Nevrodegeneracija

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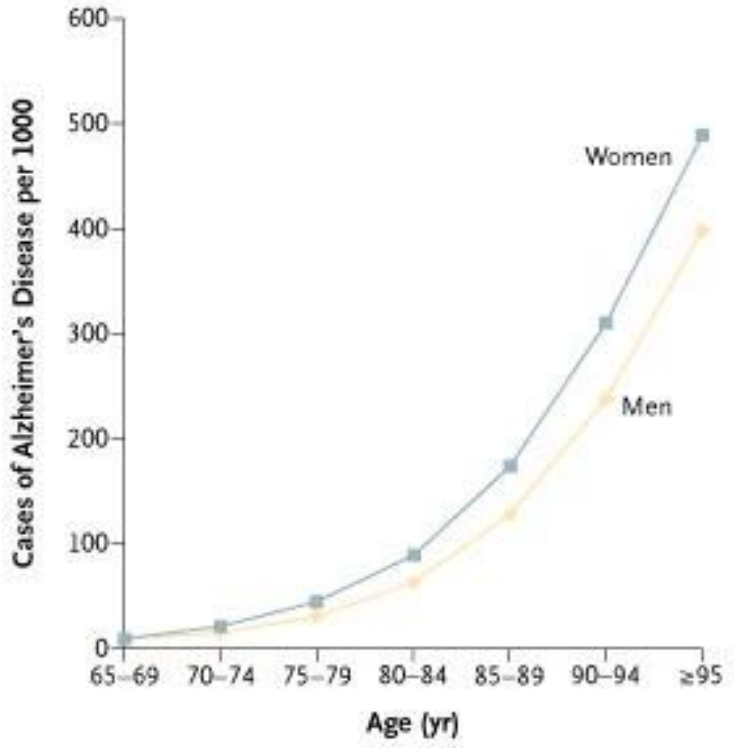
Pregled predavanja

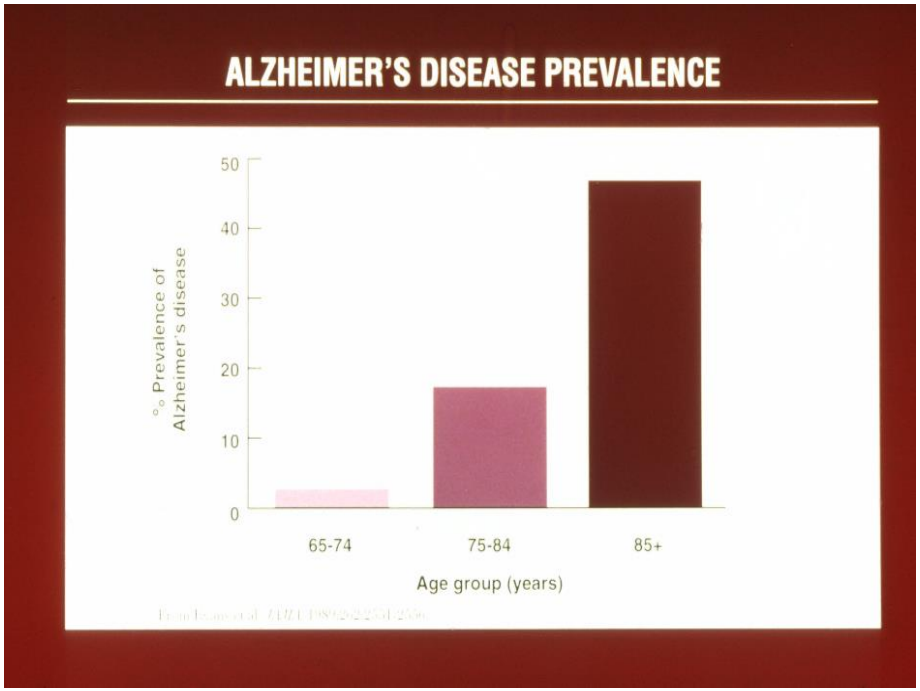
- Osnove in skupne lastnosti
- Alzheimerjava bolezen (AB)
- Parkinsonova bolezen (PB)
- Huntingtonova bolezen (HB)
- Frontotemporalna demenca (FTD)
- Amiotrofična lateralna skleroza (ALS)

Osnovne lastnosti nevrodegenerativnih bolezni

- Bolezen nastopi na zahrbten način
- Napredujoča bolezni
- Živčne celice umirajo ali ne delujejo
- Etiologija ni jasna
- Vpliva na:
  - Spomin
  - Razmišljanje
  - Orientacijo
  - Razumevanje
  - Računanje
  - Učenje
  - Presojanje
  - Jezikovne sposobnosti…

Neurodegenerativne bolezni so pogoste





Prevalenca NDB

- Alzheimerjeva bolezen
  - 1-2% v starostni dobi 65-75; 50% čez 85 let
- Parkinsonova bolezen
  - 0.5-1% v starostni dobi 60-69; 1-3% čez 80 let
- Huntingtonova bolezen
  - 1 : 10.000
- Frontotemporalna demencaa
  - 1 : 10.000
- ALS
  - 6-8 : 100.000
- Spinocerebelarne ataksije
  - 0.3-3 : 100.000

V kontekstu Evrope

(OECD Evropska skupnost, 2010)

- Prevalenca demence: ~ 7.300.000.
- Najvišja prevalenca: Švedska, Italija, Švica in Nemčija.
- Najbolj pogosti vzroki: Alzheimerjeva bolezen (50-70%), vaskularna demenca (30%).
- Obolelost se poveča s staranjem : ena tretina moških (32.4%) in polovica žensk (48.8%) pri 95 letih.

