La Sclerosi Laterale Amiotrofica

Nuovi geni e nuovi meccanismi per capire e curare la malattia

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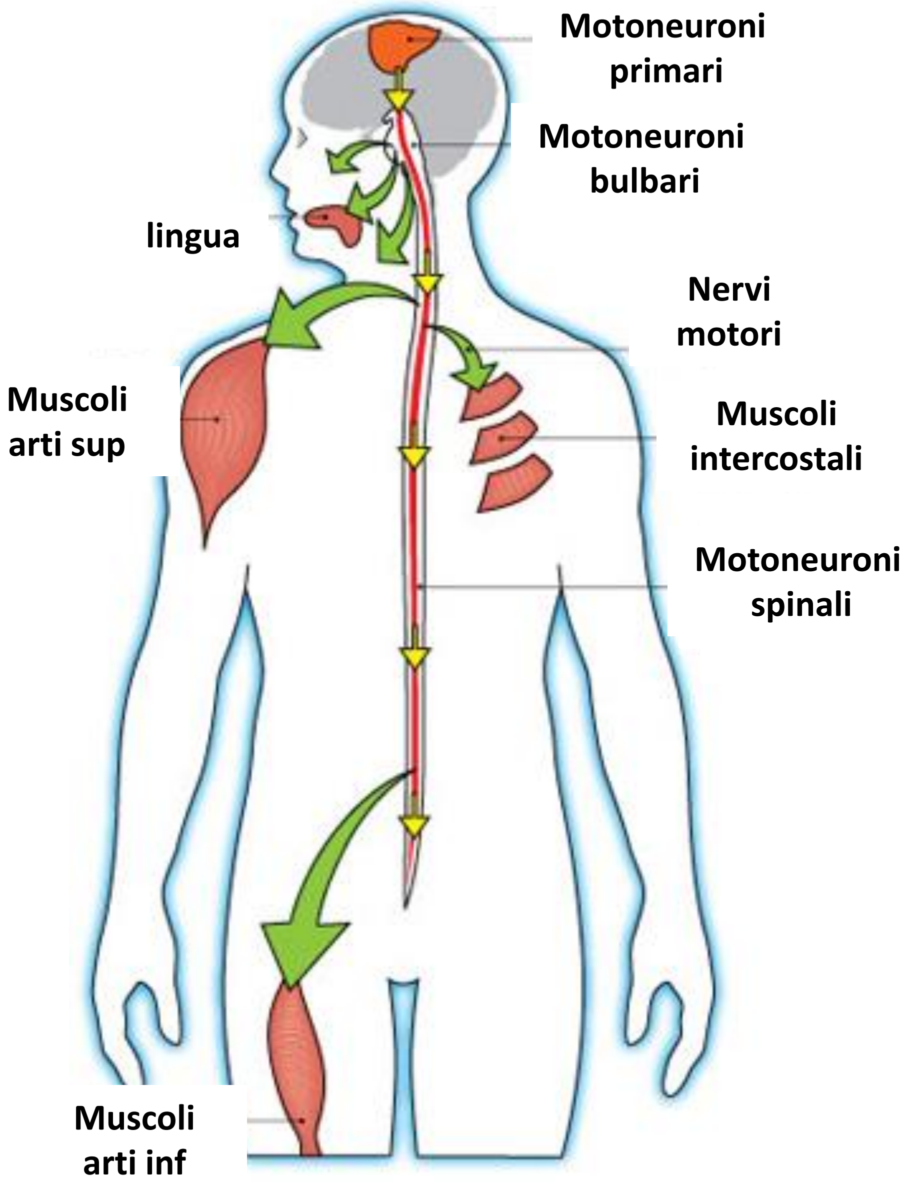
ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI

Uni-ATENeO ® "Ivana Torretta" - Anno Accademico 2014-2015

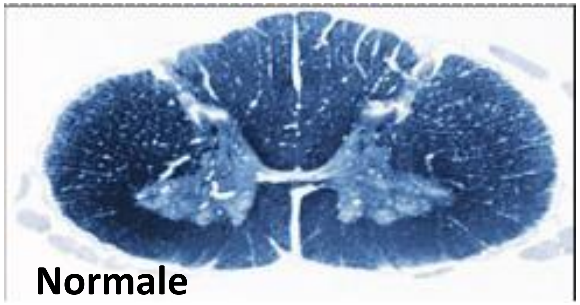
Nerviano, 30 ott 1956

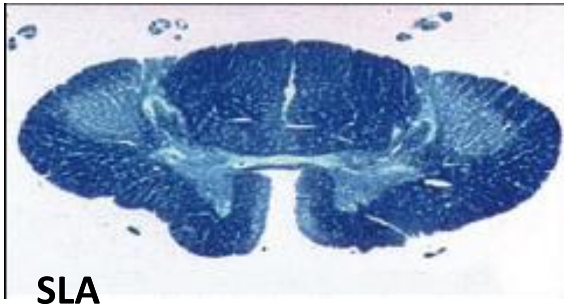
SLA

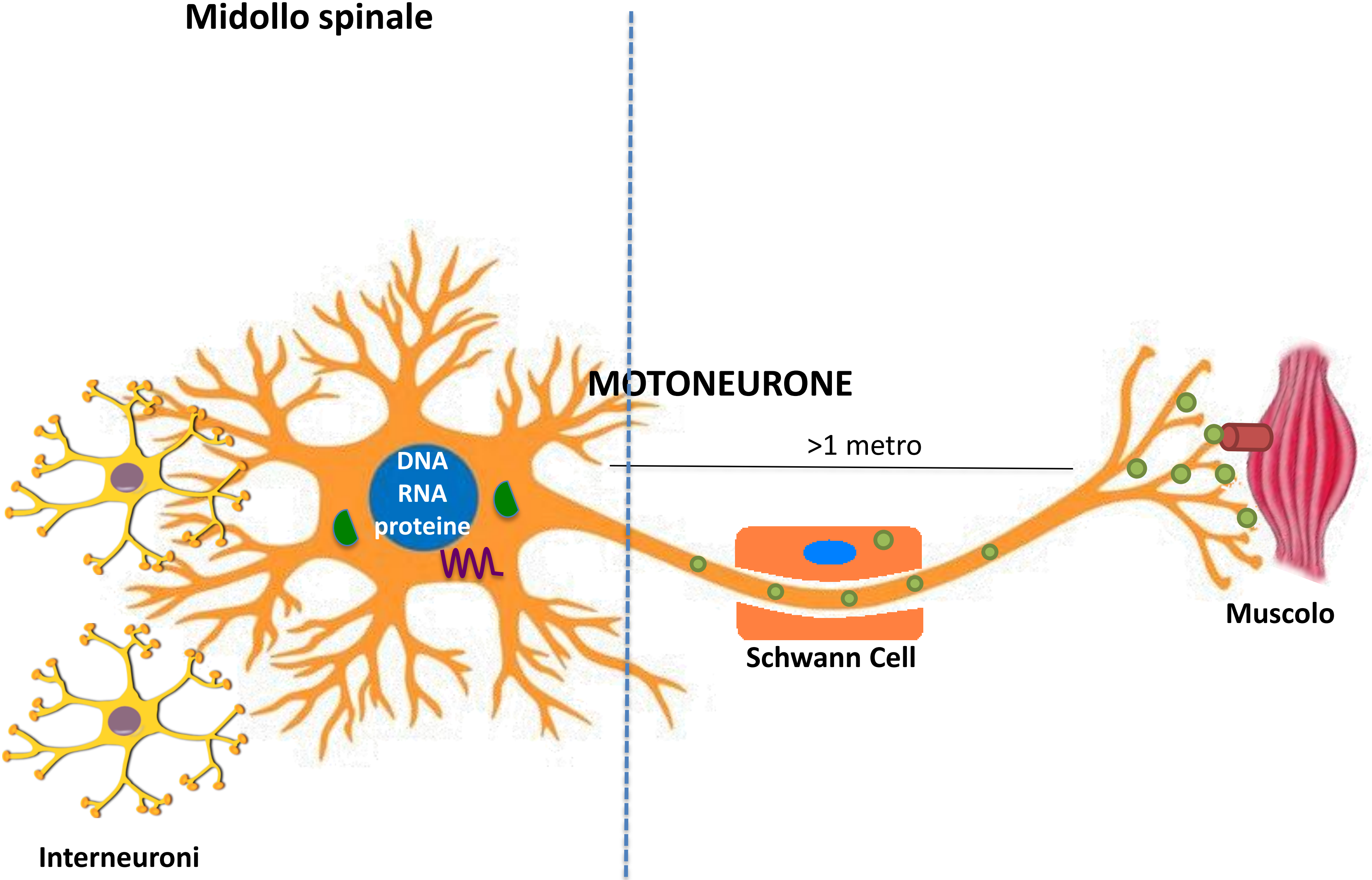
Sclerosi Laterale Amiotrofica
(Malattia del motoneurone- Malattia di Lou Gehrig)


Motoneuroni primari
Motoneuroni bulbari
lingua
Nervi motori
Muscoli arti sup
Muscoli intercostali
Motoneuroni spinali
Muscoli arti inf

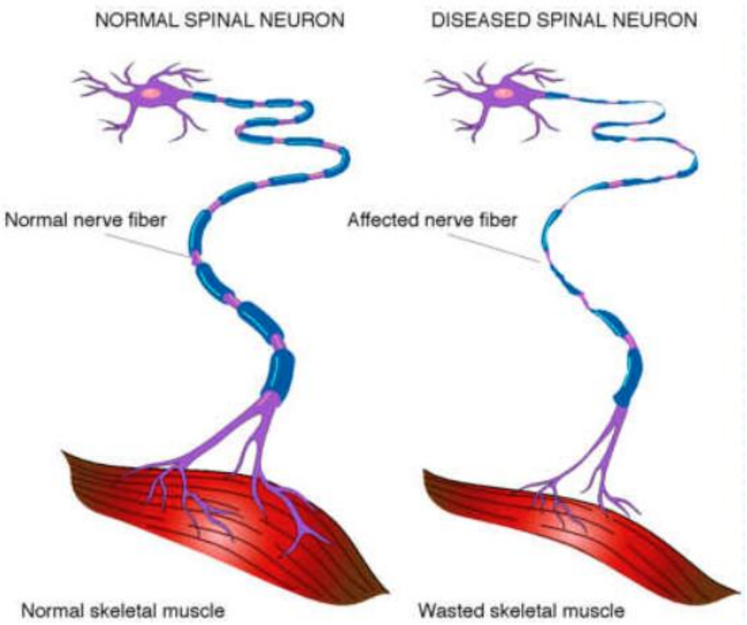
- Degenerazione selettiva dei motoneuroni primari (corticali) e secondari (bulbari e spinali)


