GENETICA DELLE PATOLOGIE NEURODEGENERATIVE

ALZHEIMER

Fabrizio Gardoni (27 Aprile 2010)

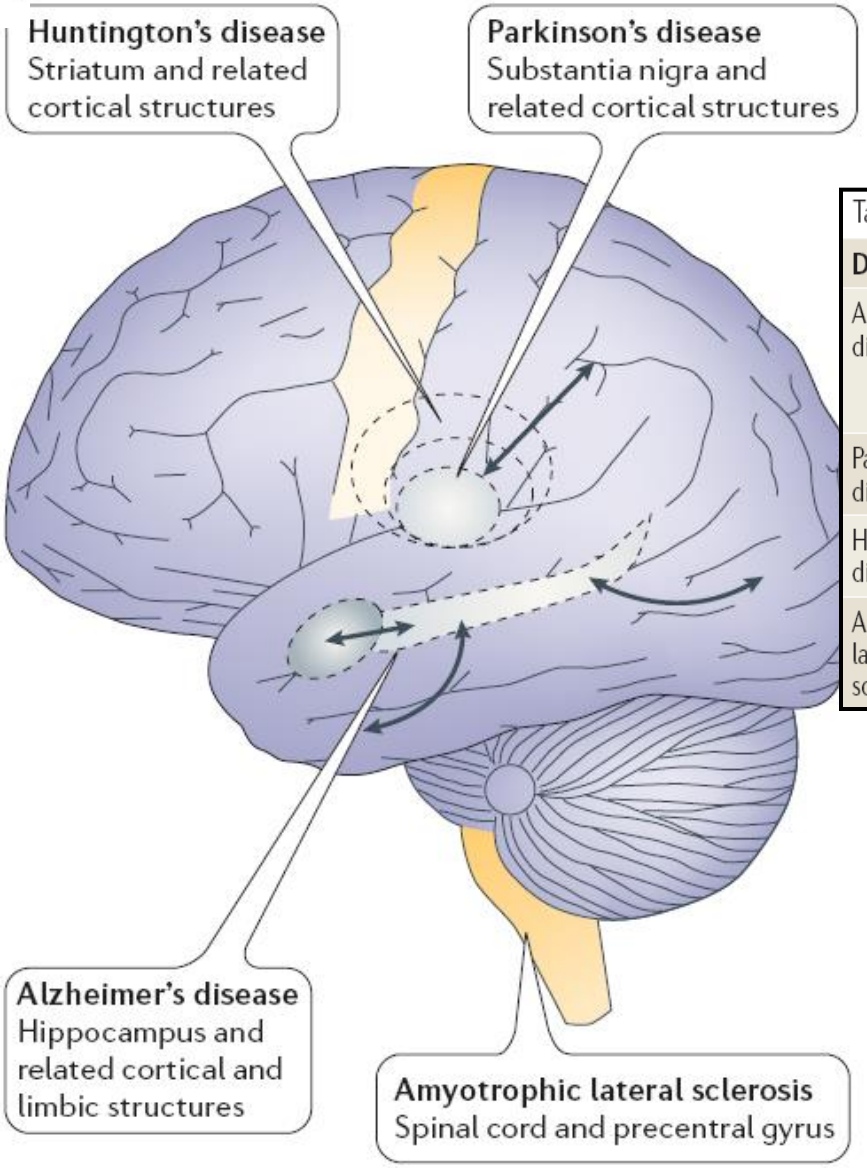
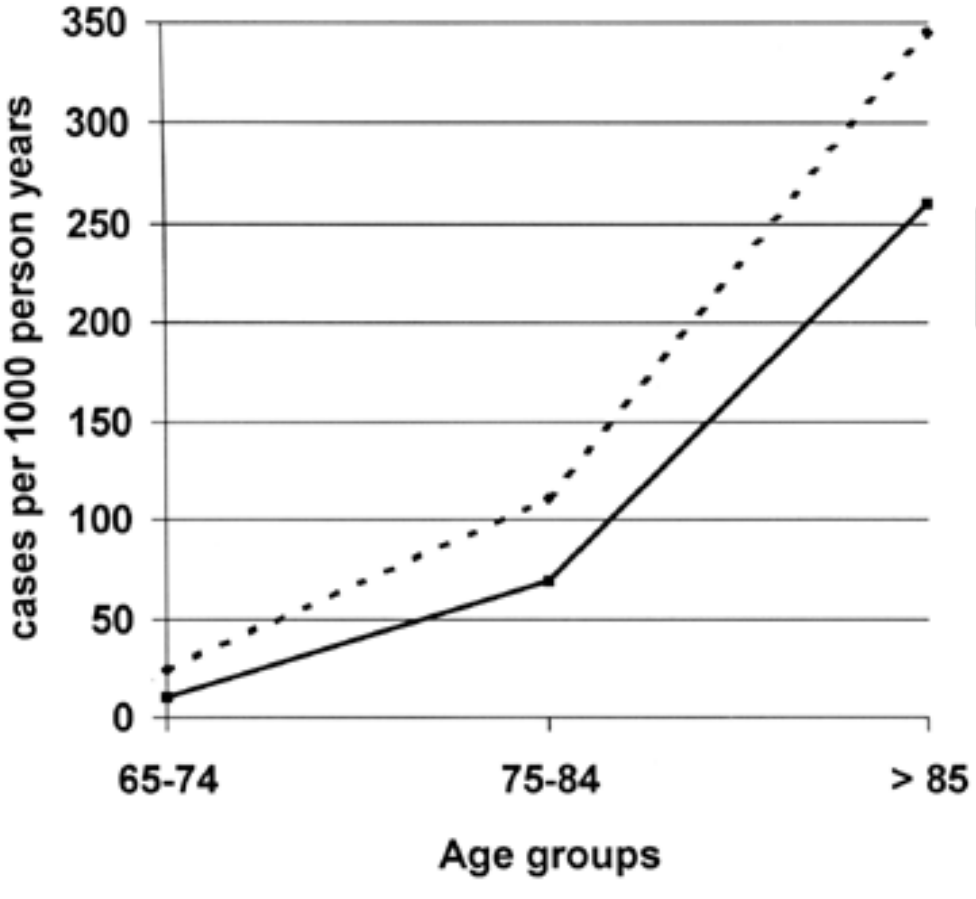


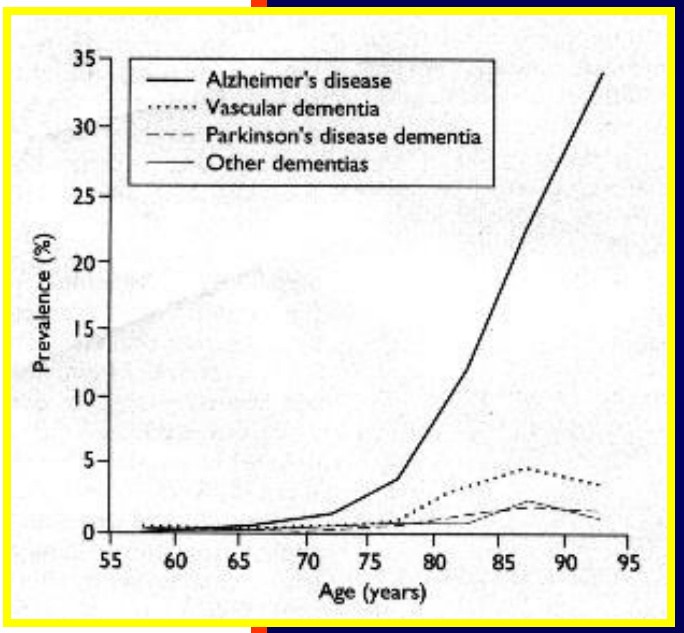
Table 1 | **Phenotypes of neurons vulnerable to age-related neurodegenerative disorders**

Disorder	Affected regions	Phenotypes
Alzheimer's disease	Entorhinal cortex, hippocampus, frontal cortex, basal forebrain, parietal lobe, occipital lobe, amygdala, locus coeruleus* and raphe nucleus*	Projection neurons; multiple transmitters (for example, glutamate, acetylcholine, noradrenaline and serotonin); and low CBPs
Parkinson's disease	Substantia nigra, frontal cortex, locus coeruleus* and raphe nucleus*	Projection neurons; primarily dopaminergic neurons; and low CBPs
Huntington's disease	Striatum, frontal cortex and locus coeruleus*	Projection neurons; and GABA-containing and glutamatergic neurons
Amyotrophic lateral sclerosis	Motor cortex and spinal cord	Projection neurons; cholinergic and glutamatergic neurons; and low CBPs

AD: più frequente e comune forma di demenza, 600.000 casi in Italia, 150.000 nuovi casi ogni anno;
5-10 anni di aspettativa media di vita dopo il primo esordio di sintomi.
>25% popolazione sopra gli 85anni è affetta dalla malattia.
>95% sporadico (SAD), <5% familiare (FAD).



Prevalence of dementia and AD, Canada



La Malattia di Alzheimer è la più frequente tra le Demenze. La percentuale cresce con l'età (50% a 65 anni; 80% a 85 anni).

Diagnosi di Demenza (DSM IV - 1994)

Perdita delle abilità intellettive di gravità tale da **interferire** con le attività lavorative e sociali

Perdita della **memoria**

Almeno **una** della seguenti alterazioni:

- **perdita del pensiero astratto**
- **perdita delle capacità di giudizio**
- **perdita di funzioni corticali superiori quali:**

- **modificazioni comportamentali**

MMSE < 27

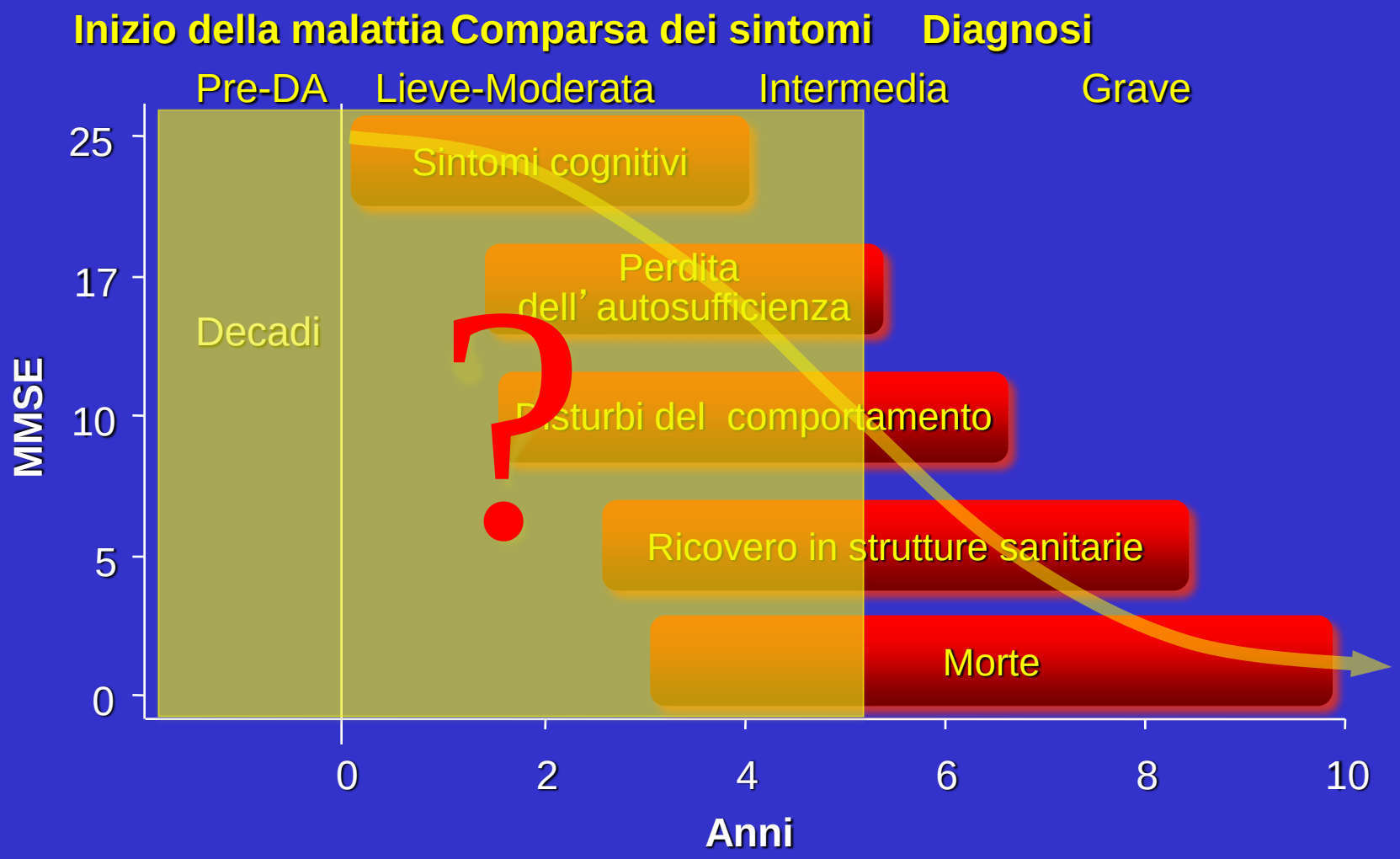
Clinical Dementia Rating > 0.5 (1-3)

Global Deterioration Scale > 3 (4-7)

Mattis Dementia Rating Scale < 123

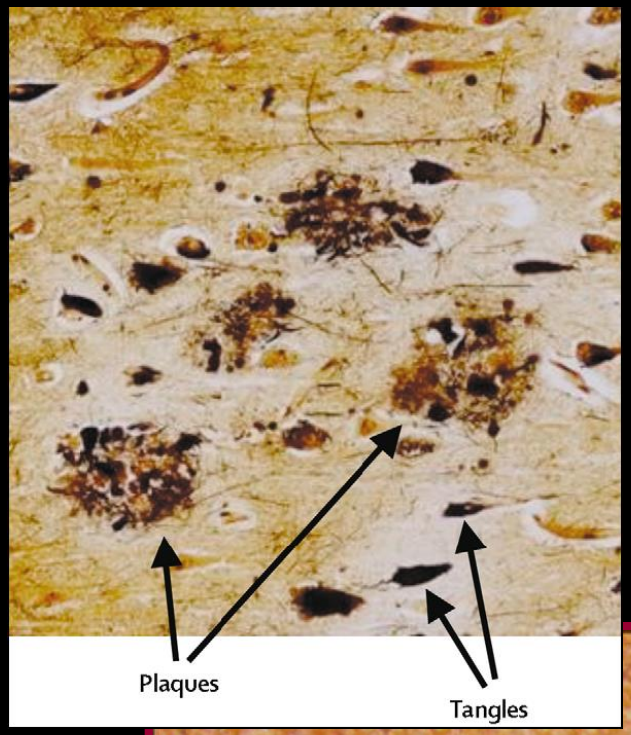
MMSE = Mini-Mental State Examination

Storia naturale della Malattia di Alzheimer



First Alzheimer's Diagnosis Confirmed

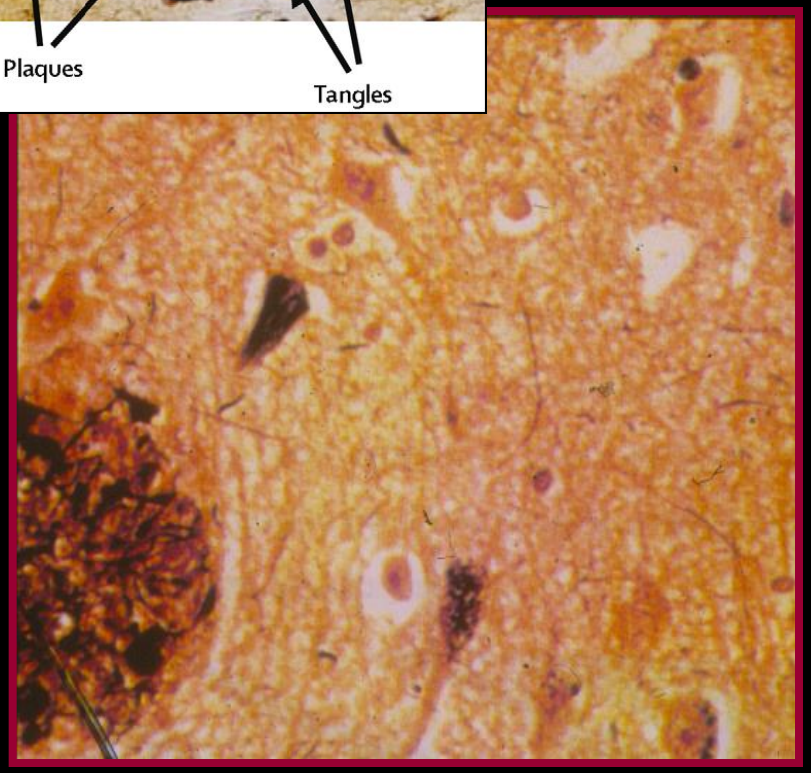
1906 Alois Alzheimer



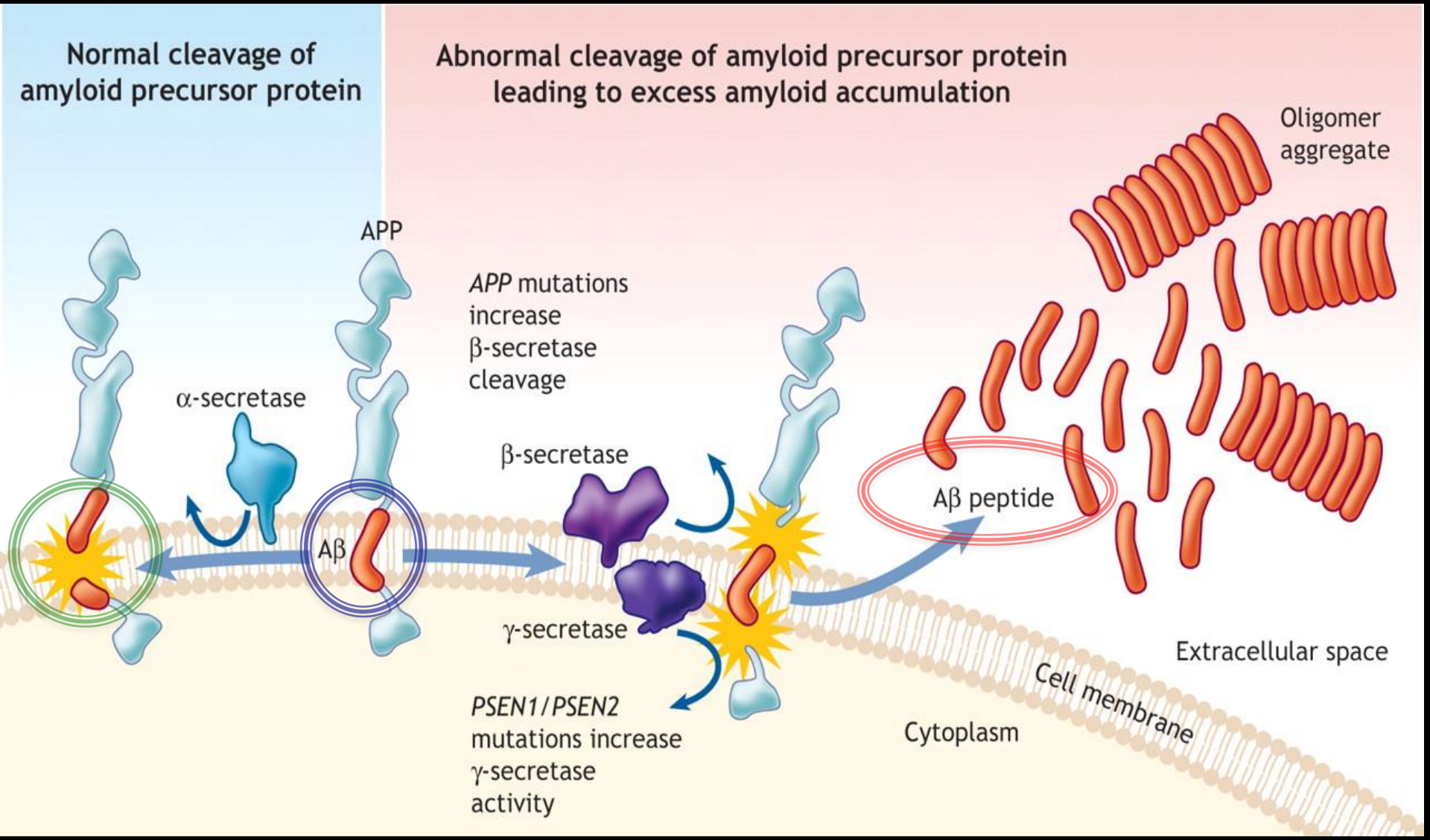
- Peptide β-Amiloide

Senile Plaques and Tangles

- Proteina Tau



Amyloid Precursor Protein metabolism



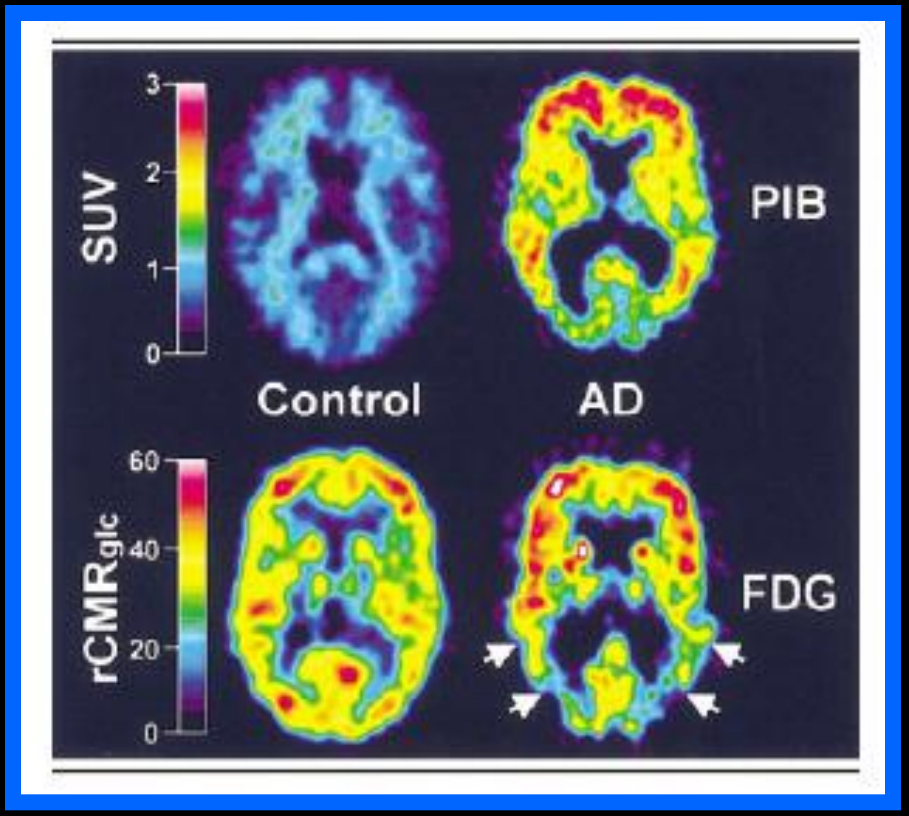
Imaging Brain Amyloid in Alzheimer's Disease with Pittsburgh Compound-B

Ann Neurol 2004;55:306–319

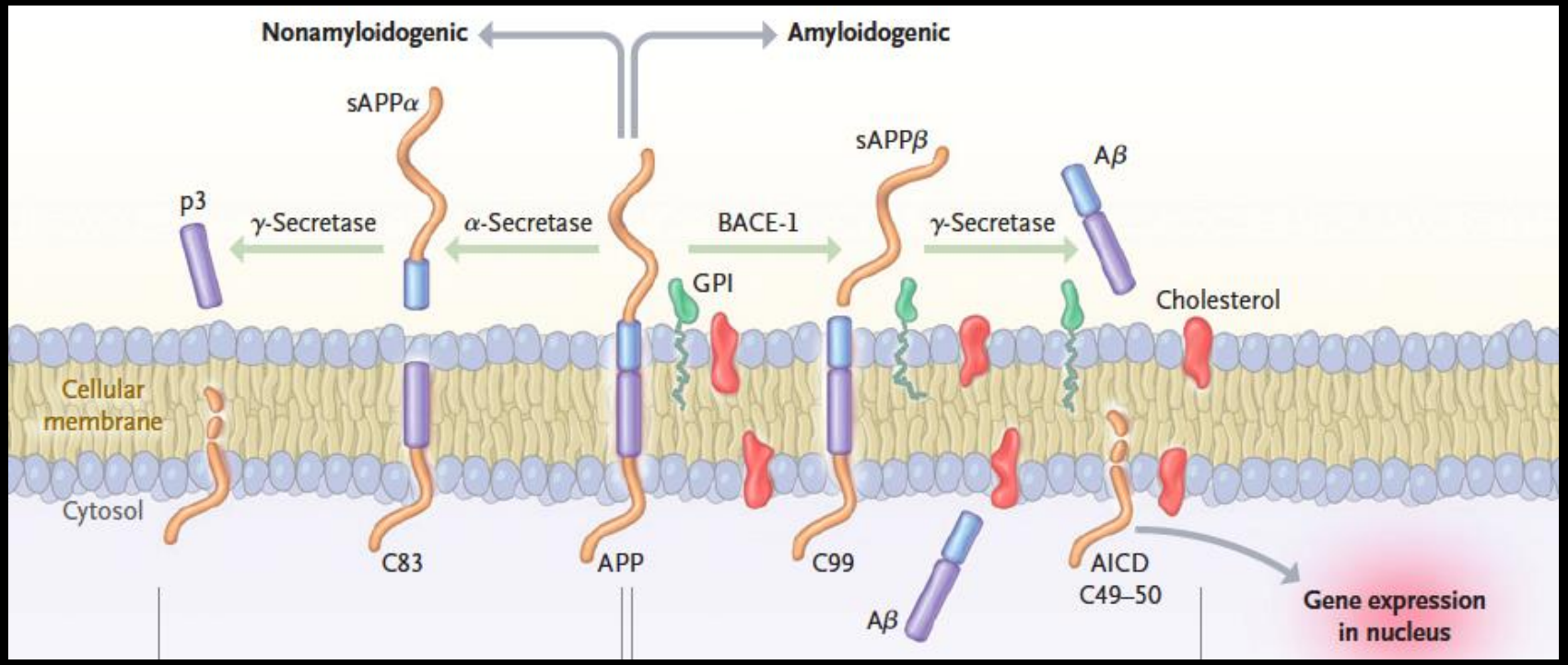
William E. Klunk, MD, PhD,[1] Henry Engler, MD,[2] Agneta Nordberg, MD, PhD,[3,4] Yanming Wang, PhD,[5] Gunnar Blomqvist, PhD,[2] Daniel P. Holt, BS,[5] Mats Bergström, PhD,[2] Irina Savitcheva, MD,[2] Guo-feng Huang, PhD,[5] Sergio Estrada, PhD,[2] Birgitta Ausén, MSCI,[4] Manik L. Debnath, MS,[1] Julien Barletta, BS,[6] Julie C. Price, PhD,[5] Johan Sandell, PhD,[2] Brian J. Lopresti, BS,[5] Anders Wall, PhD,[2] Pernilla Koivisto, PhD,[2] Gunnar Antoni, PhD,[2] Chester A. Mathis, PhD,[5] and Bengt Långström, PhD[2,6]

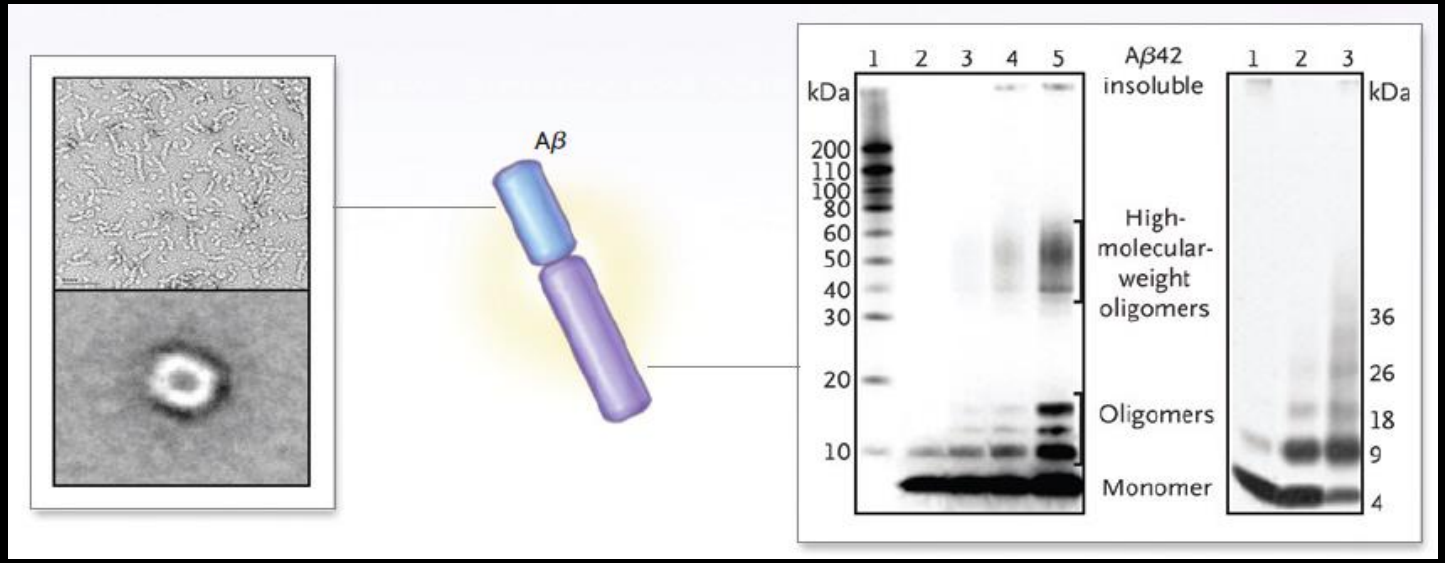
First human study of a novel amyloid-imaging positron emission tomography (PET) tracer, termed Pittsburgh Compound-B (PIB)

AD patients typically showed marked retention of PIB in areas of cortex known to contain large amounts of amyloid deposits in AD.



Processing of Amyloid Precursor Protein

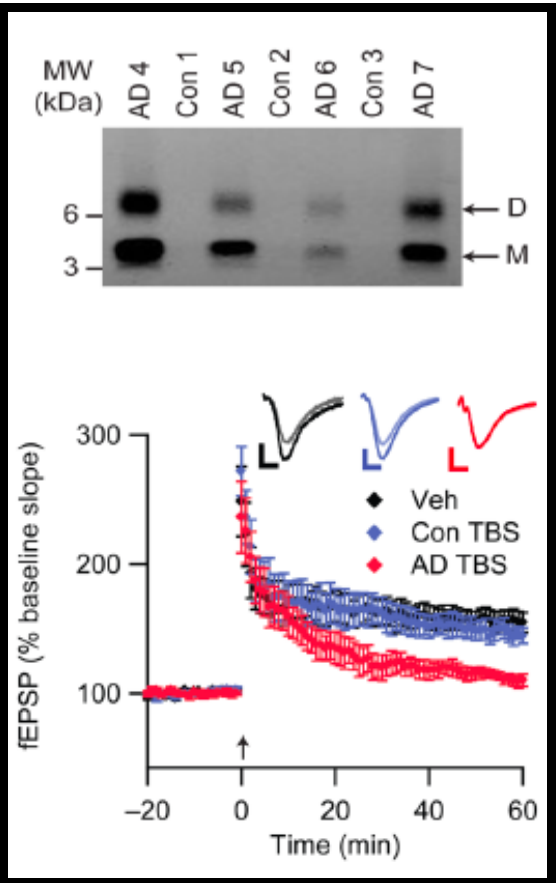




Nat Med. 2008 August ; 14(8): 837–842. doi:10.1038/nm1782.