Osebni kontekst

Demnca nas vse prizadane:

- Lahko se razvije pri nas…
- Ali pri starših ali sorodnikih…
- Ali pri življenskem partnerju ali prijatelju…
- Ali pri otrocih…

Prioriteta javnega zdravstva

EU projekti za izboljšavo razumevanja demence:

- EuroCoDe (European Collaboration on Dementia) projekt, ki ga koordinira Alzheimer Evropa.
- Podpora nacionalnim programom za preprečitev, raziskave in najboljše pristope pri zdravljenju (European Commission, 2010).

Nacionalne strategije za demenco:

- Francija, Norveška in Združeno Kraljevstvo.
- Fokus – izboljšanje zgodnje diagnoze in zdravljenja ter pomoč skrbnikom.

Metode diagnoze

- **Identifikacija bioloških markerjev**: pomembno za zgodnje odkritje bolezni. Za uporabo te metode moramo vedeti, katere molekularne kaskade se spremenijo v zgodnjih stopnjah bolezni.

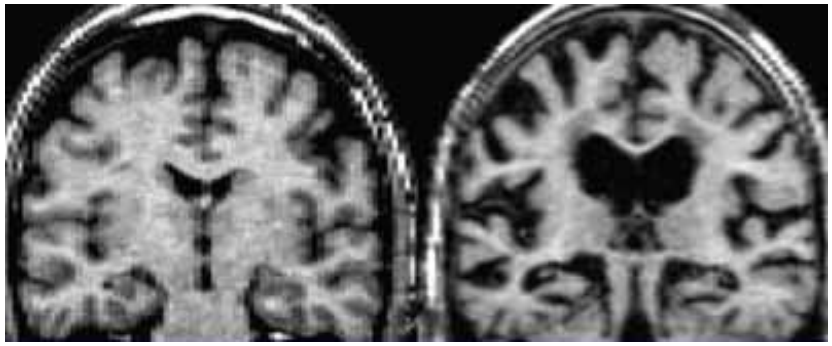
- **Vedenjski ali nevrološki testi** (PB, ALS, MS); **Kognitivni testi** (AB)

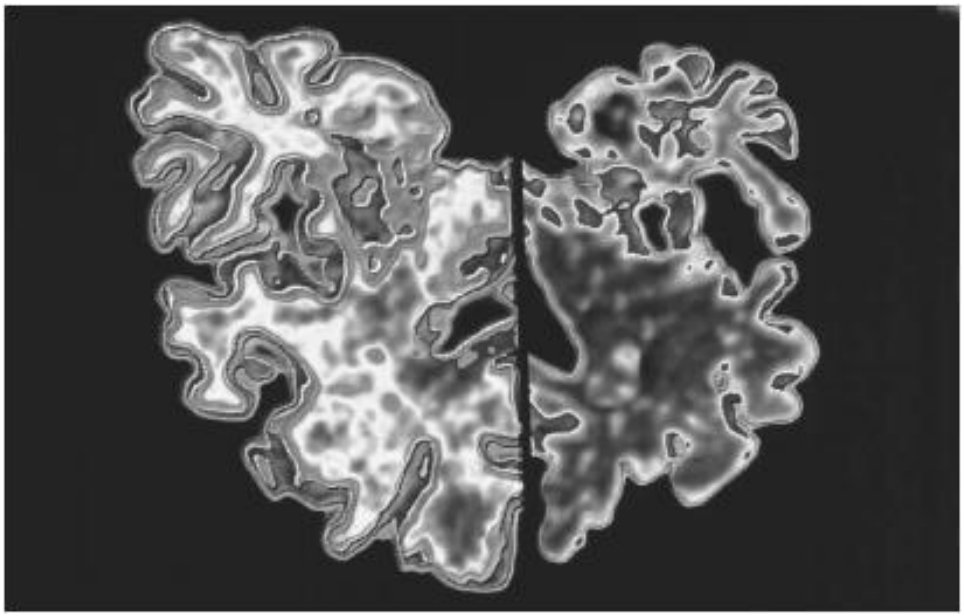
- **PET**: Vizualizacija poškodbe. Uporabljajo se radioaktivno označene molekule, ki pridejo do možganskih delov, ki so bolezensko poškodovani. Npr. L-DOPA, ki je prekurzor dopamina, se uporablja za PET pri PB; molekule, ki vežejo β-amiloid se uporabljajo pri AB. Vendar je ponavadi že prepozno za terapevtske intervencije, ko se poškodbe vidijo s PET.

- **MRI:** pregled celih možganov, ki omogoča evalvacijo sprememb volumna možganov v določenih regijah. Sicer so take spremembe bolj lastnost kasnejših stadijev bolezni.

MRI pri AB

Normal **Alzheimer's diseases**





Različne metode detekcije se lahko uporabljajo v različnih stadijih bolezni.

