Modelli animali e studio delle patologie del sistema nervoso umano.

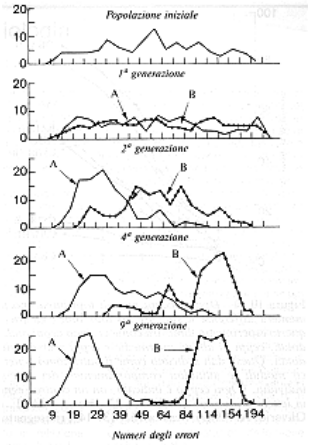
1

Francis Galton: fotografie composite

2

Genetica e comportamento.
I geni specificano un insieme di caratteristiche dei *fenotipi* somatici, ma anche di quelli comportamentali. Quale sia il fenotipo, esso dipende comunque da complesse interazioni tra *genotipo* e ambiente.

3



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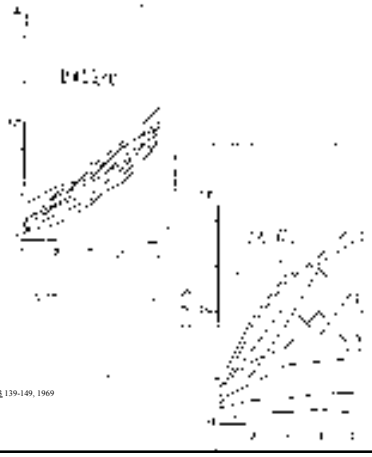
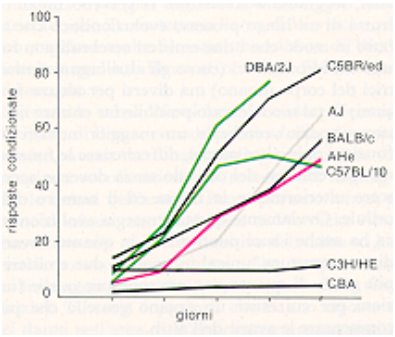


Bovet et al. Science, 163 139-149, 1969

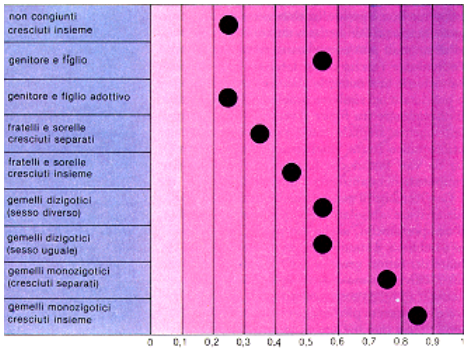
5



Bovet et al. Science, 163 139-149, 1969

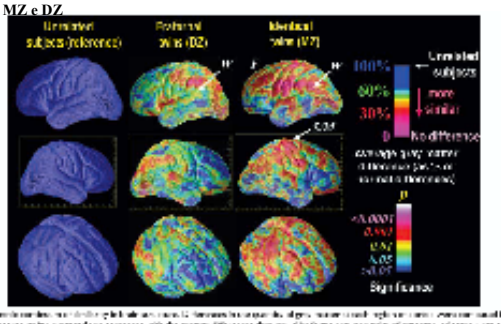
6

I primi studi gemellari



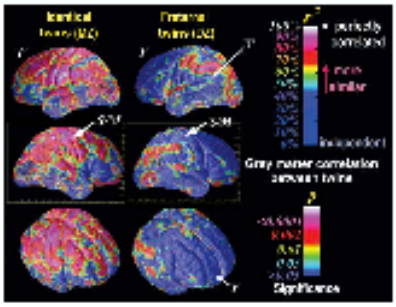
Erlenmeyer Kimling & Jarvik *Science* 1963

Differenze nella quantità di grigia nelle arre frontali, sensorimotorie e di Wernicke in gemelli MZ e DZ



Thompson P.M., Cannon T.D., Narr K.L., van Erp T., Poutanen V.P., Huttunen M., Lönnqvist J., Standertskjöld-Nordenstam C.J., Kaprio J., Khaledy N.M., Dail R., Zoumalan C.I. e Toga A. W. Genetic influences on brain structure. Nature Neuroscienze, 5 November 2001, DOI: 10.1038/nn758

