Patologia da accumulo extracellulare: l'amiloidosi

Deposizione extracellulare di una sostanza di natura proteica detta amiloide

Risultato di una serie di cambiamenti nel folding delle proteine che porta alla deposizione di fibrille amiloidi insolubili principalmente negli spazi extracellulari di organi e tessuti

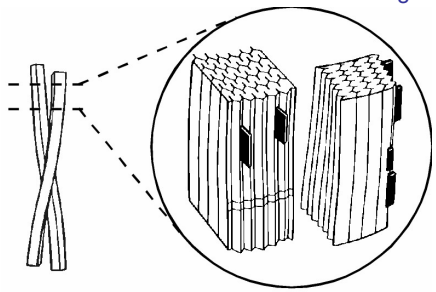
Tutte le fibrille amiloidi hanno una struttura secondaria identica, la conformazione a foglietti β ed un'ultrastruttura caratteristica

Definizione

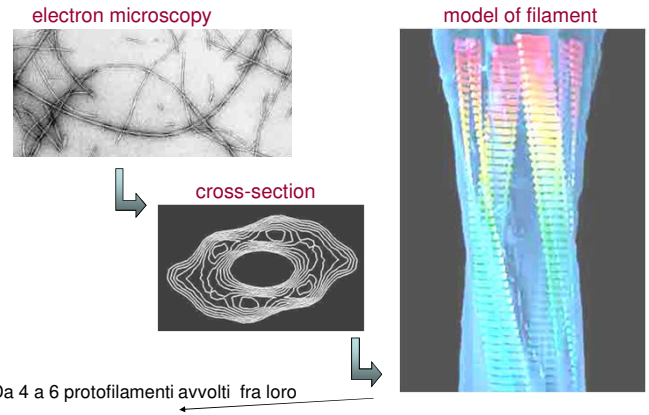
- AMILOIDE: famiglia di proteine insolubili con proprietà chimico-fisiche e strutturali analoghe ma origine molto diversa
- L'amiloide può essere prodotta digerendo qualsiasi proteina purché questa contenga, dopo la proteolisi, sequenze con configurazione a foglietti beta-ripiegati
- Orgina da proteine fibrillari, extracellulari, insolubili che derivano da precursori presenti nel sangue

- Colorabile con il Rosso Congo
- ME: filamenti di 75-100 A di spessore
- Diffrazione ai raggi X rivela che le catene polipeptidiche sono orientate trasversalmente
- La conformazione e' a foglietto beta

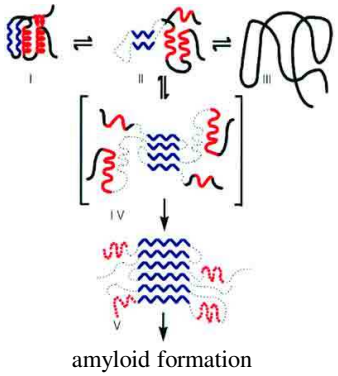
95% proteine
5% glicoproteine + componente P (proteoglicani e glicosaminoglicani solfatati)



Amyloid structure



Protein misfolding disease: amyloidosis



- at least 16 different proteins are implicated in amyloid diseases
- a number of different proteins can be induced to form fibrils *in vitro* as well

Perchè le proteine aggregano a formare fibrille amiloidi

- La proteina può avere una tendenza intrinseca ad assumere una conformazione patologica, che diviene più evidente con l'età (per accumulo, vedi transtiretina) o a concentrazioni elevate (vedi pazienti dializzati, beta2-microglobulina).
- La tendenza all'aggregazione può aumentare a causa di mutazioni (amiloidosi ereditaria)
- Questi fattori possono agire singolarmente o insieme

Nonostante le loro differenze biochimiche e cliniche, le varie amiloidosi hanno aspetti fisiopatologici comuni:

- Un precursore amiloidogenico in concentrazioni appropriate
- Un background genetico predisponente
- Anomalie nella proteolisi dei precursori fibrillari e delle fibrille amiloidi nascenti

Meccanismi di danno tissutale

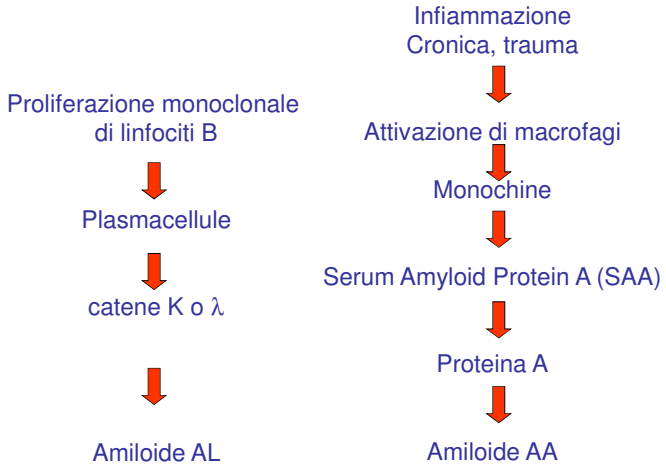
Il rapporto fra aggregazione proteica e danno tissutale è un argomento ancora molto dibattuto:

- La deposizione di grandi quantità di materiale fibrillare può sovvertire l'architettura tissutale e causare disfunzione dell'organo interessato
- L'amiloide può anche interagire a livello locale con recettori (es. RAGE*) alterando il signalling, per esempio attivando una forte risposta infiammatoria

*receptor for advanced glycation endproducts (lega proteine variamente alterate)

Table 1. Amyloid Proteins and Their Precursors.*

Amyloid Protein	Precursor	Distribution	Type	Syndrome or Involved Tissues
Aβ	Aβ protein precursor	Localized Localized	Acquired Hereditary	Sporadic Alzheimer's disease, aging Prototypical hereditary cerebral amyloid angiopathy, Dutch type
APrP	Prion protein	Localized	Acquired	Sporadic (iatrogenic) CJD, new variant CJD (alimentary?)
		Localized	Hereditary	Familial CJD, GSSD, FFI
ABri	ABri protein precursor	Localized or systemic?	Hereditary	British familial dementia
ACys	Cystatin C	Systemic	Hereditary	Icelandic hereditary cerebral amyloid angiopathy
Aβ2M	Beta$_2$-microglobulin	Systemic	Acquired	Chronic hemodialysis
AL	Immunoglobulin light chain	Systemic or localized	Acquired	Primary amyloidosis, myeloma-associated
AA	Serum amyloid A	Systemic	Acquired	Secondary amyloidosis, reactive to chronic infection or inflammation including hereditary periodic fever (FMF, TRAPS, HIDS, FCU, and MWS)
ATTR	Transthyretin	Systemic Systemic	Hereditary Acquired	Prototypical FAP Senile heart, vessels
AApoAI	Apolipoprotein A-I	Systemic	Hereditary	Liver, kidney, heart
AApoAII	Apolipoprotein A-II	Systemic	Hereditary	Kidney, heart
AGel	Gelsolin	Systemic	Hereditary	Finnish hereditary amyloidosis
ALys	Lysozyme	Systemic	Hereditary	Kidney, liver, spleen
AFib	Fibrinogen Aα chain	Systemic	Hereditary	Kidney



FEGATO ⇨ Risposta di fase acuta

↑ Concentrazione di proteine Plasmatiche sintetizzate nel fegato

Proteina C reattiva

Fibrinogeno

Aptoglobina

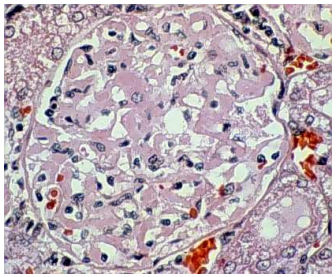
Complemento

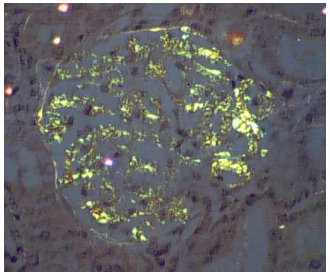
SAA amiloide sierica di tipo A

Caratteristiche istologiche dell'amiloide

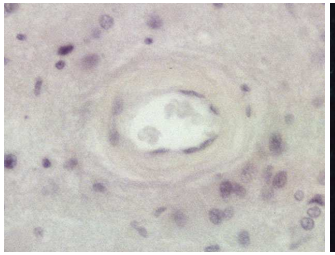
- Materiale amorfo ialino
- Scarsa proprietà tintoriali con i coloranti normali
- Tende a depositarsi a stampo negli spazi extracellulari

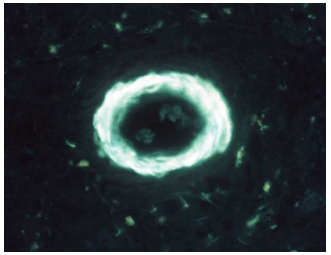
Amiloidosi renale

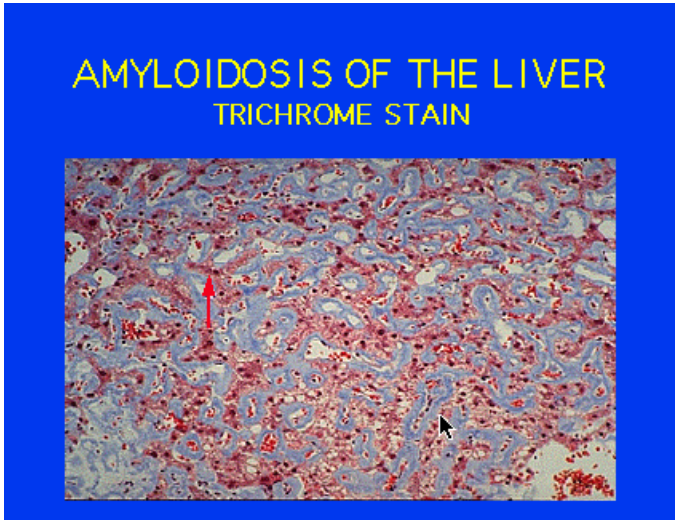




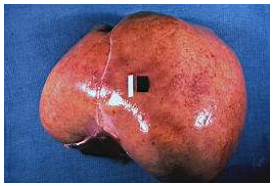
Angiopatia amiloide



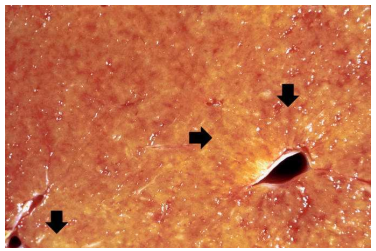




IPLAB.NET
The Interactive Pathology Laboratory

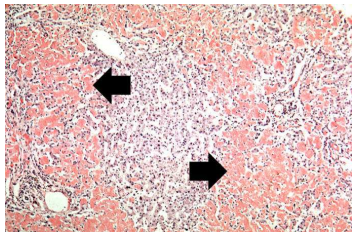


This is a gross picture of liver from this case. Note the pale, swollen appearance of this liver.

