UNIVERSITÀ DI PISA

NEUROGENOMICS

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Anno accademico	2018/19
CdS	NEUROSCIENCE
Codice	418EE
CFU	6

Moduli	Settore/i	Tipo	Ore	Docente/i
NEUROGENOMICS	BIO/18	LEZIONI	48	STEFANO LANDI ENRICA STRETTOI

Obiettivi di apprendimento

Conoscenze
Lo studente apprenderà come affrontare uno studio genetico nel campo delle neuroscienze e ne conoscerà alcune delle basi molecolari
Lo studente si confronterà con esempi di patologie del SNC che possono essere ricondotte a difetti genetici, approfondendo possibili strategie di trattamento sia sperimentali che in fasi avanzate di trial clinico.

Modalità di verifica delle conoscenze
Le conoscenze saranno verificate tramite esame scritto (orale facoltativo)
Per la parte di Physiological genomics, le conoscenze saranno verificate mediante esame orale

Capacità
Lo studente acquisirà la capacità di poter progettare (previo approfondimento) studi di tipo genetico per evincere le componenti genetiche di un dato processo (es. neuropatologico)
Verranno forniti esempi di condizioni neuropatologiche a cui applicare le conoscenze di cui sopra

Modalità di verifica delle capacità
Le capacità acquisite sono verificate tramite verifica di esame

Comportamenti
Lo studente acquisirà occhio critico su come discernere il contributo genetico da quello ambientale in patologie complesse.
Lo studente chiarirà aspetti vantaggiosi e limiti sperimentali e clinici di strategie correttive di malattie genetiche (terapia genica, cellulare etc.)

Modalità di verifica dei comportamenti
I comportamenti acquisiti sono verificati tramite verifica di esame

Prerequisiti (conoscenze iniziali)
Sono richieste le conoscenze tipiche del corso di base di Genetica del triennio.

Corequisiti
E' richiesta una buona preparazione di base nella organizzazione anatomica e funzionale del SNC

Indicazioni metodologiche
Le lezioni sono di tipo frontale

Programma (contenuti dell'insegnamento)
Introduction to the course. Types of polymorphisms in the genomes. Minisatellites, microsatellites.
DNA fingerprinting, instability of microsatellites. The Slippage-misalignment model.
Neurodisorders for aberrant expansion of triplet microsatellites. Single nucleotide polymorphisms (SNPs), micro-insertions, micro-deletions.
Discovery methods: high-resolution melting, single strand conformation polymorphisms, Sanger’s sequencing reaction. Genotyping. DOT

