HUMANITAS MEDICAL SCHOOL

Course: Molecular Medicine and Computational Biology (MMCB)

Year: 2nd

Period: 1st semester

Credits: 8 (96 hours)

Objectives

The course will merge molecular biology and medical genetics to give an up-to-date vision of molecular genetics mechanisms involved in human diseases, with a particular focus on genetic disorders. Besides the theoretical part, large space will be given to practical sessions in which the students will learn how to use genomics data present on the web and how to analyze real DNA and RNA sequencing data.
The "Molecular biology" module will be focused on understanding structure, function, and turnover of macromolecules, structure and regulation of genes and genomes, the mutability of the genome and the mechanisms of DNA repair. The impact of recombinant DNA technologies on basic and applied biomedical research will also be illustrated.
The "Medical genetics" module will illustrate the types and extent of genetic variation seen in the human genome, and how this variation affects disease susceptibility. The module will particularly emphasize how molecular and population genetics have impacted on our understanding of the mechanisms of human disease, providing us with novel diagnostic and therapeutic strategies.

Prerequisites

To be allowed to take the MMCB exam students must have passed the exam of "The Cell: Molecules and Processes" (1st year, 2nd semester).

Contents

Part 1. The mutability of the genome

Causes of mutation (Prof. Bottaro)

Spontaneous and induced causes of mutation. Agents that induce mutation. Common mutations due to DNA replication.

Learning objectives**:**

- Describe examples of the main categories of DNA damaging agents
- Describe mechanisms by which DNA can be damaged
- Discuss the relationship between DNA damage and DNA mutation
- Discuss the balance between mutation-inducing mechanisms and DNA repair and its role in evolution

Genetic variation (Prof. Asselta)

Polymorphisms and mutations: classification and functional consequences.

Learning objectives:

- Describe the different types of point mutations
- Discuss differences between germinal and somatic mutations, and between polymorphisms and mutations
- Describe the functional consequences of the different types of mutation at the RNA and protein levels

DNA repair mechanisms (Prof. Bottaro)

Main mechanisms of DNA repair and related diseases.

Learning objectives:

- Discuss the cell responses to DNA damage
- Describe the main mechanisms of DNA repair and the specific damages they are apt to
- **Clinical drop**: Inherited diseases due to defects in the DNA repair systems

Part II. Mendelian genetics: from pedigrees to mutations

Mendelian pedigree patterns and their complications (Prof. Asselta)

How to build up a pedigree. Examples of autosomal dominant, autosomal recessive, X-linked, Y-linked and mitochondrial inheritance. Incomplete penetrance, expressivity, male lethality, de-novo mutations, mosaicism, phenocopies, complementation, and mitochondrial inheritance.

Learning objectives:

- Being able to collect a genetic anamnesis and to draw a pedigree
- Describe the main patterns of inheritance
- Discuss the main complications of the classical Mendelian pattern of transmission

Genetic mapping of Mendelian traits (Prof. Asselta)

Polymorphisms as a tool for genetic mapping, recombinants and non-recombinants, two-point and multi-point mapping, the concept of LOD score.

Learning objectives:

- Understand how polymorphisms can be used to trace Mendelian traits
- Understand the meaning of LOD score in genetic analysis

Prototypic Mendelian diseases: cystic fibrosis and thalassemia (Prof. Asselta)

The quest for the gene causing cystic fibrosis. The organization of the globin loci. Sickle cell anemia. Alpha and beta thalassemia.

Learning objectives:

- Describe a typical approach to identify disease genes
- Describe the gene arrangement of the globin loci
- Illustrate the molecular mechanisms underlying sickle cell anemia and thalassemia
- **Clinical drop**: neonatal screening and genetic diagnosis of cystic fibrosis

Dynamic mutations (Prof. Asselta)

The concept of microsatellite instability. Trinucleotide repeat expansion disorders and their classification. Anticipation. Fragile X syndrome, Huntington disease, myotonic dystrophy.

