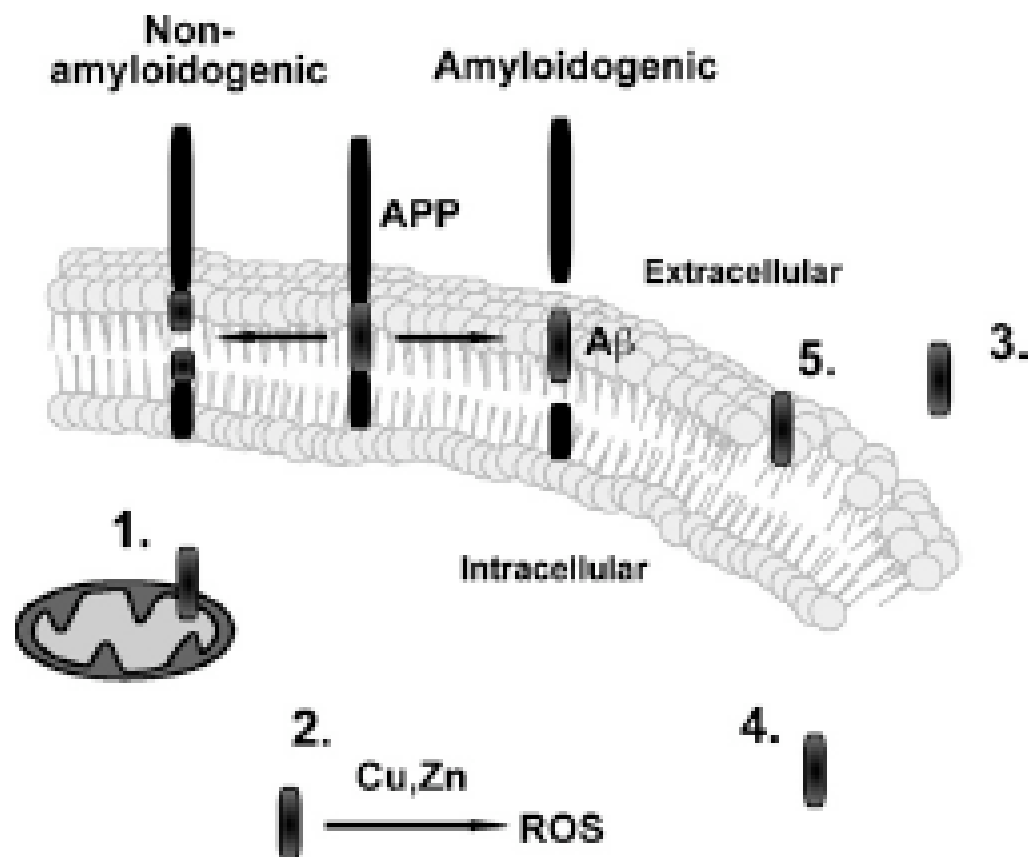




Possibili meccanismi
di degenerazione mediata
da Aβ nella malattia di
Alzheimer

1. Mitochondrial dysfunction
2. Oxidative stress
3. Synaptic transmission
4. Axonal transport
5. Membrane disruption

Sclerosi Laterale Amiotrofica (SLA)

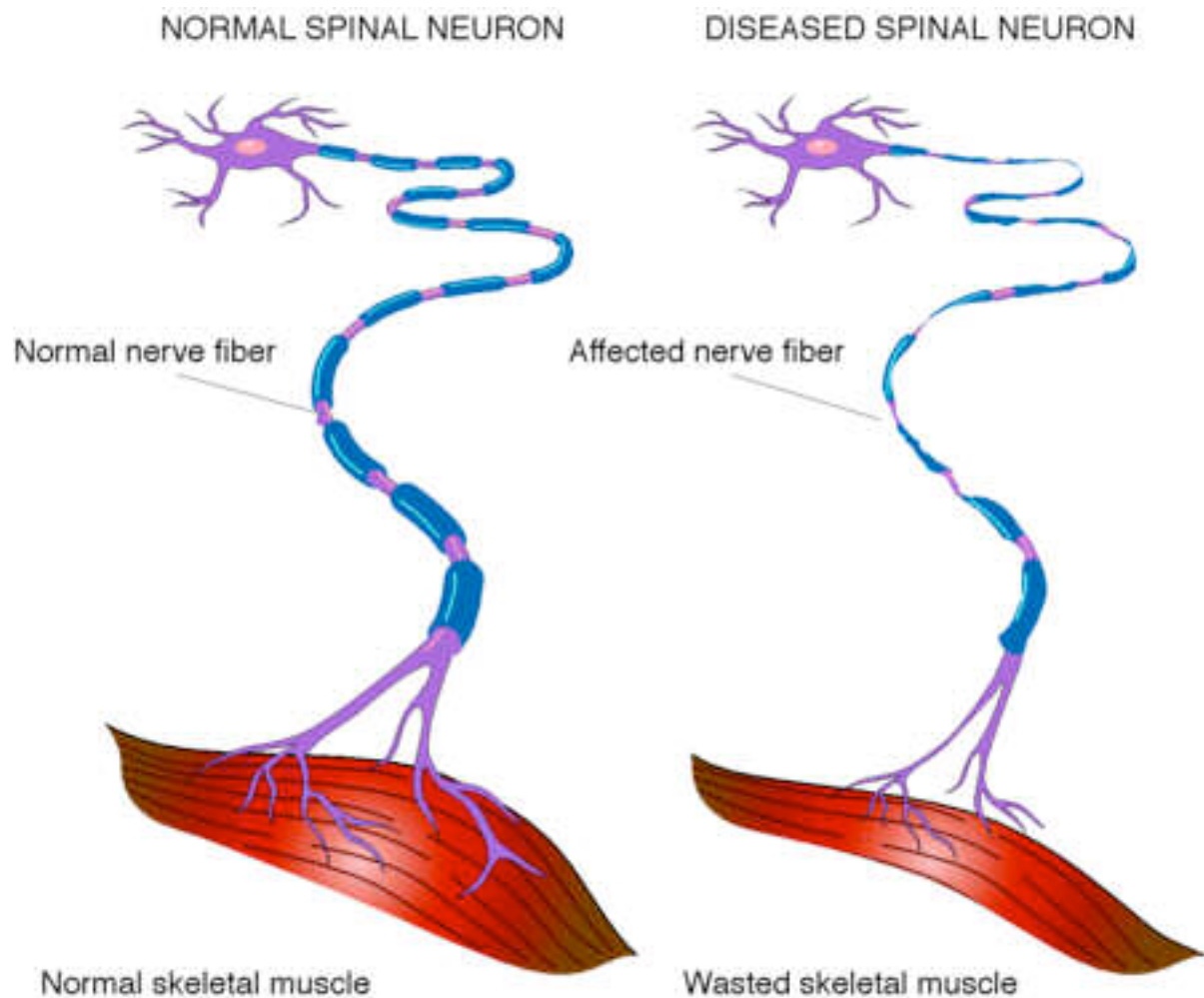
Incidenza: 1-2/100.000
Insorgenza: 50 anni
Maschi/Femmine: 1.6/1
Durata: 3 anni
Sporadica: 90%
Genetica: 10%

Non esiste alcuna
terapia per la SLA

Table 1. Recent clinical trials in amyotrophic lateral sclerosis (ALS)

