure of neural tube closure

60. In clinical assessment of multifactorial disorders, it is important to consider:

- a. the sex of the affected individual
- b. the number of affected siblings
- c. severity of the defect
- d. to ignore population differences

61. The “syndromic model” of non-syndromic orofacial clefts:

- a. includes a multifactorial type of cleft
- b. includes a purely epigenetic type of cleft
- c. includes a cleft type caused by a mutation in a single gene
- d. does not exist

62. Clinical-genetic evaluation of a patient with an orofacial cleft begins with:

- a. whole-genome analysis of the patient’s parents
- b. whole-genome analysis of the patient
- c. classification as syndromic or non-syndromic
- d. simultaneous testing of several randomly selected genes

63. Classification of an orofacial cleft as syndromic vs non-syndromic is based on:

- a. whole-genome analysis of the patient’s parents
- b. simultaneous testing of several randomly selected genes
- c. whole-genome analysis of the patient
- d. presence or absence of other dysmorphic features or malformations, with assessment of relatives with a similar phenotype

64. In the first step, clinical-genetic evaluation of a patient with a syndromic orofacial cleft requires:

- a. whole-genome analysis of the patient
- b. whole-genome analysis of relatives with the same phenotype
- c. evaluation of possible non-genetic causes
- d. evaluation of possible teratogen exposure during the first trimester

65. Cleft palate can be divided into:

- a. cleft of the soft palate
- b. cleft of the hard and soft palate
- c. cleft of the entire palate
- d. cleft uvula

66. Multifactorial inheritance differs from polygenic inheritance mainly in that it:

- a. includes only major genes
- b. includes genetic predisposition and epigenetic factors
- c. has minimal environmental influence
- d. always leads to monogenic diseases

67. The threshold model for qualitative multifactorial traits explains that:

- a. phenotype changes continuously without a boundary
- b. individuals are classified as “unaffected” and “affected” depending on whether the threshold is exceeded
- c. trait expression does not depend on the environment
- d. this is a typical feature of polygenic traits

68. Which statement about regression to the mean in quantitative traits is correct?

- a. extreme values tend to be less extreme in the next generation
- b. extreme values accumulate in every generation
- c. children always replicate the parent’s extreme values
- d. regression to the mean is typical only for qualitative traits

69. Heritability is:

- a. an individual’s risk of developing a disease
- b. the proportion of additive genetic variance in the total phenotypic variance of a trait
- c. an unchanging value that is the same in every population
- d. proof that a trait is determined only by genes

70. For empirical risks in multifactorial disorders, it holds that:

- a. affected distant relatives increase risk more than affected close relatives
- b. the more affected first-degree relatives, the higher the recurrence risk
- c. the sex of the affected individual does not matter
- d. severity of the defect has no impact on risk estimation

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. FTFF | 25. FTFT | 49. TFTF |
| 2. FTTF | 26. FTFT | 50. FTTF |
| 3. TFFF | 27. TTFT | 51. TTTF |
| 4. FTFF | 28. FTTF | 52. TTTF |
| 5. FTFT | 29. FTFT | 53. FTTF |
| 6. FTTF | 30. TFTF | 54. TTFT |
| 7. TTFT | 31. TFTT | 55. TTFT |
| 8. TFTF | 32. FTFT | 56. TFTF |
| 9. FTTF | 33. TTFF | 57. TTFT |
| 10. FTTF | 34. FFTF | 58. FTTF |
| 11. TFTT | 35. FTTF | 59. TFTF |
| 12. FTTF | 36. FTFF | 60. TTTF |
| 13. FTTF | 37. TTFF | 61. FFFT |
| 14. TFFF | 38. FTFT | 62. FFTF |
| 15. TFFF | 39. TFTF | 63. FFFT |
| 16. FTFT | 40. TFTF | 64. FFTT |
| 17. FTFT | 41. FTFT | 65. TTTT |
| 18. TFTF | 42. FTTF | 66. FTFF |
| 19. TFFT | 43. FTTF | 67. FTFF |
| 20. FTFF | 44. TFTF | 68. TFFF |
| 21. FFTF | 45. FTTF | 69. FTFF |
| 22. TFTT | 46. FTTF | 70. FTFF |
| 23. FFTF | 47. TTFT | |
| 24. TFTT | 48. TTTF | |

5. Dental aspects of congenital developmental disorders.

Congenital developmental anomalies of the dentition represent a heterogeneous group of disorders arising from genetic mutations, chromosomal aberrations, or disturbances of embryonic development of the orofacial region. Tooth development (odontogenesis) is the result of a **precisely regulated** interaction between the **ectoderm** and **ectomesenchyme**, with key roles played by the **WNT**, **SHH**, **BMP**, and **FGF** signaling pathways. Disruption of these pathways leads to abnormalities in tooth number, shape, size, and structure.

Genetically determined anomalies of tooth number include **hypodontia**, **oligodontia**, and **anodontia**. **Hypodontia**, defined as the congenital **absence of one to six permanent teeth** (excluding third molars), is the **most common** developmental anomaly of the dentition and has a predominantly **multifactorial** genetic background. Mutations in the *MSX1*, *PAX9*, *AXIN2*, and *WNT10A* genes are most frequently associated with nonsyndromic hypodontia and oligodontia. **Oligodontia**, characterized by the **absence of more than six teeth**, often has a **monogenic** basis and may be part of syndromic conditions, such as **ectodermal dysplasia**. Mutations in the *AXIN2* gene are also clinically significant due to their association with an increased risk of colorectal carcinoma, highlighting the importance of the dental phenotype as a marker of systemic disease.

Abnormalities of enamel development are collectively referred to as ***amelogenesis imperfecta*** and represent a genetically heterogeneous group of disorders with autosomal dominant, autosomal recessive, or X-linked inheritance patterns. They are caused by mutations in genes encoding **enamel proteins** or **enzymes involved in mineralization**, particularly *AMELX*, *ENAM*, *MMP20*, and *KLK4*. Clinically, ***amelogenesis imperfecta*** presents with **hypoplastic**, **hypocalcified**, or **hypomaturation** forms of enamel, accompanied by increased susceptibility to dental **caries** and dentin **hypersensitivity**. In contrast, ***dentinogenesis imperfecta*** arises from mutations in the *DSPP* gene, which encodes **dentin sialophosphoprotein**, and manifests as **opalescent discoloration of the teeth**, **bulbous crowns**, and **premature loss of tooth structure**. It is frequently associated with ***osteogenesis imperfecta***, caused by mutations in the *COL1A1* and *COL1A2* genes.

Syndromic malformations with dental manifestations constitute a significant group of congenital disorders in which dental anomalies are part of a broader phenotypic spectrum. **Ectodermal dysplasia**, most commonly the X-linked form caused by mutations in the *EDA* gene, is characterized by **hypodontia** or **anodontia**, conical-shaped teeth, and delayed eruption. **Van der Woude syndrome**, associated with mutations in the *IRF6* gene, is characterized by cleft lip and palate, **hypodontia**, and **anomalies in tooth shape**. In **cleidocranial dysplasia**, caused by mutations in the *RUNX2* gene, delayed eruption, **supernumerary** teeth, and retained permanent teeth are observed as a consequence of impaired bone remodeling.

Chromosomal aberrations may also manifest with specific dental phenotypes. Patients with **Down syndrome** frequently exhibit **hypodontia**, **microdontia**, **delayed** eruption, and an increased risk of **periodontal** disease. **Turner syndrome** is associated with a **reduced number** of teeth, **short roots**, and abnormal crown morphology, while **deletion syndromes**, such as 22q11.2 deletion syndrome (DiGeorge syndrome), are associated with enamel **hypoplasia**, **eruption disorders**, and **cleft-related anomalies**.

Disorders of mineralization and the extracellular matrix affect the quality of hard dental tissues and their mechanical resistance. **Hypophosphatasia**, caused by mutations in the *ALPL*

gene, leads to a **deficiency of alkaline phosphatase** and subsequent impairment of dentin and cementum mineralization, clinically manifested by **premature loss of primary teeth** with **intact roots**. Defects of type I collagen, as seen in osteogenesis imperfecta, directly impact dentin structure and promote the development of *dentinogenesis imperfecta*.

Pierre Robin sequence (syndrome) represents a developmental sequence characterized by **micrognathia**, **glossoptosis**, and often a **secondary cleft of the soft palate**. It arises as a result of impaired mandibular growth during early embryogenesis, which mechanically affects the closure of the palatal shelves. Although it may occur in isolation, it is frequently **part of genetic syndromes**, particularly **Stickler syndrome**, caused by mutations in the *COL2A1*, *COL11A1*, or *COL11A2* genes. Dental manifestations include **malocclusions**, **eruption disturbances**, **crowding**, and an increased risk of **enamel hypoplasia** in the cleft region.

A continuation of the previous chapter concerns **orofacial clefts**. The group of genes that may be involved includes, for example, the *TGF-alpha* and *TGF-beta 2/3* genes, the homeotic genes *MSX-1* and *MSX-2*, genes indirectly related to the development of orofacial structures, and genes involved in xenobiotic metabolism. When estimating recurrence risk, the number of previously born affected and unaffected children is emphasized, as well as sex and the severity of the defect, more severe defect and involvement of the less frequently affected sex increase the risk. Empirical risks may differ between populations; therefore, data from the population from which the patient originates should be used.

Among **external risk factors** (teratogens) associated with an increased risk of orofacial clefts are certain anticonvulsants (antiepileptic drugs), the use of systemic corticosteroids in the first trimester, and smoking during pregnancy. With regard to alcohol, study results are inconsistent, however, binge drinking or repeated consumption of approximately ≥ 5 drinks per occasion, particularly multiple times during the first trimester, has been associated with an increased risk of selected types of clefts.

Candidate genes also include those related to folate metabolism and transport (e.g., folate receptors and transporters), which aligns with current knowledge about the role of the folate pathway in craniofacial development. In **multifactorial clefts**, intergenic interaction is also important. The literature reports a marked increase in risk with the combination of *TGF-alpha* and *MSX1* variants. Gene-environment interactions have likewise been described, for example, a higher risk with maternal smoking in combination with certain variants of detoxification genes (e.g., *GST* genes) or with variants in *TGF-alpha*.

Some conditions are described as “**syndromic models**” for the study of clefts. **Van der Woude syndrome** and popliteal pterygium syndrome both belong to the spectrum of disorders caused by pathogenic variants in *IRF6*. Another example is X-linked cleft palate (CPX), which is associated with mutations in the *TBX22* gene. Among syndromes with ectodermal manifestations, EEC/EES syndrome (ectrodactyly–ectodermal dysplasia–cleft) is mentioned, typically caused by heterozygous mutations in *TP63*. A distinct entity is the autosomal recessive combined cleft lip and palate with ectodermal dysplasia, also known as the **Margarita Island type**, which is associated with mutations in *PVRL1* (nectin-1).



Model questions

1. Odontogenesis is the result of the interaction between:

- a. mesoderm and endoderm
- b. ectoderm and ectomesenchyme
- c. neural crest and mesoderm
- d. ectoderm and mesoderm

2. Which signaling pathways play a key role in tooth development?

- a. WNT
- b. SHH
- c. BMP
- d. JAK/STAT

3. Hypodontia is defined as:

- a. absence of all teeth
- b. absence of 1-6 permanent teeth (excluding third molars)
- c. absence of more than 6 teeth
- d. delayed tooth eruption

4. Which genes are most commonly associated with nonsyndromic hypodontia?

- a. *MSX1*
- b. *PAX9*
- c. *AXIN2*
- d. *TP53*

5. Oligodontia is characterized by:

- a. absence of more than 6 teeth
- b. frequent syndromic occurrence
- c. a monogenic genetic background
- d. exclusively X-linked inheritance

6. Mutations in the *AXIN2* gene are clinically significant also for:

- a. increased risk of colorectal carcinoma
- b. enamel mineralization defects
- c. disturbances of the WNT signaling pathway
- d. supernumerary teeth

7. *Amelogenesis imperfecta* is characterized by:

- a. genetic heterogeneity
- b. disturbance of enamel development
- c. exclusively autosomal dominant inheritance
- d. increased susceptibility to dental caries

8. Which genes encode proteins involved in enamel formation?

- a. *AMELX*
- b. *ENAM*
- c. *DSPP*
- d. *MMP20*

9. The hypoplastic form of *amelogenesis imperfecta* is characterized by:

- a. increased enamel formation
- b. normal mineralization
- c. impaired crystal maturation in cementum
- d. thin enamel

10. *Dentinogenesis imperfecta* most commonly arises from mutations in the gene:

- a. *DSPP*
- b. *COL1A1*
- c. *ENAM*
- d. *ALPL*

11. A typical feature of *dentinogenesis imperfecta* is:

- a. opalescent discoloration of teeth
- b. bulbous crowns
- c. short roots
- d. increased enamel transparency

12. *Dentinogenesis imperfecta* may be associated with:

- a. osteogenesis imperfecta
- b. ectodermal dysplasia
- c. type I collagen disorders
- d. hypophosphatasia

13. The most common inheritance pattern of ectodermal dysplasia is:

- a. autosomal recessive
- b. autosomal dominant
- c. X-linked
- d. mitochondrial inheritance

14. Dental manifestations of ectodermal dysplasia include:

- a. hypodontia
- b. conical-shaped teeth
- c. delayed eruption
- d. supernumerary teeth

15. The *EDA* gene is responsible for:

- a. development of ectodermal derivatives
- b. ameloblast differentiation
- c. TNF pathway signaling
- d. development of hair, sweat glands, and teeth

16. Van der Woude syndrome is associated with mutations in the gene:

- a. *IRF6*
- b. *RUNX2*
- c. *MSX1*
- d. *COL2A1*

17. Typical features of Van der Woude syndrome include:

- a. cleft lip and/or palate
- b. hypodontia
- c. lower lip pits
- d. supernumerary teeth

18. Cleidocranial dysplasia is caused by mutations in the gene:

- a. *RUNX2*
- b. *PAX9*
- c. *AXIN2*
- d. *COL1A2*

19. Dental manifestations of cleidocranial dysplasia include:

- a. delayed eruption
- b. supernumerary teeth
- c. retained permanent teeth
- d. enamel hypoplasia

20. Down syndrome is characterized by:

- a. macrodontia
- b. hypodontia
- c. microdontia
- d. increased risk of periodontitis

21. Dental features of Turner syndrome include:

- a. short roots
- b. reduced number of teeth
- c. supernumerary teeth
- d. abnormal crown shape

22. The 22q11.2 deletion syndrome is associated with:

- a. cleft anomalies
- b. enamel hypoplasia
- c. eruption disorders
- d. *amelogenesis imperfecta*

23. Hypophosphatasia results from mutations in the gene:

- a. *ALPL*
- b. *DSPP*
- c. *COL1A1*
- d. *ENAM*

24. Hypophosphatasia leads to:

- a. deficiency of alkaline phosphatase
- b. impaired dentin mineralization
- c. premature loss of primary teeth
- d. root resorption

25. The extracellular matrix of dentin is mainly composed of:

- a. type I collagen
- b. amelogenin
- c. proteoglycans
- d. elastin

26. Osteogenesis imperfecta is primarily a disorder of:

- a. enamel mineralization
- b. type I collagen synthesis
- c. ameloblast function
- d. bone remodeling

27. Pierre Robin sequence is characterized by:

- a. micrognathia
- b. glossoptosis
- c. cleft of the soft palate
- d. macrognathia

28. Pierre Robin sequence arises primarily due to:

- a. impaired mandibular growth
- b. a genetic enamel disorder
- c. mechanical interference with palatal closure
- d. neural crest defects

29. Pierre Robin sequence may be:

- a. present as an isolated condition
- b. part of a syndrome
- c. always genetically determined
- d. the result of a progressive developmental process

30. Mutations in the *PAX9* gene are most commonly associated with:

- a. hypodontia
- b. oligodontia
- c. supernumerary teeth
- d. *amelogenesis imperfecta*

31. Mutations in the *MSX1* gene may cause:

- a. hypodontia
- b. cleft lip and palate
- c. dentinogenesis imperfecta
- d. supernumerary teeth

32. Mutations in the *WNT10A* gene are associated with:

- a. nonsyndromic hypodontia
- b. ectodermal dysplasia
- c. *osteogenesis imperfecta*
- d. *amelogenesis imperfecta*

33. Dentin disorders may be caused by:

- a. *DSPP* mutations
- b. extracellular matrix defects
- c. *ENAM* mutations
- d. hypophosphatasia

34. Mutations in the *COL2A1* gene are associated with:

- a. Stickler syndrome
- b. Pierre Robin sequence
- c. Van der Woude syndrome
- d. *osteogenesis imperfecta*

35. Supernumerary teeth most commonly occur in:

- a. cleidocranial dysplasia
- b. ectodermal dysplasia
- c. trisomy 21
- d. crowding syndrome

36. Bulbous crowns are typical of:

- a. *dentinogenesis imperfecta*
- b. *amelogenesis imperfecta*
- c. Turner syndrome
- d. *osteogenesis imperfecta*

37. X-linked ectodermal dysplasia causes:

- a. hypodontia
- b. delayed eruption
- c. supernumerary teeth
- d. conical-shaped teeth

38. Mutations in the *ENAM* gene cause:

- a. hypoplastic *amelogenesis imperfecta*
- b. hypomaturational *amelogenesis imperfecta*
- c. *dentinogenesis imperfecta*
- d. *osteogenesis imperfecta*

39. Mutations in the *KLK4* gene are associated with:

- a. hypomaturational *amelogenesis imperfecta*
- b. hypoplastic *amelogenesis imperfecta*
- c. dentin dysplasia
- d. delayed eruption

40. Disorders of dentin mineralization may be caused by:

- a. hypophosphatasia
- b. *ALPL* mutations
- c. *COL1A1* mutations
- d. ectodermal dysplasia

41. Disorders of enamel mineralization may be caused by:

- a. *amelogenesis imperfecta*
- b. deficiency of FGF signaling
- c. *MMP20* mutations
- d. *AXIN2* mutations

42. Mutations in *COL11A1* and *COL11A2* cause:

- a. Stickler syndrome
- b. Pierre Robin sequence
- c. ectodermal dysplasia
- d. Turner syndrome

43. Delayed tooth eruption is frequently observed in:

- a. cleidocranial dysplasia
- b. ectodermal dysplasia
- c. amelogenesis imperfecta
- d. trisomy 21

44. Disorders of the extracellular matrix in teeth may affect:

- a. dentin
- b. enamel
- c. cement
- d. pulp

45. Cleft lip and palate are typical of:

- a. Van der Woude syndrome
- b. cleidocranial dysplasia
- c. 22q11.2 deletion syndrome
- d. Stickler syndrome

46. Microdontia is defined as:

- a. reduced tooth size
- b. decreased number of teeth
- c. altered crown shape
- d. delayed eruption

47. Malocclusion is often a consequence of:

- a. Pierre Robin sequence
- b. hypodontia
- c. Stickler syndrome
- d. amelogenesis imperfecta

48. Cleft-related anomalies may lead to:

- a. delayed tooth eruption
- b. enamel hypoplasia
- c. malocclusion
- d. supernumerary teeth

49. Teeth crowding may result from:

- a. Pierre Robin sequence
- b. cleidocranial dysplasia
- c. oligodontia
- d. micrognathia

50. What is the opalescent coloration of teeth?

- a. yellowish discoloration caused by an increased dentin content
- b. bluish discoloration resulting from fluorescence under UV light
- c. glass-like, completely transparent coloration of the incisal edges
- d. milky pearly coloration with a subtle bluish hue resembling opal

51. Examples of genes responsible for the development of orofacial clefts are:

- a. genes encoding the folic acid receptor
- b. genes involved in xenobiotic metabolism
- c. the development of orofacial clefts cannot be caused by defective genes
- d. homeotic genes *MSX-1* and *MSX-2*

52. An example of a syndromic model of non-syndromic orofacial clefts is:

- a. Down syndrome
- b. Klinefelter syndrome
- c. Van der Woude syndrome
- d. Turner syndrome

53. An example of a syndromic model of non-syndromic orofacial clefts is:

- a. popliteal pterygium syndrome
- b. Klinefelter syndrome
- c. Edwards syndrome
- d. Turner syndrome

54. An example of a syndromic model of non-syndromic orofacial clefts is:

- a. sickle cell anemia
- b. Down syndrome
- c. Turner syndrome
- d. combined cleft lip and palate with ectodermal dysplasia (Margarita Island ectodermal dysplasia)

55. An example of a syndromic model of non-syndromic orofacial clefts is:

- a. EES (ectrodactyly, ectodermal dysplasia, CLP) syndrome
- b. Klinefelter syndrome
- c. Turner syndrome
- d. Edwards syndrome

56. An example of a syndromic model of non-syndromic orofacial clefts is:

- a. cri-du-chat syndrome
- b. Jacobs syndrome
- c. X-linked cleft palate (CPX)
- d. Turner syndrome

57. X-linked cleft palate (CPX) is caused by:

- a. trisomy 21
- b. tetraploidy
- c. Philadelphia chromosome
- d. mutation in the *TBX22* gene

58. Autosomal recessive combined cleft lip and palate with ectodermal dysplasia (Margarita Island ectodermal dysplasia) is caused by:

- a. homozygous mutation in the *TP53* gene
- b. mutation of multiple genes with a small effect
- c. mutation in the gene encoding nectin-1 (surface receptor for α -herpes viruses)
- d. mutation of multiple major genes

59. X-linked cleft palate (CPX) is caused by:

- a. absence of the F body
- b. mutation in the *TBX22* gene
- c. X chromosome inactivation
- d. gonosomal aberrations

60. EES (ectrodactyly, ectodermal dysplasia, CLP) syndrome is caused by:

- a. heterozygous mutation in the *TP63* gene
- b. homozygous mutation in the *TP53* gene
- c. deletion of the terminal segment of the short arm of chromosome 5
- d. deletion of one band on the short arm of chromosome 4

61. Van der Woude syndrome is caused by:

- a. homozygous mutation in the *TP53* gene
- b. deletion of the terminal segment of the short arm of chromosome 5
- c. mutation in the *IRF6* gene
- d. mutation in the *TBX22* gene

62. Factors demonstrably associated with an increased risk of orofacial clefts are:

- a. anticonvulsants
- b. corticosteroids
- c. heavy alcohol consumption
- d. smoking

63. The risk of cleft lip and palate increases if a woman smokes during pregnancy, and she:

- a. did not transmit any mutation to the fetus
- b. transmitted a predisposition for cleft lip and palate to the fetus
- c. transmitted a *GSTT1* null polymorphism to the fetus
- d. transmitted a rare variant of the *TGF- α* gene to the fetus

64. The risk of cleft palate increases up to 10-fold with the combination of rare variants of *TGF-α* and *MSX1*. This fact highlights the importance of:

- a. maternal smoking during the periconceptional period
- b. intergenic interaction
- c. adequate folic acid intake
- d. use of multivitamins

65. Which statements about *dentinogenesis imperfecta* are correct?

- a. it affects exclusively primary teeth
- b. it may be associated with osteogenesis imperfecta
- c. teeth have an opalescent, bluish-gray, or brownish appearance
- d. it is caused exclusively by vitamin D deficiency

66. Which statements about *amelogenesis imperfecta* are correct?

- a. it is a genetically heterogeneous disorder with different types of inheritance
- b. it arises exclusively from a mutation in the *DSPP* gene
- c. it is caused only by vitamin D deficiency
- d. it may be caused by mutations in genes encoding enamel proteins or mineralization enzymes

67. Which statements about hypophosphatasia are correct?

- a. it leads to alkaline phosphatase deficiency
- b. it is typically caused by mutations in the *IRF6* gene
- c. clinically, it may present with premature loss of primary teeth with intact roots
- d. it is primarily a disorder of AMELX/ENAM enamel protein formation

68. Which genes are associated with Stickler syndrome?

- a. *COL2A1*
- b. *COL11A1*
- c. *RUNX2*
- d. *IRF6*

69. Which statements about hypodontia are correct?

- a. it is the most common developmental anomaly of dentition
- b. it always has a monogenic genetic background
- c. it predominantly has a multifactorial genetic background
- d. it is defined as the absence of more than six teeth

70. Which statements about *dentinogenesis imperfecta* are correct?

- a. *DSPP* encodes dentin sialophosphoprotein
- b. it is caused by mutations in the *COL2A1*/*COL11A1* genes
- c. it typically presents with premature loss of tooth structure
- d. it affects exclusively primary teeth

Answer Key to Model Questions

- | | | |
|-----------|----------|-----------|
| 1. FTFF | 25. TFTF | 49. TFTT |
| 2. TTTF | 26. FTFT | 50. FFFT |
| 3. FTFF | 27. TTTF | 51. TTFT |
| 4. TTTF | 28. TFTF | 52. FFTF |
| 5. TTTF | 29. TTFT | 53. TFFF |
| 6. TFTF | 30. TTFF | 54. FFFT |
| 7. TTFT | 31. TTFF | 55. TFFF |
| 8. TTFT | 32. TTFF | 56. FFTF |
| 9. FTFT | 33. TTFT | 57. FFFT |
| 10. TFFF | 34. TTFF | 58. FFTF |
| 11. TTTF | 35. TFFT | 59. FTFF |
| 12. TFTF | 36. TFFT | 60. TFFF |
| 13. FFTF | 37. TTFT | 61. FFTF |
| 14. TTTF | 38. TTFF | 62. TTTT |
| 15. TFFT | 39. TFFF | 63. FT TT |
| 16. TFFF | 40. TTFF | 64. FTFF |
| 17. TTTF | 41. TFTF | 65. FTTF |
| 18. TFFF | 42. TTFF | 66. TFFT |
| 19. TTTF | 43. TTFF | 67. TFFT |
| 20. FT TT | 44. TFTF | 68. TTFF |
| 21. TTFT | 45. TFTF | 69. FTFT |
| 22. TTTF | 46. TFTF | 70. TFTF |
| 23. TFFF | 47. TFTF | |
| 24. TTTF | 48. TTTF | |

6. Cytogenetics. Methods in Cytogenetics.

Cytogenetics deals with the study of the organization of genetic material at the level of chromosomes (chromatin), specifically their number, structure, and segregation during cell division under normal and pathological conditions, as well as the relationship of these findings with phenotype. It is used for the diagnosis of numerical and structural chromosomal aberrations in various pathological conditions.

The human genome is organized into 46 chromosomes forming 23 pairs, of which 22 pairs are autosomes and one pair consists of sex chromosomes (XX in females, XY in males). A human chromosome consists of a **short arm** (p) and a **long arm** (q), with a **centromere** located between them, where the chromosome attaches to the spindle apparatus during mitosis and meiosis. Telomeres are located at the terminal regions of chromosomes and protect DNA from shortening during cell division.

Depending on the degree of spiralization, chromosomes have different morphological appearances (they look different in interphase and in metaphase, when they are the thickest and most visible). Cytogenetics focuses on selected phases of the cell cycle in which chromosomes are morphologically best distinguishable and allow identification of structural and numerical aberrations. Particularly important is prophase of meiosis, during which potential abnormalities arising during the pairing of homologous chromosomes are observed. Another important phase is metaphase, in which chromosomes are maximally condensed, allowing their detailed analysis and the creation of a karyotype (cells treated with colchicine are often used). In anaphase, the process of chromosome segregation can be observed; a defect in chromosome separation may lead to numerical chromosomal aberrations (aneuploidies).

Chromosomes are composed of euchromatin and heterochromatin. **Euchromatin** is a loosely condensed region of the chromosome that is rich in genes and transcriptionally active. **Heterochromatin** represents a highly condensed region of the chromosome that is relatively poor in genes and genetically inactive. According to location and mechanism of origin, two types of heterochromatin are distinguished: **constitutive heterochromatin**, which is present in all cells of an individual and in all stages of development, and **facultative heterochromatin**, which occurrence depends on the cell type and its developmental stage.