Correlazioni nella distribuzione della grigia in gemelli MZ e DZ



Thompson P.M., Cannon T.D., Narr K.L., van Erp T., Poutanen V.P., Huttunen M., Lönnqvist J., Standertskjöld-Nordenstam C.J., Kaprio J., Khaledy N.M., Dail R., Zoumalan C.I. e Toga A. W. Genetic influences on brain structure. Nature Neuroscienze, 5 November 2001, DOI: 10.1038/nn758

L'approccio genetico alle malattie del sistema nervoso

Nella specie umana vi sono numerose malattie genetiche che comportano alterazioni del sistema nervoso e del comportamento.

Classification

- 3 groups of genetic diseases
- 1. Disorders with multifactorial inheritance (polygenic)
- 2. Monogenic (mendelian) disorders
- 3. Chromosomal aberrations

ALCUNE MALATTIE GENETICHE UMANE

Carattere	Modalità di trasmissione	Effetti sull'efficienza relativa
Lobo dell'orecchio	Autosoma recessivo	Nessuno evidente
Anemia falciforme	Autosoma recessivo	Letali
Galattosemia	Autosoma recessivo	Letali
Fenilchetonuria	Autosoma recessivo	Ritardo mentale, riduzione della longevità
Morbo di Tay-Sachs	Autosoma recessivo	Degen. e morte dei motoneuroni
Daltonismo	Recessivo, legato al sesso	Nessuno evidente
Distrofia muscolare di Duchenne	Recessivo, legato al sesso	Letali
Emofilia	Recessivo, legato al sesso	Emorragie incontrollabili, spesso fatali
Efelidi	Autosoma dominante	Nessuno evidente
M. di Huntington	Autosoma dominante	Si esprime dopo l'età riproduttiva
Retinoblastoma	Autosoma dominante	Tumori oculari, di solito fatali

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Modelli animali di malattie del sistema nervoso su base genetica.

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Modelli per le basi genetiche di un tratto

Models for Genetic Analysis of Behavior

Model	Description
Single gene	One gene controls a defined behavior
Polygenic trait	Additive model that has two or more genes One or more major genes with other genes contributing to phenotype
Multiple genes	Interaction of alleles at different loci generates a unique phenotype

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Modelli Animali

- Indicano che il comportamento dipende da fattori genetici
- Sono state identificate le basi molecolari degli effetti di un singolo gene su
- Esempi
  - –Open-field nel topo
  - –Animali Transgenici

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Malattie genetiche

- Ereditarie – non sempre tramite genetica classica
  - Non sempre tramite DNA (gene silencing)
- Specie specifiche
- Tramite linea germinale (progerie, disordini neurodegenerativi, sindrome di Down etc.) o acquisiti (LMC -leucemia mielogena cronica-, altri tumori). Differenze cellulari (moltiplic. o a riposo)
- In organismi multicellulari complessi

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Malattie neurodegenerative

- *Sono progressive e comportano una perdita fatale di funzioni nervose*
- *Sono stati identificati singoli geni per*
  - –Huntington
  - –Atassia Spinocerebellare
- *Esempi di tratti complessi:*
  - –Alzheimer
  - – Sclerosi laterale amiotrofica (ALS)
  - – Parkinson

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Aspetti relativi agli animali

• Differenze fondamentali con gli umani
– Quantità (raccolta del campione)
– Durata vita media
– Regolazione dell'espressione genica
– Proteine codificate dai geni
– Sistemi immunitari
– Assorbimento, distribuzione, metabolismo e eliminazione dei farmaci.