Drug	Phase	Design	No. of patients	Follow-up (months)	Daily dose	Primary end-p.o.ints	Results	Refs
Immunosuppressive approaches								
Cyclosporin	II	Randomized DB, PC 2 parallel groups	74	48	10 mg	Appel scale Muscle strength Respiratory function	No benefit	51
Total lymphoid irradiation	II	Randomized DB, Sham-radiation controlled 2 parallel groups	61	24	1.8 Gy daily 5 day weekly	Dynamometry MRC Scale Survival	No benefit	52
Neurotrophic approaches								
rhGH	II	Randomized DB, PC 2 parallel groups	75	12–18	0.3 mg kg ⁻¹ im weekly	Survival	No benefit	81
rhCNTF	II/III	Randomized DB, PC 3 parallel groups	720	9	15, 30 µg kg ⁻¹ s.c. thrice weekly	Muscle strength	No benefit	54
rhIGF-I	II/III	Randomized DB, PC 4 parallel groups	570	6	0.5, 2, 5 µg kg ⁻¹ s.c.	Muscle strength Respiratory function	No benefit Increased mortality	55
	II/III	DB, PC, 3 mo. run-in 3 parallel groups	266	9	50, 100 µg kg ⁻¹ s.c.	Appel scale	Decreased rate of decline	57
rhBDNF	II/III	Randomized DB, PC 2 parallel groups	183	9	100 µg kg ⁻¹ s.c.	Appel scale	Non-significant beneficial trend	58
	II	Randomized DB, PC 6 parallel groups	283	6	10, 25, 50, 100, 150 µg kg ⁻¹ s.c.	Respiratory function	Decreased rate of decline	59
	III	Randomized DB, PC 3 parallel groups	1100	9	25, 100 µg kg ⁻¹ s.c.	Respiratory function Survival	No benefit	60
Neuroprotective								
N-acetylcysteine	II	Randomized DB, PC 2 parallel groups	110	12	50 mg kg ⁻¹ s.c.	Survival	No benefit	62
Selegiline	II	Randomized DB, PC Cross-over	133	6	10 mg p.o.	Appel scale	No benefit	63
	II	Randomized DB, PC 2 parallel groups	10	3	10 mg p.o.	Norris scale	No benefit	64
	II	Randomized DB, PC 2 parallel groups	111	6	10 mg p.o.	MRC, Norris scales Survival	No benefit	65
BCAA	II	Randomized DB, PC 2 parallel groups	22	12	12 g Leu, 8 g Ile, 6.4 g Val p.o.	Muscle strength Bulbar function	Decreased rate of decline in strength	66
	III	Randomized DB, PC 2 parallel groups	90	12	12 g Leu, 8 g Ile, 6.4 g Val p.o.	Muscle strength Bulbar function	Nonsignificant beneficial trend	67
	III	Randomized DB, PC 2 parallel groups	32	12	12 g Leu, 8 g Ile, 6.4 g Val p.o.	Norris scale	No benefit	68
	III	Randomized DB, PC 2 parallel groups	126	12	12 g Leu, 6 g Ile, 6 g Val p.o.	Treatment failure MRC, Norris and Appel scales	No benefit Increased mortality	69
	III	Randomized DB, PC 2 parallel groups	573	12	12 g Leu, 8 g Ile, 6.4 g Val p.o.	Muscle strength Survival	No benefit	70
Nimodipine	II	Randomized DB, PC Cross-over	87	3	180 mg p.o.	Muscle strength Respiratory function	No benefit	82
Verapamil	II	Randomized DB, Modified cross-over	72	3	240 mg p.o.	Muscle strength Respiratory function	No benefit	83
L-threonine	II	Randomized DB, PC 2 parallel groups	15	12	2 g p.o.	Muscle strength	No benefit	84
Dextromethorphan	II	Randomized DB, PC Cross-over	14	3	150 mg p.o.	Muscle strength Norris scale	No benefit	71
	II	Randomized DB, PC Cross-over	18	6	120 mg p.o.	Not indicated	No benefit	72
	II	Randomized DB, PC 2 parallel groups	49	12	1.5 mg kg p.o.	Norris scale	No benefit	73
Riluzole	II	Randomized DB, PC 2 parallel groups	45	12	150 mg p.o.	Survival	No benefit	74
	II	Randomized DB, PC 2 parallel groups	155	12	100 mg p.o.	Survival	Increased survival	76
	III	Randomized DB, PC 4 parallel groups	959	18	50, 100, 200 mg p.o.	Survival	Increased survival	77
Lamotrigine	II	Randomized DB, PC 2 parallel groups	67	18	100 mg p.o.	Survival	No benefit	78
Gabapentin	II	Randomized DB, PC 2 parallel groups	152	6	2.4 g p.o.	Muscle strength	Nonsignificant beneficial trend	80

riluzolo



degenerazione dei
motoneuroni di

- ✓ Corteccia
- ✓ Bulbo
- ✓ Midollo spinale

- ✓ Occhi
- ✓ Vescica

sintomi

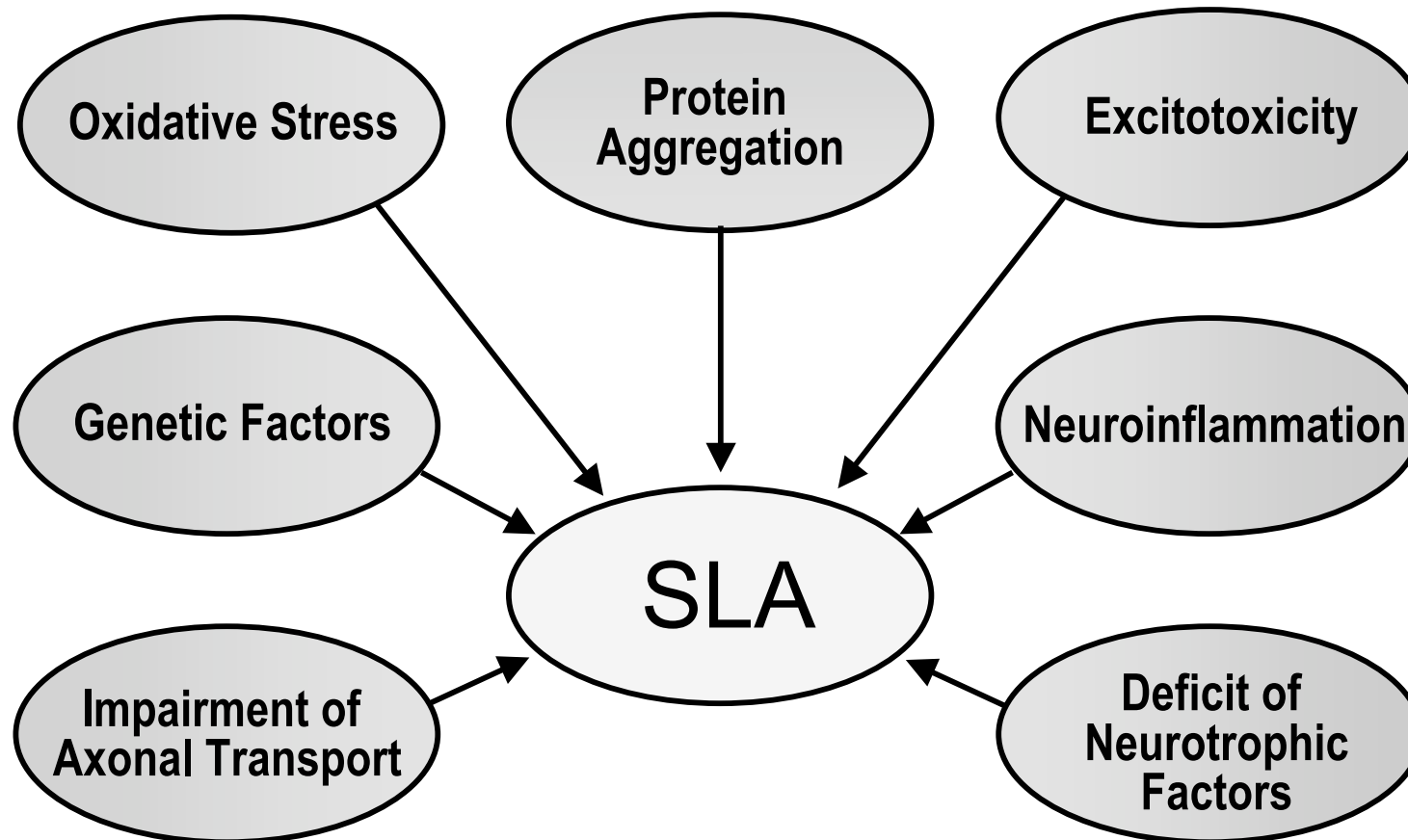
Sintomi:

✓ Motori:

Atrofia
Crampi
Fascicolazioni
Debolezza arti
Difficoltà nella masticazione
Difficoltà nella deglutizione