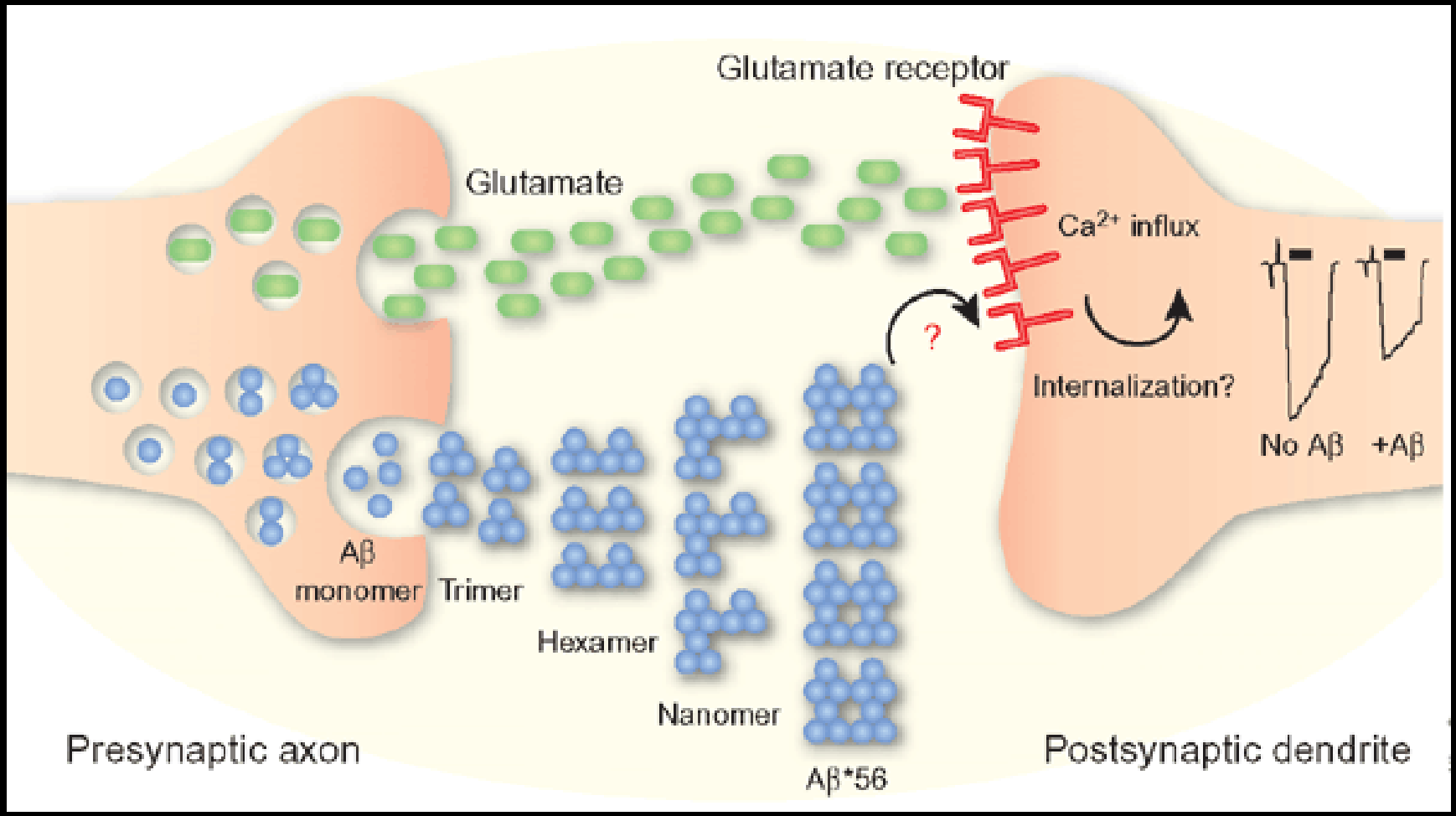
Amyloid β-Protein Dimers Isolated Directly from Alzheimer Brains Impair Synaptic Plasticity and Memory

Ganesh M. Shankar[1,6], Shaomin Li[1], Tapan H. Mehta[1], Amaya Garcia-Munoz[2], Nina E. Shepardson[1], Imelda Smith[3], Francesca M. Brett[4], Michael A. Farrell[4], Michael J. Rowan[5], Cynthia A. Lemere[1], Ciaran M. Regan[2], Dominic M. Walsh[3], Bernardo L. Sabatini[6], and Dennis J. Selkoe[1]

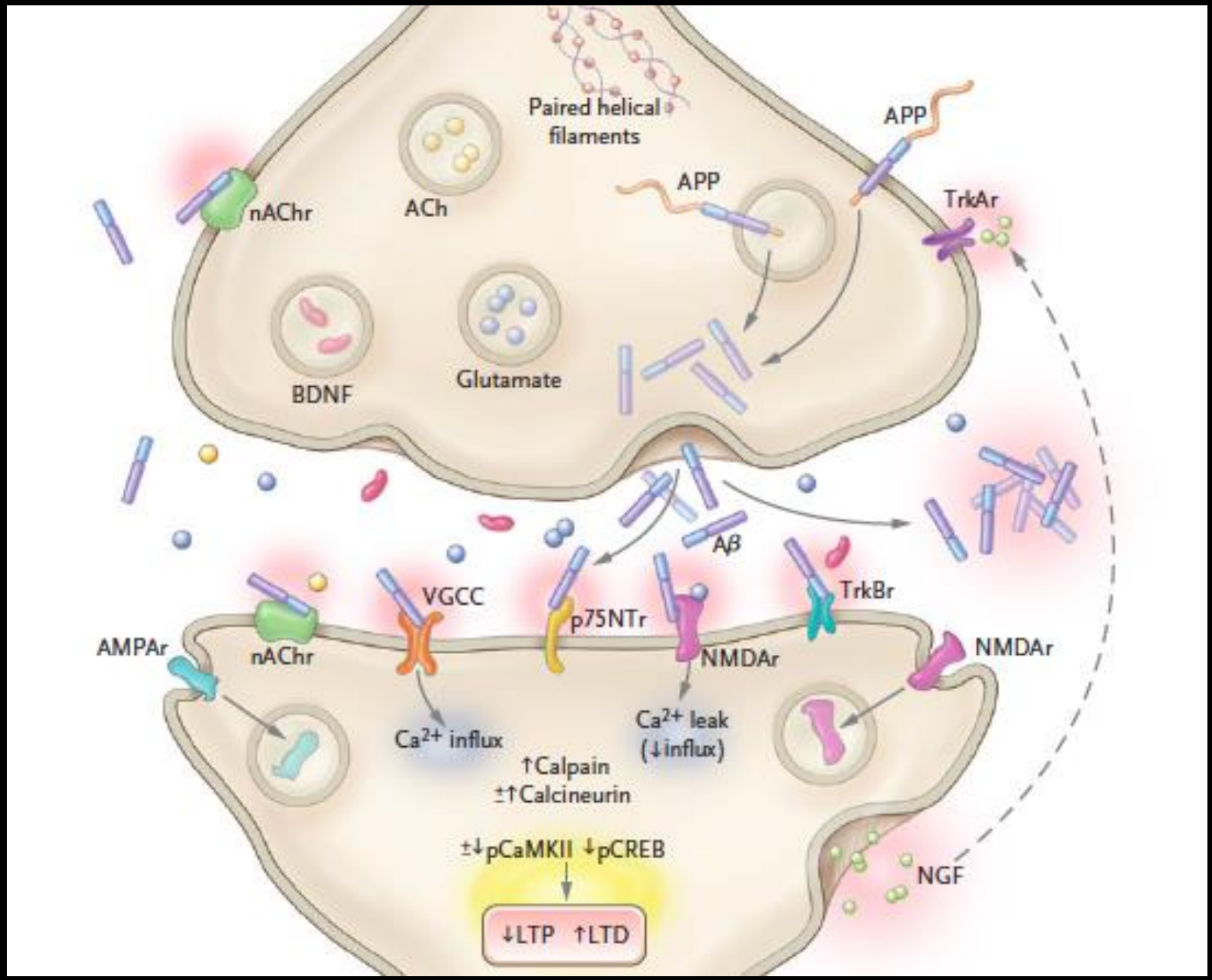


A role for Aβ soluble oligomeric and protofibrillar forms in dysregulation of synaptic function

(Walsh et al., Nature 2002; Lesne et al, Nature, 2006)



Synaptic Dysfunction in Alzheimer's Disease



Complexity of AD molecular pathogenesis

Several molecular pathways are involved

1906

1984
Aβ
sequence

1986 -tau

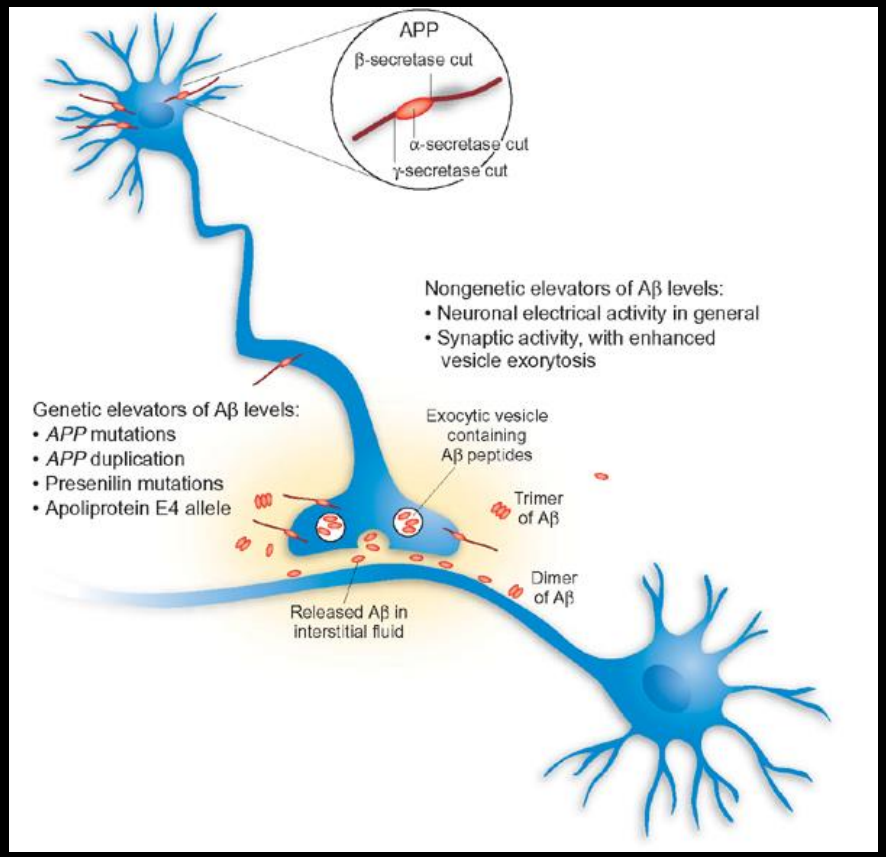
1987
APP gene

1991
synapse loss is the major correlate of cognitive impairment.

1995
PS1-PS2

1999
BACE, ADAM10

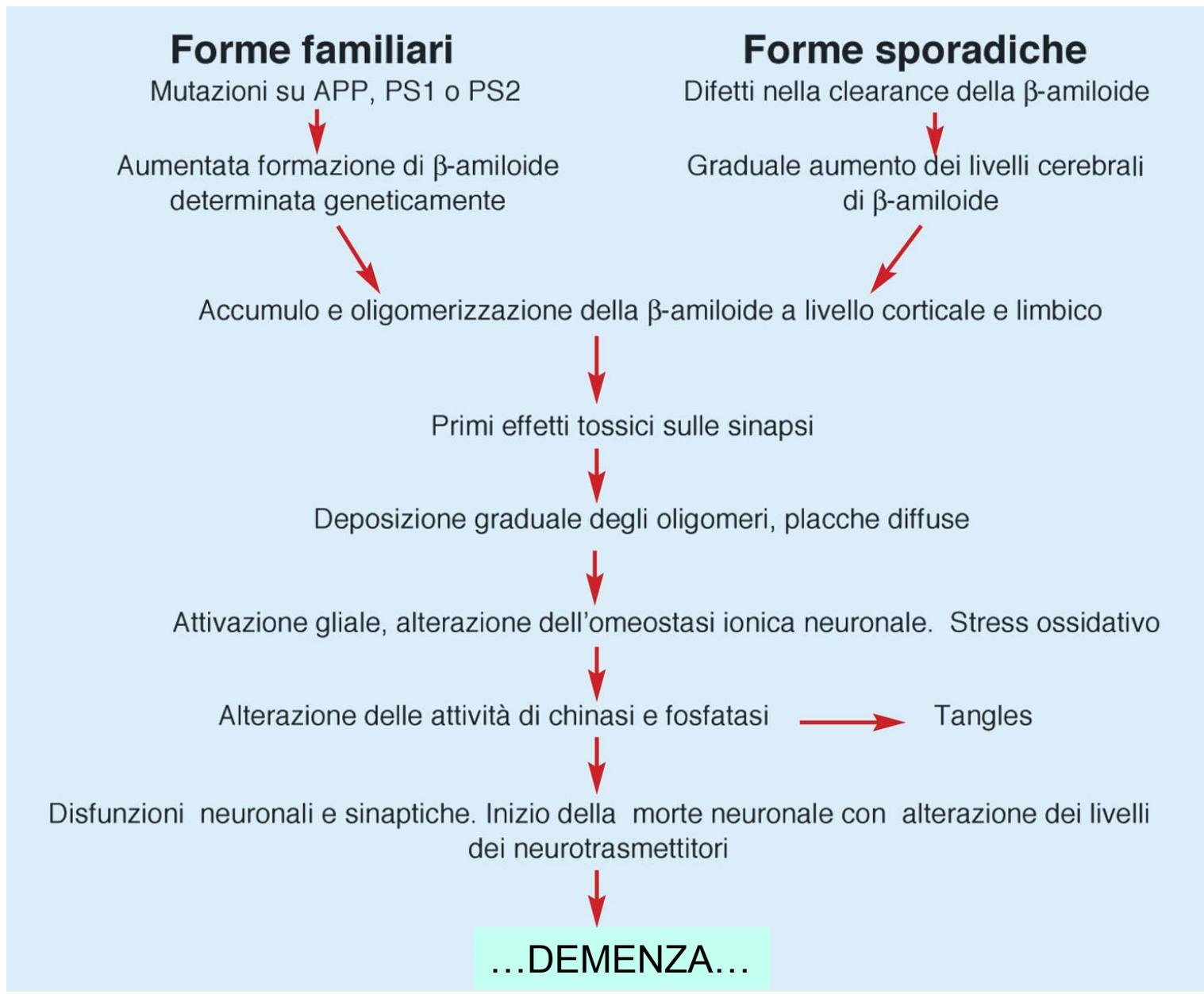
2002
AD is a synaptic failure



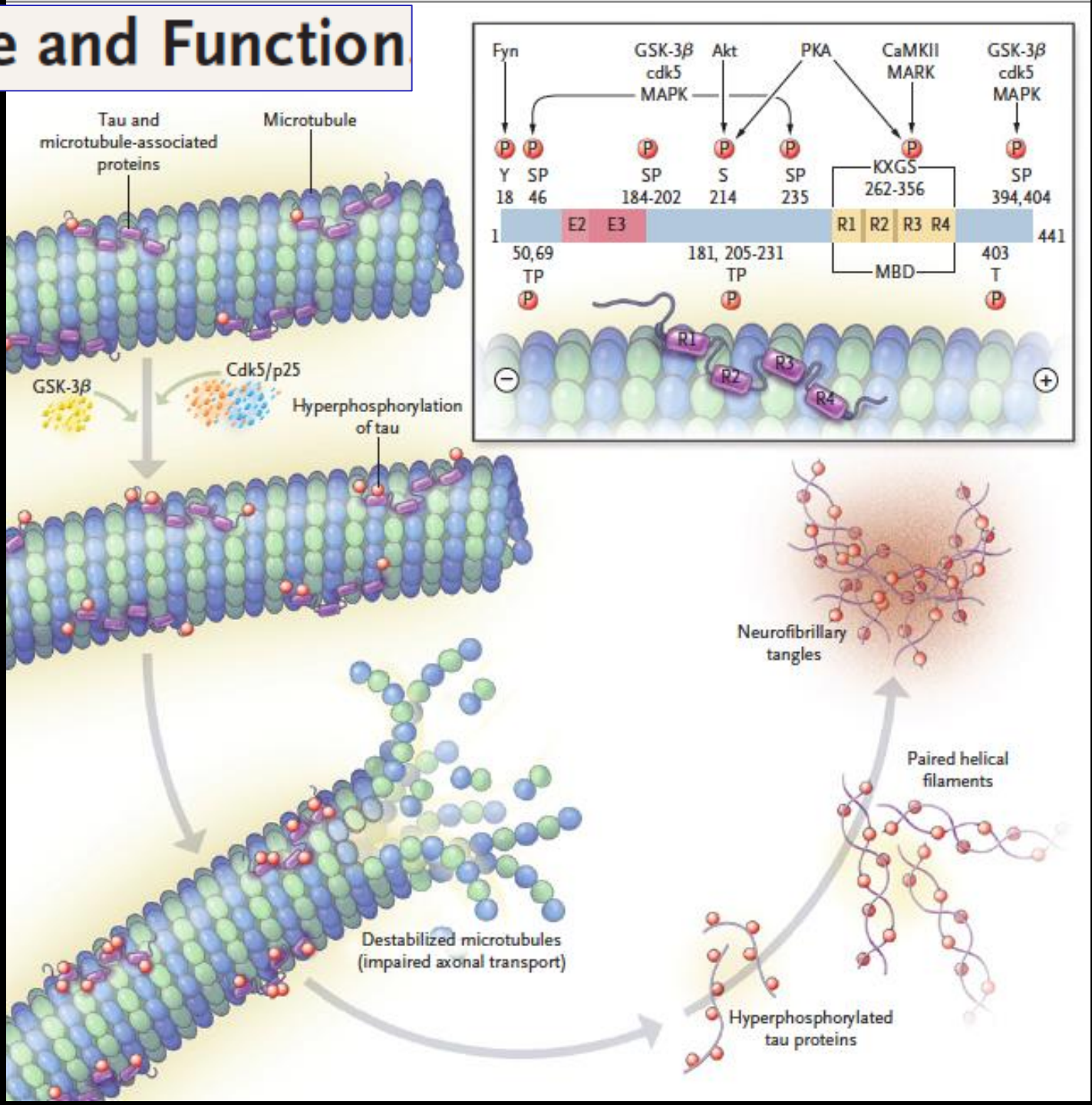
Alzheimer disease as a synaptopathology

The ups and downs of Aβ
Dennis J Selkoe, Nat Med 2006

The amyloid cascade hypothesis:



Tau Structure and Function

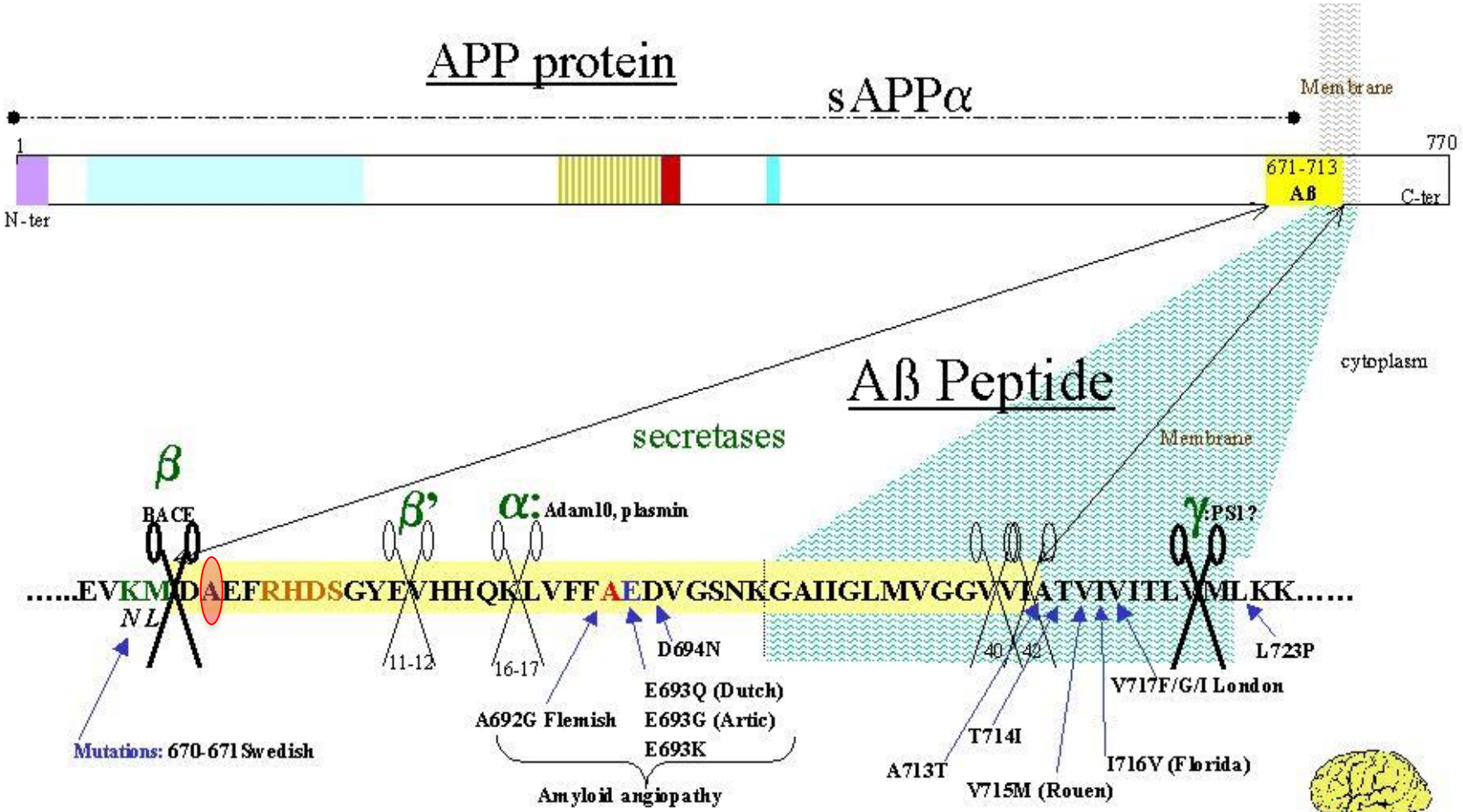


Genetica della Malattia di Alzheimer

- Mutazioni identificate:
  - APP – Amyloid precursor protein
  - γ-Secretase (PS1 – Presenilin 1 and PS2 – Presenilin 2)

Mutations in APP cluster:
- around the β-secretase cleavage site (*e.g. Swedish mutation),*
- *in* key amino acids affecting its ability to aggregate (e.g. Arctic and Dutch mutations)
- around the γ-secretase cleavage site, which increases production of the longer Abeta42 peptide (e.g. London mutation).

PS mutations play a similar role by favoring production of Abeta42 at the expense of Abeta40.



Catabolism of APP, overproduction and clearance of Aβ

VCDN INSERM 422

Table 1 | **Genes associated with Alzheimer's disease**

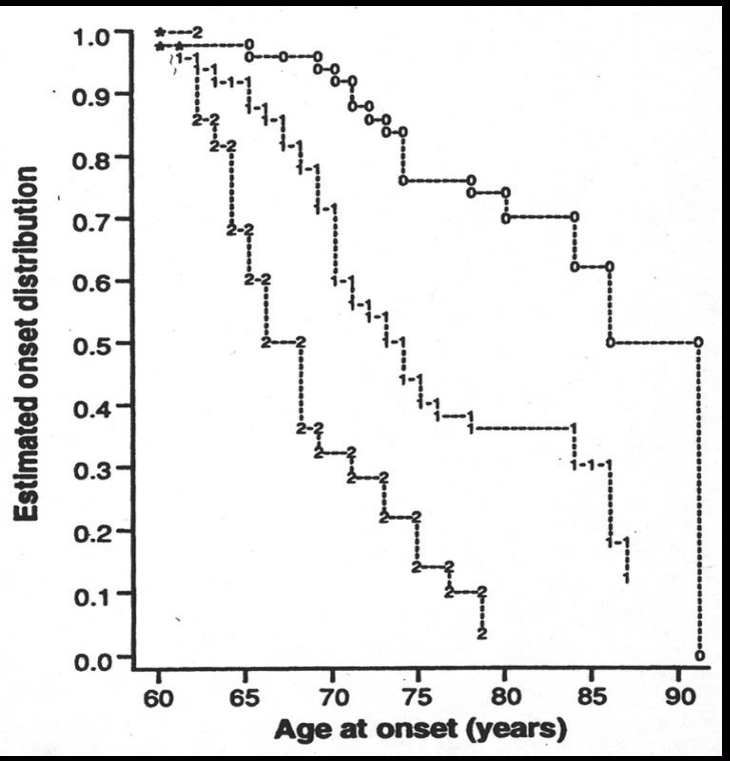
Disease onset	Gene product	Disease association	Chromosome
Early	Amyloid precursor protein (APP)	++	21
	Presenilin 1 (PS1)	++	14
	Presenilin 2 (PS2)	++	1
Late	Apolipoprotein E	++	19
	LDL receptor-related protein (LRP)	+	12
	α_2-Macroglobulin (α_2M)	+	12
	FE65	+	11
	Chromosome 12 gene product distinct from LRP and α_2M	+	12

Dominant mutations that cause early-onset AD have been identified in APP, PS1 and PS2, and cause a relative increase in APP processing to amyloid-β_{1-42}. The ApoE $\varepsilon 4$ allele has been linked to late-onset AD in a large number of studies. LRP, a multifunctional receptor that binds ApoE and numerous other ligands[20,21,59], has been found to be weakly linked to late-onset AD by several studies[12,93]. Genetic association of α_2M, a ligand of LRP, has been reported by Blacker *et al.*[9] and supported by some, but not all, studies. FE65 is a cytoplasmic scaffold protein that interacts with the APP[36,94] and LRP[31] cytoplasmic domains. Association of an intronic biallelic polymorphism in this gene with sporadic AD has been reported[95] and FE65 has been shown to modulate APP processing[55,56]. Evidence for a third late-onset AD gene on chromosome 12 has been found in other genetic linkage studies[96], but the gene has not been identified. Disease association: ++, firmly established; +, weak association or independent confirmation lacking.

Polimorfismi della ApoE

ε2	NH2-----Cys112------Cys158-----COOH
ε3	NH2-----Cys112-----Arg158-----COOH
ε4	NH2-----Arg112-----Arg158-----COOH

Allele ε_4 : influenza sull'età di esordio



Età medie di esordio

84.3±1.3 no ε4

75.5±1.0 con 1 ε4

68.4±1.1 con 2 ε4

Frequenza degli alleli di APO E

Genotypes	AD Patients (n = 234) (%)	Controls (n = 304) (%)
2/2	0	0
3/2	3.4	12.5
3/3	38.5	59.9
4/2	4.3	4.9
4/3	41.0	20.7
4/4	12.8	0.7

DRUGS APPROVED BY FDA AS AD TREATMENT

Drug	Manufacturer	Mechanism of action	Launched	US sales (2005)	Side effects
Donepezil (Aricept)	Eisai Inc.	Cholinesterase inhibitor; prevents the breakdown of acetylcholine in the brain	1997	$1.1 billion	Nausea, diarrhea, insomnia, vomiting, muscle cramps, fatigue, anorexia
Memantine (Namenda)	Forest Pharmaceuticals	NMDA receptor antagonist; blocks toxic effects associated with excess glutamate and regulates glutamate activation	2003	$498 million	Dizziness, headache, confusion, constipation
Rivastigmine (Exelon)	Novartis Pharmaceuticals Corporation	Cholinesterase inhibitor; prevents the breakdown of acetylcholine and butyrylcholine in the brain	2000	$226 million	Nausea, vomiting, loss of appetite, indigestion, weakness/ lack of energy, dizziness, diarrhea, headache, stomach pain
Galantamine (Razadyne)	Ortho-McNeil Neurologics Inc.	Cholinesterase inhibitor; prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine	2001	$223 million	Nausea, vomiting, diarrhea, anorexia, weight loss
Galantamine (Razadyne ER)	Ortho-McNeil Neurologics Inc.	Cholinesterase inhibitor; prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine	2005	$24 million	Nausea, vomiting, diarrhea, anorexia, weight loss

Source: IMS Health

Weak weapons: The drugs available for Alzheimer disease have shown only modest benefits.

Table 3. Summary of Comparable Data from All Donepezil Randomized Clinical Trials

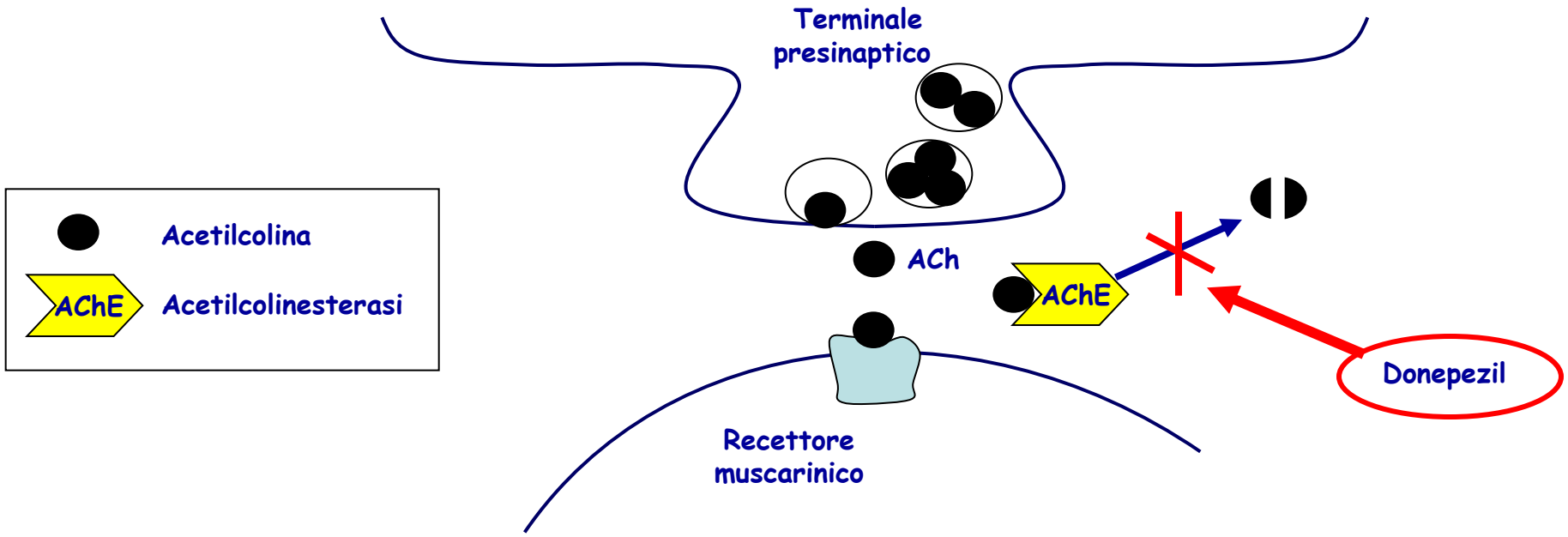
Results	Alzheimer's Disease Assessment Scale–Cognitive Subscale	Mini-Mental State Examination	Clinical Global Impression of Change	Clinician's Interview-Based Impression of Change	Clinical Dementia Rating—Sum of the Boxes	Quality of Life–Patient Rated	Neuro-psychiatric Inventory
Number of points in scale	70	30	7	7	18	350	144
Vendor-sponsored trails							
Trials, n	6	11	2	4	8	4	5
Range of significant results	1.5–3.2	0.68–1.8	9.0–23%*	0.34–0.54	0.4–0.85†		1.7–5.6
Negative trials, n	—	2	—	—	4	3	3
Non-vendor-sponsored trials							
Trials, n	1	2	2	—	—	—	2
Range of significant results	2.2	0.8–1.55	—	—	—	—	—
Negative trials, n	—	—	2	—	—	—	2

Note: Treatment effects for measurement scales used in at least three trials are presented. If multiple doses of donepezil were used, the best result is presented.

* Percentage difference in number of patients scoring in 4–7 range (better).

† Five-mg dose not different, 10-mg dose significantly worse (8 points).

Donepezil: inibizione dell'acetilcolinesterasi



AChEI (Inibitori Acetilcolinesterasi)

Amplificazione della funzionalita' colinergica (Krall et al.,1999; Rogers et al., 1998).