An example of facultative heterochromatin is the heterochromatin of the X chromosome in females, which arises from the inactivation of one of the two X chromosomes at the earliest stage of embryogenesis, approximately between the 9th and 12th day of development. This process involves condensation and extensive methylation of one of the pair of X chromosomes (including its euchromatic regions), leading to functional inactivation of genes located on this chromosome. According to **Lyon's theory**, this represents a mechanism ensuring **gene dosage compensation** – in females, only one X chromosome remains active, just as in males. The second X chromosome in females is partially condensed and inactive (in interphase cells it forms sex chromatin, X-heterochromatin), visible as the so-called **Barr body**. X-chromosome inactivation occurs randomly; in individual embryonic cells either the maternal or paternal X chromosome may be inactivated. This inactivation remains stable and permanent in the given cell clone during further development, with the exception of oogonia. From the perspective of expression of X-linked genes, a female represents a **physiological mosaic**. This mechanism explains the occurrence of phenotypic manifestations in carriers of X-linked recessive diseases, which may appear if cells with an active X chromosome carrying the pathological allele predominate in the target tissue. However, X-chromosome inactivation is not complete, as not

all genes located on this chromosome are subject to silencing. An example is the *XIC* gene (X-inactivation centre), located on the X chromosome, which controls the process of its inactivation. The product of this gene is the RNA molecule XIST (X-inactive specific transcript), which mediates X-chromosome inactivation through methylation of GC dinucleotides.

Methods in Cytogenetics

Based on different methodological approaches, cytogenetics can be divided into classical cytogenetics and molecular cytogenetics.

Classical Cytogenetics

Classical cytogenetics consists of microscopic evaluation and staining of chromosomes visualized during mitosis (mainly metaphase chromosomes are analyzed) and also during interphase (sex heterochromatin is examined). Chromosomes are best observed in the so-called **c-metaphase**, which can be achieved by adding the mitotic poison colchicine to the cell culture. Colchicine inhibits depolymerization of tubulin in kinetochore microtubules, preventing the separation of chromosomes into anaphase and stopping the entire process of mitosis.

The metaphase of mitosis represents the most suitable stage for the analysis of both numerical and structural chromosomal aberrations. At this stage, chromosomes are maximally condensed and arranged in a single plane – **the metaphase plate** (equatorial plane), which facilitates the preparation of a karyological preparation. Preparation of the karyological slide for analysis of metaphase chromosomes involves several steps: trypsinization, hypotonic treatment, fixation, and staining. By arranging chromosomes into homologous pairs according to standard classification, a **karyotype** is obtained.

Conventional (diffuse) staining belongs to the oldest cytogenetic techniques. The dye **Giemsa** (or Giemsa-Romanowski) is used, which has an affinity for chromatin. Classification of such diffusely stained chromosomes is performed according to the **Denver classification**, where their relative size (large, medium, small) and the position of the centromere are evaluated. According to this classification, all chromosomes are divided into seven groups labeled **A–G**. In humans, chromosomes are classified as **metacentric** (centromere approximately in the middle), **submetacentric** (centromere shifted closer to one telomere), and **acrocentric** (centromere very close to one telomere).

Identification staining – G, Q, C, R, and T banding techniques. The principle of these methods is that after the action of an external factor on chromosomes, heterochromatin and euchromatin regions become differentially highlighted. Subsequent staining reveals darker and lighter regions on chromosomes, called **bands**. The **Paris classification** evaluates the number, size, and position of these bands on the short (p) and long (q) arms of each chromosome. It also allows precise identification and description of individual chromosomes (1–22, X, Y) and detection of structural chromosomal aberrations.

Molecular Cytogenetics

The analysis is based on the identification of chromosomes or their parts after hybridization of their DNA with a labeled probe. Hybridization of a probe (a labeled single-stranded DNA molecule with a known nucleotide sequence) with the complementary part of denatured analyzed DNA occurs directly in the cell nucleus or in a karyological preparation, i.e., **in situ**. The **FISH method (fluorescence *in situ* hybridization)** represents a connection between

molecular genetics and cytogenetics. Its principle is the complementary binding of a fluorescently labeled probe (a fluorochrome dye) to a specific target DNA sequence in chromosomes in intact, non-homogenized material. This method can be applied to metaphase chromosomes as well as to the observation of nuclei in interphase.

Types of probes used in FISH depend on the region to which they bind. **Centromeric probes (satellite probes)** hybridize with satellite sequences of the centromere that are unique for each chromosome. **Gene probes** (locus-specific or minilocus probes) hybridize with unique DNA sequences. **Whole chromosome probes** (painting probes) hybridize with an entire specific chromosome or a large part of it. **Telomeric probes** are used to determine the number of terminal chromosomal regions, the telomeres (in humans: TTAGGG repeats).

Modifications of FISH include multicolor FISH, spectral karyotyping, multicolor banding, comparative genomic hybridization, and microarray technology. **Multicolor FISH (mFISH)** enables visualization of all chromosomes in different colors, allowing differentiation of each chromosome and identification of structural chromosomal rearrangements. **Spectral karyotyping (SKY)** displays each chromosome in a different spectral color, allowing unambiguous identification of all chromosomes in a cell and detection of complex chromosomal rearrangements. **Multicolor banding (mBanding)** uses multiple-colored probes for a single chromosome, creating detailed band-like resolution along its length. This method allows detailed analysis of chromosomal structure and precise identification of intrachromosomal rearrangements. **Comparative genomic hybridization (CGH)** compares the amount of DNA between a test sample and a reference sample using fluorescently labeled DNA. Based on differences in fluorescence, small changes in DNA quantity in the analyzed tissue can be detected (i.e., loss or gain of short DNA segments). **Microarray technology (array CGH)** uses unique single-stranded DNA probes fixed on a DNA chip. DNA from the test sample is compared with reference DNA using a microchip containing thousands to millions of probes representing different genomic regions. The DNA samples are labeled with different fluorescent dyes and simultaneously hybridized to the chip. After scanning, signal intensity is evaluated – a shift toward the color of the test sample indicates DNA gain (duplication), whereas a shift toward the reference color indicates DNA loss (deletion).

Overview of cytogenetic methods.

Applied technique	Specification
Conventional (diffuse) Giemsa staining	Chromosomes arranged according to size and centromere position (A–G); basic chromosome identification
Banding techniques (G-, Q-, C-, R-, T-banding)	Visualization of dark and light bands; enables precise identification of chromosomes and structural aberrations
FISH	Identification of specific chromosomes or DNA segments
Multicolor FISH (mFISH)	Each chromosome has a different color; identification of structural chromosomal rearrangements
Spectral karyotypization (SKY)	Each chromosome in a different color spectrum; detection of complex rearrangements
Multicolor banding (mBanding)	Multiple colored probes on a single chromosome; detailed analysis of intrachromosomal rearrangements
Comparative genomic hybridization (CGH)	Comparison of DNA amounts between the test and reference samples; detection of duplications and deletions
Microarray CGH	Use of thousands to millions of probes; reveals DNA changes at high resolution



Model questions

1. On a metaphase chromosome, we can distinguish:

- a. short arms (q) and long arms (p)
- b. centromere region and telomere regions
- c. two chromatids
- d. short arms (p) and long arms (q)

2. What is needed to obtain a sufficient number and quality of metaphase chromosomes?

- a. cell culture
- b. obtaining a sufficient number of dividing cells
- c. obtaining a sufficient number of non-dividing cells
- d. *in vivo* culture

3. What is required for the visualization of the chromosomes we want to analyze?

- a. chromosome staining
- b. chromosome hypotonic treatment
- c. addition of mitogen
- d. subculturing the culture

4. For the classification of chromosomes in cytogenetic analysis, the decisive factor is:

- a. staining techniques
- b. culture methods
- c. culture media
- d. culture vessels

5. For visualization and subsequent classification of chromosomes, the following is used:

- a. conventional staining
- b. identification staining
- c. Gram staining
- d. homogeneous staining

6. Which staining method is used in the Denver classification of chromosomes?

- a. conventional
- b. identification
- c. homogeneous
- d. banding

7. How do Q-banding and G-banding chromosome staining techniques differ?

- a. in Q-banding, chromosomes are observed under a light microscope
- b. in Q-banding, chromosomes must be partially digested
- c. in G-banding, chromosomes are observed under a light microscope
- d. in G-banding, chromosomes must be partially digested

8. The evaluation criterion in the Denver classification of chromosomes is:

- a. relative length of the chromosome and centromere position
- b. chromosome structure
- c. total length of chromosome arms
- d. structure of the telomeric region

9. In the Denver classification, we distinguish in the human karyotype:

- a. large, medium, and small chromosomes according to centromere position
- b. metacentric, submetacentric, and acrocentric chromosomes according to relative chromosome length
- c. large, medium, and small chromosomes
- d. metacentric, submetacentric, and acrocentric chromosomes

10. By arranging chromosomes into pairs according to standard classification, we obtain:

- a. karyotype
- b. chromosome map
- c. metaphase figure
- d. metaphase plate

11. What is the role of colchicine in karyotype preparation?

- a. binds microtubules and prevents their depolymerization, stopping cells in interphase
- b. binds microtubules and prevents their depolymerization, stopping cells in metaphase
- c. binds microtubules and prevents their polymerization, stopping cells in metaphase
- d. binds microtubules and prevents their polymerization, stopping cells in interphase

12. Classical cytogenetic methods allow the study of:

- a. condensed chromatin
- b. euchromatin in interphase
- c. chromosomes of dividing cells
- d. presence of target DNA

13. Molecular cytogenetics:

- a. allows determination of chromosome number even in interphase, eliminating the need for cell culture
- b. allows chromosome analysis from archival material
- c. most commonly uses fluorescence in situ hybridization – FISH
- d. uses identical methods as classical cytogenetics

14. Fluorescence *in situ* hybridization (FISH) allows:

- a. identification of genes or specific DNA sequences
- b. rapid evaluation of a large number of cells
- c. analysis of archival material
- d. analysis of non-dividing cells

15. Regarding modifications of FISH:

- a. there are metaphase forms of FISH diagnostics
- b. allow simultaneous visualization of all chromosomes in different colors
- c. do not require successful hybridization
- d. allow whole-genome screening

16. Modifications of FISH include:

- a. multicolor FISH
- b. in situ hybridization
- c. spectral karyotyping
- d. comparative genomic hybridization

17. In comparative genomic hybridization:

- a. it involves quantitative evaluation of signals from differently labelled DNA from normal tissue and test tissue
- b. balanced structural chromosomal aberrations can also be detected
- c. metaphase chromosomes are not required
- d. unbalanced chromosomal changes can be detected

18. In FISH, the following probes are used:

- a. locus/gene-specific
- b. centromeric
- c. telomere-specific
- d. whole chromosome (painting)

19. DNA chips (microarrays) are used for:

- a. comparison of gene expression
- b. lymphocyte separation
- c. analysis of reference DNA
- d. DNA sequencing

20. Disadvantage of FISH:

- a. only diploid cells can be analyzed
- b. cannot be used to detect aneuploidies in polar bodies after meiosis I
- c. exact sequence of the gene of interest must be known
- d. light microscope is needed for visualization

21. Molecular cytogenetics:

- a. allows determination of chromosome number in interphase, eliminating the need for cell culture
- b. allows chromosome analysis from archival material
- c. commonly uses FISH
- d. uses methods identical to classical cytogenetics

22. Arresting cell division in metaphase is achieved by:

- a. adding a mitotic poison to the culture
- b. adding colchicine to the culture
- c. adding a mitotic activator to the culture
- d. subculturing the cell culture

23. Simultaneous analysis of X and Y heterochromatin allows the determination of:

- a. the gonosomal complement of the cell
- b. the number and type of sex chromosomes in the cell
- c. the total number of chromosomes in the cell
- d. the number of chromosome sets in the cell

24. X-sex heterochromatin:

- a. is one of the X chromosomes in a female that is genetically inactivated
- b. is visible as a Barr body
- c. is visible as a Barr body, where the number of Barr bodies in a cell = number of X chromosomes – 1
- d. is one of the X chromosomes in a female that is genetically active

25. The normal female karyotype is:

- a. 46 XX
- b. 46 XY
- c. dependent on cell developmental stage
- d. dependent on the environment

26. FISH modifications include:

- a. multicolor FISH
- b. spectral karyotyping
- c. multicolor banding
- d. comparative genomic hybridization

27. What does multicolor FISH (mFISH) allow?

- a. display all chromosomes in the same color
- b. display all chromosomes in different colors
- c. differentiate each chromosome
- d. identify structural rearrangements

28. What does spectral karyotyping (SKY) allow?

- a. display each chromosome in a different color spectrum
- b. identify all chromosomes in the cell
- c. identify only nuclei in the cell
- d. detect complex chromosomal rearrangements

29. Principle of multicolor banding (mBanding):

- a. allows detailed analysis of chromosome structure
- b. display all chromosomes in the same size
- c. uses only one-color probe per chromosome
- d. uses multiple-colored probes on a single chromosome

30. Comparative genomic hybridization (CGH):

- a. uses fluorescently labeled DNA
- b. compares DNA amounts between test and reference samples
- c. depends on cell developmental stage
- d. detects complex chromosomal rearrangements

31. Microarray CGH uses:

- a. microarrays
- b. unique double-stranded RNA probes
- c. unique single-stranded DNA probes
- d. unique double-stranded DNA probes

32. Principle of FISH:

- a. DNA amplification using PCR
- b. hybridization of labeled probes to complementary sequences in the cell
- c. sequencing of the entire genome
- d. nucleic acid electrophoresis

33. Which FISH probe type is used for detecting aneuploidies?

- a. locus-specific probe
- b. unique double-stranded RNA probes
- c. centromeric probe
- d. gene probes

34. Target sequences of centromeric (satellite) FISH probes:

- a. telomeric sequences TTAGGG
- b. unique gene sequences
- c. satellite sequences of the centromere
- d. entire chromosome

35. Gene probes (locus-specific or minilocus) bind to:

- a. entire chromosome or a large part
- b. unique DNA sequences
- c. satellite sequences of the centromere
- d. telomeric sequences

36. Whole-chromosome (painting) probes are used for:

- a. detection of telomeres
- b. detection of satellite sequences
- c. hybridization with gene loci
- d. hybridization with the entire specific chromosome or a significant part

37. Telomeric probes are used for:

- a. determining the number of chromosome terminal regions (telomeres)
- b. detection of the entire chromosome
- c. detection of centromeric satellite sequences
- d. identification of unique gene loci

38. What does the Paris classification evaluate?

- a. number of centromeres
- b. type of telomeres
- c. number, size, and position of bands on short (p) and long (q) chromosome arms
- d. number of genes on the chromosome

39. Which chromosomes can be precisely identified using banding techniques?

- a. only sex chromosomes (X, Y)
- b. all chromosomes (1–22, X, Y)
- c. only chromosomes 1–22
- d. only chromosomes with translocations

40. Principle of chromosome identification staining:

- a. telomere detection
- b. hybridization of probes to specific genes
- c. DNA amplification using PCR
- d. differential highlighting of heterochromatin and euchromatin regions

41. Types of heterochromatin according to location and mechanism of formation:

- a. constitutive
- b. facultative
- c. euchromatin
- d. locus-specific

42. Chromosomes are composed of:

- a. only constitutive heterochromatin
- b. only facultative heterochromatin
- c. only telomeres and centromeres
- d. euchromatin and heterochromatin

43. Which chromosome region is lightly condensed, gene-rich, and transcriptionally active?

- a. heterochromatin
- b. euchromatin
- c. constitutive heterochromatin
- d. facultative heterochromatin

44. Constitutive heterochromatin is present:

- a. in all cells of the individual and at all stages of development
- b. only in sex cells
- c. only in selected cells by type
- d. only during mitosis

45. Constitutive and facultative heterochromatin are characterized by:

- a. constitutive heterochromatin is relatively gene-poor
- b. facultative heterochromatin is genetically active
- c. facultative heterochromatin depends on cell type and developmental stage
- d. both constitutive and facultative heterochromatin are usually transcriptionally inactive

46. Which chromosome region is highly condensed, gene-poor, and genetically inactive?

- a. euchromatin
- b. heterochromatin
- c. telomeres
- d. centromeres

47. What is the partially condensed and inactive X chromosome visible in interphase cells called?

- a. Barr body
- b. euchromatic complex
- c. centromere
- d. Lyon body

48. According to Lyon's theory, why is one X chromosome inactivated?

- a. to increase gene transcription
- b. to ensure the same gene dosage in males and females
- c. to ensure gene dosage compensation in females
- d. to activate heterochromatin in sex cells

49. Mechanism leading to functional inactivation of the X chromosome:

- a. condensation and extensive methylation of the chromosome
- b. gene amplification on the X chromosome
- c. histone modification (e.g., histone deacetylation)
- d. XIST RNA

50. Females regarding expression of X-linked genes:

- a. express genes equally from maternal and paternal X chromosomes
- b. form a physiological mosaic
- c. express genes only from the maternal X chromosome
- d. express genes only from the paternal X chromosome

51. X-chromosome inactivation occurs:

- a. randomly
- b. either maternal or paternal X chromosome is inactivated
- c. systematically
- d. exclusively in oogonia

52. When does inactivation of one X chromosome occur during embryogenesis?

- a. between days 1 and 3
- b. between days 9 and 12
- c. after birth
- d. during puberty

53. What applies to the X chromosome in females?

- a. only one X chromosome remains active
- b. both X chromosomes are always active
- c. the second X chromosome is partially condensed
- d. the second X chromosome is completely removed from the cell

54. *XIC* gene:

- a. completely inactivated during lyonization
- b. located on the Y chromosome
- c. controls X-chromosome inactivation
- d. located on autosomes

55. X-chromosome inactivation:

- a. is complete and affects all genes
- b. is not complete – not all genes are silenced
- c. occurs only in males
- d. occurs only in male somatic cells

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. FTTT | 20. FFTF | 39. FTFF |
| 2. TTFF | 21. TTTF | 40. FFFT |
| 3. TFFF | 22. TTFF | 41. TTFF |
| 4. TFFF | 23. TTFF | 42. FFFT |
| 5. TTFT | 24. TTTF | 43. FTFF |
| 6. TFTF | 25. TFFF | 44. TFFF |
| 7. FFTT | 26. TTTT | 45. TFFT |
| 8. TFFF | 27. FTTT | 46. FTFF |
| 9. FFTT | 28. TTFT | 47. TFFF |
| 10. TFFF | 29. TFFT | 48. FTTF |
| 11. FTFF | 30. TTFF | 49. TFFT |
| 12. TFTF | 31. TFTF | 50. FTFF |
| 13. TTTF | 32. FTFF | 51. TTFF |
| 14. TTTT | 33. FFTF | 52. FTFF |
| 15. TTFT | 34. FFTF | 53. TFTF |
| 16. TFFT | 35. FTFF | 54. FFFT |
| 17. TFFT | 36. FFFT | 55. FTFF |
| 18. TTTT | 37. TFFF | |
| 19. TFFF | 38. FFTF | |

7. Chromosomal aberrations.

In cytogenetic analysis, various deviations can be detected in the karyotype (an image of metaphase chromosomes arranged according to their relative length and the position of the centromere), which in clinical genetics are called **aberrations**. They involve changes in the structure of chromosomes or in their number and most often arise as a result of mutation. They cause significant changes in the phenotype, most often of a pathological nature. The severity of hereditary diseases associated with these aberrations is determined by the location of the mutation, its nature, and its extent. **Chromosomal aberrations** are classified according to the nature of the deviation into **numerical** and **structural**. Depending on which chromosomes are affected by the aberration, we distinguish aberrations of autosomes and gonosomes.

Numerical chromosome aberrations

Numerical chromosomal aberrations are **changes in the number of chromosomes in a cell**. They most often arise as a result of **nondisjunction** (failure of chromosomes or chromatids to separate) during meiosis or mitosis. In these aberrations, the number of chromosomes in a somatic cell differs from the diploid number ($2n$). They are divided into several types, namely polyploidy and aneuploidy.

Polyplody represents the presence of an excess of complete sets of chromosomes. In humans, triploidy and tetraploidy occur most frequently; they are relatively often detected during examinations of early spontaneous miscarriages and are incompatible with life. **Triploidy** ($3n - 69$ chromosomes) is usually the result of multiple fertilization, but it may also arise as a consequence of pathological gametogenesis (formation of a diploid oocyte or sperm, or failure of the second polar body to be expelled from the fertilized oocyte). **Tetraploidy** ($4n - 92$ chromosomes) most often arises by endoreduplication, i.e., a repeated S-phase during the first mitotic division of the zygote, or it may also occur after multiple fertilization.

Aneuploidy represents a condition in which a cell does not have an exact multiple of the haploid number of chromosomes. The resulting number of chromosomes is most often 46 ± 1 . The most common type of aneuploidy is **trisomy** ($2n+1$), which means the presence of an extra chromosome in the cells, leading to severe genetic disorders. If there is a reduced number of chromosomes, we refer to it as **monosomy** ($2n-1$).

Aneuploidies can arise by **nondisjunction** (failure of sister chromatids to separate) during anaphase I or II of meiosis (chromosomes fail to separate—one cell ends up with 24 chromosomes and the other with 22), by **anaphase lag** during the first or second meiotic division (during anaphase, one chromosome of a homologous pair fails to reach the centrosome and is lost in the cytoplasm, resulting in one cell with 22 and the other with 23 chromosomes), or by **defects in the pairing of homologous chromosomes** during prophase I of meiosis. Aneuploidies can affect any chromosome. In general, an organism tolerates an excess of genetic material better than a deficit, with autosomal aneuploidies usually having more severe consequences than gonosomal aneuploidies.

Autosomal monosomies in humans are incompatible with life already in the early stages of embryogenesis. The only monosomy compatible with life in humans is monosomy X, which causes Turner syndrome. Trisomy's of large chromosomes (with a high number of genes) generally have more severe negative consequences than trisomy's of smaller chromosomes.

Numerical aberrations of autosomes include **Down syndrome** (trisomy of chromosome 21), **Patau syndrome** (trisomy of chromosome 13), and **Edwards syndrome** (trisomy of chromosome 18). Numerical aberrations of other autosomes are rare (it is known that trisomy of chromosome 16 occurs most frequently, but the fetus does not survive to birth). When they do occur, they are mostly found in mosaic form, because the presence of a normal cell line dilutes the severity of phenotypic manifestations. The severity of the condition is related to the tissue distribution and the proportion of the pathological cell line. A mosaic form of a chromosomal aberration can arise during mitotic division in the postzygotic period, as a result of nondisjunction or anaphase lag. **Numerical aberrations of the sex chromosomes** include **Turner syndrome** (monosomy X, karyotype 45,X0), **Triple X syndrome** (trisomy X, karyotype 47,XXX), **Klinefelter syndrome** (karyotype 47,XXY), and **XYY syndrome** (karyotype 47,XYY).

Structural chromosome aberrations

Structural chromosomal aberrations represent **changes in the structure of chromosomes**. They can arise by **chromosomal break** (disruption of the continuity of the chromosome) or by **errors during crossing-over**. In unequal (non-reciprocal) crossing-over, segments of unequal length are exchanged between chromosomes, resulting in the duplication of a specific segment on one chromosome and its absence (deletion) on the other chromosome. Structural chromosomal aberrations have severe consequences for the survival of the individual. Their effects depend on the type of aberration, its location on the chromosome, and the extent of the affected segment.

In **balanced chromosomal rearrangements**, such as inversions and translocations, there is **no change in the amount of genetic information**, and carriers may not exhibit phenotypic manifestations. In contrast, **unbalanced chromosomal rearrangements** are associated with a **change in the amount of genetic material**, and carriers show phenotypic effects. They arise either de novo or as a result of incorrect segregation of chromosomes during gametogenesis in carriers of a balanced rearrangement. Carriers of a balanced rearrangement can produce normal gametes (gametes without the rearrangement), gametes with the balanced rearrangement, as well as gametes with the unbalanced rearrangement.

We distinguish several types of structural chromosomal aberrations. **Deletion** is the loss of a specific segment of a chromosome. It can be terminal (missing the end segment) or interstitial (missing a segment from the internal part of the chromosome). **Duplication** is the doubling of a specific segment of a chromosome. **Inversion** is the rotation of an internal segment of a chromosome by 180°. It can be paracentric (the inverted segment does not include the centromere) or pericentric (the inverted segment includes the centromere). A **ring chromosome** forms when both ends of a chromosome (telomeres) fuse to create a closed circle, which can lead to gene loss and problems with cell division. An **isochromosome** arises from improper division of a chromosome during cell division, where sister chromatids separate transversely rather than longitudinally at the centromere. The resulting chromosome has two identical arms (either two long or two short), leading to duplication of genes on one arm and loss of genes on the other. **Translocation** is a change in the position of a chromosome fragment. It can be a simple translocation (where a part of a chromosome moves to a new location on the same or another chromosome) or a reciprocal translocation (where two non-homologous chromosomes exchange DNA segments). A specific type of chromosomal rearrangement is **Robertsonian translocation**, where two acrocentric chromosomes (groups D and G) fuse by their long arms to form a single chromosome, while the short arms are often lost.

Genetic disorders associated with changes in chromosome structure include **Cri du chat syndrome** (deletion of part of the short arm of chromosome 5), **Prader-Willi syndrome** (deletion of part of the long arm of chromosome 15), **chronic myeloid leukemia** (reciprocal translocation between chromosomes 9 and 22), **Burkitt lymphoma** (reciprocal translocation between chromosomes 8 and 14), **fragile X chromosome** (multiple DNA duplications on the long arm of the X chromosome), and **myotonic dystrophy** (multiple DNA duplications on the long arm of chromosome 19).

Table: Numerical chromosomal aberrations.

Type	Subtype and Description	Most Common Mechanism of Origin	Examples / Notes
Polyploidy	Triploidy (3n) – an extra complete set of chromosomes (69)	Multiple fertilization, pathological gametogenesis	Incompatible with life, often seen in early miscarriages
	Tetraploidy (4n) – two extra complete sets of chromosomes (92)	Endoreduplication after fertilization, multiple fertilization	Incompatible with life
Aneuploidy	Trisomy (2n+1) – one extra chromosome	Nondisjunction, anaphase lag, pairing defects in prophase I	Syndromes – Down (21), Edwards (18), Patau (13)
	Monosomy (2n-1) – one missing chromosome	Nondisjunction / anaphase lag	Autosomal monosomies are incompatible with life; Turner (45,X0) is compatible
Mosaicism	Two or more cell lines in one individual	Postzygotic mitotic nondisjunction or anaphase lag	Often milder phenotype depending on % and distribution of the cell line
Numerical gonosomal aberrations	Change in number of X/Y chromosomes	Most often nondisjunction	Turner 45,X0; 47,XXX; 47,XXY; 47,XYY

Table: Structural chromosomal aberrations.