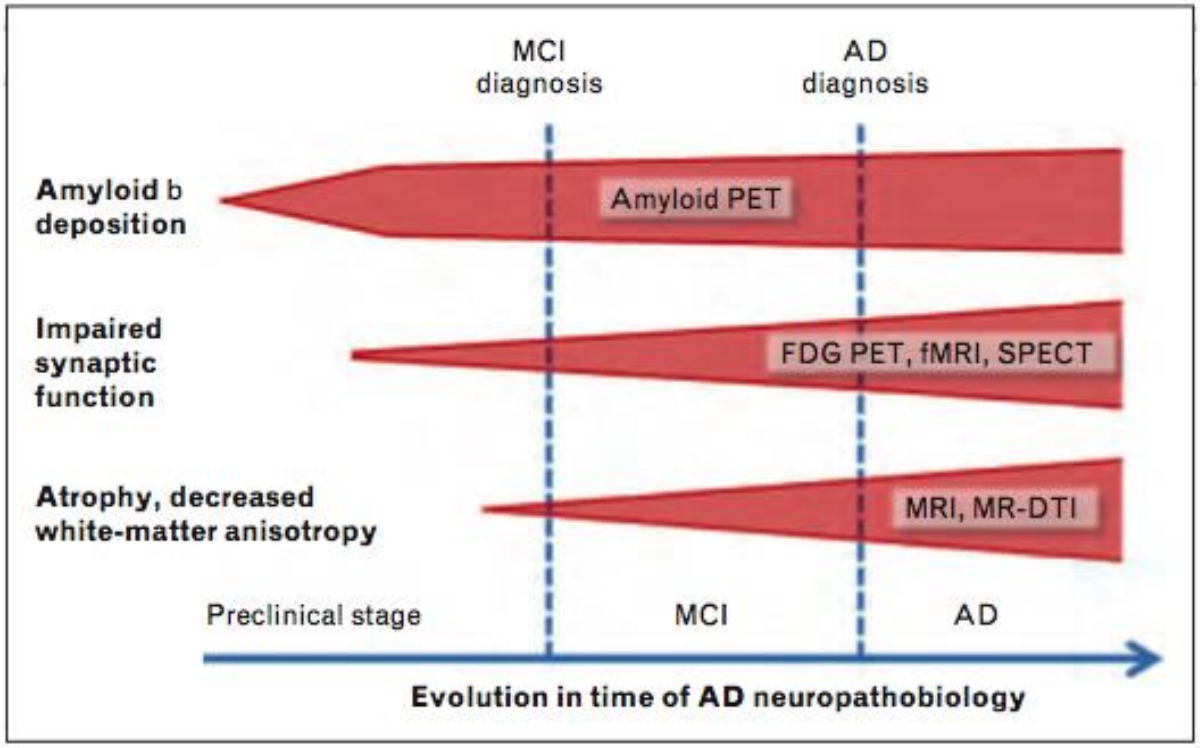


FIGURE 1. Neurobiological changes in the various stages in the development of Alzheimer disease, illustrated by specific neuroimaging techniques. A larger area in red indicates a greater degree of the neurobiological disorder (abeta deposition, impaired synaptic function or atrophy and decreased white-matter anisotropy). AD, Alzheimer disease; Amyloid PET, ^{11}C-PiB positron emission tomography; FDG PET, ^{18}F fluoro-deoxy-glucose PET; fMRI, functional MRI; MCI, mild cognitive impairment; MR-DTI, diffusion tensor imaging performed with functional MRI; SPECT, single-photon emission computed tomography.

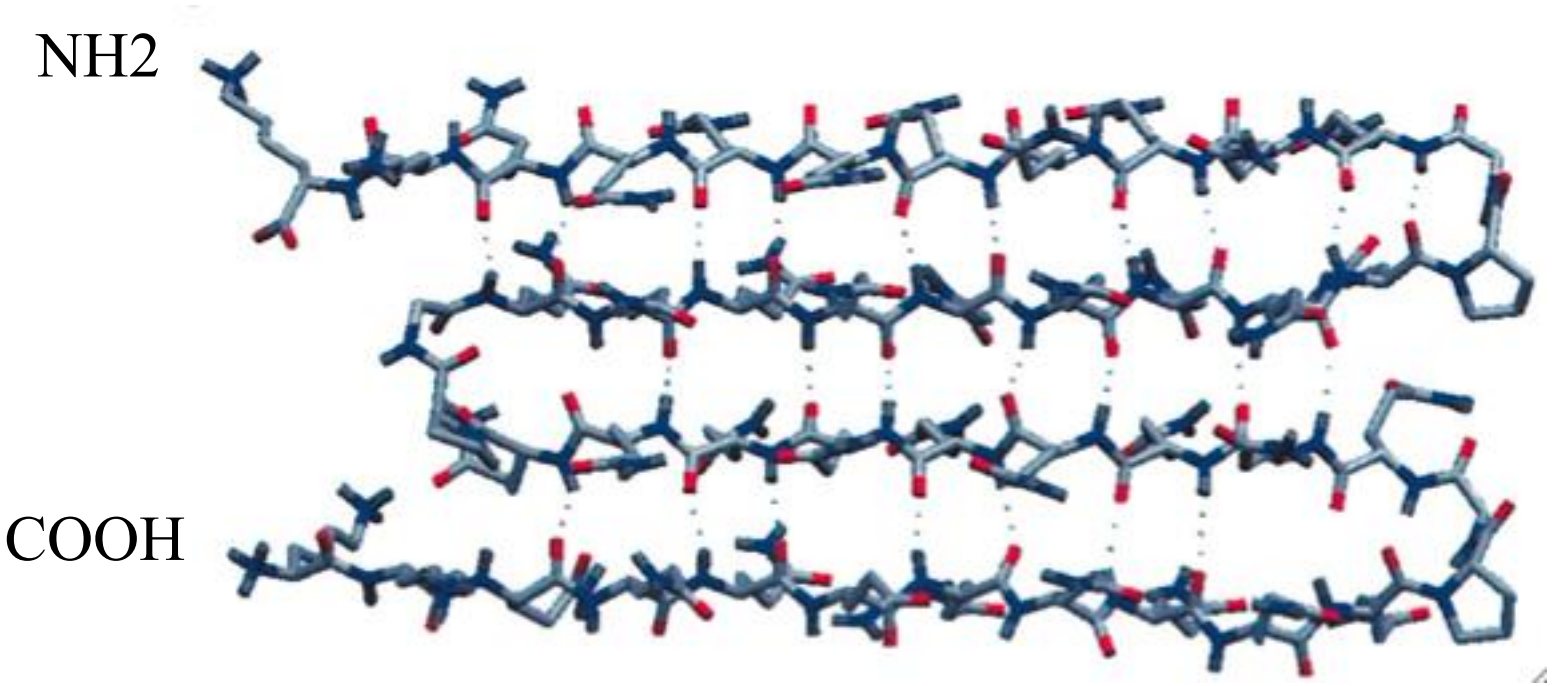
Skupne lastnosti nevrodegenrativnih bolezni