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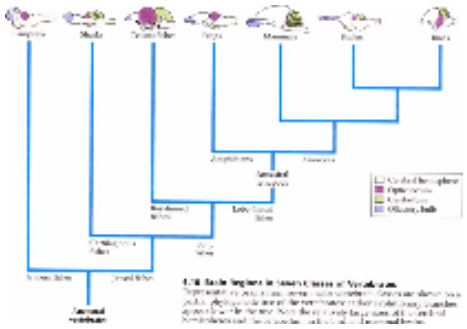
Differenze e somiglianze filogenetiche

20

Somiglianze e differenze



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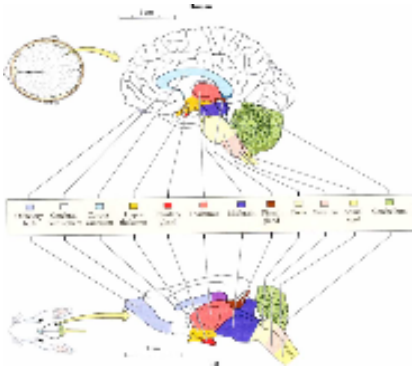
Somiglianze e differenze



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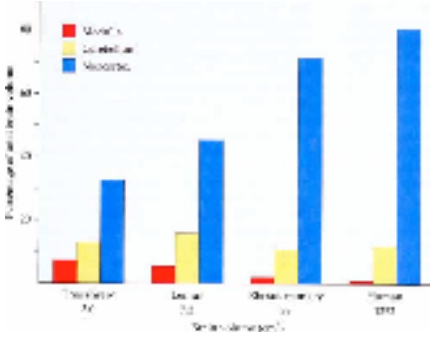
Differenze



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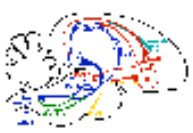
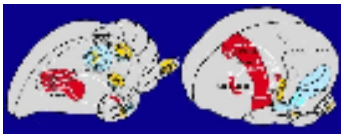
Analogie e omologie

Dalla fisiologia alla neuropatologia

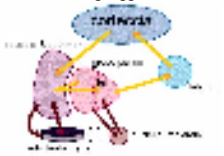
24

Circuiti canori: Zebra finch Teniopigia, estrilidi)





Circuiti linguaggio



25

Le chimere

Produrre animali con caratteri umani

26

Perché creare chimere animale/uomo usando cellule staminali?

-Per esplorare il potenziale di differenziamento delle staminali umane in tessuti animali
-Per testare la sopravvivenza delle staminali umane e la loro integrazione funzionale dopo il trapianto in modelli animali di malattie
-Per generare topi "umanizzati" che possano essere testati in termini di rigetto di staminali umane trapiantate.
-Per generare topi "umanizzati" con organi su cui testare nuovi farmaci (tossicità e efficacia).

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In che fase dello sviluppo si possono trapiantare staminali umane embrionali??

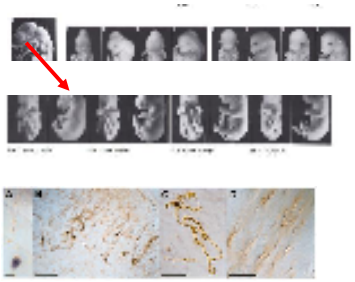
Topo

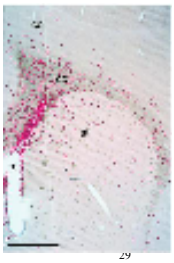
"Trapianto di hES in blastocisti non umane (scimmie escluse) solo in circostanze in cui questa informazione è essenziale "

"Trapianto di cellule hES in feti non umani
Solleva problemi specifici.
Il cervello dovrebbe essere il punto centrale".
Le cellule hES assumono un fenotipo murino?
Le cellule hES generano tutti gli strati germinali?

28

Nature Biotechnology **16**, 1040 - 1044 (1998)
Chimeric brains generated by intraventricular transplantation of fetal human brain cells into embryonic rats
Oliver Brüstle, Khalid Choudhary, Khalad Karram, Anita Hüttner, Kerren Murray, Monique Dubois-Dalcq & Ronald D.G. McKay





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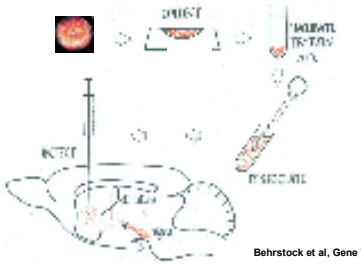
Generazione di chimere topo/umano: Capacità delle staminali di ridurre l'apotosi nel Parkinson