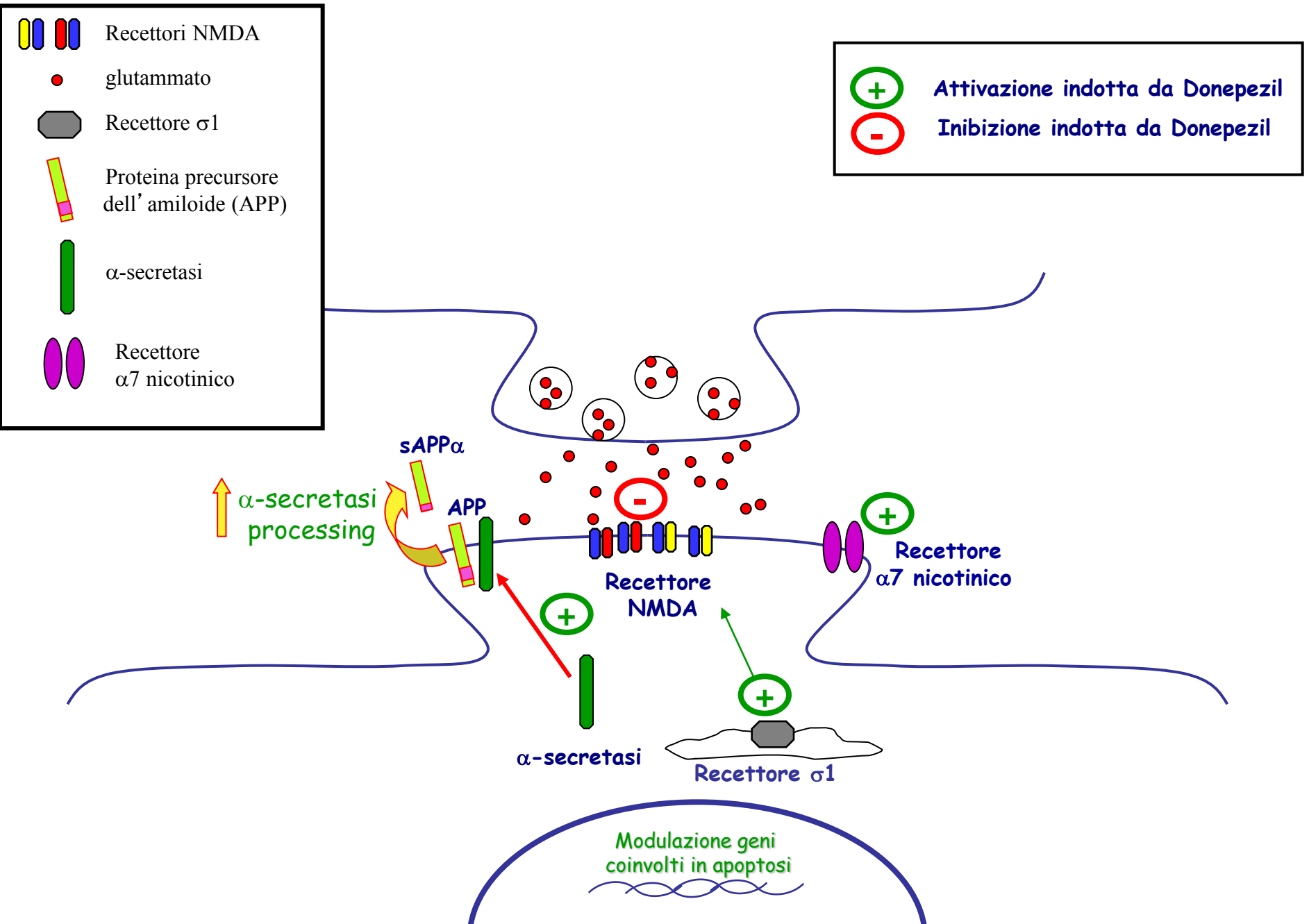
AChEI agiscono *anche* modificando i meccanismi molecolari alla base della malattia di Alzheimer:

Influenzano il metabolismo di APP

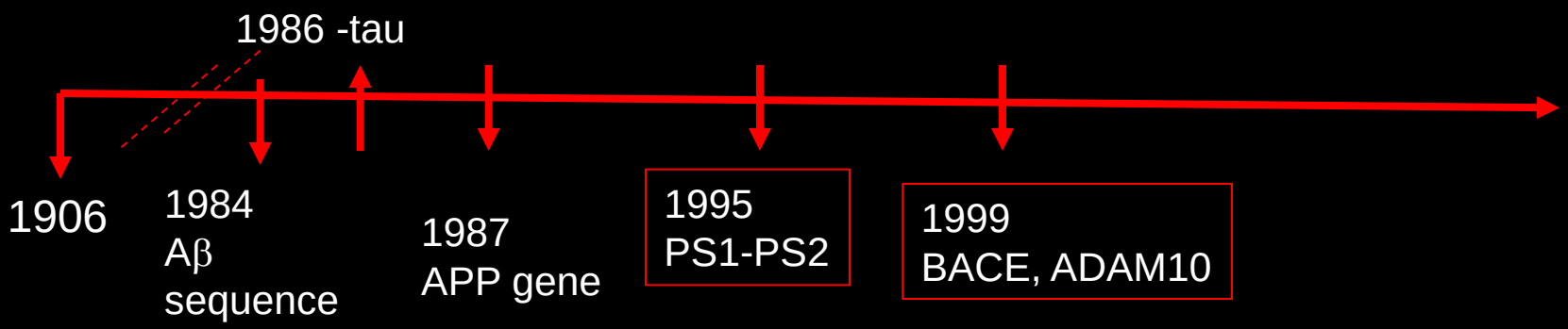
- Stimolando il recettore muscarinico e la sua trasduzione del segnale (Nitsch et al. 1996, Racchi et al., 1999)
- Riducendo la traduzione di mRNA di APP (Shaw et al., 2001)
- Aumentando la traslocazione di ADAM10 alla membrana (Zimmermann et al., 2004, 2005)

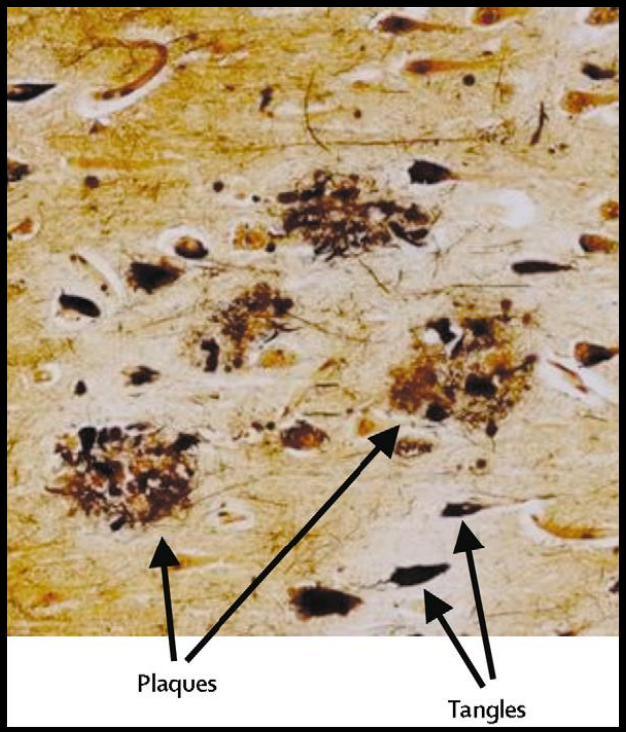
Questi risultati suggeriscono un nuovo meccanismo d' azione e forniscono un razionale all' uso di AChEI nella malattia di Alzheimer

Donepezil e Neuroprotezione: possibili meccanismi molecolari

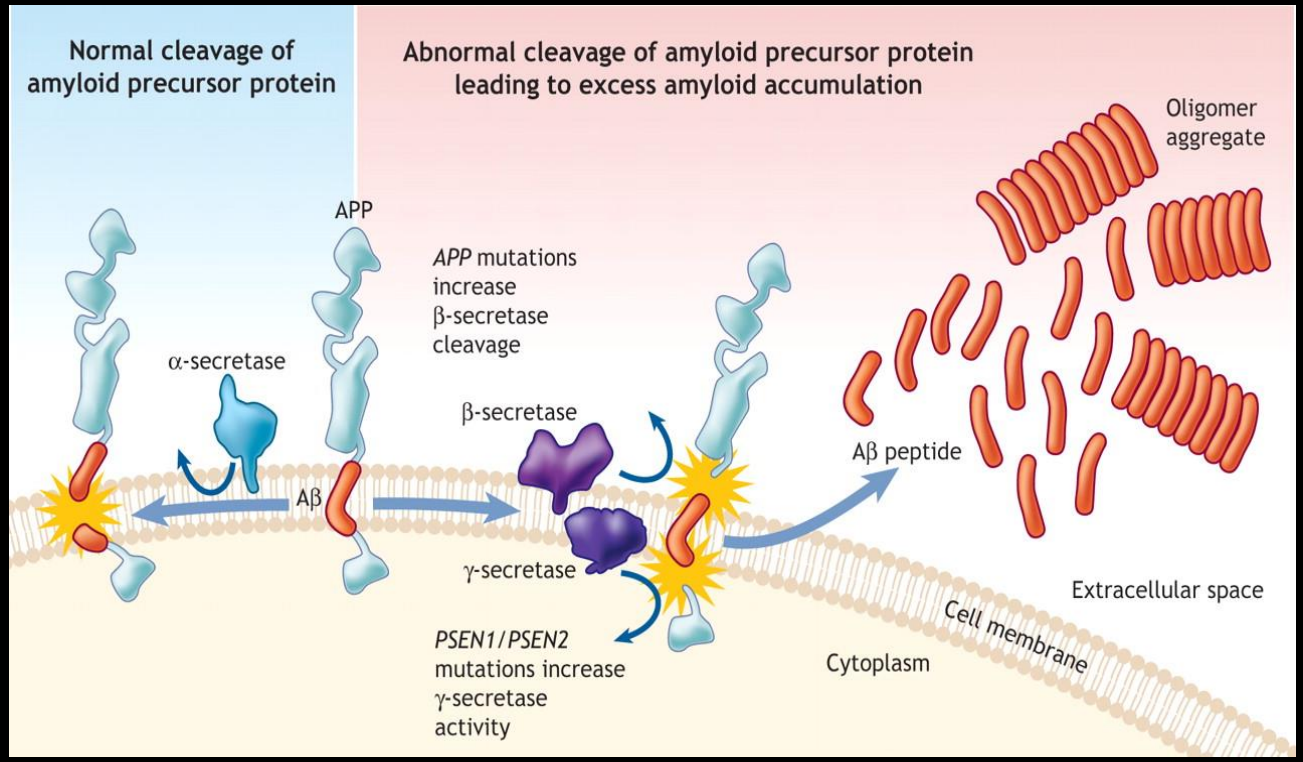


Farmaci: Prospettive future





Farmaci: Prospettive future



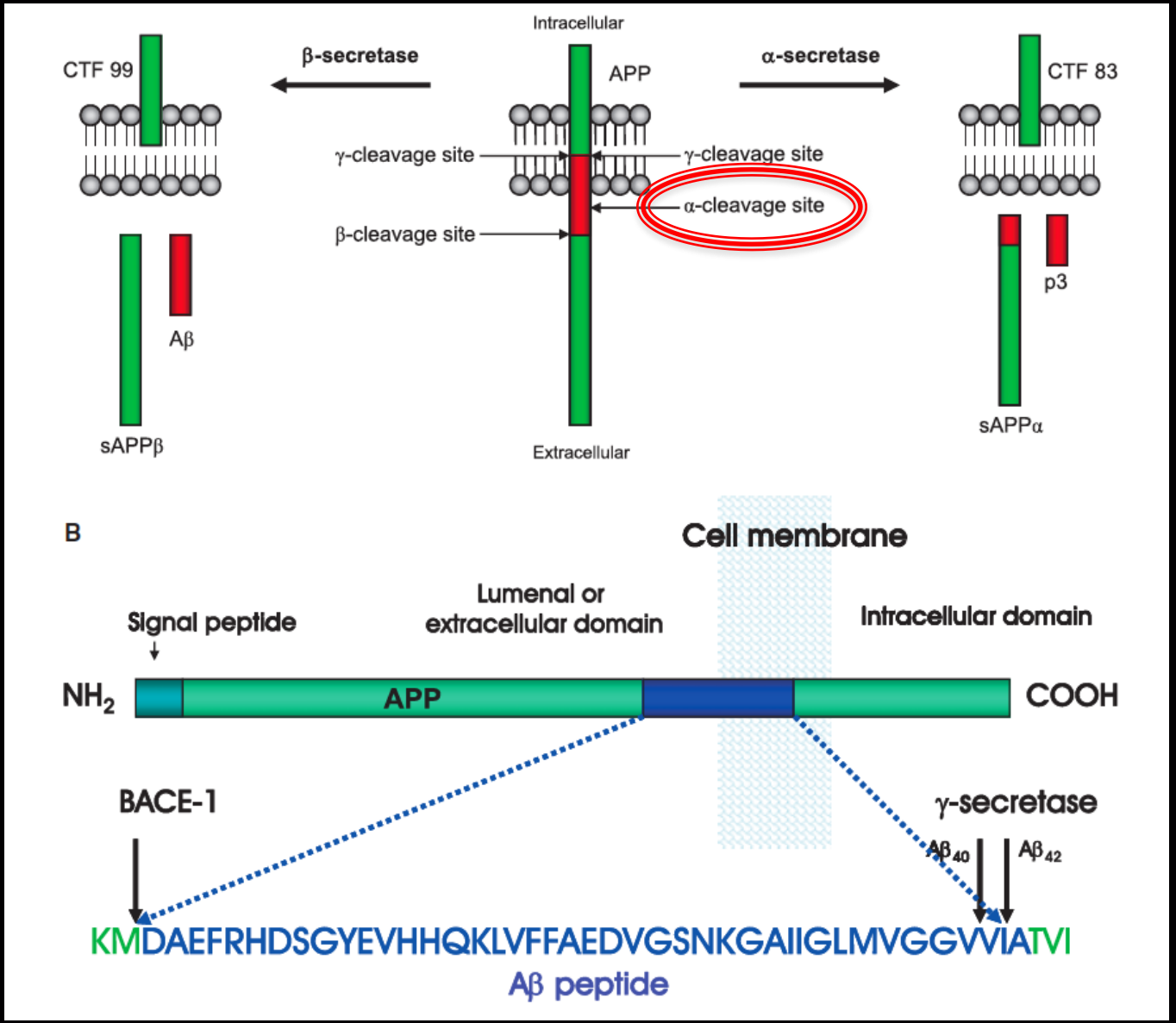
***To inhibit the production of Aβ* through:**

BACE inhibitors
γ-secretase inhibitors
Enhancing α-secretase activity
Enhancing activity of specific endopeptidases

***Block the aggregation and toxicity of Aβ* through:**

Avoiding the conversion of protofibrils to fibrils
Avoiding the interaction of oligomers with normal cellular components

APP metabolism: role of secretases



ADAM 10

- A member of Disintegrin And Metalloprotease family
- proteina di membrana di tipo I
- peso molecolare apparente di 68 e 85 kDa
- contiene un dominio autoinibitorio, un dominio disintegrinico, un dominio ricco in cys, un dominio citosolico e TM

Human Molecular Genetics, 2009, Vol. 18, No. 20 **3987–3996**
doi:10.1093/hmg/ddp323
Advance Access published on July 15, 2009

Potential late-onset Alzheimer's disease-associated mutations in the *ADAM10* gene attenuate α-secretase activity

Minji Kim[1], Jaehong Suh[1], Donna Romano[1], Mimy H. Truong[1], Kristina Mullin[1], Basavaraj Hooli[1], David Norton[1], Giuseppina Tesco[1], Kathy Elliott[2], Steven L. Wagner[2], Robert D. Moir[1], K. David Becker[2] and Rudolph E. Tanzi[1,*]

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Neurobiology of Aging xx (2010) xxx

www.elsevier.com/locate/neuaging

Evidence against a role for rare *ADAM10* mutations in sporadic Alzheimer Disease

Guiqing Cai[a], Gil Atzmon[d], Adam C. Naj[e], Gary W. Beecham[e], Nir Barzilai[d], Jonathan L. Haines[f], Mary Sano[a,g], Margaret Pericak-Vance[e], Joseph D. Buxbaum[a,b,c,*]

Curr Alzheimer Res. 2007 Sep;4(4):412-7.

Alpha-secretase as a therapeutic target.

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ADAM10- and ADAM17-mediated shedding events in CNS, inflammation and cancer.

Substrate	Involved ADAM[a]	Inducer[b]	Function	Evidence[c]
CNS				
APP	10, 17 and other	PMA, PDBu	Suppression of β-amyloid formation, neuroprotective, memory enhancing effects	siRNA, overexpression, DN ADAM10
PrP	10 (c), 17 (i) and other	PDBu	Neuroprotective	Overexpression, *adam10*−/− and *17*−/− MEF
Ephrins	10	PMA	Increased neurite outgrowth	Overexpression, siRNA, cleavage resistant substrate
L1	10 (c, i), 17 (i) and other	PMA, NMDA, MβCD	Increased neuronal migration, increased neurite outgrowth, increased tumor cell migration	Inhibitors (GI, GW), DN ADAM10, *adam10*−/− and *17*−/− MEF, siRNA
N-cadherin	10	Calcium influx, NMDA	Decreased neuronal cell adhesion, increased neurite outgrowth	Inhibitors (GI, GW), *adam10*−/− and *17*−/− MEF, siRNA
Pcdhγ	10	AMPA, PMA, staurosporin, calcium-influx, glutamate, KCl	Decreased neuronal cell-cell adhesion	Inhibitors (GI, GW), *adam10*−/− and *17*−/−MEF, siRNA
Delta	10, (17)	PMA	Hematopoesis	*adam10*−/− and *17*−/− MEF

ADAM10- and ADAM17-mediated shedding events in CNS, inflammation and cancer.

Substrate	Involved ADAM[a]	Inducer[b]	Function	Evidence[c]
Inflammation				
Notch	17, 10	Ligand binding	Neurogenesis, hematopoesis	*adam17*−/− *MEF*, *adam10*−/− MEF
TNFα	17, (10)	PMA, LPS	Soluble agonist	*adam17*−/− MEF and leukocytes, purified ADAM17/10
TNFR	17	PMA. fMLP, C5a, GM-CSF, calcium-influx, LPS	Soluble TNFα antagonist, reduced TNFR signaling	*adam17*−/− MEF, cleavage resistant substrate
IL-6R	10 (c, i), 17 (i)	PMA, cholesterol extraction, pore forming toxins, chemokines, LPS, apoptosis	Soluble agonist of gp130, trans-signaling by complex of IL-6 and sIL-6R	*adam10*−/− and *17*−/− MEF
IL-15R	17	PMA	Soluble IL-15 antagonist, enhanced proinflammatory cytokines	Inhibitors (GW), *adam10*−/− and *17*−/− MEF, overexpression
L-selectin	17 and other	PMA, chemotactic factors (C5a, LTB4), antibody binding, LPS, aspirin, selenium apoptosis	Reduced neutrophil rolling velocities, reduced neutrophil transmigration and recruitment into inflamed tissue	Inhibitors (GW), *adam17*−/− leukocytes, adoptive transfer, cleavage resistant substrate antisense ODN
CX3CL1	10 (c, i), 17 (i) and other	PMA, calcium-influx, streptolysin O, calmodulin, cholesterol depletion	Neuroprotective, soluble chemoattractant, decreased endothelial adhesiveness, de-adhesion of bound leukocytes	Inhibitors (GI, GW), *adam10*−/− and *17*−/− MEF, siRNA, DN ADAM10
CXCL16	10 (c, i), 17 (i)	Calcium-influx, PMA	Soluble chemoattractant, decreased scavenger receptor activity, decreased endothelial adhesiveness	Inhibitors (GI, GW), *adam10*−/− MEF and endothelial cells, siRNA, DN ADAM10
JAM-A	10 (c), 17(c, i)	PMA, TNFα/IFNγ, PAF	Soluble JAM-A and LFA-1antagonist, reduced leukocyte transmigration and recruitment into inflamed tissue	Inhibitors (GI, GW), *adam10*−/− and *17*−/−MEF, siRNA
VE-cadherin	10	Calcium influx, thrombin, apoptosis	Increased endothelial permeability	Inhibitors (GI, GW), *adam10*−/− and *17*−/− MEF, siRNA

ADAM10- and ADAM17-mediated shedding events in CNS, inflammation and cancer.

Substrate	Involved ADAM[a]	Inducer[b]	Function	Evidence[c]
Cancer				
EGF	17, 10 (c)	AMPA, pervanadate, calcium-influx	Soluble agonist of EGFR	*adam10*–/– and *17*–/– MEF, overexpression
Epigen	17	PMA, pervanadate, calcium-influx	Soluble agonist of EGFR	Inhibitors (GI, GW), *adam17*–/– MEF
Neuregulin	17 and other	PMA	Soluble agonist of ErbB3 and ErbB4	*adam17*–/– MEF, overexpression
HB-EGF	17 and other	PMA	Soluble agonist of EGFR and ErbB4	*adam17*–/– MEF
TGFα	17	PMA	Soluble agonist of EGFR	*adam17*–/– MEF
Amphiregulin	17	PMA	Soluble agonist of EGFR	*adam17*–/– MEF
Betacellulin	10 (i)	AMPA, calcium- influx	Soluble agonist of EGFR and ErbB4	*adam17*–/– MEF, overexpression
Epiregulin	17 and other	PMA	Soluble agonist of EGFR and ErbB4	*adam10*–/– and *adam17*–/– MEF
ErbB2/HER2	10	Pervanadate	Constitutive receptor activity	siRNA
ErbB4/HER4	17	PMA, pervanadate		*adam10*–/– and *17*–/– MEF, overexpression
CD44	10 (c, i), 17 (i) and other	Calcium influx, CaM inhibitor, PMA, anti-CD44 antibody	Increased tumor cell migration	*adam10*–/– and *17*–/– MEF, siRNA
Des 2	10	EGF	Reduced tumor cell-cell contacts	*adam10*–/– and *17*–/– MEF, overexpression
ALCAM	17	Pervanadate, PMA, EGF	Reduced tumor cell-cell contacts	*adam10*–/– and *17*–/– MEF, overexpression
MICA	10 (c), 17 (i)	PMA	Tumor immune escape	Inhibitors (GI, GW), cleavage resistant substrate, siRNA, overexpression
CD30	17 (i), 10 (i)	PMA, Ki1-antibody binding, calcium influx, MβCD	Soluble ligand of CD153	Inhibitors (GI, GW), recombinant ADAM17, adam10–/– and 17–/– MEF

Potential effect of ADAM10 inhibitors in CNS, inflammation and cancer

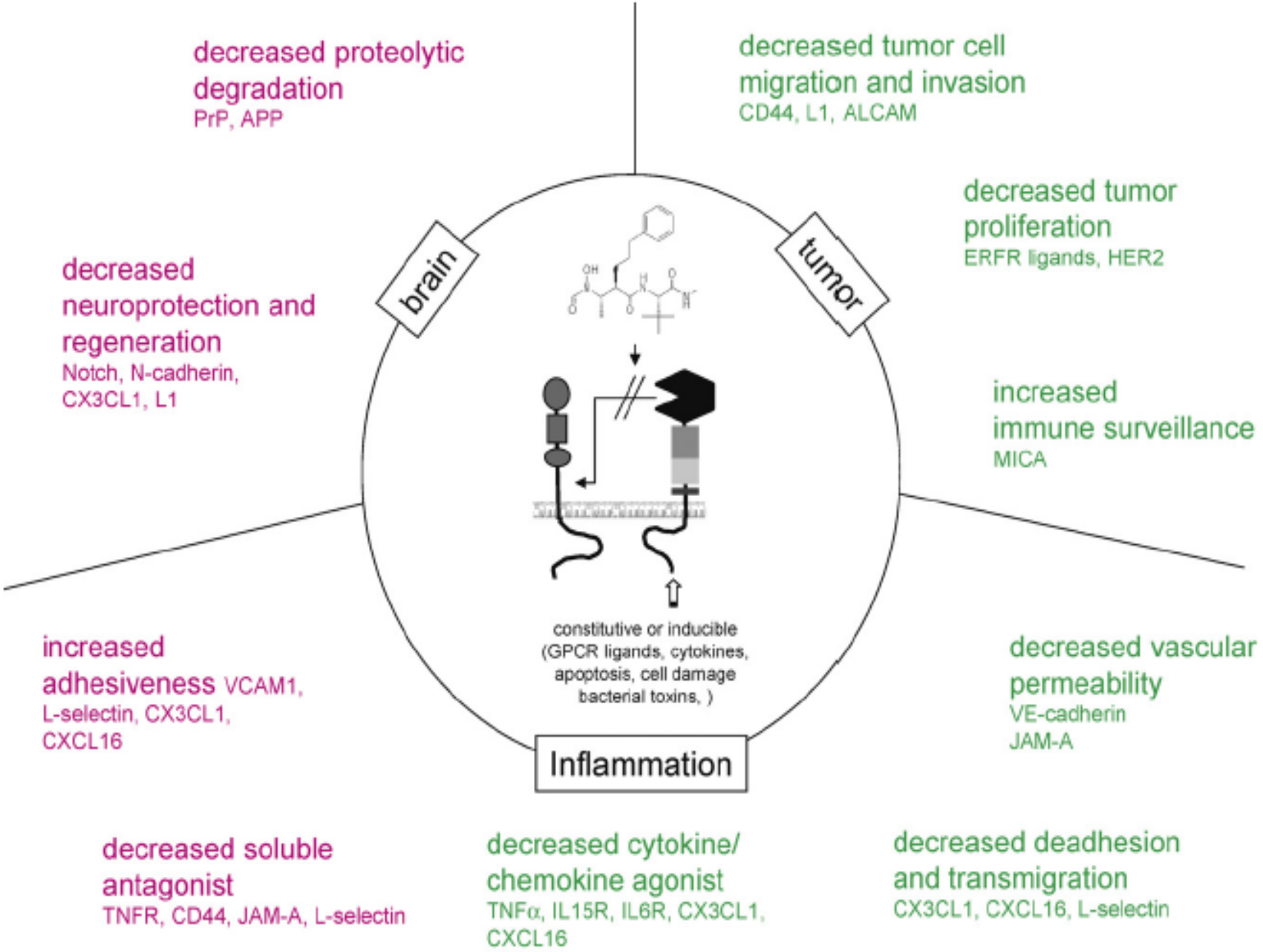
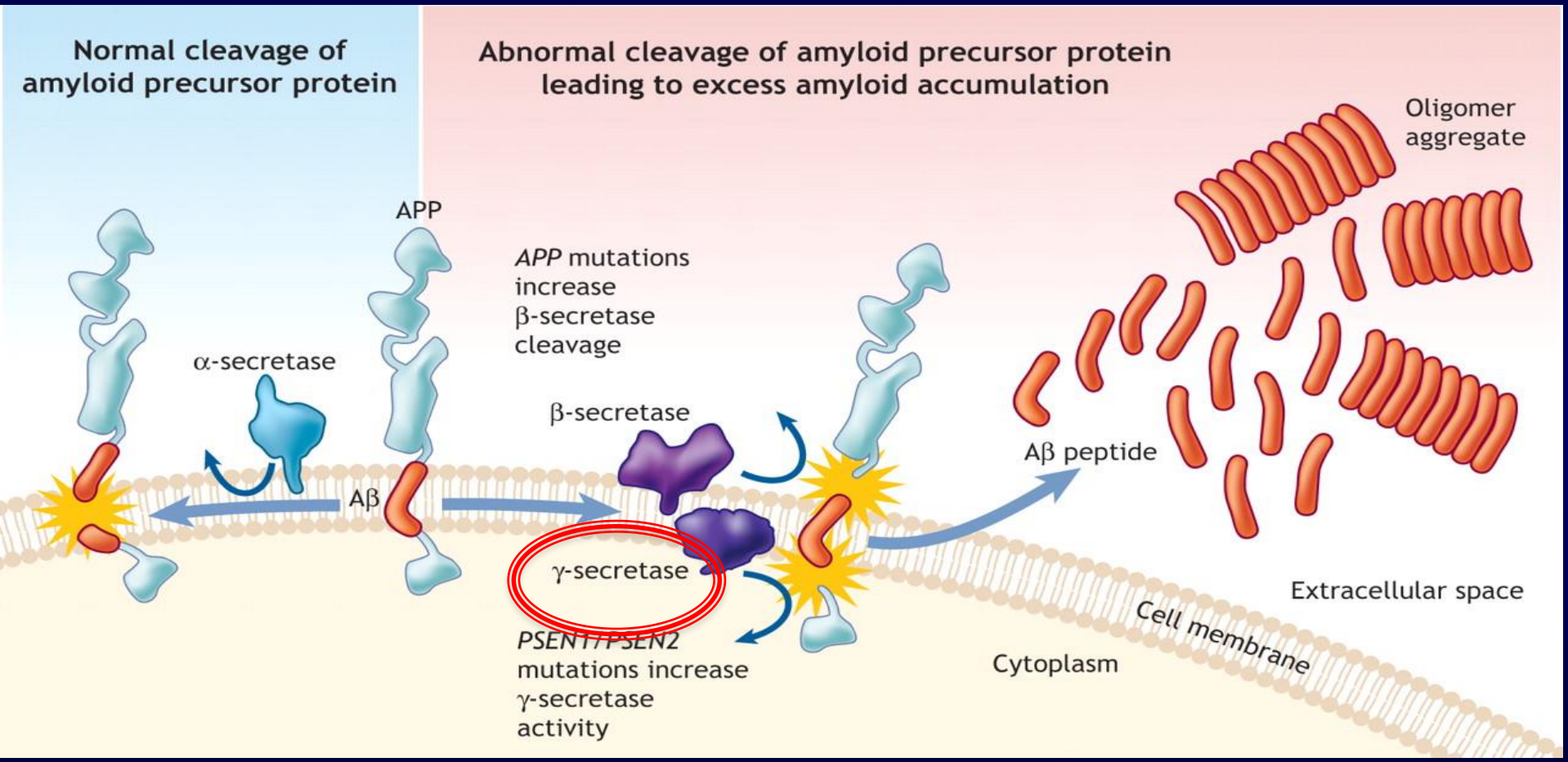


Fig. 1. Potential effects of ADAM10 and ADAM17 inhibitors in CNS, inflammation and cancer. Constitutive and inducible shedding by ADAM10 and ADAM17 can be targeted

APP metabolism: role of secretases



Mutations in the γ-secretase (presenilins)

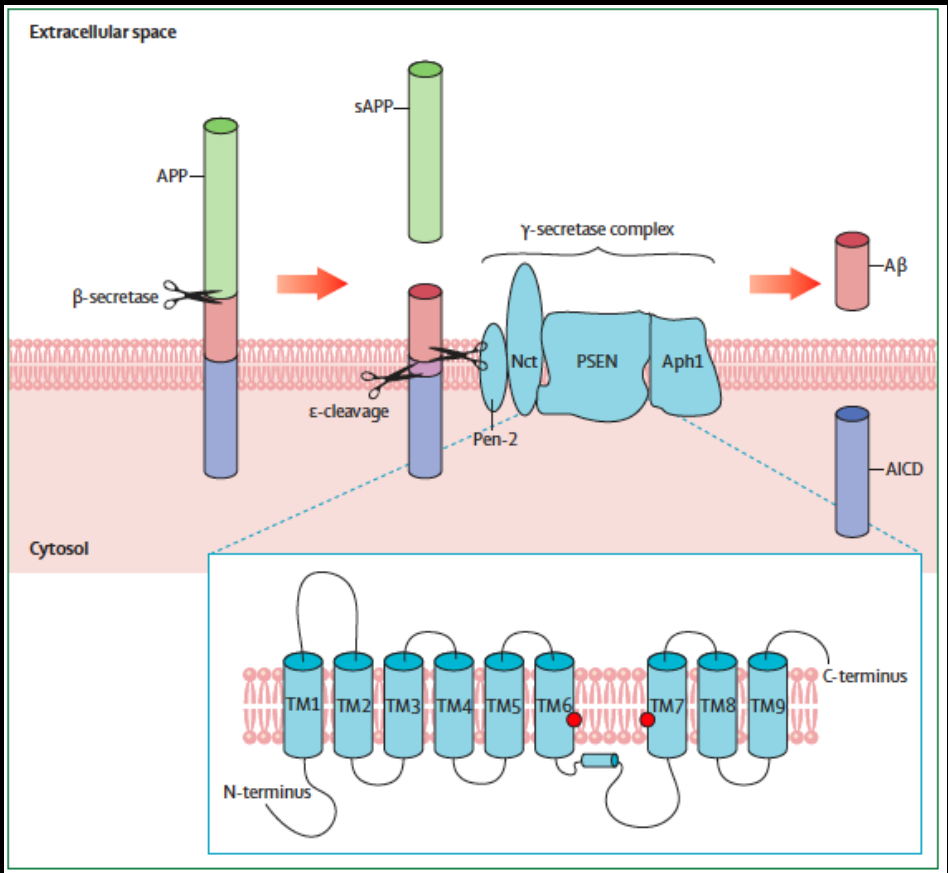


Figure 1: **Presenilins, the γ-secretase complex, and APP processing**
After release of sAPP by β-secretase, the remaining APP segment is further cleaved by γ-secretase. First, ε-cleavage by γ-secretase releases the AICD. Second, γ-secretase cleavage releases the Aβ peptide, of which there can be longer and shorter forms (eg, $A\beta_{40}$ and $A\beta_{42}$), depending on the exact cleavage site by γ-secretase. The γ-secretase enzyme consists of four components: PSEN, Aph1, Nct, and Pen-2.[20] The magnified area shows the nine TM domains of PSEN.[21] The two red circles indicate the catalytic aspartates. Aβ=β-amyloid. AICD=APP intracellular domain. Aph1=anterior pharynx defective. APP=amyloid precursor protein. Nct=nicastrin. Pen-2=presenilin 2 enhancer. PSEN=presenilin. sAPP=soluble APP. TM=transmembrane.