1. Intracelulalrno in/ali ekstracelularno nalaganje fibrilarnih proteinskih agregatov.
2. Disfunkcija mitohondrijev, povečan oksidativni stres in proizvodnja reaktivne oblike kisika (ROS – reactive oxygen species).
3. Povečanje apoptoze.
4. Zmanjšana razgradnja proteinov zaradi poškodbe proteasoma.
5. Zmanjšana avtofagija in razgradnja proteinov v lizosomih.
6. Eksocitotoksičnost.

Proteinski agregati

Nalaganje proteinskih fibrilov in tvorba agregatov

Amiloid: amiloidni fibrili so hidrofobni filamenti, širine ~10nm in dolžine 0,1-10mM. Traku podobna β-struktura se tvori iz β-verig in zavojev. Taki fibrili so pogosti pri večini NDB.

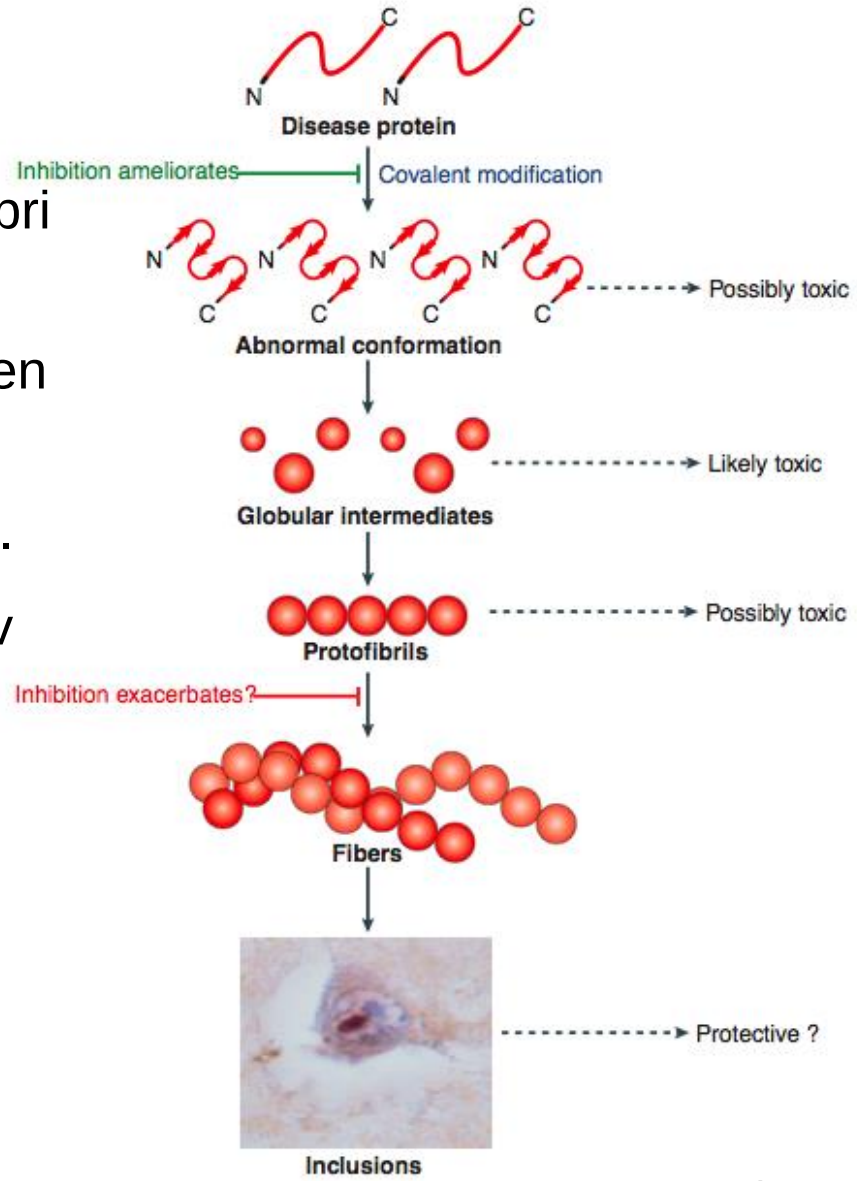


Ross et al., Nat Med 2004

Kako proteini agregirajo in tvorijo amiloide/netopne fibrile?

Dejavniki, ki lahko sprožijo agregacijo proteinov:

1. Oksidacija proteinov (α-sinuklein pri PB).
2. Kelacija s kovino (Prionska bolezen in AB).
3. Specifično rezanje proteinov (AB).
4. Neučinkovita razgradnja proteinov z β-strukturo / poškodba proteasoma (PB, AB, ALS, Prionska bolezen, HB).
5. Spremembe v zunajceličnem pH (AB).