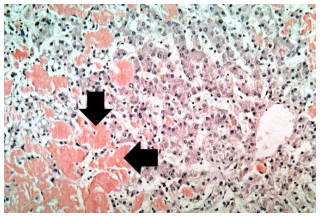


This is a closer view of the cut surface of this liver. The pale waxy material can be seen within the hepatic tissue (arrows).

IPLAB.NET
The Interactive Pathology Laboratory

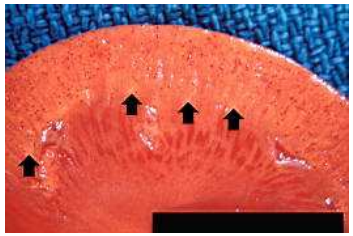


This is a low-power photomicrograph of liver tissue stained with Congo red (orange color in slide). Congo red reacts with amyloid and gives it an orange color (arrows).

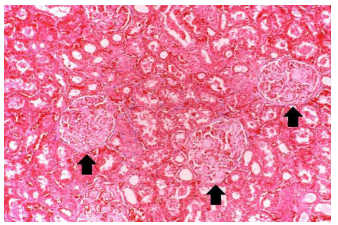


This is a high-power view of liver tissue stained with Congo red. The orange amyloid material (arrows) is seen clearly between liver parenchymal cells.

IPLAB.NET
The Interactive Pathology Laboratory



This is a gross photograph of kidney from this case. Note the pale yellow material within the cortex (arrows). This is indicative of amyloid within the cortex and the glomeruli. Also note that there are multiple red spots in the cortex. These represent congested glomeruli due to the vascular compromise produced by the amyloid.



This photomicrograph of kidney demonstrates the amyloid deposits (arrows) within glomeruli.

Proteostasi

e

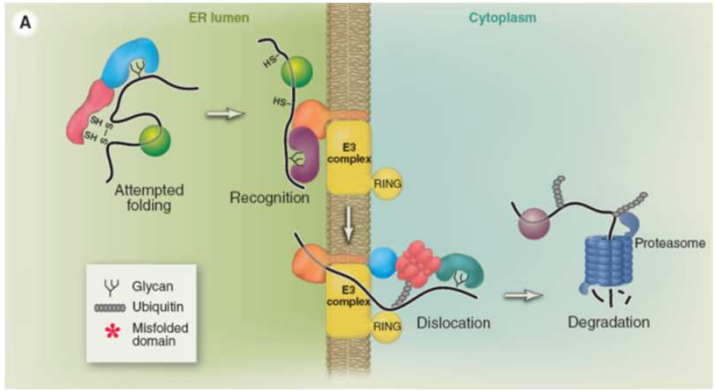
Proteotossicità

Road to Ruin: Targeting Proteins for Degradation in the Endoplasmic Reticulum

Melanie H. Smith,[1] Hidde L. Ploegh,[2] Jonathan S. Weissman[1]*

Some nascent proteins that fold within the endoplasmic reticulum (ER) never reach their native state. Misfolded proteins are removed from the folding machinery, dislocated from the ER into the cytosol, and degraded in a series of pathways collectively referred to as ER-associated degradation (ERAD). Distinct ERAD pathways centered on different E3 ubiquitin ligases survey the range of potential substrates. We now know many of the components of the ERAD machinery and pathways used to detect substrates and target them for degradation. Much less is known about the features used to identify terminally misfolded conformations and the broader role of these pathways in regulating protein half-lives.

1086 25 NOVEMBER 2011 VOL 334 SCIENCE



Occasional misfolded protein that are synthesized in the ER are exported to the cytosol by ERAD system and are degraded by the proteasome system.

In the event that many misfolded or unfolded proteins accumulate in ER they trigger a response to restore chaperone content and to reduce protein synthesis. The process is called **Unfolded Protein Response** and involves……

The Unfolded Protein Response: From Stress Pathway to Homeostatic Regulation

Peter Walter[1] and David Ron[2]

The vast majority of proteins that a cell secretes or displays on its surface first enter the endoplasmic reticulum (ER), where they fold and assemble. Only properly assembled proteins advance from the ER to the cell surface. To ascertain fidelity in protein folding, cells regulate the protein-folding capacity in the ER according to need. The ER responds to the burden of unfolded proteins in its lumen (ER stress) by activating intracellular signal transduction pathways, collectively termed the unfolded protein response (UPR). Together, at least three mechanistically distinct branches of the UPR regulate the expression of numerous genes that maintain homeostasis in the ER or induce apoptosis if ER stress remains unmitigated. Recent advances shed light on mechanistic complexities and on the role of the UPR in numerous diseases.

SCIENCE VOL 334 25 NOVEMBER 2011 1081

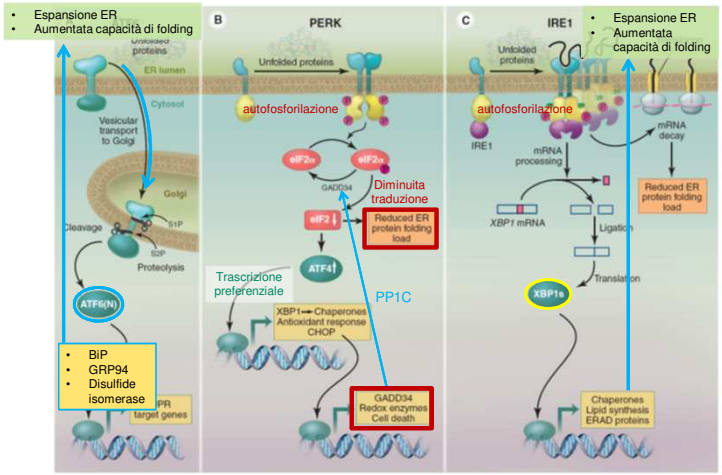


Fig. 2. (A to C) The three branches of the UPR. Three families of signal transducers (ATF6, PERK, and IRE1) sense the protein-folding conditions in the ER lumen and transmit that information, resulting in production of bZIP transcription regulators that enter the nucleus to drive transcription of UPR target genes. Each pathway uses a different mechanism of signal transduction: ATF6 by regulated proteolysis, PERK by translational control, and IRE1 by nonconventional mRNA splicing. In addition to the transcriptional responses that largely serve to increase the protein-folding capacity in the ER, both PERK and IRE1 reduce the ER folding load by down-tuning translation and degrading ER-bound mRNAs, respectively.

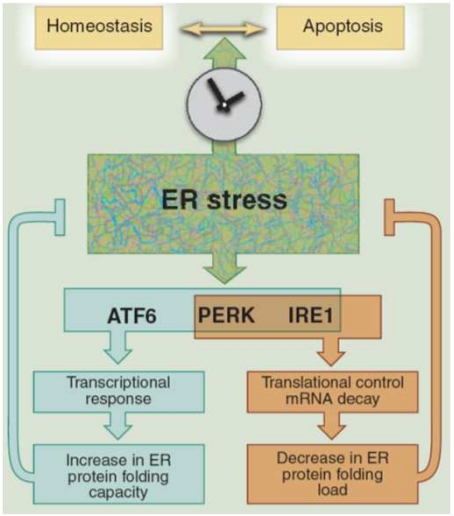
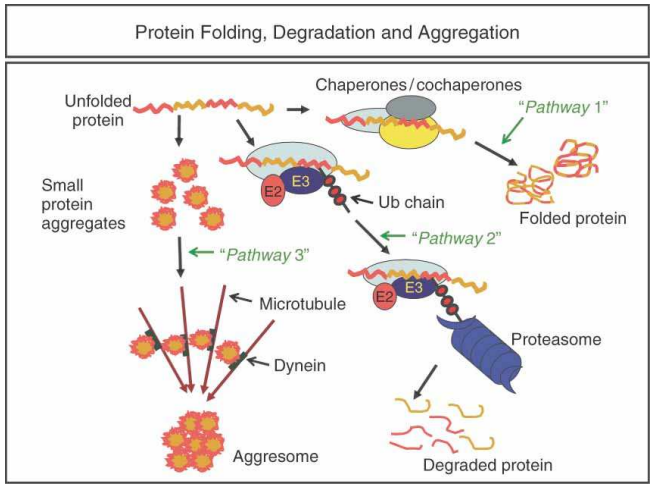


Fig. 1. A simplified wiring diagram of the core elements of the UPR signaling network. ER stress activates the stress sensors ATF6, IRE1, and PERK, representing the three branches of the UPR. Activation of each sensor produces a transcription factor [ATF6(N), XBP1, and ATF4, respectively] that activates genes to increase the protein-folding capacity in the ER. IRE1 (via RIDD) and PERK (via eIF2α phosphorylation) also decrease the load of proteins entering the ER. Both outcomes work as feedback loops that mitigate ER stress. If cells cannot reestablish homeostasis but continue to experience prolonged and unmitigated ER stress (depicted by the timer), they apoptose.