- There are over 70 mutations in PSEN1 and 2 mutations in PSEN2
- PSEN mutations are fully penetrant, autosomal-dominant mutations
- PSEN1 mutations are associated with very early-onset FAD (25-60 years)
- PSEN2 mutations are associated with early-onset FAD (45-80)
- PSEN mutations increase the formation of longer Aß variants (abeta42 vs Abeta40).
- PSEN mutations account for a small percentage of AD cases

Journal of Neurochemistry, 2001, **76**, 173–181

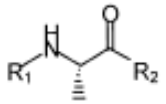
Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain

H. F. Dovey, V. John, J. P. Anderson, L. Z. Chen, P. de Saint Andrieu, L. Y. Fang, S. B. Freedman, B. Folmer, E. Goldbach, E. J. Holsztynska, K. L. Hu, K. L. Johnson-Wood, S. L. Kennedy, D. Kholodenko, J. E. Knops, L. H. Latimer, M. Lee, Z. Liao, I. M. Lieberburg, R. N. Motter, L. C. Mutter, J. Nietz, K. P. Quinn, K. L. Sacchi, P. A. Seubert, G. M. Shopp, E. D. Thorsett, J. S. Tung, J. Wu, S. Yang, C. T. Yin and D. B. Schenk

Elan Pharmaceuticals, Inc., South San Francisco, USA

P. C. May, L. D. Altstiel, M. H. Bender, L. N. Boggs, T. C. Britton, J. C. Clemens, D. L. Czilli, D. K. Dieckman-McGinty, J. J. Droste, K. S. Fuson, B. D. Gitter, P. A. Hyslop, E. M. Johnstone, W-Y. Li, S. P. Little, T. E. Mabry, F. D. Miller, B. Ni, J. S. Nissen, W. J. Porter, B. D. Potts, J. K. Reel, D. Stephenson, Y. Su, L. A. Shipley, C. A. Whitesitt, T. Yin and J. E. Audia

Eli Lilly and Company, Lilly Research Labs, Lilly Corporate Center, Indianapolis, USA



Compound	R_1	R_2	IC_{50} (μM)
1	3,4-dichlorophenyl	ethoxy	0.90
2	3,4-dichlorophenyl	*i*-butoxy	0.44
3	2-naphthyl	*i*-butoxy	1.52
4	phenylacetyl	*i*-butoxy	5.08
5	3,4-dichlorophenyl	L-valine ethyl ester	0.52
6	3,5-difluorophenylacetyl	L-phenylalanine amide	0.11
7 (**DAPT**)	3,5-difluorophenylacetyl	(S)-phenylglycine *t*-butyl ester	0.02
8	enantiomer of 7		>30

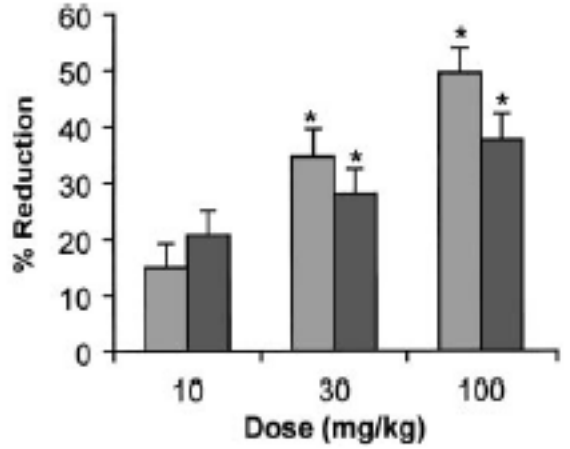
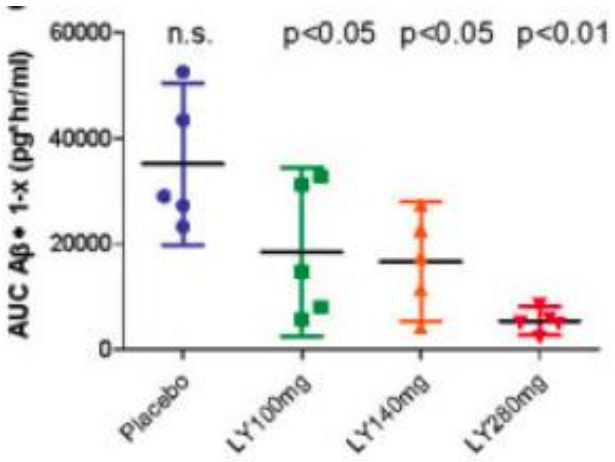


Fig. 8 The effect of DAPT on total Aβ (shaded gray) and AB42 (shaded black) levels

LY450139 (semagacestat) is currently being studied for Alzheimer's disease in a phase 3 clinical trial



LY450139

inibizione γ-secretasi

APP

↓ Aβ40 – 42
(risposta bifasica)

NOTCH

↑ rischio leucemie, linfomi, neoplasie gastrointestinali, altre neoplasie

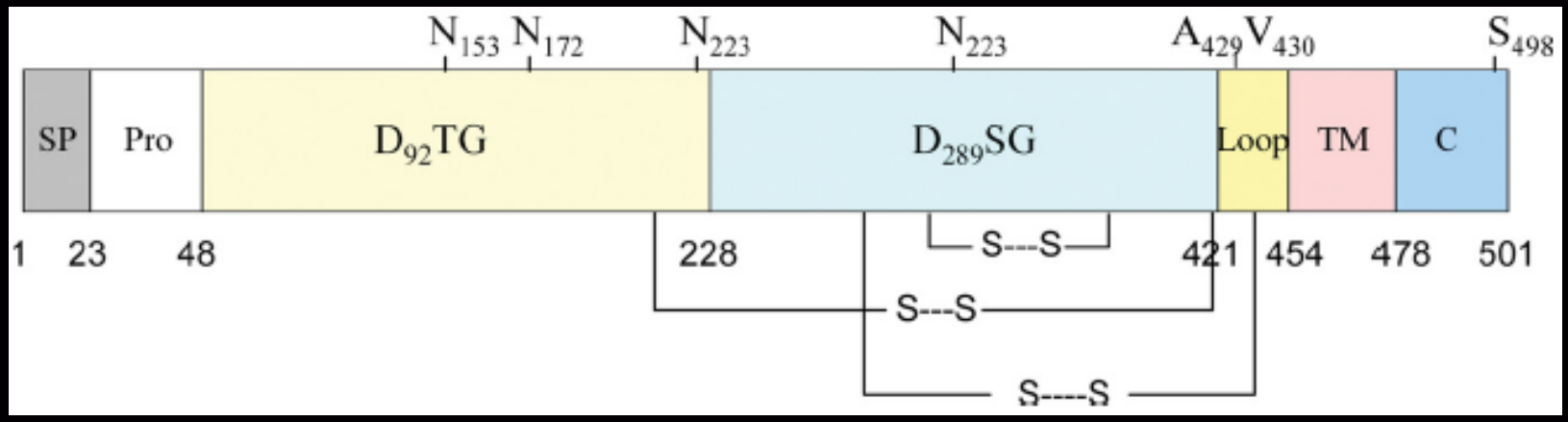
LY450139

Effect of LY450139 on the Long Term Progression of Alzheimer's Disease

- **fase III**
- **60 o 140 mg / die vs placebo**
- **1100 soggetti arruolati (recruiting)**
- **completamento atteso per marzo 2012**

http://www.clinicaltrials.gov/ct2/show/record/NCT00762411?term=semagacestat

BACE (β-secretase)



- Aspartic proteinase: endopeptidases which use two aspartic acid residues to catalyse the hydrolysis of a peptide bond.
- it's a glycoprotein with an apparent MW of 50-70 kDa
- is an ubiquitous enzyme
- BACE activity shows an optimum at pH 4.5
- has preferentially an intracellular localization:

  colocalizes with APP in Golgi /ER/endosomes →BACE

  overexpression induces β-secretase activity in these compartments

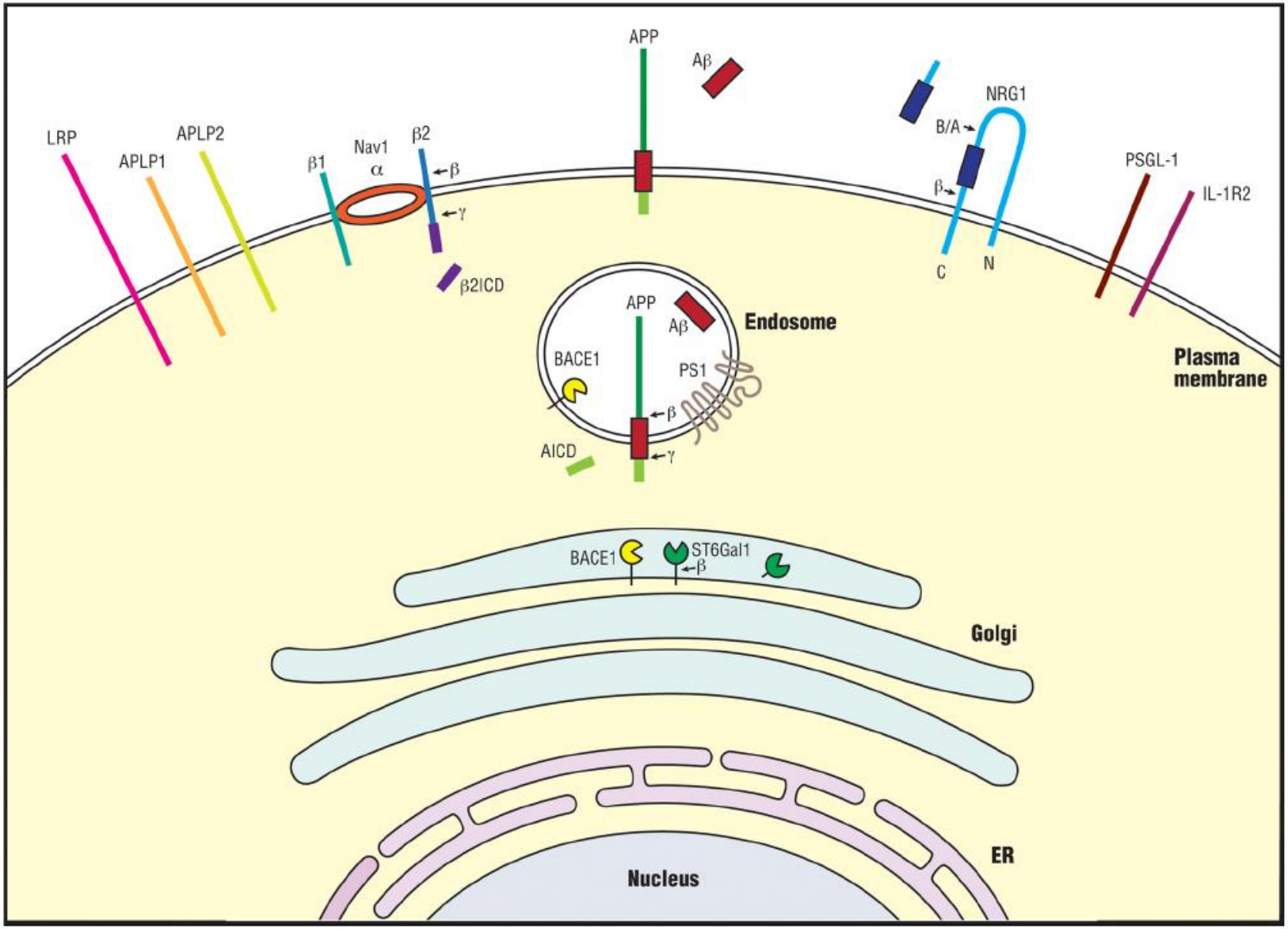
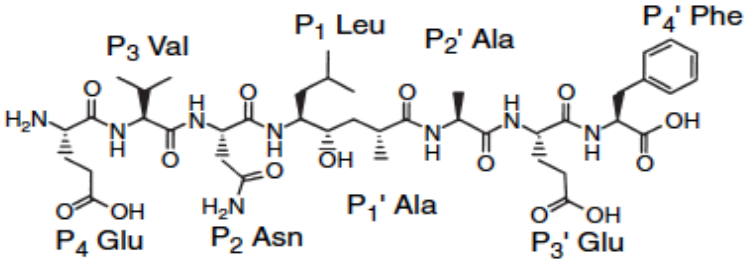


Figure 2. BACE1 substrate processing in the cell. The major known BACE1 substrates are depicted as colored stick figures in the membrane: APP, APLP2, APLP2, IL-1R2, LRP, $Na_v\beta2$, NRG1, PSGL-1, and ST6Gal1. Cleavage of APP by BACE1 ($\leftarrow\beta$) in the endosome is shown as a typical example of BACE1 substrate processing. Most, if not all, BACE1 substrates are also processed by γ-secretase ($\leftarrow\gamma$), which in the case of APP releases Aβ (red rectangle) for secretion. APP intracellular domain (AICD) is also released and acts as a cotransactivator of gene transcription in the nucleus, similar to the function of $Na_v\beta2$ intracellular domain ($\beta2$ICD) in $Na_v1\alpha$ gene expression. Type III NRG1 is cleaved by BACE1 at a site adjacent to the transmembrane domain ($\beta\rightarrow$). The second cut may occur by either BACE1 or ADAM cleavage (B/A$\rightarrow$) and will release an EGF-like domain (blue rectangle) that signals through ErbB4 in adjacent glial cells to regulate axon myelination. Type I NRG1 and NRG3 are also cleavable by BACE1. ST6Gal1 is processed by BACE1 in the *trans*-Golgi network. PS1, Presenilin-1 component of γ-secretase; N and C, N terminus and C terminus of NRG1, respectively.

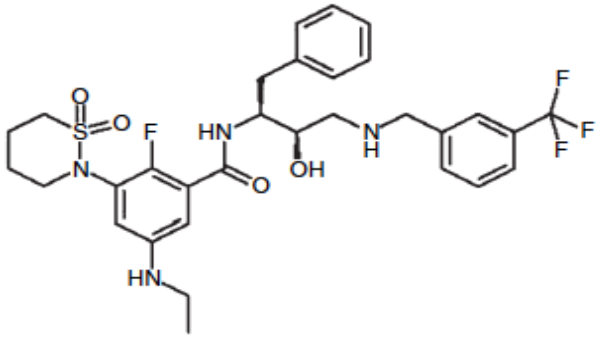
BACE-1 inhibitors

Table 1. Potential strategies to inhibit β-secretase processing of APP by BACE-1.

Active site-directed (competitive) inhibition of enzyme activity. small-molecule inhibitors; peptidic or non-peptidic
Non-competitive or allosteric inhibition, e.g. targeting protein processing, conformational changes
Modulation of oligomeric state and hence activity of the enzyme
Modulation of protein–protein interactions affecting localization and/or activity
Modulation of lipid environment of the enzyme
Immunization with BACE-1



OM99-2



GSK 188909

Fig. 2. BACE-1: from peptide-based to non-peptidic BACE-1 inhibitors. Examples of two BACE-1 inhibitors: the first reported compound OM99-2 (reproduced from [108] with permission of the American Association for the Advancement of Science) and a recently described orally active, non-peptidic inhibitor GSK 188909 (reproduced from [117] with permission of the International Society for Neurochemistry). In OM99-2, the constituent amino acids and their subsite designations are indicated. The hydroxyethylene transition state isostere is between P_1-Leu and P_1'-Ala. Figure reproduced from Hussain *et al.* [117] by kind permission.

Considerable efforts have been directed towards the identification of low-molecular-mass, specific and stable non-peptide analogues as BACE-1 inhibitors that can lead to the development of a successful therapeutic.

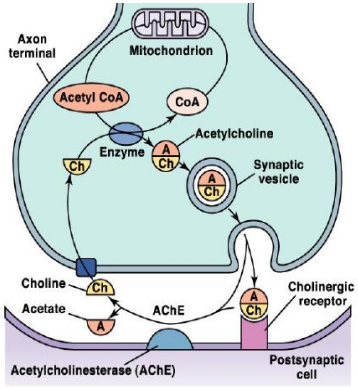
To date, the screening of extensive libraries for non-peptide-based BACE- 1 inhibitors has resulted in the discovery of relatively few, generally low-affinity, compounds, indicating that this is not an easy protein target to inhibit effectively in vivo.

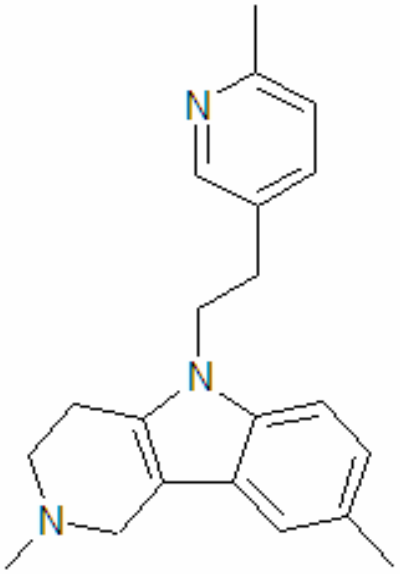
BACE1 participates in the proteolytic processing of Neuroregulin-1 (NRG1; Hu et al., 2006; Willem et al., 2006), a ligand for members of the ErbB family of receptor-tyrosine kinases.

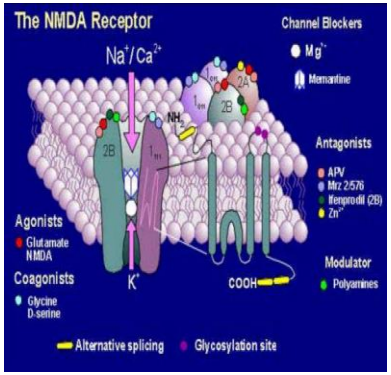
This signaling pathway has numerous roles in the CNS, including synapse formation, plasticity, neuronal migration, myelination of central and peripheral axons, and the regulation of neurotransmitter expression and function (Falls, 2003; Michailov et al., 2004).

In addition to these physiological roles, *NRG1 is one of the* first genes that have been linked to an increased risk of schizophrenia (Stefansson et al., 2002). *BACE1/ mice exhibit a sensorimotor-* gating deficiency, behavioral signs of glutamatergic hypofunction, and other typical endophenotypes of schizophrenia (Savonenko et al., 2008).

Dimebon







debole inibizione acetil- e butirril-colinesterasi

Debole blocco NMDA

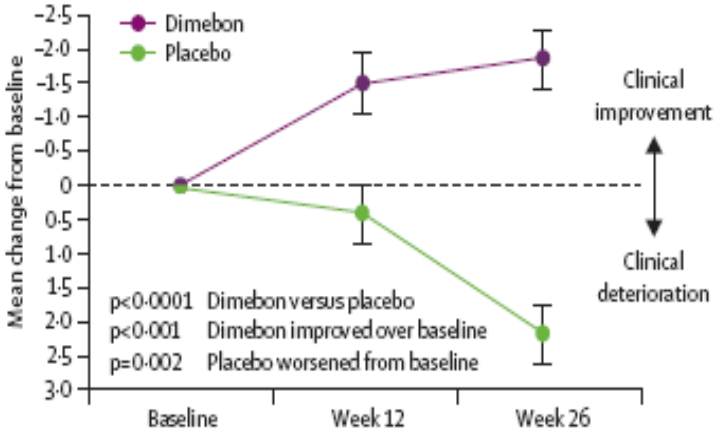
inibizione permeabilità mitocondriale

blocco canali Ca++

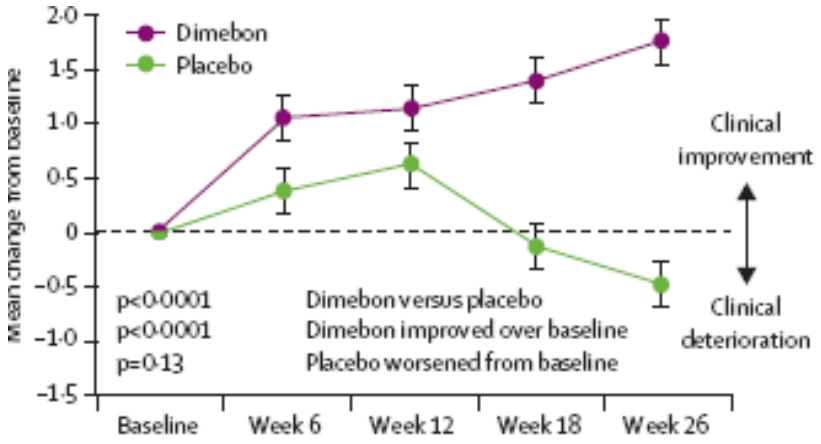
SINTOMATICO + NEUROPROTETTIVO

Dimebon

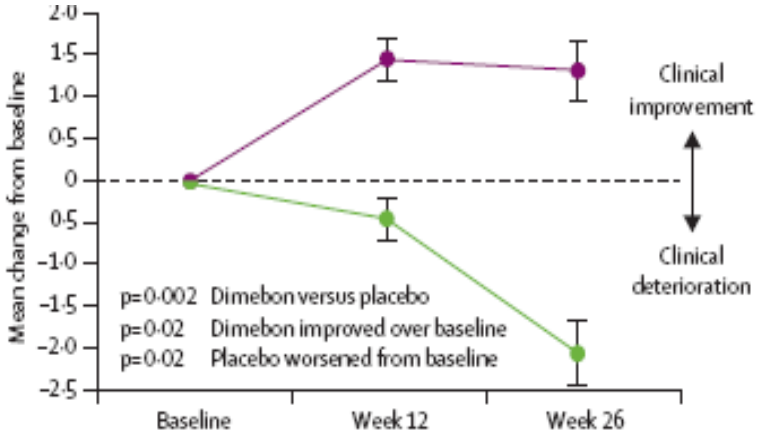
Mean change from baseline to week 26:



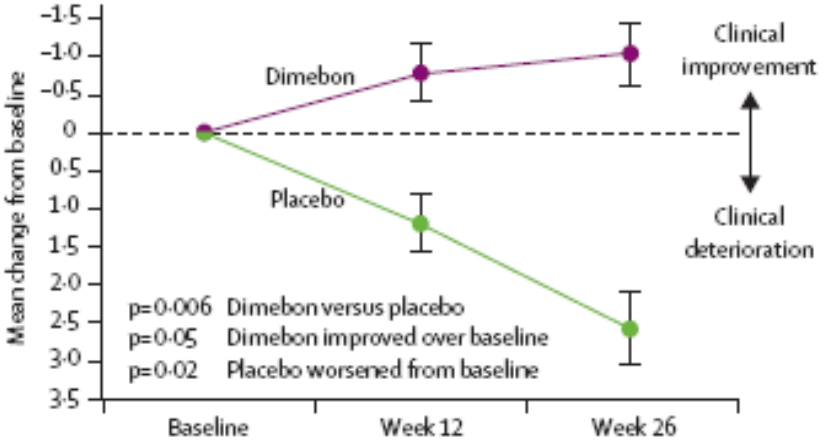
ADAS-cog



MMSE



ADL

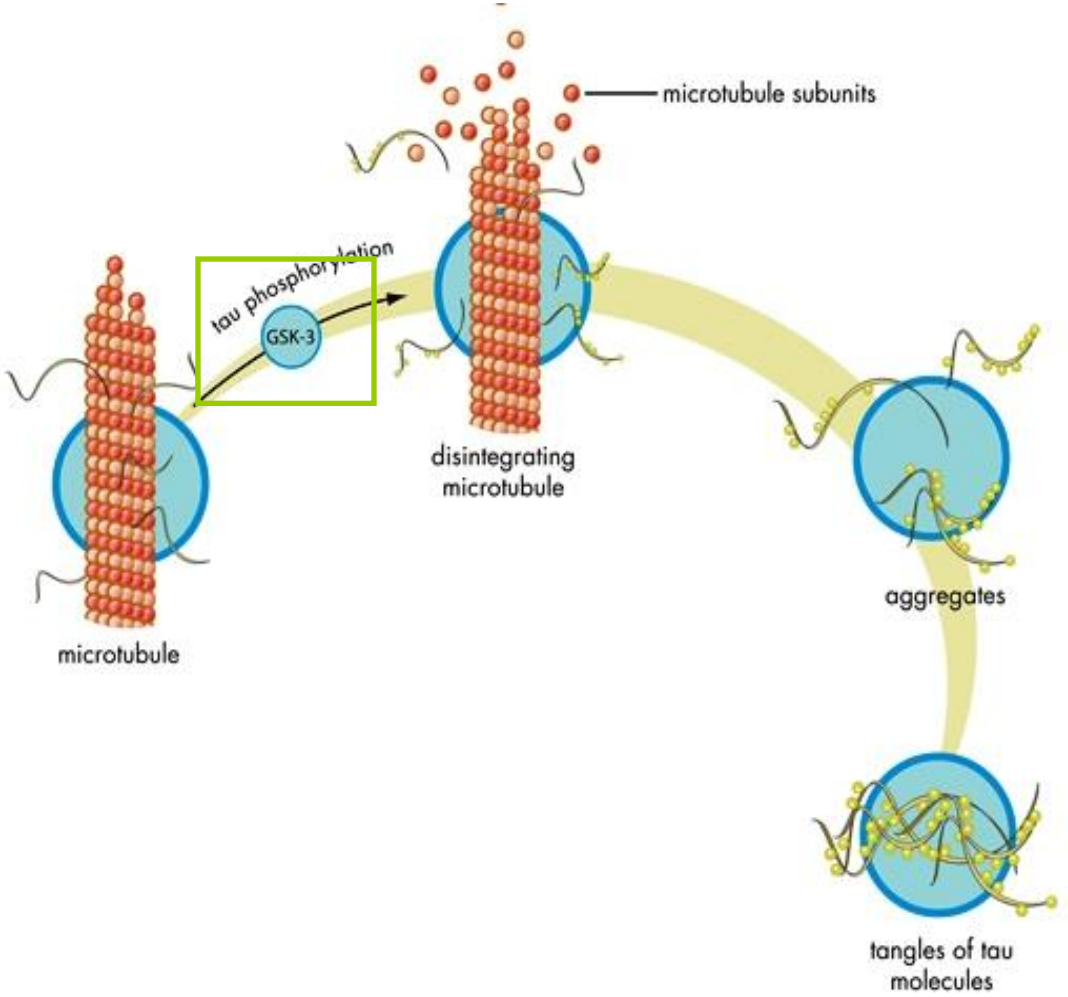


NPI

Doody et al, Lancet 2008

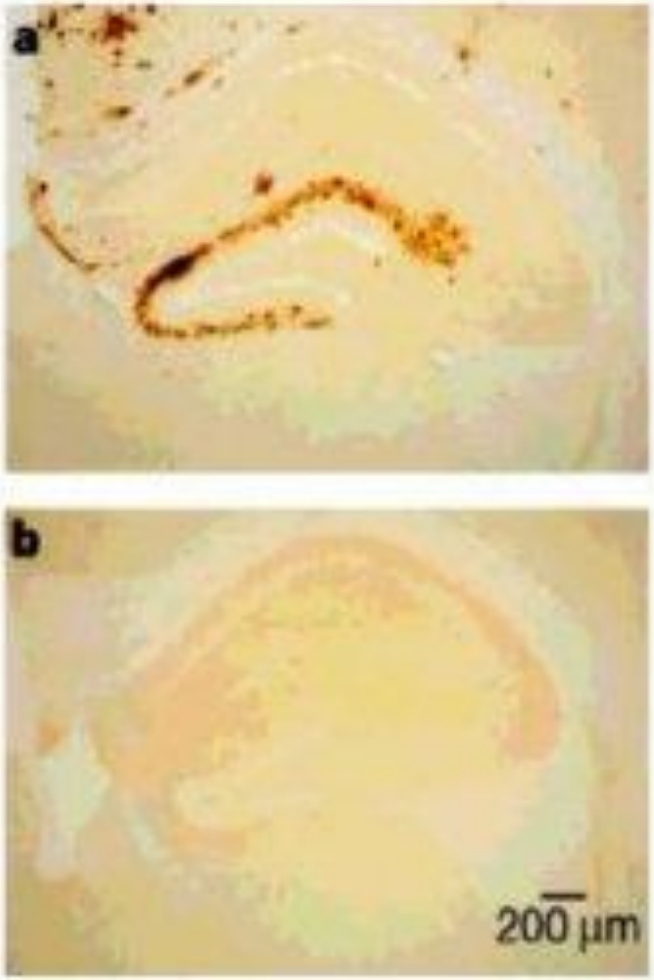
AD: nuove terapie?

❖ **Inibitori GSK-3: Litio, Acido Valproico**



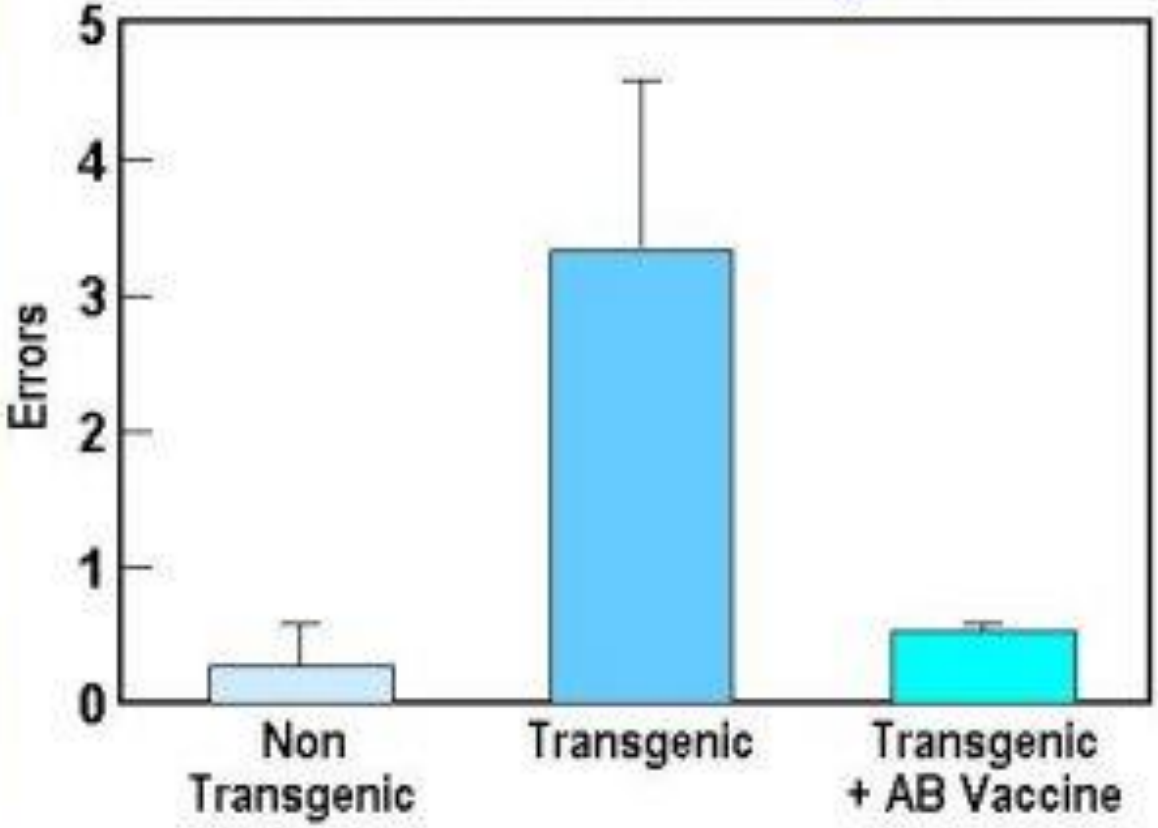
- **neuroprotezione**
- **miglioramento disturbi comportamentali**
- **riduzione fosforilazione di APP**
- **protezione dall' eccitotossicità**

Immunization with Aβ Reduces Aβ Deposition in Hippocampus of Mice and Prevents Memory Deficit



Schenk et al., 1999

Immunized Mice Perform Better than Control in Memory Tests



Morgan, et al, Nature, 408:932-935,2000.

Prospettive future: l'immunizzazione

In 1999, Schenk and colleagues demonstrated that vaccination of a mouse model of Alzheimer's disease (AD) with amyloid-β1–42 peptide (Aβ1–42) and adjuvant resulted in striking mitigation of AD-like pathology – giving rise to the field of AD immunotherapy.

Later studies confirmed this result in other mouse models of AD and additionally showed cognitive improvement after Aβ vaccination.

Based on these results, early developmental clinical trials ensued to immunize AD patients with Aβ1–42 plus adjuvant (so-called "active" Aβ immunotherapy; trade name AN-1792; Elan Pharmaceuticals). However, the phase IIa trial was halted after 6 % of patients developed aseptic meningoencephalitis.

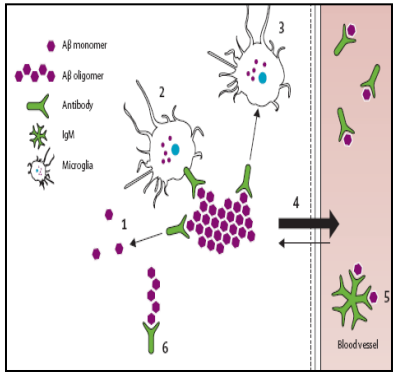
Despite occurrence of this adverse event, many individuals demonstrated high serum antibody titres to Aβ and histological evidence of clearance of the hallmark AD pathology, β-amyloid plaques.

While raising justifiable safety concerns, these important results nonetheless demonstrated the feasibility of the active Aβ immunotherapy approach (Schenk et al., 1999; Janus et al., 2000; Orgogozo et al., 2003).

IMMUNOTERAPIA:

stimolare il sistema immune a riconoscere ed eliminare Aβ o introdurre anticorpi preformati per prevenire la deposizione in placche di Aβ o aumentare l'eliminazione delle placche

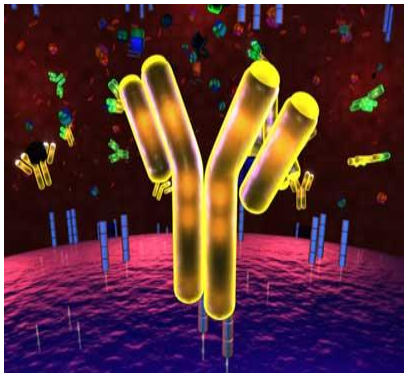
ATTIVA



Induzione di eccessiva risposta autoimmune proinfiammatoria

Compromissione degli eventuali benefici derivanti dalla rimozione delle placche

PASSIVA

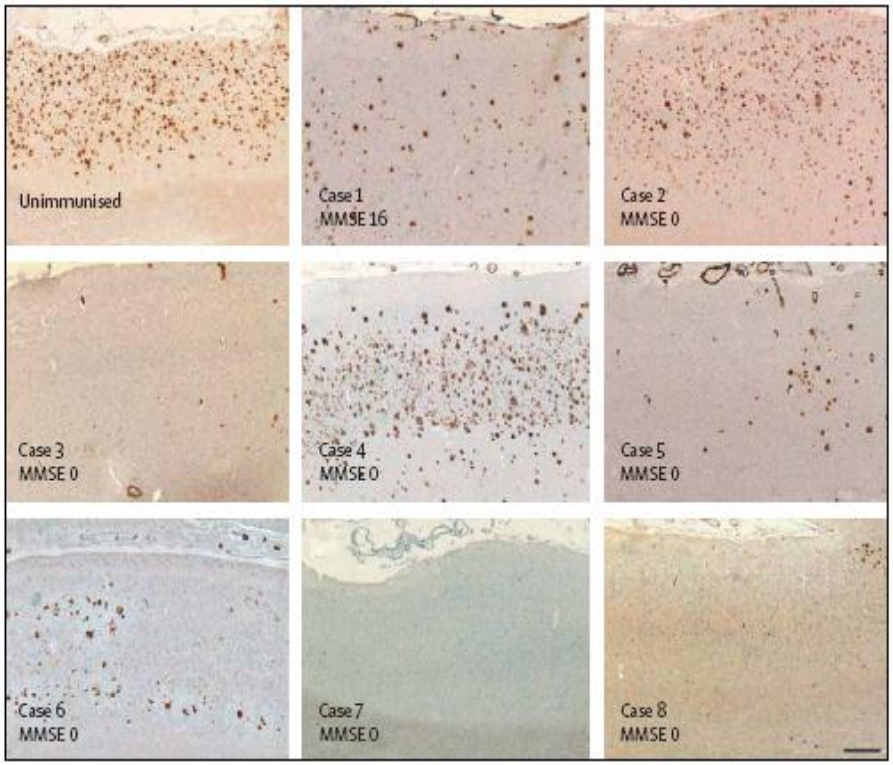


La somministrazione passiva di anticorpi può aggirare la risposta T-cellulare indesiderata associata alla vaccinazione attiva, mantenedo le importanti attività biologiche correlate all'efficacia

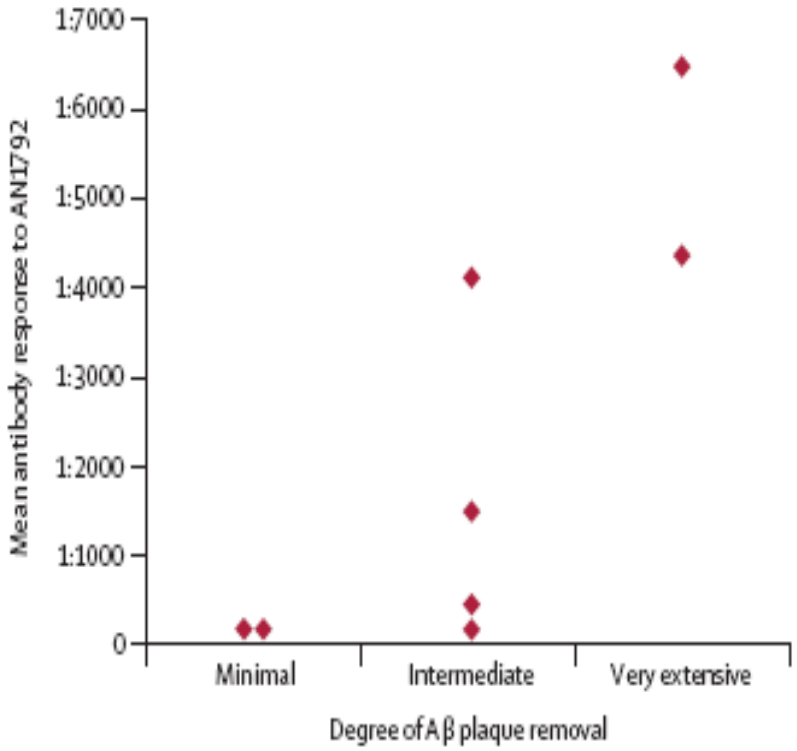
Amiloide: evento patogenetico centrale di AD?