Normale


SLA


Midollo spinale
DNA
RNA
proteine
MOTONEURONE
>1 metro
Schwann Cell
Muscolo
Interneuroni

SINTOMI DELLA MALATTIA


NORMAL SPINAL NEURON
DISEASED SPINAL NEURON
Normal nerve fiber
Affected nerve fiber
Normal skeletal muscle
Wasted skeletal muscle

- Debolezza agli arti
- Crampi muscolari
- Fascicolazioni muscolari
- Ipotonia muscolare
- Atrofia muscolare

CONSEGUENZE DELLA MALATTIA

POLO JEANS COMPANY
RL
RALPH LAUREN
SMS
562 e dona 2

Stefano Borgonovo

- **perdita della normale capacità di deglutizione (disfagia)**
- **perdita dell'articolazione della parola (disartria)**
- **perdita del controllo dei muscoli scheletrici (paralisi)**
- **perdita della capacità respiratoria**
- **morte (ipossia, infezioni respiratorie)**
- ***non altera le funzioni cognitive, sensoriali, sfinteriali e sessuali***

EPIDEMIOLOGIA

INCIDENZA: 2-3 nuovi casi/100.000 ab./anno

PREVALENZA: 6-8 casi/100.000 ab. (circa 4000 casi in Italia)

Frequenza leggermente superiore nei maschi (rapporto M/F 1.4-2.5)

DISTRIBUZIONE: ubiquitaria e uniforme

ORIGINE: 10% familiare, 90% sporadica (pressochè indistinguibili)

ETA’ MEDIA DI INSORGENZA: 60-70 anni

DURATA MEDIA: 2-5 anni (20% vive >5 anni e il 10% vive >10 anni)

TEMPO DI DIAGNOSI DAI PRIMI SINTOMI: 17 mesi

FATTORI DI RISCHIO PER LA SLA

DIFETTI GENETICI

- 13 geni associati alla forma familiare (SOD1, Alsin, TDP43…..
- 7-10 geni/loci associati alla forma sporadica (VEGF,NF…..

AMBIENTALI

- Esposizione a metalli (Pb,Al)
- Esposizione a tossine ambientali
  -(cyanobacteria neurotoxin, erbicidi, pesticidi)
- Infezioni virali (HIV; HTLV-1)

STILE DI VITA

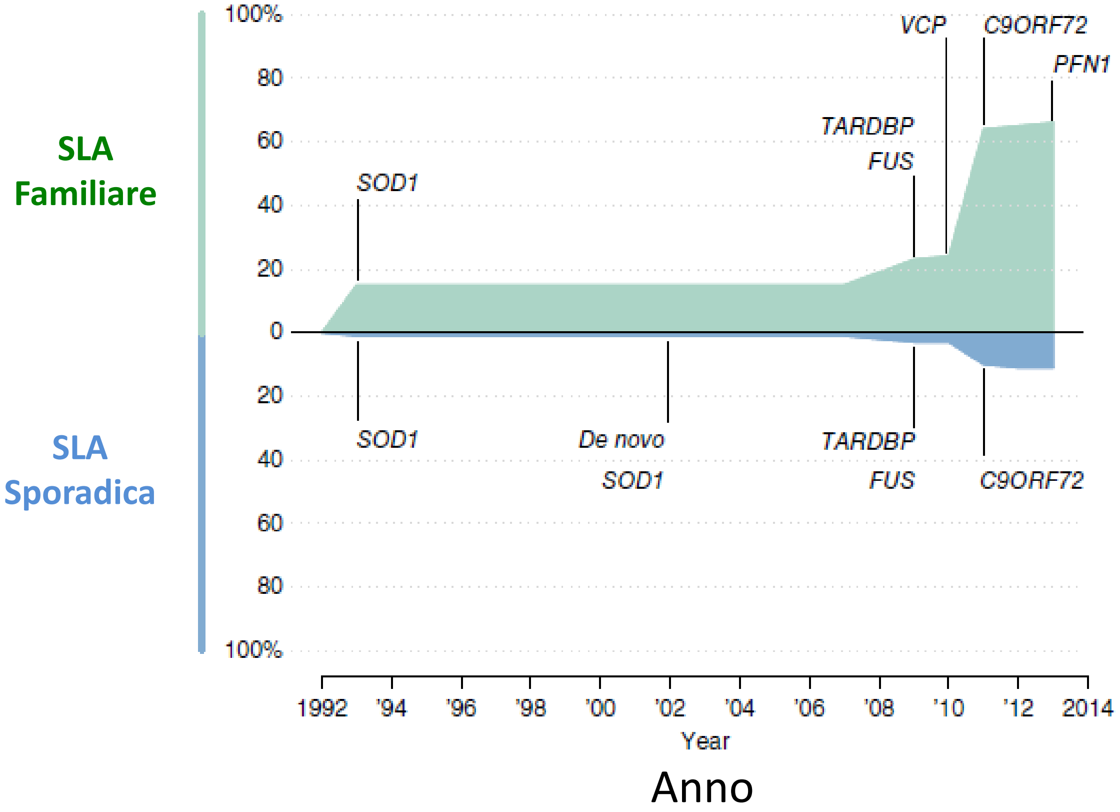
- Calcio professionistico (rischio 8 volte maggiore)
- Intensa attività fisica
- Traumi ripetuti (testa, muscoli degli arti)
- Fumo

TERAPIA

Riluzolo (Rilutek) prolunga la sopravvivenza di 3-6 mesi

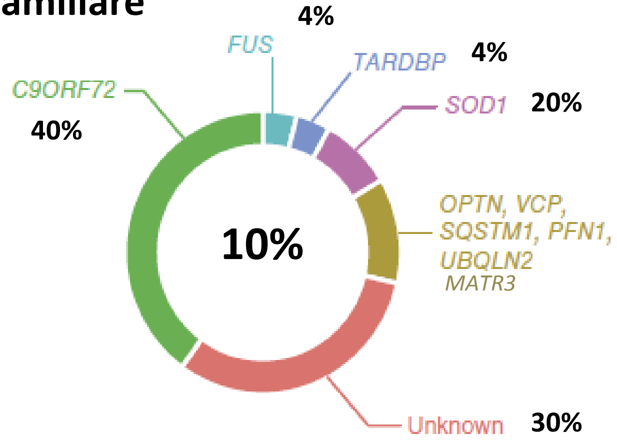
GENETICA DELLA SLA

% delle mutazioni genetiche individuate dal 1992

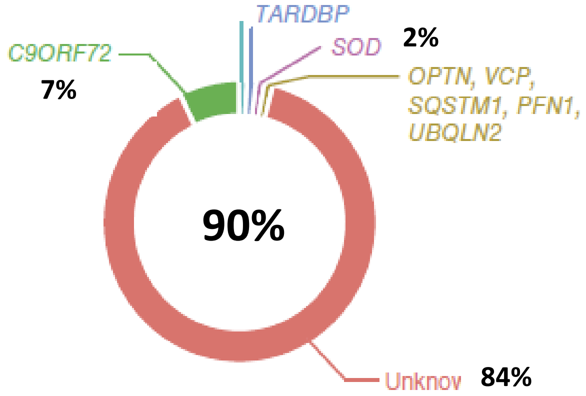


(Modificato da Renton, et al. 2014)