✓ Difficoltà respiratorie sono la causa della morte

La SLA è una malattia multifattoriale



genetica

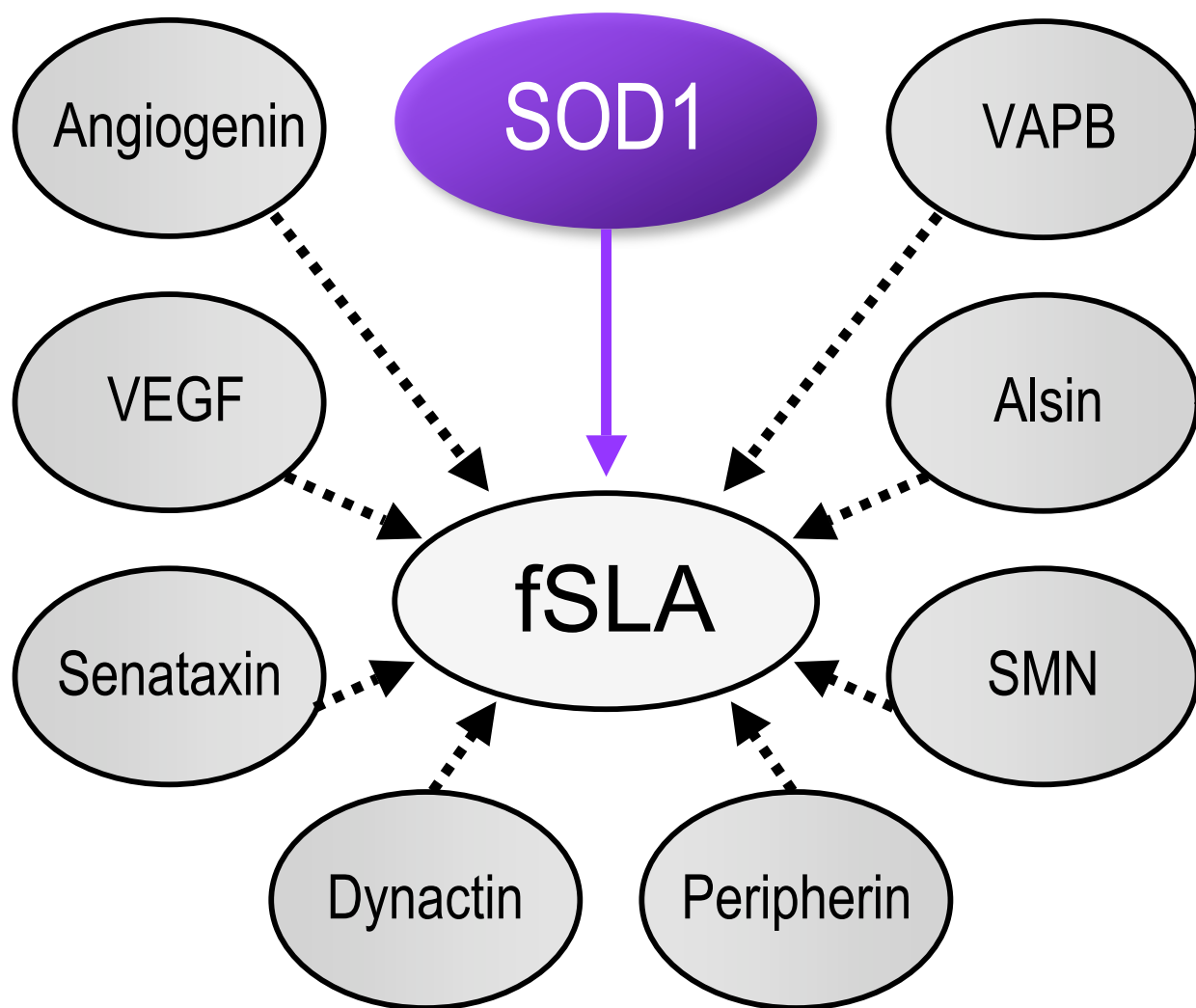
Table 1 | Genes that predispose to ALS

ALS disease type	Gene	Chromosome	Inheritance	Clinical features
<i>Mendelian genes</i>				
ALS1	SOD1	21q22	AD	Typical ALS
ALS2	ALS2	2q33	AR	Juvenile onset, slowly progressive, predominantly corticospinal
ALS4	SETX	9q34	AD	Adult onset, slowly progressive
ALS8	VAPB	20q13	AD	Typical ALS
ALS	Dynactin	2p13	AD	Adult onset, slowly progressive, early vocal cord paralysis
<i>Mendelian loci</i>				
ALS5	?	15q15–21	AR	Juvenile onset, slowly progressive
ALS6	?	16q12	AD	Typical ALS
ALS7	?	20p13	AD	Typical ALS
ALS-FTD	?	9q21–22	AD	ALS, frontotemporal dementia
ALS-FTD	?	9p	AD	ALS, frontotemporal dementia
ALS-X	?	Xcen	XD	Typical ALS
<i>Mitochondrial genes</i>				
ALS-M	COX1	mtDNA	Maternal	Single case, predominantly corticospinal
ALS-M	IARS2	mtDNA	Maternal	Single case, predominantly lower motor neuron

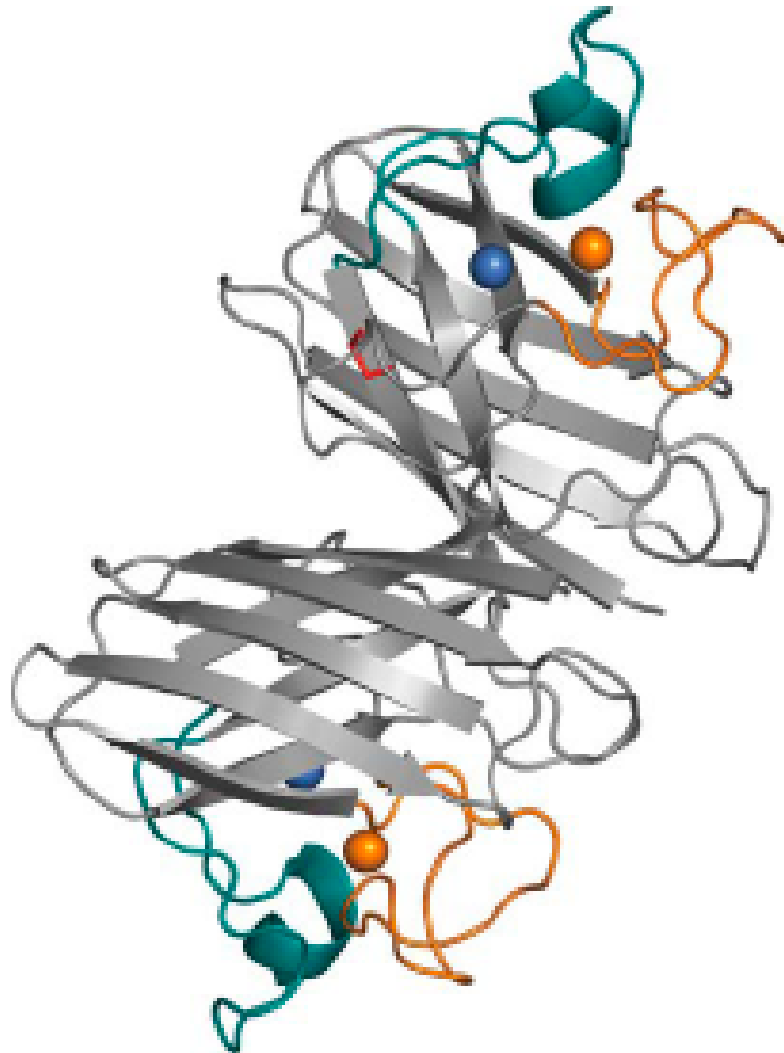
Mutations in the *FUS/TLS* Gene on Chromosome 16 Cause Familial Amyotrophic Lateral Sclerosis

T. J. Kwiatkowski Jr.,^{1*} D. A. Bosco,^{1,2} A. L. LeClerc,^{1,2} E. Tamrazian,¹ C. R. Vanderburg,³
C. Russ,^{1,4} A. Davis,¹ J. Gilchrist,⁵ E. J. Kasarskis,⁶ T. Munsat,^{7†} P. Valdmanis,⁸ G. A. Rouleau,⁸
B. A. Hosler,¹ P. Cortelli,⁹ P. J. de Jong,¹⁰ Y. Yoshinaga,¹⁰ J. L. Haines,¹¹ M. A. Pericak-Vance,¹²
J. Yan,¹³ N. Ticozzi,^{1,2,14} T. Siddique,¹³ D. McKenna-Yasek,¹ P. C. Sapp,^{1,15} H. R. Horvitz,¹⁵
J. E. Landers,^{1,2} R. H. Brown Jr.^{1,2*}

Science 27 February 2009



Struttura/funzione SOD1



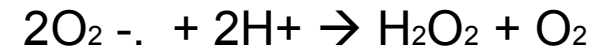
● rame

● zinco

Struttura:

omodimero
struttura a cilindro β
legame con i metalli
disolfuro C57-C146

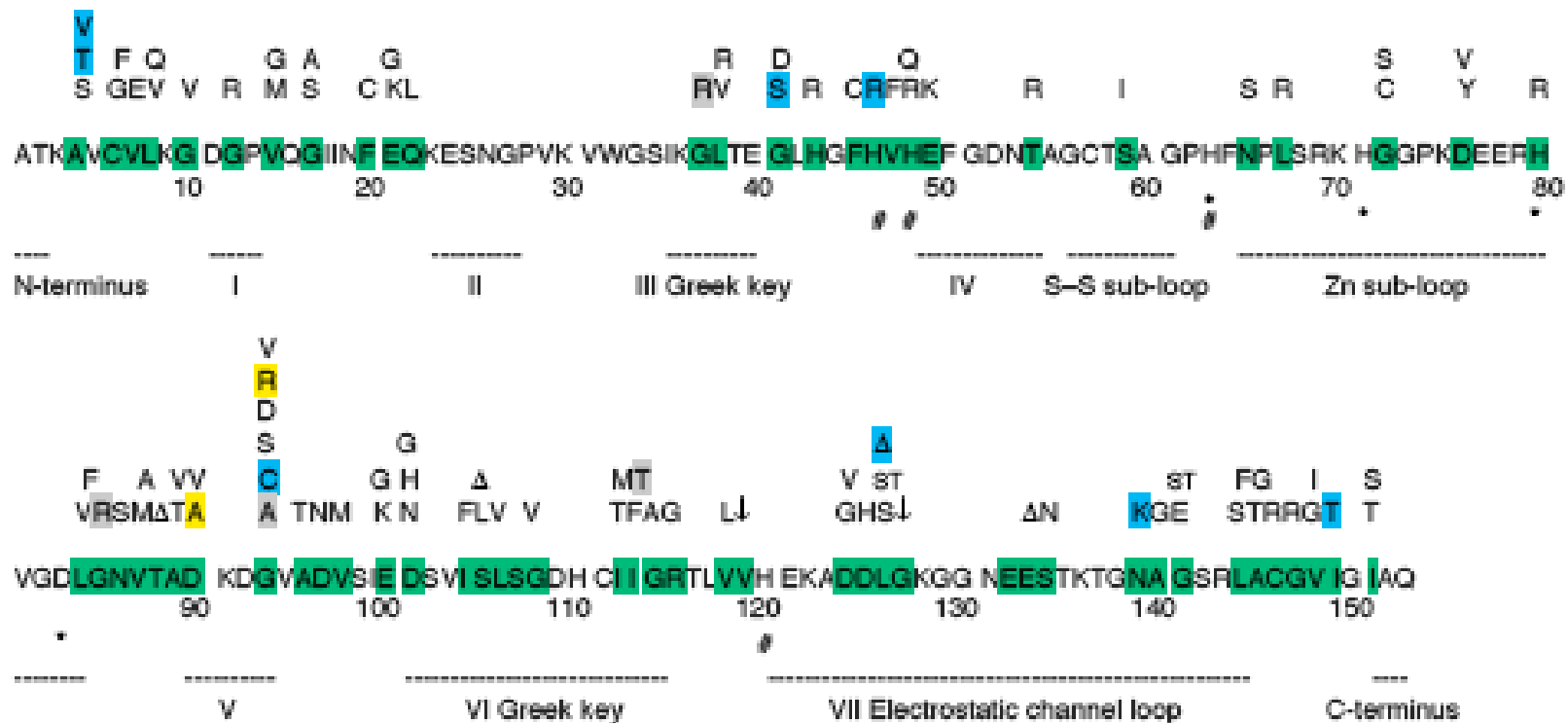
Funzione:



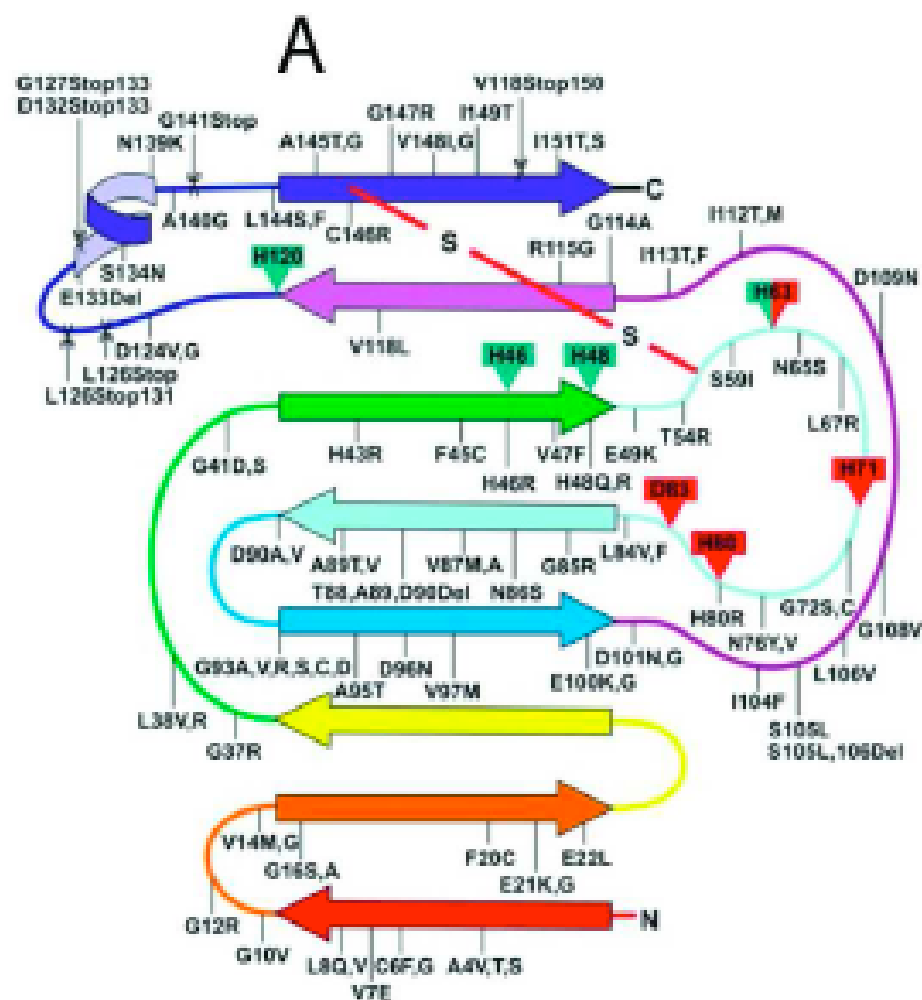
Localizzazione:

citosol
nucleo
mitocondri
ubiquitaria

>100 mutazioni
>70 posizioni



 at KAVCVLK_g dGPV_gGIINF_g eqkesngpV_k vwGsIk_gGLTE_g GIHGFHvHeF_g GDNT_aGC_TSA_g Gp_gHFNP_gLSrK_g HG_gGPkDeERH_g
 β1a β2b β3c β6d S
 Zn
 vGDLGNVTAd_g kdGvAdV_gSi_ge D_gsvISL_gSGdh_g cIIGRTlVvH_g EK_aDDL_gGKGG_g NEESTKTGNA_g GsRLACGVIG_g Iaq_g
 β5e β4f β7g β8h
 largest deletion by frame shift



- ✓ Mutanti “Wild-type like”: struttura/funzione ± normali
- ✓ Mutanti nel sito di legame dei metalli: struttura/funzione alterate

TABLE 1 Isolated fALS mutant SOD1 proteins

Wild-type-like (WTL) mutants				Metal-binding region (MBR) mutants
A4V ^{a,b}	G72S ^a	G93V ^b	N139K	H46R ^{a,b}
V7E ^b	D76Y ^a	E100G ^b	L144F ^b	H48Q ^{a,b}
L8Q ^b	L84V ^b	E100K ^b	L144S ^b	H80R ^b
G37R ^b	N86S ^b	D101N ^b	A145T ^b	G85R ^{a,b}
L38V ^{a,b}	D90A ^a	D101G ^b	V148G ^b	D124V ^{a,b}
G41D ^b	G93A ^{a,b}	I113T ^b	I149T ^b	D125H ^{a,b}
G41S ^a	G93R ^b	R115G ^b		S134N ^{a,b}
H43R ^b	G93C ^b	E133Del ^a		C146R ^b

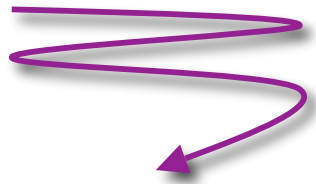
^aIsolated from Sf21 insect cell lines (39–41).

^bIsolated from *S. cerevisiae* (97, 97a).

- ✓ Topi knock-out SOD1 sono normali

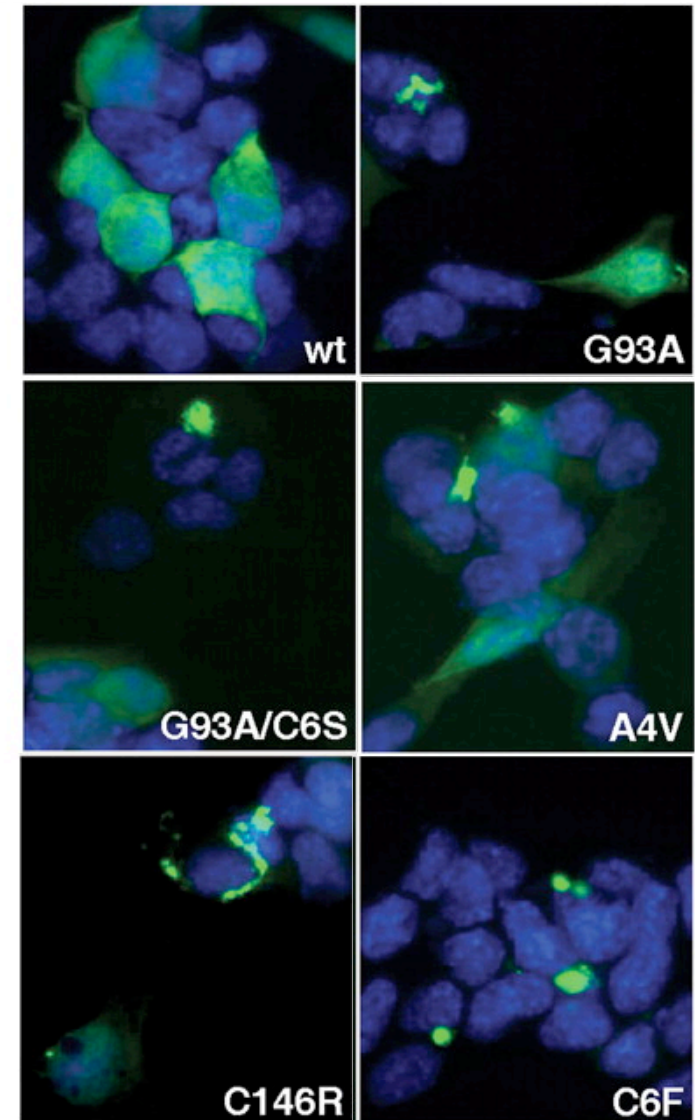
acquisto di funzione tossica

Proprietà comune di tutti i mutanti?