Long-term effects of Aβ42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial

Holmes et al, Lancet Neurol 2008



Histological patterns of Aβ in the temporal lobe neocortex after immunisation with AN1792



Mean antibody response to AN1792 and Aβ plaque removal

Immunoterapia : problemi aperti

Riscontro di modesti o assenti benefici a livello cognitivo, non aumento della sopravvivenza, non rallentamento della progressione della malattia nonostante evidenza di rimozione delle placche

LA FORMAZIONE DI AGGREGATI DI Aβ E' REALMENTE L'EVENTO PATOLOGICO CENTRALE DI AD?

Amiloide: evento patogenetico centrale di AD?

Necessità della presenza delle placche per l'inizio, ma non per il mantenimento della neurodegenerazione

Rimozione troppo lenta delle placche nei soggetti immunizzati

Rimuovere le placche Aβ non basta ad impedire la progressione della neurodegenerazione

Eccessiva risposta proinfiammatoria

Ruolo delle Aβ oligomeriche come causa immediata di disfunzione sinaptica: innocue o addirittura protettive le forme aggregate in placche

Current randomised, double-blind, parallel-assignment studies of immunotherapy in Alzheimer's disease (Wisniewski T, Konietzko U. *Lancet Neurol 2008*)

	Phase	Intervention	Primary outcomes	Size	Duration
Active immunisation					
NCT00498602	2	ACC-001+QS21 vs ACC-001 vs placebo	Safety, tolerability	228	Nov 2007 to March 2012
NCT00411580	1	CAD106	Safety, tolerability	60	June 2005 to April 2008
NCT00464334	1	V950	Safety	70	April 2007 to Sep 2011
Passive immunisation					
NCT00575055	3	Bapineuzumab	Cognitive, functional	800	Dec 2007, to Dec 2010
NCT00667810	3	Bapineuzumab	Cognitive, functional	1250	May 2008 to April 2011
NCT00749216	2	Solanezumab	Safety, tolerability, pharmacokinetics	30	Sep 2008 to Aug 2009
NCT00329082	2	LY2062430	Safety, tolerability	25	May 2006, to May 2008
NCT00299988	2	Intravenous immunoglobulin	ADAS-cog, ADAS-CGIC	24	Start Feb 2006; ongoing
NCT00455000	1	PF-04360365	Safety, tolerability, pharmacokinetics	36	March, 2007 to June 2008
NCT00174525	2	AAB-001	Safety		April 2005 to April 2008
NCT00531804	1	R1450	Adverse events, laboratory measures, vital signs	80	Dec, 2006 to Jan 2009

TABLE 1. *Current Drugs in Clinical Development for the Treatment of AD*

Drug Name	Probable Mechanism of Action	Company	Probable Clinical Phase
SAM-315	5-HT_6 receptor antagonist	Wyeth	Phase 1
LY-451395	AMPA receptor agonist	Lilly	Phase 1
S-18986	AMPA receptor agonist	Servier	Phase 1
GSK-189254	H3 receptor antagonist	GlaxoSmithKline	Phase 1
MEM-1003	L-type calcium channel blocker	Memory	Phase 1
MEM-3454	nAch receptor agonist	Memory & Roche	Phase 1
MEM-1414	PDE4 inhbitor	Memory & Roche	Phase 1 (recently terminated)
Humanized m266	Anti-Aβ antibody	Lilly	Phase 1
LY-450139	γ-Secretase inhibitor	Lilly	Phase 1
Anti-Aβ fragment	Active anti-Aβ immunization	ENKAM Pharma	Phase 1
PAZ-417	Activator of Aβ catabolism	Wyeth	Phase 1
GSI-953	γ-Secretase inhibitor	Wyeth	Phase 1
ACC-001	Active anti-Aβ immunization	Wyeth & Elan	Phase 1
Anti-Aβ fragment	Active anti-Aβ immunization	Novartis & Cytos	Phase 1
Apan	Antiamyloid fibril agent	Praecis	Phase 1
CERE-10	NGF gene therapy	Ceregene	Phase 1
PTI-00703	Antiamyloid fibril agent	ProteoTech	Phase 1
TAK-070	β-Secretase inhibitor	Takeda	Phase 1
PBT-2	Antiamyloid fibril agent	Prana & Schering	Phase 1
NS-2330	Biogenic amine transport blocker	Neurosearch & Boehringer Ingleheim	Phase 2
Avandia	PPARγ receptor agonist	GlaxoSmithKline	Phase 2
GSK-742457	5-HT_6 receptor antagonist	GlaxoSmithKline	Phase 2
S-8510	$GABA_A$ receptor inverse agonist	Shinogi & GlaxoSmithKline	Phase 2 (likely terminated)
SRA-333	5-HT_{1A} receptor antagonist	Wyeth	Phase 2
SL-650155	5-HT_4 receptor antagonist	Sanofi-Aventis	Phase 2
CX-717	AMPA receptor agonist	Cortex & Servier & Organon	Phase 2 (successor to recently terminated CX-516)
P-58	M_1 receptor antagonist	Phytopharm & Yamanouchi	Phase 2
SGS-742	$GABA_B$ receptor agonist	Saegis	Phase 2
AC-3933	$GABA_A$ receptor inverse agonist	SanofiAventis & Dainippon	Phase 2
ABT-089	nAch receptor agonist	Abbott	Phase 2
TC-1734	nAch receptor agonist	Targacept	Phase 2
SR-57667	Growth factor modulator	Sanofi-Aventis	Phase 2
AAB-001	Passive anti-Aβ immunotherapy	Wyeth & Elan	Phase 2
PBT-1	Fibrillization inhbitor	Prana & Schering	Phase 2 (terminated due to impurities in drug substance)
Avicor	HMG Co-A reductase inhibitor	Andrx	Phase 2
SR-57746	5-HT_{1a} receptor agonist	Sanofi-Aventis	Phase 3
R-flurbiprofen	γ-Secretase inhibitor	Myriad	Phase 3 (but failed to meet primary Ph 2 end points)
Phenserine	APP modulating cholinesterase inhibitor	Axonyx	Phase 3 (but failed to meet Ph 3 end points)
NC-531	Fibrillization inhibitor	Neurochem	Phase 3
Lipitor	HMG Co-A reductase inhibitor	Pfizer	Phase 3
Zocor	HMG Co-A reductase inhibitor	Merck	Phase 3

Shaded areas highlight drugs that have potential to be disease modifying.

Study Phase

Most clinical trials are designated as phase I, II, III, or IV, based on the type of questions that study is seeking to answer:

In Phase I clinical trials, researchers test a new drug or treatment in a small group of people (20-80) for the first time to evaluate its safety, determine a safe dosage range, and identify side effects.

In Phase II clinical trials, the study drug or treatment is given to a larger group of people (100-300) to see if it is effective and to further evaluate its safety.

In Phase III clinical trials, the study drug or treatment is given to large groups of people (1,000-3,000) to confirm its effectiveness, monitor side effects, compare it to commonly used treatments, and collect information that will allow the drug or treatment to be used safely.

In Phase IV clinical trials, post marketing studies delineate additional information including the drug's risks, benefits, and optimal use.

These phases are defined by the Food and Drug Administration in the Code of Federal Regulations.

Mini-Mental State Examination (MMSE)

Test di screening ideato per rilevare il deterioramento cognitivo, valutarne quantitativamente la severità e documentarne le modificazioni nel tempo.
E' costituito da 22 prove in parte verbali e in parte di performance, diverse funzioni cognitive:

ORIENTAMENTO TEMPORALE;
ORIENTAMENTO SPAZIALE;
MEMORIA IMMEDIATA (registrazione di tre parole);
ATTENZIONE E CALCOLO (serie di "7", scansione di parola al contrario);
MEMORIA DI RICHIAMO (rievocazione delle tre parole);
LINGUAGGIO (denominazione, ripetizione, comprensione e esecuzione di comandi orali e scritti, capacità di scrivere una frase);

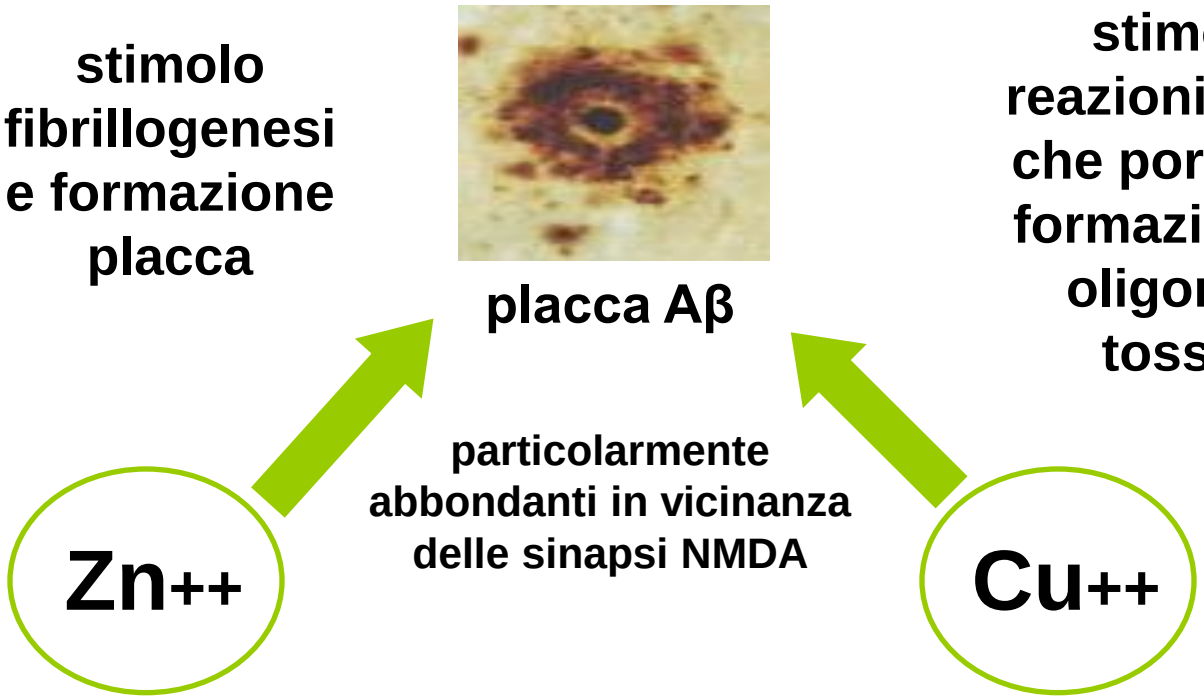
Il punteggio soglia ai fini della diagnosi di disturbi dell'efficienza intellettiva è 23 (la maggior parte delle persone anziane non dementi ottiene punteggi superiori a tale soglia).

24 – 30 ASSENZA DI DECADIMENTO COGNITIVO;
18 – 23 DECADIMENTO COGNITIVO da LIEVE a MODERATO;
0 – 17 DECADIMENTO COGNITIVO GRAVE;

AD: nuove terapie?

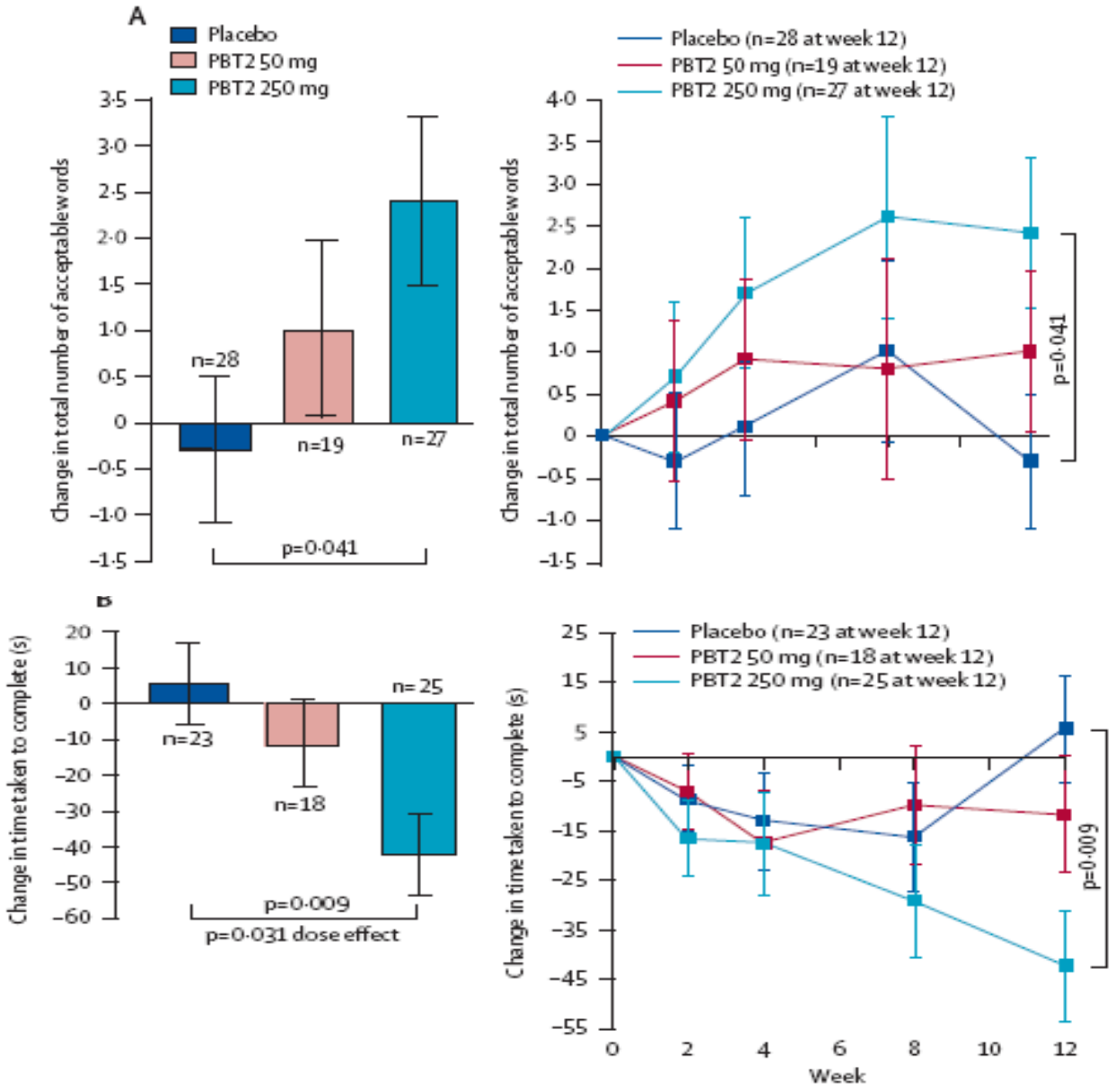
Safety, efficacy, and biomarker findings of PBT2 in targeting Aβ as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial

Lannfelt et al, Lancet Neurol 2008



PBT2 is a metal-protein attenuating compound (MPAC) that affects the Cu2(+)-mediated and Zn2(+)-mediated toxic oligomerisation of Abeta seen in Alzheimer's disease (AD).

AD: nuove terapie?



Effect of PBT2 (50mg and 250mg) and placebo on the change from pretreatment at 12 weeks

(A) Category fluency test

(B) trail making test part B

LE ESPANSIONE DI TRIPLETTE

•SONO IMPLICATE IN OLTRE 40 MALATTIE NEURODEGENERATIVE

•LE RIPETIZIONI SI LOCALIZZANO IN REGIONI CODIFICANTI (ESONI) O IN REGIONI NON CODIFICANTI (INTRONI)

•TRIPLETTE ESPANSE POSSONO ESSERE PIÙ O MENO STABILI

•L'ESPANSIONE CAUSA PATOLOGIA CON DIFFERENTI MECCANISMI: PERDITA DI FUNZIONE O ACQUISIZIONE DI FUNZIONE

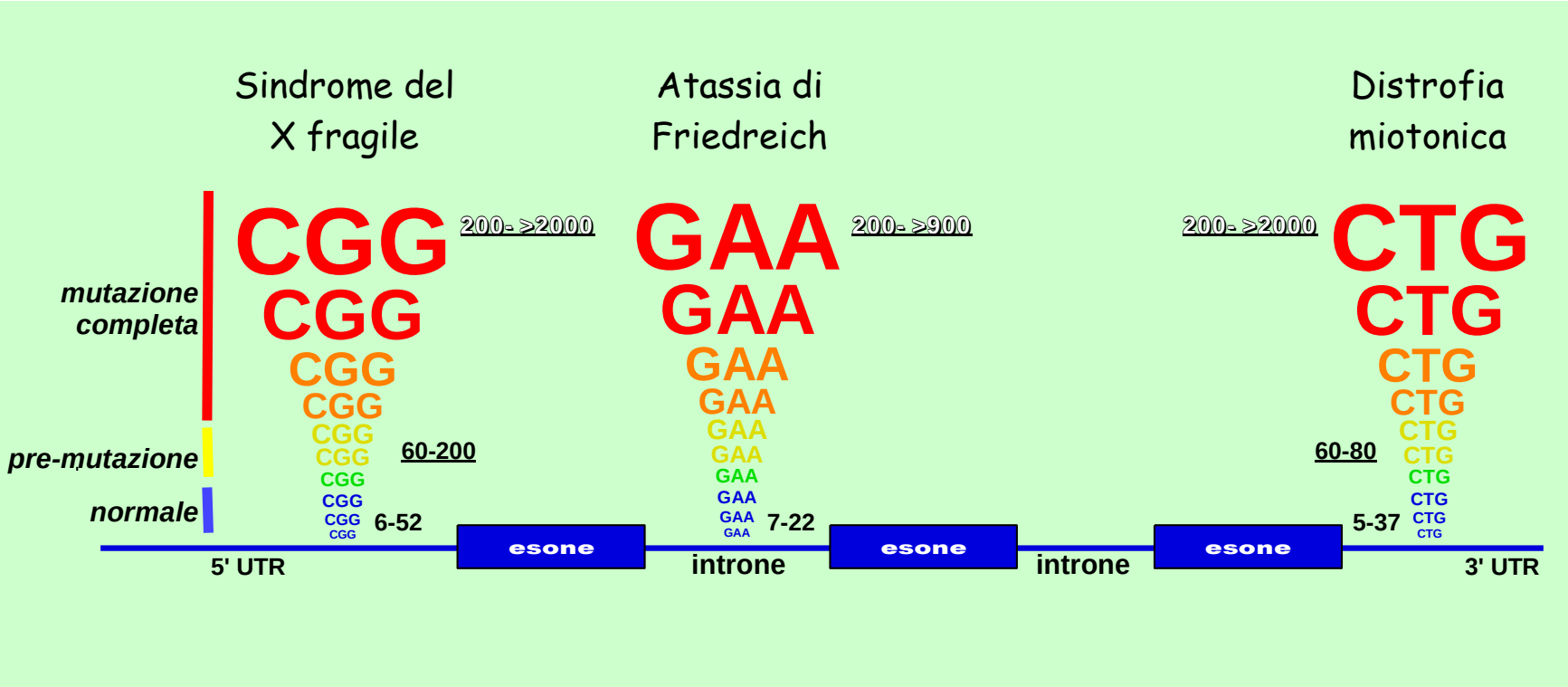
•L'ESPANSIONE AVVIENE TIPICAMENTE NEGLI INDIVIDUI COSIDETTI PREMUTATI E PUÒ RIGUARDARE PREFERENZIALMENTE LE TRASMISSIONI MATERNE (PER ES. FRAXA, DISTROFIA MIOTONICA) O PATERNE (PER ES COREA DI HUNTINGTON)

•LA PATOLOGIA MANIFESTA COMPARE DOPO UN CERTO NUMERO DI RIPETIZIONI TIPICO DI CIASCUNA PATOLOGIA

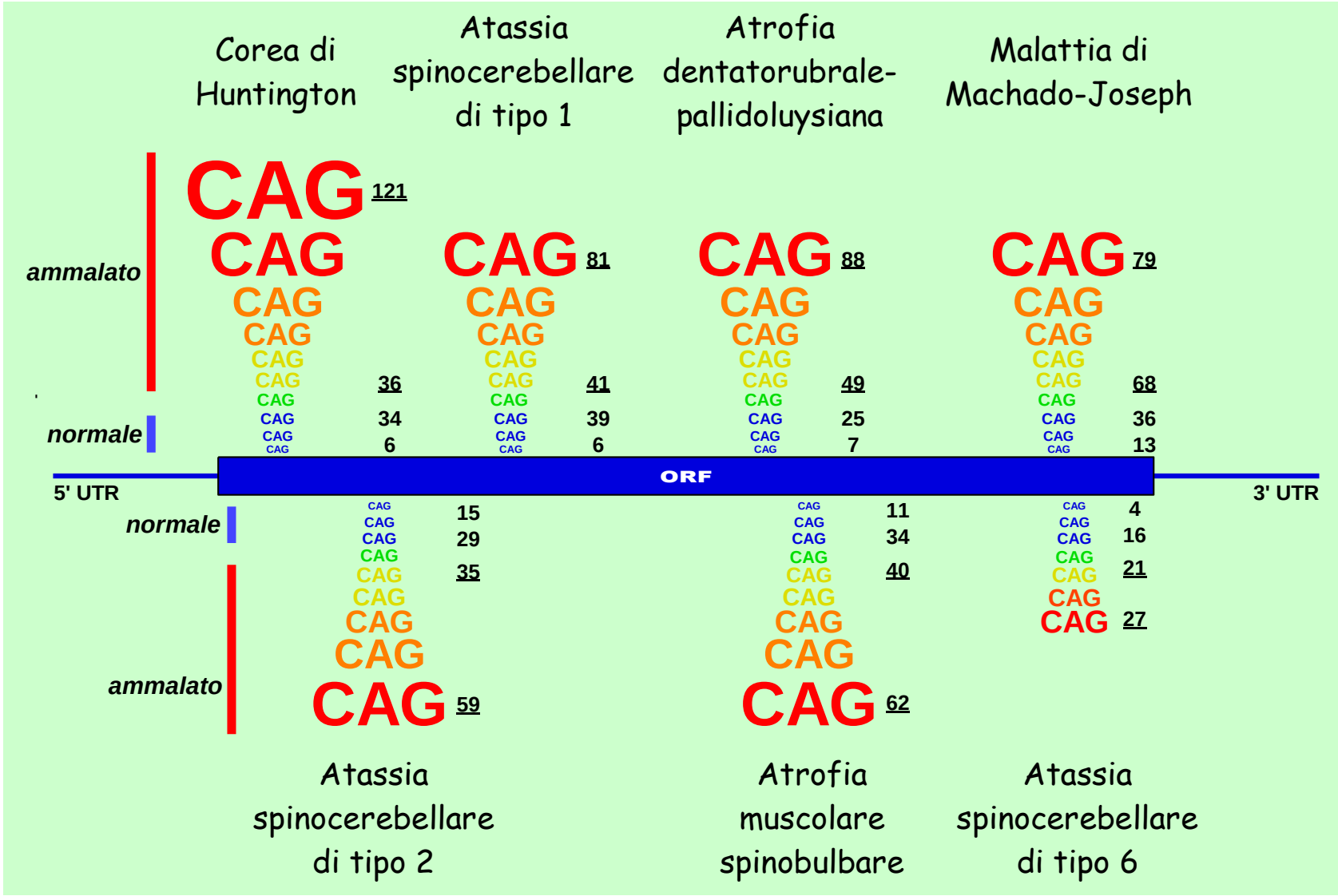
MALATTIE DA TRIPLETTE RIPETUTE

Disease	Gene Locus/Protein	Repeat	Location
Fragile X syndrome	Xq27.3/FMR-1 protein	CGG	Noncoding
Fragile XE syndrome	Xq28/FMR-2 protein	GCC	Noncoding
Friedreich ataxia	9q13-9q21.1/frataxin	GAA	Noncoding
Myotonic dystrophy 1	19q13/myotonic dystrophy protein kinase	CTG	Noncoding
Myotonic dystrophy 2	3q21	CCTG	Noncoding
Spinobulbar muscular atrophy	Xq13-Xq21/androgen receptor	CAG	Coding
Huntington disease	4p16.3/huntington	CAG	Coding
Dentatorubralpallidoluysian atrophy	12p13.31/atrophin-1	CAG	Coding
SCA type 1	6p23/ataxin-1	CAG	Coding
SCA type 2	12q24/ataxin-2	CAG	Coding
SCA type 3 (Machado-Joseph disease)	14q32.1/ataxin-3	CAG	Coding
SCA type 6	19p13/α-1A (voltage-ependent calcium channel subunit)	CAG	Coding
SCA type 7	3p12-3p13/ataxin-7	CAG	Coding
SCA type 8	13q12/none identified	CTG	?
SCA type 12	5q31-5q33	CAG	Noncoding

MALATTIE DA TRIPLETTE RIPETUTE
IN REGIONI NON CODIFICANTI



MALATTIE DA TRIPLETTE RIPETUTE
IN REGIONI CODIFICANTI



PROBLEMATICHE DELLE MALATTIE DA MUTAZIONI DINAMICHE

GRANDEZZA DELLA RIPETIZIONE

NORMALE: INTERVALLO DELLA VARIAZIONE NON ASSOCIATA CON L'INSORGENZA DI PATOLOGIA

INTERMEDIA: NESSUNA ALTERAZIONE CLINICA. SOLITAMENTE DEFINITE PREMUTAZIONI, PERCHÉ POSSONO PREDISPORRE AD INCREMENTI DI LUNGHEZZA TIPICI DELLE MUTAZIONI PATOGENE

BORDERLINE: PUÒ PRODURRE MALATTIA CON RIDOTTA PENETRANZA O FENOTIPO PIÙ LIEVE

GRANDE (O **FULL**): SOLITAMENTE ASSOCIATA AD ESPRESSIONE DELLA MALATTIA. PUÒ ESSERE STABILE O PREDISPORRE AD ULTERIORE ESPANSIONE O CONTRAZIONI

ESPANSIONI INTERGENERAZIONALI

PIÙ FREQUENTI SE LA SEQUENZA È PERFETTA (SENZA INTERRUZIONI NELLA RIPETIZIONE)

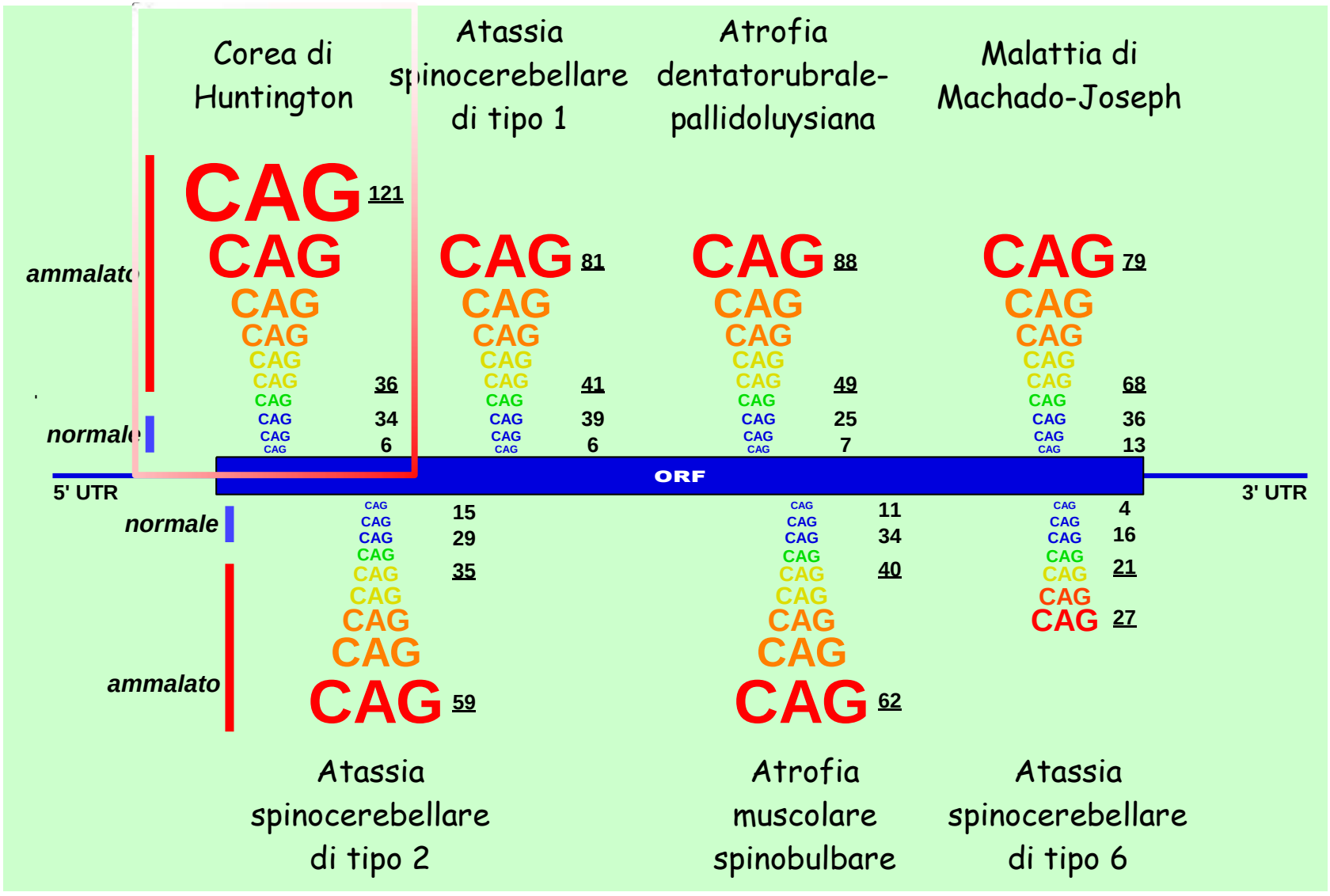
TALORA LEGATE ALL'ETÀ DEL GENITORE:
HD (corea): ++ CON AUMENTO ETÀ PATERNA
SCA1: ++ CON AUMENTO ETÀ MATERNA

BIAS PARENTALE

DM: TRASM. MATERNA → ESPANSIONE SENZA LIMITI (FORMA CONGENITA)

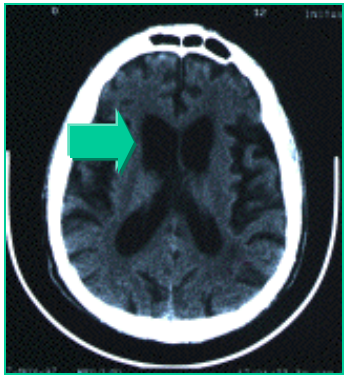
HD: TRASM. PATERNA → ESPANSIONI MAGGIORI, ESORDIO PRECOCE

MALATTIE DA TRIPLETTE RIPETUTE
IN REGIONI CODIFICANTI

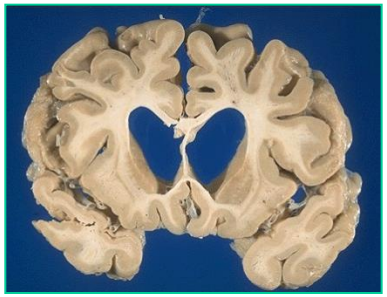


MALATTIA DI HUNTINGTON - LA CLINICA

malattia neurodegenerativa dovuta a degenerazione dei neuroni colinergici del nucleo caudato e del putamen



QUADRO CLINICO
-DISTURBI PSICHIATRICI
(AGGRESSIVITÀ,DEPRESSIONE, SUICIDIO)
-DISTURBI DEL MOVIMENTO
(IPERCINESIE COREO-ATETOSICHE → IPOCINESIA)
-DETERIORAMENTO MENTALE
-ESORDIO NELLA 4° DECADE



AUTOSOMICA DOMINANTE

PENETRANZA COMPLETA età-correlata
ESPRESSIVITÀ VARIABILE
frequenza: 3-7:100.000 nati

MALATTIA DI HUNTINGTON - L'ESPANSIONE CAG

GENETICA MOLECOLARE

ESPANSIONE DI UNA SEQ. RIPETUTA **CAG** NELLA REGIONE CODIFICANTE DEL GENE IT-15 SU CROM. 4P16

range normale → 6-26 CAG normal alleles

forma intermedia →27-35 CAG ("mutable" normal alleles)

ridotta penetranza → 36-39 CAG (reduced penetrant alleles)