Type	Subtype and Description	Balanced vs. Unbalanced	Examples / Notes
Deletion	Terminal / interstitial – loss of a chromosome segment	Unbalanced	Cri du chat (del 5p), Prader-Willi (del 15q)
Duplication	Duplication of a chromosome segment	Unbalanced	Fragile X, Myotonic dystrophy
Inversion	Paracentric / pericentric – rotation of a segment by 180°	Often balanced	Carriers may be asymptomatic
Ring chromosome	Ring – fusion of chromosome ends (telomeres) into a circle	Usually unbalanced (often material lost)	May cause cell division disorders
Isochromosome	Two identical arms (p/p or q/q)	Unbalanced	Duplication of one arm + loss of the other arm
Translocation	Simple / reciprocal – movement of a segment, or exchange between chromosomes	Reciprocal often balanced, but risk of unbalanced gametes	CML: t(9;22), Burkitt: t(8;14)
Robertsonian translocation	Fusion of two acrocentric chromosomes by their long arms	Often balanced in the carrier, but risk of unbalanced offspring	Involves acrocentric chromosomes (D, G)



Model questions

1. Structural chromosomal aberrations include:

- a. deletion
- b. terminal or interstitial loss of a chromosome segment
- c. trisomy
- d. inversion

2. Polyploidies:

- a. are a relatively common finding in spontaneous miscarriages
- b. often arise from fertilization disorders
- c. cause the formation of mosaicism
- d. do not occur in the prenatal period

3. Structural chromosomal aberrations include:

- a. duplication
- b. partial monosomy
- c. ring chromosome
- d. partial trisomy

4. Triploidy:

- a. can result from pathological gametogenesis
- b. can result from failure to expel the second polar body from a fertilized oocyte
- c. can result from double fertilization of a single oocyte
- d. is compatible with postnatal development

5. Structural chromosomal aberrations include:

- a. isochromosome – transverse division of the chromosome instead of longitudinal
- b. translocation
- c. isochromosome – longitudinal division of the chromosome instead of transverse
- d. aberration causing Cri du chat syndrome

6. Chromosomal aberrations:

- a. are, in a broader sense, deviations in chromosome number or structure
- b. numerical – deviations in the number of chromosomes
- c. structural – deviations in chromosome structure
- d. are deviations in the morphology of the nucleus

7. Structural chromosomal aberrations can be classified:

- a. according to whether they involve a change in the amount of genetic material or not
- b. into balanced and unbalanced
- c. into reciprocal and non-reciprocal
- d. according to whether they involve a change in chromosome structure or not

8. The most common autosomal aneuploidies include:

- a. Down syndrome
- b. Turner syndrome
- c. Edwards syndrome
- d. Klinefelter syndrome

9. Which three forms of Down syndrome exist?

- a. trisomy 21, mosaic form, translocation-related Down syndrome
- b. trisomy 18, trisomy 21, mosaic form of Down syndrome
- c. trisomy 21, X-linked Down syndrome, translocation-related Down syndrome
- d. mosaic form of Down syndrome, partial trisomy 21, deletion form of Down syndrome

10. Structural chromosomal aberrations include:

- a. deletion
- b. inversion
- c. trisomy
- d. terminal or interstitial loss of a chromosome segment

11. In numerical chromosomal aberrations:

- a. the number of chromosomes in a somatic cell differs from the normal diploid number
- b. the number of chromosomes differs from the normal haploid number $2n$
- c. a human somatic cell may contain 69 chromosomes
- d. a human somatic cell may contain 45 chromosomes

12. If structural chromosomal aberrations involve a change in the amount of genetic material:

- a. it is an unbalanced chromosomal rearrangement
- b. the carrier shows phenotypic manifestations
- c. it is a balanced chromosomal rearrangement
- d. the carrier may not show phenotypic manifestations

13. Cri du Chat syndrome is caused by a deletion in:

- a. 4p
- b. 4q
- c. 5q
- d. 5p

14. Polyploidy:

- a. is an excess of complete chromosome sets
- b. includes, for example, triploidy ($3n - 69$)
- c. includes, for example, tetraploidy ($4n - 92$)
- d. does not occur in humans at any stage of ontogeny

15. If structural chromosomal aberrations do not involve a change in the amount of genetic material:

- a. it is an unbalanced chromosomal rearrangement
- b. the carrier must show phenotypic manifestations
- c. it is a balanced chromosomal rearrangement
- d. the carrier may not show phenotypic manifestations

16. In aneuploidy:

- a. the number of chromosomes is not an exact multiple of the haploid number
- b. the resulting number of chromosomes is usually 46 ± 1
- c. it can be a trisomy or monosomy
- d. it involves exclusively the presence of an extra chromosome

17. Unbalanced structural chromosomal rearrangements can arise as:

- a. a de novo mutation
- b. the result of chromosome segregation in a carrier of a balanced rearrangement during gametogenesis
- c. a consequence of nondisjunction
- d. a consequence of vegetative reproduction

18. Tetrads consist of:

- a. 4 chromatids in 2 conjugated chromosomes in the zygotene stage
- b. 4 chromosomes
- c. 2 chromatids in 4 conjugated chromosomes
- d. 4 chromatids in 2 conjugated chromosomes in the pachytene stage

19. A mosaic form of a chromosomal aberration can arise:

- a. from a normal zygote – nondisjunction during mitotic division in the postzygotic period
- b. from a normal zygote – anaphase lag during mitotic division in the postzygotic period
- c. from a gametic mutation
- d. from a point mutation

20. Aneuploidies can arise from:

- a. nondisjunction in anaphase I of meiosis
- b. nondisjunction in anaphase II of meiosis
- c. mitotic errors
- d. endoreduplication

21. Phenotypic consequences of structural chromosomal aberrations:

- a. depend on the type of aberration, its location on the chromosome, and its extent
- b. are always severe with respect to the carrier's phenotype
- c. vary; carriers may show no phenotypic manifestations
- d. vary; however, carriers must always show phenotypic manifestations

22. Which chromosomal aberration arises from the movement of a chromosome segment to a different location?

- a. duplication
- b. deletion
- c. inversion
- d. translocation

23. What changes in chromosomal aberrations?

- a. the arrangement of genes on the chromosome
- b. the morphology of a gene
- c. the amount of RNA in the chromosome
- d. the gene linkage group

24. Which belong to numerical chromosomal aberrations?

- a. polyploidy
- b. duplication
- c. inversion
- d. trisomy

25. Which belong to structural chromosomal aberrations?

- a. polyploidy
- b. duplication
- c. inversion
- d. trisomy

26. What happens to an acrocentric chromosome during anaphase of mitosis?

- a. it fuses with another chromosome
- b. it is segregated to a daughter cell
- c. it remains in the cell and is degraded
- d. it induces polyploidy

27. Genetic disorders associated with changes in chromosome structure include:

- a. Cri du chat syndrome
- b. Down syndrome
- c. Prader–Willi syndrome
- d. Turner syndrome

28. Triploidy:

- a. is designated $3n$
- b. consists of 69 chromosomes
- c. is often the result of multiple fertilization
- d. can also arise as a consequence of pathological gametogenesis

29. Tetraploidy

- a. is designated $4n$
- b. consists of 92 chromosomes
- c. can also arise as a consequence of multiple fertilization
- d. most often arises by endoreduplication

30. Deletion:

- a. is a duplication of a specific chromosome segment
- b. is the loss of a specific chromosome segment
- c. can be terminal
- d. can be interstitial

31. Duplication:

- a. is the doubling of a specific chromosome segment
- b. is the absence of a specific chromosome segment
- c. can be terminal or interstitial
- d. most often arises in germ cells

32. Inversion:

- a. causes triploidy
- b. the inverted segment may include the centromere
- c. is the rotation of an internal chromosome segment by 180°
- d. is the rotation of an internal chromosome segment by 90°

33. Ring chromosome:

- a. forms when both ends of the chromosome (telomeres) fuse to create a closed circle
- b. forms when telomeres fuse to create a closed circle
- c. can lead to gene loss
- d. causes the formation of aneuploidy

34. Isochromosome:

- a. can lead to gene loss
- b. the resulting chromosome has one “p” and one “q” arm
- c. arises from improper division of a chromosome during cell division
- d. the resulting chromosome has two identical arms

35. Translocation:

- a. is a change in the position of a chromosome fragment
- b. involves loss of the centromere
- c. involves loss of only telomeric sequences
- d. leads to duplication of the same genes

36. What is the phenomenon called when a part of a chromosome moves to a new location on the same chromosome?

- a. reciprocal translocation
- b. duplication
- c. simple translocation
- d. deletion

37. What is the phenomenon called when two non-homologous chromosomes exchange DNA segments?

- a. reciprocal translocation
- b. duplication
- c. simple translocation
- d. deletion

38. Robertsonian translocation occurs when:

- a. the X and Y chromosomes fuse
- b. two acrocentric chromosomes fuse
- c. two metacentric chromosomes fuse
- d. two submetacentric chromosomes fuse

39. Which disorders belong to autosomal numerical aberrations:

- a. trisomy of chromosome 13
- b. Prader–Willi syndrome
- c. trisomy of chromosome 18
- d. Down syndrome

40. Trisomy of chromosome 18 is a disorder:

- a. called Patau syndrome
- b. called Edwards syndrome
- c. belonging to autosomal numerical aberrations
- d. belonging to autosomal structural aberrations

41. Trisomy of chromosome 13 is a disorder:

- a. called Patau syndrome
- b. called Edwards syndrome
- c. belonging to autosomal numerical aberrations
- d. belonging to autosomal structural aberrations

42. Trisomy of chromosome 21 is a disorder:

- a. called Patau syndrome
- b. called Down syndrome
- c. belonging to autosomal numerical aberrations
- d. belonging to autosomal structural aberrations

43. Numerical aberrations of sex chromosomes include:

- a. Klinefelter syndrome
- b. Down syndrome
- c. Edwards syndrome
- d. Turner syndrome

44. Which monosomy is compatible with life in humans?

- a. monosomy X
- b. monosomy Y
- c. Turner syndrome
- d. Klinefelter syndrome

45. Karyotype 47, XXY is called:

- a. Klinefelter syndrome
- b. Turner syndrome
- c. Fragile X syndrome
- d. XYY syndrome

46. What are structural chromosomal aberrations?

- a. changes in the structure of chromosomes
- b. changes arising exclusively from gene mutations
- c. changes arising from chromosome breaks
- d. changes arising from errors during crossing-over

47. Chromosome break:

- a. means duplication of genetic material
- b. causes disruption of chromosome continuity
- c. results in complete loss of the entire chromosome
- d. can cause the formation of a structural aberration

48. In unequal (non-reciprocal) crossing-over:

- a. duplication of part of a chromosome
- b. deletion of part of a chromosome
- c. exchange of equally long chromosome segments
- d. change in the number of chromosomes in the cell

49. Duplication and deletion arise as a result of:

- a. equal crossing-over
- b. unequal crossing-over
- c. exchange of unequally long chromosome segments
- d. chromosome break

50. The severity of the consequences of structural chromosomal aberrations depends on:

- a. the type of aberration
- b. the location of the aberration on the chromosome
- c. the extent of the affected segment
- d. the sex of the individual

51. Balanced chromosomal rearrangements:

- a. cause a change in the amount of genetic information
- b. include translocations
- c. may have no phenotypic manifestations
- d. are always lethal

52. Unbalanced chromosomal rearrangements:

- a. always arise *de novo*
- b. are associated with a change in the amount of genetic material
- c. are associated with phenotypic manifestations
- d. can arise from incorrect segregation of chromosomes

53. Phenotypic consequences of structural chromosomal aberrations:

- a. depend on the type of aberration, its location on the chromosome, and its extent
- b. are always severe with respect to the carrier's phenotype
- c. vary; carriers may show no phenotypic manifestations
- d. vary; however, carriers must always show phenotypic manifestations

54. When does inactivation of one X chromosome occur during embryogenesis?

- a. between the 1st and 3rd day of development
- b. between the 9th and 12th day of development
- c. after birth
- d. during puberty

55. Chromosomes can be:

- a. autosomes
- b. homologous
- c. sex chromosomes
- d. heterologous

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. TTFT | 20. TTTF | 39. TFTT |
| 2. TTFF | 21. TFTF | 40. FTTF |
| 3. TTTT | 22. FFFT | 41. TFTF |
| 4. TTTF | 23. TFFT | 42. FTTF |
| 5. TTFT | 24. TFFT | 43. TFFT |
| 6. TTTF | 25. FTTF | 44. TFTF |
| 7. TTFF | 26. FTFF | 45. TFFF |
| 8. TFTF | 27. TFTF | 46. TFTT |
| 9. TFFF | 28. TTTT | 47. FTFT |
| 10. TTFT | 29. TTTT | 48. TTFF |
| 11. TFTT | 30. FTTT | 49. FTTF |
| 12. TTFF | 31. TFFF | 50. TTTF |
| 13. FFFT | 32. FTTF | 51. FTTF |
| 14. TTTF | 33. TTTF | 52. FTTT |
| 15. FFTT | 34. TFTT | 53. TFTF |
| 16. TTTF | 35. TFFF | 54. FTFF |
| 17. TTFF | 36. FFTF | 55. TTTT |
| 18. FFFT | 37. TFFF | |
| 19. TTFF | 38. FTFF | |

8. Gametogenesis. Ontogenesis. Stages of intrauterine development.

Ontogeny (ontogenetic development) is the set of biological, morphological, and functional changes that occur in an organism throughout its life. In sexually reproducing organisms, including humans, ontogeny begins with the process of gametogenesis, continues with fertilization, embryonic and post-embryonic development of the individual, and ends with death. Ontogenetic development includes periods of growth, differentiation, maturation, reproduction, and subsequent aging of the organism.

Gametogenesis is a biological process during which sex cells – **gametes** are formed, and it occurs exclusively in the gonads. Meiosis itself also takes place here, consisting of two consecutive divisions, with **DNA replication occurring only once** (before the first meiotic division). During gametogenesis, the **chromosome number is reduced by half** (to the haploid level), ensuring that sexual reproduction maintains a constant amount of genetic information within a species. Gametogenesis is an important source of **genetic variability**, leading to the formation of new combinations of alleles and traits, which is determined mainly by two mechanisms accompanying meiosis. The first is the **recombination** of genetic material through **crossing-over** in prophase I of meiosis. The second is the **random segregation** of homologous chromosomes and chromatids during anaphase I and II of meiosis.

Meiosis

Meiosis is a specific type of division of sex cells in eukaryotic organisms, in which one diploid parent cell ($2n$) produces four haploid cells (n). Meiosis consists of two consecutive divisions, with DNA replication occurring only once (before the first meiotic division).

The **first meiotic division**, also called the reductional or heterotypic division, results in two daughter cells with a haploid number of chromosomes. During **prophase I** of meiosis, homologous chromosomes pair and exchange genetic information. Prophase I is divided into several stages. In the **preleptotene** stage, chromosomes begin to condense. During **leptotene**, condensation of the chromosomes continues, and the long, thin chromosomes remain attached to the nuclear membrane by their telomeres. In the **zygotene** stage, homologous chromosomes pair, the synaptonemal complex forms, and bivalents, consisting of four chromatids, are created. In **pachytene**, the bivalents condense further, and segments of DNA are exchanged between non-sister chromatids of homologous chromosomes in a process called **crossing-over**, occurring at the chiasmata. During **diplotene**, the synaptonemal complex disassembles, and homologous chromosomes begin to separate, but they remain connected at the chiasmata. In the final stage, **diakinesis**, the chromatids condense further, chiasmata move toward the ends of the chromosomes, the nucleolus and nuclear membrane disintegrate, and the spindle apparatus forms.

After the completion of prophase I of meiosis, metaphase I, anaphase I, and telophase I follow. After this, a short interphase occurs, which does not include an S phase, so no DNA replication takes place. The result of the first meiotic division is the segregation of homologous chromosomes, with their distribution being random. Cells are produced with only one set of chromosomes (n), but the chromosomes still consist of two chromatids.

The **second meiotic division** (also called the equational or homeotypic division) immediately follows the first meiotic division and proceeds similarly to mitotic division. Sister chromatids separate (segregate randomly) into the daughter cells - gametes.

The **result of meiosis** is a reduction in the chromosome number by half ($2n \rightarrow n$) and the creation of new genetic combinations through random segregation of parental chromosomes and crossing-over. The product of the first meiotic division is haploid (n) secondary spermatocytes and oocytes. These enter the second meiotic division, resulting in an oocyte or four spermatids, which during maturation (spermiogenesis) differentiate into mature sperm (developing a flagellum).

Meiosis disorders during gametogenesis

Disorders of meiosis during gametogenesis can lead to chromosomal aberrations in the offspring, which are in most cases severe and often incompatible with life, whereas normal gametogenesis ensures the production of healthy offspring and the continuity of the species.

The causes of faulty gametogenesis can be diverse. One cause is a **defect in the pairing of homologous chromosomes**, which can lead to the formation of univalents or partially asynaptic bivalents, potentially resulting in aneuploid gametes. Non-homologous or lateral pairing of univalents can trigger structural chromosomal aberrations. Another cause is **unequal crossing-over during prophase I of meiosis**, which leads to the exchange of unequal segments of homologous chromosomes, resulting in the duplication of a segment on one chromosome and the deletion of the same segment on the other. These structural chromosomal aberrations can have severe phenotypic consequences. Another cause is **nondisjunction** (failure to separate) **of chromosome pairs in anaphase I of meiosis**, which produces aneuploid gametes - two disomic (with an extra chromosome) and two nullisomic (lacking a chromosome) - or **nondisjunction of chromatids in anaphase II**, producing one disomic and one nullisomic gamete. Another type is **anaphase lag** during anaphase I or II, where a chromosome fails to reach the cell pole in time due to a kinetochore-microtubule defect and is excluded from the newly forming nucleus.

The development of sex cells can be generally divided into three phases. The first is the **proliferation phase**, which involves repeated mitotic divisions of gametogonia (spermatogonia or oogonia), increasing their number. The second is the **growth phase**, during which gametogonia increase in size. Diploid gametogonia produce cellular organelles and prepare for division. After this phase, primary gametocytes (primary spermatocytes or oocytes), also called first-order gametocytes, are formed; they are still diploid. The third is the **maturation phase**, which involves meiotic divisions that produce haploid sex cells. In the development of sperm, this phase also includes spermiogenesis.

Spermiogenesis

Spermiogenesis (the development of male sex cells—sperm) in humans occurs continuously from puberty until late adulthood. The process takes 64–72 days. During spermiogenesis, four equivalent sperm are produced from a single spermatogonium. The cells resulting from the second meiotic division are called spermatids, which, during spermiogenesis, transform into mature sperm without further division by forming a head, neck, connecting piece, and flagellum.

Oogenesis

Oogenesis (the development of female sex cells—oocytes) begins during the early stages of intrauterine development. All oocytes enter prophase I of meiosis before birth, where they

remain arrested in the diplotene stage until puberty. During puberty, individual oocytes are gradually activated in monthly cycles and complete the first meiotic division within the Graafian follicle. They then enter prophase and metaphase of the second meiotic division, during which ovulation occurs. The second meiotic division is completed only after fertilization. The first meiotic division produces two unequal cells—a secondary oocyte and a polar body (which may divide). The second meiotic division produces the ovum and another polar body.

Stages of intrauterine development

In mammals, intrauterine development occurs in four stages – the pre-implantation stage, blastogenesis, embryogenesis, and the fetal period. Each stage differs in the type of genetic programming and cell differentiation. After fertilization, a totipotent zygote is formed, which through mitotic divisions produces blastomeres, followed by the morula and the blastocyst. During embryogenesis, cells differentiate into the ectoderm, mesoderm, and endoderm based on regulated gene expression and the action of signaling molecules. The fetal period is primarily focused on growth and maturation of the fetus. Developmental regulation is evolutionarily conserved, and disruptions in regulatory genes can lead to severe developmental disorders (e.g., holoprosencephaly, polydactyly, syndactyly).

The main regulatory genes that control embryonic development and cell differentiation are **SHH (Sonic Hedgehog)**, **HOX genes**, and the **PAX gene family**. The **SHH protein** forms concentration gradients that determine cell proliferation and specification according to the local signal concentration. Disruptions in its expression can lead to severe developmental defects, such as holoprosencephaly, cleft lip, or cyclopia. **HOX genes** encode homeodomain transcription factors and regulate the anterior-posterior development of the embryo. Once activated, their proteins bind to DNA and control the expression of other genes, determining the sequential specification of individual structures. Mutations in HOX genes can cause limb malformations such as polydactyly, syndactyly, or synpolydactyly. **PAX family** genes have specific functions. **PAX1** controls the formation of embryonic segments along the cranio-caudal axis. **PAX2** promotes differentiation of kidney cells. **PAX3** is involved in neurogenesis, and its defects cause Waardenburg syndrome. **PAX5** regulates the development of B lymphocytes, the central nervous system, and spermiogenesis. **PAX6** is crucial for the development of the eyes and nose, and its mutations can lead to aniridia or partial absence of the iris.

In addition to the genes themselves, the diversity of proteins and differentiated cells is ensured by mechanisms of variable gene expression. **Alternative transcription start site** allow a single gene to generate different mRNAs with distinct N-termini, producing proteins of different lengths (e.g., dystrophin). **Alternative splicing** enables exons and introns to be cut in different ways, resulting in protein isoforms (e.g., the calcitonin gene produces different mRNAs in the thyroid and in nervous tissue). **mRNA editing** through enzymatic modification changes nucleotide sequences, which can create truncated proteins (e.g., the apoB gene produces apoB100 in the liver and apoB48 in the intestine). **Post-translational modifications** of proteins, such as glycosylation, phosphorylation, or proteolytic cleavage, allow further adaptation of proteins to the needs of specific cells. All these mechanisms enable a single gene to produce multiple proteins that contribute specifically to the function of cells and tissues, while mutations in these systems can lead to severe developmental disorders.

Table: Genes and regulatory proteins important in intrauterine development.

Gene	Main function	Mechanism of action / Effect on cells
<i>SHH</i>	Induction of differentiation, formation of concentration gradients	Signals bound to receptors (Patch) regulate cell proliferation and specification according to molecule concentration.
HOX gene	Encode homeodomain transcription factors, which control the anteroposterior development of the embryo	Once activated, proteins bind to DNA and regulate the expression of other genes; they create a sequence of structural specifications
<i>PAX1</i>	Formation of the segmental structure of the embryo	Transcription factor, regulates gene expression in the cranio-caudal axis
<i>PAX2</i>	Differentiation of kidney cells	Transcription factor, promotes kidney cell specification
<i>PAX3</i>	Development of the nervous system	Activates genes for neurogenesis
<i>PAX5</i>	B-lymphocyte differentiation, CNS development, and spermatogenesis	Regulates gene expression in immune and nerve cells
<i>PAX6</i>	Development of the eye and nose	Regulates gene expression for eye structures



Model questions

1. The first meiotic division is called:

- a. homeotypic
- b. diploid
- c. reductional
- d. equational

2. During the first maturation division:

- a. chromosome segregation occurs
- b. chromatids separate
- c. diploid cells are formed
- d. gametogonia are formed

3. The result of meiotic division is:

- a. cells with a diploid number of chromosomes
- b. primordial germ cells
- c. cells with a haploid number of chromosomes
- d. blastomeres

4. Development of male gametes:

- a. is called spermiogenesis
- b. is called oogenesis
- c. occurs in the convoluted seminiferous tubules of the testes
- d. occurs in the cortical layer of the ovary

5. Development of female gametes:

- a. is called spermiogenesis
- b. is called oogenesis
- c. occurs in the convoluted seminiferous tubules of the testes
- d. occurs in the cortical layer of the ovary

6. Gametogenesis proceeds in phases (in correct chronological order):

- a. proliferation phase, growth phase, maturation phase
- b. growth phase, proliferation phase, maturation phase
- c. maturation phase, proliferation phase, growth phase
- d. meiosis, proliferation phase, maturation phase

7. During the proliferation phase of gametogenesis:

- a. the number of diploid gametogonia increases
- b. the number of haploid gametogonia increases
- c. mitotic division of diploid gametogonia occurs
- d. mitotic division of haploid gametogonia occurs

8. During the proliferation phase of spermiogenesis:

- a. the number of diploid spermatogonia increases
- b. the number of haploid spermatogonia increases
- c. mitotic division of diploid spermatogonia occurs
- d. mitotic division of haploid spermatogonia occurs

9. During the proliferation phase of oogenesis:

- a. the number of diploid oogonia increases
- b. the number of haploid oogonia increases
- c. mitotic division of diploid oogonia occurs
- d. mitotic division of haploid oogonia occurs

10. Meiosis:

- a. consists of two consecutive divisions, between which the S phase occurs
- b. consists of two consecutive divisions, between which the S phase does not occur
- c. is identical to a single reductional division
- d. occurs in all tissues

11. In nondisjunction during the second meiotic division:

- a. four aberrant cells are formed from one primary gametocyte
- b. two aberrant cells are formed from one primary gametocyte
- c. no aberrant cells are formed
- d. half of the cells resulting from such meiosis are aneuploid

12. During anaphase lag in meiosis:

- a. one of the pair of homologous chromosomes fails to reach the centriole during anaphase
- b. one cell has 22 chromosomes, and the other has 23 chromosomes
- c. one cell has 45, and the other has 47 chromosomes
- d. nondisjunction occurs

13. A recessive X-linked disorder can manifest in a healthy woman if:

- a. it can never manifest because she has a dominant allele that makes her healthy
- b. it can manifest if most X chromosomes with the recessive allele are inactivated
- c. it can manifest if most X chromosomes with the dominant allele are inactivated
- d. it cannot manifest because the woman is a mosaic

14. How many blastomeres make up the human morula?

- a. 12
- b. 22
- c. 32
- d. 42

15. The significance of meiosis lies in ensuring:

- a. division of somatic cells
- b. genetic variability of offspring
- c. genetic identity of offspring
- d. and the formation of haploid gametes

16. Meiosis consists of:

- a. a single division
- b. reductional division
- c. heterotypic division
- d. meiosis I and meiosis II

17. The result of meiosis is:

- a. 4 prokaryotic cells
- b. 4 somatic cells
- c. 4 gametes
- d. 2 cells capable of further division

18. The genetic significance of meiosis lies in:

- a. the production of gametes
- b. reducing the chromosome number by half during gamete formation
- c. recombination, which increases genetic variability
- d. segregation and random distribution of chromosomes

19. In which stage of meiotic division are oocytes before birth?

- a. Prophase I
- b. Prophase II
- c. Metaphase I
- d. Metaphase II

20. When is the second meiotic division of oocytes completed?

- a. in the diplotene stage
- b. at ovulation
- c. after fertilization
- d. after completion of the first meiotic division

21. What cells are formed by the first meiotic division?

- a. 4 equivalent cells
- b. haploid cells
- c. gametes
- d. diploid cells

22. What is the result of the first meiotic division?

- a. segregation of homologous chromosomes
- b. random distribution of chromosomes
- c. formation of cells with only one set of chromosomes
- d. chromosomes are composed of two chromatids

23. What is the result of the second meiotic division?

- a. formation of 4 haploid gametes
- b. formation of 4 diploid gametes
- c. formation of 2 haploid gametes
- d. formation of 2 diploid gametes

24. What cells are produced by the second meiotic division in oogenesis?

- a. 2 equivalent cells
- b. secondary oocyte and polar body
- c. primary and secondary oocyte
- d. egg and second polar body

25. What can cause faulty gametogenesis?

- a. failure of homologous chromosome pairing
- b. unequal crossing-over in prophase I of meiosis
- c. nondisjunction
- d. unequal crossing-over in prophase II of meiosis

26. What can be caused by failure of homologous chromosome pairing?

- a. formation of duplications and deletions of chromosome segments
- b. formation of structural chromosomal aberrations
- c. formation of numerical chromosomal aberrations
- d. formation of faulty gametogenesis

27. What can nondisjunction in anaphase I cause?

- a. structural chromosomal aberrations
- b. formation of healthy offspring
- c. formation of disomic gametes
- d. formation of nullisomic gametes

28. What is anaphase lag?

- a. a chromosome fails to reach the pole of the cell
- b. a kinetochore microtubule defect
- c. increased speed of division
- d. a consequence of faulty gametogenesis