Familiare

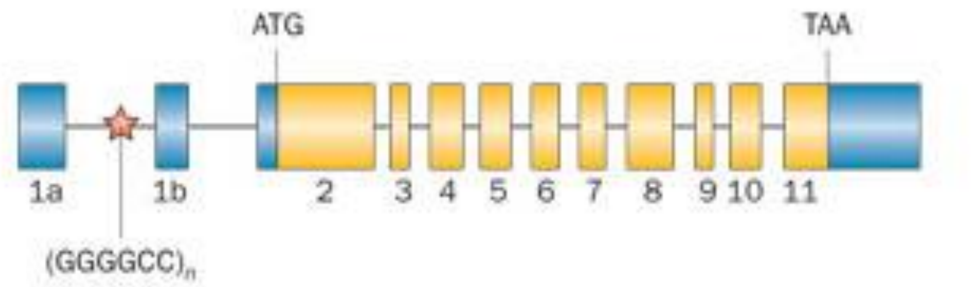


Sporadica

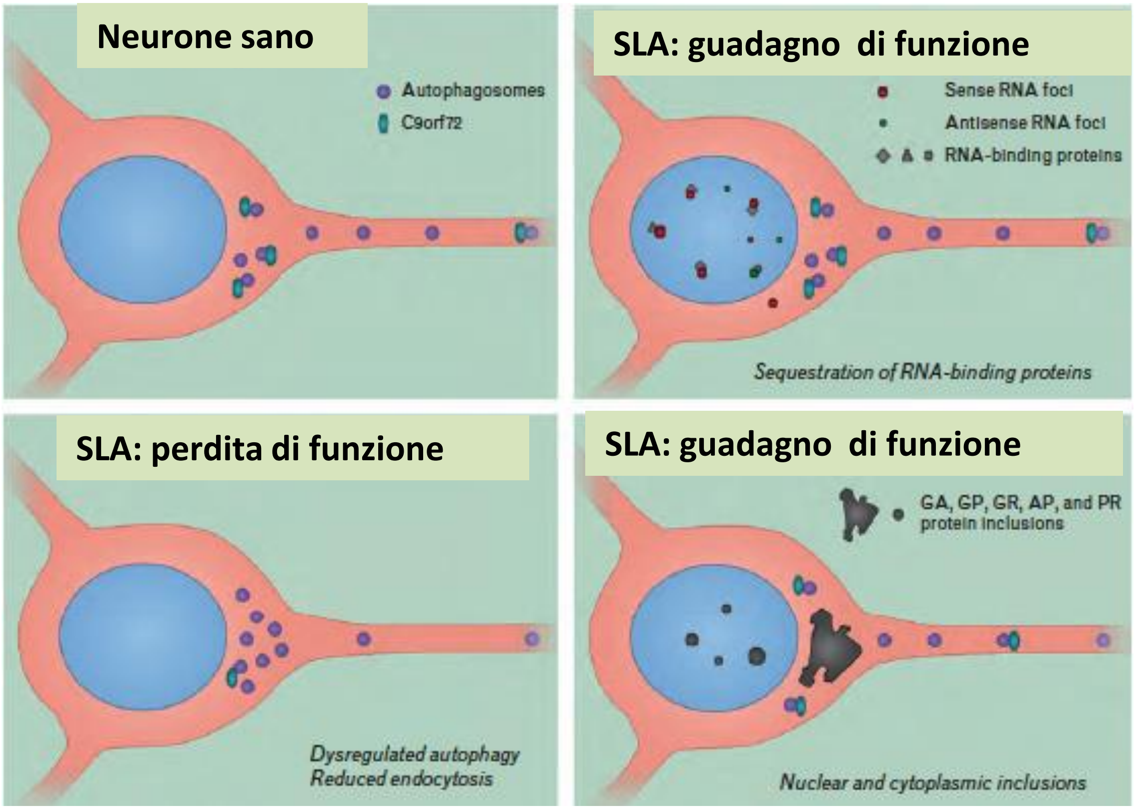


Tipo di SLA	Cromosoma	Gene	Proteina	Funzione fisiologica
ALS1	21q22.1	SOD1	Cu/Zn superoxide dismutase	**protegge dallo stress ossidativo**
ALS2	2q33-35	ALS2	Alsin	**regola il trasporto di vescicole**
ALS4	9q34	SETX	senataxin	**regola la funzione del DNA e RNA**
ALS5	15q15-21	SPG11	spatacsin	**regola il trasporto negli assoni**
ALS6-FTD	16p11.2	FUS	Fused in Sarcoma	**regola la funzione del DNA e RNA**
ALS8	20p13.33	VAPB	VAMP-associated protein	**regola il trasporto di vescicole**
ALS9	14q11	ANG	Angiogenin	**regola il tRNA**
ALS10-FTD	1q36	TARDBP	TAR-DNA binding protein	**regola la funzione del DNA e RNA**
ALS11	6q21	FIG4	PI(3,5)P(2)5 phosphatase	**regola il trasporto delle proteine**
ALS12	10p15-p14	OPTN	Optineurin	**regola la degradazione delle proteine**
ALS13	Xp11.23	UBQLN2	Ubiquitin like protein ubiquilin 2	**regola la degradazione delle proteine**
ALS14-FTD	9p21	C9ORF72	unknown	**regola la degradazione delle proteine ?**
ALS	5q31.2	MATR3	Matrin 3	**regola la funzione del DNA e RNA**

Il gene C9orf72 mutato nella SLA


ATG
TAA
1a
1b
2
3
4
5
6
7
8
9
10
11
(GGGGCC)n

n= 2-23 nei controlli , n=100-1000 nei pazienti SLA



Neurone sano

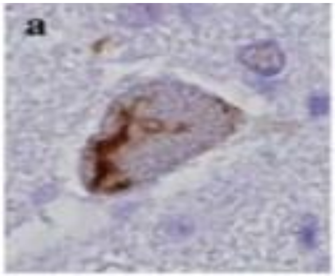
SLA: guadagno di funzione

SLA: perdita di funzione

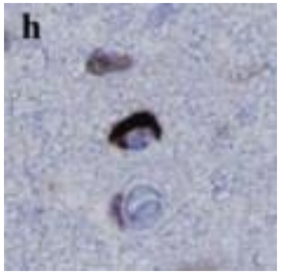
SLA: guadagno di funzione

Autophagosomes
C9orf72
Sense RNA foci
Antisense RNA foci
RNA-binding proteins
Sequestration of RNA-binding proteins
Dysregulated autophagy
Reduced endocytosis
GA, GP, GR, AP, and PR protein inclusions
Nuclear and cytoplasmic inclusions

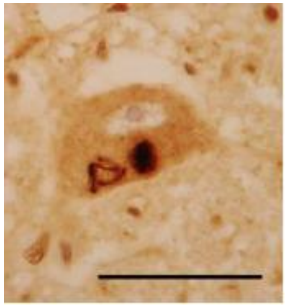
SL-TDP43 (sALS)



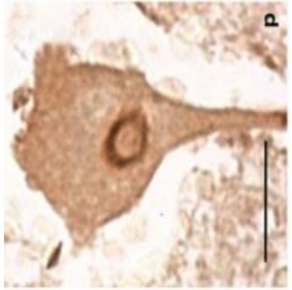
TDP43 Glial (sALS)



HI+SL-TDP43 (sALS)

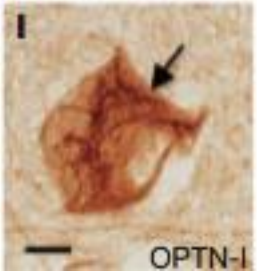


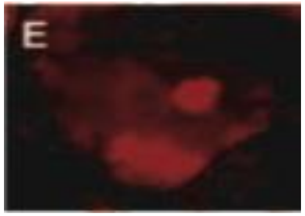
LBI-SOD1 (fALS)



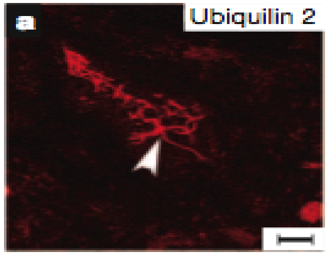
SLA, una proteinopatia?

SL-OPTN (sALS)

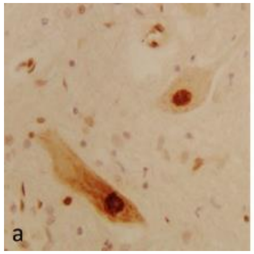




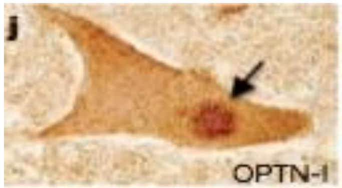
LBI-VCP (sALS)


a
Ubiquilin 2

UBQL2(fALS)



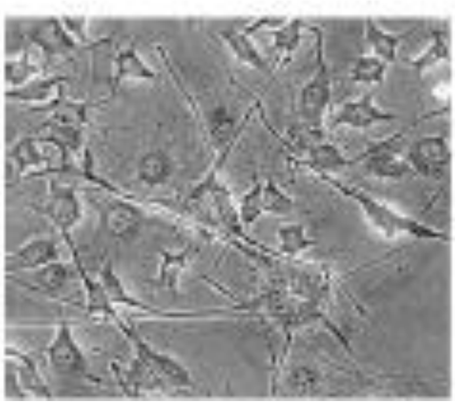
SL-FUS (fALS)



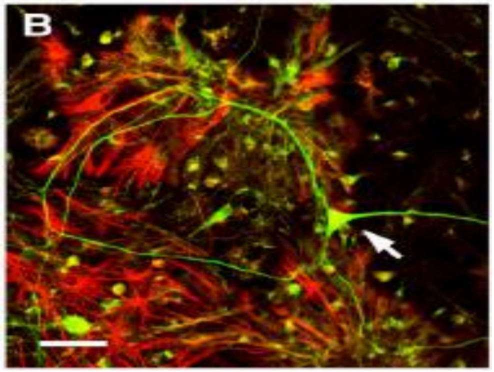
HI-OPTN (sALS)

Modelli sperimentali per lo studio della SLA

Cellulari



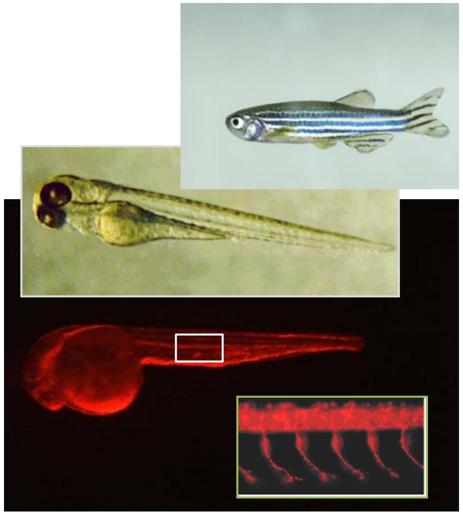
Linee cellulari MN(NSC34)