HD classica → 40-55 CAG (full penetrant alleles)

HD giovanile → > 60 CAG

ANTICIPAZIONE

```
  1 ttg ctg tgt gag gca gaa cct gcg ggg gca
    ggg gcg ggc tgg ttc cct ggc cag cca ttg
 61 gca gag tcc gca ggc tag ggc tgt caa tca
    tgc tgg ccg gcg tgg ccc cgc ctc cgc cgg
121 cgc ggc ccc gcc tcc gcc ggc gca cgt ctg
    gga cgc aag gcg ccg tgg ggg ctg ccg gga
181 cgg gtc caa gat gga cgg ccg ctc agg ttc
    tgc ttt tac ctg cgg ccc aga gcc cca ttc
241 att gcc ccg gtg ctg agc ggc gcc gcg agt
    cgg ccc gag gcc tcc ggg gac tgc cgt gcc
301 ggg cgg gag acc gcc atg gcg acc ctg gaa
    aag ctg atg aag gcc ttc gag tcc ctc aag
361 tcc ttc cag cag cag cag cag cag cag cag cag
    cag cag cag cag cag cag cag cag cag cag
421 cag cag cag caa cag ccg cca ccg ccg ccg
    ccg ccg ccg ccg cct cct cag ctt cct cag
```

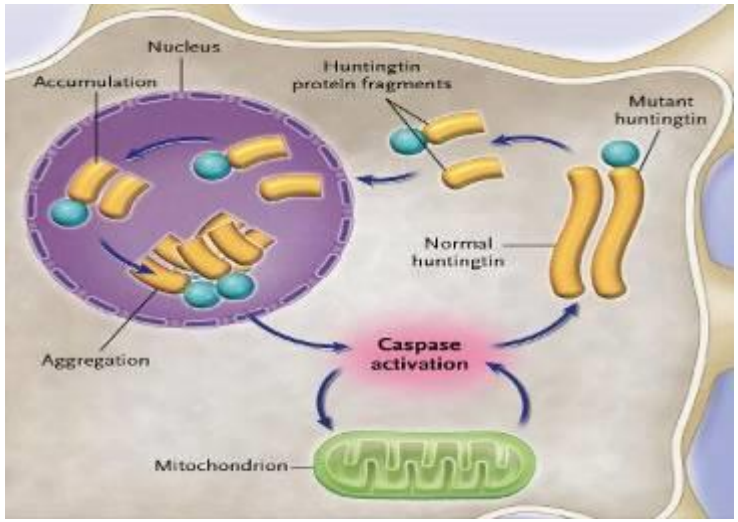
21 triplette CAG ripetute in un gene HTT "normale"

- LA HUNTINGTINA CON POLIGLUTAMMINA FORMA AGGREGATI NEI NEURONI CAUSANDONE LA MORTE

- ALL'INIZIO SINTOMI PSICHIATRICI QUALI DEPRESSIONE, IRRITABILITÀ, DIFFICOLTÀ A PRENDERE DECISIONI, POI PRESENTA MOVIMENTI INCONTROLLATI SIMILI A UNA DANZA E DEMENZA

- PUR CON UNA GRANDE VARIABILITÀ INDIVIDUALE, LA MALATTIA AVANZA INESORABILMENTE FINO ALLA MORTE

- TENTATIVI TERAPEUTICI SONO IN CORSO CON LA CISTAMINA CHE INIBISCE LA TRANSGLUTAMINASI COINVOLTA NELLA FORMAZIONE DEGLI AGGREGATI



MALATTIA DI HUNTINGTON – LA CONSULENZA GENETICA

Probabilità di avere ereditato la mutazione nel gene IT15 in adulti asintomatici in relazione all'età

20 anni	49.6%
30 anni	47.6%
40 anni	42.5%
50 anni	31.5%
60 anni	22.1%
70 anni	6.2%

un test genetico positivo non consente di predire con esattezza l'età di esordio → tabelle empiriche di correlazione

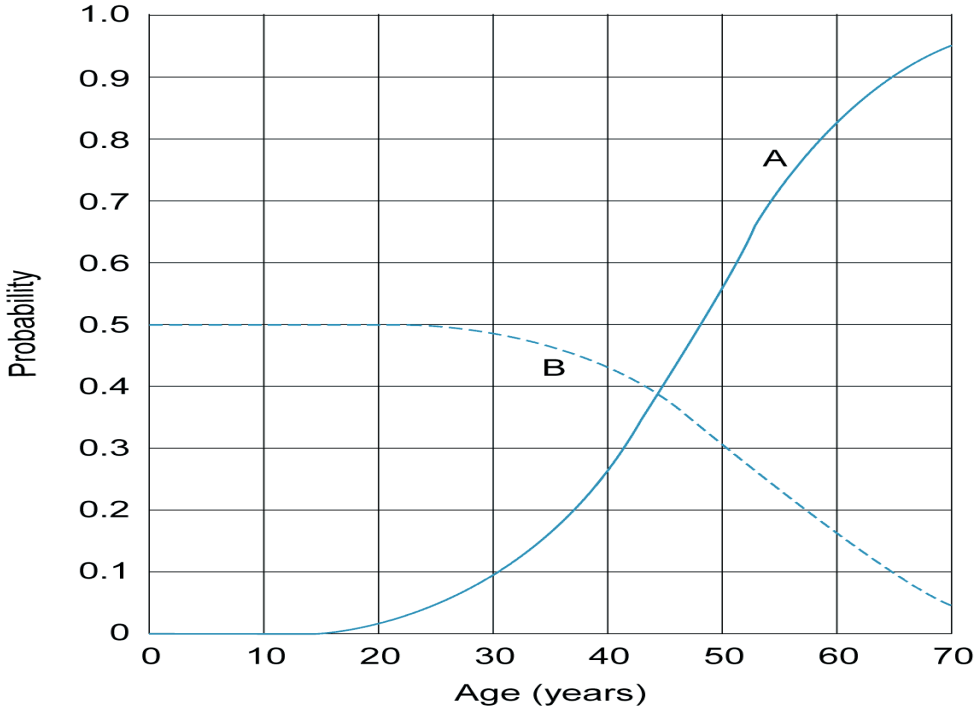
39 CAG	66 aa	45 CAG	37 aa
40 CAG	59 aa	46 CAG	36 aa
41 CAG	54 aa	47 CAG	33 aa
42 CAG	49 aa	48 CAG	32 aa
43 CAG	44 aa	49 CAG	28 aa
44 CAG	42 aa	50 CAG	27 aa

Brinkman et al, 1997

aspetti psicologici importanti → è necessario seguire un iter formale per la consulenza genetica del test presintomatico → linee guida

ogni soggetto con test presintomatico positivo va seguito nel tempo, e soprattutto al momento dell'esordio dei sintomi

Età di insorgenza della Corea di Huntington



a) Probabilità che un individuo portatore del gene mutato abbia sviluppato i sintomi ad una data età

b) Rischio che il figlio sano di un soggetto affetto sia portatore del gene mutato ad una determinata età.

TEST PRESINTOMATICO– LINEE GUIDA

IL TEST È OFFERTO SOLTANTO A SOGGETTI CHE ABBIANO >18 ANNI

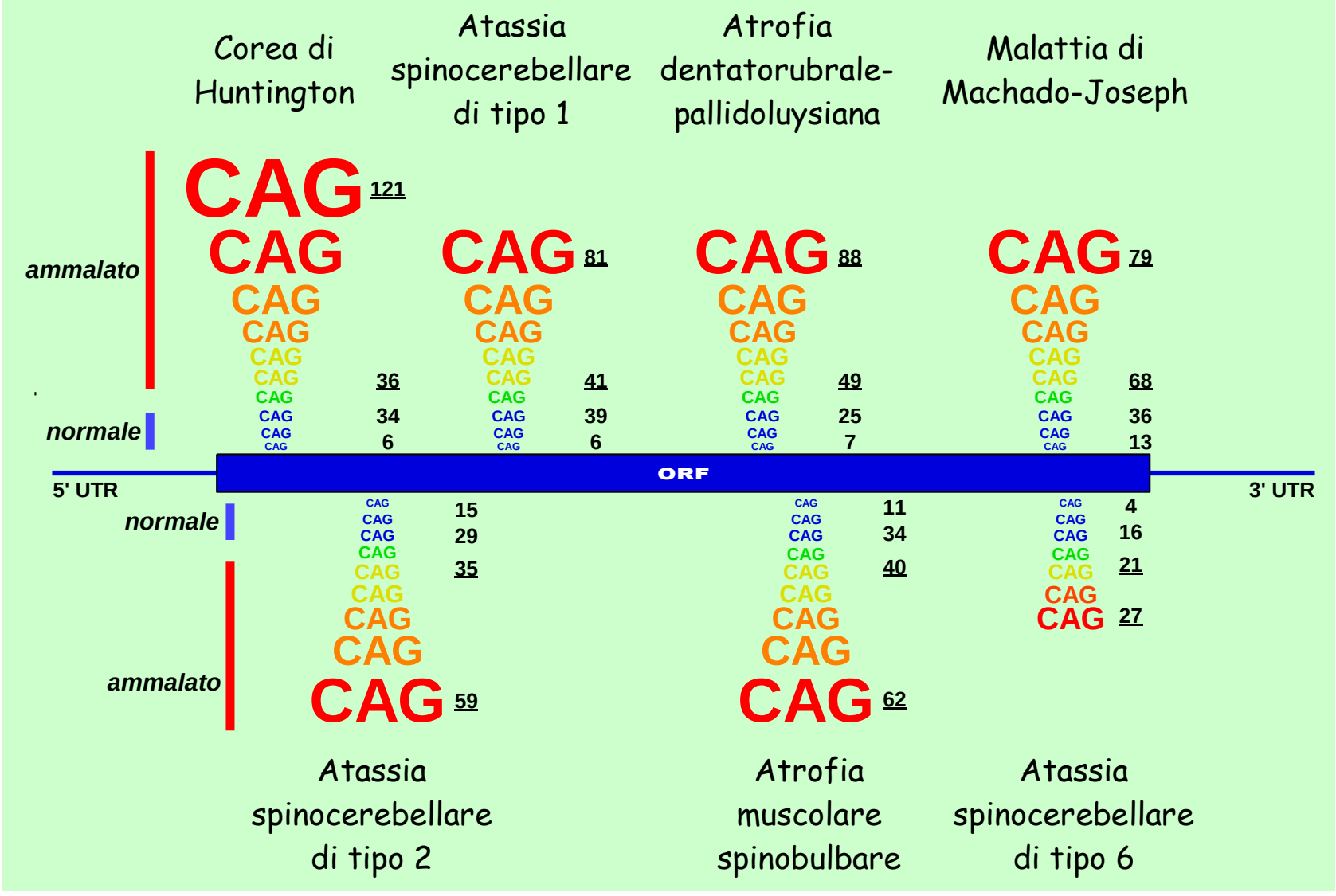
OTTENIMENTO DEL CONSENSO INFORMATO (“CONSAPEVOLE”) DOPO CONSULENZA GENETICA, RIGUARDANTE I POTENZIALI BENEFICI E RISCHI DEL TEST GENETICO PER L’INDIVIDUO E I SUOI FAMILIARI

PREVISTE SESSIONI MULTIPLE (DISTANZIATE NEL TEMPO) CON VARIE FIGURE PROFESSIONALI (GENETISTA, PSICOLOGO, NEUROLOGO)

VIENE SUGGERITO UN PERIODO DI ATTESA (DI ALMENO UN MESE) TRA LA CONSULENZA GENETICA E LA DECISIONE DI PROCEDERE AL TEST GENETICO

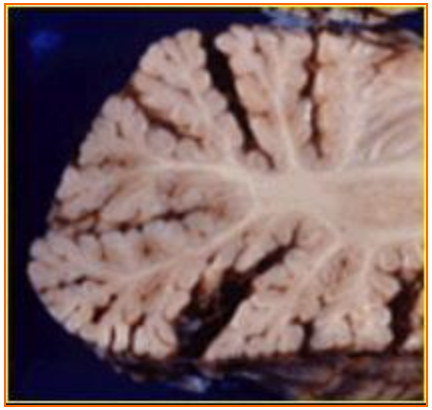
IL RISULTATO DEL TEST GENETICO VIENE DATO PERSONALMENTE AL SOGGETTO A RISCHIO

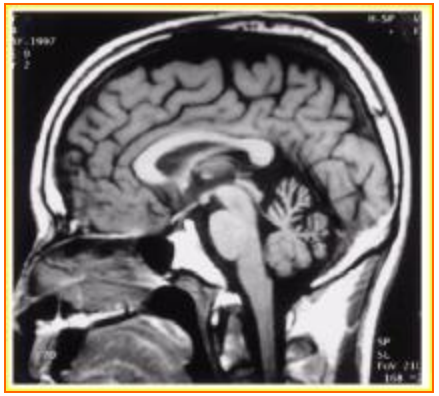
MALATTIE DA TRIPLETTE RIPETUTE
IN REGIONI CODIFICANTI



ATASSIE SPINOCEREBELLARI EREDITARIE- LA CLINICA

PATOLOGIE NEURODEGENERATIVE CARATTERIZZATE DA ATROFIA CEREBELLARE ± ASSOCIATA A DEGENERAZIONE COLONNE POSTERIORI MIDOLLO SPINALE E PARTE DEL TRONCOENCEFALO





QUADRO CLINICO
esordio ad età variabile (3° -5° decade) di atassia del tronco e degli arti, disartria, nistagmo, con andamento lentamente progressivo e possibile associazione di altri segni neurologici in alcune forme.

Patologie a PENETRANZA COMPLETA
ma ESPRESSIONE VARIABILE
prevalenza: 3/100.000 (variabile in diverse popol.)

Establishing the Diagnosis of Hereditary Ataxia

Establishing the diagnosis of hereditary ataxia requires the following:

- Detection on neurologic examination of typical clinical symptoms and signs including poorly coordinated gait and finger/hand movements, often associated with dysarthria and nystagmus
- Documenting the hereditary nature by the presence of:
    - A positive family history of ataxia;
    - A causative (i.e., pathogenic) allelic variant or variants one of the many genes associated with hereditary ataxia;
    - A clinical phenotype characteristic of a genetic form of ataxia.
      Note: In some individuals with no family history of ataxia it may not be possible to establish a genetic cause if results of all available genetic tests are normal.

Differential Diagnosis of Hereditary Ataxia

Differential diagnosis of hereditary ataxia includes acquired, non-genetic causes of ataxia, such as alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, primary or metastatic tumors, or paraneoplastic diseases associated with occult carcinoma of the ovary, breast, or lung.

The possibility of an acquired cause of ataxia needs to be considered in each individual with ataxia because a specific treatment may be available [Shakkottai & Fogel 2013].

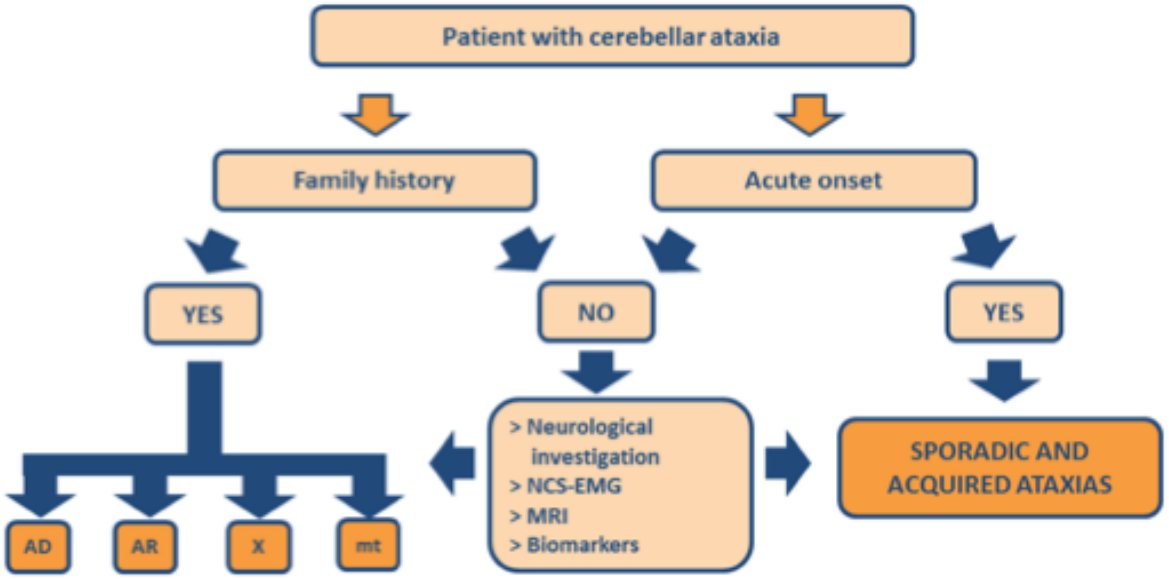


Fig. 1 Discrimination of acquired and hereditary conditions. *AD* autosomal dominant, *AR* autosomal recessive, *MRI* magnetic resonance imaging, *mt* mitochondrial, *NCS–EMG* nerve conduction studies and electromyography, *X* X-linked

GENETICA MOLECOLARE

La modalità di trasmissione può essere:
- autosomica dominante
- autosomica recessiva
- X-linked
- mitocondriale

ETEROGENEITA' GENETICA

ETEROGENEITÀ MOLECOLARE

-ESPANSIONE DI SEQUENZE RIPETUTE (++CAG) IN GENI DIVERSI
-MUTAZIONI PUNTIFORMI/DELEZIONI IN ALTRI GENI

REVIEW | Genetics inMedicine

Hereditary ataxias: overview

Suman Jayadev, MD[1] and Thomas D. Bird, MD[1–3]

	Polyglutamine expansion SCAs	Conventional mutation SCAs
Age at onset	Variable (intrafamilial); anticipation	Variable (often in childhood); no anticipation
Disease progression	Progressive, often fatal and juvenile cases are more severe than late-onset cases	Slowly progressive, childhood onset is not associated with a more severe disease course
Clinical features	Multisystemic, neurodegenerative widespread lesions that increase with disease duration	Pure cerebellar, congenital features such as stable mental retardation
Neuropathology	Widespread neuronal loss	Predominant Purkinje cell loss
Cerebral imaging	Brainstem>cerebellar atrophy	Pure and global cerebellar atrophy

SCA=spinocerebellar ataxia.

Table 3: Phenotypic differences between autosomal dominant cerebellar ataxias caused by polyglutamine expansions and conventional mutations

	Gene	Mutations	Key symptom in addition to cerebellar ataxia
Polyglutamine expansions SCAs			
SCA1	*ATXN1*	CAG repeat	Early swallowing and respiratory signs
SCA2	*ATXN2*	CAG repeat	Slow eye movements
SCA3	*ATXN3*	CAG repeat	--
SCA6	*CACNA1A*	CAG repeat	--
SCA7	*ATXN7*	CAG repeat	Visual loss
SCA17	*TBP*	CAG repeat	Dementia
DRPLA	*ATN1*	CAG repeat	Epilepsy
Non-coding expansion SCAs			
SCA8	*ATXN8* and *ATXN8OS*	CTG repeat	--
SCA10	*ATXN10*	ATTCT	--
SCA12	*PPP2R2B*	CAG repeat	--
SCA31=16qlinked	*BEAN-TK2*	TGGAA repeat	--
Conventional mutations SCAs			
SCA5	*SPTBN2*	Missense, in-frame deletion	--
SCA11	*TTBK2*	Frameshift	--
SCA13	*KCNC3*	Missense	Mental retardation
SCA14	*PRKCG*	Missense	Myoclonus
SCA15/16	*ITPR1*	Missense, deletion	--
SCA20	--	Duplication	Dysphonia
SCA27	*FGF14*	Missense, frameshift	--
SCA28	*AFG3L2*	Missense	Ptosis
Loci (test unavailable)			
SCA4	--	--	Sensory neuropathy
SCA18	--	--	Sensory neuropathy
SCA19	--	--	--
SCA21	--	--	Mental retardation
SCA22	Allelic to SCA19?	--	Mental retardation
SCA23	--	--	--
SCA25	--	--	Sensory neuropathy
SCA26	--	--	--
SCA30	--	--	--

SCA=spinocerebellar ataxia. --=unknown.

Table 1: **Genes and mutations that cause autosomal dominant cerebellar ataxias, according to locus and mutation type**

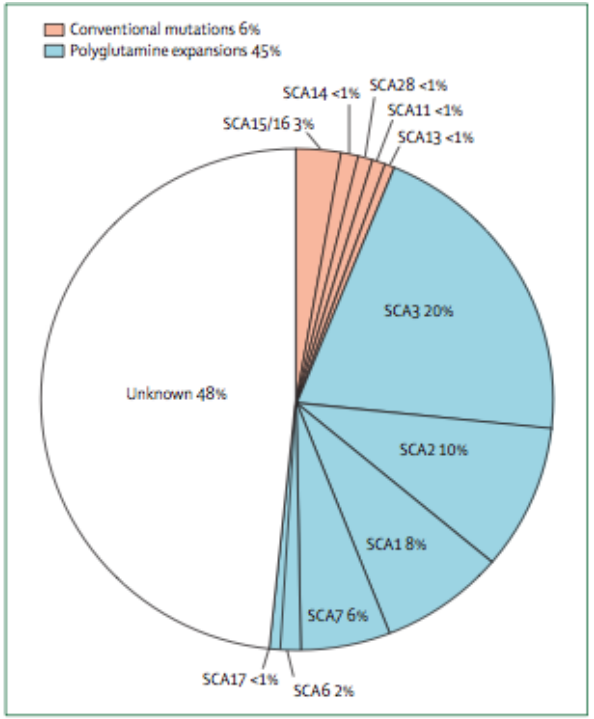


Figure 1: **Relative prevalence of SCA subtypes of autosomal dominant cerebellar ataxia**

826 index patients, mostly of French origin, were studied. Data from reference 20.

	Small repeat	Medium repeat	Large repeat	Very large repeat
SCA1	..	Cerebellar ataxia, pyramidal syndrome	Amyotrophic lateral sclerosis-like disorders	Developmental delay
SCA2	Postural tremor	Cerebellar ataxia, decreased reflexes	Cerebellar ataxia, chorea, dementia	Myoclonus, dystonia, cardiac failure, retinal degeneration
SCA3	Axonal neuropathy, dopa-responsive parkinsonism	Cerebellar ataxia, diplopia	Dystonia, pyramidal signs	Rare cases, predominant dystonia
SCA6	Episodic ataxia	..	Few associated signs after 10-years of disease course	..
SCA7	Cerebellar ataxia without visual loss	Cerebellar ataxia, macular degeneration	Visual loss before cerebellar syndrome	Cardiac failure
SCA17	Huntington's disease-like phenotype, parkinsonism	Ataxia, dementia, chorea and dystonia, pyramidal signs	Ataxia, dementia, spasticity, epilepsy	Growth retardation
DRPLA	Chorea, ataxia, psychiatric manifestations	..	Progressive myoclonus, epilepsy, developmental delay, mild ataxia	Myoclonic epilepsy, chorea, cognitive impairment

..=unknown. SCA=spinocerebellar ataxia. DRPLA=dentatorubro-pallidoluysian atropy.

Table 2: **Clinical features in polyglutamine expansion SCAs, according to size of CAG repeat**

ETEROGENEITA' GENETICA

Table 1 Autosomal dominant hereditary ataxias

Disease Name[a]	Gene	Average onset (range in years)	Average duration (range in years)	Distinguishing features[b]
SCA1	*ATXN1*	3rd–4th decade (<10 to >60)	15 years (10–28)	Pyramidal signs, peripheral neuropathy
SCA2	*ATXN2*	3rd–4th decade (<10 to >60)	10 years (1–30)	Slow saccadic eye movements, peripheral neuropathy, decreased DTRs, dementia
SCA3	*ATXN3*	4th decade (10–70)	10 years (1–20)	Pyramidal and extrapyramidal signs; lid retraction, nystagmus, decreased saccade velocity; amyotrophy fasciculations, sensory loss
SCA4	16q22.1	4th–7th decade (19–72)	Decades	Sensory axonal neuropathy, deafness; may be allelic with 16q22-linked SCA
SCA5	*SPTBN2*	3rd–4th decade (10–68)	>25 years	Early onset, slow course; first reported in descendants of Abraham Lincoln
SCA6	*CACNA1A*	5th–6th decade (19–71)	>25 years	Sometimes episodic ataxia, very slow progression
SCA7	*ATXN7*	3rd–4th decade (0.5–60)	20 years (1–45; ea y onset correlates wi h shorter duration)	Visual loss with retinopathy
SCA8	*ATXN8/ ATXN80S*	4th decade (1–65)	Normal life span	Slowly progressive, sometimes brisk DTRs, decreased vibration sense; rarely, cognitive impairment
SCA10	*ATXN10*	4th decade (12–48)	9 years	Occasional seizures; most families are of Native American background
SCA11	*TTBK2*	Age 30 years (15–70)	Normal life span	Mild, remain ambulatory
SCA12	*PPP2R2B*	4th decade (8–62)		Slowly progressive ataxia; action tremor in the 30s; hyperreflexia; subtle Parkinsonism possible; cognitive/psychiatric disorders including dementia
SCA13	*KCNC3*	Childhood or adulthood	Unknown	Mild intellectual disability, short stature
SCA14	*PRKCG*	3rd–4th decade (3–70)	Decades (1–30)	Early axial myoclonus
SCA15	*ITPR1*	4th decade (7–66)	Decades	Pure ataxia, very slow progression
SCA16	*SCA16*	Age 39 years (20–66)	1–40 years	Head tremor; one Japanese family
SCA17	*TBP*	4th decade (3–55)	>8 years	Mental deterioration; occasional chorea, dystonia, myoclonus, epilepsy; Purkinje cell loss, intranuclear inclusions with expanded polyglutamine
SCA18	7q22–q32	Adolescence (12–25)	Decades	Ataxia with early sensory/motor neuropathy, nystagmus, dysarthria, decreased tendon reflexes, muscle weakness, atrophy, fasiculations, Babinski responses
SCA19/22	*KCND3*	4th decade (10–51)	Decades	Slowly progressive, rare cognitive impairment, myoclonus, hyperreflexia
SCA20	11q12.2–11q12.3	5th decade (19–64)	Decades	Early dysarthria, spasmodic dysphonia, hyperreflexia, bradykinesia; calcification of the dentate nucleus
SCA21	*SCA21*	6–30	Decades	Mild cognitive impairment
SCA23	*PDYN*	5th–6th decade	>10 years	Dysarthria, abnormal eye movements, reduced vibration and position sense; one Dutch family; neuropathology[c]
SCA25	*SCA25*	(1.5–39)	Unknown	Sensory neuropathy; one French family
SCA26	*EEF2*	(26–60)	Unknown	Dysarthria, irregular visual pursuits; one Norwegian-American family; MRI: cerebellar atrophy
SCA27	*FGF14*	Age 11 years (7–20)	Decades	Early-onset tremor; dyskinesia, cognitive deficits; one Dutch family
SCA28	*AFG3L2*	Age 19.5 years (12–36)	Decades	Nystagmus, ophthalmoparesis, ptosis, increased tendon reflexes; two Italian families
SCA29	3p26	Early childhood	Lifelong	Learning deficits
SCA30	4q34.3–q35.1	(45–76)	Lifelong	Hyperreflexia

Table 1 Continued

Disease Name[a]	Gene	Average onset (range in years)	Average duration (range in years)	Distinguishing features[b]
SCA31	*BEAN1*	5th–6th decade	Lifelong	Normal sensation
SCA35	*TGM6*	43.7 ± 2.9 (40–48) years	15.9 ± 8.8 (5–31) years	Hyperreflexia, Babinski responses; spasmodic torticollis
SCA36	*NOP56*	52.8 ± 4.3 years	Decades	Muscle fasiculations, tongue atrophy, hyperreflexia
DRPLA	*ATN1*	3rd–4th decade (8–20 or 40–60s)	Early onset correlates with shorter duration	Chorea, seizures, dementia, myoclonus; often confused with Huntington disease
EA1	*KCNA1*	1st–2nd decade (2–15)	Attenuates after 20 years	Myokymia; attacks lasting seconds to minutes; startle or exercise induced; no vertigo
EA2	*CACNA1A*; *CACNB4*	2–32	Lifelong	Nystagmus; attacks lasting minutes to hours; posture-change induced; vertigo; later, permanent ataxia
EA5	*CACNB4*	20–30	Lifelong	Nystagmus; acetazolamide helpful
EA6	*SLC1A3*	Childhood	Lifelong	Headache; nausea; photophobia; rare alternating hemiplegia/ seizures
SPAX1	*VAMP1*	10–20	Normal life span	Initial progressive leg spasticity; similar to ARSACS

ARSACS, autosomal recessive spastic ataxia of Charlevoix-Saguenay; DRPLA, dentatorubral-pal doluysian atrophy; DTR, deep tendon reflex; EA, episodic ataxia; MRI, magnetic resonance imaging; SCA, spinocerebellar ataxia; SPAX1, autosomal dominant spastic taxia 1.

[a]SCA9 has not been assigned. [b]All have gait ataxia. [c]Purkinje cell loss, demyelination of the poste ior and lateral columns of the spinal cord, and neuronal intranuclear inclusions in the substantia nigra.

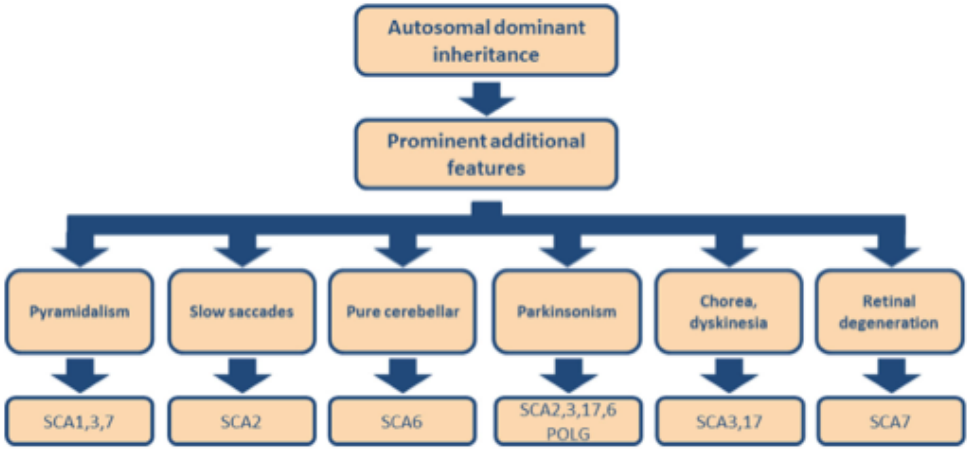


Fig. 2 Differential neurological signs in autosomal dominant cerebellar ataxia. *SCA* spinocerebellar ataxia (types 1–3, 6, 7, 17), *POLG* mitochondrial DNA polymerase gamma

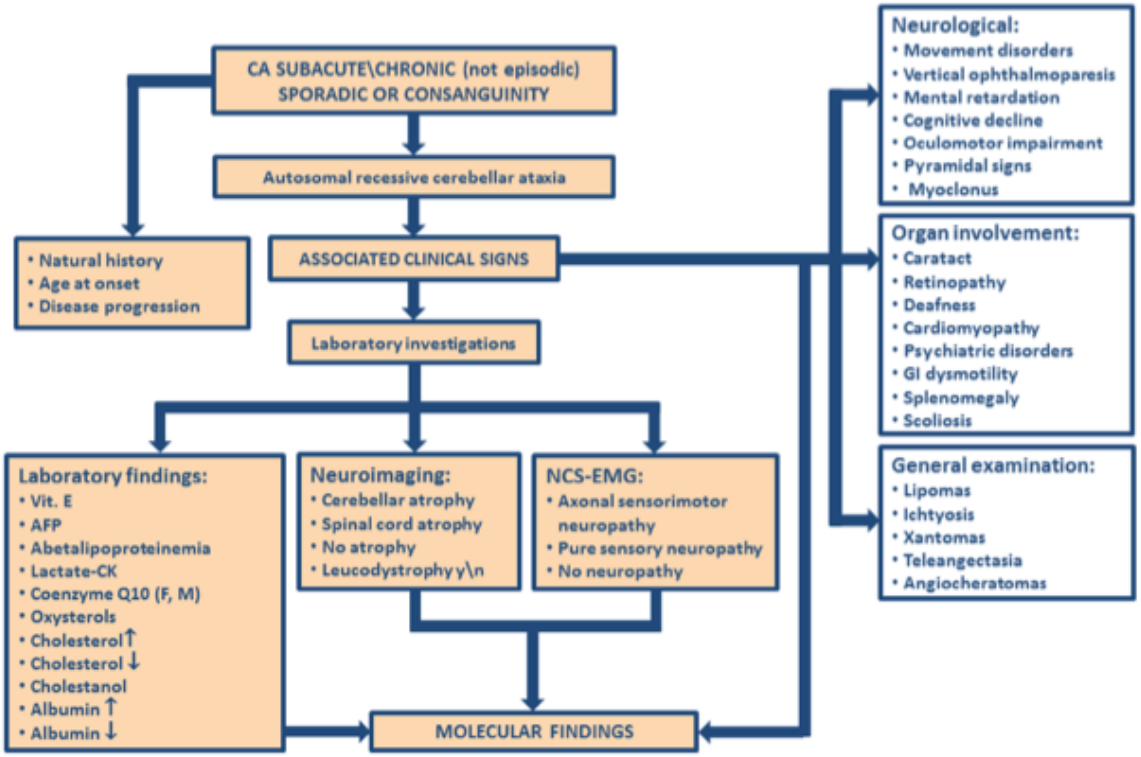


Fig. 3 Multidisciplinary approach to differential diagnosis of cerebellar ataxia. *AFP* alpha feta protein, *CA* cerebellar ataxia, *F* fibroblasts, *M* skeletal muscle, *NCS–EMG* nerve conduction studies and electromyography

ETEROGENEITA' GENETICA

Table 2 Autosomal recessive ataxias

Disease name	Gene symbol/ protein name	Population frequency	Onset (range in years)	Duration in years	Distinguishing features
Friedreich ataxia (FRDA)	*FXN*/frataxin	1–2:50,000	1st–2nd decade (4–40)	10–30	Hyporeflexia, Babinski responses, sensory loss, cardiomyopathy
Ataxia-telangiectasia	*ATM*	1:40,000 to 1:100,000	1st decade	10–20	Telangiectasia, immune deficiency, cancer, chromosomal instability, increased α-fetoprotein
Ataxia with vitamin E deficiency	*TTPA*	Rare	Age 2–52 years, usually <20	Decades	Similar to FRDA, head titubation (28%)
Ataxia with oculomotor apraxia type 1	*APTX*/ aprataxin	Unknown	Childhood	Decades	Oculomotor apraxia, choreoathetosis, mild intellectual disability, hypoalbuminemia
Ataxia with oculomotor apraxia type 2	*SETX*	Unknown	Age 10–22 years	Decades	Cerebellar atrophy, axonal sensorimotor neuropathy, oculomotor apraxia
IOSCA	*C10orf2*/ twinkle	Rare (Finland)	Infancy	Decades or less	Peripheral neuropathy, athetosis, optic atrophy, deafness, ophthalmoplegia, seizures
Marinesco–Sjögren syndrome	*SIL1*	Rare	Infancy	Decades	Intellectual disability, cataract, hypotonia, myopathy
Autosomal recessive spastic ataxia of Charlevoix-Saguenay	*SACS*/sacsin	Rare	Childhood	Decades	Spasticity, peripheral neuropathy, retinal striation
Refsum disease	*PHYH; PEX7*	Rare	1st–6th decade	Decades	Neuropathy, deafness, ichthyosis, retinopathy
CoQ10 deficiency	*CABC1; COQ2; COQ9; PDSS1; PDSS2*	Rare	Childhood	Decades	Seizures, cognitive decline, pyramidial signs, myopathy
MIRAS	*POLG1*	Rare (Finland and Norway)	Childhood to young adulthood	Decades	Nystagmus, dysarthria, epilepsy, cerebellar atrophy on MRI
SANDO	*POLG1*	Rare	Childhood to young adulthood	Decades	Abnormal eye movements, ragged red fibers, myopathy, dysphagia, neuropathy, myopathy
Cerebrotendinous xanthomatosis	*CYP27A1*	1:50,000	Childhood to young adulthood	Decades	Thick tendons, cognitive decline, dystonia, white matter disease, cataract

CoQ10, coenzyme Q10; IOSCA, infantile-onset spinocerebellar ataxia; MIRAS, mitochondrial recessive ataxia syndrome; MRI, magnetic resonance imaging; SANDO, sensory ataxia, neuropathy, dysarthria and ophthalmoplegia.