Proteostasi

e

neurotossicità

1. Alterazioni della funzione cognitiva

Malattia di Alzheimer (AD)

2. Alterazioni della funzione motoria

Morbo di Parkinson (PD)
Corea di Huntington (HD)

Comparsa: età adulta o senile, quindi sono espressione di un danno progressivo in una popolazione cellulare post-mitotica

Malattia di Alzheimer (AD)

Morbo di Parkinson (PD)

Idiopatiche; < 10% familiari (AD, PD)

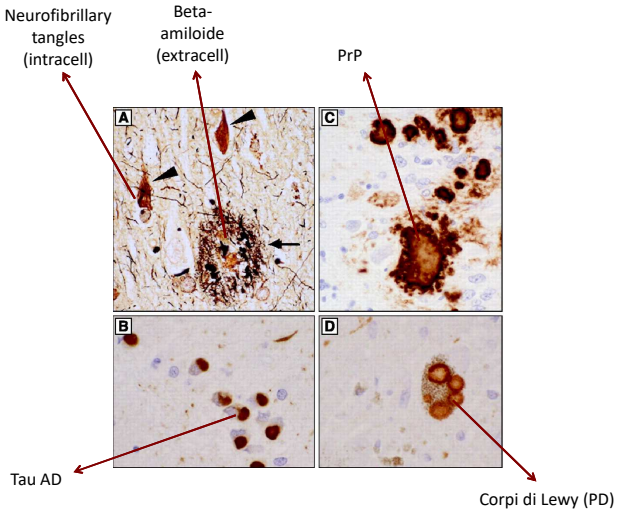
1. Alterazioni della funzione cognitiva

Malattia di Alzheimer (AD)

2. Alterazioni della funzione motoria

Morbo di Parkinson (PD)
Corea di Huntington (HD)

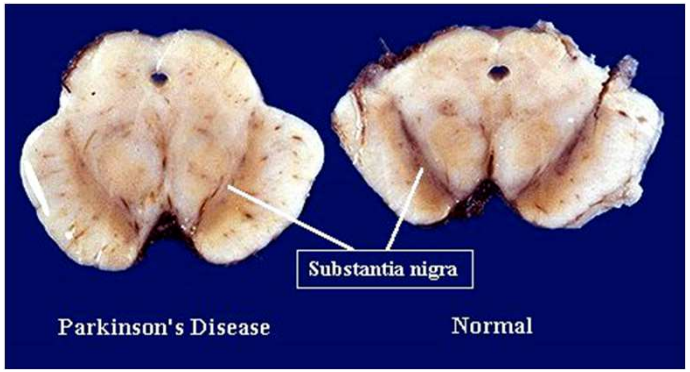
Caratterizzate da accumulo di proteine

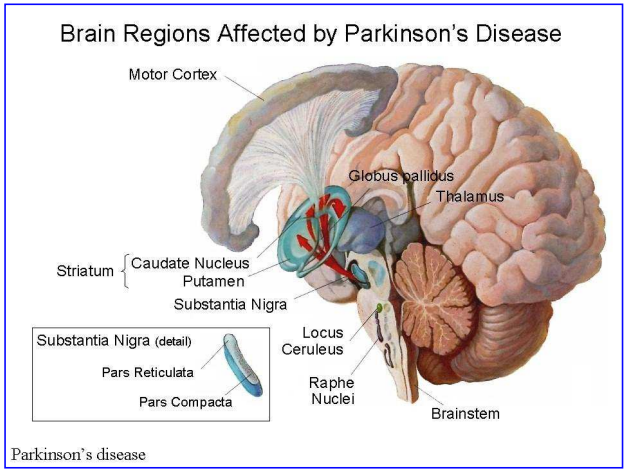


Morbo di Parkinson (PD)

- ✓ Malattia neurodegenerativa cronica e progressiva a eziologia sconosciuta
- ✓ Malattia idiopatica; sono rari i casi di eredità mendeliana con specifico difetto genetico (<10%)
- ✓ La malattia è dovuta alla perdita di neuroni dopaminergici della *pars compacta* della *substantia nigra* (SN) e alla degenerazione delle terminazioni nervose nello *striatum* del SNC

Sezione di mesencefalo





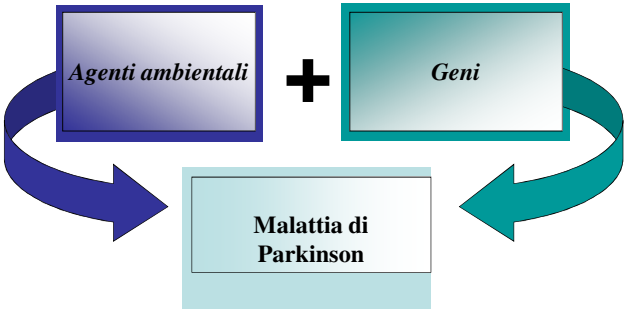
Scientific American Ediz.italiana. Aprile 2001;199

Sintomi principali della PD

- ✓ Rigidità: braccia, gambe, collo
- ✓ Tremore
- ✓ Bradicinesia
- ✓ Instabilità posturale

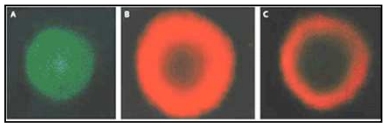
Si accompagna a sintomi non-motori
- autonomici
- sensoriali
- cognitivi
- ciclo sonno/veglia
- psichiatrici

L'eziologia



Marcatori della malattia

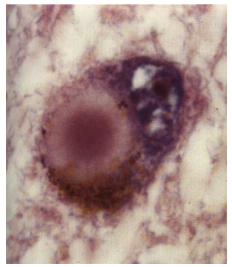
CORPI DI LEWY (LBS)



A ubiquitina

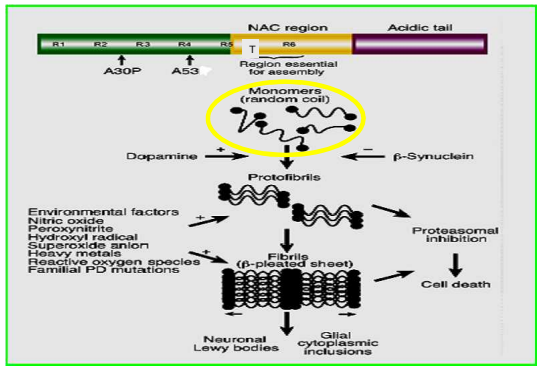
B α-sinucleina

C sovrapposiz. fluorescenze



N Engl J Med.April2003;vol.348

alfa-sinucleina



La sovraespressione e mutazioni patogeniche favoriscono lo stato protofibrillare, permettendo ad uno stimolo aggiuntivo (lo stress ossidativo) di stabilizzarle in aggregati non rimuovibili

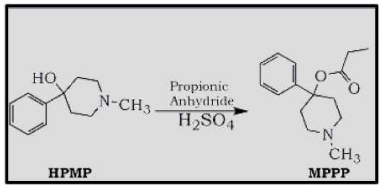
J Clin Invest. 2003 vol 111 n.2

Malattia neurodegenerativa cronica e progressiva a eziologia sconosciuta, eppure ……..

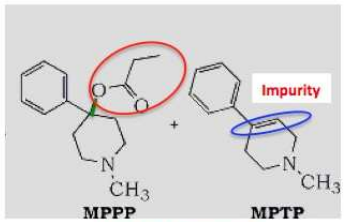
A Frozen Addict from 1982.
Source: Neurology Update.

Da un incidente a un possibile meccanismo

In the 1970s a street narcotic was put out that was not created properly, and led to intravenous drug users in San Francisco having a rapid onset of Parkinson Disease. MPTP was identified as the contaminant, and the toxic metabolite was found to be methylphenyl pyridinium (MPP^+). MPP^+ has a similar chemical structure to the herbicide Paraquat (a redox cycler) which has been linked to onset of (sporadic) Parkinson's. It is important to note that MPTP is metabolized by the enzyme MAO B

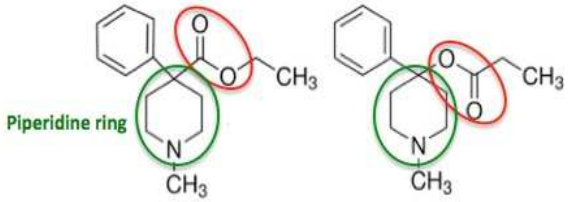


Synthesis of MPPP- Reaction temperature is kept below 30° C (Source erowid.org)



At elevated temperature (Dehydration)

Da un incidente a un possibile meccanismo



ELSEVIER

Psychiatry Research

Volume 1, Issue 3, December 1979, Pages 249-254

Chronic parkinsonism secondary to intravenous injection of meperidine analogues

Glenn C. Davis [ast; 1], Adrian C. Williams [*, 2], Sanford P. Markey [*, 3], Michael H. Ebert [*, 4], Eric D Caine [*, 5], Cheryl M. Reichert [*, 6], Irwin J. Kopin [*, 7]

Critica: il Parkinson da MPTP è una lesione acuta; non sono stati riscontrati i corpi di Lewy

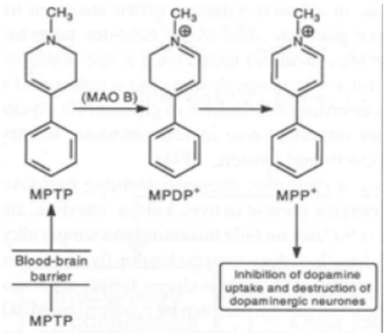
PNAS

Parkinson-like syndrome induced by continuous MPTP infusion: Convergent roles of the ubiquitin-proteasome system and α-synuclein 2005

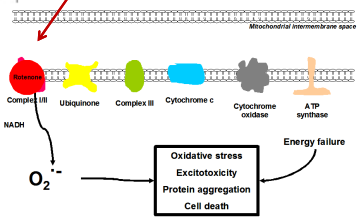
Francesco Fornai*†‡, Oliver M. Schlüter§, Paola Lenzi*, Marco Gesi*, Riccardo Ruffoli*, Michela Ferrucci*, Gloria Lazzeri*, Carla L. Busceti†, Fabrizio Pontarelli†, Giuseppe Battaglia†, Antonio Pellegrini*, Ferdinando Nicoletti†¶, Stefano Ruggieri†¶, Antonio Paparelli*, and Thomas C. Südhof‖

*Department of Human Morphology and Applied Biology, University of Pisa, 56126 Pisa, Italy; †Laboratory of Neurobiology of Movement Disorders, Istituto Ricovero e Cura a Carattere Scientifico, Instituto Neurologico Mediterraneo, Neuromed Pozzilli (IS), 86077 Pozzilli, Italy; §Max-Planck-Institut für Experimentelle Medizin, 37075 Göttingen, Germany; ¶Department of Neurological Sciences, University of Rome "La Sapienza," 00100 Rome, Italy; and ‖Center for Basic Neuroscience, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390

Nella somministrazione cronica di MPTP ai topi si sviluppa una sindrome di Parkinson con i corpi di Lewy!