Ross et al., Nat Med 2004

Ali so agregati toksični ali zaščitni???

Različne hipoteze pri različnih nevrodegeneracijah.

AB: Plaki in topna Aβ so v korelaciji z napredovanjem bolezni. Vendar naj bi bili oligomeri Aβ najbolj toksična oblika.

PB: inkluzijska telesca ne sledijo razvoju bolezni.

HD: možno je, da so agregati prisotni samo v preživelih nevronih.

Termodinamika zvijanja proteinov

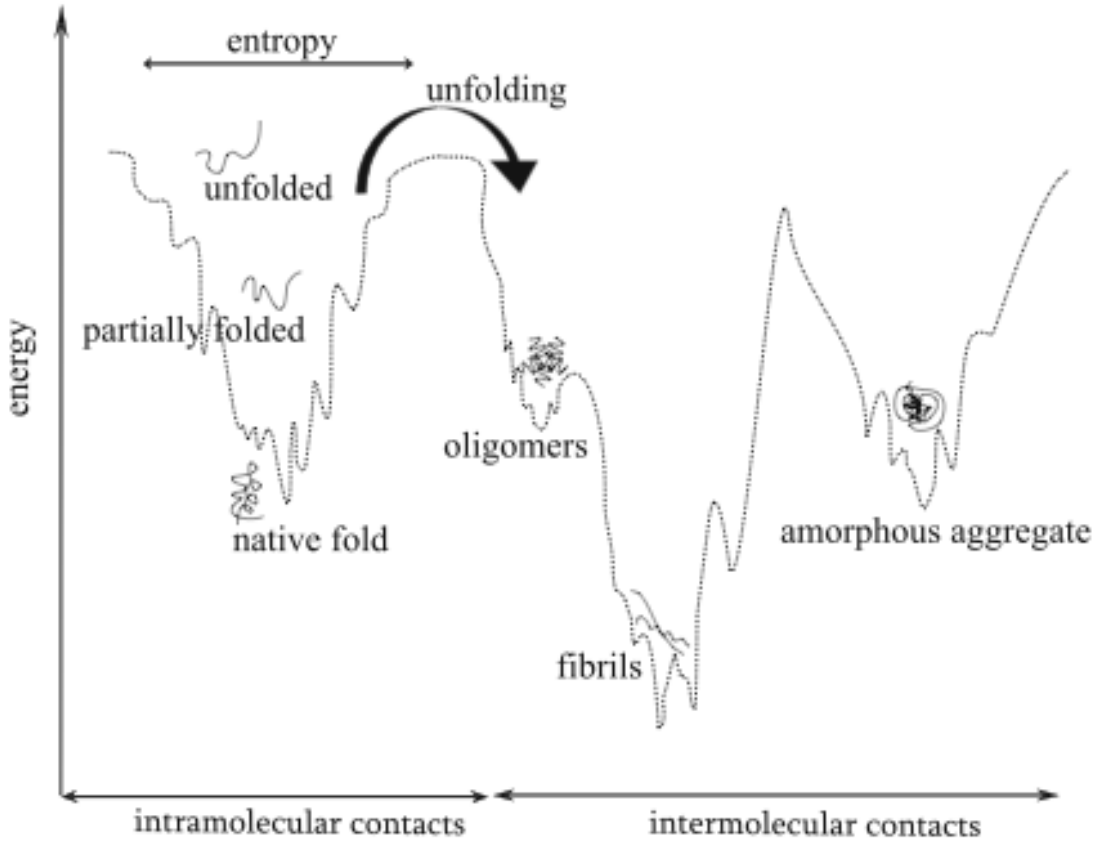


Figure 2. Energy state of protein folding under physiological and misfolding conditions. The shape of the graph shows energy state of the protein conformations moving toward its native or misfolded condition through multiple inter- and intramolecular contact arrangements.

Herczenik et al., FASEB J 2008

Koraki, ki vodijo k tvorbi agregatov

Razvitje

Nukleacija: proteini se reverzibilno vežejo na rastoče jedro.

Agregacija: proteini se ireverzibilno vežejo na jedro in tvorijo velike agregate.

Dejavnki, ki vplivajo na denaturacijo in razvijanje proteinov:

1. Mutacije.
2. Oksidacija.
3. Vezava ionov.
4. Nivo glukoze.
5. Spremembe v pH.
6. Koncentracija monomera.

Shematski prikaz napačnega zvijanja proteinov

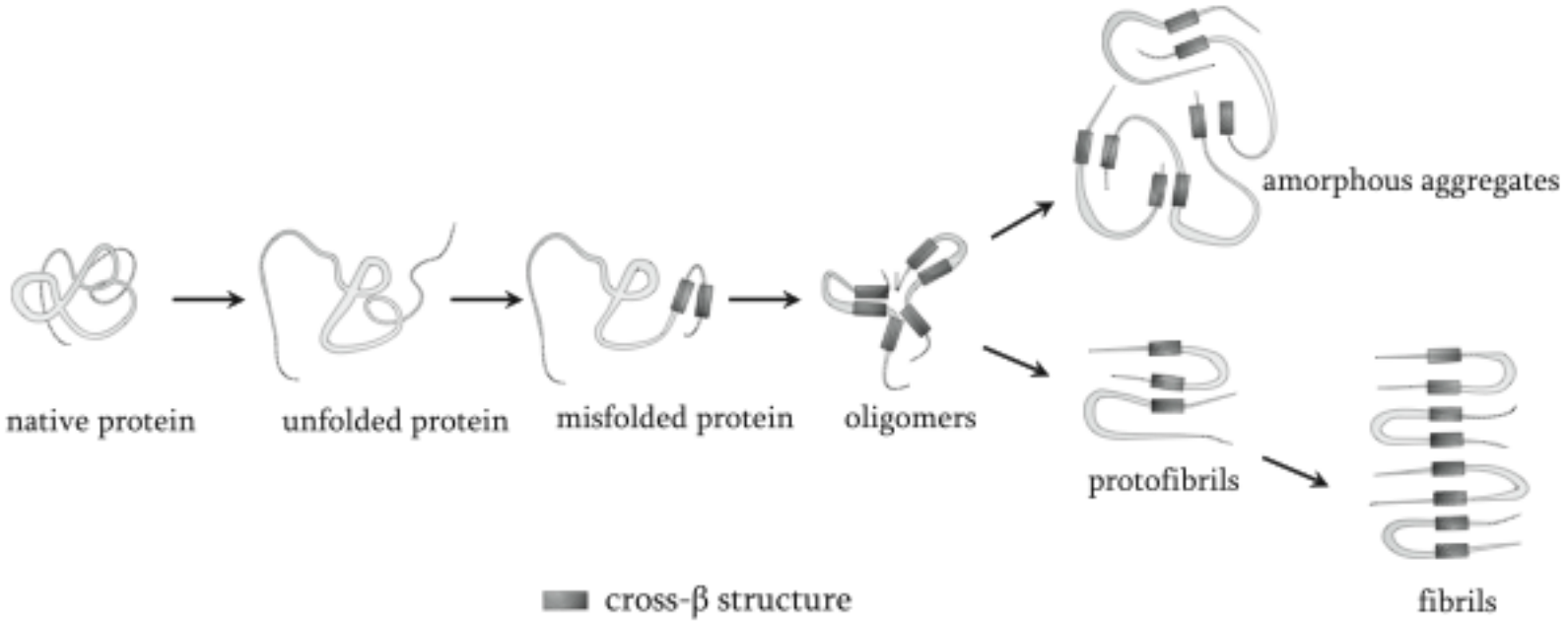


Figure 3. Protein misfolding and aggregation. Under certain circumstances such as pH or temperature change, mechanical stress, glycation, or oxidation, proteins undergo conformational changes that result in unfolding and partial misfolding that is associated with the tendency to aggregate. During aggregation, proteins can obtain a range of different structural appearances, which are generally enriched in cross-β structure, including intermediates varying from unordered amorphous aggregates to ordered fibrils that are called amyloid.

Herczenik et al., FASEB J 2008

Tabela bolezni z napačnim zvijanjem proteinov

TABLE 2. *Summary of protein misfolding diseases and the related proteins and peptides in human.*

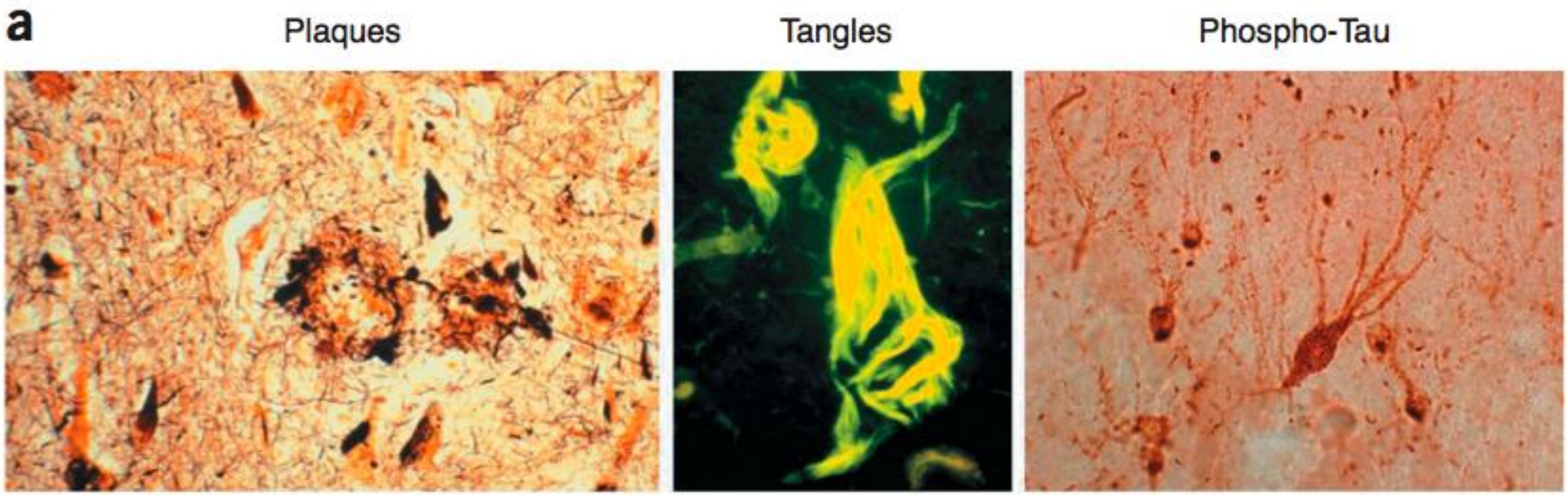
Disease	Associated proteins	Affected tissues	Reference
Amyloidosis—systemic			
Primary systemic amyloidosis	Ig light chain	Most tissues	52
Ig heavy-chain-associated amyloidosis	Ig heavy chain	Most tissues	99
Secondary (reactive) systemic amyloidosis	SAA	Most tissues	100
Senile systemic amyloidosis	Transthyretin	Microvasculature	11
Hemodialysis-related amyloidosis	β_2-Microglobulin	Osteoarticular tissues	101
Hereditary systemic ApoAI amyloidosis	ApoA-I	Liver, kidney, heart	102, 103
Hereditary systemic ApoAII amyloidosis	ApoA-II	Kidney, heart	103, 104
Finnish hereditary amyloidosis	Gelsolin	Most tissues	105
Hereditary lysozyme amyloidosis	Lysozyme	Kidney, liver	106
Hereditary cystatin C amyloid angiopathy	Cystatin C	Most tissues	107
Amyloidosis—localized			
Injection-localized amyloidosis	Insulin	Skin, muscles	108
Hereditary renal amyloidosis	Fibrinogen	Kidney	109
Senile seminal vesicle amyloid	Lactoferrin, seminogelin	Seminal vesicles	110, 111
Familial subepithelial corneal amyloidosis	Lactoferrin	Cornea	112
Cataract	Crystallin	Eye	113
Medullary thyroid carcinoma	Calcitonin	Thyroid tissues	114
Neurodegenerative diseases			
Alzheimer's disease	Amyloid-β, tau	Brain	115, 116
Parkinson's disease	α-Synuclein	Brain	117
Lewy-body dementia	α-Synuclein	Brain	118
Huntington's disease	Huntington	Brain	119
Spongiform encephalopathies	Prion	Brain, peripheral nervous system	120, 121
Hereditary cerebral hemorrhage with amyloidosis	Cystatin C	Cerebral vasculature	122, 123
Amyotrophic lateral sclerosis	Superoxide dismutase 1	Brain	124
Familial British dementia	Abri	Brain	125
Familial Danish dementia	ADan, amyloid-β	Brain	126
Familial amyloidotic polyneuropathy	Transthyretin	Peripheral nervous system	127
Frontotemporal dementias	Tau	Brain	128
Other diseases			
Diabetes mellitus	IAPP, amylin	Pancreas (islet)	129
Atherosclerosis	Modified LDL	Arteries	130
Sickle cell anemia	Hemoglobin	Erythrocytes	131, 132