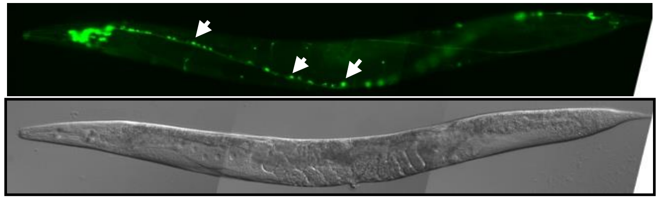
Primary motor neurons
and co-coltures

Animali

Drosophila

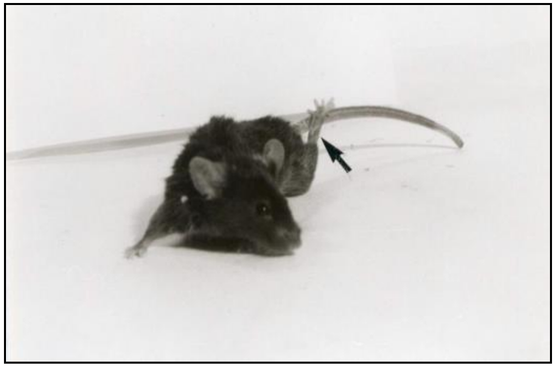


Zebrafish embryo

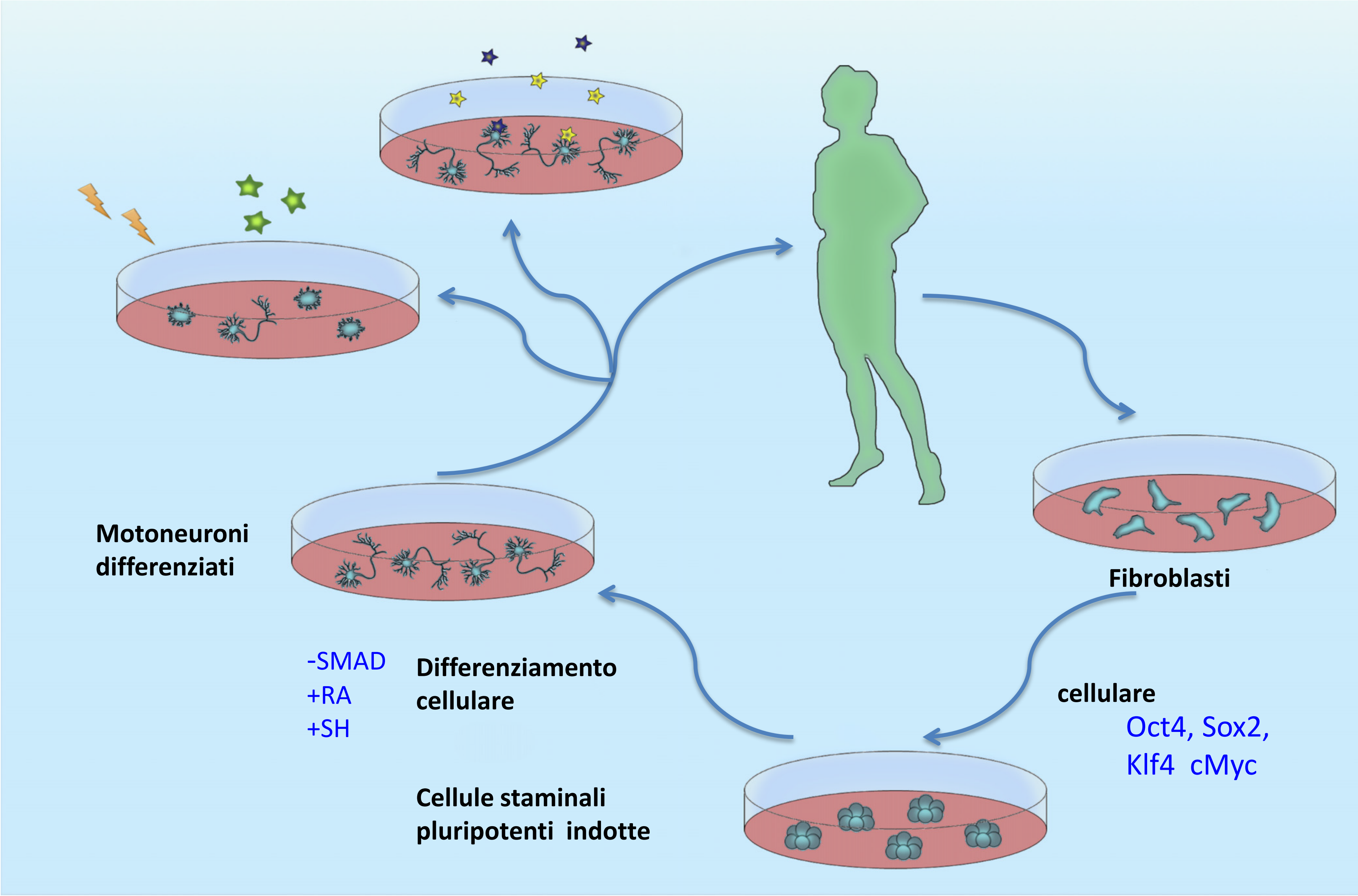


C.Elegans

Topi geneticamente
modificati



Staminali umane pluripotenti indotte (iPSC)