Learning objectives:

- Describe the general features of trinucleotide repeat expansion disorders

- Understand the concept of anticipation
- Illustrate the molecular characteristics of Fragile X syndrome, Huntington disease, and myotonic dystrophy

Part III. Molecular Biology techniques for Medicine

Essentials of DNA recombinant technology (Prof. Bottaro)

What is rDNA? Tools of rDNA technology, making a recombinant DNA molecule, DNA probes and hybridization, main applications of rDNA.

Learning objectives:

- Understand the principles of rDNA technology
- Understand the concept of genomic and cDNA library
- Be able to describe a cloning experiment
- Know what a molecular probe is and what is a hybridization experiment

Polymerase Chain Reaction (PCR) and Sanger sequencing (Prof. Bottaro)

The discovery of PCR, the principles of a PCR reaction, what can you do with PCR? Sanger sequencing

Learning objectives:

- Discuss why DNA is amplified during a PCR reaction
- Illustrate the main applications of PCR
- Describe the theory of Sanger sequencing
- **Clinical drop:** how we can diagnose a genetic disease by PCR and DNA sequencing

Experimental and Computational methods in Structural Biology (Prof. Bottaro)

Topics: Experimental methods for biomolecular structural determination. Computational approaches protein structure prediction, including machine learning.

Learning objectives:

- Discuss the different methods for determining the three-dimensional structure of proteins and nucleic acids.

- Understand the importance of detailed structural information in biology and medicine
- Illustrate the advantages and shortcomings in computational prediction in structural biology.

Forensic science and Paternity testing (Prof. Asselta)

Topics: Forensic science: kind of traces, DNA fingerprinting. Paternity testing.

Learning objectives:

- Discuss the different methods of identification adopted in the past
- Understand the basic statistics allowing the identification of individuals with high accuracy

Gene therapy (Prof. Soldà)

Overview on the main approaches used for gene therapy. Traditional approaches with their pros and cons and genome editing (CRISPR/Cas9)

Learning objectives:

- Be able to describe the concept of gene therapy
- Understand the differences between in-vivo and ex-vivo gene therapy

Part IV. Regulation of gene expression

Introduction to the multilayer regulation of gene expression (Prof. Soldà)

How cells modulate gene expression. Levels of gene expression regulation: from chemical modification of DNA to post-transcriptional and post-translational regulation. The principles of DNA-protein interaction.

Learning objectives:

- Discuss the importance of gene expression modulation in driving the processes of cellular differentiation and morphogenesis
- Discuss the principles of transcriptional regulation in prokaryotes and eukaryotes
- Describe the main protein modules interacting with DNA

The transcriptional regulation (Prof. Soldà)

The transcriptional regulation of gene expression in eukaryotes. Enhancers, silencers and insulators. The epigenetic regulation of gene expression.

Learning objectives:

- Understand the concept of in-cis and in-trans regulation
- Discuss the interaction between modulation by transcription factor and chromatin status
- Explain how epigenetic information influences gene expression

Post-transcriptional regulation (Prof. Soldà)

Transcription attenuation. Alternative splicing, differential polyadenylation, RNA editing. Control of mRNA localization and stability.

Learning objectives:

- Explain the mechanism of attenuation in Bacteria
- Understand the importance of alternative splicing regulation
- Describe examples of post-transcriptional regulation
- Understand the role on nonsense-mediated mRNA decay
- Describe mechanisms regulating mRNA stability

Noncoding RNAs (Prof. Bottaro)

Topics: The non-coding RNA revolution. Overview on the main classes of non-coding RNAs. Post-transcriptional regulation by small RNAs in prokaryotic and eukaryotic organisms. Long non-coding RNAs: structural features and mechanism of action. Competing endogenous RNAs.

Learning objectives:

- Explain the importance of RNA-mediated gene expression regulation
- Describe the main classes of noncoding RNAs (small and long)
- Illustrate the features of long noncoding RNAs
- Recognize the complexity of mechanisms of action of long noncoding RNAs

Small RNAs (Prof. Soldà)

RNA interference. Post-transcriptional regulation by small RNAs in eukaryotic organisms.
Learning objectives:

- Illustrate the mechanism of RNA interference
- Describe microRNA biogenesis and mechanism of action
- **Clinical drop:** miRNA therapeutics

Part V. Genetics of complex traits

Population genetics (Prof. Asselta)

Allele frequencies in populations. Hardy-Weinberg equilibrium. Genetic drift, population bottlenecks and founder effects. Genetic selection. The concept of the heterozygote advantage.