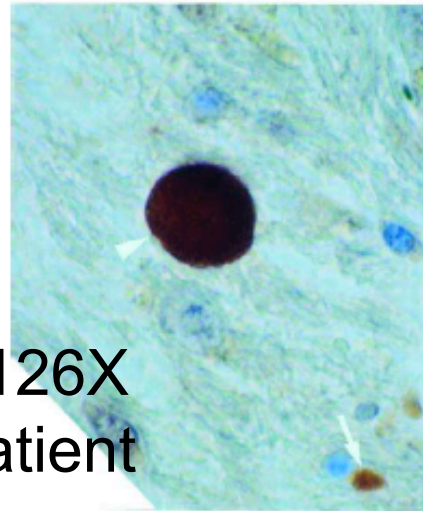
Formazione di aggregati (oligomeri)
insolubili

Le mutazioni diminuiscono la carica
netta della proteina

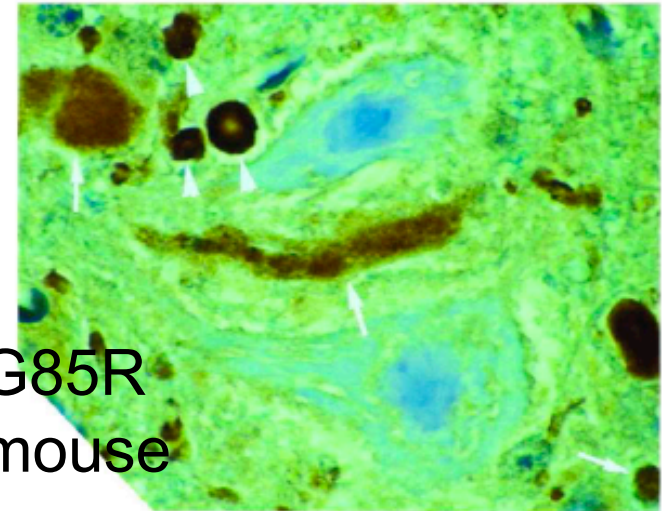


Inclusioni intraneuronali

L126X
patient

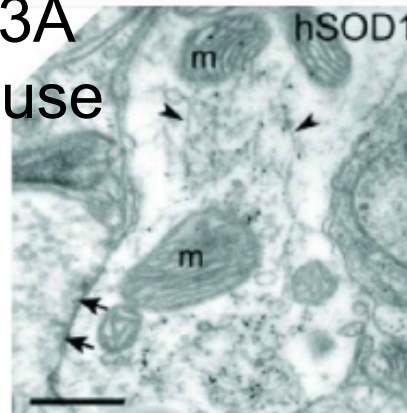


G85R
mouse



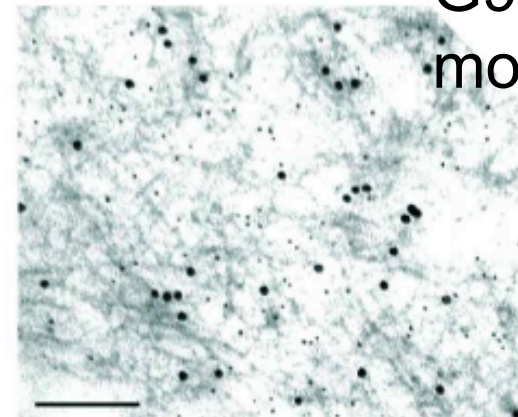
Inclusioni fibrillari

C
G93A
mouse

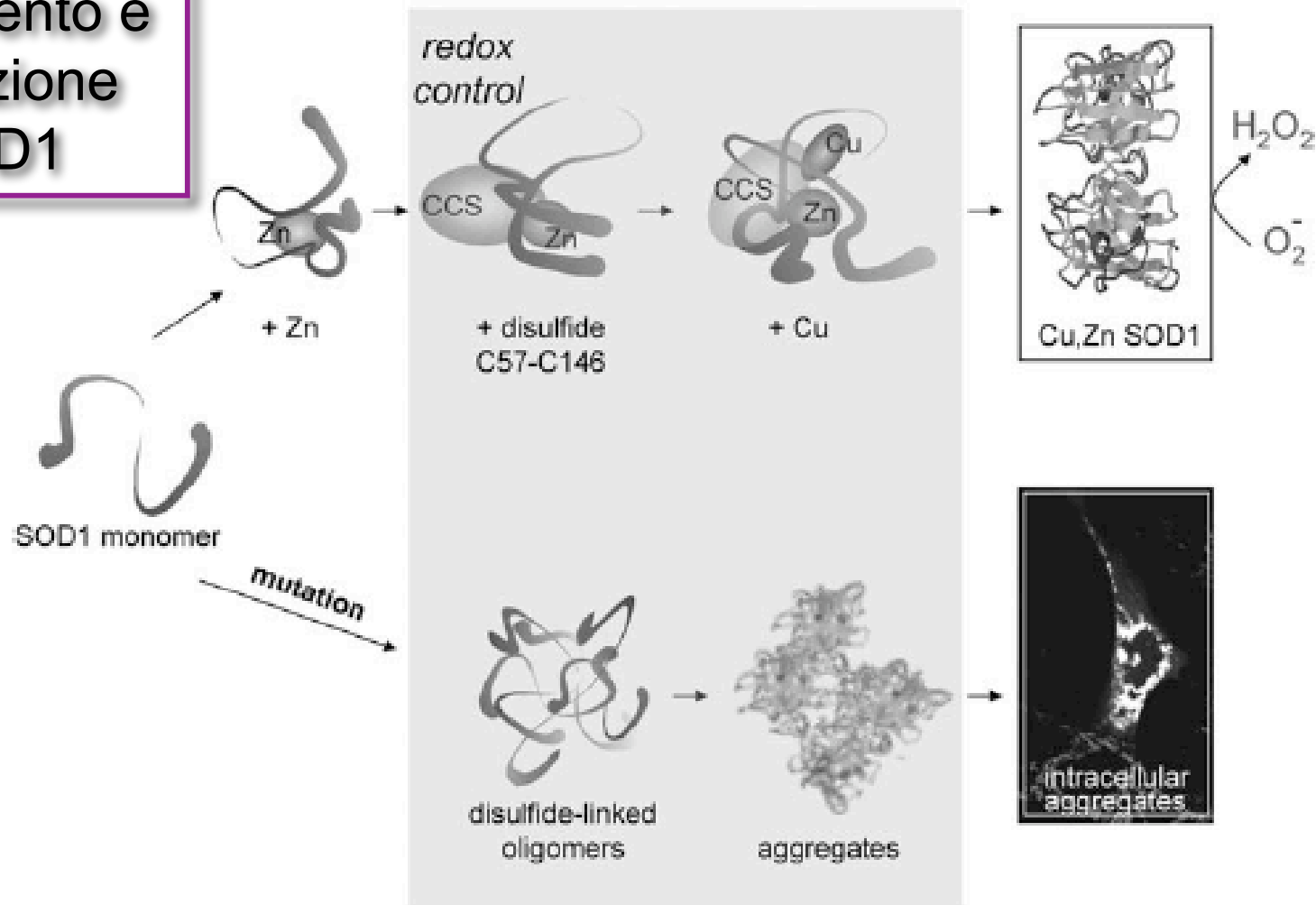


D

G93A
mouse



ripiegamento e aggregazione della SOD1



Le forme mutate associate alla fSLA acquistano conformazioni instabili che non rispecchiano il corretto ripiegamento, proprio della forma WT e, conseguentemente tendono ad oligomerizzare, formando aggregati insolubili di alto peso molecolare