Staminali Fetali umane



Behrstock et al, Gene Therapy 2006

30

L'approccio transgenico

31

Altri studi transgenici

• Studi in *Drosophila*

• Mosche portatrici del gene umano per HD o per l'atassia cerebellare 3

– Dimostrano che le proteine mutanti distruggono i neuroni

– Identificazione dei geni o sostanze che rallentano o provengono la morte cellulare

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Gene *SOD1*

•10% di tutti i casi di SLA sono ereditati come autosomico dominante

•In alcune di queste persone c'è una mutazione del gene *SOD1*

•La mutazione induce una tossicità della proteina *SOD1* nei confronti dei neuroni

•Topi transgenici con un gene mutante *SOD1* sviluppano atonia muscolare simile a quella delle persone affette da SLA

Chapter 18 *Human Heredity* by Michael Cummings ©2006 Brooks/Cole-Thomson Learning

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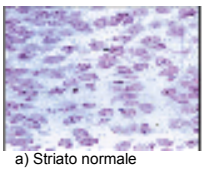
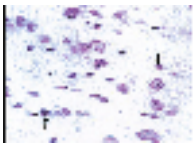
Perdita neuronale in topi transgenici per l'Huntington



a) Striato normale



b) Striato di un topo HD89

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Studi sulla drosophila

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In 2000, Mel Feany and Welcome Bender published a landmark paper, reporting a Drosophila model of Parkinson's Disease.

This paper:

- ❖reported the most accurate animal model of PD to date.
- ❖age-related neurodegeneration specific to dopaminergic cells
- ❖Produced histological hallmarks of PD that had never been observed in vertebrates
- ❖Subsequent investigators built on this model:
  - ❖PD Flies on clinically relevant drugs responded in ways similar to human patients.
  - ❖Responses were consistent with known dose-response curves
  - ❖PD flies could be used to predict human pathology!

Within 6 years, Drosophila geneticists have generated models of

- ❖Huntington's Disease
- ❖Prion/prion-like neurodegeneration
- ❖Fragile X Mental Retardation
- ❖Spinocerebellar Ataxia 1
- ❖Alzheimer's Disease
- ❖And more than 20 others.

Alice Schmid, University of Utah,Eccles Institute of Human Genetics

Why are flies so amenable to models of human neurodegeneration?

Are these models valid?

How similar to humans ARE flies?

Are they useful in drug discovery?

How efficient are they for drug development, as compared to cell-based assays?

Lo studio di un pathway metabolico

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Aging and Neurodegenerative Diseases: Models and Assessment of the Impact and Responses to ROS / RNS*

Kenneth Hensley

Free Radical Biology and Aging Research Program
Oklahoma Medical Research Foundation

*(specie radicaliche dell'ossigeno (ROS) e dell'azoto (RNS)

39

Methods for studying oxidative stress with examples
From recent human and animal studies

What animal models are beginning to tell us about the relationship of oxidative stress to neuroinflammation

40

Oxidative Damage in Neurological Disease

Implicated in:	rodent models:
Alzheimer's disease (AD)	Several genetic models *e.g.* Tg2576, APP/PS1 mice
Amyotrophic lateral sclerosis (ALS, Lou Gherig's disease)	G93A-SOD1, G85R SOD1, other SOD1 mice; ALS2 mouse peripherin mouse
Huntington's disease	R6/2 mouse, 3-nitropropionate
Parkinson's disease	MPTP induced lesions; LPS-induction models
Stroke	Gerbil, rat models for carotid and MCAO occlusion

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Alzheimer's Disease

Characterized by amyloid protein deposition in plaques and by intraneuronal inclusions of various proteins (*eg.* hyperphosphorylated tau)

Glial activation around plaques, and associated neuron damage / death

Region-specific accumulation of oxidative damage that correlates with histopathology

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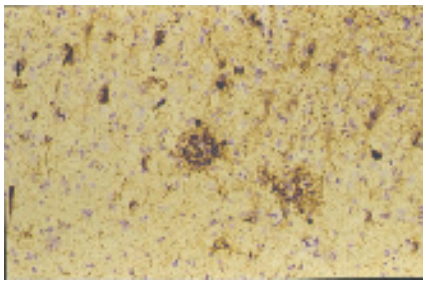
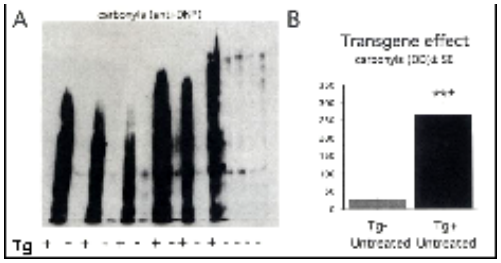
Histopathology of AD: Plaques and Tangles