L'MPP+ inibisce il complesso I della catena respiratoria



Chronic systemic pesticide exposure reproduces features of Parkinson's disease

Ranjita Betarbet, Todd B. Sherer, Gillian MacKenzie, Monica Garcia-Osuna, Alexander V. Panov and J. Timothy Greenamyre

Department of Neurology, Emory University, 1639 Pierce Drive, WMB 6000, Atlanta, Georgia 30322, USA
The first two authors contributed equally to this work
Correspondence should be addressed to J.T.G. (jgreena@emory.edu)

The cause of Parkinson's disease (PD) is unknown, but epidemiological studies suggest an association with pesticides and other environmental toxins, and biochemical studies implicate a systemic defect in mitochondrial complex I. We report that chronic, systemic inhibition of complex I by the lipophilic pesticide, rotenone, causes highly selective nigrostriatal dopaminergic degeneration that is associated behaviorally with hypokinesia and rigidity. Nigral neurons in rotenone-treated rats accumulate fibrillar cytoplasmic inclusions that contain ubiquitin and α-synuclein. These results indicate that chronic exposure to a common pesticide can reproduce the anatomical, neurochemical, behavioral and neuropathological features of PD.

nature *neuroscience* • volume 3 no 12 • december 2000

Article

Disruption of mitochondrial complex I induces progressive parkinsonism

https://doi.org/10.1038/s41586-021-04059-0
Received: 11 December 2020
Accepted: 28 September 2021
Published online: 3 November 2021
Check for updates

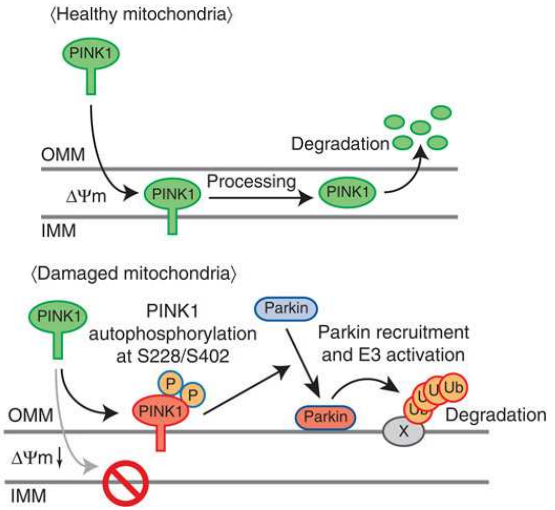
Patricia González-Rodríguez[1], Enrico Zampese[1], Kristen A. Stout[1], Jaime N. Guzman[1], Ema Ilijic[1], Ben Yang[1], Tatiana Tkatch[1], Mihaela A. Stavarache[2], David L. Wokosin[1], Lin Gao[3], Michael G. Kaplitt[2], José López-Barneo[3], Paul T. Schumacker[4] & D. James Surmeier[1]✉

Loss of functional mitochondrial complex I (MCI) in the dopaminergic neurons of the substantia nigra is a hallmark of Parkinson's disease[1]. Yet, whether this change contributes to Parkinson's disease pathogenesis is unclear[2]. Here we used intersectional genetics to disrupt the function of MCI in mouse dopaminergic neurons. Disruption of MCI induced a Warburg-like shift in metabolism that enabled neuronal survival, but triggered a progressive loss of the dopaminergic phenotype that was first evident in nigrostriatal axons. This axonal deficit was accompanied by motor learning and fine motor deficits, but not by clear levodopa-responsive parkinsonism—which emerged only after the later loss of dopamine release in the substantia nigra. Thus, MCI dysfunction alone is sufficient to cause progressive, human-like parkinsonism in which the loss of nigral dopamine release makes a critical contribution to motor dysfunction, contrary to the current Parkinson's disease paradigm[3,4].

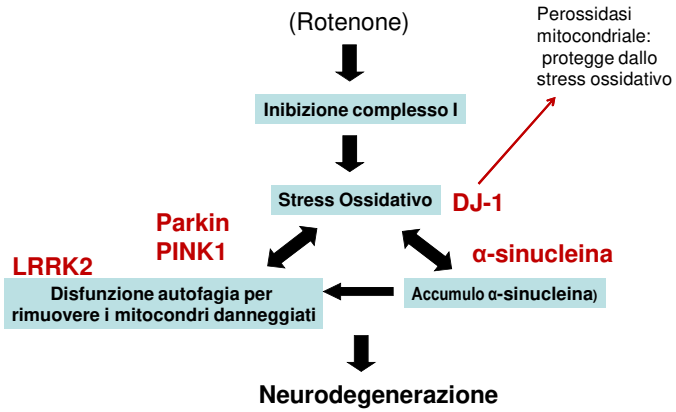
La componente genetica: le forme a trasmissione mendeliana

In un numero limitato di casi, mutazioni e/o duplicazioni di singoli geni causano PD:

- α-sinucleina
- Parkin
- PINK1 (PTEN-induced kinase)
- DJ-1
- LRRK2 (leucine-rich repeated kinase)



Interazione tra fattori ambientali e genetici: un modello patogenetico per PD



- Abbiamo visto che la somministrazione cronica di MPTP porta a formazione dei corpi di Lewy nei topi. Acclarato che il MPTP blocchi il complesso I della catena respiratoria, favorendo l'accumulo di ROS, come mai è proprio l'α-sinucleina ad accumularsi e non qualsiasi altra proteina, in assenza di mutazioni predisponenti dell'α-sinucleina stessa?

Le citazioni sono da Scott Kim et al. (2014) Alpha-synuclein biology in Lewy body diseases, Alzheimers Res. Ther. 6, 73.

1. α-Synuclein is abundantly expressed in the human brain, **making up as much as 1% of protein content in the cytosol**. Quindi il primo motivo plausibile è che ce ne è molta. This protein is expressed throughout the brain, with high levels in the neocortex, hippocampus, substantia nigra, thalamus and cerebellum. It is predominantly expressed in neurons and to a lesser extent in glial cell. In the past two decades α-synuclein has been the center of focus in understanding the etiology of a group of overlapping neurodegenerative disorders called α-synucleinopathies, which includes Parkinson's disease (PD), Parkinson's disease dementia (PDD), dementia with Lewy bodies (DLB), multiple system atrophy (MSA) and a number of less-well characterized neuroaxonal dystrophies. Quindi anche se è caratteristica e sempre presente nel morbo di Parkinson non è esclusiva in questa malattia.
2. Despite much research into α-synuclein biology, the exact function of α-synuclein is still elusive. α-synuclein is thought to play a role in maintaining a supply of synaptic vesicles in presynaptic terminals. The protein has also been **suggested to be involved in regulating the release of the neurotransmitter dopamine** in controlling voluntary and involuntary movements. Questo spiegherebbe il suo impatto sui neuroni dopaminergici.

3. Posttranslational modification of α-synuclein is prevalent and altered α-synuclein proteins impact on a number of pathological processes, including α-synuclein aggregation, Lewy body formation and neurotoxicity.

The most common posttranslational modification of α-synuclein is phosphorylation, which occurs predominantly at serine residues S129 and, to a lesser extent, S87 and at tyrosine residues Y125, Y133 and Y135.

The second most common posttranslational modification of α-synuclein is ubiquitination. Although α-synuclein contains 15 lysine residues, α-synuclein isolated from Lewy bodies shows that the protein is ubiquitinated mainly at K6, K10 and K12 residues.

Another common posttranslational modification of α-synuclein is nitration – the attachment of a nitro molecule to α-synuclein at tyrosine residues (Y39, Y125, Y133 and Y136). **High concentrations of nitrated α-synuclein are found in Lewy bodies. Nitration of α-synuclein is enhanced under conditions of elevated oxidative stress, which is widely regarded as an important factor in Lewy body diseases.** *In vitro* studies have shown that nitration of α-synuclein induced α-synuclein oligomer formation and mitochondrial impairment, leading to apoptosis via the integrin pathway. **In a PD cell model, nitration of α-synuclein (via increased nitric oxide production) caused increases in the level of neurotoxic α-synuclein species and cell death**.

La nitrazione mi sembra un meccanismo molto plausibile, a cui si aggiunge l'effetto "istruttivo" prion-like una volta che il processo di aggregazione è iniziato.