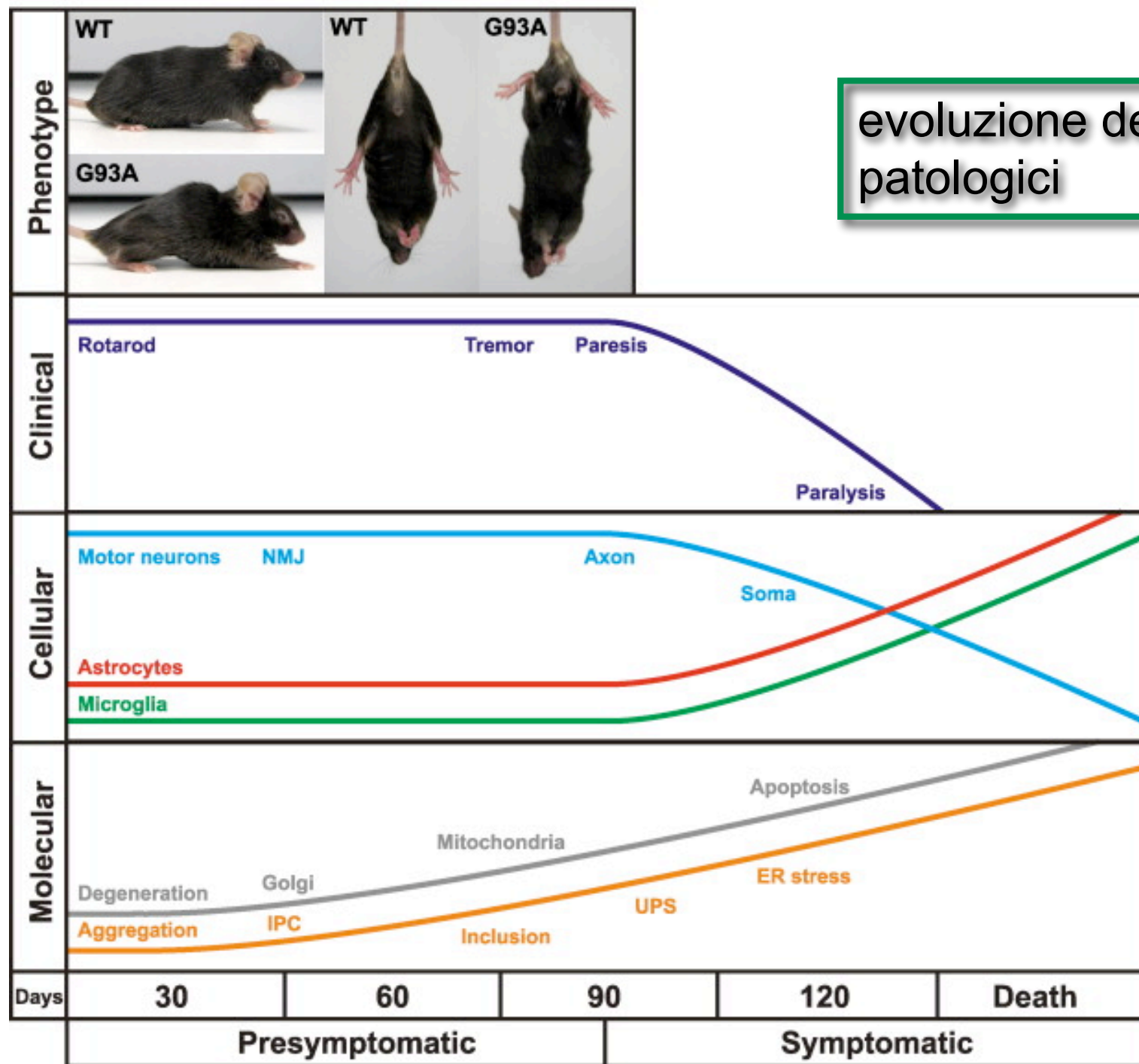
Modelli murini transgenici per la mutSOD1

Table 1
Transgenic mutant SOD1 mouse models

SOD1 mutant	SOD1 activity (fold)	Disease onset (months)	Disease duration (months)
SOD1 ^{A4Va}	nd	8	3
SOD1 ^{G37R}	14.5	4–6	nd
SOD1 ^{H46R}	nd	5	1
SOD1 ^{H46R/H48Q}	0	4–6	nd
SOD1 ^{H46R/H48Q/H63G/H120G}	0	8–12	nd
SOD1 ^{L84V}	nd	5–6	1
SOD1 ^{G85R}	0	8–14	0.5
SOD1 ^{G86Rb}	0	3–4	1
SOD1 ^{D90A}	6–8	12	2
SOD1 ^{G98A}	11	3–4	1–2
SOD1 ^{I113T}	nd	12	2
SOD1 ^{L126X}	nd	7–9	nd
	nd	11	0.75
SOD1 ^{L126delTT}	0	15	1
SOD1 ^{G127X}	0	8	0.25

nd: not described.

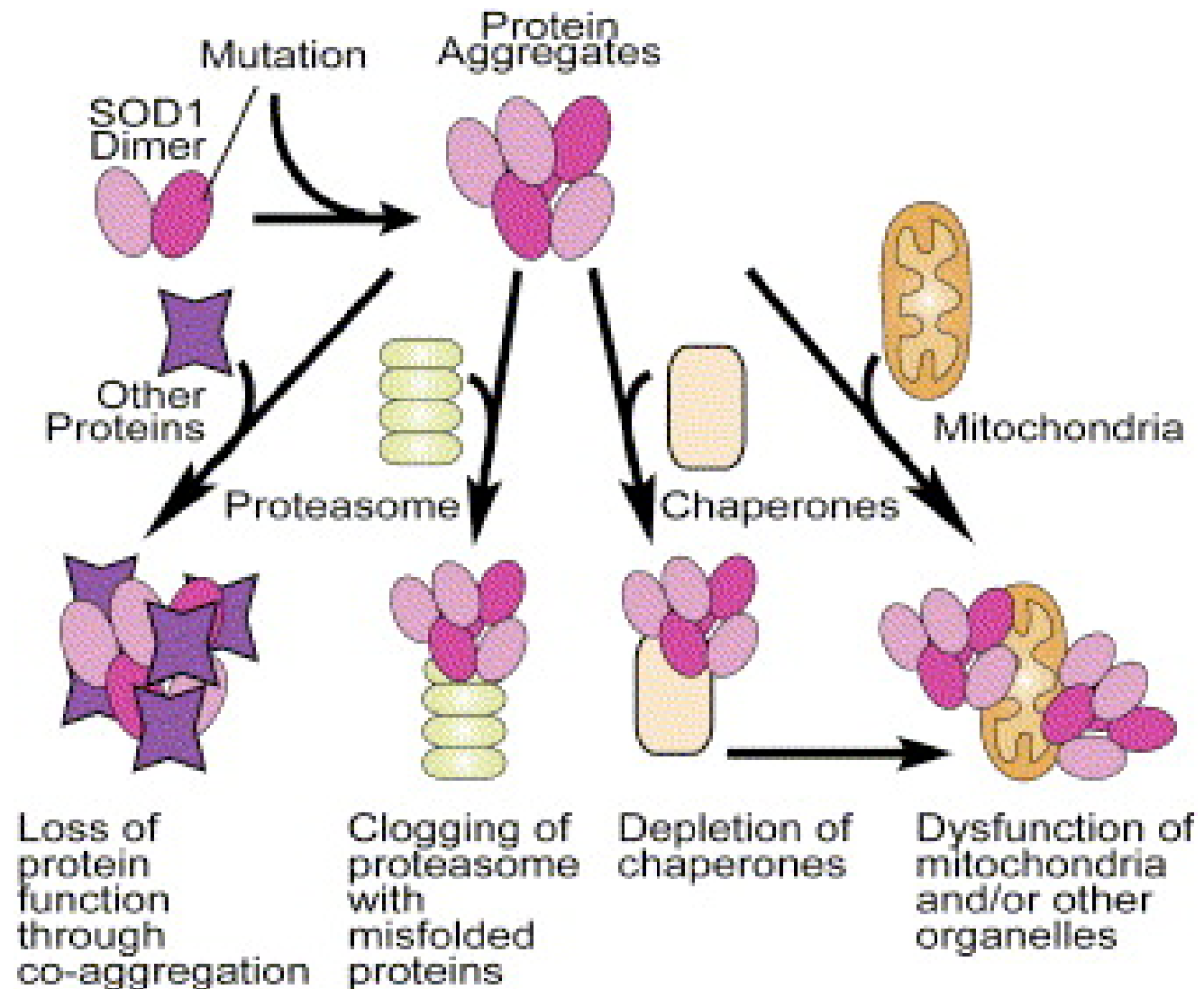
^a Doubly transgenic for SOD1^{WT}.