Atassie spinocerebellari da espansione di sequenze ripetute – problematiche

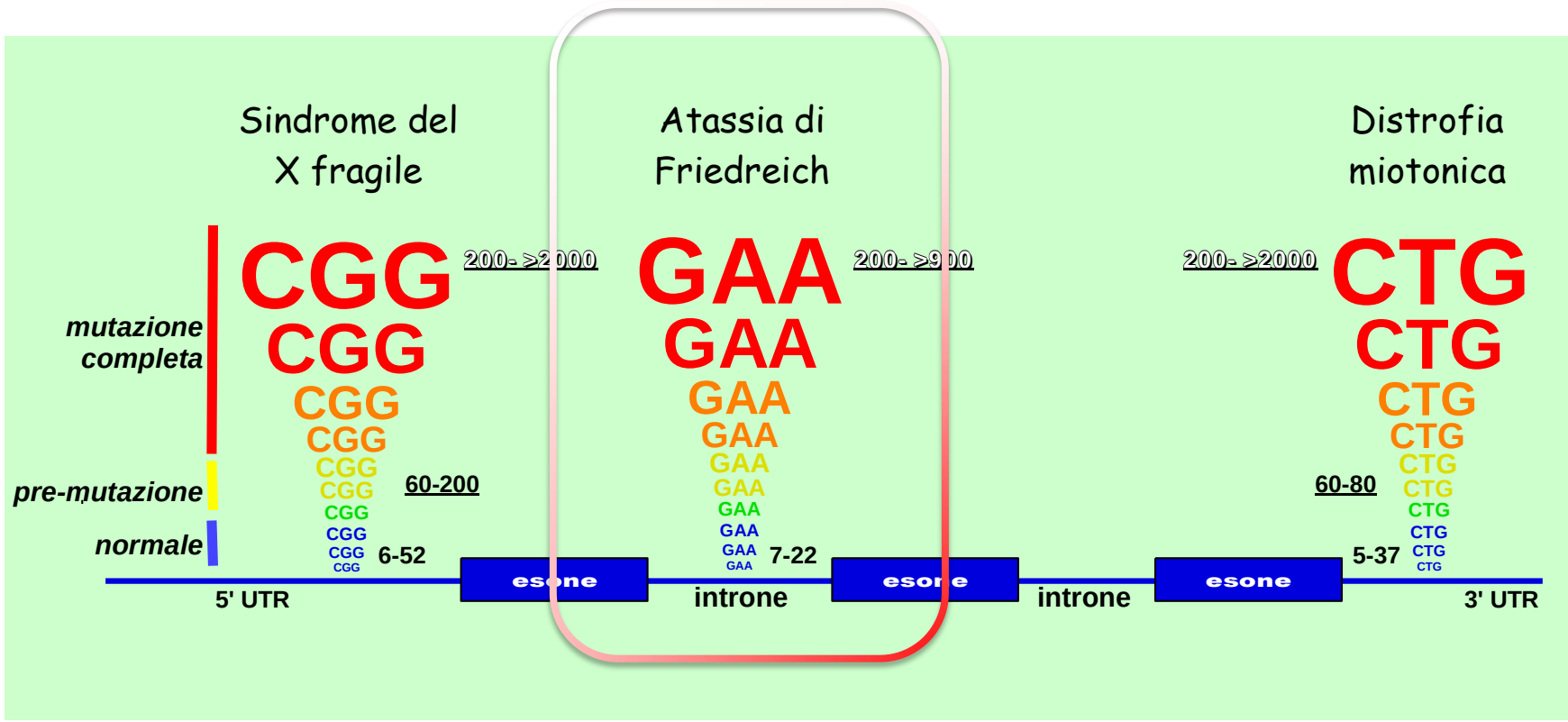
ANTICIPAZIONE → correlazione tra lunghezza della sequenza ripetuta- eta' di esordio e gravità della malattia
Alcune forme (es. SCA7) molto più instabili di altre → anticipazione più evidente (il figlio può diventare sintomatico prima del genitore, con evoluzione rapida e morte precoce)

BIAS PARENTALE → in quasi tutte le SCA, l'espansione si verifica nelle meiosi paterne, mentre in caso di trasmissione materna spesso non vi sono cambiamenti o addirittura contrazione (unica eccezione: SCA8)

ALLELI INTERMEDI → si riscontrano in numerose SCA. Quadro clinico eterogeneo.

PROBLEMI DEL TEST PRESINTOMATICO
→ non esistono terapie nè trattamenti preventivi che permettano di modificare l'insorgenza o la storia naturale della patologia
→ variabilità fenotipica → l'identificazione dell'espansione in un soggetto asintomatico non permette di predire età d'esordio e gravità della malattia

MALATTIE DA TRIPLETTE RIPETUTE
IN REGIONI NON CODIFICANTI



Friedreich ataxia (FRDA) is the most common of the autosomal recessive ataxias. It generally begins in childhood with slowly progressive ataxia associated with depressed tendon reflexes and posterior column sensory loss.

- About 25% of affected individuals have an "atypical" presentation with later onset (age >25 years), retained tendon reflexes, or unusually slow progression of disease.
- The vast majority of individuals have a GAA triplet-repeat expansion in *FXN;* however, FRDA is not associated with anticipation [Durr et al 1996]. Rare individuals have a missense mutation in one *FXN* allele with the GAA repeat in the other allele.

Suggestive Findings

Diagnosis of Friedrich ataxia (FRDA) should be suspected in individuals with a combination of the following findings:

- Progressive ataxia of gait and limbs
- Dysarthria; decrease in/loss of position sense and/or vibration sense in lower limbs; muscle weakness
- Absent muscle stretch reflexes in the legs (in atypical cases, reflexes may be preserved; see FRDA with retained reflexes)
- Onset before age 25 years (in atypical cases, onset may be delayed; see late-onset FRDA and very late-onset FRDA)
- Family history consistent with autosomal recessive inheritance

Especially if these additional findings are present:

- Pyramidal weakness of the legs, extensor plantar responses
- Scoliosis, pes cavus, hypertrophic non-obstructive cardiomyopathy
- Glucose intolerance, diabetes mellitus, optic atrophy, or deafness

Allele sizes. Four classes of alleles are recognized for the GAA repeat sequence in intron 1 of *FXN* [Cossee et al 1997, Montermini et al 1997a, Sharma et al 2004]:

- **Normal alleles.** 5-33 GAA repeats. More than 80%-85% of alleles contain fewer than 12 repeats (short normal; SN) and approximately 15% have 12-33 repeats (long normal; LN). Normal alleles with more than 27 GAA repeats are rare.
- **Mutable normal (premutation) alleles.** 34-65 GAA repeats. Although the exact frequency of these alleles has not been formally determined, they likely account for fewer than 1% of *FXN* alleles.
- **Full-penetrance (disease-causing expanded) alleles.** 66 to approximately 1700 GAA repeats. The majority of expanded alleles contain between 600 and 1200 GAA repeats [Campuzano et al 1996, Durr et al 1996, Filla et al 1996, Epplen et al 1997].
- **Borderline alleles.** 44-66 GAA repeats. The shortest repeat length associated with disease (i.e., the exact demarcation between normal and full-penetrance alleles) has not been clearly determined (see Penetrance).
- **Rare alleles of variant structure.** In contrast to the alleles discussed above in which the GAA trinucleotides are perfect repeats, in rare pathogenic alleles the GAA repeats are not in perfect tandem order but rather are interrupted by other nucleotides. Such "interrupted *FXN* alleles" differ in length and types of nucleotides in the interruption, but they are typically close to the 3' end of the GAA repeat tract (see Molecular Genetics).
  Note: (1) Molecular genetic testing does not determine presence or absence of nucleotide interruptions of the GAA tract. (2) These rare interrupted alleles may be associated with LOFA or VLOFA [Stolle et al 2008] (see Genotype-Phenotype Correlations).

CASO CLINICO
Richiesta di consulenza genetica DH neurologia

Paziente inviata alla ns attenzione per **atassia spastica**, richiesta di valutazione genetica per SCA

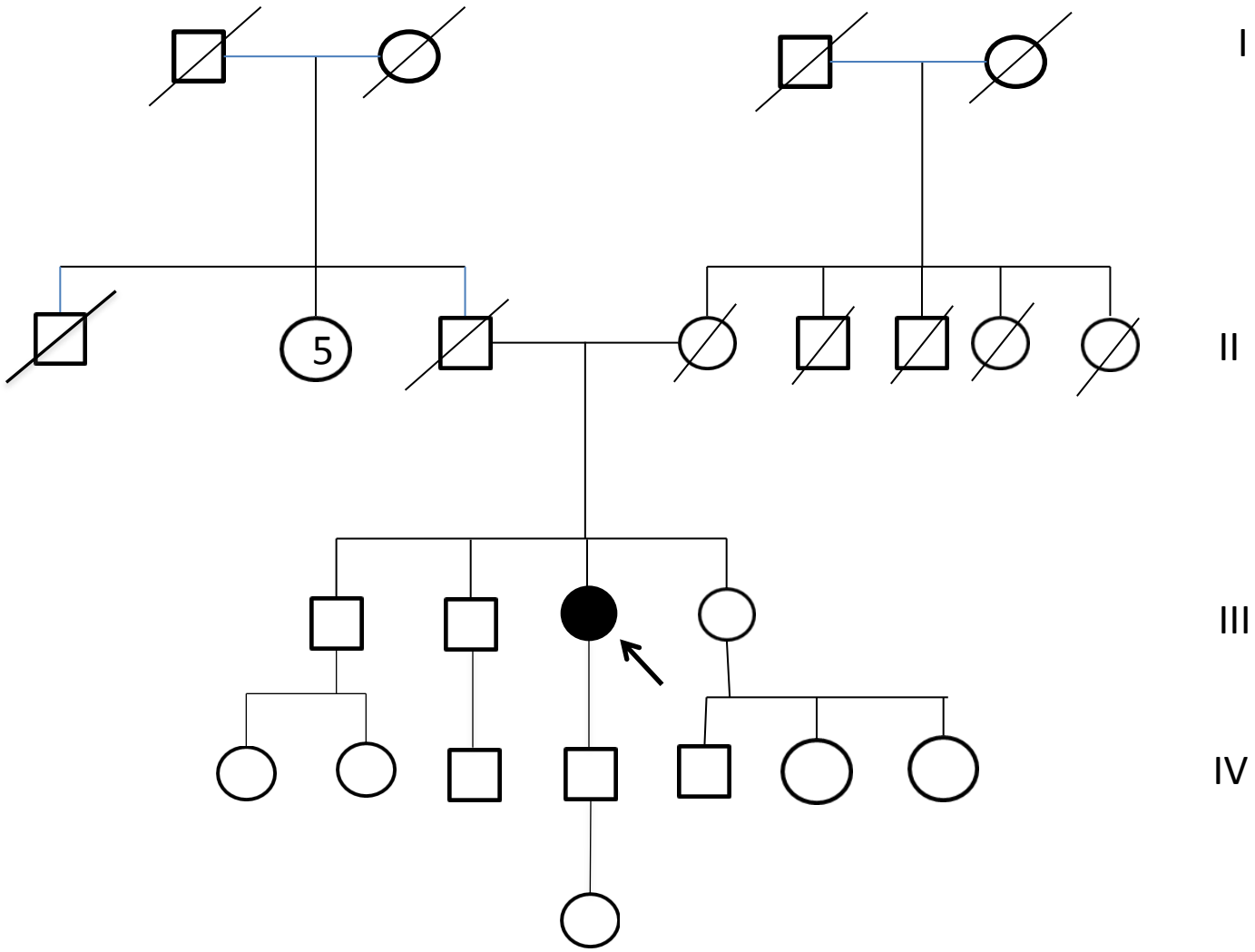
Spastic Ataxias

The combination of spasticity with signs of cerebellar ataxia is relatively common and one or the other finding may predominate [de Bot et al 2012]. For example, ataxia and cerebellar atrophy both frequently occur in spastic paraplegia 7 (a form of hereditary spastic paraplegia), caused by pathogenic variants in *SPG7* coding the protein paraplegin [van Gassen et al 2012].

Five disorders have specifically been designated spastic ataxia (SPAX) (Table 5). SPAX1 is inherited in an autosomal dominant manner and the other four are inherited in an autosomal recessive manner.

Disease	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	Neuroimaging	Additional signs (typical in bold)
SPAX5									Cerebellar atrophy	Sensorimotor pnp, ptosis, oculomotor apraxia, dystonia, **progressive myoclonus epilepsy**
SPAX4									Unknown	**Optic atrophy,** PMR, some emotional lability
FAHN									Glob. pallidus iron deposition, pontocerebellar atrophy,WMC, TCC	**Dystonia**, sometimes optic atrophy, seizures
SPAX1									Normal	Sometimes dystonia, ptosis, **eye lid retraction** and pes cavus
CTX									(Ponto)cerebellar atrophy,TCC, focal lesions, WMC	**Cataract, tendon xanthomas, neuropsychiatric symptoms**
ARSACS									Cerebellar atrophy, transverse lines in the pons+ middle cerebellar peduncles	**Sensorimotor pnp**, retinopathy, sometimes mitral valve prolapse
SPAX2									Unknown	**Pronounced dysarthria,** fasciculations
SPAX3									pWMC, cerebellar atrophy, TCC in some	Sometimes dystonia, optic atrophy, cataract, decreased hearing, MCI, scoliosis
SCA3									Pontocerebellar atrophy	Dystonic / hypokinetic syndrome, pnp, **eye lid retraction**, ophtalmoparesis, **(peri)oral fasciculations**
Alexander adult-onset									Medulla oblongata abnormalities +/- pWMC, cerebellar atrophy	**Lower brainstem signs, palatal myoclonus**, sometimes autonomic dysfunctions, sleep disorders, ocular abnormalities
SPG7									Normal or cerebellar atrophy	Sometimes sensorimotor pnp, optic atrophy, ptosis, strabismus, supranuclear palsy, decreased hearing
LOFA									Normal or mild cerebellar atrophy, cervical spinal cord atrophy	**Axonal sensory pnp**, **square wave jerks,** diabetes, cardiomyopathy, optic atrophy and/or sensorineural deafness

Typical, most frequent Possible, less frequent


I
II
5
III
IV

Atassia spastica: approccio diagnostico

La paziente è affetta da

atassia ereditaria con spasticità

o da

paraparesi spastica con segni cerebellari?

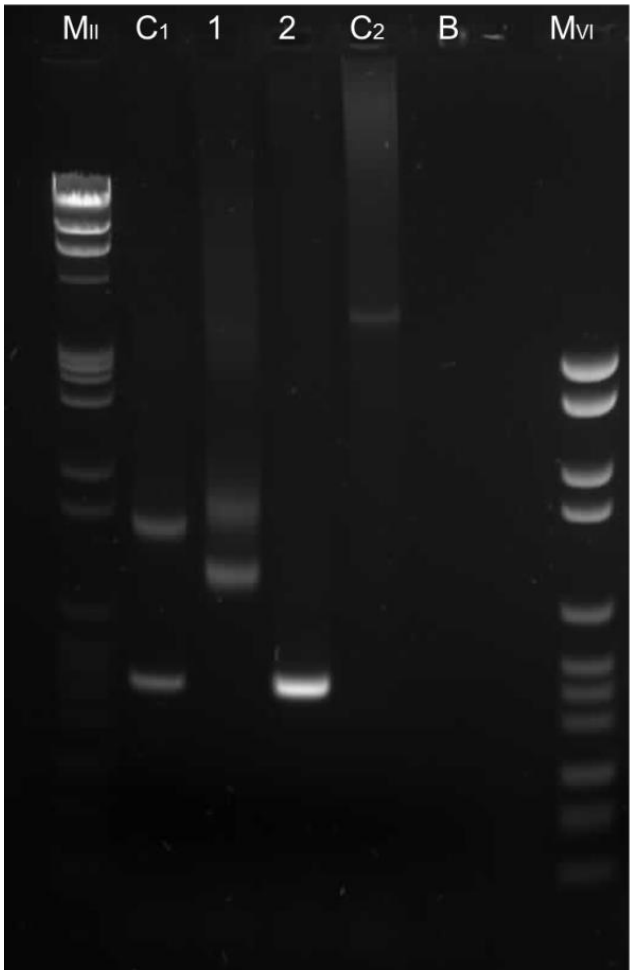
Analisi molecolare:

1. <u>Atassia di Friedreich</u>:
2. SCA 3
3. Alexander disease
4. SPG7

- Elementi clinici: scoliosi, diabete, preponderante atassia
- Familiarità negativa
- Frequenza e prevalenza
- Età di insorgenza

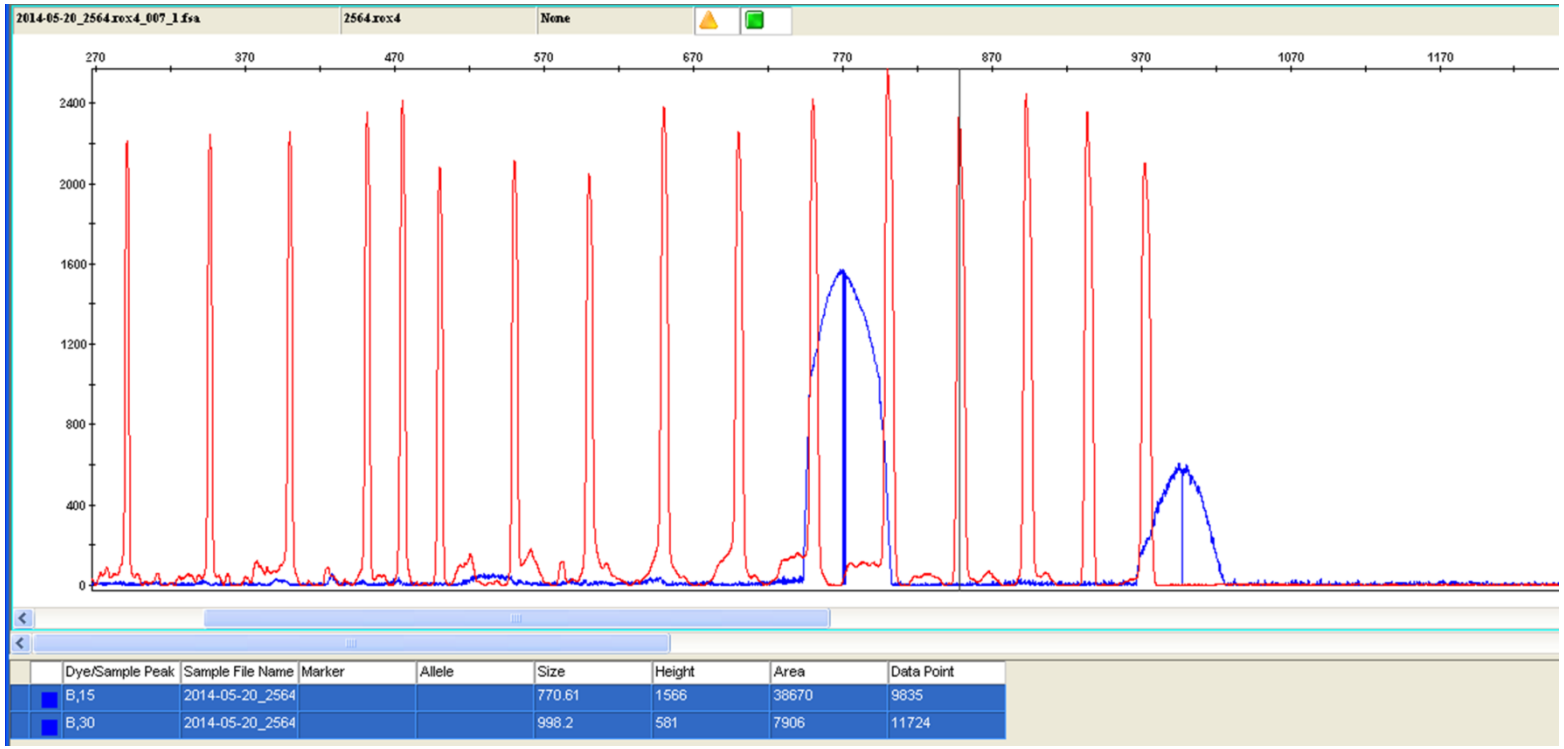
Analisi molecolare gene FXN:

amplificazione in PCR ed elettroforesi su gel



MII = marker II (Roche)
C1 = heterozygous control 1
1 = Patient PG (2564)
2 = Patient with normal *GAA* repeats
C2 = full expanded control 2
B = blank
MVI = marker VI (Roche)

Analisi molecolare gene FXN: analisi di frammenti su sequenziatore per la determinazione del numero di triplette



Calcolo per la determinazione del numero di triplette

(998-450)/3= 182 GAA

(770-450)/3= 106 GAA

Analisi molecolare gene FXN: risultati

Eterozigosi composta per l'espansione della tripletta GAA responsabile dell'**atassia di Friedreich**

Genotipo FNX: 110/180 ripetizioni.

Intervallo di normalità 5-33

Alleli con più di 66 espansioni ininterrotte sono da considerarsi patologici.

Alleli normali: 5-33 ripetizioni
Premutazione: 34-65 ripetizioni
Alleli patologici: 66-1700
Alleli border-line: 44-55 ripetizioni

Pazienti affetti da LOFA o VLOFA hanno almeno un allele con un'espansione significativamente inferiore rispetto alla forma classica

Late-Onset Friedreich Ataxia

Phenotypic Analysis, Magnetic Resonance Imaging Findings, and Review of the Literature

Roongroj Bhidayasiri, MD, MRCP(UK); Susan L. Perlman, MD; Stefan-M. Pulst, MD, DrMed; Daniel H. Geschwind, MD, PhD

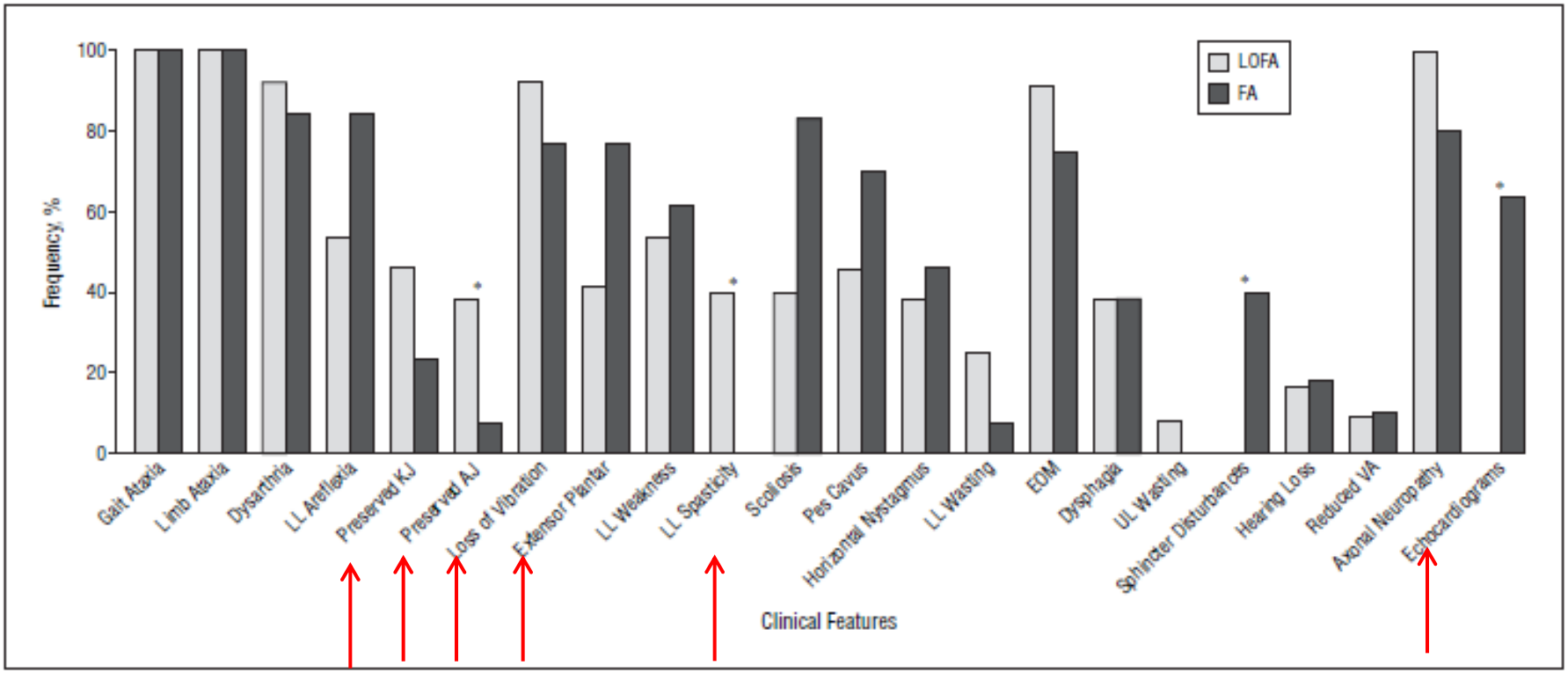
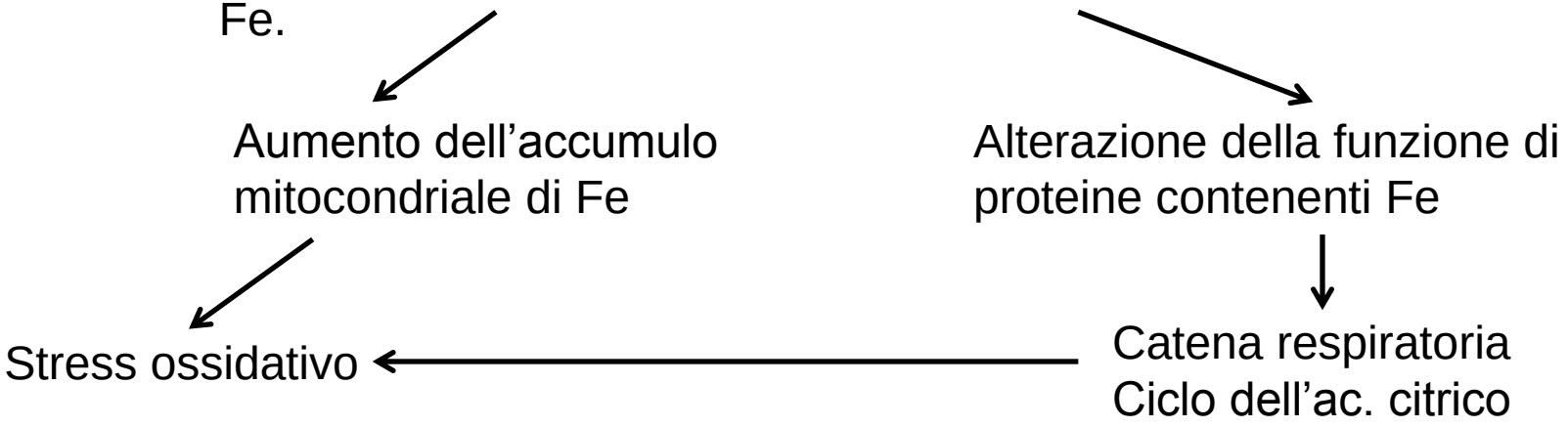


Figure 1. Frequency of various clinical features in patients with typical Friedreich ataxia (FA) vs those with late-onset FA (LOFA). Asterisk indicates $P<.05$; LL, lower limb; KJ, knee jerk; AJ, ankle jerk; EOM, extraocular movement; UL, upper limb; and VA, visual acuity.

Patogenesi molecolare dell'atassia di Friedreich:

- Patologia da mutazioni dinamiche
- Espansione del tri-nucleotide GAA all'interno dell'introne 1 del gene X25 → loss of function mutation → diminuzione dell'espressione genica
- Gene che codifica la proteina mitocondriale frataxina
- Frataxina: ruolo cruciale in funzioni mitocondriali coinvolgenti il Fe.



<u>Riduzione dell'espressione genica</u>:

•Formazione di eterocromatica (ipoacetilazione degli istoni+ipermetilazione della regione)

•Formazione di «sticky DNA»

Correlazione genotipo-fenotipo:

- Età di insorgenza, gravità e progressioni dipendenti dal numero di ripetizioni dell'allele meno espanso
  Late onset Friedreich ataxia (LOFA): 25-38 yrs
  Very late onset Friedreich ataxia (VLOFA): > 40 yrs
- Instabilità mitotica = mosaicismo somatico
- Casi limite ed eccezioni

OBSERVATION

Very Late-Onset Friedreich Ataxia Despite Large GAA Triplet Repeat Expansions

Sanjay I. Bidichandani, MBBS, PhD; Carlos A. Garcia, MD; Pragna I. Patel, PhD; Mazen M. Dimachkie, MD

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148

J Neurol (2013) 260:1408–1409
DOI 10.1007/s00415-013-6874-6

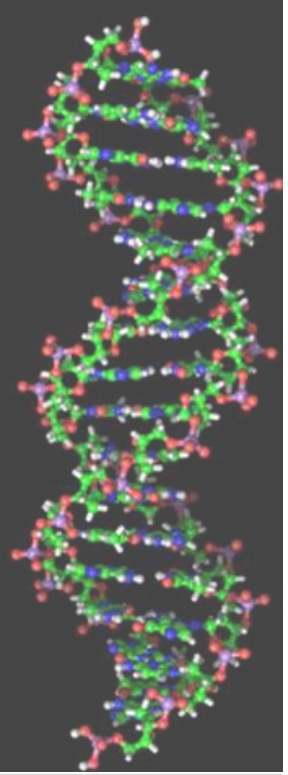
LETTER TO THE EDITORS

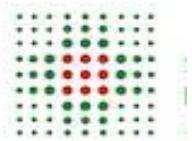
Very late-onset Friedreich ataxia: later than life expectancy?

Vincent Alvarez · Pierre Arnold · Thierry Kuntzer

In conclusione:

- La signora risulta affetta da una forma di atassia di Friedreich ad esordio tardivo (VLOFA) caratterizzata molecolarmente.
- In base alla modalità di trasmissione autosomico recessiva, la signora ha trasmesso alla prole con una probabilità del 100% uno dei due alleli mutati: i figlio risulta pertanto portatore eterozigote obbligato di una mutazione per espansione del gene FXN. Considerando la frequenza di 1/60 dei portatori sani di mutazione di tale gene nella popolazione generale, il figlio della consultanda ha un rischio a priori di prole affetta da Atassia di Friedreich pari a 1/2400.
- Vi è inoltre un rischio familiare di stato di portatore eterozigote di mutazione del gene FXN, anche per i parenti (fratelli, cugini di primo grado sia materni che paterni), in quanto la signora avrà ereditato le mutazioni da ciascuno dei genitori, portatore sano.
- Alla luce di quanto detto sopra, riteniamo appropriata consulenza genetica specifica per i familiari a rischio, per la verifica/conferma molecolare dello stato di portatore e la stima di specifici rischi di ricorrenza. Il nostro Servizio si rende disponibile, previo appuntamento telefonando al numero 0532 236491 (dal lunedì al venerdì dalle 10.30 alle 14.30).
- In considerazione infine della riferita ricorrenza familiare di lipomi multipli, in assenza di specifico inquadramento diagnostico dermatologico non risulta possibile essere conclusivi. Riteniamo appropriata valutazione specialistica e ci rendiamo disponibili a consulenza genetica specifica nei tempi e modi ritenuti più opportuni.