Learning objectives**:**

- Understand the applications and limitation of Hardy-Weinberg law
- Distinguish heterozygous advantage from founder effect
- Being able to calculate heterozygote frequency from disease prevalence

Genetics of complex (multifactorial) diseases (Prof. Asselta)

The polygenic and multifactorial nature of common diseases. Estimating the contribution of genetic and environmental factors. Linkage disequilibrium. Principle of allelic association. The example of COVID-19.

Learning objectives:

- Understand the difference between dichotomic and continuous traits
- Describe the concept of linkage disequilibrium.
- Understand the difference between linkage analysis and association analysis

Part VI. Cancer genetics **DIGITAL HEALTH**

Sporadic, Familial and Hereditary cancers (Prof. Asselta)

Epidemiology of cancer & Cancer as genetic disease. Cancer as a hereditary disease. Genetic counselling of cancer. Detecting mosaicism in cancer: from FISH to digital PCR (dPCR). Applications of dPCR to liquid biopsy and monitoring of minimal residual disease.

Learning objectives:

- Describe the basic molecular mechanism leading to cancer
- Describe the genetic landscape of cancers
- Describe the most common inherited cancer syndromes and their molecular mechanisms
- Learn the basic steps that are taken during a genetic counselling for cancer predisposition
- Being able to distinguish differences between FISH, NGS, dPCR (etc)
- Learn the concept of liquid biopsy

Part VII. Genomic Medicine DIGITAL HEALTH

Human genome organization (Prof. Soldà)

Nuclear and mitochondrial genomes. The C-value paradox. Classes of repetitive DNA elements. Gene families. Organization of repetitive elements in the human genome. Segmental duplications.

Learning objectives:

- Discuss the differences between the nuclear and mitochondrial genomes
- List the main classes of genomic sequences, their function and their origin
- Describe the concept of pseudogene and transposon

NGS sequencing (Prof. Bottaro)

DNA sequencing from Sanger to today. Next-generation sequencing (NGS) 2nd and 3rd generation DNA sequencing. Short-read and long-read approaches.

Learning objectives:

- Compare Sanger sequencing and next generation sequencing (NGS)
- Describe the concepts of clonal amplification and parallel sequencing
- List the main technologies for NGS
- Discuss the concept of sequencing depth

NGS for the identification of the molecular basis of Mendelian diseases. From genome sequence to the causative variant (Prof. Soldà)

NGS of whole genomes and exomes as a powerful tool in biomedical research and clinical diagnostics. Targeted resequencing vs whole genome sequencing. Exome sequencing: flowchart and examples on how to design a study. The big challenge to fish out the pathogenic variants. How to find a novel disease gene: from theory to practice.

Learning objectives:

- Compare different NGS-based approaches for the molecular diagnosis of inherited disease
- Illustrate what is an exome and what are the critical steps in a standard exome- sequencing experiment
- Understand the hypotheses underlying different study design choices

Transcriptomics (Prof. Soldà)

Techniques and applications for the analysis of gene expression. Expression microarrays. RNA sequencing. Overview of the pipeline for RNA sequencing and differential gene expression analyses.

Learning objectives:

- Evaluate different different methods to analyze the transcriptome and know when to use them
- Describe the steps involved in data analysis of microarray and RNA-seq experiments

Genome-wide approaches to complex diseases (Prof. Asselta)

LD structure of human genome. Genome-wide association studies (with a focus on COVID-19). Missing heritability. Burden of rare variants.

Learning objectives:

- Describe the modalities for conducting a genome-wide association analysis
- List possible genetic approaches to fill the gap of the missing heritability

What we have learned from omics approaches (Prof. Asselta)

Genomic consortia, Databases. From phenotypes to mutations, to mutations without a phenotype: redefining the classical concept of Mendelian diseases. The problem of incidental findings.