evoluzione dei fenotipi patologici

Meccanismi di tossicità

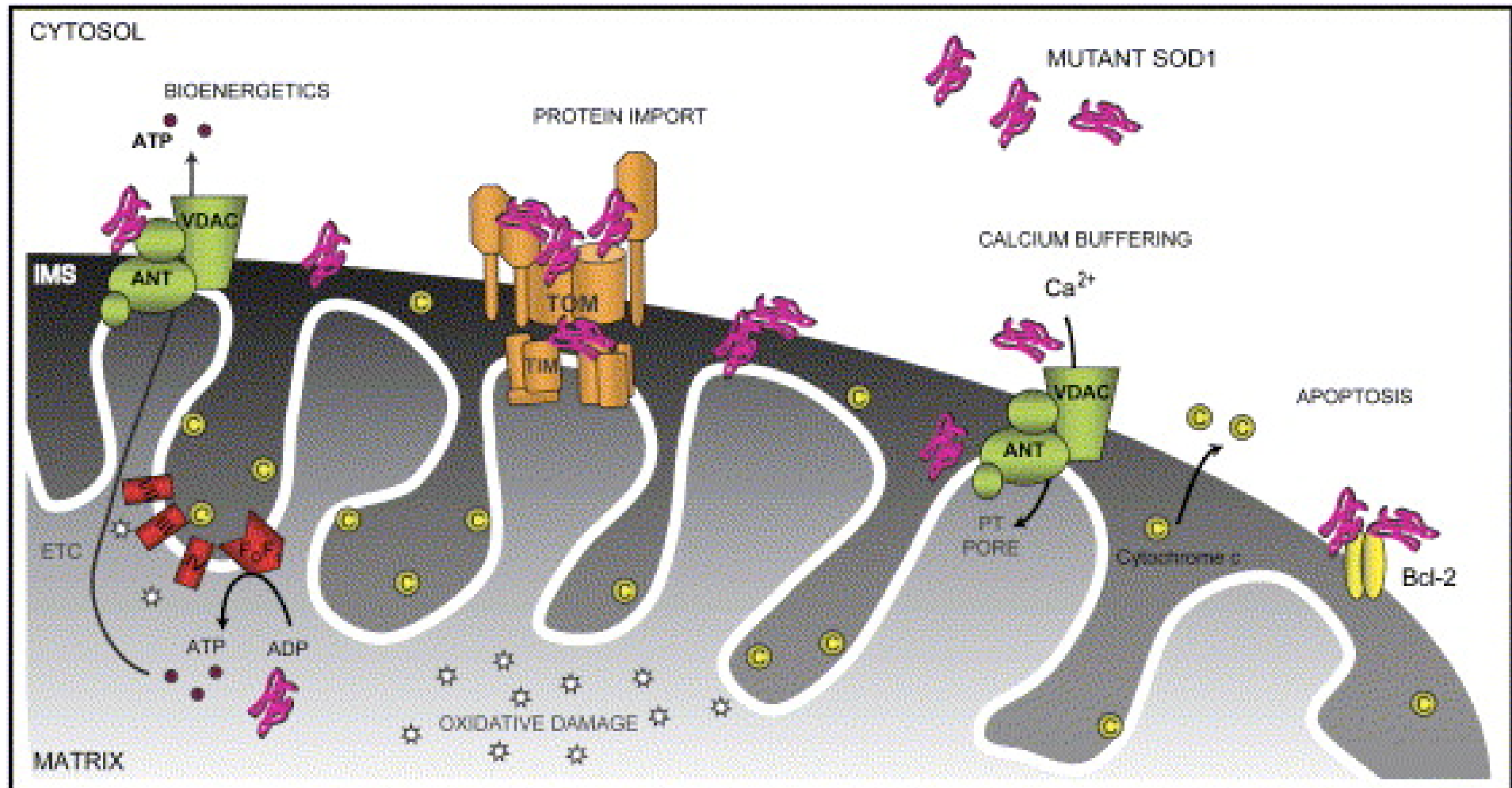
Aggregazione proteica



Disfunzioni mitocondriali

Meccanismi di tossicità

- ✓ tutti i mutanti SOD1 si accumulano nei mitocondri

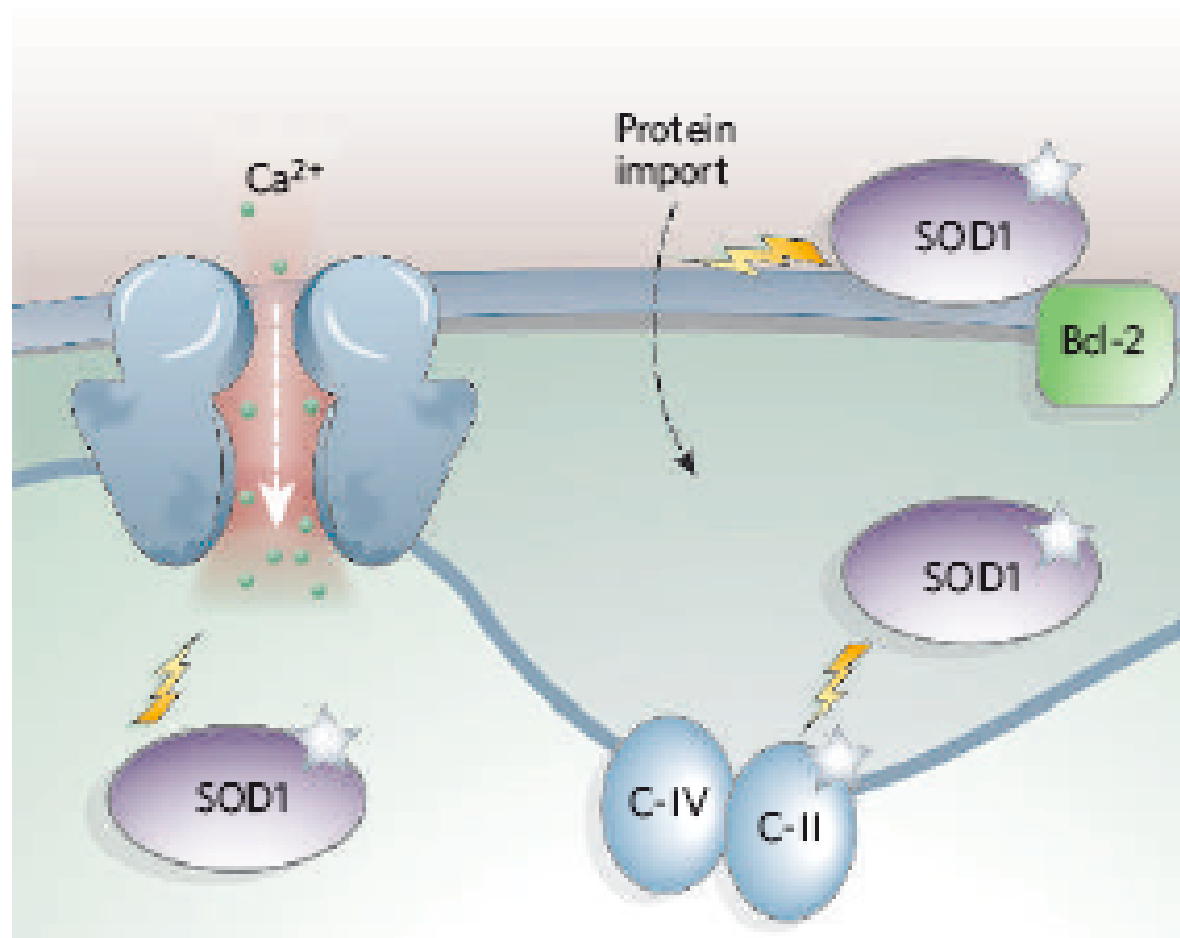


SLA e mitocondri

↓ attività del Complesso II e IV

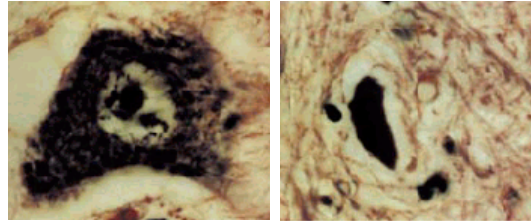
↑ stress ossidativo

Amyotrophic lateral sclerosis

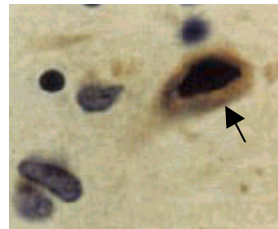


SLA e apoptosi

Morfologia



TUNEL



Inibitori apoptosi: XIAP



Famiglia di Bcl-2: Bcl-2
Bax

