



UNIVERSITÀ DI PISA

NEUROGENOMICS

STEFANO LANDI

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CdS	NEUROSCIENCE
Codice	418EE
CFU	6

Moduli	Settore/i	Tipo	Ore	Docente/i
NEUROGENOMICS	BIO/18	LEZIONI	56	ALESSANDRO CELLERINO STEFANO LANDI

Obiettivi di apprendimento

Conoscenze

Lo studente apprenderà come affrontare uno studio genetico nel campo delle neuroscienze e ne conoscerà alcune delle basi molecolari

Modalità di verifica delle conoscenze

Le conoscenze saranno verificate tramite esame scritto (orale facoltativo)

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)

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

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

Conoscenze statistiche sarebbero preferibili

Prerequisiti per studi successivi

Il corso fornirà agli studenti gli strumenti necessari per approfondire lo studio della genetica dei tratti quantitativi e dei caratteri complessi

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.



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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 Muscular Dystrophy.

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 Muscular 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.

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E NON DA STEFANO LANDI

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 "Identical 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 pedigrees (dominant model, an example).

Questa lezione non si terra'

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 screening 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

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



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GWAS.

SNO-Microarray: the choice of the correct SNPs: the haplotype tagging SNPs. Linkage disequilibrium (LD) and the calculation of the r^2 . 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.

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)

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 della prova scritta di ± 1 punto

Stage e tirocini

Non previsti

Pagina web del corso

<http://www.stefanolandi.eu>

Altri riferimenti web

Cercare su e-learning o Moodle

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