ABri, British amyloid peptide; ADan, Danish amyloid peptide; IAPP, islet amyloid polypeptide; Ig, Immunolglobulin; SAA, serum amyloid A.

Herczenik et al., FASEB J 2008

Agregati pri AB

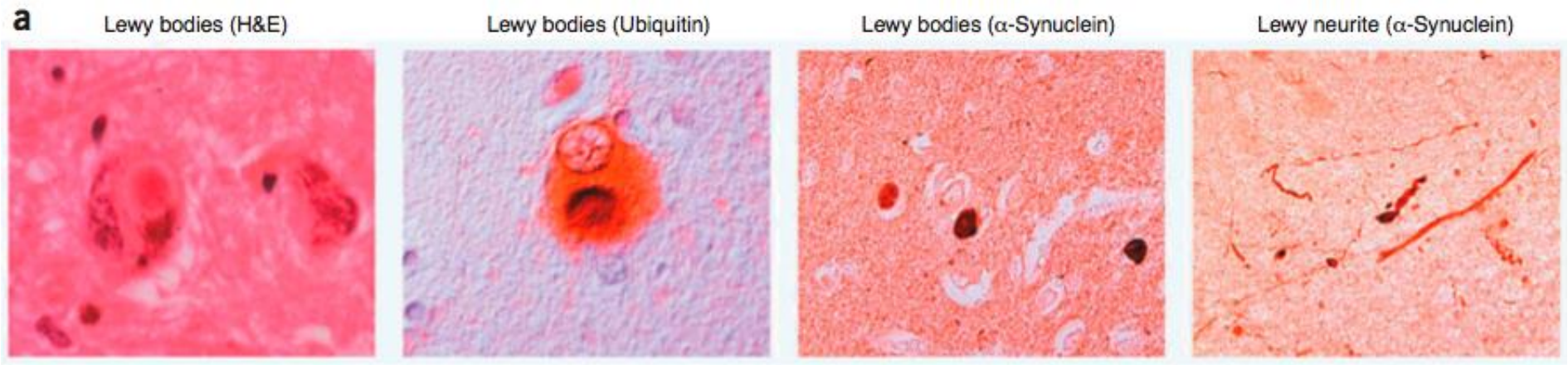
Alzheimerjava bolezen: Tvorijo se izvencelični agregati - **Aβ plaki** in znotrajcelične **nevrofibrilarne pentelje,** ki so agregati hiperfosforiliranega proteina tau.