BLOT, PCR-RFLP (restriction fragment length polymorphism), ASO-PCR/ARMS (amplification refractory mutation system). Genotyping of SNPs: oligonucleotide ligation assay (OLA), MALDI-TOF, TaqMan Allelic Discrimination Assay. Microarrays for genotyping. The original method: Single base extension (SBE) - Arrayed Primer Extension Assay (APEX). Genotyping with Illumina BeadArray. The Bead decoding. Genotyping by hybridization. Affymetrix GeneChip. PM and MM set probes. Segmental Duplications. Mandatory and optional. Mechanisms of formation: unequal crossing-over, whole-genome duplications, chromosomal rearrangements. The loci of CYP2D6, GSTM1, GSTT1, and TP53 as example of mandatory and polymorphic duplications and interstitial deletions in the human genome. The example of CYP2D6 in the metabolism of antidepressants and other drugs. Genotyping of polymorphic interstitial deletions and small insertions. The example of GSTT1, GSTM1. Analysis of interstitial deletions within the gene of the Duchenne Muscolar Distrophy. Analysis by gel electrophoresis of PCR products, analysis by Multiplex Ligation-dependent Probe Amplification (also called Multiplex Oligonucleotide Ligation Assay), analysis by TaqMan assay (Real-time quantitative PCR). Comparative Genomic Hybridization (Classical CGH). BAC arrayCGH, tiling BAC arrayCGH. SNP array CGH. Interstitial inversions. The example of 900Kbps inversion polymorphisms within 17q21.31 and susceptibility to mental retardation. The forces shaping allele frequencies in populations. How human genome is organized. Satellite DNA, Sat I, II, III, alphoid sequences, beta-sau, organization of centromeric heterochromatin, G and Q bands. Telomeric Minisatellites, multicopy genes (functional RNAs, duplicated genes, pseudogenes, processed pseudogenes). Retrotransposons: LINEs (LINE-1), SINEs (Alu dimer), Endogenous retroviruses, virus-like elements. Mechanisms of retrotransposition. DNA transposons. Mechanism of transposition with and without transposon duplication. Mapping mendelian traits. The first example: Duchenne's Muscolar Dystrophy. Cloning by subtraction (Kunkel's method). Examples of genes causative for various types of neurological disorders detected by SNP-arrays in micro-interstitial deletions. Linkage analysis. Principles. Example of LOD score calculation. Two-points mapping, multi-point mapping. The multipoint LOD score. The first high density map of genetic markers (CHLC, CEPH). Mapping homozygosity traits exploiting the autozygosity mapping. Chromosomal segments "Identitcal by Descent" (IBD). The "Identity by State" (IBS). Example of calculation of a LOD score in the offspring of second cousins. Candidate region identified with autozygosity mapping. Narrowing the candidate region exploiting a common ancestor in closed populations. Specific examples (cystic fibrosis, Nijmegen Breakage Syndrome, literature, see slides). Fine mapping in pedegrees (dominant model, an example). Identification of the candidate region by the use of ENU-mutagenized mice. Mice helping to discover the gene for human diseases: the examples of Waardenburg syndrome, and mice shaker-2. The basics of the positional cloning. Pitfalls in linkage studies. After the human genome project: gene predictions, prioritizing genes for mutation scan. The examples of Retinitis pigmentosa, marfan syndrome, Beals's syndrome, Wilson's disease, Menkes's disease. Mutation screening of exons or cDNAs? The example of Haemophilia Factor VIII. Possible landscapes following mutation screening:
1) Good correspondence between genotype and phenotype. Carriers/homozygotes must show the phenotype, healthy people within family should not be carriers or homozygotes.
2) Verify if the variant is a simple polymorphism (Genebank).
3) Go for mutation screeening of the same gene on probands of other families- (a) find the same mutation
(b) find a different mutation (in the same gene)
(c) find no mutations
4) Again: verify these mutations are not polymorphisms (Genebank)
5) Inferring a possible deleterious effect:
(a) evaluating the ORF
(b) use of in silico algorithms predicting the effect on the protein
(c) using the conservation of the region by comparing with evolutionary distant organisms (orthologues) available in Genebank
GENETICS NOW: NEXT GENERATION SEQUENCING MAPPING COMPLEX TRAITS (non mendelian diseases). Introduction to case-control association studies. Hypothesis-driven case-control studies. Selection bias. Stratification bias. The error alpha (type-I error). The error beta (power of the study). Calculation of the Odd Ratio and the 95% confidence intervals. Examples of the genetics models (linear, dominant, recessive). From candidate genes (hypothesis driven studies) to genome-wide associations studies (GWAS, hypothesis generating studies). The Manhattan plots. The problems with multiple testing: the Bonferroni's correction. A design multistep for a powerful and cost-effective GWAS. SNO-Microarray: the choice of the correct SNPs: the haplotype tagging SNPs. Linkage disequilibrium (LD) and the calculation of the r2. The forces shaping the LD. The hapmap project (www.hapmap.org). Example of extraction of htSNPs. The correct interpretations of the results: association is not causation.