Annual Review of Neuroscience

Annu. Rev. Neurosci. 2021. 44:87–108

The *Annual Review of Neuroscience* is online at neuro.annualreviews.org

https://doi.org/10.1146/annurev-neuro-100720-034518

Parkinson's Disease Genetics and Pathophysiology

Gabriel E. Vázquez-Vélez[1,2] and Huda Y. Zoghbi[1,2,3,4]

Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by degeneration of the substantia nigra pars compacta and by accumulation of α-synuclein in Lewy bodies. PD is caused by a combination of environmental factors and genetic variants. These variants range from highly penetrant Mendelian alleles to alleles that only modestly increase disease risk. Here, we review what is known about the genetics of PD. We also describe how PD genetics have solidified the role of endosomal, lysosomal, and mitochondrial dysfunction in PD pathophysiology. Finally, we highlight how all three pathways are affected by α-synuclein and how this knowledge may be harnessed for the development of disease-modifying therapeutics.

Table 1 Selected genetic loci associated with familial Parkinsonism

Locus	Locus map	Inheritance pattern	Gene	Clinical features	Reference
PARK1/4	4q22.1	AD	*SNCA*	Early onset, rigidity, cognitive impairment	Polymeropoulos et al. 1997
PARK2	6q26	AR	*PRKN*	Juvenile onset, dystonia	Kitada et al. 1998
PARK6	1p36.12	AR	*PINK1*	Early onset, dystonia	Valente et al. 2002
PARK7	1p36.23	AR	*PARK7*	Early onset, dystonia	Abou-Sleiman et al. 2003
PARK8	12q12	AD	*LRRK2*	Classic PD	Funayama et al. 2002
PARK9	1p36.13	AR	*ATP13A2*	Early onset, cognitive impairment	Di Fonzo et al. 2007
PARK14	22q13.1	AR	*PLA2G6*	Early onset, cognitive impairment, dystonia	Paisán-Ruiz et al. 2009
PARK15	22q12.3	AR	*FBXO7*	Early onset	Di Fonzo et al. 2009
PARK17	16q11.2	Unknown	*VPS35*	Adult onset, cognitive impairment, dystonia	Zimprich et al. 2011
PARK19a/b	1p31.3	AR	*DNAJC6*	Early onset, cognitive impairment	Edvardson et al. 2012
PARK20	21q22.11	AR	*SYNJ1*	Early onset, seizures	Krebs et al. 2013
PARK21	3q22	AD	*DNAJC13*	Classic PD	Vilariño-Güell et al. 2014
PARK23	15q22.2	AR	*VPS13C*	Early onset, rapid progression, cognitive impairment	Lesage et al. 2016

Table 2 Pathogenic *SNCA* variants

Variant and amino acid sequence	Age of onset	Clinical features	Pathological features	Reference(s)
c.G>209A, p.A53T	Youngest: 23 years Average: 47 years	Parkinsonian motor symptoms responsive to levodopa with early cognitive impairment, as well as myoclonus and autonomic dysfunction	Degeneration of the SNc with widespread LBs Cortical thinning with vacuoles Some patients have neurofibrillary tangles and TDP-43 inclusions	Athanassiadou et al. 1999, Bostantjopoulou et al. 2001, Ki et al. 2007, Markopoulou et al. 2008, Polymeropoulos et al. 1997, Puschmann et al. 2009, Spira et al. 2001, Xiong et al. 2016
c.G>88C, p.A30P	Average: 60 years	Tremor-dominant PD, responsive to levodopa	Degeneration of the SNc with widespread LBs	Krüger et al. 1998, Seidel et al. 2010
c.G>188A, p.E46K	Range: 50–65 years	Parkinsonian motor symptoms responsive to levodopa with early cognitive impairment	Mild degeneration of the SNc with widespread LBs	Zarranz et al. 2003
c.C>152A, p.G51D	Range: 19–61 years	Parkinsonian motor symptoms as well as pyramidal (motor neuron) signs, which include spasticity and exaggerated tendon reflexes	Massive nigral and locus coeruleus degeneration with widespread LBs or GCIs	Kiely et al. 2015, Lesage et al. 2013

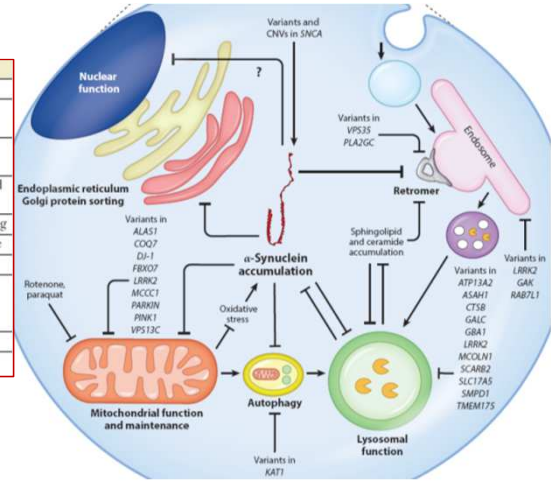
Table 3 *LRRK2* variants associated with Parkinson's disease

Mutation and amino acid sequence	Mutation consequence	Protein domain affected and proposed effect on function	Reference(s)
c.4309A>C p.N1437H	AD PD	ROC, decreases GTPase activity	Cookson 2015
c.4321C>T/G/A p.R1441C/G/H/S			
c.5096A>G p.Y1699C		COR, decreases GTPase activity	
c.6055G>A p.G2019S		Kinase domain, increases kinase activity	
c.6059T>C p.I2020T			
c.1464A>T p.A419V	Increased sporadic PD risk	Sequence in between ARM and ANK domains, effect unknown	Christensen et al. 2018, Li et al. 2015, Heckman et al. 2013, Ross et al. 2011, Tan et al. 2010, Zhang et al. 2017
c.7153G>A p. G2385R		WD40, increases kinase activity	
c.4883G>C p.R1628P		COR, increases kinase activity	
c.4937T>C p.M1646T			
c.3494T>C- c.4193G >A-c.4269G>A p.N551K-R1398H-K1423K	Decreased sporadic PD risk	Sequence in between ARM and ANK domains–ROC–ROC, reduces kinase activity	

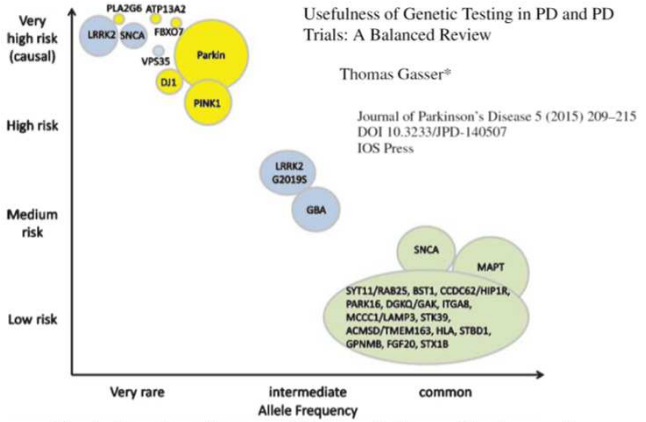
Table 4 Genes associated with single nucleotide polymorphisms that modulate Parkinson's disease risk

Category	Candidate genes
Cytoskeleton	*CAB39L, TUBG2, MAPT, DNAH17, ANK2, PDLIM2, SORBS3*
Endosomal and vesicular trafficking	*VAMP4, SIPA1L2, SNCA, CHMP2B, LRRK2, BIN3, RIMS1, DDRGK1, SYT4, ATP6V0A1, GBF1, ARHGAP27, SH3GL2*
Immune system	*FCGR2A, IL1R2, HLA-DRB6, HLA-DQA1, FYN, CD19, CD38, NOD2, TRIM40, FAM49B, ITIH3, ITIH4, TLR9, STAB1*
Ion channels, transporters, and neurotransmitter signaling	*KCNS3, KCNIP3, TMEM163, SCN3A, CHRNB1, CLCN3, GCH1, NCKIPSD, CAMK2D*
Lipid metabolism and signaling	*SPTSSB, ELOVL7, DGKQ*
Lysosome and autophagosome	*GBA, CTSB, GALC, KAT8, TMEM175*
Mitochondria	*SLC41A1, COQ7, VPS13C, BAG3, MCCC1, CRLS1, MICU3*
Nucleus and gene regulation	*NUCKS1, CCNT2, SATB1, KPNA1, MED12L, LCORL, MBNL2, MEX3C, MIR4697, TOX3, UBTF, LSM7, BRIP1, ASXL3, RPS6KL1, PSMC3IP, SREBF1, RAI1, KANSL1, RNF141, RPS12, CDC71, PHF7, NUPL2, ZNF184*
Ubiquitin pathway	*UBAP2, BAP1, KLHL7*
Miscellaneous	*ITPKB, LINC00693, DYRK1A, OGFOD2, FAM171A2, ZNF646, FAM47E, FBRSL1, MIPOL1, SCAF11, PAM, TMEM229B, CRHR1, STH, SPPL2C, DLG2, C5orf24, C8orf58, GS1–124K5-11, ALAS1, NISCH, GPNMB, FAM200B, STK39*

Category
Cytoskeleton
Endosomal and vesicular trafficking
Immune system
Ion channels, transporters, and neurotransmitter signaling
Lipid metabolism and signaling
Lysosome and autophagosome
Mitochondria
Nucleus and gene regulation
Ubiquitin pathway
Miscellaneous



Parkinson's disease (PD) pathogenesis involves vesicular trafficking and lysosomal and mitochondrial maintenance. This diagram summarizes what is currently known about PD genetics. α-Synuclein is shown in the center to highlight its importance and influence in all known signaling nodes of PD pathogenesis (mitochondrial function, vesicular trafficking, and lysosomal function).