Histochemistry: anti-phospho-p38 / cresyl violet

43

Mouse models of AD partially reproduce the oxidative damage aspect of the disease



Lim *et al.*, *J. Neurosci.* **21**: 8370-8377 (2001)

44

Oxidative damage in humans and animal models of ALS

ALS is a fatal motor neuron disease causing death of neurons in the spinal cord, brainstem and motor cortex.

It is essentially untreatable (+6 month life extension with the NMDA receptor antagonist riluzole).

Prognosis: Progressive paralysis followed by death in 3-5 years. Death is usually by pneumonia and near complete paralysis.

45

ALS may be sporadic or familial

About 20 % of all ALS cases are heritable

Of these, 20-30% are caused by gain-of-toxic Function mutations in Cu,Zn-SOD (SOD1)
[Deng *et al. Science* **20**: 1047-1051 (1993)]

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{\text{SOD1}} H_2O_2 + O_2$$

SOD1 normally detoxifies ROS; it is unclear what is the toxic gain-of-function associated with mutant SOD1.

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Cu,Zn-Superoxide Dismutase (SOD1)

Mutant SOD1 causes ALS-like disease in mice when ubiquitously expressed (Pramatarova *et al.* 2001; Lino *et al.* 2002)

47

Why do mutant SOD1 enzymes cause motor neuron disease?

SOD1 mutants have increased peroxidase activity and convert H_2O_2 to $^{\bullet}OH$ (Valentine and Bredesen 1996; Yim *et al.* 1997)

Mutant SOD1 lose metals easily. Metal deficient enzymes promote protein nitration and render neurons susceptible to apoptosis (Crow, Beckman *et al.* 1997; Estevez *et al.* 1999)

SOD1 mutants aggregate inside neurons, contribute to toxicity (Bruijn *et al.* 1998)

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Alternative Explanations

Maybe SOD1 mutants exert their pathogenic effects through non-neuronal cells.

astrocytes

microglia

→ **Neuroinflammation:**

- **ROS, RNS**
- **cytokines**
- **apoptosis initiators**
- **limited involvement of lymphocytes**

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Transgenic mice expressing the G93A-SOD1 mutation develop ALS-like disease

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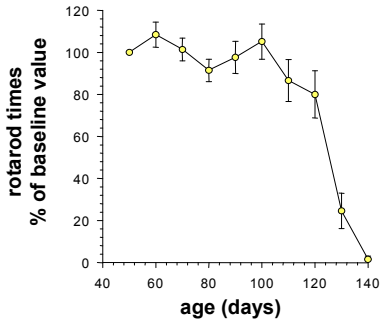
G93A-SOD1 mutant mice experience progressive decline in motor function



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Indicazioni metodologiche

I database

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NCRR Comparative Medicine Biological Resources

Invertebrate Resources
- Bloomington *Drosophila* Stock Center
- *Caenorhabditis* Genetics Center
- National Resource for *Aplysia*
- National Resource for Cephalopods

Non-human Primate Resources
- National Primate Research Centers (8)
- Baboon and Monkey Research Resources (3)
- Chimpanzee Biomedical Research Resources (6)
- Specific-Pathogen-Free Macaque Resources (4)

Zebrafish International Resource Center

Rodent Resources
- Mutant Mouse Regional Resource Centers (4)
- Induced Mutant Resource
- Special Mouse Strains Resource
- *Peromyscus* Genetic Stock Center
- Rat Resource and Research Center
- Transgenic Mice With Altered Calcium Handling Resource

Biological Materials Resources
- Adult Mesenchymal Stem Cell Resource
- National Cell Culture Center
- National Stem Cell Resource
- Viper Resource Center
- Yeast Genetic Stock Center