Motoneuroni differenziati

Fibroblasti

-SMAD
+RA
+SH

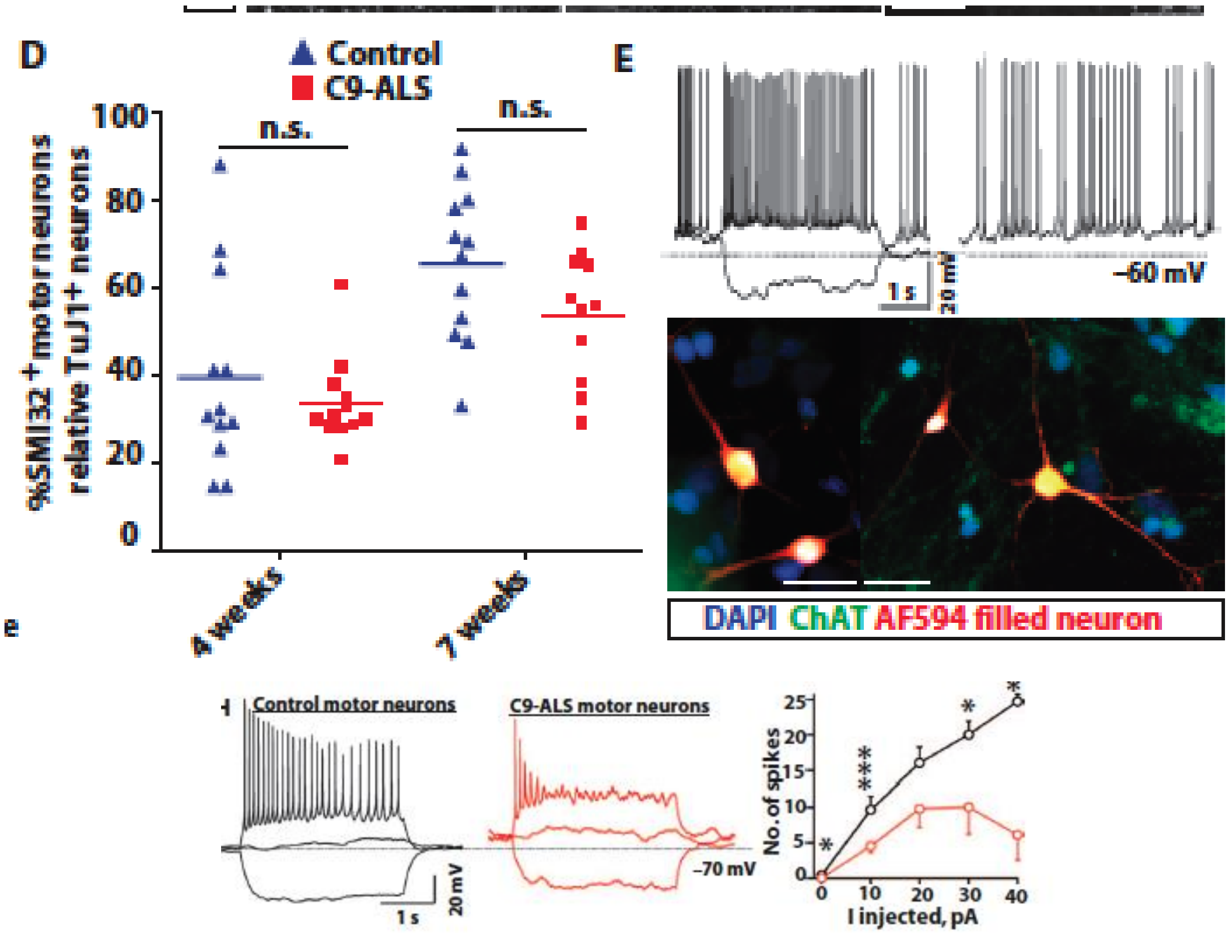
Differenziamento cellulare

cellulare

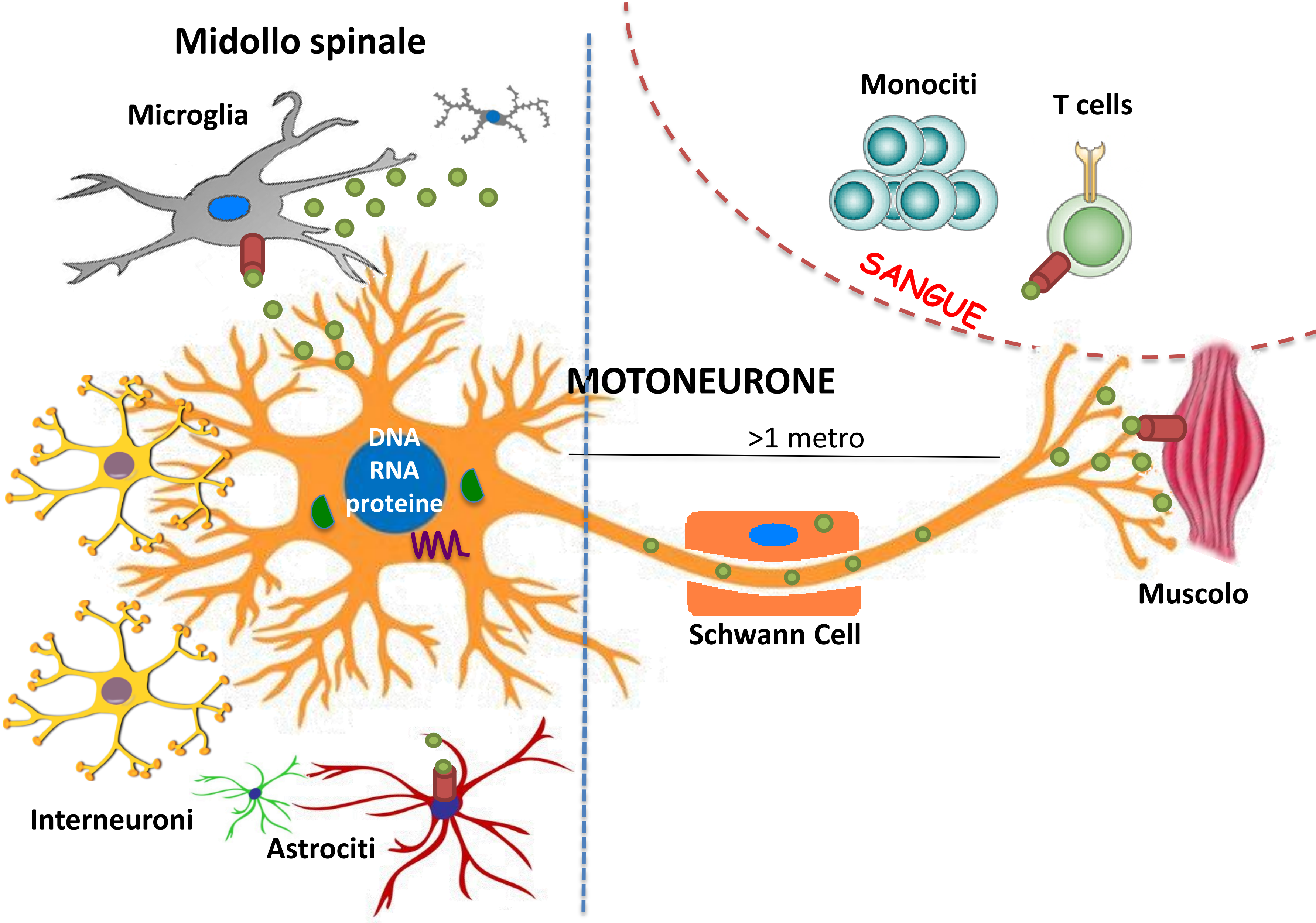
Oct4, Sox2,
Klf4 cMyc

Cellule staminali pluripotenti indotte

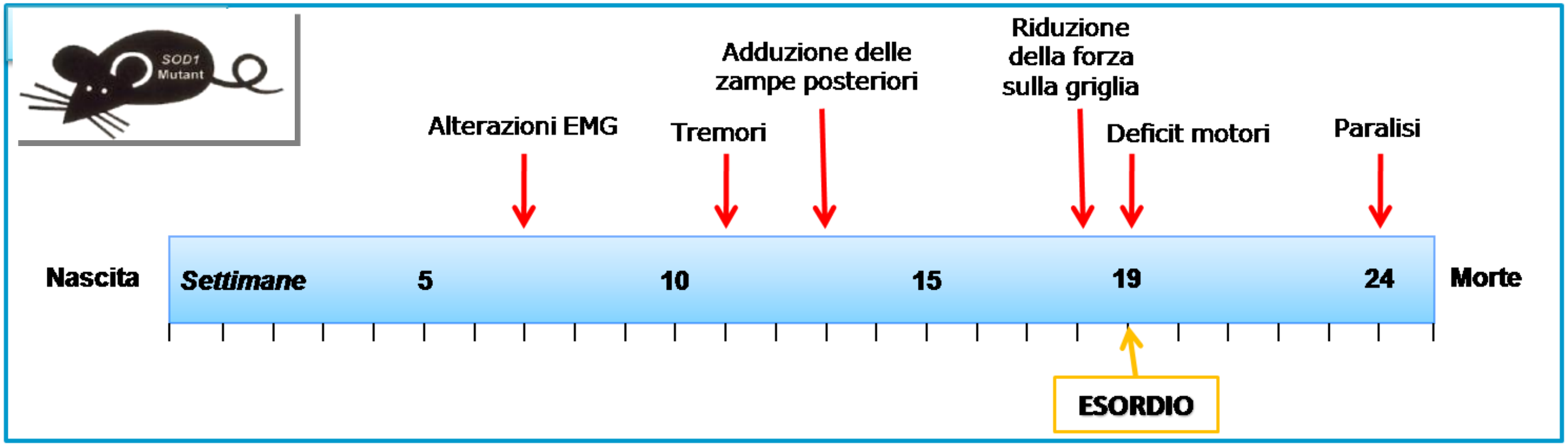
I motoneuroni ottenuti da iPSC di pazienti SLA con mutazione C9ORF72 sono più deboli



La SLA è una malattia multicellulare


Midollo spinale
Microglia
Monociti
T cells
SANGUE
MOTONEURONE
>1 metro
DNA
RNA
proteine
Schwann Cell
Muscolo
Interneuroni
Astrociti

Topo transgenico per la SOD1G93A: modello di SLA familiare


SOD1
Mutant

Alterazioni EMG

Tremori

Adduzione delle zampe posteriori

Riduzione della forza sulla griglia

Deficit motori

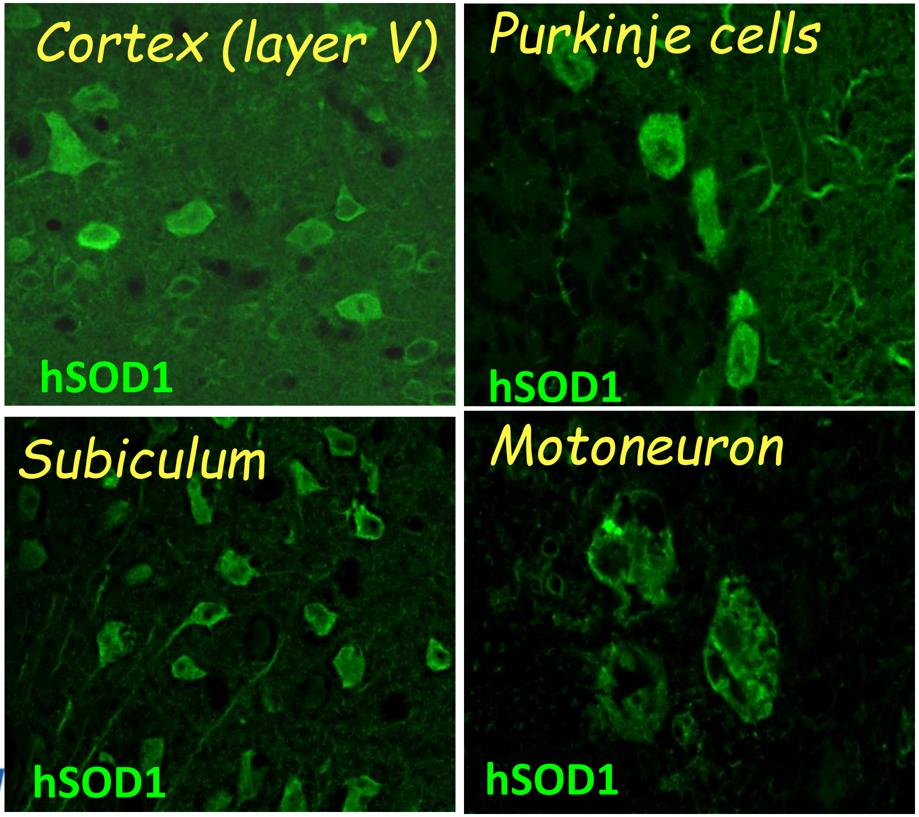
Paralisi

Nascita

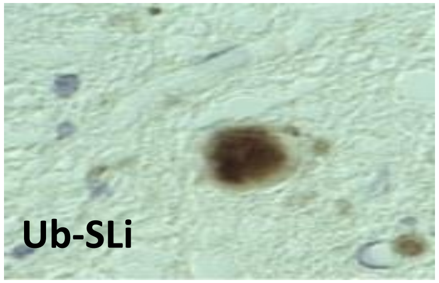
Settimane 5 10 15 19 24

Morte

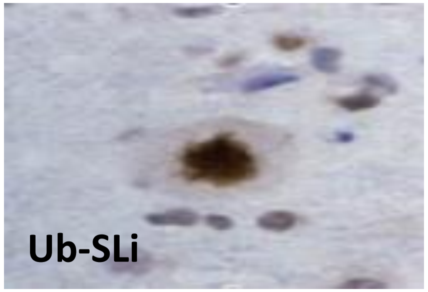
ESORDIO

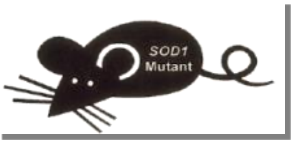

Cortex (layer V)
hSOD1
Purkinje cells
hSOD1
Subiculum
hSOD1
Motoneuron
hSOD1

Topo SODG93A

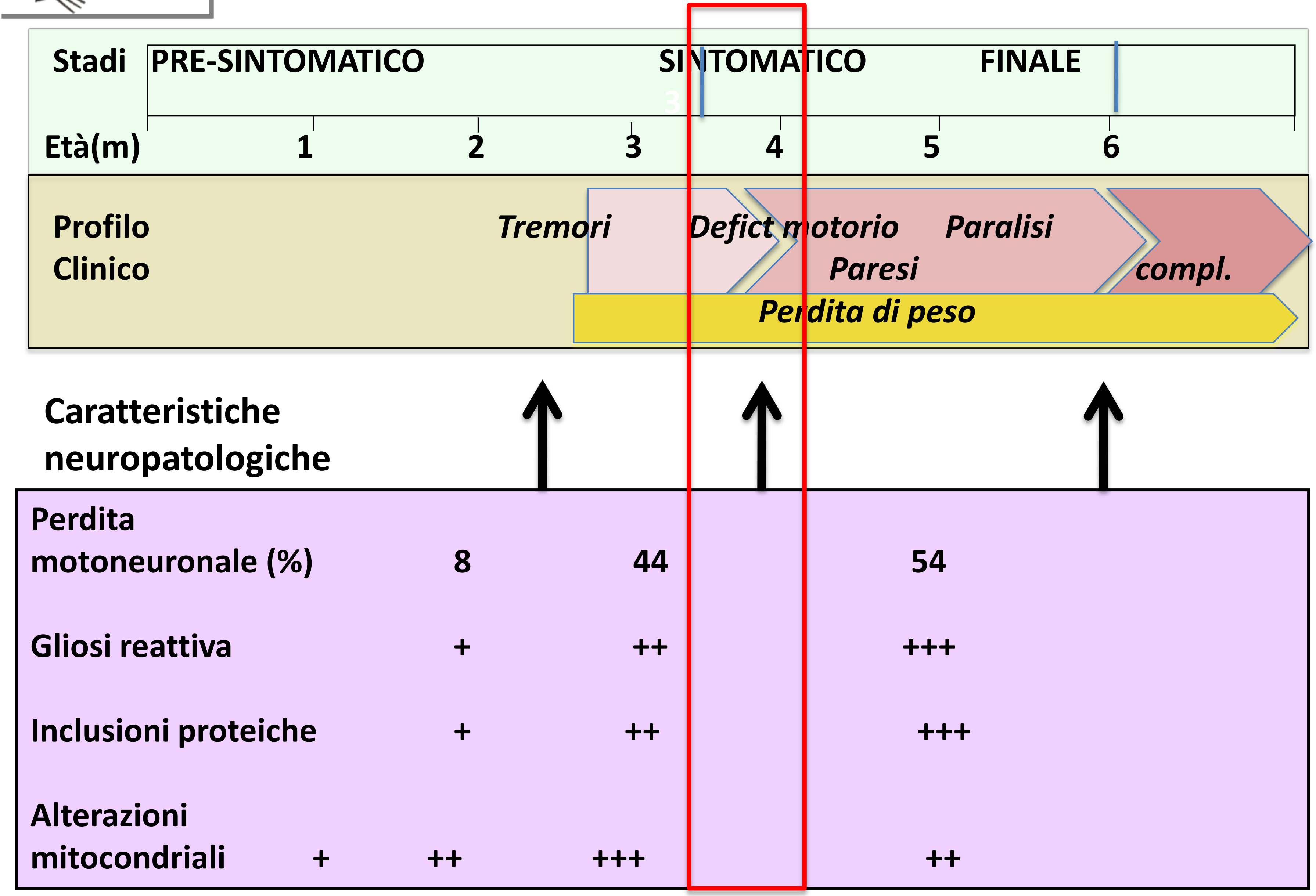


Paziente SLA





PROGRESSIONE DELLA MALATTIA NEI TOPI SOD



Stadi PRE-SINTOMATICO SINTOMATICO FINALE

Età(m) 1 2 3 4 5 6

Profilo Clinico

Tremori

Defict motorio

Paralisi

Paresi

compl.