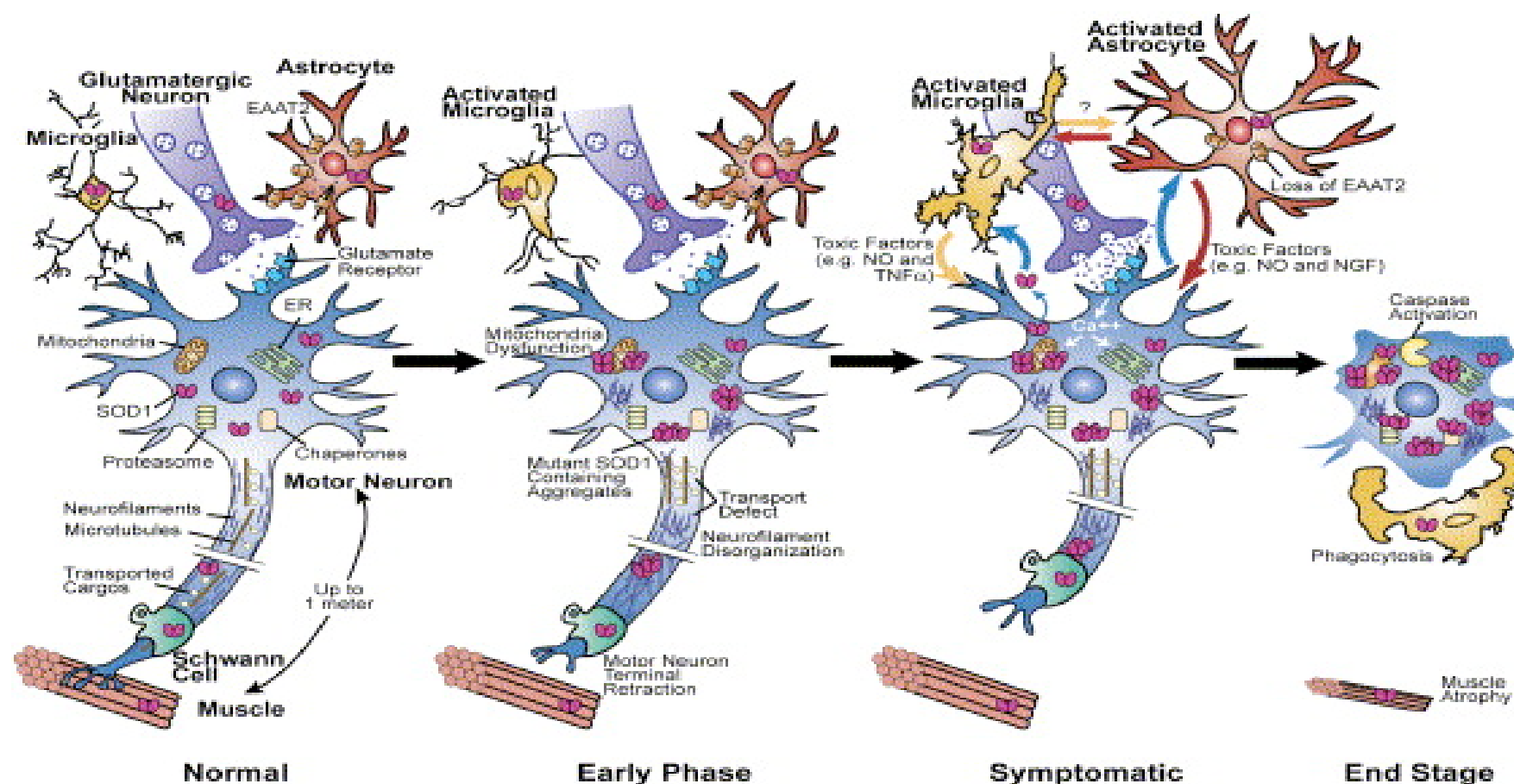


Attivazione caspasi:

caspase-1 ↑
caspase-3 ↑

La SLA è una malattia multisistemica

- ✓ Motoneuroni
- ✓ Astrociti
- ✓ Microglia
- ✓ Muscolo





microglia

CD11b Cre ricombinasi

SOD1 G37R

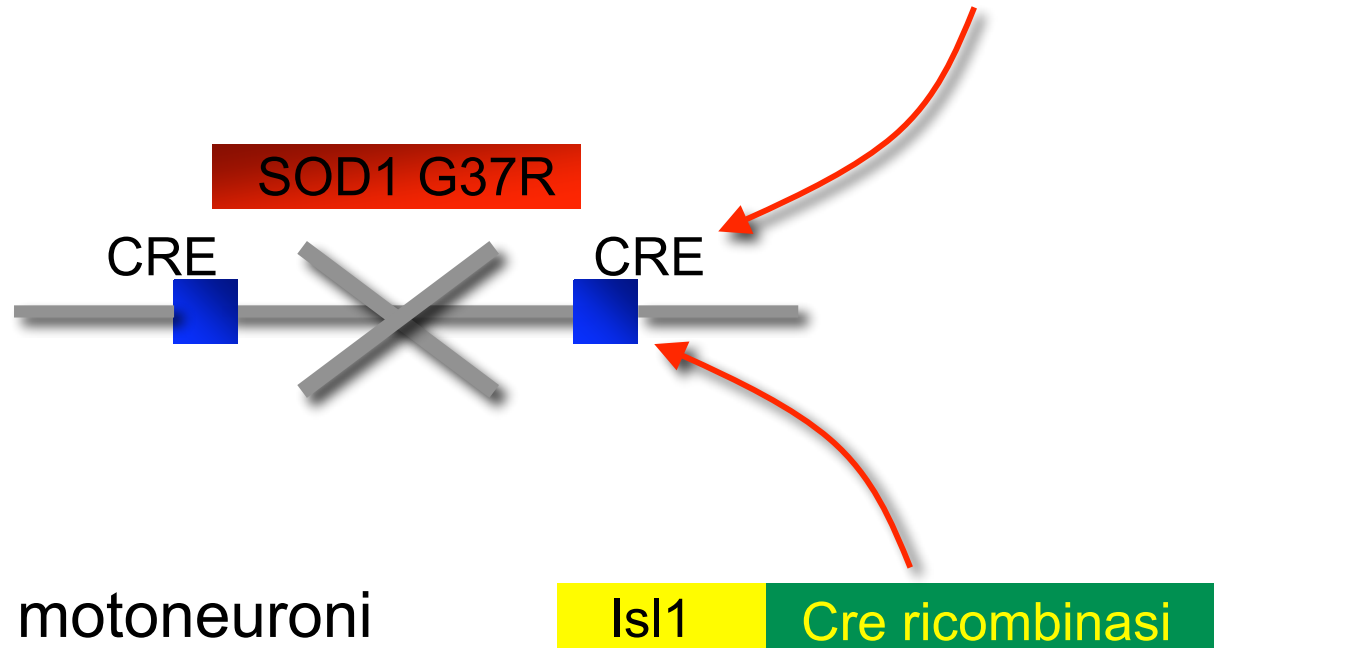
CRE

CRE

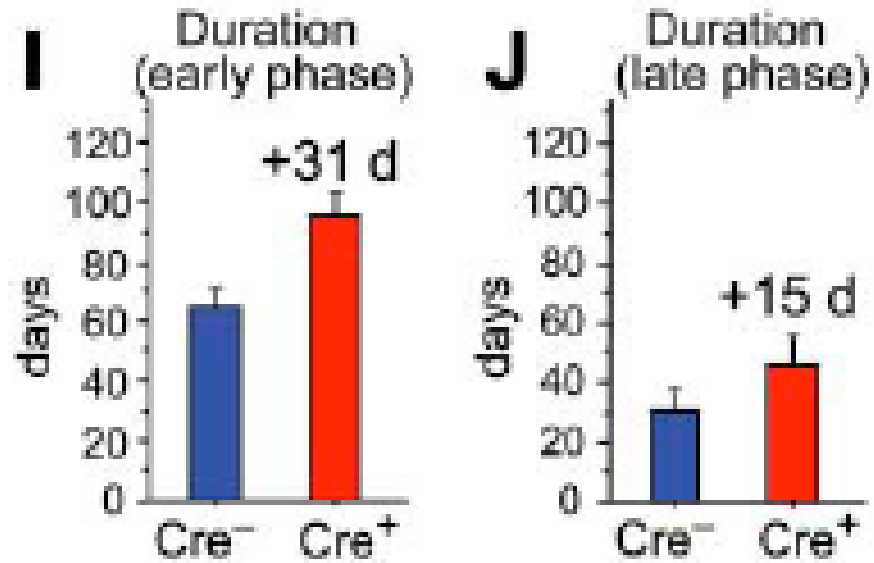
motoneuroni

Isl1

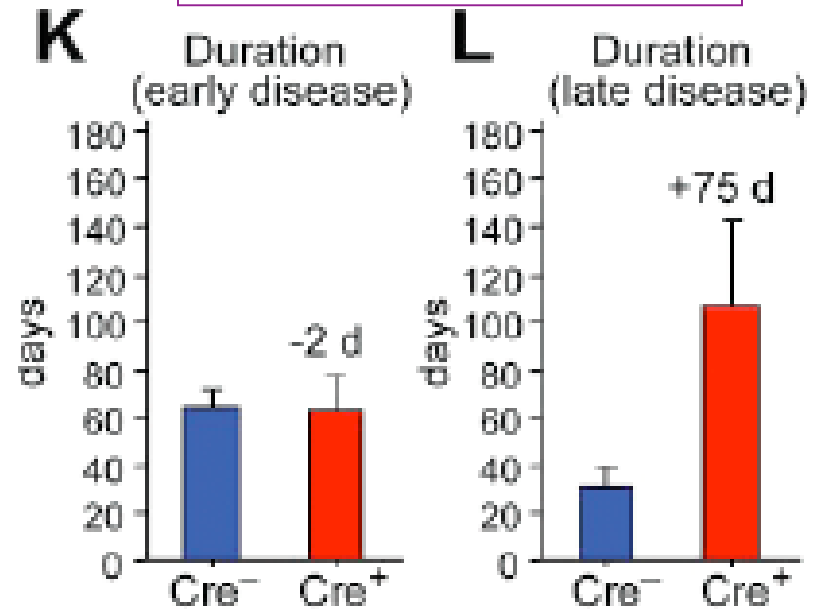
Cre ricombinasi



mutSOD1 rimossa
dai motoneuroni



mutSOD1 rimossa
dalla microglia



Espressione di mutSOD1:

motoneuroni: responsabile di insorgenza e fasi iniziali

microglia: responsabile della progressione