29. What is the effect of normal gametogenesis on offspring?

- a. production of healthy offspring
- b. unequal crossing-over
- c. ensures species continuity
- d. none

30. What can non-homologous pairing of univalents cause?

- a. numerical chromosomal aberrations
- b. structural chromosomal aberrations
- c. formation of normal offspring
- d. aneuploid gametes

31. When does spermiogenesis begin in humans?

- a. after birth
- b. from puberty
- c. after fertilization
- d. cyclically from puberty to old age

32. How many cells are produced from one spermatogonium during spermiogenesis?

- a. 4 haploid cells
- b. 4 diploid cells
- c. 4 equivalent sperm
- d. 2 equivalent sperm

33. The cells produced by the second meiotic division in spermiogenesis are called:

- a. spermatogonia
- b. spermatids
- c. secondary spermatocytes
- d. sperm

34. What happens to spermatids during spermiogenesis?

- a. they divide again
- b. they degenerate
- c. they differentiate
- d. they transform into sperm without further division

35. Regarding DNA replication during gametogenesis:

- a. it occurs only once
- b. it occurs multiple times
- c. it occurs only before the first meiotic division
- d. it occurs also before the second meiotic division

36. Which characteristics belong to the proliferation phase of gametogonia?

- a. repeated mitotic divisions
- b. increase in the number of gametogonia
- c. formation of haploid cells
- d. increase in the cell volume of gametogonia

37. Into how many main phases can gamete development be divided?

- a. 2
- b. 3
- c. 4
- d. 5

38. What happens in the growth phase of gametogonia?

- a. increase in cell size
- b. production of cell organelles
- c. preparation for division
- d. formation of haploid gametes

39. Primary gametocytes (first-order gametocytes) are:

- a. haploid
- b. diploid
- c. formed after the growth phase
- d. formed after the first meiotic division

40. What does spermiogenesis during spermiogenesis involve?

- a. transformation of spermatids into sperm
- b. mitotic division of spermatogonia
- c. increase in spermatogonia volume
- d. formation of the head, neck, and tail

41. Which processes characterize the gamete maturation phase?

- a. mitotic division
- b. meiotic division
- c. spermiogenesis
- d. increase in the number of gametogonia

42. Which cells persist in the diplotene stage until puberty?

- a. secondary oocytes
- b. primary oocytes
- c. spermatids
- d. sperm

43. Meiosis is a specific type of division in which:

- a. one diploid cell produces two haploid cells
- b. one diploid cell produces four haploid cells
- c. two successive divisions occur
- d. DNA replicates before each division

44. Before the first meiotic division:

- a. DNA replication occurs
- b. DNA replication does not occur
- c. the cell is haploid
- d. the cell is diploid

45. What happens during the first meiotic division?

- a. 2 daughter cells are formed
- b. the resulting daughter cells are haploid
- c. the resulting daughter cells are diploid
- d. segregation of homologous chromosomes occurs

46. Which processes occur in prophase I of meiosis?

- a. pairing of homologous chromosomes
- b. separation of sister chromatids
- c. crossing-over
- d. formation of the synaptonemal complex

47. Which stages belong to prophase I?

- a. proleptotene
- b. leptotene
- c. zygotene
- d. pachytene

48. What happens in the pachytene stage of prophase I?

- a. DNA is exchanged between non-sister chromatids
- b. chromosomes cross over at the site of the chiasma
- c. homologous chromosomes separate
- d. the synaptonemal complex is fully formed

49. What characterizes the diplotene stage of prophase I?

- a. completion of mitotic division
- b. breakdown of the synaptonemal complex
- c. beginning of separation of homologous chromosomes
- d. homologous chromosomes remain connected at chiasmata

50. What does diakinesis include?

- a. movement of chiasmata toward the ends of chromosomes
- b. segregation of homologous chromosomes
- c. condensation of chromatids
- d. disintegration of the nucleolus and nuclear membrane

51. After the first meiotic division:

- a. daughter cells are haploid
- b. daughter cells have chromosomes composed of two chromatids
- c. homologous chromosomes segregate
- d. DNA replication occurs

52. What is the result of meiosis?

- a. reduction of the chromosome number by half
- b. formation of new genetic combinations
- c. formation of identical copies of the parent cell
- d. formation of four diploid cells

53. Crossing-over occurs:

- a. in prophase I
- b. after the first meiotic division
- c. after the second meiotic division
- d. between non-sister chromatids

54. Why is prophase I important?

- a. reduction of the chromosome number occurs
- b. pairing of homologous chromosomes occurs
- c. the synaptonemal complex is formed
- d. crossing-over occurs

55. In the second meiotic division, the gametes produced are:

- a. genetically identical
- b. formed from oocytes and spermatocytes
- c. haploid
- d. diploid

56. Chromosome segregation in meiosis is:

- a. random
- b. ensures genetic variability
- c. always the same as in the parent cell
- d. ensured by crossing-over

57. What happens to spermatids during spermiogenesis:

- a. they differentiate into mature sperm
- b. the first meiotic division occurs
- c. the second meiotic division occurs
- d. diploid sperm are formed

58. Which are the main regulatory genes controlling embryonic development and cell differentiation?

- a. *SHH* (*Sonic Hedgehog*)
- b. HOX genes
- c. PAX genes
- d. *BRCA1*

59. What can a disorder of SHH (Sonic Hedgehog) expression lead to?

- a. polydactyly
- b. holoprosencephaly
- c. cyclopia
- d. cleft lip

60. What do mutations of HOX genes lead to?

- a. polydactyly
- b. cleft lip
- c. syndactyly
- d. holoprosencephaly

61. What does alternative splicing allow?

- a. enzymatic changes of mRNA nucleotides
- b. phosphorylation of proteins
- c. removal of exons and introns in different ways
- d. dephosphorylation of proteins

62. What do post-translational protein modifications include?

- a. proteolytic cleavage
- b. transcription of DNA
- c. glycosylation
- d. phosphorylation

63. mRNA editing:

- a. occurs at the DNA level
- b. changes the nucleotide sequence of mRNA by enzymatic action
- c. can lead to the formation of shortened proteins
- d. generally prevents translation

64. Alternative splicing:

- a. removes the entire gene from DNA
- b. allows exons and introns to be spliced in different ways
- c. occurs only in prokaryotes
- d. leads to the formation of protein isoforms

65. In which stage of prophase I are bivalents (tetrads) formed and the synaptonemal complex created?

- a. leptotene
- b. zygotene
- c. diplotene
- d. diakinesis

66. Which two mechanisms contribute most to genetic variability during meiosis?

- a. crossing-over in prophase I
- b. random segregation of homologous chromosomes/chromatids in anaphase I and II
- c. DNA replication before the second meiotic division
- d. mitotic division of gametogonia

67. What is typical for the short interphase between the first and second meiotic divisions?

- a. the S phase occurs
- b. DNA replicates for the second time
- c. the S phase does not occur
- d. bivalents are formed

68. Which cells are formed after the first meiotic division and enter the second meiotic division?

- a. primary spermatocytes and oocytes
- b. secondary spermatocytes and secondary oocytes
- c. spermatogonia and oogonia
- d. blastomeres and morula

69. Which two statements correctly describe oogenesis in humans?

- a. oocytes enter prophase I before birth
- b. the second meiotic division is completed only after fertilization
- c. oogenesis occurs continuously from puberty to old age
- d. four equivalent gametes always arise from one oogonium

70. What can be a consequence of unequal crossing-over in prophase I?

- a. duplication of a segment on one chromosome
- b. deletion of the same segment on the other chromosome
- c. formation of disomic and nullisomic gametes (as a direct consequence of crossing-over)
- d. formation of 4 genetically identical gametes

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. FFTF | 25. TTTF | 49. FTTT |
| 2. TFFF | 26. TTFT | 50. TFTT |
| 3. FFTF | 27. FFTT | 51. TTTF |
| 4. TFFF | 28. TTFF | 52. TTFF |
| 5. FFFT | 29. TFTF | 53. TFFT |
| 6. TFFF | 30. TFFT | 54. FTTT |
| 7. TFTF | 31. FTFF | 55. FTTF |
| 8. TFTF | 32. TFTF | 56. TTFF |
| 9. TFTF | 33. FTFF | 57. TFFF |
| 10. FTFF | 34. FFTT | 58. TTTF |
| 11. FTFT | 35. TFTF | 59. FTTT |
| 12. TTFF | 36. TTFF | 60. TFTF |
| 13. FFTF | 37. FTFF | 61. FFTF |
| 14. FFTF | 38. TTTF | 62. TFTT |
| 15. FTFT | 39. FTTF | 63. FTFF |
| 16. FTTT | 40. TFFT | 64. FTFT |
| 17. FFTF | 41. FTTF | 65. FTFF |
| 18. TTTT | 42. FTFF | 66. TTFF |
| 19. TFFF | 43. FTTF | 67. FFTF |
| 20. FFTF | 44. TFFT | 68. FTFF |
| 21. FTTF | 45. TTFT | 69. TTFF |
| 22. TTTT | 46. TFTT | 70. TTFF |
| 23. TFFF | 47. TTTT | |
| 24. FFFT | 48. TTFT | |

9. Regulation and deregulation of the cell cycle.

The cell cycle represents the life cycle of a cell, which begins with the formation of a daughter cell and ends with its division into two new cells.

Prokaryotic organisms, due to their simple structure, have a shortened cell cycle. The cell divides immediately after DNA replication through direct division - **amitotic division**. The next round of DNA replication can begin even before cytokinesis is fully completed. If differentiation occurs at the beginning of the cell cycle, a prokaryotic cell enters an anabiotic stage - a spore. The cell cycle resumes only when the spore encounters favorable conditions, germinates, and transforms into a vegetative form capable of division.

Eukaryotic organisms are more complex, and their cell cycle is regulated by an intricate system of control and regulatory mechanisms that must be balanced. Together, these mechanisms ensure monitoring and repair of all DNA errors - before, during, and after replication. The cell cycle is divided into multiple stages, each containing a checkpoint where the cycle can be halted for repair of damaged DNA. If the extent of mutations is too great, cell cycle arrest triggers cell death through apoptosis.

Cell cycle regulation

For cells and the entire organism to function properly, their division must be carefully controlled and regulated. A normal somatic cell will divide only after receiving the appropriate external stimulus - a mitogen (growth factor). The signal for progression through the cell cycle is transmitted within the cell via **signaling pathways**, which lead to targeted gene expression or influence the activity (activation/inhibition) of specific proteins. **Proto-oncogenes** and **tumor suppressor genes** regulate the cell cycle through regulatory proteins, and mutations in these genes can lead to cell cycle deregulation, uncontrolled cell division, and the development of cancer.

The proper progression of the cell cycle depends on balanced interactions of regulatory proteins, as their imbalance can lead to deregulation and an increased risk of malignant transformation. This balance is maintained through several mechanisms. One of them is **regulated gene expression**, which ensures the correct timing of the presence of specific proteins in the nucleus or cytoplasm. Another mechanism is the **activation and inactivation of proteins** that control the cell cycle. Proteins also undergo **enzymatic modifications** and the formation of enzymatic complexes. Additionally, cell cycle regulation is influenced by the binding of activators, such as **cyclins** and **cyclin-dependent kinases (CDKs)**.

Cyclins are proteins whose role is to activate CDKs. They are synthesized in the nucleus and are active only during specific phases of the cell cycle. The cyclins involved in cell cycle regulation include A, B, C, D, E, and H. **Cyclin-dependent kinases (CDKs)** are active only when bound to a cyclin and drive transitions between the different phases of the cell cycle. CDKs are constantly present in the cell, and in humans, the key CDKs for cell cycle regulation are CDK1, CDK2, CDK4, CDK6, and CDK7. Activation of a CDK can sometimes occur simply by binding to its cyclin, but in other cases it requires activating phosphorylation or the association with an additional regulatory component. Phosphatases remove phosphate groups from sites where kinases have added them (for example, cdc25, a phosphatase that also ensures the transition from G2 to M phase).

Cell cycle regulation also involves targeted protein degradation, including **ubiquitination**, which is a specific, directed process for eliminating unnecessary proteins via proteolytic enzymes.

Ubiquitin ligases (SCF and APC/C) play key roles in cell cycle regulation. **SCF** is responsible for degrading inhibitory molecules that slow down (p27) or block (p21) the transition from G1 to S phase. **APC/C** is part of the anaphase-promoting complex at the mitotic checkpoint of the cell cycle. **Inhibitors p27 and p21** are crucial regulators, as they prevent the activity of cyclin D–CDK4 and cyclin E–CDK2 complexes. This inhibition stops these complexes from phosphorylating Rb1, ensuring that cells progress correctly from G1 to S phase.

The products of **tumor suppressor genes act oppositely to proto-oncogenes**. They inhibit the cell cycle and promote cell differentiation (examples include p53 and Rb1). The **p53 protein**, called the “guardian of the genome,” is a specific transcription factor that first activates the expression of the gene encoding the inhibitory protein p21. p21 binds to the active site of cyclin–CDK complexes, blocking their activity when the cell contains damaged DNA or when chromosome telomeres are depleted. If the damage persists, p53 triggers transcription of the p73 (or p78) gene, leading to apoptosis.

Checkpoints in the cell cycle

The **cell cycle** is regulated by four main **checkpoints**, which ensure proper cell division and protect the genome. In the G1 phase, DNA damage is monitored, and a decision is made whether to enter the S phase. During the S phase, the checkpoint ensures accurate and one-time DNA replication. The G2 phase checkpoint allows entry into mitosis only after successful repair of replication errors. The metaphase checkpoint ensures correct attachment of chromosomes to the mitotic spindle and the even distribution of genetic information between daughter cells.

Table: Control nodes of the cell cycle.

Control node	Cycle phase	Main role	Key molecules	Consequence of failure
1. Control node (G1/S)	End of G1	DNA damage checkpoint, permission to enter the S phase	rb1, E2F, cyclins D/E/A, CDK4/2, c-myc, p21, p27	Cycle arrest or apoptosis
2. Control node (S)	S - phase	Checking correct and single-round DNA replication	cyclin A-cdk2, telomeres, telomerase, ORC, cdc6, cdt1, MCM	Telomere shortening, cell death, genomic instability
3. Control node (G2/M)	G2 -phase	Permission to enter mitosis after DNA repair	cdc25, cyclin B-cdk1 (MPF), ATR/ATM, Chk1/Chk2, p53, p21	Cessation of mitosis or apoptosis
4. Control node (Metaphase)	Metaphase of mitosis	Uniform distribution of chromosomes	SAC, APC/C, cdc20, Mad2, separase, cohesins	Nondisjunction, chromosome loss, aneuploidy

The **G1/S checkpoint** (the main checkpoint) monitors DNA damage and the availability of growth signals, allowing entry into the S phase only when Rb1 is inactivated by phosphorylation and the family of specific transcription factors E2F can initiate the synthesis

of replication proteins. If irreparable DNA damage is detected, the cell cycle is halted, and the cell undergoes apoptosis.

The **S-phase checkpoint** ensures that DNA is replicated exactly once and accurately, through monitoring telomeres and replication origins (ORIs). It verifies that DNA replication has occurred without errors and repairs any mistakes.

The **G2/M checkpoint** allows entry into mitosis only after complete inspection and repair of replication errors, through activation of the cyclin B–CDK1 complex (MPF) by the cdc25 phosphatase, which removes inhibitory phosphates from CDK1.

The **metaphase checkpoint** ensures the correct attachment of all chromosomes to the mitotic spindle and only then permits the start of anaphase by activating APC/C and enabling the separation of sister chromatids.

Proto-oncogenes and tumor suppressor genes and their role in cell cycle regulation and deregulation

The regulation of the **cell cycle** involves two main groups of genes: **proto-oncogenes** and **tumor suppressor genes**. Products of proto-oncogenes encode **regulatory proteins** that push the cell into the next phase of the cycle, promote **proliferation**, and simultaneously inhibit **cellular differentiation**. Key examples include **cyclins** and **cyclin-dependent kinases (CDKs)**. In contrast, products of tumor suppressor genes act oppositely—they **slow down or halt the cell cycle**, promote **differentiation**, and monitor the **integrity of genetic information**. Typical representatives are **p53** and **Rb1**.

The functions of proto-oncogenes and tumor suppressor genes are relatively **antagonistic**, yet during the cell cycle, they interact closely. Their products influence each other and maintain a **balance between proliferation and DNA integrity control**. Mutations or deregulation of these genes lead to **loss of cell cycle control**, **uncontrolled cell division**, and can cause **malignant transformation**, i.e., the development of **cancer**.

Examples of **tumor suppressor genes** include *RBI*, *TP53*, *WT1*, *NF1*, *APC*, *DCC*, *BRCA1*, *BRCA2*, *MLH1*. Examples of **proto-oncogenes** (oncogenes) include *c-myc*, *K-ras*, and *BCR–ABL*.

Cell cycle control is carried out at each phase through **checkpoints**, which assess DNA status and decide whether the cell can proceed to the next phase, must repair damage, halt the cycle, or enter apoptosis. Proper cell cycle regulation, therefore, requires a balance between the stimulatory effects of proto-oncogenes and the inhibitory actions of tumor suppressors.

The **conversion of a proto-oncogene into an oncogene** is a key step in the initiation of tumorigenesis. It leads to **cell cycle deregulation** and, together with the loss of tumor suppressor genes, forms the basis of multistep carcinogenesis. This can occur via mutation (e.g., formation of the BCR–ABL fusion gene) or overexpression (regulatory overexpression or amplification), resulting in protein overproduction and further cell cycle deregulation.

Table: Key genes regulating the cell cycle and their function.

Gene / protein	Brief function
<i>RBI</i> / <i>rb1</i>	Repressor E2F, prevents transition G1 → S
<i>TP53</i> / p53	The "guardian of the genome" halts the cycle, induces p21 formation and apoptosis in cases of irreparable DNA damage
<i>WT1</i>	Tumor suppressor regulating gene transcription, crucial in kidney and genital development
<i>NF1</i>	Tumor suppressor inactivating Ras G-proteins and controlling cell division signaling
<i>APC</i> / FAP	Tumor suppressor regulating intestinal epithelial proliferation and intercellular junction formation
<i>DCC</i>	Tumor suppressor responsible for adhesive cell connections
<i>BRCA1</i>	Controls the G2 phase, repairs DNA, and slows down the proliferation of breast and ovarian epithelia
<i>BRCA2</i>	Works with BRCA1 to repair double-strand DNA breaks
<i>MLH1</i>	Mutator gene involved in DNA repairs
<i>PDGF</i>	Extracellular growth factor stimulating the proliferation of connective tissue, smooth muscle, and neuroglia
<i>EGF</i>	Stimulates the proliferation of epithelial cells and neuroglia via EGFR
<i>IGF-I</i>	It promotes the proliferation of various types of cells and is produced mainly in the liver
<i>EGFR</i> / HER1	Tyrosine kinase receptor for EGF and TGF α ; activation triggers signaling to the nucleus
<i>Ras</i> / K-Ras	G-protein mediating signals from receptors to the nucleus
<i>c-myc</i>	Transcription factor activating genes for G1 → S transition
<i>bcr-abl</i> / <i>abl</i>	Constantly active kinase arising from translocation of chromosomes 9 and 22



Model questions

1. Cell cycle:

- a. in prokaryotes, it is not regulated at all
- b. its regulation is not necessary
- c. checkpoints play an important role
- d. transition between individual phases is ensured by cyclin-dependent kinases

2. Ubiquitination is defined as:

- a. a sexual type of cell division
- b. targeted destruction of the cytoplasmic membrane
- c. targeted degradation of unnecessary proteins
- d. degradation of mitochondria

3. The first checkpoint:

- a. allows the cell to pass from the G1 phase to the S phase
- b. persists until the G2 phase
- c. allows the initiation of mitosis
- d. is also called the main checkpoint

4. Cyclin D:

- a. its synthesis is ensured by c-myc
- b. together with Cdk4 phosphorylates the Rb1 protein
- c. increases degradation of the p27 protein
- d. is part of the signaling pathway that allows the cell to enter the S phase

5. The fourth checkpoint:

- a. is present in prophase
- b. ensures the identity of viral genomes
- c. plays an important role in anaphase
- d. ensures the identity of the genomes of daughter cells

6. Cyclin-dependent kinases:

- a. are constantly present in the cell
- b. have no significance in ensuring the transition between the phases of the cell cycle
- c. Cdk2 and Cdk4 play an important role in the cell cycle
- d. their activation requires binding with a cyclin

7. The main mechanisms controlling the cell cycle are:

- a. spontaneous gene expression
- b. targeted degradation of proteins
- c. activation and inactivation of specific proteins
- d. binding of immunoglobulins

8. What are the genes that control the progression of the cell through the cell cycle?

- a. *p73* gene
- b. tumor suppressor genes
- c. proto-oncogenes
- d. checkpoint genes

9. Tumor suppressor genes:

- a. ensure control of the cell cycle
- b. loss of their function can lead to malignant transformation of the cell
- c. include the *RB1* gene
- d. are also known as proliferative genes

10. From which two basic phases does the cell cycle consist?

- a. metaphase and prophase
- b. S phase and M phase
- c. interphase and M phase
- d. interphase and G phase

11. Mutation of which gene causes retinoblastoma?

- a. mutation of a proto-oncogene
- b. mutation of the *RB1* gene
- c. mutation of a tumor suppressor gene
- d. mutation of the *p73* gene

12. What does LOH indicate?

- a. loss of homozygosity
- b. loss of hemizyosity
- c. loss of heterozygosity
- d. a condition that can lead, for example, to retinoblastoma

13. *BRCA1* gene:

- a. together with *BRCA2* belongs to tumor suppressor genes
- b. belongs to the group of proto-oncogenes
- c. its mutations cause genetically predisposed breast cancer
- d. its mutation does not cause a serious diagnosis

14. Conversion of a proto-oncogene to an oncogene:

- a. is a basic mechanism of cell cycle regulation
- b. can occur through overexpression of the proto-oncogene
- c. its conversion (activation) can lead to cell cycle deregulation
- d. underlies the development of malignancies

15. What are enzymes that remove phosphate groups called?

- a. phosphorylases
- b. kinases
- c. amylases
- d. phosphatases

16. Which tumor suppressor gene is called the “guardian of the genome”?

- a. *RB1*
- b. *WT1*
- c. *TP53*
- d. *APC*

17. DNA polymerase III:

- a. has a main checkpoint function in interphase
- b. participates only in DNA replication
- c. plays a crucial role during the S phase of the cell cycle
- d. can replicate DNA and also detect replication errors

18. What are enzymes that add phosphate groups called?

- a. kinases
- b. kinetases
- c. acutases
- d. phosphatases

19. By which mechanisms are proteins regulating the cell cycle activated?

- a. dephosphorylation
- b. phosphorylation
- c. mutation
- d. assembly of enzyme subunits

20. What is the “device” used for degradation of unnecessary proteins called?

- a. cyclin-dependent complex
- b. apoptotic body
- c. proteasome
- d. lipoma

21. Which statements about cell cycle regulation are true?

- a. a normal somatic cell starts dividing without the need for an external signal
- b. mitogens are growth factors that trigger cell division
- c. cells divide only if a growth factor is translated in the nucleus
- d. malignant cells divide independently of mitogens

22. Which statements about enzymes regulating phosphorylation are correct?

- a. kinases transfer phosphate from ATP or GTP to amino acids with –OH groups
- b. phosphatases like cdc25 remove phosphate groups and can activate Cdks
- c. phosphatases activate signaling pathways by transferring phosphate to DNA
- d. phosphorylation of Cdks is always inhibitory and blocks cell cycle progression

23. Which statements about the activity of the cdc20-APC/C complex are correct?

- a. it is activated only after all microtubules are attached to all kinetochores
- b. it is inhibited by proteins such as Mad2 and RASSF1A during prometaphase
- c. it is responsible for phosphorylation of kinetochore proteins
- d. it is activated when each chromatid is attached to two microtubules from one side

24. Which statements about proto-oncogenes are correct?

- a. under normal conditions, they regulate cell proliferation and differentiation
- b. they always inhibit cell division
- c. after mutation or overexpression, they can lead to malignant transformation of the cell
- d. they encode only nonfunctional or harmful proteins

25. Which statements about growth factors are correct?

- a. they are ligands for receptors with kinase activity in intracellular domains
- b. they act exclusively through endocrine regulation
- c. they can be specific for certain cell types or universal
- d. growth factors bind exclusively to the nuclei of target cells

26. How does activation and signal transduction via EGFR occur?

- a. ligand binding converts the receptor into an active dimeric form
- b. EGFR activation leads to autophosphorylation of tyrosines inside the cell
- c. ligand binding inhibits the receptor's protein kinase activity
- d. phosphorylated EGFR domains serve as binding sites for other signaling proteins

27. Which statements about K-ras and its role in cell signaling are correct?

- a. K-ras is a G-protein activated by exchanging GDP for GTP
- b. activated K-ras triggers a signaling pathway via the serine-threonine kinase Raf
- c. K-ras mutations increase its GTPase activity, accelerating inactivation
- d. accessory proteins SOS and GBR2 inhibit K-ras activation

28. The development of cancer, i.e., carcinogenesis, is:

- a. transformation of normal cells into tumor cells
- b. a multi-step process
- c. only hereditary
- d. only sporadic

29. Carcinogenesis:

- a. is a process by which cancer cells turn into normal cells
- b. is also called oncogenesis
- c. can start with a mutation in a proto-oncogene
- d. can start with activation of tumor suppressor genes

30. Which of the following are examples of activated oncogenes?

- a. *C-MYC*
- b. *RBI*
- c. *TP53*
- d. *BCR-ABL*

31. Which of the following are examples of tumor-suppressor genes?

- a. *C-MYC*
- b. *RBI*
- c. *TP53*
- d. *BCR-ABL*

32. Regulators of cell division include:

- a. stimulators
- b. inhibitors
- c. hormones
- d. chemical substances

33. Uncoordinated cell division leads to:

- a. tumors
- b. tissue cultures
- c. cancer
- d. nuclear cells

34. The main checkpoint of the cell cycle is at the end of:

- a. G1 phase
- b. S phase
- c. G2 phase
- d. interphase

35. Which groups of genes are involved in cell cycle regulation?

- a. mitochondrial genes
- b. tumor suppressor genes
- c. proto-oncogenes
- d. ribosomal genes

36. Why is the balance between proto-oncogenes and tumor suppressor genes essential for the cell cycle?

- a. to ensure continuous activation of all cyclins
- b. to maintain proper control of the cell cycle and prevent uncontrolled cell division
- c. to allow DNA to replicate multiple times before mitosis
- d. to prevent the formation of mitochondria

37. What is the main function of proto-oncogenes?

- a. affect cell differentiation
- b. push the cell into the next phase of the cell cycle
- c. stop the cell cycle
- d. inhibit cell differentiation

38. Which of the following are products of proto-oncogenes?

- a. cyclins
- b. cyclin-dependent kinases
- c. p53
- d. RB1

39. What is the function of tumor suppressor genes and their encoded proteins?

- a. slow down or stop the cell cycle
- b. control the integrity of genetic information
- c. promote cell differentiation
- d. stimulate uncontrolled cell division

40. Which genes belong to tumor suppressor genes?

- a. *TP53*
- b. *c-myc*
- c. *RBI*
- d. *K-ras*

41. What is the relationship between proto-oncogenes and tumor suppressor genes?

- a. they act completely independently
- b. they have antagonistic functions
- c. their products interact with each other
- d. they maintain a balance between proliferation and DNA control

42. What can be a consequence of mutations in proto-oncogenes or tumor suppressor genes?

- a. loss of cell cycle control
- b. increased apoptosis without pathological consequences
- c. malignant transformation of cells
- d. uncontrolled cell division

43. Which of the following genes are tumor suppressor genes?

- a. *BRCA1*
- b. *BRCA2*
- c. *APC*
- d. *c-myc*

44. Which genes are proto-oncogenes (oncogenes)?

- a. *BRCA1*
- b. *BRCA2*
- c. *K-ras*
- d. *c-myc*

45. What is checked in the G1 phase of the cell cycle?

- a. DNA damage
- b. attachment of chromosomes to the mitotic spindle
- c. decision to enter the S phase
- d. separation of sister chromatids

46. What is the main role of the checkpoint in S phase?

- a. ensures one-time DNA replication
- b. control of replication origins (ORI)
- c. control of telomeres
- d. control of damaged DNA

47. When does the G2 checkpoint allow entry into mitosis?

- a. immediately after DNA replication
- b. after successful repair of post-replication errors
- c. after activation of APC/C
- d. after chromosome separation

48. G1/S checkpoint:

- a. ensures separation of chromatids
- b. is the main checkpoint of the cell cycle
- c. monitors DNA damage
- d. monitors availability of growth signals

49. What happens if DNA damage is detected at the G1/S checkpoint?

- a. the cell enters mitosis
- b. DNA replicates with errors
- c. the cell cycle is halted
- d. the cell may enter apoptosis

50. The metaphase checkpoint:

- a. monitors attachment of chromosomes to the mitotic spindle
- b. ensures even distribution of genetic information
- c. allows the start of anaphase by separating sister chromatids and activating APC/C
- d. monitors DNA damage after replication

51. What does activation of APC/C lead to?

- a. allows entry of the cell into S phase
- b. ensures separation of sister chromatids
- c. permits transition from metaphase to anaphase
- d. inhibits cyclin B

52. Cell cycle control occurs:

- a. assesses the state of DNA
- b. decide on ribosome synthesis
- c. can halt the cell cycle
- d. are active only in tumor cells

53. Checkpoints:

- a. assesses the state of DNA
- b. decide on ribosome synthesis
- c. can halt the cell cycle
- d. are active only in tumor cells

54. If DNA is damaged, the cell can:

- a. continues unchecked into the next phase
- b. repair the damage
- c. halt the cell cycle
- d. enters apoptosis

55. What is most important for proper cell cycle regulation?

- a. activation of only growth factors
- b. predominance of proto-oncogenes
- c. absence of tumor suppressor genes
- d. balance between proto-oncogenes and tumor suppressor genes

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. FFTT | 20. FFTF | 39. TTTF |
| 2. FFTF | 21. FTFT | 40. TFTF |
| 3. TFFT | 22. TTFF | 41. FTTT |
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| 6. TFFT | 25. TFTF | 44. FFFT |
| 7. FTTF | 26. TTFT | 45. TFTF |
| 8. FFTF | 27. TTFF | 46. TTTF |
| 9. TTTF | 28. TTFF | 47. FTFF |
| 10. FFTF | 29. FTTF | 48. FTTT |
| 11. FTTF | 30. TFFT | 49. FFFT |
| 12. FFFT | 31. FTTF | 50. TTTF |
| 13. TFTF | 32. TTTT | 51. FTTF |
| 14. FTTT | 33. TFTF | 52. TFTF |
| 15. FFFT | 34. TFFF | 53. TFTF |
| 16. FFTF | 35. FTTF | 54. FTTT |
| 17. FFFT | 36. FTFF | 55. FFFT |
| 18. TFFF | 37. TTFT | |
| 19. TTFT | 38. TTFF | |

10. Mutagenesis and Repair Mechanisms.

Mutagenesis

Mutations are sudden changes in genetic material (DNA, or RNA in RNA viruses) that alter the nucleotide sequence. They always represent a change in the genotype, but they do not necessarily manifest in the phenotype. Whether a mutation is replicated and expressed in the phenotype depends on the site of its origin, the type of mutation, and its consequences.