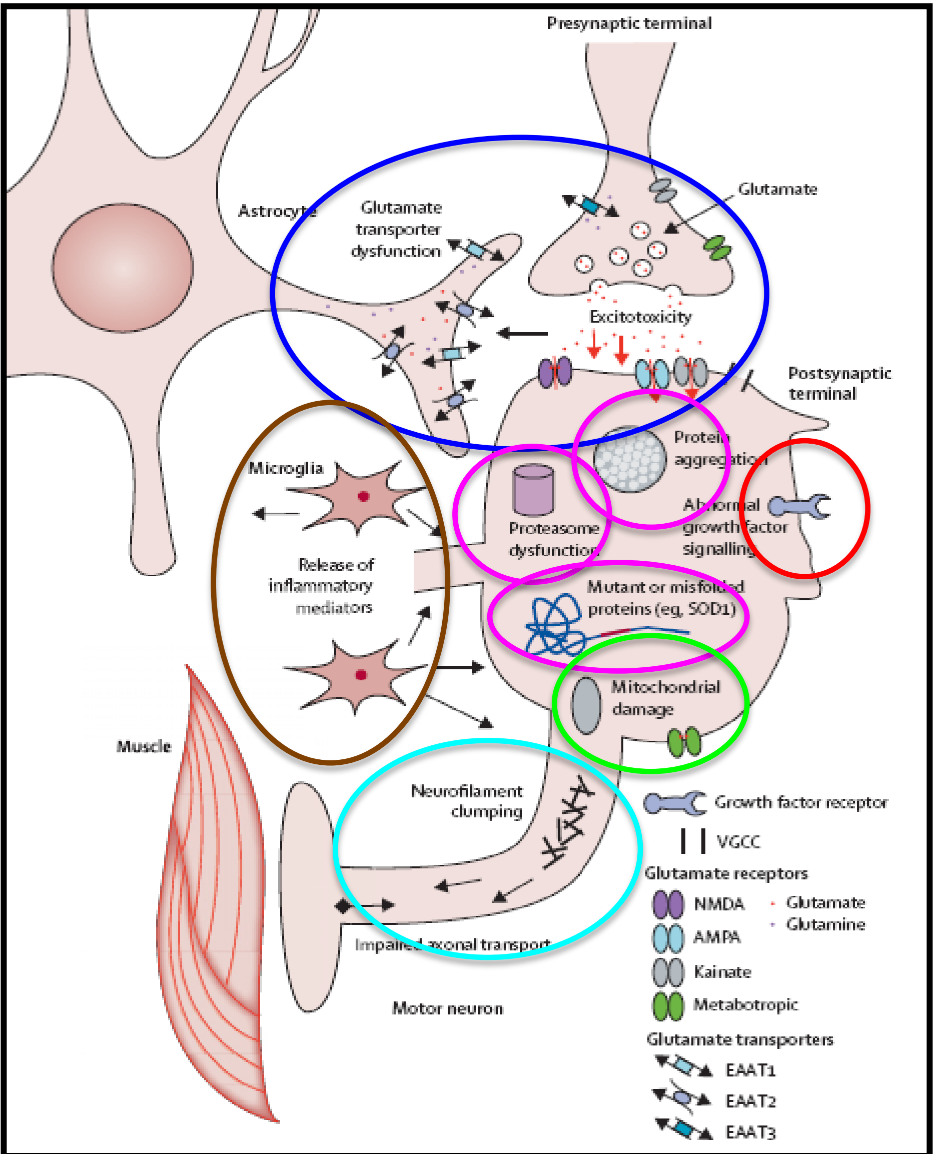
Perdita di peso

Caratteristiche neuropatologiche

Perdita motoneuronale (%)		8	44	54
Gliosi reattiva		+	++	+++
Inclusioni proteiche		+	++	+++
Alterazioni mitocondriali	+	++	+++	++

Bendotti & Carri, Trends Mol.Med., 2004

EZIOLOGIA: la complessità della SLA


Presynaptic terminal
Astrocyte
Glutamate transporter dysfunction
Glutamate
Excitotoxicity
Postsynaptic terminal
Microglia
Protein aggregation
Proteasome dysfunction
Abnormal growth factor signalling
Release of inflammatory mediators
Mutant or misfolded proteins (eg, SOD1)
Mitochondrial damage
Muscle
Neurofilament clumping
Growth factor receptor
VGCC
Glutamate receptors
NMDA
AMPA
Kainate
Metabotropic
Glutamate
Glutamine
Impaired axonal transport
Motor neuron
Glutamate transporters
EAAT1
EAAT2
EAAT3

Malattia *MULTIFATTORIALE*

- Eccitotossicità
- Aggregazione proteica
- Alterata degradazione proteica
- Disifunzione mitocondriale
- Omeostasi del Ca^{2+}
- Alterazioni trasporto assonale
- Gliosi reattiva

DANNO MOTONEURONALE

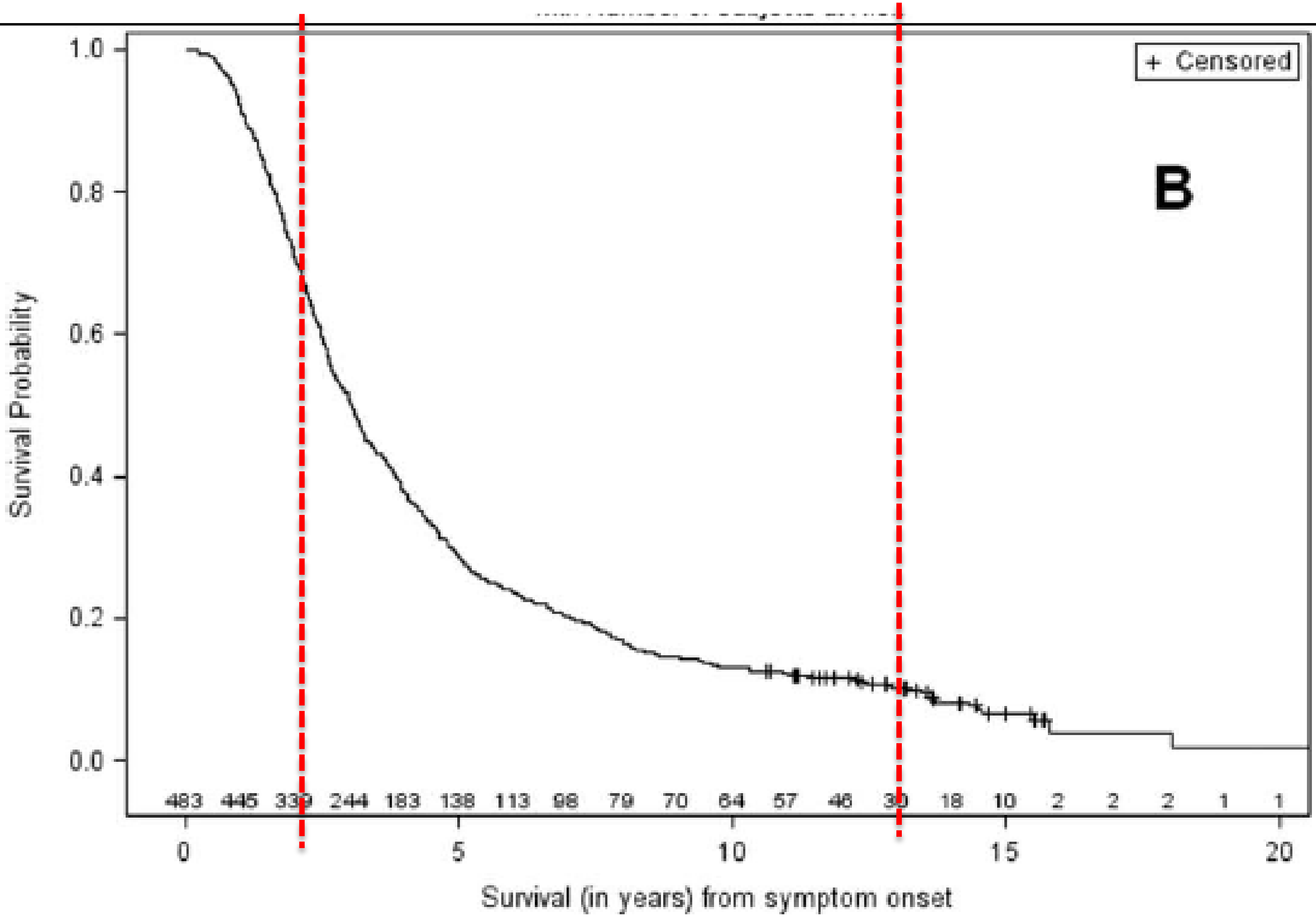
Test preclinici nei topi transgenici SOD1 in 20 anni

Totale studi : **234**
Effetto positivo sulla sopravvivenza:**154** (66%)

Meccanismo d'azione :

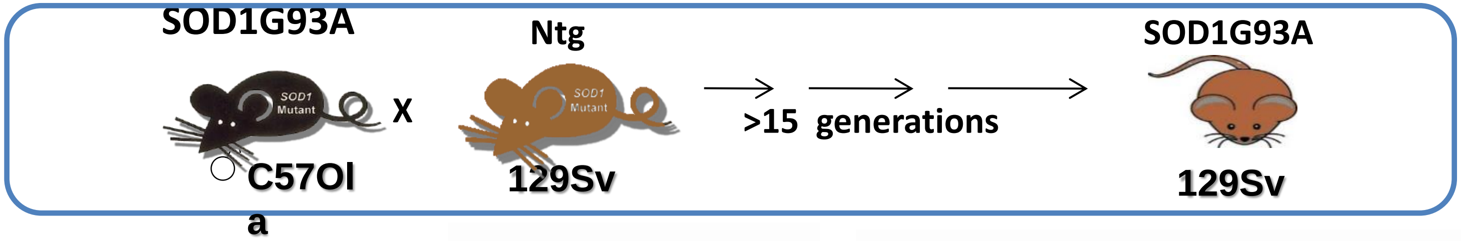
- Antiossidante : **52**
- Anti-eccitotossicità: **20**
- Anti-aggregazione: **25**
- Anti-infiammazione /immunomodulatori : **33**
- Anti-apoptotici: **14**
- Neurotrofici: **35**
- Combinazione: **20**
- Cellule staminali: **25**
- Altri: **10**

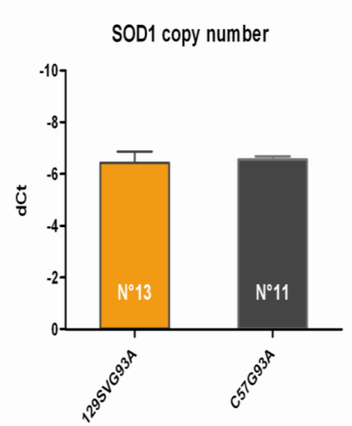
Variabilità di sopravvivenza nei pazienti SLA