Usefulness of Genetic Testing in PD and PD Trials: A Balanced Review

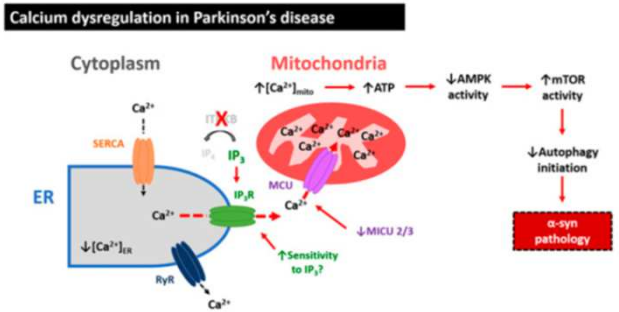
Thomas Gasser*

Journal of Parkinson's Disease 5 (2015) 209–215
DOI 10.3233/JPD-140507
IOS Press

Fig. 1. Genetic architecture of Parkinson's disease. Continuum of variants of different effect strengths and allele frequencies. The size of the bubbles roughly corresponds to population allele frequencies. Colors symbolize modes of inheritance: dominant (blue), recessive (yellow), risk loci (green). Modified from [35] and [36].

The Parkinson's disease-associated gene ITPKB protects against α-synuclein aggregation by regulating ER-to-mitochondria calcium release

Daniel J. Apicco[a,b,1], Evgeny Shlevkov[a], Catherine L. Nezich[a], David T. Tran[a], Edward Guilmette[c], Justin W. Nicholatos[a,b], Collin M. Bantle[a,b], Yi Chen[a], Kelly E. Glajch[a], Neeta A. Abraham[a], Lan T. Dang[a], G. Campbell Kaynor[d], Ellen A. Tsai[e], Khanh-Dung H. Nguyen[e], Joost Groot[f], YuTing Liu[g], Andreas Weihofen[a], Jessica A. Hurt[b], Heiko Runz[e], and Warren D. Hirst[a,1]



PNAS 2021 Vol. 118 No. 1 e2006476118 https://doi.org/10.1073/pnas.2006476118

Inositol-1,4,5-triphosphate (IP_3) kinase B (ITPKB) is a ubiquitously expressed lipid kinase that inactivates IP_3, a secondary messenger that stimulates calcium release from the endoplasmic reticulum (ER). Genome-wide association studies have identified common variants in the ITPKB gene locus associated with reduced risk of sporadic Parkinson's disease (PD). Here, we investigate whether ITPKB activity or expression level impacts PD phenotypes in cellular and animal models. In primary neurons, knockdown or pharmacological inhibition of ITPKB increased levels of phosphorylated, insoluble α-synuclein pathology following treatment with α-synuclein preformed fibrils (PFFs). Conversely, ITPKB overexpression reduced PFF-induced α-synuclein aggregation. We also demonstrate that ITPKB inhibition or knockdown increases intracellular calcium levels in neurons, leading to an accumulation of calcium in mitochondria that increases respiration and inhibits the initiation of autophagy, suggesting that ITPKB regulates α-synuclein pathology by inhibiting ER-to-mitochondria calcium transport. Furthermore, the effects of ITPKB on mitochondrial calcium and respiration were prevented by pretreatment with pharmacological inhibitors of the mitochondrial calcium uniporter complex, which was also sufficient to reduce α-synuclein pathology in PFF-treated neurons. Taken together, these results identify ITPKB as a negative regulator of α-synuclein aggregation and highlight modulation of ER-to-mitochondria calcium flux as a therapeutic strategy for the treatment of sporadic PD.

Malattia di Alzheimer

Fa parte delle demenze senili

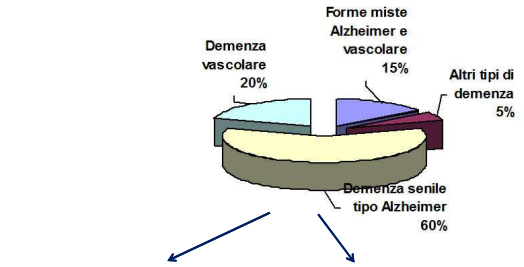
PRIMARIE

- corticali: Alzheimer, fronto-temporale (Pick)
- sottocorticali: PD, HD, altre forme più rare

SECONDARIE:

- vascolari
- prioni
- infettive/infiammatorie
- tossiche/dismetaboliche
- post-traumatiche
- idrocefalo
- neoplasie

Cause di demenza

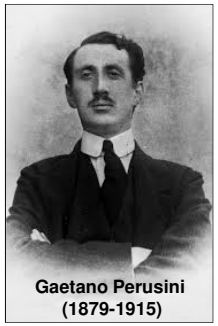


5% dei >65 a
20% dei >80 a

Sopravvivenza 8±4 a

Nel 1906 Alois Alzheimer per la prima volta descrisse i sintomi della patologia che oggi porta il suo nome

- perdita della memoria
- disorientamento topografico
- ridotte capacità lessicali e parafasie
- alterato riconoscimento di volti familiari
- disturbi nella comprensione


Gaetano Perusini (1879-1915)

European Journal of Neurology (1997), 4, 210–213

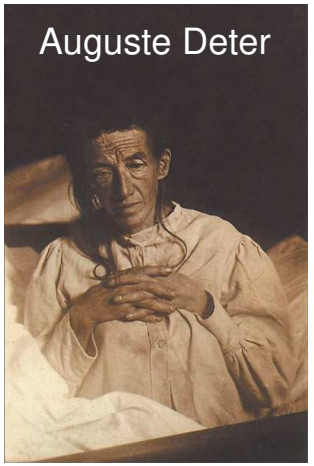
Alois Alzheimer and Gaetano Perusini: should man divide what fate united?

G. Macchi[1], C. Brahe[2] and M. Pomponi[3]

"It is very likely that the plaques I have outlined are the same as those reported by Fischer and Hübner. Nevertheless it should be stressed that neither Fischer nor Hübner outlined the quite unique fibrillary changes. In my case histories, on the other hand, the concurrence of the fibrillary changes with the formation of the plaques is clearly an essential finding. … What distinguishes findings in our cases from those of senile dementia are primarily quantitative differences … even though we are partly dealing with pre-senile diseases … ".

However in 1911 Perusini was more explicit: " … in the other cases, outlined by both Alzheimer and Perusini, the histopathological changes always were in agreement with those detected in the severest forms of senile dementia … ". This comment and the older patients of his study show that Perusini believed that presenile dementia and senile dementia were one disease.

Perusini G (1910) Über klinisch und histologisch eigenartige psychische Erkrankungen des späteren Lebensalters. *Histologische und Histopathologische Arbeiten*, III, Heft 2, 297–351.



On November 25, 1901 Dr. Alzheimer told her to write her name. She tried to, but would forget the rest and repeat:

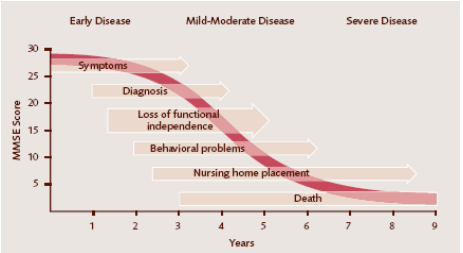
"I have lost myself"
(Ich hab mich verloren)

To be the world's greatest philosopher in the prime of life is no guarantee against developing the ravages of dementia in old age.

Immanuel Kant (1724-1804)

In 1799 memory loss first became evident, especially for recent events, and he would recount the same story many times in a single day. He became weak, intolerant, lost his perception of time, had several falls which led him to abandon his walks, became spatially disorientated, confused, later aphasic, and continued to exhibit mental deterioration. He died in 1804 at the age of eighty. Neurologists are inclined to rule out the pseudo-dementia of depression, Parkinson's disease or other afflictions of the extrapyramidal system, subdural hematoma, as well as infectious, vascular, and substance-induced dementia. We are left with a senile dementia of the Alzheimer's type, a condition described a century after Immanuel Kant's death by his fellow countryman Alois Alzheimer.

Decorso della patologia di Alzheimer



Primi sintomi

Disturbi dell'umore

Perdita di memoria

Disorientamento temporale e spaziale

Con l'aggravarsi della malattia

Amnesia anterograda

Incapacità di svolgere attività quotidiane

Disturbi del comportamento (agitazione, a volte aggressività)

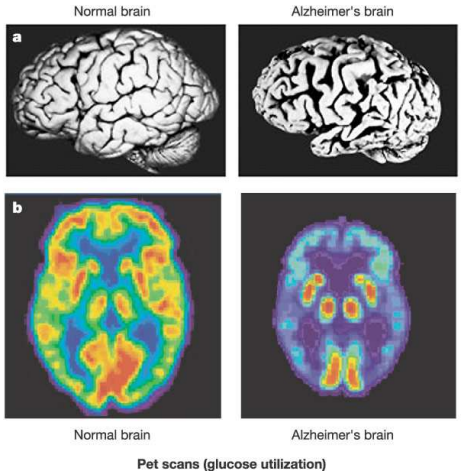
Afasia

In 5-10 anni

Grave demenza

Perdità del linguaggio, locomozione e funzioni intellettive elevate

Perdita dell'autosufficienza



Pet scans (glucose utilization)

Atrofia corticale

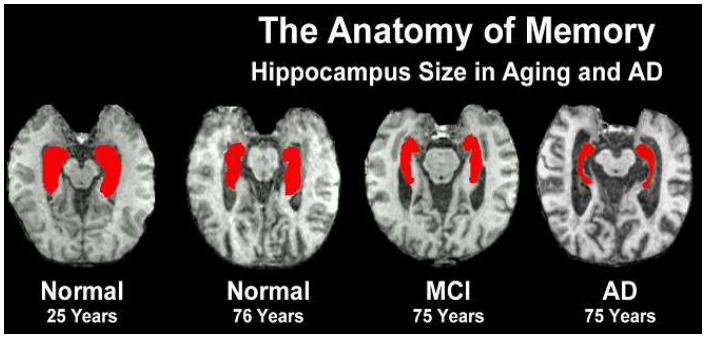
Ingrandimento ventricolare per perdita di parenchima