The formation of mutations is associated with the action of **mutagens**, which may be physical, chemical, or biological factors. **Physical mutagens** include various types of radiation whose biological effects depend on the mechanism of action, intensity, absorbed dose, and duration of exposure. Ionizing radiation, such as X-rays or gamma radiation, causes DNA ionization, the formation of free radicals, strand breaks, and chromosomal rearrangements. Ultraviolet radiation induces the formation of thymine dimers, which interfere with DNA replication and transcription. **Chemical mutagens** have diverse mechanisms of action. They may directly damage DNA (modify the DNA structure), act as analogs of nitrogenous bases, or reduce the activity of repair enzymes. **Biological mutagens** primarily include mobile genetic elements and retroviruses, which can integrate randomly into genes and cause insertional mutations.

Depending on where mutations occur and what effect they have on the cell and on the entire organism or its offspring, several types of mutations can be distinguished (see Table).

Table: Distribution of mutations

Category	Type of mutation	Characteristics
According to the type of cells	Germline/gametic	Affect germ cells; are heritable; arise during spermatogenesis or oogenesis
	Somatic	Affect somatic cells; are not heritable; may cause cancer
According to origin	Spontaneous	Random phenomenon
	Induced	Induced (intentionally caused, e.g., in a laboratory)
According to the extent of the genetic information affected by the mutation	Gene	Affect the nucleotide sequence of a single gene
	Chromosomal	Affect the structure of chromosomes or their parts
	Genomic	Change in the number of chromosomes or entire chromosome sets
According to localization	Nucleic	In nuclear DNA
	Extranucleic	In mitochondrial or chloroplast DNA
According to the direction of mutation	Direct	A normal allele is changed into a mutated allele
	Reverse (back) mutation	A mutated allele reverts to the original normal allele
According to viability	Vital	Do not threaten the survival of the individual
	Lethal	Mutations in essential genes; incompatible with survival
According to the degree of phenotypic expression	Dominant	Mutated allele is dominant over the normal allele
	Recessive	Mutated allele is expressed only in the homozygous recessive state

Gene mutations are small changes in DNA affecting only a few nucleotides, often just a single nucleotide. They are also called **point mutations** and are classified according to their effect on DNA, amino acids, the reading frame, or protein function (deletions, insertions, substitutions, inversions, and frameshift mutations) (see Table). The effect of such a mutation on a gene can vary – the mutated gene may retain full function, or a single nucleotide change can completely inactivate the encoded protein, leading to a **genetic disease** and its manifestation in the offspring.

Table. Classification of Gene/Point Mutations

Mutation	Effect	Example
Deletion	Loss of base(s), shift in the reading frame	GATC → GTC
Addition (insertion)	Insertion of base(s), reading frame shift, duplication	GATC → GGATC; trinucleotide expansion CAG CAG → CAG CAG CAG
Substitution	Substitution of one base for another	GATC → CATC
– no change in meaning (silent)	Amino acid remains unchanged	UAU → UAC (Tyr → Tyr)
– change in meaning (missense)	Amino acid is changed	UAU → UCC (Tyr → Ser)
– nonsense	Formation of a stop codon	UAU → UAA (Tyr → STOP)
– mutations at the (splice site)	Incorrect intron splicing	ATP7A mutation → Menkes disease
Inversion	Reversal of the order of bases by 180°	GATC → GTAC
Frameshift mutácia	Shift in reading (1–2 bases)	ΔF508, nt317(insA)

To elucidate a gene's function, knowing only its sequence or protein structure is insufficient, because many proteins function only within complexes or participate in multiple processes.

Targeted mutagenesis is used to create gene mutants that inactivate a specific protein or part of it, allowing researchers to study the role of that particular protein. **Recombinant techniques** enable modification of a gene's coding or regulatory sequence, production of partially functional or nonfunctional proteins, and observation of their effects in the organism. Organisms with modified genes are called **transgenic** and are used to study protein structure, interactions, and enzymatic activity; similar approaches are applied in **gene therapy**.

The goal of **mutagen testing** is to identify environmental factors that can damage DNA and pose risks to humans or ecosystems. An organism carrying a mutation is called a **mutant**, while the standard reference is the **wild type**. A direct mutation converts a wild type into a mutant type, whereas the reverse leads to a back mutation.

Mutagen testing uses experimental systems with bacteria, yeast, *Drosophila*, mammals, or human cells and monitors gene, chromosome, and genome damage. Tests must be sensitive, accurate, and reproducible, allowing risk assessment for humans. They are classified as mandatory (gene and chromosome mutations, DNA repair), confirmatory, or supplementary (dominant lethal test, sperm abnormalities, recombination). Each test involves different doses of the substance, along with negative and positive controls to properly evaluate the effect.

DNA Repair Mechanisms

DNA damage arises from both external and internal factors. Every cell repairs thousands of errors daily through specialized repair pathways. Accurate DNA replication and effective repair are essential for life, mediated by repair enzymes present in all cells, often activated only after DNA damage occurs. Failure of these mechanisms leads to impaired cell function, aging, cancer, apoptosis, and genetic instability.

Repair during DNA replication occurs through several mechanisms. DNA polymerase itself greatly reduces mutation rates due to its high fidelity and **proofreading activity**, correcting mispaired bases. Errors that escape this proofreading are immediately corrected after replication by the **mismatch repair (MMR) system**. For more severe damage, such as DNA strand breaks, **recombinational repair** restores missing segments either through **homologous recombination** (precise repair using the second DNA copy) or **non-homologous recombination** (joining broken DNA ends without a template, which may lead to DNA loss or changes).

Repair outside DNA replication focuses on correcting mispaired bases in already replicated or mutated DNA. Extensive damage often triggers **apoptosis** rather than risking malignant transformation. Repair occurs only if genes controlling repair are not affected and the damage does not exceed ~1,000 base pairs. Smaller lesions are repaired before replication, while larger ones are repaired afterward. DNA repair outside replication occurs through **direct repair**, **excision repair**, or **SOS repair**.

DNA is repaired by various mechanisms depending on the type and extent of damage. **Direct repair – photoreactivation** uses light to cleave thymine dimers formed by UV radiation, with photolyase as the key enzyme. In the case of damage by alkylating agents, O6-methylguanine-DNA methyltransferase repairs O6-guanine and O4-thymine bases and becomes irreversibly inactivated after transferring the alkyl group. Another type is **excision repair**, in which a small damaged segment of DNA is removed provided the complementary strand is intact; the process involves excision, separation, and filling in new nucleotides. **Base excision repair (BER)** removes individual damaged bases, for example during oxidative damage or deamination, after which DNA polymerase fills in the correct nucleotide and DNA ligase seals the strand.

Nucleotide excision repair (NER) removes larger damaged segments of DNA, often pyrimidine dimers caused by environmental factors; DNA polymerase fills in the missing nucleotides, and the process ends with joining the DNA strands via ligase. Defects in nucleotide excision repair lead to xeroderma pigmentosum. In cases of extensive DNA damage in bacteria, **SOS repair** is initiated, during which the cell cycle is halted and SOS genes are activated, with enzymes and proteins synthesized in a coordinated manner for emergency repair. The process works such that LexA blocks SOS genes in undamaged cells, while RecA, upon DNA damage, binds to single-stranded regions, inactivates LexA, and triggers SOS gene activation.

Chromosomal instability syndromes result from mutations in genes encoding repair enzymes, including xeroderma pigmentosum, Fanconi anemia, Bloom syndrome, ataxia-telangiectasia, and Cockayne syndrome. These are autosomal recessive and very rare. Affected individuals are extremely sensitive to mutagens. Accumulation of mutations in their DNA increases the risk of malignant transformation and cancer, and no effective therapy currently exists.



Model questions

1. Spontaneous mutations:

- a. their occurrence frequency is low
- b. are an important part of evolution
- c. their occurrence frequency is high
- d. are the cause of many pathological conditions

2. About spontaneous mutations, it is true that:

- a. we do not know the cause of their occurrence
- b. they are rare
- c. their frequency is 10^{-5} to 10^{-10} per gene per generation
- d. their frequency is 10^{-10} to 10^{-20} per gene per generation

3. Mutagens are:

- a. chemical
- b. technical
- c. physical
- d. biological

4. Induced mutations:

- a. we know the cause of their occurrence
- b. we do not know the cause of their occurrence
- c. we deliberately induce them in model organisms
- d. occur randomly without human intervention

5. Complete or partial repair of damaged DNA by enzymes is carried out by:

- a. recombination techniques
- b. restriction enzymes
- c. repair mechanisms
- d. DNA cloning

6. According to the type of affected cell, mutations are divided into:

- a. induced
- b. germline
- c. somatic
- d. spontaneous

7. Chemical mutagens include:

- a. substances that reduce the activity of repair enzymes
- b. substances that increase the activity of repair enzymes
- c. analogs of nitrogenous bases
- d. substances that damage DNA

8. Consequences of somatic mutations in an adult individual may be:

- a. apoptosis of affected cells
- b. cell death
- c. malignant transformation of the cell
- d. congenital developmental defect

9. According to the level of organization of genetic information they affect, mutations are divided into:

- a. genomic
- b. gametic
- c. gene
- d. chromosomal

10. Point mutations include:

- a. inversion
- b. duplication
- c. insertion
- d. substitution

11. Chemical mutagens include:

- a. nitrous acid
- b. acridine dyes
- c. 2-aminopurine
- d. cisplatin

12. Define mutation:

- a. sudden change in genetic information
- b. change in nucleotide sequence, which may consist of deletion, substitution, inversion
- c. specifically predicted change in genetic information
- d. sudden change in the expression of a polygenic trait in older people

13. What is a transition?

- a. purine replaced by purine
- b. pyrimidine replaced by purine
- c. pyrimidine replaced by pyrimidine
- d. purine replaced by pyrimidine

14. What is the function of repair mechanisms?

- a. prophylaxis of damaged DNA
- b. facultative repair of damaged DNA
- c. enzymatic repair of damaged DNA
- d. complete or partial enzymatic repair of damaged DNA (mutations)

15. What are spontaneous mutations?

- a. mutations only in mtDNA
- b. mutations with unknown cause but known frequency
- c. mutations with known cause but unknown frequency
- d. mutations only in rRNA

16. Main types of DNA repair processes are:

- a. base excision repair
- b. photoreactivation
- c. mismatch repair
- d. nucleotide excision repair

17. The most important enzyme in photoreactivation is:

- a. DNA photokinase
- b. photophylase
- c. photolyase
- d. photodeactivase

18. Photoreactivation is:

- a. error-free repair
- b. faulty repair
- c. repair of thymine dimers
- d. repair of misincorporated bases

19. Mutations of one or more nucleotides are called:

- a. chromatin mutations
- b. point mutations
- c. gene mutations
- d. chromosomal mutations

20. What is the role of ligase in DNA repair?

- a. joining DNA strands
- b. forming phosphodiester bonds between sugars (pentoses)
- c. forming phosphotriester bonds between sugars (pentoses)
- d. separating DNA strands

21. Define mutagen:

- a. factor inducing mutagenesis (mutation)
- b. mutated gene
- c. only an external factor inducing mutagenesis (mutation)
- d. only an internal factor inducing mutagenesis (mutation)

22. Which repair mechanism fixes thymine dimers?

- a. homologous recombination
- b. nucleotide excision repair
- c. photoreactivation
- d. none of the above

23. Physical mutagens are:

- a. ethanol
- b. UV radiation
- c. ionizing radiation
- d. nicotine

24. What is the difference between a mutation and DNA polymorphism?

- a. mutation is present in <1% of the population, polymorphism in >1%
- b. mutation is present in >1% of the population, polymorphism in <1%
- c. polymorphism is present in <1% of the population, mutation in >1%
- d. polymorphism is present in >1% of the population, mutation in <1%

25. Which repair mechanism fixes DNA with a larger number of errors (error-proof)?

- a. translesion DNA synthesis
- b. SOS repair
- c. PPT mechanisms
- d. none of the above

26. Main repair mechanisms include:

- a. SOS repair
- b. base excision repair
- c. photoreactivation
- d. heterologous recombination

27. Which property of the genetic code causes some point mutations to be silent?

- a. universality of the genetic code
- b. degeneracy of the genetic code
- c. redundancy of the genetic code
- d. overlapping sequences

28. Which mutations cause a reading frame shift (frame-shift mutations)?

- a. deletions
- b. chromosomal translocations
- c. insertions
- d. duplications

29. Which repair mechanism fixes uracil incorporated in DNA?

- a. photoreactivation
- b. SOS repair
- c. nucleotide excision repair
- d. base excision repair

30. Which mutations are called nonsense mutations?

- a. mutations where base substitution changes a codon for an amino acid to a stop codon
- b. mutations where base substitution changes a codon for an amino acid to a start codon
- c. mutations that do not change the coded protein
- d. mutations causing a frameshift that alters all codons

31. Which mechanism repairs mispaired bases during DNA replication?

- a. photoreactivation
- b. SOS
- c. mismatch repair
- d. all of the above

32. What do we call mutations occurring in somatic cells?

- a. autosomal mutations
- b. somatic mutations
- c. body mutations
- d. gametic mutations

33. What is the principle of recombinational DNA repair?

- a. repair using the correct complementary strand
- b. replication
- c. Holliday-junction repair
- d. D-loop

34. What do we call mutations affecting sperm or eggs?

- a. gametic mutations
- b. non-disjunction mutations
- c. somatic mutations
- d. gametophyte mutations

35. According to the extent of genetic material affected, mutations are divided into:

- a. genomic mutations
- b. gene mutations
- c. spontaneous and induced mutations
- d. chromosomal mutations

36. Which mutations are called silent – do not change meaning?

- a. mutation resulting in incorporation of a different amino acid into the protein
- b. mutation causing a premature stop codon in the protein sequence
- c. mutation that does not change the amino acid sequence of the protein
- d. mutation causing a frameshift that changes the protein sequence

37. Which mechanism repairs DNA damage caused by UV light?

- a. base excision repair
- b. nucleotide excision repair
- c. photoreactivation
- d. mismatch repair

38. Which mutations are called missense – changing meaning?

- a. mutation resulting in incorporation of a different amino acid into the protein
- b. mutation causing a premature stop codon in the protein sequence
- c. mutation that does not change the amino acid sequence of the protein
- d. mutation causing a frameshift, changing the protein sequence

39. Diseases caused by trinucleotide repeat expansions are:

- a. ataxia telangiectasia
- b. FRAXA
- c. Huntington's disease
- d. spinocerebellar ataxia

40. Which enzymes are involved in nucleotide excision repair?

- a. helicase
- b. ligase
- c. endonuclease
- d. protease

41. Frameshift mutations most frequently arise as a result of:

- a. inversion
- b. duplication
- c. insertion
- d. deletion

42. Mandatory mutagen detection tests are divided into:

- a. *in vitro* and *in vivo* tests
- b. chemical and physical
- c. biochemical and genetic
- d. detection of damage and RNA repair

43. Bloom syndrome is caused by a mutation in the gene for:

- a. DNA polymerase
- b. RNA polymerase
- c. DNA helicase
- d. DNA ligase

44. Ataxia telangiectasia is caused by a mutation in the gene:

- a. *ATM*
- b. *ATR*
- c. *BRCA1*
- d. for ligase

45. Somatic mutations:

- a. occur in gametes
- b. are transmitted to all cells of the affected individual
- c. occur in somatic cells at different stages of postzygotic development
- d. are present in part of the cells of the affected individual

46. Hereditary cancer syndromes:

- a. are hereditary
- b. are caused by mutations in tumor suppressor genes
- c. are caused by mutations in DNA repair genes
- d. are transmitted according to Mendelian autosomal dominant rules

47. Mutations that change structure but not genes or chromosome number are:

- a. chromosomal
- b. gene
- c. genomic
- d. gametic

48. Mutagens include:

- a. various types of radiation
- b. some drugs
- c. some viruses
- d. some chemical substances

49. According to origin, mutations are:

- a. gametic
- b. somatic
- c. genomic
- d. spontaneous

50. Mutations:

- a. have negative importance in breeding
- b. are a prerequisite for selection
- c. are one source of heritable variability
- d. are a fundamental prerequisite for evolution

51. The purpose of mandatory mutagen tests is to:

- a. determine chemical composition
- b. increase gene expression
- c. detect the ability of substances to cause DNA mutations
- d. detect the biological composition of a mutagen

52. Which changes can occur in gene mutations?

- a. substitution of one nucleotide for another
- b. insertion of extra nucleotides
- c. loss of a single nucleotide
- d. loss of multiple nucleotides

53. Which mutation may lead to premature termination of protein translation?

- a. nonsense mutation
- b. missense mutation
- c. silent mutation
- d. neutral mutation

54. Mutations:

- a. are a prerequisite for selection
- b. are a fundamental prerequisite for evolution
- c. are an internal source of heritable variability
- d. have negative importance in breeding

55. Mutations are classified according to:

- a. type of affected cell
- b. type of nucleic acid
- c. mode of disappearance
- d. mode of origin

56. Mutations are:

- a. sudden
- b. random
- c. caused by DNA changes
- d. changing the genotype

57. How do we classify mutagens?

- a. chemical
- b. spontaneous
- c. induced
- d. biological

58. Mutations can be divided according to:

- a. type of mutagen used
- b. mode of origin
- c. type of affected cell
- d. extent of genetic information affected

59. Mutations can cause:

- a. crossing-over
- b. congenital diseases
- c. cancer development
- d. genetic disorders

60. What causes syndromes with spontaneous chromosomal instability?

- a. mutations in genes for repair enzymes
- b. excessive UV radiation
- c. vitamin D deficiency
- d. viral infections

61. Which of the following diseases belong to syndromes with spontaneous chromosomal instability?

- a. Ataxia telangiectasia
- b. Xeroderma pigmentosum
- c. Fanconi anemia
- d. Bloom syndrome

62. Individuals affected by syndromes with spontaneous chromosomal instability are:

- a. resistant to mutagens
- b. extremely sensitive to mutagens
- c. immune to cancer
- d. prone to accumulating DNA mutations

63. Which enzyme performs direct repair of thymine dimers after UV exposure?

- a. DNA ligase
- b. Photolyase
- c. AP endonuclease
- d. RecA

64. Which type of repair removes individual damaged bases and inserts the correct nucleotide?

- a. base excision repair
- b. nucleotide excision repair
- c. direct repair
- d. SOS repair

65. What can be repaired by base excision repair?

- a. pyrimidine dimers caused by UV radiation
- b. extensive DNA breaks
- c. damaged SOS genes
- d. oxidative DNA damage

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. TTFT | 23. FTTF | 45. FFTT |
| 2. FFFF | 24. TFFT | 46. TTTT |
| 3. TFTT | 25. TTFF | 47. TFFF |
| 4. TFTF | 26. TTTF | 48. TTTT |
| 5. FFTF | 27. FTFF | 49. FFFT |
| 6. FTTF | 28. TFFT | 50. FTTT |
| 7. TFTT | 29. FFFT | 51. FFTF |
| 8. TTTF | 30. TFFF | 52. TTTT |
| 9. TFFT | 31. FFTF | 53. TFFF |
| 10. TTTT | 32. FTFF | 54. TTTF |
| 11. TTTT | 33. TFFT | 55. TFFT |
| 12. TTFF | 34. TFFF | 56. TTTT |
| 13. TFTF | 35. TTFT | 57. TFFT |
| 14. FFTT | 36. FFTF | 58. TTTT |
| 15. FTFF | 37. FTTF | 59. FTTT |
| 16. TTTT | 38. TFFF | 60. TFFF |
| 17. FFTF | 39. FTTT | 61. TTTT |
| 18. TFTF | 40. TTTF | 62. FTFT |
| 19. FTTF | 41. FFTT | 63. FTFF |
| 20. TTFF | 42. TFFF | 64. TFFF |
| 21. TFFF | 43. FFTF | 65. FFFT |
| 22. FTTF | 44. TFFF | |

11. Gene therapy.

Gene therapy is currently a rapidly developing area of modern medicine, offering a real therapeutic option for patients with genetic disorders, oncological diagnoses, and other serious diseases. Significant advances achieved in recent years have enabled its gradual introduction into clinical practice. The development of gene therapy takes place under strict regulatory oversight, with emphasis on safety and efficacy. Clinical studies are carefully designed, and long-term patient monitoring serves to minimize potential risks. Gene therapy is defined as a therapeutic approach that uses **gene engineering methods to supplement a missing gene, modify a gene, or remove a gene with pathological function.**

Genetic disorders arise from qualitative or quantitative changes in DNA, such as point mutations, deletions, or duplications. These changes often lead to reduced or complete loss of gene function, resulting in insufficient production or absence of a functional protein. The **principle** of gene therapy involves **introducing a functional copy of the gene** into a cell using a **suitable vector**, thereby restoring the disrupted biological function. However, implementing gene therapy is technologically and biologically challenging. It requires precise targeting of the correct gene to specific cells, its regulated expression at the right time, and long-term stability of effect. Equally important is ensuring an appropriate amount of protein production, as both excess and deficiency can be undesirable. The main goal of gene therapy is to offer new therapeutic options where conventional treatments fail or are insufficient. In many cases, even partial restoration of the missing protein production leads to significant clinical improvement or slows disease progression, including inhibition of tumor cell growth.

Clinical and experimental research in gene therapy focuses on so-called **model genetic disorders**, which can be divided into four main groups. The **first group** comprises **monogenic** diseases caused by a mutation in a single gene, such as **sickle cell anemia**. The **second group** includes **multigenic** disorders, in which multiple genes in combination with environmental factors contribute to disease development, such as **cardiovascular diseases, diabetes mellitus, or cancer**. The **third group** consists of mitochondrial disorders resulting from mutations in **mitochondrial DNA**, which are often lethal and lead to severe organ dysfunction. The **final group** comprises **chromosomal aberrations**, including deletions, duplications, and translocations of chromosomes.

Despite intensive research, gene therapy remains in most cases at an experimental stage or in clinical testing, and only a limited number of products have reached routine clinical use. One of the main **limiting factors** is the occurrence of **side effects**, as well as **high economic costs**. Historically, the **first approved** gene therapy was **Gendicine** in China in 2003, intended for the treatment of brain and skin tumors. In the European Union, **Glybera** was approved in 2012, based on the use of a genetically modified viral vector carrying the human gene for **lipoprotein lipase**. This therapy was intended for patients with an extremely rare lipid metabolism disorder, accompanied by severe pancreatitis and increased risk of atherosclerosis and type 2 diabetes. Despite proven efficacy, Glybera was discontinued due to extremely high costs.

In May 2016, after more than twenty years of research, the first gene therapy **Strimvelis** (GlaxoSmithKline) was approved for the treatment of severe combined immunodeficiency (**SCID**). This is an extremely rare inherited disease caused by a mutation in the **ADA** gene, which encodes the enzyme **adenosine deaminase**. Its dysfunction leads to the accumulation of toxic purine metabolites, inhibition of DNA synthesis, and subsequent failure of cell division, especially in T-lymphocytes. The result is severe immunodeficiency with recurrent and often

life-threatening infections from early childhood. Enzyme replacement therapy (Adagen) had been introduced, improving patient survival but not providing a definitive solution. ADA deficiency thus became an ideal model for developing gene therapy. Strimvelis employs **ex vivo gene transfer**. The functional ADA gene is introduced into the patient's **hematopoietic stem cells** using a retroviral vector, and these genetically modified cells are reinfused into the body. These cells acquire the ability to permanently produce the missing enzyme and restore immune system function.

In addition to SCID, gene therapy is applied in **oncology**. Approved products include **Kymriah** for acute lymphoblastic leukemia, **Yescarta** for certain lymphomas, **Imlygic** for malignant melanoma, **Provenge** for prostate cancer, and **Zalmoxis** for hematological malignancies. Approved gene therapies for genetic disorders include **Luxturna**, designed to treat mutations in the **RPE65** gene, which is essential for proper **retinal photoreceptor function**. Defects in this gene lead to progressive vision loss up to blindness. The therapy involves local application of a genetically modified viral vector directly into the patient's eye.