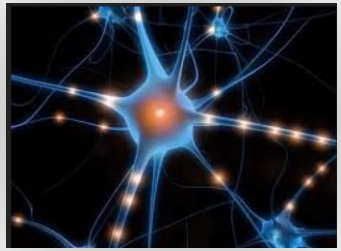


HSP (Hereditary Spastic Paraparesis)

Ad oggi sono stati identificati 72 LOCI malattia e sono stati clonati 55 GENI responsabili di paraparesi spastica ereditaria (HSP)

HSP può essere trasmessa con modalità autosomica dominante (AD), autosomica recessiva (AR), legata al cromosoma X (XL), o per via mitocondriale.

Vi possono essere casi sporadici dovuti a mutazioni de novo, mutazioni AD con penetranza ridotta, trasmissione AR in assenza di consanguineità e ricorrenza in altri fratelli/cugini



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Review Article

Molecular aspects of hereditary spastic paraplegia

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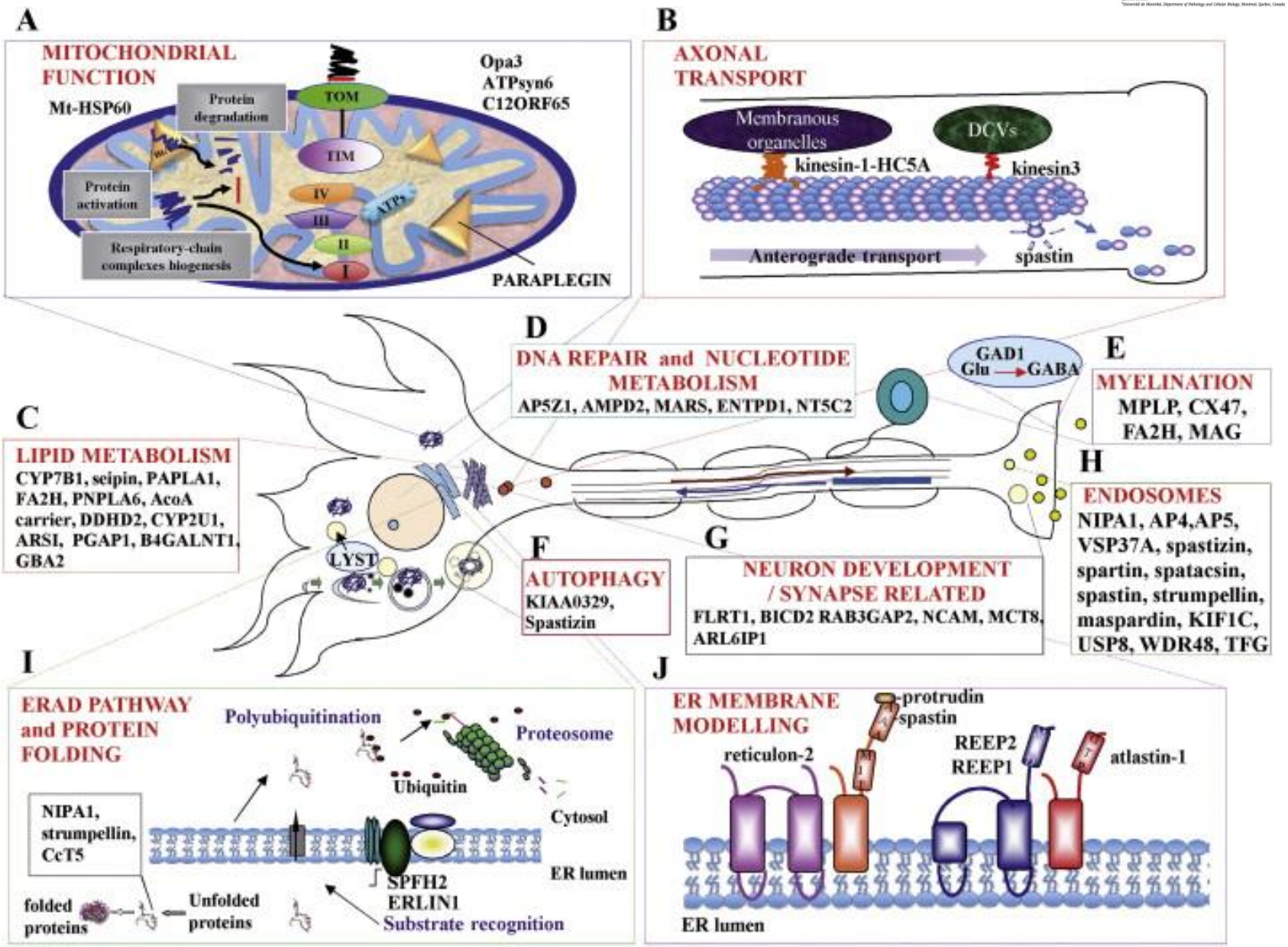
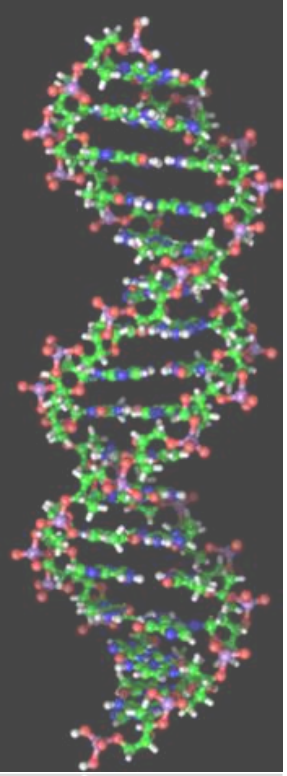
[b]Université de Montréal, Department of Pathology and Cellular Biology, Montreal, Quebec, Canada





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Review

Hereditary spastic paraplegia: Clinical-genetic characteristics and evolving molecular mechanisms

Temistocle Lo Giudice [a,b], Federica Lombardi [a], Filippo Maria Santorelli [c], Toshitaka Kawarai [d], Antonio Orlacchio [a,b,*]

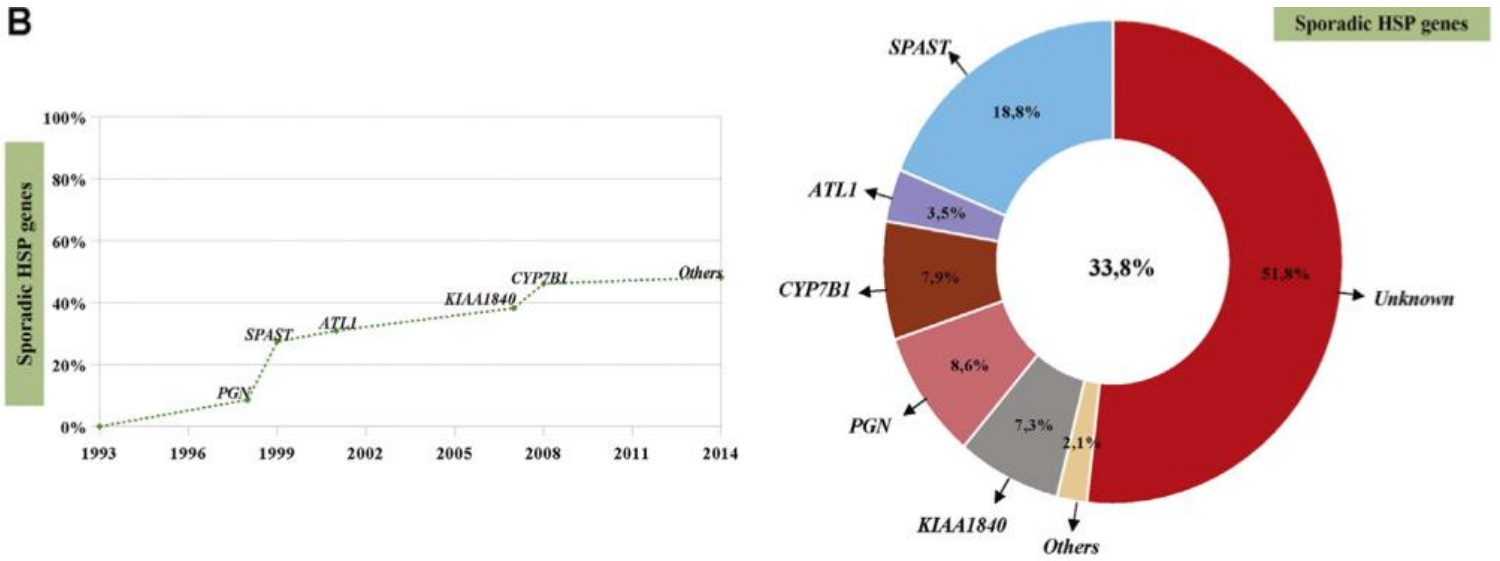
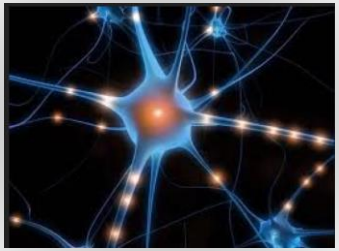


Fig. 1. Timeline of gene discoveries and frequencies of the most frequent causative genes in familial (A) and sporadic (B) HSP. Epidemiological data of the HSP Italian network (total of 1707 affected subjects from all over the world).



J. Finsterer et al. / Journal of the Neurological Sciences 318 (2012) 1–18

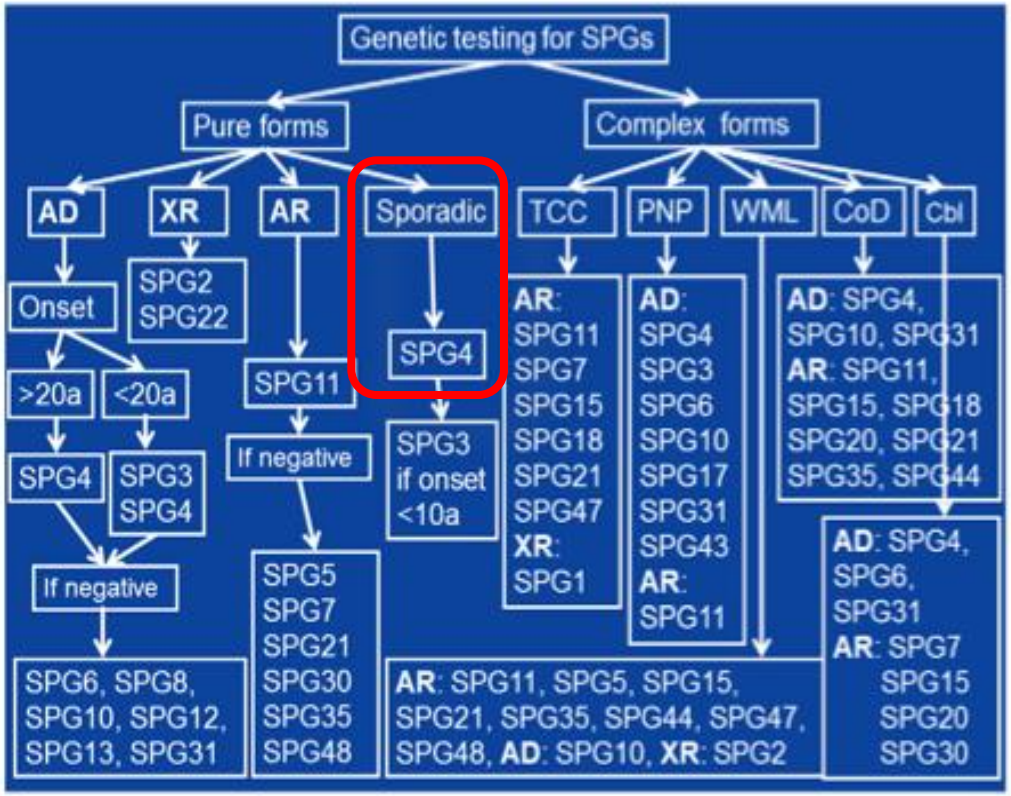
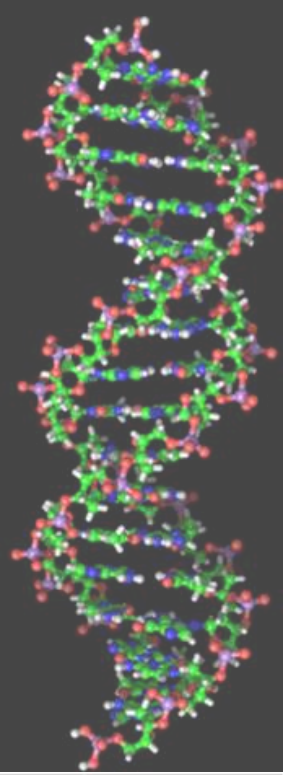


Fig. 1. Work-up for genetic testing. The procedures required depend on the clinical presentation and the trait of inheritance. TCC: thin corpus callosun, PNP: polyneuropathy, WML: white matter lesions, CoD: cognitive decline, Cbl: cerebellar abnormalities. Testing for AR HSP11 is indicated in the presence of associated neuropathy (ALS-like presentation).



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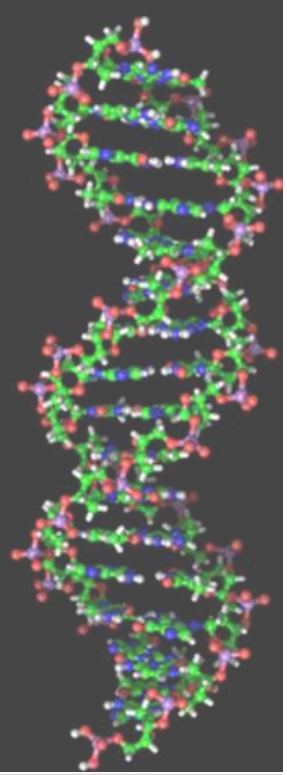
Review

Hereditary spastic paraplegia: Clinical-genetic characteristics and evolving molecular mechanisms

Temistocle Lo Giudice [a,b], Federica Lombardi [a], Filippo Maria Santorelli [c], Toshitaka Kawarai [d], Antonio Orlacchio [a,b,*]

Table 2
Inheritance and clinical features of HSP patients.

Type	MI	NOF	Onset	Phenotype	Additional features
SPG1	X-linked	~20	EO	C	Mental retardation, aphasia, shuffling gait, and adducted thumbs, hydrocephalus, ACC
SPG2	X-linked	~10	VO	P or C	Seizures, mental retardation, nystagmus, ataxia, WMLs, PNP
SPG3A	AD	~35	EO	P or C	Lower limb muscle atrophy, seizures, ataxia, optic atrophy, spasticity in the upper limbs, sensorimotor axonal neuropathy, cognitive and cranial nerve impairment, intellectual disability, pes cavus, TCC
SPG4	AD	~130	VO	P or C	Cognitive decline, epilepsy, ataxia, psychosis, upper limb spasticity, pes cavus, posterior fossa abnormalities, PNP, hand tremor, WMLs, amyotrophy of small hand muscles
SPG5A	AR	~35	VO	P or C	Optic atrophy, WMLs, cerebellar ataxia
SPG6	AD	11	TO	P or C	Idiopathic generalized epilepsy (IGE), dysarthria, PNP, facial dystonia, atrophy of the small hand muscles and upper limbs spasticity, pes cavus
SPG7	AR	~30	VO	P or C	Cerebellar signs, cerebellar atrophy, PNP, optic atrophy, supranuclear palsy, cognitive impairment of attention and executive functions, TCC, scoliosis, pes cavus
SPG8	AD	10	AO	P	-
SPG9	AD	1	TO	C	Cataracts, motor neuropathy, skeletal abnormalities, gastrooesophageal reflux
SPG10	AD	17	EO	P or C	Distal amyotrophy in the upper extremities, cognitive decline, PNP, dysautonomia, parkinsonism, deafness, retinitis pigmentosa
SPG11	AR	~35	VO	C	Cerebellar signs, PNP, WMLs, cerebellar atrophy, TCC, seizures, cognitive decline, abnormal eye signs, amyotrophy, parkinsonism, maculopathy, action tremor, mental retardation, upper limbs weakness
SPG12	AD	4	EO	P	-
SPG13	AD	2	VO	P or C	Dystonia
SPG14	AR	1	AO	C	Motor PNP, mental retardation
SPG15	AR	20	EO	C	Pigmentary retinopathy, cerebellar signs, PNP, amyotrophy, seizures, mental retardation, TCC
SPG16	X-linked	2	EO	P or C	Aphasia, ipovision, nystagmus, mental retardation
SPG17	AD	13	TO	C	Amyotrophy of small hand and feet muscles, lower motor neuron disease
SPG18	AR	3	EO	C	Epilepsy, mental retardation, congenital hip dislocation, multiple joint contractures
SPG19	AD	1	AO	P	-
SPG20	AR	~25	EO	C	Mental retardation, dysarthria, upper limbs spasticity, cerebellar signs, euphoria, crying, WMLs
SPG21	AR	2	EO	C	Dementia, TCC, WMLs, cerebellar signs, extrapyramidal features, callosal disconnection syndrome
SPG22	X-linked	~10	EO	C	Mental retardation, muscle atrophy, distal wasting, dyskinesia, nystagmus, ataxia
SPG23	AR	4	EO	C	Cognitive impairment, pigmentary abnormalities, facial and skeletal dysmorphism, tremor
SPG24	AR	1	EO	C	Pseudobulbar signs
SPG25	AR	1	AO	C	Cataracts, neuropathy, disc emiation
SPG26	AR	5	EO	C	Intellectual disability, cortical atrophy, peripheral neuropathy, distal atrophy. cerebellar ataxia, WMLs
SPG27	AR	2	VO	C	Dysarthria, mental retardation, polineuropathy
SPG28	AR	3	EO	P or C	Saccadic eye pursuit, axonal neuropathy
SPG29	AD	1	TO	C	Pes cavus, hearing loss, hiatal hernia, hyperbilirubinaemia
SPG30	AR	3	TO	P or C	Sensory PNP, cerebellar signs, hypoacusis, distal muscle wasting
SPG31	AD	~30	EO	P or C	PNP, cerebellar ataxia, tremor, dementia, amyotrophy of small hand muscles, pes cavus
SPG32	AR	1	EO	C	Mental retardation, pontine dysraphism, TCC
SPG33	AD	1	AO	C	Pes equines
SPG34	X-linked	1	VO	P	–
SPG35	AR	2	EO	P or C	Cognitive decline, epilepsy
SPG36	AD	1	VO	C	Sensory polyneuropathy
SPG37	AD	1	VO	P	-
SPG38	AD	1	VO	C	Amyotrophy of small hand muscles, PNP
SPG39	AR	2	EO	C	Distal wasting in all four limbs, atrophy of the spinal cord, axonal neuropathy
SPG40	AD	1	AO	P or C	Hyperreflexia of upper limbs, cognitive impairment
SPG41	AD	1	TO	P	-
SPG42	AD	1	VO	P	-
SPG43	AR	1	VO	C	Amyotrophy of small hand muscles, bilateral optic atrophy, axonal sensory and motor neuropathy, brain iron deposits in the globus pallidus
SPG44	AR	1	AO	C	Mild cognitive impairment, moderate cerebellar dysfunction, dysarthria, WMLs, pes cavus, TCC, scoliosis, upper limb involvement
SPG45	AR	1	EO	C	Mental retardation, pendular nystagmus, optic atrophy
SPG46	AR	4	EO	C	Mental impairment, cataract, cerebellar atrophy, TCC, hypogonadism in males
SPG47	AR	2	EO	C	Periventricular WMLs, TCC, microcephaly, epilepsy, waddling gait, joint hyperlaxity
SPG48	AR	1	AO	P or C	Spinal cord hyperintensities
SPG49	AR	3	EO	C	Delayed psychomotor development, mental retardation, TCC, cerebral and cerebellar dysfunction, dysmorphic features, central apnea
SPG50	AR	1	EO	C	Tetraplegic cerebral palsy, mental retardation, reduction of cerebral white matter, atrophy of the cerebellum
SPG51	AR	2	EO	C	Microcephaly, growth and intellectual retardation
SPG52	AR	1	EO	C	Delayed speech, stereotypic laughter, growth retardation
SPG53	AR	3	EO	C	Spasticity in upper extremities, delays in cognition and speech, kyphosis, pectus carinatum, hypertrichosis
SPG54	AR	6	EO	C	Mental retardation, strabismus, dysarthria, dysphagia, optic-nerve hypoplasia, short stature, TCC, laterally deviated feet, abnormal lipid peak on brain spettroscopy, WMLs
SPG55	AR	1	EO	C	Optic atrophy, neuropathy, pes equinovarus
SPG56	AR	5	EO	P or C	TCC, cognitive decline, upper limbs involvement, basal-ganglia calcification. dystonic postures, WMLs
SPG57	AR	1	EO	C	Optic atrophy, PNP
SPG58	AR	3	EO	P or C	Chorea, myoclonus, ataxia, hypodontia, deafness, short stature, pes planus, ptosis, developmental delay, mental retardation, WMLs
SPG59	AR	1	EO	C	Nystagmus, borderline intelligence
SPG60	AR	1	EO	C	Neuropathy in lower limbs, nystagmus
SPG61	AR	1	EO	C	Loss of terminal digits, acromutilation, PNP
SPG62	AR	3	EO	P	-
SPG63	AR	1	EO	C	TCC, WMLs, underweight, short stature
SPG64	AR	2	EO	C	Pes equinovarus, aggressiveness, delayed puberty, microcephaly, borderline intelligence



SPG65	AR	5	EO	P or C	TCC, defective myelination, small bilateral cystic occipital leukomalacia, learning disability, pes equinovarus
SPG66	AR	1	EO	C	Corpus callosum and cerebellar hypoplasia, colpocephaly, borderline intelligence, PNP, pes equinovarus
SPG67	AR	1	EO	C	Distended abdomen, borderline intelligence, ACC, vermis hypoplasia, defective myelination
SPG68	AR	1	EO	C	Nystagmus, optic atrophy, PNP, amyotrophy, foot drop
SPG69	AR	1	EO	C	Intellectual disability, deafness, cataract
SPG70	AR	1	EO	C	Scoliosis, bilateral Achilles contracture, borderline intelligence, nephrotic syndrome
SPG71	AR	1	EO	C	TCC
GAD1 gene	AR	1	EO	C	Spastic cerebral palsy, mental retardation
SPOAN	AR	2	EO	C	Optic atrophy, PNP, dysarthria, exacerbated acoustic startle response, joint retractions, spine deformities, fixation nystagmus, distal amyotrophy, extrapiramidal signs
Cct5 gene	AR	1	EO	C	Mutilating sensory neuropathy
ATPase6 gene	Maternal	1	AO	C	Diabetes mellitus, hypertrophic cardiomyopathy, supraventricular arrhythmia, cerebellar syndrome
OPA3 gene	AR	1	EO	C	Optic atrophy, chorea, cerebellar ataxia, dementia
BICD2 gene	AR	1	EO	P	-
MAG gene	AR	1	EO	C	Nystagmus, poor school achievement
REEP2 gene	AR	3	EO	P	-
LYST gene	AR	1	AO	C	PNP, cerebellar ataxia

ACC = agenesis corpus callosum; AO = adult onset; AD = autosomal dominant; AR = autosomal recessive; C = complicated; EO = early onset (infancy); IGE = idiopathic generalized epilepsy; MI = mode of inheritance; NOF = number of families; P = pure; PNP = polyneuropathy; TCC = thin corpus callosum; TO = teenage onset; VO = variable onset (infancy, teenage, or adult); WMLs = white matter lesions.

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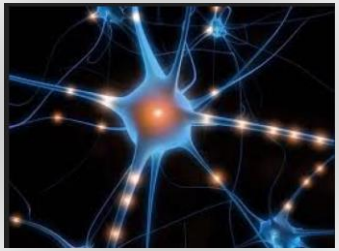
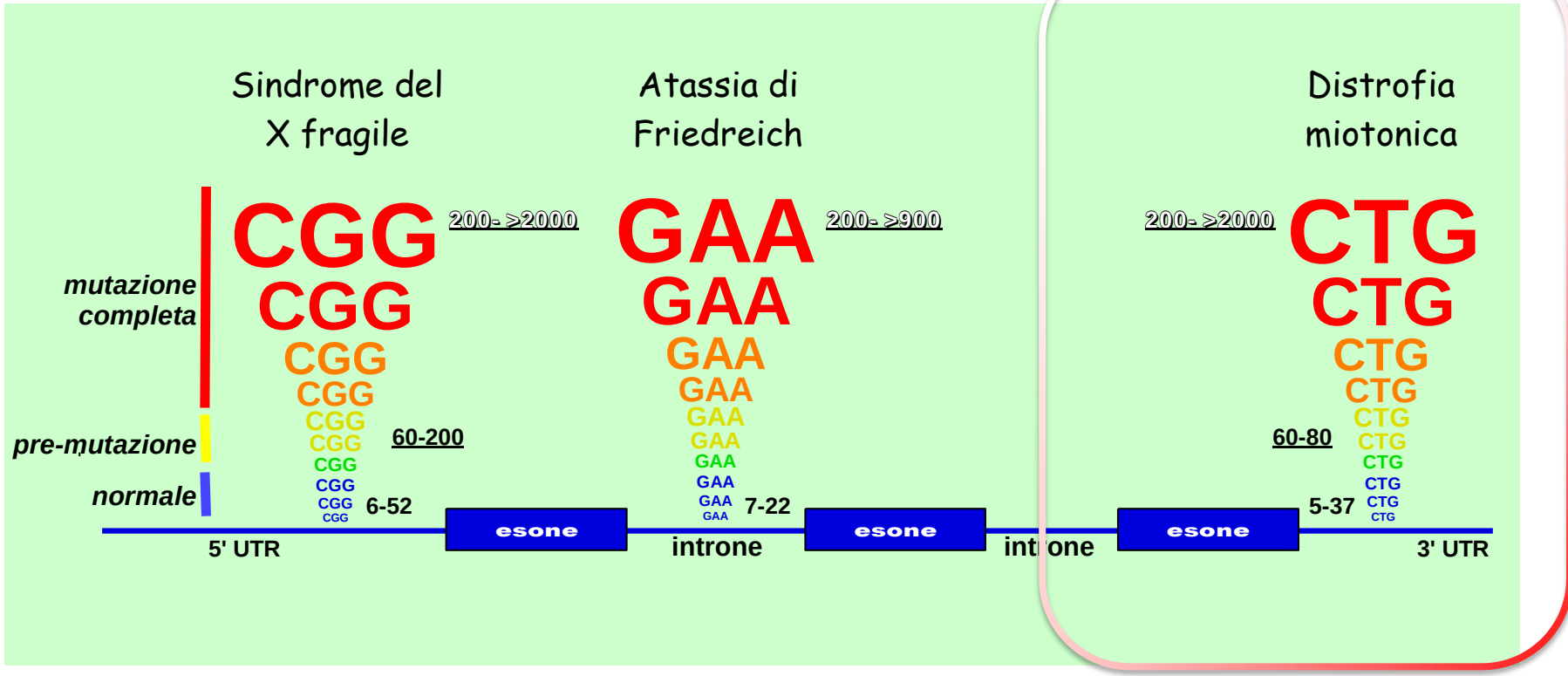
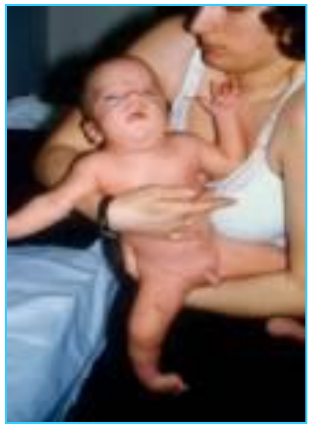


MALATTIE DA TRIPLETTE RIPETUTE IN REGIONI NON CODIFICANTI



DISTROFIA MIOTONICA – LA CLINICA

malattia multisistemica → interessamento di vari organi



QUADRO CLINICO

froma lieve: lieve ipostenia prossimale, cataratta, (diabete mellito) → normale durata e qualità di vita

forma classica: esordio 2° -3° decade con miotonia e debolezza muscolare, cataratta, anomalie di conduzione cardiaca, (diabete mellito, ipogonadismo, lieve ritardo mentale, ipotiroidismo)

forma congenita: grave ipotonia alla nascita, difficoltà respiratorie, debolezza generalizzata, ritardo mentale → ++ mortalità precoce

autosomica dominante - penetranza completa

frequenza: 1/8000 nati vivi

follow-up utile per alcuni aspetti clinici (es. cardiopatia, diabete)

DISTROFIA MIOTONICA

GENETICA MOLECOLARE
Due loci genici:

- DM1 (98% famiglie) cr. 19q13.3 (DMPK → esp. CTG)
- DM2 (PROMM) raro cr. 3q21 (ZNF9 → esp. CCTG)

Instabilità nella trasmissione meiotica → anticipazione

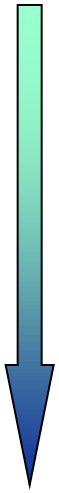
range normale → 5-35 CTG

premutazione → 36-49 CTG

DM lieve → 50-150 CTG

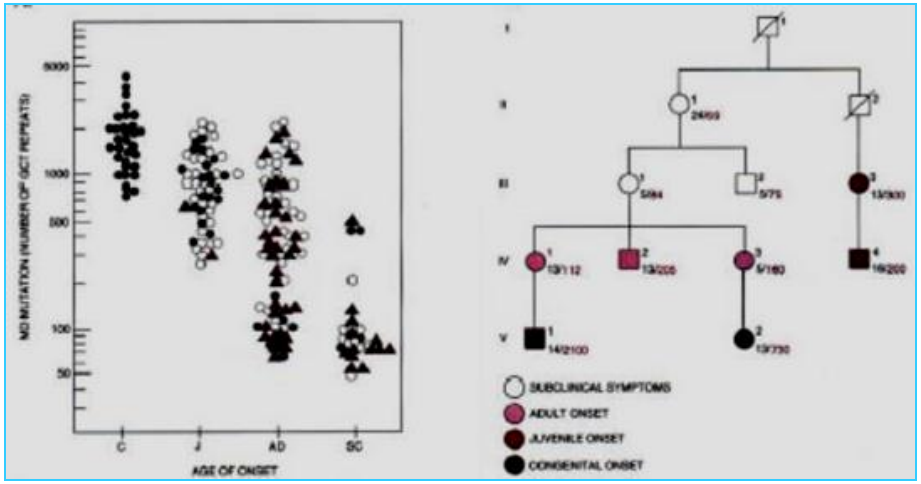
DM classica → 100-1500 CTG

DM congenita → 1000 – 4000 CTG



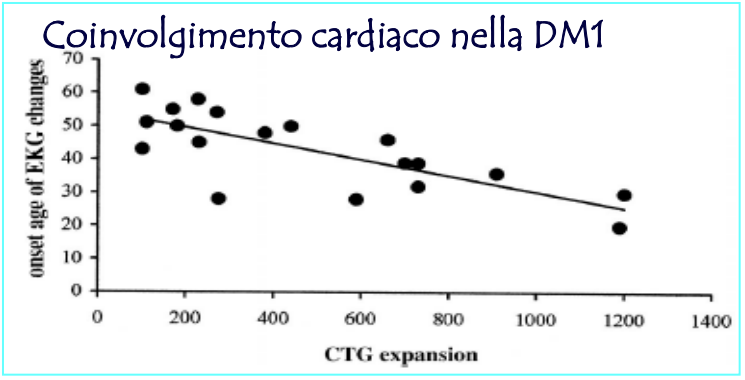
DISTROFIA MIOTONICA: PROBLEMATICHE

correlazione diretta tra lunghezza dell'espansione CTG nei linfociti ed età di esordio / gravità del fenotipo clinico



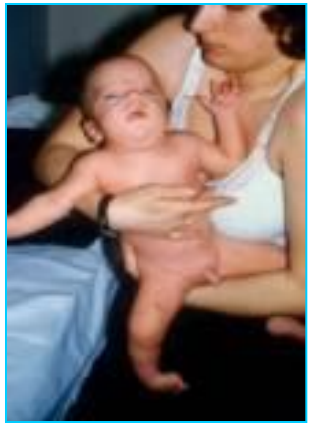
Instabilità nella trasmissione mitotica → mosaicismo somatico nei diversi tessuti (espressione variabile)

Coinvolgimento cardiaco nella DM1



DISTROFIA MIOTONICA CONGENITA

forma molto grave di DM con esordio alla nascita → ipotonia e grave debolezza generalizzata, spesso con insufficienza respiratoria e morte precoce. Ritardo mentale spesso presente.



trasmissione materna della forma congenita
gli oociti di una madre DM1 rimangono vitali anche se possiedono espansioni CTG molto estese, mentre gli spermatozoi di un padre DM1 con elevate espansioni CTG non sopravvivono oppure sono sterili

La possibilità di avere figli affetti dalla forma congenita CDM aumenta con la lunghezza dell'espansione CTG nelle madri DM1 (generalmente > 300-600)

Table 2 Risk of CDM with different cut offs of expansion size

Maternal expansion cut off (kb)	Chance of child having CDM if expansion >cut off (%)	Chance of a child having CDM if expansion ≤cut off (%)
0.5	49	16
1	59	17
2	71	24
3	75	29
4	100	31

PROBLEMATICHE DELLE MALATTIE NEURODEGENRATIVE AD ESORDIO TARDIVO

PENETRANZA

DISPONIBILITÀ DI TERAPIE O TRATTAMENTI PREVENTIVI

ESPRESSIONE VARIABILE

DIAGNOSI DIFFERENZIALE CON FORME NON-GENETICHE

ETEROGENEITÀ GENETICA

TEST PRESINTOMATICO

ASPETTI PSICOLOGICI

ASPETTI FAMILIARI

ASPETTI SOCIALI