Causata da perdita di neuroni (soprattutto colinergici)

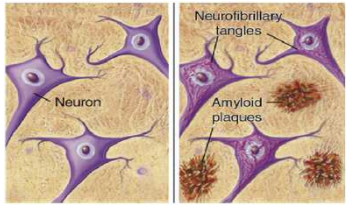
Forte riduzione del metabolismo

Diminuzione dell'uptake di glucosio

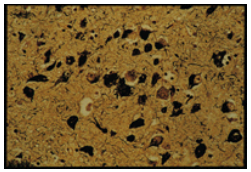
The change is particulary marked in hippocampus, a key area for new memory formation



Alterazioni morfologiche a livello cellulare

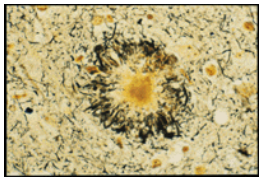


Normale **Alzheimer**

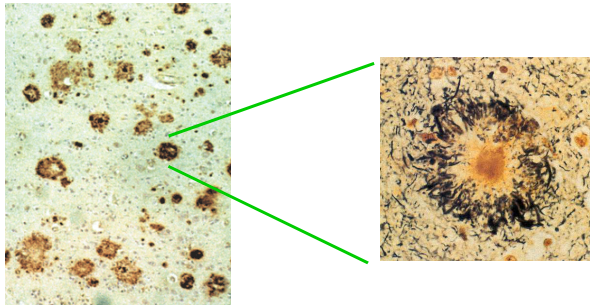


Ammassi neurofibrillari
(proteina *Tau*) intracellulari

Formazione **placche senili**
per deposizione di **β-amiloide**,
soprattutto $A\beta_{42}$



A livello tissutale: formazione di placche senili



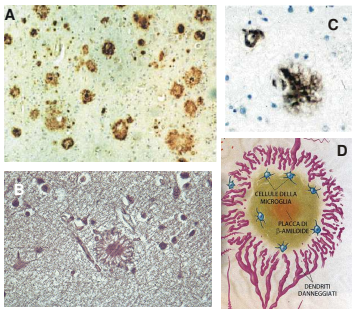
Presenti in varie zone cerebrali (ippocampo, corteccia entorinale, neocorteccia)

Dimensioni delle placche da 20 a 200 μm

Placche senili: macchie dense e scure a livello della corteccia cerebrale

Formate da un nucleo di β-amiloide circondato da un alone di terminazioni nervose che contengono i filamenti neri di tau.

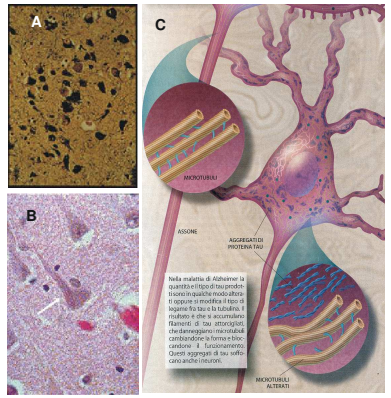
Tutt'intorno si riconoscono cellule infiammatorie della microglia.

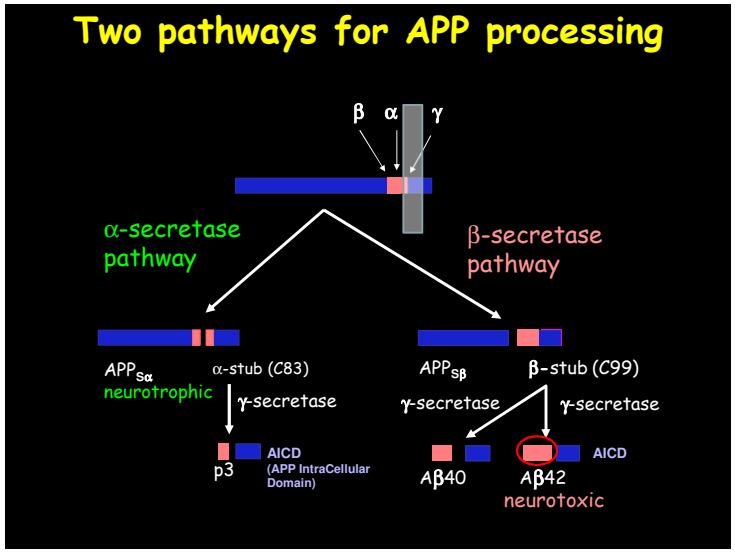


Ammassi neurofibrillari: aggregati di proteina tau nel citoplasma di neuroni che compaiono tardivamente durante la malattia

Tau è una proteina di 55 kDa associata ai microtubuli espressa abbondantemente nel cervello. La sua porzione C-term lega la tubulina facilitandone l'assemblaggio

La proteina tau negli ammassi neurofibrillari è iper-fosforilata (HP-tau) e non è in grado di legarsi alla tubulina compromettendo la stabilità dei microtubuli e quindi interferendo con il trasporto assonico con perdita di sinapsi funzionali





La produzione e l'accumulo di peptide β-amiloide (Aβ) ha un ruolo centrale nella patogenesi dell'Alzheimer

- Mutazioni nel precursore APP determinano forme genetiche precoci di Alzheimer
- Tutte le mutazioni finora caratterizzate portano ad un aumento della produzione di Aβ
- Pazienti con trisomia 21 (sindrome di Down), e quindi tre copie del gene APP per la proteina precursore dell'amiloide, sviluppano relativamente presto le caratteristiche neuropatologiche dell'AD
- Aβ e' neurotossico *in vitro* e provoca morte cellulare
- La sovra-espressione di huAPP in topi transgenici induce lesioni simili a quelle osservabili in pazienti AD
- Questi topi hanno deficit di apprendimento e memoria che correlano con l'accumulo di amiloide

FAD: Familial Alzheimer's Disease

Rappresenta meno del 5% dei casi di Alzheimer

Caratterizzato da:
precoce età di insorgenza (< 65 anni)
ereditarietà autosomica-dominante

Associato a mutazioni puntiformi nei geni di:

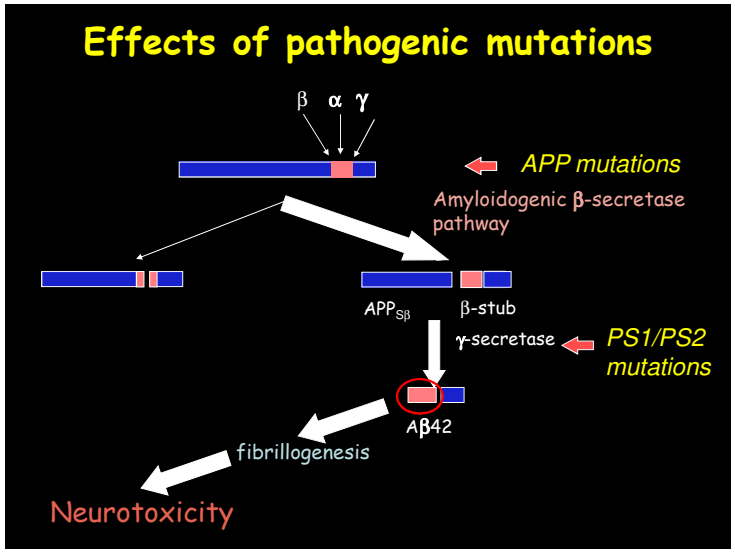
Proteina Precursore dell'Amiloide (*app*) - cromosoma 21
Presenilina 1 (*psen1*) - cromosoma 14
Presenilina 2 (*psen2*) - cromosoma 1

Tutte le mutazioni portano ad un'aumentata produzione di Aβ42

APP/PS → $\frac{A\beta_{42}}{A\beta_{40}}$

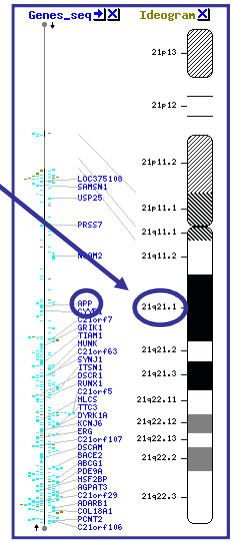
APP/PS mutate (FAD) → $\frac{A\beta_{42}}{A\beta_{40}}$ → **Placche Senili**

Le mutazioni in APP o PSs associate a FAD sono mutazioni che alterano l'attività γ-secretasica. Viene prodotta maggiormente l'isoforma contenente 42 AA



CLONAGGIO DEL GENE APP

- Il gene APP mappa sul braccio lungo del cromosoma 21 (21q21.1).
- Il clonaggio del gene APP risale al 1987.



CLONAGGIO DEL GENE APP

TAPPE

Individui con sindrome Down (trisomia 21) spesso sviluppavano lesioni istopatologiche di AD se vivevano più di 30 anni

Nel cromosoma 21 possibile locus della malattia

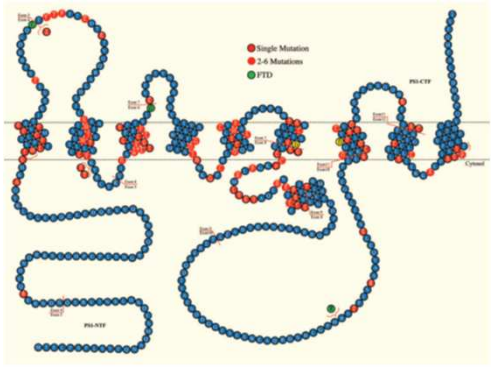
1987 (St George-Hyslop et al.) 1° studio di linkage, usando marker polimorfici del braccio lungo del chr 21, in famiglie con FAD ad insorgenza precoce, si individuò una regione 21q11.2-q21.1

MUTAZIONI DEL GENE APP

- Le mutazioni dell'APP sono rare (solo il 3-5% di tutti i casi di FAD e 0,5% di tutti i casi AD) e causano FAD ad esordio precoce (35-50 anni).
- Si ereditano con modalità autosomica dominante.
- Per il momento identificate 23 mutazioni. Sono tutte mutazioni "senso" a livello del sito di taglio delle β,α o γ secretasi che ne alterano l'attività.