In 2019, the U.S. Food and Drug Administration (FDA) approved **Zolgensma** gene therapy for children under two years of age with spinal muscular atrophy (**SMA**). This is an autosomal recessive disorder caused by a mutation in the **SMN1** gene, which is critical for motor neuron survival. SMA manifests in early childhood as rapid muscle weakness and, without treatment, often leads to death from respiratory failure within the first years of life.

In the European Union, **Zynteglo** gene therapy was approved for the treatment of **beta-thalassemia**, a disease characterized by **impaired hemoglobin synthesis**. The therapy is based on **ex vivo** genetic modification of the patient's hematopoietic stem cells using a viral vector, followed by reinfusion. The modified cells engraft in the bone marrow and produce functional erythrocytes capable of synthesizing hemoglobin.

Successful gene therapy requires several **essential conditions**. The most important is precise knowledge of the **molecular cause** of the disease, the responsible **gene**, its **DNA sequence**, **product**, and **mechanism of action**. The second condition is **suitable target cells**, ideally **hematopoietic** stem cells, which can be harvested, expanded, genetically modified, and incorporated so that the corrected gene remains active in daughter cells. Modern techniques, such as **CRISPR-Cas9**, allow precise replacement of a defective gene. The third key element is an **appropriate vector**, which may be viral (e.g., **retroviruses**, **lentiviruses**, **AAV**), non-viral (**naked DNA**), or **bacterial** vectors. The aim is to efficiently **deliver** the genetic information into cells with minimal side effects, often combined with mechanical, chemical, or physical methods (electroporation, liposomes, nanoparticles). The fourth requirement is **safety**. The genetic information must not cause mutations, inactivate tumor suppressor genes, or activate proto-oncogenes. Therapy is applied only after successful testing, regulatory approval, and informed patient consent. Gene therapy is investigated or applied in diseases such as hemophilia, cystic fibrosis, hemoglobinopathies, Parkinson's and Alzheimer's disease, muscular dystrophies, liver cirrhosis, AIDS, and various cancers. Thorough patient monitoring is essential after therapy.

Gene therapy is divided into **somatic and germline**. **Germline** therapy alters the **genome of an egg or sperm** and is **inherited** by future generations. **Somatic** therapy targets the correction or supplementation of a mutated gene **in patients**, either by replacing it or delivering extra copies to target cells, for example, to protect against the toxic effects of treatment. Modern techniques, especially CRISPR-Cas9, enable precise genetic modification, ranging from single-

gene correction to immune cell modification for cancer therapy. In oncology, “killer” genes are used to directly kill tumor cells, or they are activated only in the presence of specific drugs (“suicide gene therapy”). A significant advance is **CAR-T therapy**, in which a patient’s **T cells** are **engineered** to effectively **target cancer cells**. A gene can also be specifically “turned off” (ablated) at the DNA, RNA, or protein level, or activated only in tumor cells using specific promoters. Gene therapy can be **administered *in vivo*, directly into the patient**, or ***ex vivo***, where cells are **harvested, genetically modified** in the laboratory, and then **reinfused**. The *ex vivo* approach allows control, selection, and expansion of cells, whereas *in vivo* is used when cells cannot be cultured or reinfused. The choice of method depends on the type of disease and treatment goals.

Gene therapy has advantages but also significant limitations. Its effect is often **short-term**, especially if the transgene is not integrated into the genome, and **immune responses can reduce efficacy** or prevent repeated administration. The use of viral vectors carries risks of **toxicity, inflammatory reactions, and random genomic integration**, which can potentially trigger malignant transformation. Moreover, most diseases are multigenic, so simultaneous **correction of multiple genes is currently impossible**. Gene therapy is technically complex, extremely expensive, and raises ethical questions regarding its appropriateness and safety.

Gene therapy research focuses primarily on rare genetic diseases and metabolic disorders. Key development projects expected to gain approval in 2026 include Tabelecleucel (tab-cel, Atara Biotherapeutics/Pierre Fabre), an allogeneic T-cell immunotherapy for EBV-positive PTLID; Clemidsogene lanparvovec (RGX-121, REGENXBIO), an AAV9-based gene therapy for Hunter syndrome; DTX401 (pariglasgene breccaparvovec, Ultragenyx) for glycogen storage disease type Ia; MCO-010 (Nanoscope Therapeutics), an optogenetic therapy for retinitis pigmentosa; Isaralgagene civaparvovec (ST-920, Sangamo Therapeutics) for Fabry disease; Sura-vec (ABBV-RGX-314, REGENXBIO) for wet age-related macular degeneration; and GENE202 (Genespire), a one-time lentiviral gene therapy for methylmalonic acidemia.

CRISPR-Cas9

CRISPR-Cas9 is a **genome-editing** technology derived from **bacterial** immune mechanisms. It uses a **guide RNA (gRNA)** to direct the **Cas9 endonuclease** to a precisely defined DNA sequence. The Cas9 enzyme creates a **double-strand break** at the target DNA site. The cell then **repairs** this break through two main mechanisms. Non-homologous end joining (NHEJ), which is **error-prone** and often leads to **gene inactivation**, or homology-directed repair (HDR), which allows **precise incorporation** or correction of a gene according to a **template**. The **main components** of the CRISPR-Cas9 system are the **Cas9 enzyme**, which **cuts DNA** at a specific site; the **guide RNA**, which **binds Cas9 and directs it** to a precise 20-nucleotide target sequence; and the **PAM sequence** (Protospacer Adjacent Motif), a short 2–6 nucleotide DNA motif adjacent to the target **that Cas9 must recognize to bind and cleave DNA**. The CRISPR-Cas9 mechanism involves **three steps**. First, the **gRNA-Cas9 complex binds to the target DNA and verifies the presence of the PAM sequence**; second, **Cas9 induces a double-strand break in the DNA**; and third, the **cell repairs this break** via NHEJ or HDR.

In January 2026, researchers at the University of New South Wales introduced a **new generation** of CRISPR technology that allows **regulation of gene activity without direct DNA cleavage**. Traditional CRISPR systems work by creating a double-strand break, which

can lead to unwanted mutations or other complications. The new approach instead targets epigenetic marks that influence gene activity. It can “turn on” genes or activate already existing beneficial genes without altering the DNA sequence itself. Research has shown that these **epigenetic marks** directly regulate gene activity rather than being passive markers of inactive genes. This mechanism enables fine, precise control of gene expression and minimizes the risk of side effects associated with classic DNA breaks. In medicine, this technology has the potential to enable safer therapies for genetic disorders, such as sickle cell anemia, in which fetal globin can be activated to partially compensate for a defective gene without genome-level intervention. This opens a new avenue for gene therapies combining high efficacy with lower risk, enhancing safety and clinical potential.



Model questions

1. Gene therapy is defined as:

- a) pharmacological treatment of genetic disorders
- b) a therapeutic approach using gene engineering to modify genes
- c) organ transplantation with genetic modification
- d) organ transplantation without genetic modification

2. Which of the following mechanisms most often leads to loss of gene function?

- a) enhancer duplication
- b) point mutation causing a missense change
- c) frameshift mutation
- d) CpG island duplication

3. The main goal of gene therapy is:

- a) a complete replacement of the patient's genome
- b) an increased expression of all genes
- c) a restoration or compensation of missing biological function
- d) a prevention of all genetic disorders

4. Model monogenic diseases suitable for gene therapy include:

- a) epilepsy
- b) sickle cell anemia
- c) Alzheimer's disease
- d) hypertension

5. Multigenic disorders are characterized by:

- a) being caused by a mitochondrial DNA mutation
- b) always having a lethal course
- c) arising from a combination of genetic and environmental factors
- d) being easily treated by gene therapy

6. Glybera was intended to treat:

- a) SCID
- b) hemophilia A
- c) lipoprotein lipase deficiency
- d) SMA

7. Why was Glybera withdrawn from the market?

- a) insufficient efficacy
- b) severe adverse effects
- c) ethical reasons
- d) extremely high price

8. Strimvelis is a gene therapy intended for:

- a) SCID caused by ADA deficiency
- b) beta-thalassemia
- c) hemophilia
- d) SMA

9. Strimvelis uses the principle of:

- a) *in vivo* gene transfer
- b) epigenetic modulation
- c) *ex vivo* gene transfer
- d) CRISPR knock-out

10. Which enzyme is deficient in ADA-SCID?

- a) adenylate cyclase
- b) adenosine deaminase
- c) DNA polymerase
- d) adenosine dehydrogenase

11. CAR-T therapy is an example of:

- a) *in vivo* gene therapy
- b) chemotherapy
- c) *ex vivo* genetic modification of immune cells
- d) enzyme replacement therapy

12. Kymriah and Yescarta are primarily used to treat:

- a) solid tumors
- b) autoimmune diseases
- c) hematologic malignancies
- d) metabolic disorders

13. Luxturna is a gene therapy intended for:

- a) SMA
- b) retinitis pigmentosa caused by *RPE65* mutation
- c) Alzheimer's disease
- d) glaucoma

14. Zolgensma is indicated for patients with:

- a) Duchenne muscular dystrophy
- b) SMA
- c) hemophilia
- d) SCID

15. The *SMN1* gene is crucial for:

- a) photoreceptor function
- b) insulin production
- c) motor neuron survival
- d) T-lymphocyte activation

16. Zynteglo is used to treat:

- a) hemophilia B
- b) beta-thalassemia
- c) sickle cell anemia
- d) Fabry disease

17. Which cell is the ideal target for *ex vivo* gene therapy?

- a) erythrocyte
- b) neuron
- c) hematopoietic stem cell
- d) lymphocyte

18. Viral vectors used in gene therapy include:

- a) plasmid
- b) lentivirus
- c) nanoparticle
- d) liposome

19. The main risk of random transgene integration is:

- a) reduced gene expression
- b) activation of proto-oncogenes
- c) decreased immunity
- d) epigenetic inactivation

20. Somatic gene therapy:

- a) affects germline cells
- b) is heritable
- c) affects only the patient
- d) is prohibited

21. Germline gene therapy:

- a) is routinely used in clinical practice
- b) does not affect future generations
- c) alters the genome of gametes
- d) is ethically uncontroversial

22. The CRISPR-Cas9 system consists of:

- a) Cas9 and guide RNA
- b) Cas9 and tRNA
- c) PAM and mRNA
- d) DNA ligase

23. The PAM sequence is:

- a) RNA polymerase binding site
- b) necessary for Cas9 binding to DNA
- c) part of the guide RNA
- d) a repair enzyme

24. The NHEJ mechanism most often leads to:

- a) a precise gene repair
- b) a gene activation
- c) an insertion-deletion mutations
- d) an epigenetic regulation

25. The HDR mechanism allows:

- a) random DNA integration
- b) precise gene repair according to a template
- c) RNA degradation
- d) gene silencing

26. The new generation of CRISPR technologies allows:

- a) only DNA cleavage
- b) regulation of gene expression without DNA breaks
- c) complete genome removal
- d) gene regulation via epigenetic marks

27. Epigenetic CRISPR therapy:

- a) alters the DNA sequence
- b) activates or inhibits genes without changing DNA
- c) is more toxic than classical CRISPR
- d) has no therapeutic potential

28. Activation of fetal globin is therapeutically advantageous in:

- a) hemophilia
- b) sickle cell anemia
- c) SMA
- d) SCID

29. *In vivo* gene therapy means:

- a) modification of cells outside the patient's body
- b) direct application of genetic material into the body
- c) modification of only germline cells
- d) exclusive use of CRISPR

30. Major limitations of gene therapy include:

- a) a short-term effect
- b) immune response
- c) high financial cost
- d) a long-term effect

31. Main goals of gene therapy include:

- a) replacement of a missing gene
- b) modification of a gene with pathological function
- c) removal or silencing of a pathological gene
- d) random mutagenesis

32. Genetic disorders can be caused by:

- a) point mutations
- b) DNA deletions
- c) gene duplications
- d) chromosomal aberrations

33. Major challenges of gene therapy include:

- a) precise targeting of the gene to correct cells
- b) regulation of gene expression over time
- c) long-term stability of therapeutic effect
- d) ensuring the correct level of expressed protein

34. For successful gene therapy, it is essential to:

- a) know the exact molecular cause of the disease
- b) identify target cells
- c) choose a suitable vector
- d) completely eliminate the patient's immune system

35. What is true about mitochondrial diseases:

- a) they are caused by mitochondrial DNA mutations
- b) often lead to severe organ dysfunction
- c) can have a lethal course
- d) are inherited exclusively through the paternal line

36. Approved gene therapies in oncology include:

- a) Kymriah
- b) Yescarta
- c) Imlygic
- d) Provenge

37. Luxturna gene therapy:

- a) is intended for inherited retinal diseases
- b) is applied locally to the eye
- c) is based on the CAR-T principle
- d) treats glaucoma

38. Zolgensma:

- a) is intended for children with SMA
- b) is mainly indicated in early childhood
- c) delivers a functional copy of the *SMN1* gene
- d) requires repeated lifelong administration

39. Ex vivo gene therapy is characterized by:

- a) cells being genetically modified outside the patient's body
- b) allowing selection and control of modified cells
- c) often using hematopoietic stem cells
- d) always being less safe than in vivo therapy

40. Viral vectors used in gene therapy include:

- a) retroviruses
- b) lentiviruses
- c) adeno-associated viruses
- d) plasmids

41. Non-viral gene delivery methods include:

- a) naked DNA
- b) liposomes
- c) nanoparticles
- d) electroporation

42. Risks of gene therapy include:

- a) immune response
- b) random integration into the genome
- c) activation of proto-oncogenes
- d) inflammatory reactions

43. The CRISPR-Cas9 system is naturally found in:

- a) prokaryotes
- b) viruses
- c) bacteria
- d) subcellular organisms

44. After creating a DNA double-strand break by CRISPR, the cell can use:

- a) NHEJ mechanism
- b) HDR mechanism
- c) LTR mechanism
- d) DDR mechanism

45. The NHEJ mechanism:

- a) is error-prone
- b) often leads to insertions or deletions
- c) requires a repair template
- d) allows precise gene repair

46. The HDR mechanism:

- a) enables precise gene repair
- b) requires a homologous template
- c) is dominant in the G0 phase of the cell cycle
- d) leads to random mutations

47. Provenge gene therapy is used in:

- a) pernicious anemia
- b) cervical cancer
- c) lung cancer
- d) prostate cancer

48. One of the main technological challenges of gene therapy is:

- a) uncontrolled gene expression at any time
- b) precise targeting of the therapeutic gene to specific cells
- c) exclusive use of mitochondrial DNA
- d) complete elimination of the need for vectors

49. Which statement about vectors in gene therapy is correct?

- a) bacterial vectors are the only group used
- b) AAV belongs to viral vectors
- c) liposomes are viral vectors
- d) naked DNA cannot be used for gene transfer

50. “Suicide gene therapy” in oncology means:

- a) activation of a gene only in healthy cells
- b) use of a gene that is activated in the presence of a specific drug
- c) removal of all proto-oncogenes from the genome
- d) bone marrow transplantation without cell modification

51. Which of the following products is listed as a gene therapy used for malignant melanoma?

- a) Provenge
- b) Luxturna
- c) Imlygic
- d) Zynteglo

52. Mitochondrial disorders:

- a) include type 2 diabetes mellitus
- b) are often lethal and lead to organ dysfunctions
- c) include sickle cell anemia
- d) are caused by mutations in mitochondrial DNA

53. In CRISPR-Cas9, the “guide RNA” is important because it:

- a) repairs the double-strand DNA break
- b) guides Cas9 to a specific target DNA sequence
- c) serves as the PAM sequence
- d) is the enzyme that cleaves DNA

54. During epigenetically targeted CRISPR technology, the main effect achieved is:

- a) permanent alteration of the DNA sequence
- b) regulation of gene activity without direct DNA cleavage
- c) random integration of a transgene into the genome
- d) degradation of mitochondrial DNA

55. Which statement about the difference between ex vivo and in vivo gene therapy is correct?

- a) *ex vivo* means modification of cells and direct application into the patient’s body
- b) *in vivo* means direct application of genetic material into the patient’s body
- c) *ex vivo* allows control, selection, and expansion of modified cells
- d) *in vivo* genetic material cannot be administered directly into the patient’s body

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. FTFF | 20. FFTF | 39. TTTF |
| 2. FFTF | 21. FFTF | 40. TTTF |
| 3. FFTF | 22. TFFF | 41. TTTT |
| 4. FTFF | 23. FTFF | 42. TTTT |
| 5. FFTF | 24. FFTF | 43. TFTF |
| 6. FFTF | 25. FTFF | 44. TTFF |
| 7. FFFT | 26. FTFT | 45. TTFF |
| 8. TFFF | 27. FTFF | 46. TTFF |
| 9. FFTF | 28. FTFF | 47. FFFT |
| 10. FTFF | 29. FTFF | 48. FTFF |
| 11. FFTF | 30. TTTF | 49. FTFF |
| 12. FFTF | 31. TTTF | 50. FTFF |
| 13. FTFF | 32. TTTT | 51. FFTF |
| 14. FTFF | 33. TTTT | 52. FTFT |
| 15. FFTF | 34. TTTF | 53. FTFF |
| 16. FTFF | 35. TTTF | 54. FTFF |
| 17. FFTF | 36. TTTT | 55. FTTF |
| 18. FTFF | 37. TTFF | |
| 19. FTFF | 38. TTTF | |

12. Teratology, teratogenesis.

Teratology is an interdisciplinary scientific field that studies congenital developmental defects, including their causes and mechanisms of origin. The term was coined in the 18th century and is derived from the Greek *teraton* (“monster”). In clinical terms, a congenital developmental defect (**congenital anomaly/birth defect**) is a postnatally detected deviation of intrauterine development that exceeds the normal range of phenotypic variability of a given structure or its function. Variability is influenced by epigenetic factors that act with randomly fluctuating intensity and magnitude. Therefore, the boundary between an “extreme of variability” and a “defect” also depends on what is considered a congenital anomaly in a given clinical setting and population. In our population, congenital anomalies are diagnosed in approximately 2% of newborns, although this figure is influenced by the reporting system, the definitions used, and regional differences in both frequency and the distribution of affected organ systems.

From the perspective of the “direction” of change, we distinguish **minus** forms, in which part of a structure or the entire structure is missing, and **plus** forms, in which there is an additional part or an excess (for example, extra fingers). In clinical practice, diverse terms are used, such as malformations, anomalies, developmental deviations, deformities, and similar labels. Therefore, it is useful to use terminology that distinguishes the mechanism underlying the developmental disturbance.

A **malformation** is a major structural abnormality caused by abnormal development (that is, an intrinsic “programming error” in structure formation). A **deformation** is a change in the shape or position of a previously normally formed part, typically due to mechanical conditions (for example, pressure or limited space). **Disruption** means breakdown or damage of previously normal tissue. In other words, the structure formed correctly, but was subsequently destroyed or interrupted. **Dysplasia** is abnormal tissue organization (a disturbance of tissue “architecture”).

In addition, terms such as **agenesis** (failure of an organ, part of an organ, or a tissue to develop) and **hypoplasia** (insufficient development, reduced volume, and/or reduced cell number) are used. A separate chapter includes severe disturbances of intrauterine development leading to spontaneous miscarriage or stillbirth. These are often referred to broadly as developmental defects because live birth does not occur. The causes of disturbed intrauterine development can be divided, in a practical way, into genetically determined causes and non-genetic causes (**teratogenesis**), although in reality these causes often combine. Genetically determined diseases and syndromes, whether inherited or arising from a **de novo** mutation, are estimated to account for approximately one quarter of congenital anomalies.

The second large group is teratogenesis, meaning the action of factors on cells and their interactions in an originally normal embryo. This includes situations in which a mutagen affects some embryonic cells, when gene expression in cells is altered, or when a factor negatively affects intercellular communication and developmental regulation. This group includes, for example, energy resource deficiency, inhibition of enzyme synthesis or function, disturbances of osmotic balance, disruption of membrane integrity, impaired cell communication (both physical and **paracrine**), as well as mechanical compression injuries such as amniotic bands.

Another part involves maternal causes (for example, certain endocrine disorders or congenital uterine anomalies) and infectious diseases during pregnancy, especially primary infections with certain agents (for example, rubella virus or the protozoan *Toxoplasma gondii*). Although these

are medically very important factors, identifiable external causes (maternal factors, infections, chemical substances, and similar) make up a smaller proportion of the overall etiology, and in a large share of congenital anomalies, the cause remains unrecognized in practice.

A **teratogen** is a factor from the external (**extraembryonic**) environment that, after passing through a **route of transfer (exposure pathway)**, causes an undesirable influence on ontogenesis **in utero**, resulting in a congenital anomaly. The effect of a teratogen depends on several parameters, of which the most important are timing of exposure during development, embryonic susceptibility, dose and duration of exposure, mechanism of action, and the **route of transfer (exposure pathway)**. Timing is crucial because a “younger” embryo generally reacts more severely. In the very early period, the “**all-or-none**” **principle** is often described, where exposure may lead either to embryo loss or to continued development without an obvious defect.

During organogenesis, by contrast, the risk of malformations is highest because the basic organ structures are being formed. Embryonic susceptibility is determined by genetic constitution and by intraspecies and interspecies variability. Therefore, the same factor may not produce the same effect in all individuals. Dose and duration of exposure influence the probability and extent of damage. With increasing ontogenetic age, the relative effect of the same dose decreases, in part due to the increasing body mass of the mother and the fetus. The mechanism of action also includes the possibility that combinations of factors may potentiate or inhibit one another. The **route of transfer (exposure pathway)** is the path by which a factor reaches target structures in the embryo. For chemical and biological teratogens, maternal metabolism and the maternal immune system are decisive, as is the substance's ability to cross the placenta.

Teratogens are traditionally divided into physical, chemical, and biological categories. Physical teratogens include, for example, penetrating and ionizing radiation. Chemical teratogens include drugs, xenobiotics, toxic substances, and mutagens. Biological teratogens include viruses, bacteria, and protozoa. In clinical teratology, it is practical to present typical examples and the characteristic defects associated with them.

A classic example of a drug teratogen is thalidomide, which can cause abnormal development or absence of limbs, as well as systemic abnormalities. Valproic acid (an antiepileptic drug) is associated mainly with craniofacial malformations and **neural tube defects (NTDs)**, and other congenital anomalies may also be reported, including some cardiac and skeletal defects. Phenytoin is associated with the pattern known as fetal hydantoin syndrome, in which growth disturbances and several craniofacial and limb features are described. With trimethadione, developmental delay and typical dysmorphic features are described, including cleft lip and/or cleft palate. Among biological teratogens, toxoplasmosis is associated, for example, with hydrocephalus, intracranial calcifications, and chorioretinitis, and it can also lead to severe outcomes, including hearing or vision loss, or miscarriage. In congenital rubella, typical findings include congenital heart defects, cataract, and hearing impairment, often with growth retardation. Such examples are most meaningful when linked to a specific period of pregnancy, because the risk of a particular defect is closely tied to critical periods of development.

To understand why a **pattern of multiple defects** appears in one case and only an isolated defect in another, a useful concept is that **basic morphogenetic processes** are taking place in the embryo and that these processes differ in activity across time and across tissues. The more active these processes are, the more sensitive the tissue is to teratogenic disruption. The periods in which individual organ systems are most sensitive to teratogens are called **critical periods**.

In humans, high sensitivity of the central nervous system is described mainly from week 3 to week 6, with sensitivity potentially decreasing only gradually up to birth. The cardiovascular system is sensitive roughly from the middle of week 3 to week 6, and sensitivity decreases until about the middle of week 8. The lip and palate are critical approximately between weeks 6 and 12, and the limbs between the middle of week 4 and week 7. From a practical point of view, it is helpful to think in terms of a simple timeline: the very early period after fertilization is risky mainly with respect to embryo loss (or, alternatively, continued development without defects), the period of organogenesis carries the highest risk for major malformations, and the later fetal period is more associated with growth disturbances, functional consequences, and deformities if mechanical or systemic influences are present.

Table: Risk periods by organ system

Period (approx.)	Typical rule/risk	Most sensitive organs/systems (examples of “windows”)
From fertilization to the start of organogenesis	“all-or-none” principle (either loss or no obvious consequences)	
Organogenesis	The highest risk of major structural malformations (critical period)	CNS: week 3–6; CVS: mid-week 3–6; Lip and palate: week 6–12; Limbs: mid-week 4–7
Later fetal period	rather a risk of growth disturbances and functional insufficiency	CNS remains sensitive longer (up to birth)

The basic postulates of teratology emphasize that susceptibility depends on the embryo’s genotype and on how it interacts with the environmental factor; that susceptibility changes according to developmental stage and timing of exposure (**critical periods**); that a teratogen acts through a specific mechanism on cells and tissues and thereby induces abnormal embryogenesis; that the route by which a harmful factor reaches the embryo depends on the nature of the agent; and that manifestation of developmental disturbance increases with dose. The final picture of disturbed development may present as death, malformation, growth retardation, or functional insufficiency. The same factor can lead to different outcomes depending on timing and dose.

For medicine and prevention, a key question is how to identify teratogens and assess their risk. **Teratoepidemiology** monitors the occurrence of congenital anomalies in the population and seeks to identify possible links to exposures, drugs, infections, or other factors. **Experimental teratology** tests the teratogenic potential of factors in experiments, from screening assays to complex reproductive studies. When identifying teratogens in humans, there are practical paradoxes: the human embryo, the pregnant woman, and the **fetoplacental unit** are, to a large extent, unique, so there is no fully adequate non-human model with perfect transferability of results. At the same time, the societal need to test new substances, especially medicines, is very high. Therefore, practice often proceeds by compromise: the ability of a substance to cross the placenta is assessed (**this information is given high priority**) and, in parallel, teratogenic potential is evaluated in models. If placental transfer is demonstrated and models also suggest a teratogenic effect, the substance must not be given to pregnant women.

Models in experimental teratology include both in vivo and in vitro approaches. In vivo studies use various organisms, from simpler **invertebrates** to vertebrates. From a practical standpoint,

laboratory animals are considered the most suitable because they provide a complete mother–placenta–embryo/fetus system. In vitro approaches include cultured cells to study mechanisms of action, cultured embryonic parts, or cultured whole preimplantation embryos in screening tests.

The organization of embryotoxicity and teratogenicity testing is based on selection of an appropriate model, precise determination of the start of pregnancy and embryogenesis, a method of substance administration corresponding to expected human exposure, dosing with multiple groups (typically at least three doses plus a control), a time-based dosing schedule, and termination of the experiment according to the standard of the given methodology. Parameters are then monitored, including female body weight and behaviour, the number of corpora lutea, implantations, resorptions, the number of live and dead fetuses, fetal weight, and the occurrence of visceral and skeletal malformations, and these are evaluated statistically. The aim is to distinguish non-specific toxicity, which can mask specific developmental effects, from true teratogenicity.

Teratology is therefore both a theoretical and a highly practical field. It explains why a particular developmental phase is vulnerable, how different types of factors can disrupt the regulation of embryogenesis, and how to translate this knowledge into prevention. In clinical practice, its principles are applied when assessing the risk of exposures during pregnancy, in prenatal counselling, when interpreting combinations of congenital defects, and when deciding which factors are likely causes and which rather increase susceptibility or act as cofactors.

Table: Examples of teratogens.