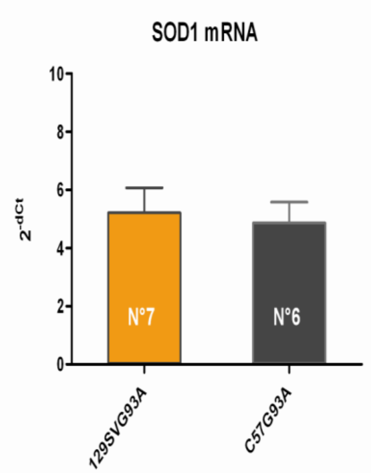


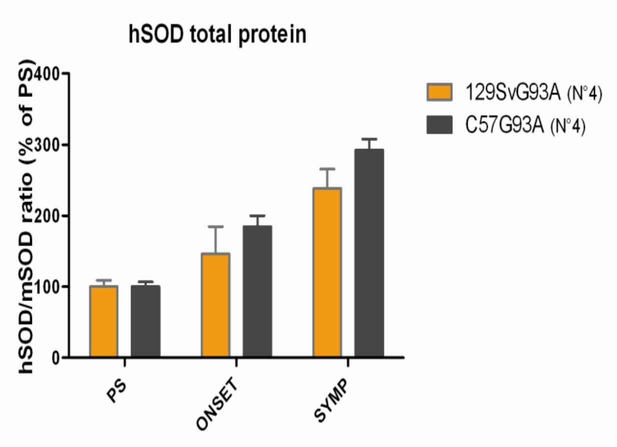
Pupillo et al. Ann Neurol 75:287-297

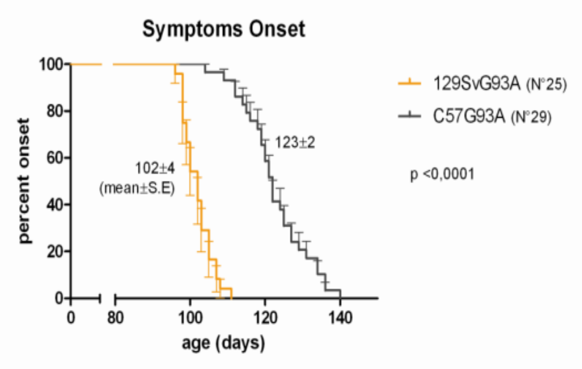
Variabilità nella progressione della malattia in due topi transegnici per SOD1

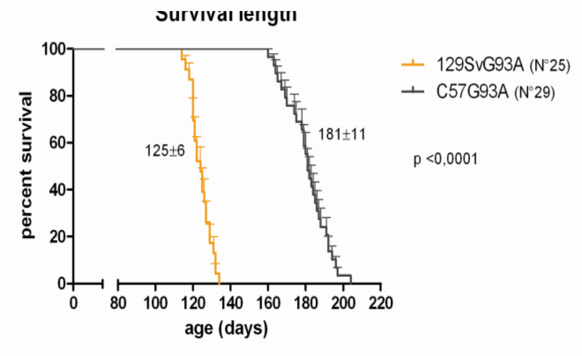

SOD1G93A
SOD1 Mutant
X
C57Ol
a
Ntg
SOD1 Mutant
129Sv
>15 generations
SOD1G93A
129Sv

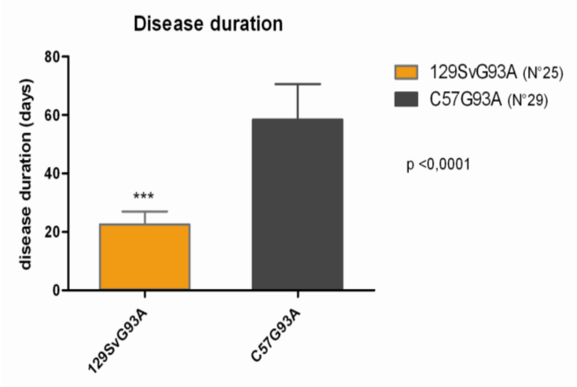

SOD1 copy number


SOD1 mRNA


hSOD total protein


Symptoms Onset

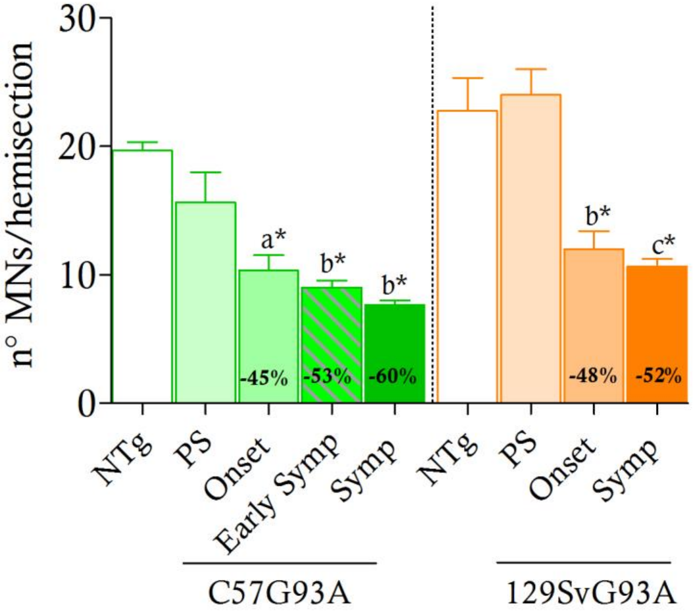

Survival length


Disease duration

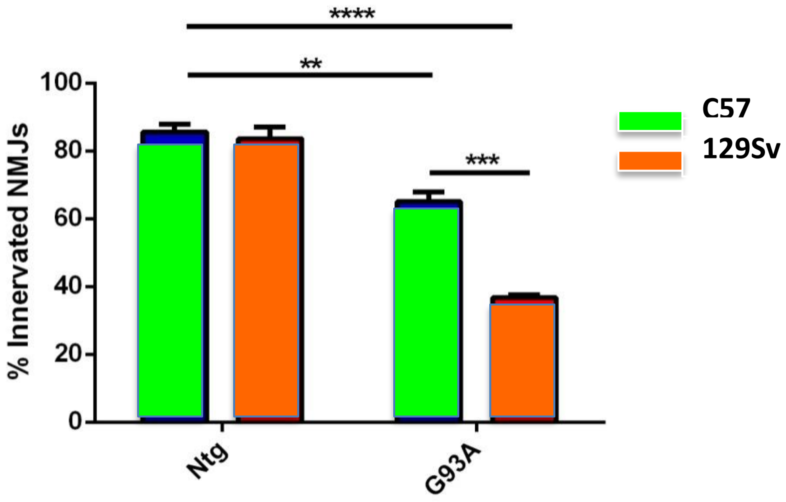
Pizzasegola et al. ALS 2009; Nardo, 2013, Marino 2014

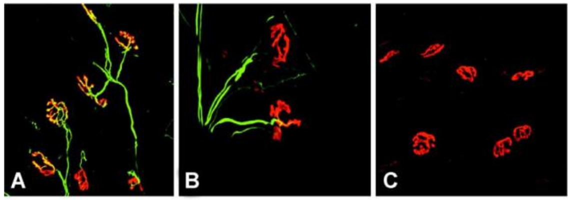
La differenza nella progressione della malattia non dipende dalla perdita dei motoneuroni ma dalla denervazione dei muscoli