Aumento produzione Aβ 42

PRESENILINE (PS1 e PS2)



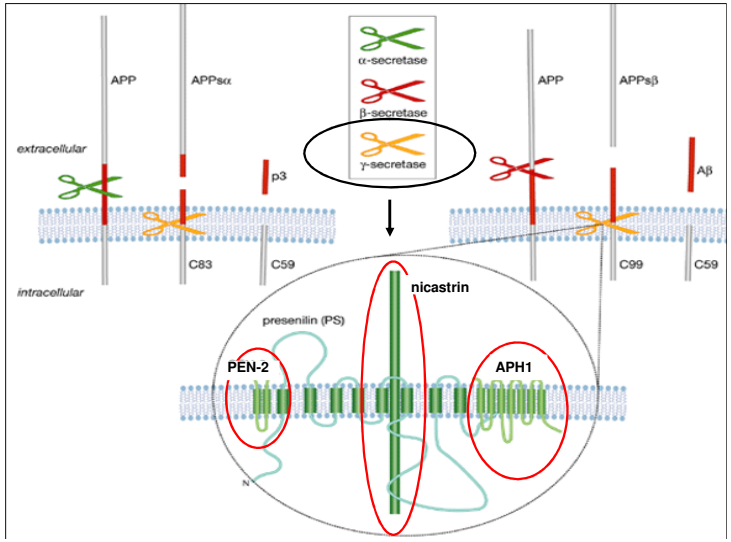
Mutazioni FAD (più di 160 in PS1, una dozzina in PS2)

Polytopic transmembrane proteins mainly present in ER and Golgi apparatus

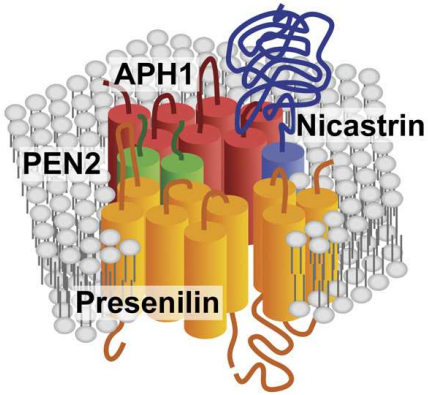
PS1 and PS2 share an overall 63% homology

9 TM domains proteins

key components of the γ-secretase complex



Le PS sono il core catalitico della γ-secretasi



γ-secretasi: proteasi aspartiche in grado di catalizzare un taglio transmembrana

La γ-secretasi è responsabile della maturazione anche di altre proteine:

Notch: recettore transmembrana coinvolto nella trasduzione del segnale intracellulare durante lo sviluppo dell'asse dorsale, nell'embriogenesi, e nella maturazione di vari tipi cellulari nel periodo post-natale

ErbB4: recettore tirosin-chinasico implicato nella regolazione della proliferazione e nel differenziamento

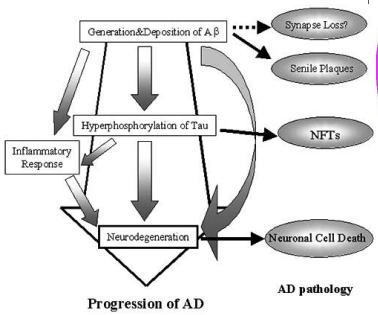
CD44: molecola di adesione cellulare

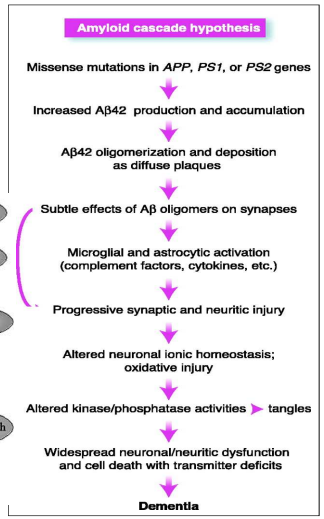
Genetic forms of Alzheimer's Disease

Gene	Chr		
APP	21	Transmembrane protein physiologically cleaved and secreted. Generation of β-amyloid	Mutation 100% penetrant for early-onset FAD. β-amyloid deposition
PS1	14	Transmembrane protein with 9 hydrophobic domains. Mainly localized in intracellular organelles membranes	Major early-onset FAD gene
PS2	1	63% homology to PS1	Early-onset FAD gene
APOE	19	Lipid particles associated protein. Three isoforms: ApoE e2, e3 and e4	e4 isoform confers risk for late-onset AD

Principale ipotesi patogenetica:

LA DEPOSIZIONE DI $A\beta_{42}$ E' L'EVENTO CAUSALE DELLA MALATTIA

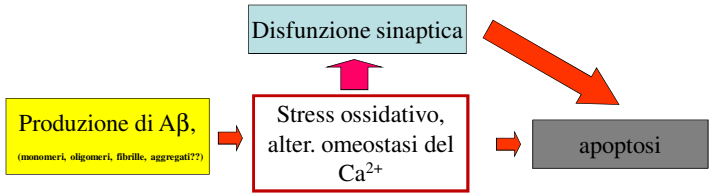




Quale forma del peptide Aβ è tossica per il neurone?

- **Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability** (J Biol Chem. 2002 Aug 30;277(35):32046-53. Epub 2002 Jun 10)

Sono state dimostrate varie alterazioni della permeabilita' della membrana plasmatica neuronale



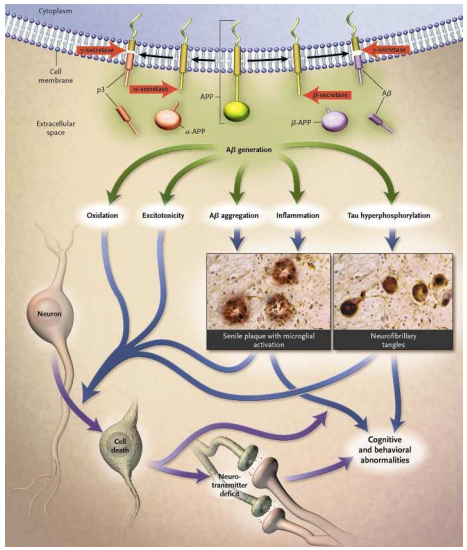
Nature Medicine **14**, 1097 - 1105 (2008)
Published online: 21 September 2008 | doi:10.1038/

Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease

Heng Du[1], Lan Guo[1], Fang Fang[1], Doris Chen[1], Alexander A Sosunov[2], Guy M McKhann[2], Yilin Yan[3], Chunyu Wang[3], Hong Zhang[4,5], Jeffery D Molkentin[6], Frank J Gunn-Moore[7], Jean Paul Vonsattel[4], Ottavio Arancio[4,5], John Xi Chen[8] & Shi Du Yan[1,4,5]

regulator

Cyclophilin D (CypD, encoded by *Ppif*) is an integral part of the mitochondrial permeability transition pore, whose opening leads to cell death. Here we show that interaction of CypD with mitochondrial amyloid-β protein (Aβ) potentiates mitochondrial, neuronal and synaptic stress. The CypD-deficient cortical mitochondria are resistant to Aβ- and Ca^{2+}-induced mitochondrial swelling and permeability transition. Additionally, they have an increased calcium buffering capacity and generate fewer mitochondrial reactive oxygen species. Furthermore, the absence of CypD protects neurons from Aβ- and oxidative stress–induced cell death. Notably, CypD deficiency substantially improves learning and memory and synaptic function in an Alzheimer's disease mouse model and alleviates Aβ-mediated reduction of long-term potentiation. Thus, the CypD-mediated mitochondrial permeability transition pore is directly linked to the cellular and synaptic perturbations observed in the pathogenesis of Alzheimer's disease. Blockade of CypD may be a therapeutic strategy in Alzheimer's disease.



Pharmacological Treatments:

- Cholinesterase Inhibitors (Donepezil; Galantamine) to increase Ach levels
- NMDA-Receptor antagonist (Memantine) to target excitotoxicity induced by glutamate
- Anti-oxidants and anti-inflammatory agents to contrast the inflammatory response

Therapeutic Strategies:

- Nerve growth factor
- Aβ–related disease-modifying strategies (secretase inhibitors-modulators
- anti-Aβ immunotheraphy (active: vaccination; passive: IgG); Aβ plaque inhibitors)
- Anti-tangle therapies (inhibitors of tau aggregation; microtubule stabilizers)

The New York Times

Scientists Question Data Behind an Experimental Alzheimer's Drug

Studies linked to Cassava Sciences, once a stock market favorite, have been retracted or challenged by medical journals.

Give this article

Ilana Panich-Linsman for The New York Times

By Apoorva Mandavilli

April 18, 2022

A small biotech company that trumpeted an exciting new treatment for Alzheimer's disease is now under fire for irregularities in its research results, after several studies related to its work were retracted or questioned by scientific journals.

The company, Cassava Sciences, based in Austin, Texas, announced last summer that its drug, simufilam, improved cognition in Alzheimer's patients in a small clinical trial, describing it as the first such advance in treatment of the disease. Cassava later initiated a larger trial.

The drug's potential garnered enormous attention from investors. Alzheimer's disease affects roughly six million Americans, a number that is expected to double by 2050, and an effective treatment would be lucrative. Cassava's stock soared, by more than 1,500 percent at one point. The company was worth nearly $5 billion last summer.