Teratogen (examples)	Typical effects/defects (examples)
Isotretinoin (13-cis-retinoic acid)	craniofacial abnormalities, neural tube defects (NTDs), cardiovascular defects
Lithium carbonate	various malformations of the heart and great vessels
Methotrexate	multiple malformations, especially of the skeleton, face, skull, limbs, and spine
Phenytoin	“fetal hydantoin syndrome”: IUGR, microcephaly, intellectual disability, characteristic facial dysmorphism, phalangeal hypoplasia
Thalidomide	abnormal development or absence of limbs, systemic abnormalities
Trimethadione	developmental delay, characteristic dysmorphic features (e.g. V-shaped eyebrows), low-set ears, cleft lip/palate
Valproic acid	craniofacial malformations, NTDs (often also hydrocephalus), heart and skeletal defects
Alcohol	CNS and skeletal defects, fetal alcohol syndrome
Smoking	miscarriage, stillbirth, placental abruption, low birth weight, also reported: limb deformities, polycystic kidneys, cleft lip and palate
Marijuana (cannabis)	cardiac malformations, CNS malformations, ocular, gastrointestinal, genitourinary, and musculoskeletal malformations; lower birth weight and preterm birth



Model questions

1. Teratology is a scientific field that:

- a. studies congenital developmental defects, their causes, and mechanisms of origin
- b. deals exclusively with the inheritance of monogenic disorders
- c. studies only postnatal growth disorders
- d. addresses only infectious diseases in newborns

2. The term “teraton” (origin of the word teratology) means:

- a. fetus
- b. monster
- c. gene
- d. placenta

3. In the clinical sense, a congenital developmental defect (congenital anomaly / birth defect) is:

- a. a developmental deviation detected prenatally by ultrasound
- b. a postnatally detected deviation of intrauterine development exceeding normal variability
- c. a developmental deviation arising after birth
- d. an exclusively genetically determined disorder

4. The boundary between an “extreme of variability” and a “defect” depends mainly on:

- a. what is considered a congenital anomaly in a given clinical practice and population
- b. only on the mother’s genotype
- c. only on fetal sex
- d. exclusively on placental size

5. Genetically determined diseases and syndromes contribute to congenital anomalies approximately:

- a. 90%
- b. 50%
- c. 25%
- d. 5%

6. Teratogenesis is best described as:

- a. damage to an originally normal embryo caused by environmental factors
- b. congenital anomalies arising exclusively through de novo mutation
- c. a developmental defect caused only by a chromosomal aberration
- d. a genetic predisposition formed by minor genes

7. Which statements about minus and plus forms are correct?

- a. minus forms: part or all of a structure is missing
- b. plus forms: there is an extra part or excess of a structure
- c. plus forms always mean disruption
- d. minus forms are always deformations

8. A malformation is:

- a. a change in the shape of a normally formed part due to pressure
- b. a major structural abnormality caused by an abnormal developmental “program”
- c. breakdown/damage of normal tissue after correct formation
- d. abnormal tissue organization without a structural defect

9. A deformation is typically a consequence of:

- a. an intrinsic error in organ formation
- b. mechanical conditions (pressure, limited space)
- c. breakdown of already formed tissue by a toxin
- d. chromosomal aneuploidy

10. Disruption means:

- a. insufficient organ development from the start
- b. breakdown/damage of previously normally formed tissue
- c. abnormal tissue architecture
- d. excess structures

11. Dysplasia is:

- a. a disorder of tissue “architecture”, abnormal organization
- b. always a result of mechanical pressure
- c. a synonym of agenesis
- d. exclusively a disorder of neural crest cell migration

12. Agenesis means:

- a. excessive organ growth
- b. failure of an organ or part of an organ to develop
- c. damage to already formed tissue
- d. displacement of an organ to another position

13. Hypoplasia means:

- a. absence of an entire structure
- b. insufficient development with reduced volume and/or cell number
- c. extra fingers
- d. abnormal tissue arrangement without a change in volume

14. Severe disturbances of intrauterine development may lead to:

- a. spontaneous miscarriage
- b. stillbirth
- c. always fully compensable defects after birth
- d. exclusively isolated deformations

15. Mechanisms of teratogenesis include:

- a. inhibition of enzyme synthesis or function
- b. disturbances of osmotic balance
- c. disruption of membrane integrity
- d. increased recombination frequency in meiosis

16. Disruptions of cell communication include:

- a. impaired physical cell–cell communication
- b. impaired paracrine communication
- c. increased expression of all genes without exception
- d. exclusively mechanical deformations without a molecular basis

17. Maternal causes of congenital anomalies may include:

- a. certain maternal endocrine disorders
- b. congenital uterine anomalies
- c. chronic paternal diseases as the main direct cause of congenital anomalies
- d. only blood pressure changes in the third trimester

18. The infectious agents mentioned include:

- a. rubella virus
- b. *Toxoplasma gondii*
- c. influenza virus as a typical teratogen
- d. *Plasmodium falciparum* as a typical teratogen

19. Which statement is correct?

- a. identifiable external causes make up the large majority of the etiology of congenital anomalies
- b. in a large proportion of congenital anomalies, the cause remains unrecognized in practice
- c. all congenital anomalies can be explained by one teratogen
- d. infections during pregnancy are not important

20. The “transport channel” of a teratogen is:

- a. the sum of the fetus’s genetic predispositions
- b. the route by which the factor reaches the target structures of the embryo
- c. only blood flow in the umbilical cord
- d. exclusively fetal metabolism without maternal influence

21. For chemical/biological teratogens, transport depends mainly on:

- a. maternal metabolism and immune system
- b. the ability of the substance to cross the placenta
- c. only fetal sex
- d. exclusively the mother’s blood group

22. The “all-or-none” principle is typically stated for:

- a. organogenesis
- b. the very early period after fertilization
- c. the late fetal period
- d. the neonatal period

23. During organogenesis, the highest risk is for:

- a. functional disorders without structural changes
- b. major structural malformations
- c. exclusively pressure-related deformations
- d. only growth retardation without defects

24. In the later fetal period, risk is more typically associated with:

- a. “new” major malformations
- b. growth disturbances and functional consequences
- c. exclusively aneuploidies
- d. only plus forms

25. Critical periods are:

- a. periods when organ systems respond most sensitively to teratogens
- b. periods with no effect of exposure
- c. only the postnatal period
- d. periods defined exclusively by fetal weight

26. Which period of greatest susceptibility to harmful exposure applies to the CNS?

- a. weeks 1–2
- b. weeks 3–6
- c. weeks 10–12
- d. only weeks 20–24

27. CNS sensitivity may decrease:

- a. until mid-week 8
- b. up to birth
- c. only until the end of week 6
- d. only until mid-week 3

28. The critical period for embryonic development of the cardiovascular system is approximately:

- a. weeks 6–12
- b. mid-week 3 to week 6
- c. mid-week 4 to week 7
- d. only after week 12

29. Sensitivity of embryonic cardiovascular development decreases approximately:

- a. until mid-week 8
- b. without change until birth
- c. until week 20
- d. only after birth

30. The critical period for development of the lip and palate is:

- a. weeks 3–6
- b. weeks 6–12
- c. mid-week 4 to week 7
- d. weeks 12–20

31. The critical period for the development of the limbs is:

- a. mid-week 4 to week 7
- b. weeks 6–12
- c. weeks 3–6
- d. weeks 1–2

32. Teratogens are traditionally divided into:

- a. physical
- b. chemical
- c. biological
- d. genetic (chromosomal)

33. Physical teratogens include:

- a. ionizing radiation
- b. toxoplasmosis
- c. thalidomide
- d. lithium carbonate

34. Biological teratogens include:

- a. viruses
- b. bacteria
- c. protozoa
- d. drugs only

35. Thalidomide is mainly associated with:

- a. absence or abnormal development of limbs
- b. cataract only
- c. systemic abnormalities
- d. isolated hearing impairment without other defects

36. Valproic acid is mainly associated with:

- a. craniofacial malformations
- b. neural tube defects (NTDs)
- c. kidney defects only
- d. cardiac or skeletal defects

37. Phenytoin is associated with:

- a. “fetal hydantoin syndrome”
- b. isolated aortic coarctation as the typical single sign
- c. growth disturbances
- d. craniofacial and limb features

38. Trimethadione is associated with:

- a. developmental delay
- b. typical dysmorphic features
- c. cleft lip and/or cleft palate
- d. neural tube defects only without other features

39. Congenital toxoplasmosis is associated with:

- a. hydrocephalus
- b. intracranial calcifications
- c. chorioretinitis
- d. isolated polydactyly as a typical sign

40. Congenital rubella is typically associated with:

- a. congenital heart defects
- b. cataract
- c. hearing impairment
- d. cleft lip and palate only

41. Isotretinoin (13-cis-retinoic acid) is associated with:

- a. craniofacial abnormalities
- b. neural tube defects (NTD)
- c. cardiovascular defects
- d. isolated hearing impairment only

42. Lithium carbonate is mainly associated with:

- a. various malformations of the heart and great vessels
- b. fetal alcohol syndrome
- c. chorioretinitis
- d. hypoplasia of phalanges as a typical sign

43. Methotrexate is mainly associated with:

- a. multiple malformations
- b. malformations of the skeleton, face, skull, limbs, and spine
- c. microcephaly only without other defects
- d. hearing disorders only

44. In experimental teratology, after the experiment is completed, the following may be assessed, for example:

- a. number of corpora lutea
- b. number of implantations and resorptions
- c. occurrence of visceral and skeletal malformations
- d. fetal sex only, without other parameters

45. A congenital developmental defect can represent:

- a. only an abnormal organ structure
- b. only abnormal organ function
- c. abnormal organ structure or function due to a disturbance of intrauterine development
- d. abnormal organ structure or function due to a postnatal developmental disturbance

46. A teratogen is:

- a. an extraembryonic factor that undesirably affects intrauterine development
- b. an abnormal number of chromosomes in the embryo
- c. a factor that leads to Patau syndrome
- d. a genetic predisposition formed by small-effect genes

47. Congenital developmental defects may be caused by:

- a. some chronic maternal diseases (e.g., endocrine disorders)
- b. genetically determined diseases
- c. infection with *Toxoplasma gondii* in the first trimester of pregnancy
- d. chronic paternal diseases

48. Congenital defects in which some body part is missing are referred to as:

- a. minus forms
- b. plus forms
- c. hypertrophies
- d. dysplasias

49. In our population, congenital anomalies are diagnosed in approximately what proportion of newborns?

- a. 26%
- b. 1 in 1000
- c. 1 in 10000
- d. 2%

50. The effect of a teratogen depends on:

- a. the developmental period
- b. embryonic susceptibility
- c. the dose of the teratogen
- d. the duration of exposure

51. Chemical teratogens include:

- a. some drugs
- b. xenobiotics
- c. protozoa
- d. ionizing radiation

52. For in vivo testing in experimental teratology, the following models are used:

- a. *Xenopus laevis* frogs
- b. chicken embryos
- c. cultured cells
- d. cultured parts of embryos

53. Major structural congenital malformations arise as a result of teratogen exposure mainly:

- a. during the fetal period
- b. during the embryonic period
- c. during the neonatal period
- d. all answers are correct

54. Teratoepidemiology focuses on:

- a. monitoring the occurrence of congenital anomalies and searching for potential teratogens
- b. counselling families with congenital anomalies
- c. testing teratogens in animal models
- d. testing teratogens in cultured cells

55. The effect of a teratogen depends on:

- a. the route of transfer (exposure pathway)
- b. mainly molecular weight
- c. embryonic susceptibility
- d. the developmental period

56. Models used for testing in experimental teratology include, for example:

- a. cultured parts of the fetus
- b. FETAX
- c. cultured parts of embryos
- d. CHEST

57. What is a teratogen?

- a. a factor from the extraembryonic environment that causes an undesirable influence on ontogenesis in utero, resulting in congenital anomalies
- b. a factor from the intraembryonic environment that causes an undesirable influence on ontogenesis in utero, resulting in congenital anomalies
- c. for example, valproic acid
- d. a gene whose mutation causes a congenital anomaly

58. In humans, periods in which individual organ systems are most sensitive to teratogens include:

- a. CNS development
- b. genital development
- c. cardiovascular system development
- d. lip and palate development

59. What are the types of teratogens?

- a. biological
- b. mutagenic
- c. chemical
- d. physical

60. Basic postulates of teratology include:

- a. susceptibility to teratogenesis depends on the embryo's genotype and on how it interacts with an external factor
- b. susceptibility to teratogenic agents varies with the developmental stage and timing of exposure (critical periods)
- c. a teratogen acts through a specific mechanism on embryonic cells and tissues and induces abnormal embryogenesis (teratogenesis)
- d. the route by which exogenous harmful factors reach developing structures depends on the nature of the agent

Answer Key to Model Questions

1. TFFF	21. TTFF	41. TTTF
2. FTFF	22. FTFF	42. TFFF
3. FTFF	23. FTFF	43. TTFF
4. TFFF	24. FTFF	44. TTTF
5. FFTF	25. TFFF	45. FFTF
6. TFFF	26. FTFF	46. TFFF
7. TTFF	27. FTFF	47. TTTF
8. FTFF	28. FTFF	48. TFFF
9. FTFF	29. TFFF	49. FFFT
10. FTFF	30. FTFF	50. TTTT
11. TFFF	31. TFFF	51. TTFF
12. FTFF	32. TTTF	52. TTFF
13. FTFF	33. TFFF	53. FTFF
14. TTFF	34. TTTF	54. TFFF
15. TTTF	35. TFTF	55. TFTT
16. TTFF	36. TTFT	56. FTTT
17. TTFF	37. TFTT	57. TFTF
18. TTFF	38. TTTF	58. TFTT
19. FTFF	39. TTTF	59. TFTT
20. FTFF	40. TTTF	60. TTTT

13. Epigenetics, Alternative Use of Genetic Information, Mechanisms of Differentiation.

Epigenetics is a field of molecular biology focusing on the regulation of gene expression **without altering the nucleotide sequence** of DNA itself. While genetic information is encoded in the DNA base sequence, epigenetic mechanisms determine which genes are active in a given cell, at what time, and to what extent. These mechanisms function as an additional regulatory layer above the genetic code and enable cells with identical DNA to acquire distinct functions and phenotypes. Epigenetic regulation thus explains how morphologically and functionally diverse cell types, such as neurons, muscle cells, or epithelial cells, can arise from a single zygote.

The fundamental substrate of epigenetic regulation is chromatin, a complex of DNA and histone proteins that ensures extremely efficient packaging of genetic information within the cell nucleus. A human cell contains approximately two meters of DNA, which must be organized within a nucleus only a few micrometers in diameter. DNA is therefore wrapped around a **histone octamer** to form a **nucleosome**, the basic structural unit of chromatin. **Histones** carry a **positive** charge that allows them to **interact electrostatically** with negatively charged DNA. The organization of chromatin critically influences gene accessibility to the transcriptional machinery. Less condensed euchromatin is associated with active or potentially active gene expression, whereas highly condensed heterochromatin is typically transcriptionally inactive.

One of the most important epigenetic mechanisms is **DNA methylation**. This modification involves the covalent attachment of a methyl group to **cytosine**, most commonly within **CpG** islands. DNA methylation is **catalyzed** by **DNA methyltransferases**, with **DNMT3A and DNMT3B** responsible for **de novo methylation** during development, while **DNMT1** maintains existing methylation patterns during **DNA replication**. Methylation of gene **promoter** regions is generally associated with **transcriptional repression**, as it interferes with transcription factor binding or recruits repressive protein complexes. In this way, DNA methylation acts as a stable yet dynamically regulatable mechanism of gene expression control. In epigenetics, it is essential to strictly distinguish between DNA methylation, which affects cytosines in CpG islands and is typically linked to gene repression, and **histone amino acid methylation**, whose functional outcome is context-dependent and may result in either **transcriptional activation or repression**. For example, methylation of histones **H3K4 and H3K36** is generally associated with **active gene expression**, whereas methylation of **H3K9** is typically linked to **repressive chromatin**, with the final effect depending on the **degree of methylation and cellular context**.

In addition to DNA methylation, **post-translational modifications of histones** play a crucial role in epigenetic regulation. Histone proteins possess amino-terminal “tails” that can undergo chemical modifications such as **acetylation, methylation, phosphorylation, or ubiquitination**. Histone **acetylation** is usually associated with **activation** of gene expression, as it reduces the positive charge of histones, weakens their interaction with DNA, and leads to chromatin relaxation. In contrast, certain forms of histone **methylation** are associated with **repressive chromatin**, although the effect depends on the **specific amino acid residue** and the **degree of methylation**. The combination of histone modifications constitutes the so-called **histone code**, which is recognized by specific proteins and determines the functional state of chromatin.

Non-coding RNA molecules are also an important component of epigenetic regulation. **MicroRNAs** regulate gene expression primarily at the **post-transcriptional** level by promoting

mRNA degradation or inhibiting translation. Long non-coding RNAs can act directly at the chromatin level by serving as guiding molecules for epigenetic complexes. A classical example is the **lncRNA Xist**, which plays a key role in the **inactivation of one X chromosome in female mammals**. This process ensures dosage compensation between the sexes and is stably maintained through all subsequent cell divisions.

Epigenetics plays a fundamental role throughout **development**, starting from fertilization, when extensive **genome reprogramming** occurs. After formation of the zygote, most epigenetic marks, particularly **DNA methylation, are globally erased**, allowing the restoration of **totipotency in embryonic cells**. This process occurs **actively in the paternal genome and passively in the maternal genome** during the first mitotic divisions. Subsequently, during the **blastocyst stage**, **de novo DNA methylation** mediated by DNMT3A and DNMT3B establishes new, developmentally specific methylation patterns. **An exception** to this global erasure is represented by **imprinted genes**, whose methylation marks are preserved and ensure parent-of-origin-specific gene expression, such as in the case of *IGF2* or *H19*.

During further development, epigenetic mechanisms govern cell differentiation through stable regulation of key transcription factors. Activation or repression of genes such as *OCT4*, *SOX2*, and *NANOG* determines whether pluripotency is maintained or cells enter differentiation pathways. In **somatic cells**, these genes are epigenetically **silenced** by promoter methylation and repressive histone marks, particularly **trimethylation of lysine 27 on histone H3** (H3K27me3). Conversely, genes essential for specific cell lineages, such as *MYOD1* in muscle cells or *PAX6* in neural precursors, are associated with activating marks, including H3K4me3 and histone acetylation.

Epigenetic regulation is also critical for the **spatial and temporal control of gene expression during organogenesis**. Homeobox (**HOX**) genes, which define the **anteroposterior** body axis, are regulated through epigenetic mechanisms that ensure their sequential activation. **Polycomb repressive complexes** (PRC1 and PRC2) maintain **silencing of HOX genes** in cells where they should not be expressed, while their release allows precisely defined expression at the correct developmental time and location. Disruptions in this regulation can lead to severe developmental abnormalities.

The importance of epigenetics is particularly evident in studies of **monozygotic twins**, who share identical genetic material yet often display differences in phenotype and health status. Epigenomic analyses have shown that young monozygotic twins have nearly identical DNA methylation and histone modification profiles, whereas older twins exhibit substantial divergence in epigenetic patterns. Studies using methylation microarrays and CpG island sequencing have revealed differences in the methylation of genes involved in immune response, metabolism, and the cell cycle.

Specific epigenetic differences between monozygotic twins have been identified, for example, in genes such as *BRCA1*, *GSTM1*, or in loci regulating cytokine genes, potentially contributing to different risks of cancer or autoimmune diseases. Differences in promoter methylation of genes involved in dopaminergic signaling have been associated with varying susceptibility to neuropsychiatric disorders. These epigenetic changes arise as a result of **differing environmental exposures**, such as smoking, diet, physical activity, or psychosocial stress, as well as from stochastic epigenetic drift during cell division.

Epigenetic **differences** between monozygotic twins may arise already during **embryonic** development, for instance due to **unequal distribution of epigenetic marks during early mitotic divisions** or differential exposure to signaling molecules *in utero*. These differences subsequently accumulate over the lifespan and lead to distinct regulation of gene expression in individual tissues. The study of monozygotic twins therefore provides a unique model for distinguishing genetic and epigenetic influences and clearly demonstrates that an individual's phenotype is the result of a complex interaction between the genome, epigenome, and environment.

The significance of epigenetics is particularly pronounced in the context of human disease. Aberrant DNA methylation patterns and disturbances in histone modifications are characteristic features of many cancers, where tumor suppressor genes are silenced or oncogenes aberrantly activated. Epigenetic alterations also play a role in neurological, metabolic, and autoimmune disorders. Because epigenetic modifications are potentially reversible, they represent attractive targets for therapeutic intervention, leading to the development of epigenetic drugs such as DNA methyltransferase inhibitors or histone deacetylase inhibitors.



Model questions

1. Epigenetics mainly deals with:

- a) changes in the nucleotide sequence of DNA
- b) regulation of gene expression without changing the DNA sequence
- c) mutations arising during replication
- d) mechanisms influencing gene activity

2. Epigenetic mechanisms cause cells with identical DNA to:

- a) have the same phenotype
- b) acquire different functions
- c) express different sets of genes
- d) lose genetic information

3. The basic structural unit of chromatin is:

- a) chromosome
- b) histones H1
- c) nucleosome
- d) CpG island

4. A nucleosome is composed of:

- a) one histone and DNA
- b) a histone octamer and DNA
- c) RNA and histones
- d) DNA without proteins

5. The positive charge of histones is important mainly for:

- a) DNA replication
- b) electrostatic interaction with DNA
- c) chromatin degradation
- d) inhibition of transcription by RNA polymerase

6. Euchromatin is typically characterized by:

- a) high condensation
- b) low transcriptional activity
- c) higher accessibility for transcription factors
- d) potentially active gene expression

7. DNA methylation most commonly occurs on:

- a) adenine in CpG islands
- b) guanine in CpG islands
- c) cytosine in CpG islands
- d) thymine in CpG islands

8. *De novo* DNA methylation is mainly carried out by the enzymes:

- a) DNMT1
- b) DNMT3A
- c) DNMT3B
- d) HDAC

9. DNMT1 plays a main role in:

- a) removal of methyl groups
- b) maintenance of methylation during replication
- c) *de novo* methylation in the embryo
- d) histone acetylation

10. Methylation of gene promoters is usually associated with:

- a) activation of transcription
- b) repression of transcription
- c) blocking the binding of transcription factors
- d) recruitment of repressive protein complexes

11. Histone acetylation most often leads to:

- a) an increase in the positive charge of histones
- b) chromatin relaxation
- c) activation of gene expression
- d) formation of heterochromatin

12. The effect of histone methylation depends on:

- a) the specific amino acid residue
- b) the degree of methylation
- c) the cell type
- d) none of the above

13. The histone code represents:

- a) the genetic code for histones
- b) a set of post-translational modifications of histones
- c) the base sequence of histone genes
- d) a mechanism for recognizing the functional state of chromatin

14. MicroRNAs regulate gene expression mainly:

- a) at the transcriptional level
- b) post-transcriptionally
- c) by degradation of mRNA
- d) by inhibition of translation

15. The lncRNA Xist participates in:

- a) activation of the X chromosome
- b) inactivation of one X chromosome
- c) gene dosage compensation among sexes
- d) regulation of imprinted genes

16. After fertilization, the following occurs:

- a) global removal of epigenetic marks
- b) immediate stabilization of DNA methylation
- c) restoration of totipotency of embryonic cells
- d) epigenetic reprogramming of the genome

17. Global erasure of DNA methylation after fertilization occurs:

- a) actively in the paternal genome and passively in the maternal genome
- b) passively in both the paternal and maternal genomes
- c) through identical mechanisms in both genomes
- d) exclusively during later mitotic divisions of the embryo

18. Which genes escape global demethylation?

- a) HOX genes
- b) imprinted genes
- c) *IGF2*
- d) *H19*

19. The genes *OCT4*, *SOX2*, and *NANOG* are associated with:

- a) pluripotency
- b) differentiation of muscle cells
- c) embryonic stem cells
- d) epigenetic silencing in somatic cells

20. The repressive histone mark H3K27me3 is typically associated with:

- a) active transcription
- b) gene silencing
- c) Polycomb repressive complexes
- d) the pluripotent state

21. *MYOD1* and *PAX6* are examples of genes:

- a) associated with pluripotency
- b) specific to certain cell lineages
- c) activated by activating epigenetic marks
- d) silenced in all somatic cells

22. HOX genes are important mainly for:

- a) regulation of the cell cycle
- b) determination of the anteroposterior body axis
- c) organogenesis
- d) spatial and temporal regulation of development

23. Polycomb repressive complexes (PRC1, PRC2):

- a) maintain gene silencing
- b) activate transcription of HOX genes
- c) ensure proper timing of gene expression
- d) their dysfunction leads to developmental abnormalities

24. In monozygotic twins, the following is observed with age:

- a) a decrease in genetic identity
- b) divergence of epigenetic patterns
- c) differences in DNA methylation
- d) differences in phenotype and disease susceptibility

25. Epigenetic differences between monozygotic twins may arise due to:

- a) environmental influences
- b) epigenetic drift during cell division
- c) differences in the intrauterine environment
- d) mutations in the nucleotide sequence of DNA

26. Which amino acid residues of histones can be methylated?

- a) only serine
- b) only threonine
- c) lysine and arginine
- d) cysteine and glycine

27. Which histone H3 methylations are associated with transcriptional activation?

- a) H3K9 and H3K27
- b) H3K4 and H3K36
- c) H3K9 and H3K36
- d) H3K27 and H4K20

28. Which enzyme catalyzes histone acetylation?

- a) HDAC
- b) DNMT
- c) HAT
- d) HMT

29. What is the main effect of histone acetylation on chromatin?

- a) increased chromatin condensation
- b) inhibition of transcription
- c) increased chromatin relaxation
- d) formation of heterochromatin

30. Which of the following histone post-translational modifications are well characterized?

- a) phosphorylation
- b) ubiquitination
- c) acetylation
- d) none of the above

31. Epigenetic signals are considered dynamic because they:

- a) can change over time depending on developmental stage and environmental factors
- b) are potentially reversible and respond to cellular signals
- c) are fixed by the DNA sequence and do not change during a cell's lifetime
- d) can be actively maintained or removed by specific enzymes

32. Epigenetic differences between monozygotic (MZ) twins change over the course of life such that:

- a) in early childhood, epigenetic profiles of young MZ twins are almost identical
- b) older MZ twins show pronounced divergence of the epigenome
- c) changes involve global content and genomic distribution of 5-methylcytosine in DNA
- d) changes in histone acetylation affect their gene expression profiles

33. During embryonic development, the cellular environment plays a key role because:

- a) signals from surrounding embryonic cells can induce transcriptional changes in the target cells
- b) interactions between cells lead to differentiation of specialized cell types
- c) the cellular environment has no influence on gene regulation during development
- d) inductive signals mediate cellular responses to the local environment

34. Which statement about stem cell differentiation is correct?

- a) totipotent cells can differentiate into any tissue but cannot form a whole organism
- b) pluripotent cells can differentiate into all cells and form a whole organism
- c) totipotent cells can form any tissue and a whole organism, whereas pluripotent cells can form only cells of the three germ layers
- d) unipotent cells can differentiate into multiple tissue types

35. What do Prader-Willi and Angelman syndromes have in common, and how do they differ?

- a) both are associated with a missing region on chromosome 15, but Prader-Willi results from loss of the paternal gene and Angelman from loss of the maternal gene
- b) both cause obesity and hyperactivity in newborns
- c) both are associated with a missing region on chromosome 15, but Prader-Willi results from loss of the maternal gene and Angelman from loss of the paternal gene
- d) both have identical clinical manifestations and genetic origin

36. Which syndrome belongs to imprinting disorders?

- a) Down syndrome
- b) Beckwith-Wiedemann syndrome
- c) Wiedelstein syndrome
- d) Duchenne-Becker muscular dystrophy

37. Which molecular method can be used to detect differentiation of maternal and paternal alleles of the *SNRPN* gene in the diagnosis of Prader-Willi syndrome?