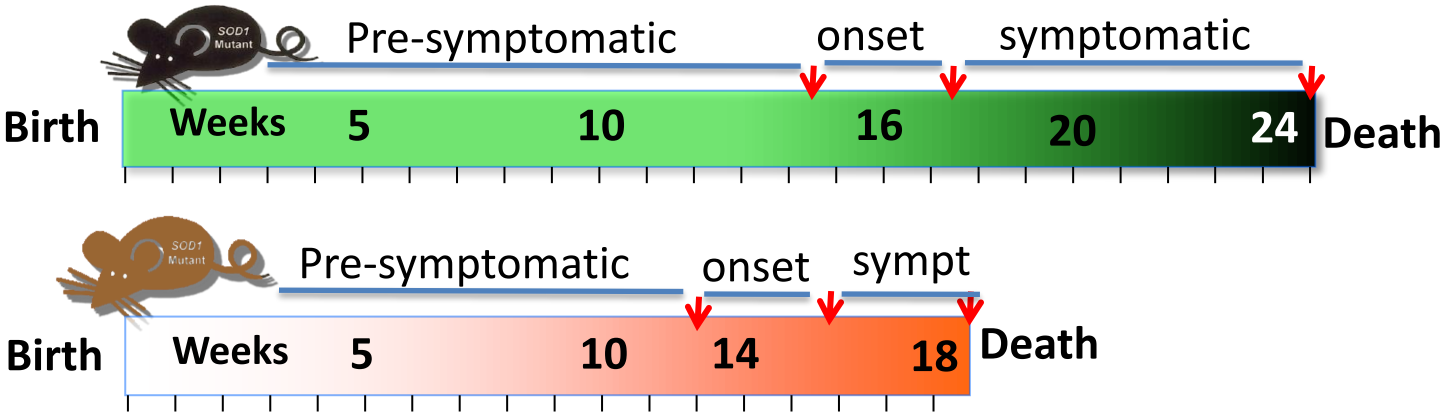
Spinal motor neuron

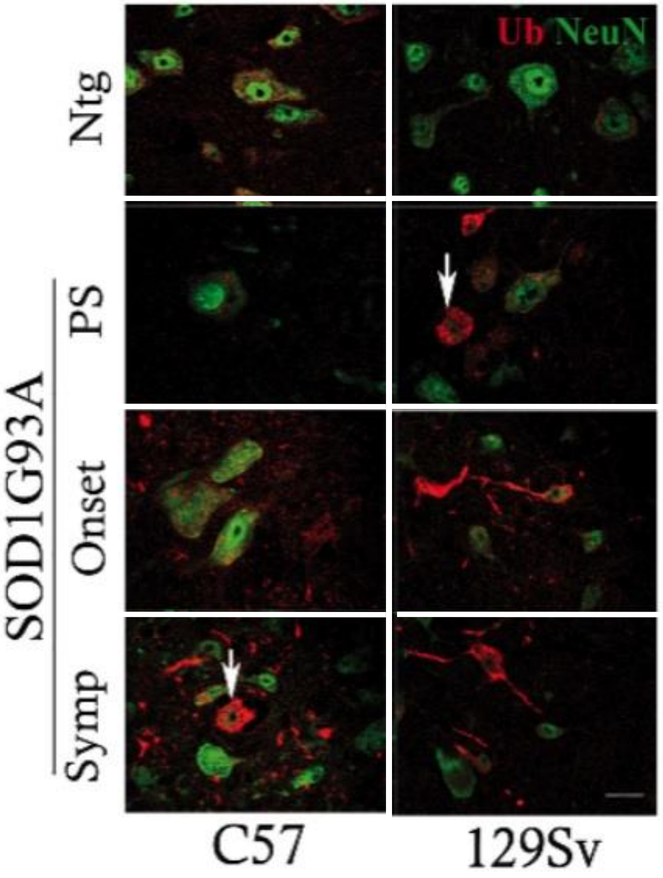


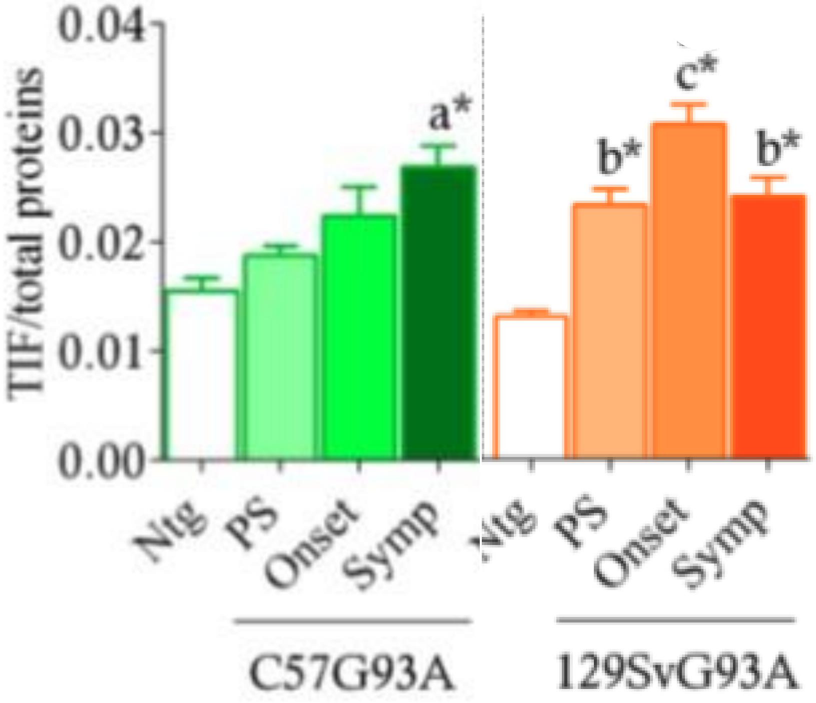
Neuromuscular junction






SOD1 Mutant
Pre-symptomatic
onset
symptomatic
Birth
Weeks
5
10
16
20
24
Death
SOD1 Mutant
Pre-symptomatic
onset
sympt
Birth
Weeks
5
10
14
18
Death

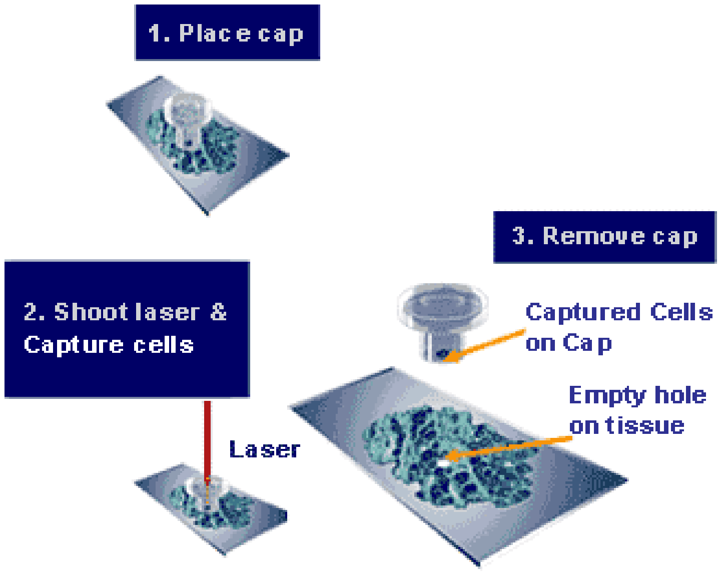

Ub NeuN
Ntg
PS
Onset
Symp
SOD1G93A
C57
129Sv



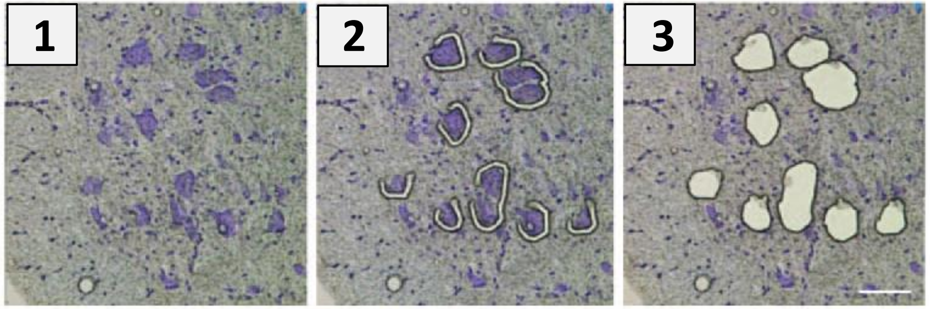
Cheroni et al Human Mol. Gen. 2009
Bendotti et al. Progress Neurobiol,2012
Marino et al. Neurobiol Aging 2014

METHODS 1

Laser Capture Microdissection


1. Place cap
3. Remove cap
2. Shoot laser & Capture cells
Captured Cells on Cap
Empty hole on tissue
Laser

Lobsiger et al. 2007



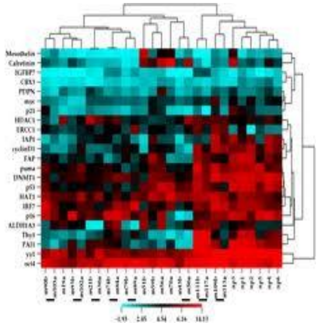
LUMBAR VENTRAL SPINAL CORD
(1000 MN each mouse)

RNA EXTRACTION AND PURIFICATION
cDNA AMPLIFICATION

→

Hybridization to Gene Mouse Genome 430 2.0 Affymetrix GenChip System

→



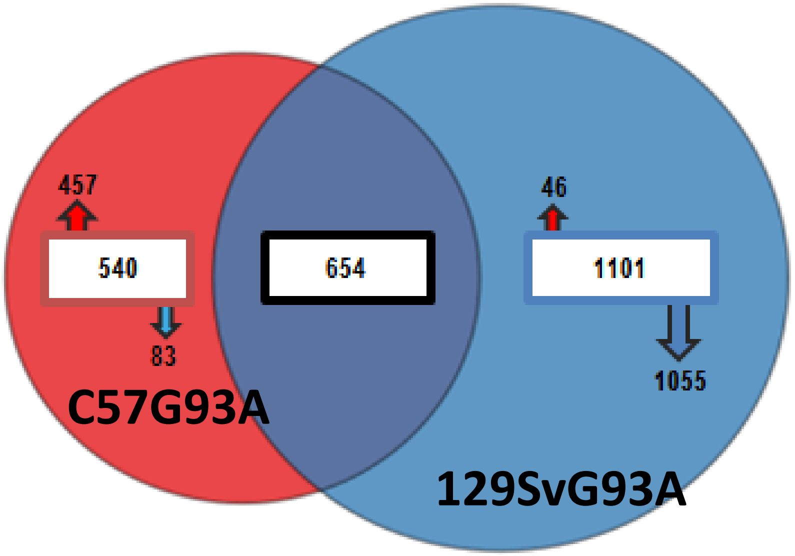
hybridizaton signal detection
By Gene Chip Scanner 300

→

System Biology Analysis

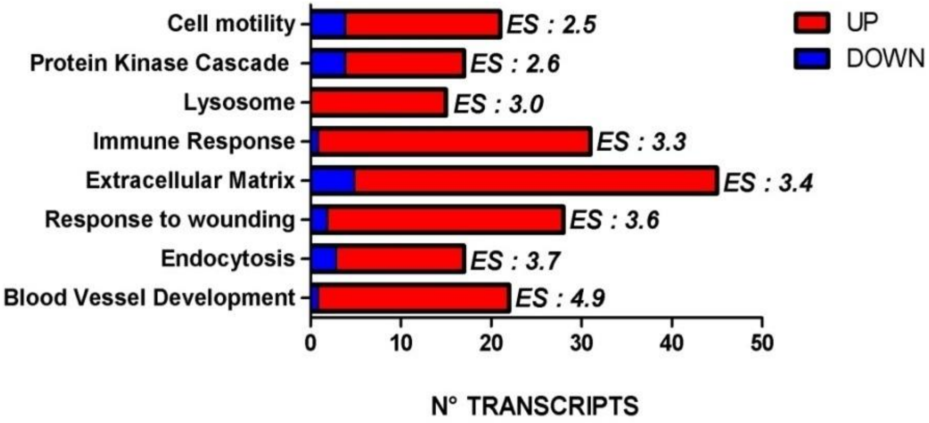
Microarray Analysis

SYSTEM BIOLOGY ANALYSIS

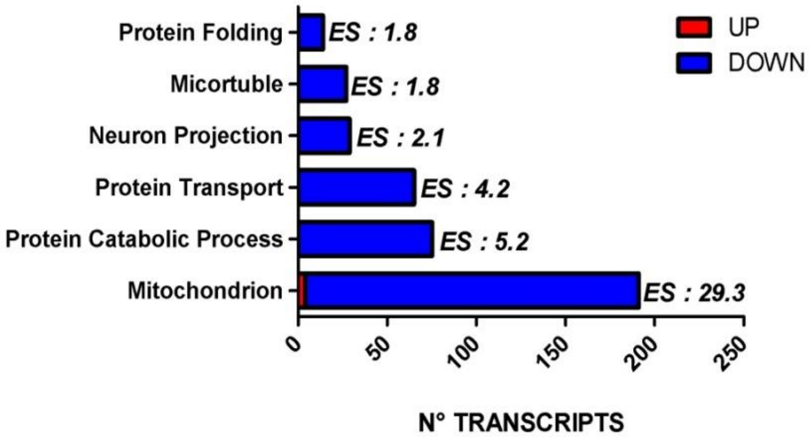

457
540
83
654
46
1101
1055
C57G93A
129SvG93A

ONSET

Malattia a progressione lenta



Malattia a progressione rapida



Conclusioni

✓La SLA è una malattia complessa che dipende nelle forme familiari dalla mutazione di vari geni con funzioni diverse

✓Sono implicati diversi meccanismi che coinvolgono non solo il motoneurone ma anche le cellule che gli stanno intorno nel sistema nervoso e nel sangue.

✓Data tale complessità occorre agire con terapie farmacologiche ad azione multipla e non mediante interventi su singoli meccanismi