Learning objectives:

- Illustrate the key experiments that lead to the discovery of the LD structure of human genome
- List the principal consortia and publicly available databases with genomic data

Part VIII. An additional level of complexity: Epigenetics

Epigenetics (Prof. Asselta)

Introduction to epigenetics. DNA methylation during development & Genomic imprinting. Chromatin structure & spatial organization of chromosomes. Regulatory RNAs: X-inactivation and sex determination. Environment & Epigenome

Learning objectives:

- Describe apparently unexplained phenomena now enclosed in the term Epigenetics
- Describe the Waddington epigenetic landscape
- Learn the difference between epigenetic and genetic modification
- Describe different functions of DNA methylation
- Describe the developmental process from the DNA methylation point of view
- Describe how genomic imprinting works
- Learn the most common genetic diseases associated with genomic imprinting problems
- Understand the different mechanisms leading to UPD
- Learn the most common genetic diseases associated with aberrant pattern of histone modifications
- Describe the organization of the nucleus, in terms of pores, lamina, and internal structures/granules
- Describe chromosome territories and TADs
- Learn the most common genetic diseases associated with a dys-organization of the nucleus structure
- Learn the basic modes of sex determination
- Describe the molecular mechanisms leading to X inactivation
- Describe how environmental factors can influence the epigenome

RNA-based pathogenic mechanisms (Prof. Asselta)

MicroRNA-mediated pathogenic mechanisms: implications in Mendelian and complex diseases. The ceRNA hypothesis

Learning objectives:

- Describe the 4 main mechanisms leading to miRNA-based genetic disorders
- Learn how a ceRNA network works, and how they can be related to genetic disorders

Practical Activities

Surfing the genome (Prof. Bottaro)

These practical lessons will allow to acquire confidence with online tools for medical doctors and researchers in the field of molecular genetics. Students will become able to get information on genes, mutations, and associated diseases starting from an anonymous sequence of DNA.

Bioinformatics tools to study miRNA biological activity (Prof. Soldà)

These practical lessons to acquire confidence with online tools and problems that scientists are facing when they start working in the miRNA field. Students will be able to get information on microRNA, their expression and predicted targets, and the effect of variation in the miRNA or miRNA recognition elements.

Teaching Methods

Frontal instructions and group work.

- Questions are always welcome during and after the lectures.
- We strongly encourage you to take notes during lessons, to share materials, and, when possible, to study together, helping each other.
- Slides of the lectures will be uploaded in the LMS platform, where additional material (video, papers, animations) will be posted by lecturers.

Assessment

Students' evaluation will be assessed through a written examination with multiple choice questions. The exam will include 33 questions, proportionally distributed among the different topics. Each question will score 1 point; no penalties will be applied for wrong answers. The

threshold score for passing the exam will be 18 points. In addition, up to +1 point to the final exam grade will be assigned based on participation to practical activities ("Surfing the genome" and "Understanding miRNA biological activity") , provided that the final exam is passed (score >=18).

Textbooks

- Strachan & Read. Human Molecular Genetics 5th edition, Garland, 2018
- Alberts B. et al. Essential Cell Biology 5th edition, Norton, 2018
- Watson J et al. Molecular biology of the gene. 7th edition, Pearson, 2013
- Thompson & Thompson. Genetics in medicine. 8th edition, Elsevier, 2015
- Alberts B et al. Molecular biology of the cell. 6th edition, Garland Science, 2014

Code of conduct

- Students who falsify attendance to classes or any teaching activities will not be allowed to register for any of the dates published for the first exam session of the semester. They will receive an official warning letter from the Dean of the degree program and the Rector of the University. This breach of honor will be officially communicated to all the members of the Teaching Committee. If such behavior is repeated after receiving the first warning, the student will be required to repeat the academic year and the breach will be added to his/her academic career record. Additionally, the student will not be allowed to graduate with laude or with honors.

- Students who do not reach 75% of attendance will be required to pass an allocated part of the exam prior to being admitted to the final exam of the course. This part of the exam must be taken on one of the dates of the session preceding the date they intend to take the final exam. The student will receive a pass or fail evaluation, however, the result will not affect the final grade. Students who do not reach 50% of attendance in all the courses of the semester will have to repeat the academic year unless otherwise decided in the light of specific and well documented reasons, submitted to a commission of the Teaching Committee.