- a) classical PCR-RFLP
- b) immunohistochemical method
- c) allele-specific real-time PCR
- d) methylation-specific PCR-RFLP

38. How does the paternal genome behave after fertilization?

- a) it is actively demethylated
- b) methylation stays unchanged
- c) it is passively demethylated during subsequent mitotic divisions
- d) only imprinted genes are demethylated

39. During puberty and gametogenesis, methylation in germ cells:

- a) decreases to a minimum and remains low
- b) is re-established in a developmentally specific manner (*de novo* methylation)
- c) disappears completely and is not regained
- d) is the same as in the embryo

40. What is the most common cause of Beckwith-Wiedemann syndrome (BWS)?

- a) change in chromosome number
- b) nucleotide substitution
- c) change in DNA methylation
- d) change in RNA methylation

41. Can a mother's lifestyle during pregnancy influence the epigenome of her grandchild?

- a) yes, if the fetus is male, because his testes already contain primordial germ cells of future sperm
- b) no, epigenetic changes are transmitted only directly from parent to child
- c) no, the epigenome of the next generation cannot be influenced by environment or lifestyle
- d) yes, if the fetus is female, because her ovaries already contain primary oocytes that may undergo epigenetic changes

42. What is the difference between CpG island methylation in DNA and methylation of amino acids in histone tails?

- a) CpG island methylation has the same effect on gene expression as histone methylation
- b) CpG island methylation suppresses gene expression
- c) histone methylation can either activate or repress gene expression
- d) histone methylation only represses gene activity

43. Which of the following can lead to long-term epigenetic changes in humans?

- a) diet and lifestyle during pregnancy
- b) several hours of stress in an adult
- c) long-term exposure to toxins
- d) short physical activity

44. Gene imprinting means:

- a) a gene is expressed from only one allele, depending on parental origin
- b) a gene is always expressed equally from both alleles
- c) a gene changes its sequence during embryonic development
- d) imprinting is only a temporary change during adolescence

45. Small non-coding RNAs (e.g., miRNAs) can influence gene expression by:

- a) degrading mRNA or blocking its translation
- b) increasing CpG island methylation
- c) activating histone acetylation
- d) changing the DNA sequence

46. Which of the following situations is an example of environmentally induced epigenetic change?

- a) increased maternal stress during pregnancy affecting the fetal epigenome
- b) mutation in the DNA of the *p53* gene
- c) short-term physical activity in an adult
- d) change in pH in the digestive tract

47. What of the following is true about CpG islands in the genome? They:

- a) are often located in gene promoters
- b) mainly contain cytosines and adenines
- c) are regions of histones with increased acetylation
- d) are found only in mitochondrial DNA

48. Which of the following factors does not cause epigenetic modifications?

- a) diet and lifestyle
- b) stress and hormonal changes
- c) direct change in DNA sequence
- d) exposure to toxins

49. Which enzyme removes a methyl group from DNA?

- a) HAT
- b) deubiquitinase
- c) TET
- d) Dicer

50. Which enzyme adds a methyl group to DNA?

- a) DMTN
- b) DNMT
- c) TET
- d) HAT

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. FTFT | 18. FTTT | 35. TFFF |
| 2. FTTF | 19. TFTT | 36. FTFF |
| 3. FFTF | 20. FTTF | 37. FFTT |
| 4. FTFF | 21. FTTF | 38. TFFF |
| 5. FTFF | 22. FTTT | 39. FTFF |
| 6. FFTT | 23. TFTT | 40. FFTF |
| 7. FFTF | 24. FTTT | 41. FFFT |
| 8. FTTF | 25. TTFF | 42. FTTF |
| 9. FTFF | 26. FFTF | 43. TFTF |
| 10. FTTT | 27. FTFF | 44. TFFF |
| 11. FTTF | 28. FFTF | 45. TFFF |
| 12. TTTF | 29. FFTF | 46. TFFF |
| 13. FTFT | 30. TTTF | 47. TFFF |
| 14. FTTT | 31. TTFT | 48. FFTF |
| 15. FTTF | 32. TTTT | 49. FFTF |
| 16. TFTT | 33. TTFT | 50. FTFF |
| 17. TFFF | 34. FFTF | |

14. Biological Aspects of Regenerative Medicine. Biology and Its Application in Advanced Therapies.

Biological Aspects of Regenerative Medicine

Regenerative medicine is an interdisciplinary field focused on the restoration or replacement of damaged tissues and organs. It is closely related to **tissue engineering**, which combines **cells**, **biomaterials** (scaffolds), and **bioactive molecules** to create functional **tissue substitutes**. These components (cells, scaffolds, and bioactive molecules) are commonly referred to as the “triad” of tissue engineering and regenerative medicine.

Scaffolds represent three-dimensional (3D) matrices composed of **biocompatible** and **biodegradable** materials that primarily provide **mechanical support** for cells. They enable cell attachment, proliferation, and differentiation, while also ensuring nutrient transport. If cells successfully adapt to the scaffold and are provided with a suitable environment, they are able, over time, to cover the entire scaffold surface and form an *in vitro* **tissue construct**. For scaffolds to be applicable in regenerative medicine, they must fulfill several criteria: **biocompatibility**, **controlled degradation** (biodegradation), appropriate **mechanical properties**, and a **porous structure** (which ensures diffusion of nutrients and oxygen as well as removal of metabolic waste). Ideally, the mechanical-physical, biochemical, and biological properties of scaffolds should mimic the key characteristics of the **extracellular matrix** of the tissue intended to be repaired through regenerative medicine. In this way, an optimal microenvironment for seeded cells can be achieved.

The **extracellular matrix** represents a complex **network of macromolecules** located within the intercellular space of tissues. It is produced by the cells themselves, and its **most important components** include collagen, elastin, glycoproteins, proteoglycans, and glycosaminoglycans. Its functional importance lies in providing **mechanical support** and **structural integrity** to the tissue. From a biological perspective, it is essential in modulating cell behavior, **regulating** adhesion, migration, proliferation, and differentiation. It also serves as a **reservoir** of growth factors and plays a key role in tissue healing and regeneration. **Specialized materials** are used for scaffold fabrication, which can generally be divided into five main groups: **synthetic** polymers (e.g., polycaprolactone, polyvinyl alcohol, polyethylene glycol), **natural** polymers (e.g., collagen, hyaluronic acid, polysaccharides), **ceramics** (e.g., aluminum oxide, tricalcium phosphate), **hybrid materials** (combining synthetic and natural polymers), and **hydrogels**. The selection of a specific material depends on the type of regenerated tissue and the intended clinical application, taking into account mechanical properties, biocompatibility, degradation rate, and the ability to support cell adhesion and differentiation. For example, ceramic-based scaffolds have proven effective in the regeneration of hard tissues. Synthetic materials offer excellent mechanical properties and controllable degradation; however, their biological performance is often less favorable compared to natural materials.

In contrast, natural polymers provide a favorable biological response; however, their mechanical and physicochemical properties are often inferior. For this reason, **hybrid material**-based scaffolds are currently highly attractive, as they combine the advantageous properties of both polymer groups. Hydrogels are defined as **3D polymer networks** capable of binding large amounts of water. They provide highly suitable conditions for cells and also serve as a key component in bioprinting. **Bioprinting** represents a scaffold fabrication technology in which cells suspended in hydrogels are printed into a desired 3D structure. Among other technological approaches, **3D printing** is used to fabricate the scaffold alone in the required

shape, which is subsequently colonized with cells after printing. Both bioprinting and 3D printing are classified as **additive manufacturing** techniques. Other scaffold **fabrication processes** include electrospinning, gas foaming, lyophilization, decellularization of native tissues, and others.

Cells represent another key element of regenerative medicine and tissue engineering. One of the most critical factors determining the successful development of a tissue construct is the ability to generate a **sufficient number** of cells under laboratory conditions. It is also essential that cells maintain their **phenotype** and **biological functionality** after implantation into a living organism. Following *in vivo* application, cells should be capable of producing a properly organized extracellular matrix and responding to biochemical and physical stimuli. **Various cell types** are used in regenerative medicine and tissue engineering, **differing** in their origin, differentiation potential, and clinical applicability. The fundamental group consists of **stem cells**, which possess the capacity for **self-renewal** and **differentiation** into multiple cell lineages.

According to their **differentiation potential**, cells are classified as totipotent, pluripotent, multipotent, oligopotent, and unipotent. **Totipotent cells** (e.g., the zygote) have the capacity to generate an **entire organism**, including extraembryonic structures. However, their use is limited by ethical and legislative considerations. **Pluripotent** stem cells, which include embryonic stem cells and induced pluripotent stem cells (iPSCs), are capable of differentiating into cells of **all three germ layers** (ectoderm, mesoderm, and endoderm), and therefore into virtually any cell type of the body. iPSCs are generated by **reprogramming** somatic cells (e.g., fibroblasts) back into a pluripotent state, thereby circumventing the ethical concerns associated with embryonic stem cells and enabling autologous applications. The most frequently utilized cell population in clinical practice consists of **multipotent mesenchymal stem cells** (MSCs), which are primarily found in bone marrow, adipose tissue, and umbilical cord blood. MSCs are capable of differentiating predominantly into mesenchymal lineage cells, such as **osteoblasts**, **chondrocytes**, and **adipocytes**. In addition, they exhibit significant immunomodulatory and paracrine effects. Their ability to modulate the microenvironment through the secretion of growth factors and cytokines is crucial for promoting tissue regeneration. Differentiated **somatic cells** derived from specific tissues are also commonly used in tissue engineering applications, for example, chondrocytes for cartilage regeneration, keratinocytes for burn treatment, myoblasts for muscle regeneration, or hepatocytes for liver tissue modeling. The advantage of autologous somatic cells lies in the **reduced risk** of immune rejection.

A specific category is represented by cells with **immunological** or **regenerative potential**, such as genetically modified T lymphocytes used in advanced therapy medicinal products (ATMPs), or cells modified by gene editing to enhance their therapeutic efficacy. Overall, the **selection of a cell source** in regenerative medicine and tissue engineering depends on the **target tissue**, the required functional outcome, cell availability, immunological considerations, and safety profile. **Autologous** stem cells and iPSC technology appear particularly promising, as they enable a **personalized** therapeutic approach while minimizing the risk of graft rejection.

Bioactive molecules constitute the third, **regulatory component** of the triad of regenerative medicine and tissue engineering. These are proteins or peptides that bind to specific receptors on the cell surface and **activate intracellular signaling pathways**. Through these mechanisms, they influence cell proliferation, differentiation, migration, angiogenesis, and extracellular matrix synthesis. Among the most frequently used growth factors are transforming growth factor beta (**TGF- β**), bone morphogenetic proteins (**BMPs**), vascular endothelial growth factor

(VEGF), and fibroblast growth factor (FGF). The importance of biologically active molecules lies in their ability to **mimic** the natural **regenerative processes** occurring in the body. Following tissue injury, cytokines and growth factors are released at the site of damage, where they regulate the inflammatory response, activate stem and progenitor cells, and initiate subsequent tissue repair. In tissue engineering, these signals are applied in a **targeted manner** - either incorporated directly into scaffolds or delivered via cells that produce them. Without biological signals, cells would lack the necessary instructions regarding the direction of differentiation and the specific function they are expected to perform. Growth factors, therefore, ensure **control** over the quality and type of newly formed tissue. Moreover, they promote **vascularization**, which is essential for the survival of the implanted construct. For these reasons, bioactive molecules represent an integral part of the tissue engineering triad. They function as the regulatory element that connects cells and scaffolds into a functional unit, enabling **controlled, efficient, and biologically relevant tissue regeneration**.

Biology and Its Application in Advanced Therapies

Biology plays a crucial role in the development of **modern** therapeutic approaches. An in-depth understanding of physiological and pathological processes at the cellular and molecular levels enables the **identification** of disease causes, **clarification** of underlying mechanisms, and the design of targeted treatment strategies. The study of genetic information, regulatory and signaling pathways, as well as the use of cellular and tissue models, has led to the identification of diagnostic **biomarkers** and the development of **innovative therapies** for serious diseases, including oncological, genetic, and autoimmune disorders. As a result, there has been a shift toward **personalized medicine**, which takes into account the individual biological characteristics of each patient. Among the so-called **advanced therapies** are gene therapy, cell therapy, regenerative medicine, and immunotherapy. In regulatory terms, however, Advanced Therapy Medicinal Products (ATMPs) primarily refer to gene therapy medicinal products, somatic cell therapy medicinal products, tissue-engineered products, and combined ATMPs. These represent innovative therapeutic strategies based on the use of cells, genes, bioactive molecules, advanced materials, or their combinations, with the aim of improving patient health.

Gene therapy (see Chapter 13) is a medical technology designed to achieve a therapeutic effect through the manipulation of gene expression or the modification of the biological properties of cells. Genes are introduced, repaired, or replaced within cells in order to eliminate the cause of disease at the **genetic level**, rather than merely alleviating its symptoms. The fundamental approaches in gene therapy include **gene replacement, gene silencing, gene addition, and genome editing**. The outcome may involve mutation correction, targeted inhibition of gene expression, or conversely, increased expression of a desired gene. A critical step in gene therapy is the delivery of genetic information into the target cell. For this purpose, **vectors** are employed, which may be either viral or non-viral.

Viral vectors (e.g., lentiviruses, adenoviruses, or adeno-associated viruses—AAV) are genetically modified to be non-pathogenic while efficiently transferring genetic material into cells. Their advantage lies in high gene transfer efficiency; however, potential disadvantages include immunogenicity or the risk of unintended genetic alterations. **Non-viral** vectors include plasmid DNA, lipofection, electroporation, and sonoporation. They are characterized by lower immunogenicity and simpler production, but often exhibit reduced gene expression efficiency. Consequently, ongoing research focuses on improving their targeting capacity and stability. From a **methodological perspective**, gene therapy can be classified as *in vivo*, where genetic material is administered directly into the patient's body, or *ex vivo*, where patient-derived cells

are genetically modified outside the body and subsequently reintroduced. A typical example of *ex vivo* gene therapy is **CAR-T cell therapy** used in the treatment of certain leukemias.

Cell therapy represents a modern therapeutic approach based on the administration of living cells in order to restore, replace, or modulate damaged tissues and biological functions of the organism. Unlike conventional pharmacotherapy, where the active substance is a chemical molecule, in cell therapy the cell itself serves as the therapeutic agent. It constitutes a dynamic biological system capable of responding to the microenvironment, producing bioactive molecules, communicating with surrounding cells, and participating in **regeneration** or **immunomodulation**. From a clinical perspective, cell therapy is particularly significant in areas where traditional treatment cannot ensure the restoration of functional tissue. It is applied in hematology (hematopoietic stem cell transplantation), oncology (e.g., adoptive cell immunotherapy), orthopedics and traumatology (support of cartilage and bone regeneration), cardiology (attempts to restore myocardium after infarction), neurology, and in the treatment of chronic wounds.

In addition to direct cell replacement, the **paracrine effect** plays a crucial role—cells secrete growth factors, cytokines, and extracellular vesicles that modulate inflammation, promote angiogenesis, and stimulate endogenous regenerative mechanisms. From the perspective of medical biology, cell therapy represents a direct application of fundamental knowledge regarding cellular structure, function, and regulation.

It is based on an understanding of the cell cycle, differentiation, signaling pathways, cell–cell and cell–extracellular matrix interactions, as well as mechanisms of apoptosis and immune responses. The success of therapy depends on the ability of transplanted cells to survive, integrate into host tissue, and **respond** to local biological signals. Therefore, a thorough understanding of the molecular mechanisms regulating cell proliferation, migration, and differentiation is essential for safe clinical application. Medical biology also provides the framework for assessing the risks associated with cell therapy, such as **adverse immune reactions, uncontrolled proliferation, or tumorigenicity**.

Knowledge of genetic stability, epigenetic alterations, and the influence of the microenvironment on cellular phenotype is equally important. Modern methods of molecular biology, cytometry, imaging techniques, and advanced culture systems enable detailed characterization of cells prior to clinical use. Cell therapy thus represents a bridge between experimental biology and clinical medicine. Both **somatic** cells and **stem** cells, including **induced pluripotent stem cells**, may be applied.

Immunotherapy is a therapeutic approach that deliberately modulates the patient's immune system. It may have either an **activating** or **suppressive** character and is primarily used in oncology, autoimmune diseases, and immunodeficiencies. Among the most important forms of activating immunotherapy are checkpoint inhibitors, monoclonal antibodies, CAR-T cell therapy, cytokine therapy, and therapeutic vaccines. These therapies enable targeted intervention against tumor cells with the potential for long-term effects. Conversely, immunosuppressive therapy is applied in organ transplantation and autoimmune diseases, where excessive immune activity must be suppressed. It includes the use of glucocorticoids, cytostatics, immunophilin inhibitors, and biological agents.

Advanced therapies represent a significant advancement in modern medicine and open new possibilities for the treatment of diseases that were previously considered incurable. Owing to

the rapid progress in biological sciences and gene and cell technologies, medicine is increasingly moving toward **individualized** and **targeted treatment** approaches with the potential to substantially improve patients' quality of life.

Table: Summary of advanced therapies.

Therapy	Basic principle	Advantages	Risks / limitations	Clinical application
Gene therapy	Modification or regulation of gene expression to eliminate the cause of disease	Causal therapy, long-term effect, high specificity	Immune response, off-target effects, high technical demands	Monogenic diseases, tumors, hereditary diseases
<i>Ex vivo</i> gene therapy (CAR-T)	Genetic modification of cells outside the patient's body followed by their re-administration	High efficacy and precise targeting	Cytokine storm and high costs	Leukemias and lymphomas
Cell therapy	Replacement or support of the function of damaged cells	Tissue regeneration and immunomodulation	Short lifespan of cells and variable therapeutic effect	Degenerative and inflammatory diseases
Mesenchymal stem cells (MSCs)	Paracrine, anti-inflammatory, and regenerative effects	Low immunogenicity and broad therapeutic potential	Dependence on the microenvironment	Regenerative medicine and autoimmune diseases
Induced pluripotent stem cells (iPSCs)	Reprogramming of adult cells into a pluripotent state	Ethically acceptable with high differentiation potential	Risk of genetic instability	Disease modeling and personalized medicine
Regenerative medicine/tissue engineering	Replacement or restoration of tissues using cells and biomaterials	Restoration of both tissue structure and function	Technological and financial complexity	Skin, cartilage, and urinary bladder
Activation immunotherapy	Activation of the immune system against disease	Target specificity and long-term effect	Autoimmunity and inflammatory reactions	Oncological diseases
Immuno-suppression therapy	Suppression of excessive immune response	Prevention of rejection and control of autoimmunity	Infections and systemic adverse effects	Transplantation and autoimmune diseases



Model questions

1. Regenerative medicine focuses on:

- a. the diagnosis of genetic diseases
- b. the restoration or replacement of damaged tissues and organs
- c. the development of antibiotics
- d. pain suppression

2. The triad of tissue engineering consists of:

- a. cells, genes, vaccines
- b. cells, scaffolds, bioactive molecules
- c. cells, cytokines, antibodies
- d. DNA, RNA, proteins

3. The main function of a scaffold is:

- a. production of growth factors
- b. mechanical support for cells
- c. induction of an immune response
- d. providing a suitable surface for cell attachment

4. The porous structure of a scaffold ensures:

- a. higher immunogenicity
- b. diffusion of nutrients and oxygen
- c. removal of metabolites
- d. apoptosis

5. The extracellular matrix is produced by:

- a. only fibroblasts
- b. the tissue cells themselves
- c. only epithelial cells
- d. only chondrocytes

6. Major components of the extracellular matrix include:

- a. hemoglobin
- b. myosin
- c. collagen
- d. insulin

7. The extracellular matrix regulates:

- a. only mechanical support
- b. cell adhesion
- c. cell migration
- d. cell differentiation

8. A synthetic polymer used for scaffold fabrication is:

- a. collagen
- b. polycaprolactone
- c. elastin
- d. fibronectin

9. Ceramic scaffolds are mainly used for the regeneration of:

- a. skin
- b. muscles
- c. hard tissues
- d. bone

10. Hydrogels are:

- a. used in bioprinting
- b. three-dimensional polymer networks
- c. inorganic crystals
- d. capable of binding large amounts of water

11. Bioprinting belongs to:

- a. subtractive manufacturing
- b. additive manufacturing
- c. fermentation processes
- d. electrophoresis

12. Electrospinning is:

- a. a gene therapy method
- b. a method for scaffold fabrication
- c. immunotherapy
- d. a type of cell differentiation

13. A totipotent cell is capable of:

- a. forming only mesoderm
- b. differentiating only into blood cells
- c. forming an entire organism
- d. proliferating, differentiating, and forming extraembryonic structures

14. Pluripotent cells can differentiate into:

- a. only one cell type
- b. cells of all three germ layers
- c. only mesenchymal cells
- d. only epithelial cells

15. Induced pluripotent stem cells are generated:

- a. under laboratory conditions
- b. by reprogramming somatic cells
- c. by mutation of stem cells
- d. by gene therapy

16. The most commonly used stem cells in clinical practice are:

- a. totipotent
- b. embryonic
- c. mesenchymal
- d. multipotent

17. Mesenchymal stem cells are mainly found in:

- a. pancreas
- b. bone marrow and adipose tissue
- c. retina
- d. spleen and small intestine

18. Stem cells exhibit strong:

- a. cytotoxic effects
- b. immunomodulatory effects
- c. mutagenic effects
- d. paracrine effects

19. The advantage of autologous cells is:

- a. higher immunogenicity
- b. lower risk of immune reaction
- c. the donor and recipient are the same patient
- d. shorter lifespan

20. TGF- β belongs to:

- a. enzymes
- b. growth factors
- c. lipids
- d. bioactive molecules

21. VEGF promotes:

- a. ingrowth of new blood vessels into tissue
- b. osteogenesis
- c. angiogenesis
- d. inhibition of proliferation

22. Bioactive molecules bind to:

- a. nuclear DNA
- b. specific receptors on the cell surface
- c. mitochondria
- d. ribosomes

23. Advanced therapies in medicine:

- a. enable personalized, patient-specific treatment
- b. are also based on knowledge from biology
- c. differ from classical pharmacotherapy
- d. include regenerative medicine

24. Gene therapy is aimed at:

- a. symptomatic treatment
- b. manipulation of gene expression
- c. causal treatment of disease
- d. production of stem cells

25. Gene replacement means:

- a. gene silencing
- b. replacement of a defective gene with a functional one
- c. DNA degradation
- d. blockade of translation

26. Gene therapy vectors:

- a. must be safe for the patient
- b. are divided into viral and non-viral
- c. must be produced with high purity and quality
- d. do not need to be targeted

27. A viral vector is:

- a. plasmid
- b. lipofection
- c. adenovirus
- d. electroporation

28. Non-viral vectors have:

- a. lower immunogenicity
- b. lower gene expression efficiency
- c. higher risk of integration
- d. higher toxicity

29. CAR-T therapy is an example of:

- a. in vivo gene therapy
- b. ex vivo gene therapy
- c. immunodeficiency
- d. passive immunization

30. Cell therapy uses:

- a. molecular therapy
- b. living cells as a therapeutic tool
- c. both somatic and stem cells
- d. in certain cases also embryonic stem cells

31. Paracrine effect means:

- a. autocrine apoptosis
- b. the action of cells through secretion of factors affecting neighboring cells
- c. gene mutation
- d. inhibition of immunity

32. Cell therapy is used in:

- a. hematology
- b. orthopedics
- c. oncology
- d. forensic medicine

33. Cells in regenerative medicine:

- a. only stem cells are applied
- b. should interact well with the scaffold
- c. undergo apoptosis on the scaffold surface
- d. both somatic and embryonic stem cells may be applied

34. Immunotherapy can be:

- a. only suppressive
- b. activating or suppressive
- c. regenerative
- d. only cell-based

35. Regenerative medicine:

- a. is based on genes, biomaterials, and cells
- b. growth factors have a regulatory function
- c. interaction of cells with the scaffold determines the formation of a tissue construct
- d. is applicable only in pediatrics

36. Personalized medicine:

- a. is synonymous with pharmacotherapy
- b. aims to provide patients with tailored therapy
- c. includes gene therapy and regenerative medicine
- d. is applicable only under laboratory conditions

37. Immunosuppressive therapy is used in:

- a. treatment of tumors by activating immunity
- b. organ transplantation
- c. promotion of angiogenesis
- d. differentiation of MSCs

38. Glucocorticoids belong to:

- a. checkpoint inhibitors
- b. immunosuppressants
- c. growth factors
- d. drugs that suppress the activity of the immune system

39. Personalized medicine takes into account:

- a. patient's age
- b. individual biological characteristics of the patient
- c. sex
- d. cost of treatment

40. The advantage of induced pluripotent stem cells is:

- a. high immunogenicity
- b. ethical acceptability
- c. high differentiation potential
- d. low plasticity

41. Mesenchymal stem cells mainly differentiate into:

- a. neurons and retinal cells
- b. osteoblasts, chondrocytes, adipocytes
- c. hepatocytes and spleen cells
- d. erythrocytes and lymphocytes

42. A scaffold should be:

- a. compatible with cells
- b. biodegradable
- c. rigid without pores
- d. non-toxic

43. Decellularization means:

- a. genetic mutation
- b. removal of cells from native tissue
- c. apoptosis *in vitro*
- d. RNA translation

44. In regenerative medicine:

- a. the application of embryonic stem cells is strictly regulated ethically and legislatively
- b. somatic cells exhibit a high differentiation potential
- c. somatic cells show differentiation potential after reprogramming
- d. stem cells are capable of self-renewal

45. Scaffold fabrication:

- a. 3D printing can print both cells and material simultaneously
- b. uses both synthetic and natural polymers
- c. bioprinting uses cell-laden hydrogels
- d. additive manufacturing does not allow scaffold production

46. Regenerative medicine restores:

- a. only tissue structure
- b. only tissue function
- c. both tissue structure and function
- d. genetic information

47. Bioactive molecules:

- a. activate intracellular signaling pathways
- b. are carbohydrates
- c. participate in wound healing *in vivo*
- d. do not regulate the formation of new tissue

48. Induced pluripotent stem cells:

- a. can be used to establish a cell culture
- b. are specifically reprogrammed somatic cells
- c. are multipotent
- d. represent a component of personalized medicine

49. Hydrogel is a key component of:

- a. pharmacotherapy of burns
- b. bioprinting
- c. cytometry
- d. PCR

50. Advanced therapies represent:

- a. classical pharmacotherapy
- b. innovative biological therapeutic approaches
- c. exclusively surgical procedures
- d. only experimental treatment without clinical use

Answer Key to Model Questions

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| 1. FTFF | 18. FTFT | 35. FFTF |
| 2. FTFF | 19. FTTF | 36. FTTF |
| 3. FTFT | 20. FTFT | 37. FTFF |
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| 5. FTFF | 22. FTFF | 39. TTTF |
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| 7. FTTT | 24. FTTF | 41. FTFF |
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| 9. FFTT | 26. TTTF | 43. FTFF |
| 10. TTFT | 27. FFTF | 44. TFTT |
| 11. FTFF | 28. TTFF | 45. FTTF |
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| 13. FFTT | 30. FTTT | 47. TFTF |
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| 15. TTFF | 32. TTTF | 49. FTFF |
| 16. FFTT | 33. FTFF | 50. FTFF |
| 17. FTFF | 34. FTFF | |

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