Program of Dr. Enrica Strettoi course

The module will focus on various diseases of the CNS with recognized underlying genetic defects and will illustrate genotype-phenotype correlations and experimental and clinical stage approaches to improve the disease outcome. The visual system will be often used as a paradigm to illustrate concepts and tools that can be extended to the CNS in general. Lectures involving specific CNS areas (commissural system and callosal body; motor areas pertinent to the mirror system; the hippocampus) will be introduced by brief summaries of the main anatomical organization, relevant morphological features, basic nomenclature and interrelations between structures and the rest of the brain.
Specific topics:

- Cell death in development and disease. Genes that control apoptosis. Bcl-2. Manipulation of neuronal survival and regenerative capacities of the CNS by interference with apoptosis. Neurotrophic theory.
- Regeneration and sprouting in the nervous system. Central and peripheral glia. Inhibitory factors for CNS regeneration (Nogo, Mag, OMGP etc.). Contribution of transcriptomic analysis to identification of extrinsic and intrinsic factors. Visual system, optic nerve transection and damage as experimental paradigms for CNS regeneration studies.
- Connecting two parts of the brain. Commissural system of the brain organization and function. The callosal body. Complete Agenesis of the Callosal Body, pathophysiology and examples of underlying genetic defects.
- The hippocampus. Fundamental organization and functions. Paradigm for experimental studies. Temporal lobe epilepsy. Possible genetic causes.
- Mirror system organization in primates and birds. Autism. Possible genetic causes.
- Gene therapy from bench to bedside. Ongoing gene therapy and open clinical trials for Retinitis Pigmentosa, Leber Congenital Amaurosis, Achromatopsia, X-linked RP. Comparisons with gene therapy for approaches and outcome for Alzheimer and Parkinson diseases.
- From mutations to phenotype: known and (many) unknown cellular pathways to photoreceptor death starting from hundreds of

mutations in 66 different genes. Prevalence of mutations according to geographic distribution.
- Viral Vectors for ocular gene therapy; evolutions, limitations, targeting of specific cells. Optogenetics to restore light sensitivity. State of the art of cell-specific promoters and extension to other genetic diseases.
- Alternatives to gene therapy for neuronal rescue in genetic diseases: Organoids as recent tools for cell-based repair therapy and diagnosis of genetic defects. IPSC organoids from patients with genetic defects. Brain organoid. The brain in a dish. The eye in a dish. Experimental advantages, perspectives and limitations.
- Repairing a genetic disease by using small molecules. Delivering molecules to the eye, delivering molecules to the brain. Brain and retinal blood barriers and the CNS immune privilege. Classical concepts and recent findings: consequences for gene therapy.

Bibliografia e materiale didattico

1) Text book (suggested):
"Genetica molecolare umana ", by Tom Strachan & Andrew P. Read (Zanichelli)
"Introduzione alla Genomica", by Greg Gibson & Spencer Muse (Zanichelli)
"Principles of Neural Science", by Eric R. Kandel, J.H. Schwartz and other Authors (5th edition suggested, but previous editions are also valid)

2) Original articles, recommended websites for specific studies, video material will be provided to the students through direct access to a common site created ad hoc.

Articoli originali, indicazioni di siti web raccomandati per lo studio di argomenti specifici, materiale video etc. saranno forniti agli studenti mediante accesso a un link comune creato per la condivisione di materiale didattico.

Indicazioni per non frequentanti

Tutte le info si trovano nei siti web del corso

Modalità d'esame

L'esame è scritto e prevede una parte di esercizi, una parte come domanda aperta, e una relazione su un articolo scientifico scelto anticipatamente. Una prova orale è facoltativa con possibilità di modificare la votazione dlela prova scritta di -/+ 1 punto

Per la parte di Physiological Genomics (Dott. E. Strettoi) l'esame è orale

Altri riferimenti web

Cercare su e-learning o Moodle
Per il materiale di Physiological Genomics è stato creato un gruppo aperto a tutti gli iscritti dove è raccolto tutto il materiale didattico

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