Bossy-Wetzel et al., Nat Med 2004

Agregati pri PB

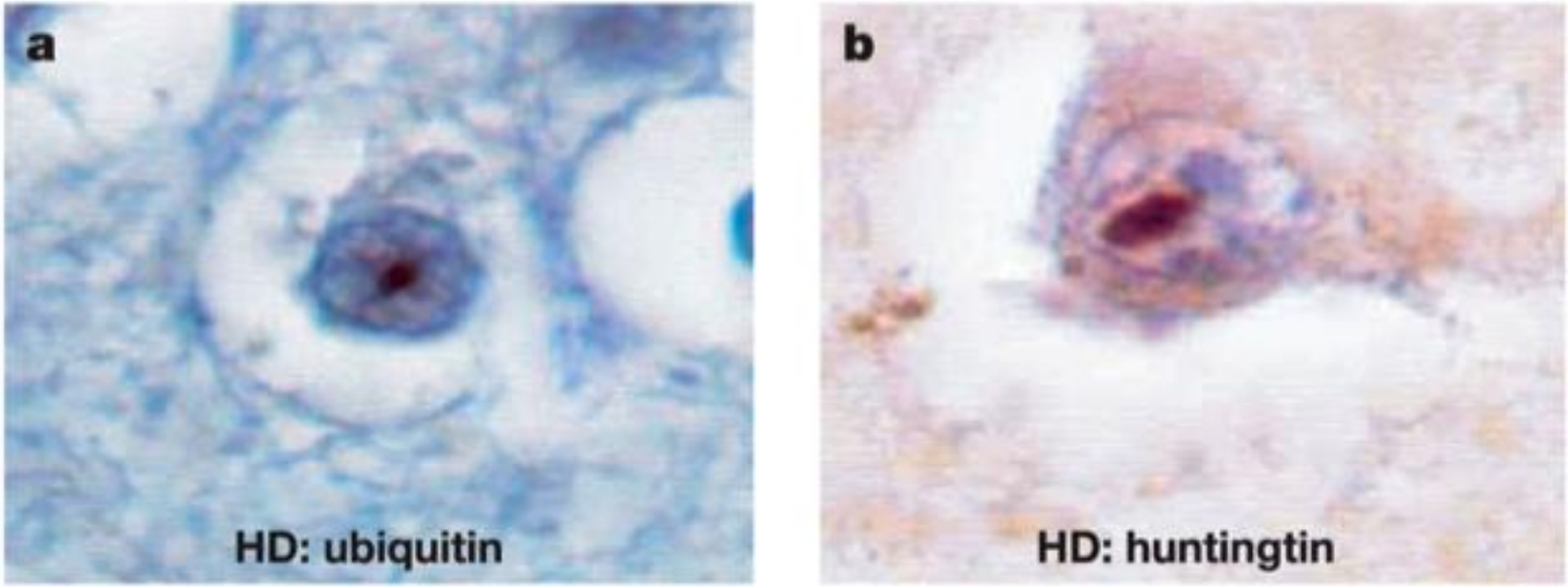
Parkinsonova bolezen: karakterizirana z izgubo dopaminergičnih nevronov in z intracelularnimi agregati - **Lewyjevimi telesci**. Ta so večinoma sestavljena iz α-sinukleina in ubikvitina. Druge komponente Lewyjevih telesc so proteasom, citoskeletni proteini in drugi proteini, ki se vežejo z α-sinukleinom.



Bossy-Wetzel et al., Nat Med 2004

Agregati pri HB

Huntingtonova bolezen: je progresivna NDB, karakterizirana s CAG ponovitvami, ki nosijo zapis za poly Q motiv v N-koncu proteina **huntingtina**. Nastop bolezni obratno korelira s številom CAG ponovitev. Huntingtin tvori inkluzijska telesca.



Ross et al., Nat Med 2004

Agregati pri CJB

Prionska bolezen: povzročijo jo prioni s pomočjo zunanjih stimulusov ali genetskih mutacij. Spememebe v prionih vodijo v zunaj- in znotraj-celično kopičenje amiloidnih agregatov – plakov, podobnih tistim pri AB, in pozitivnih na barvanje prionov. Replikacija in kopičenje PrP^{sc} , ki ni občutljiva na proteaze, verjetno povzročita tvorbo fibrilov in odlaganje plakov.

AB CJB

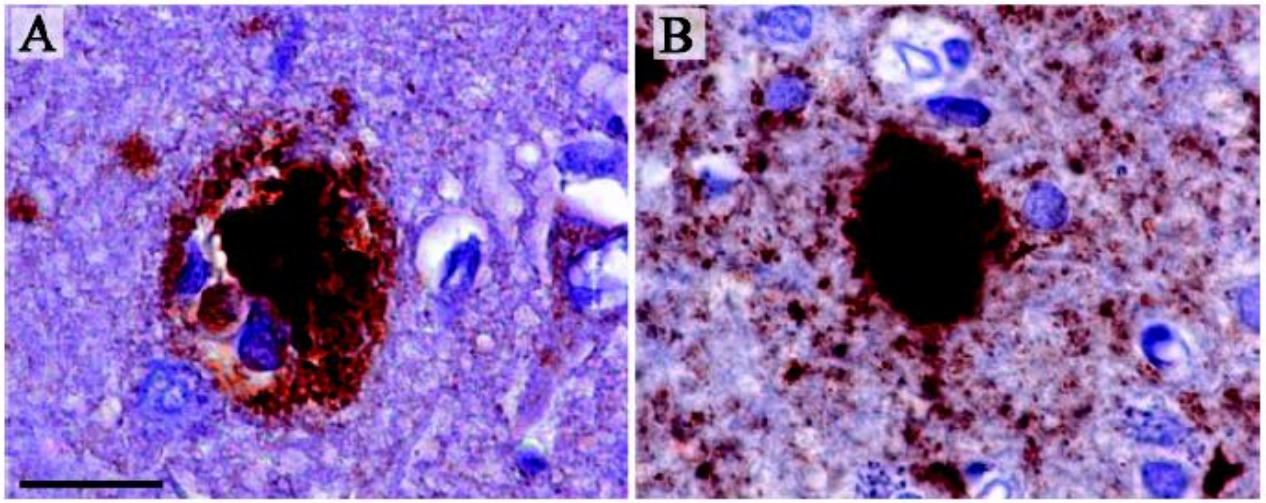
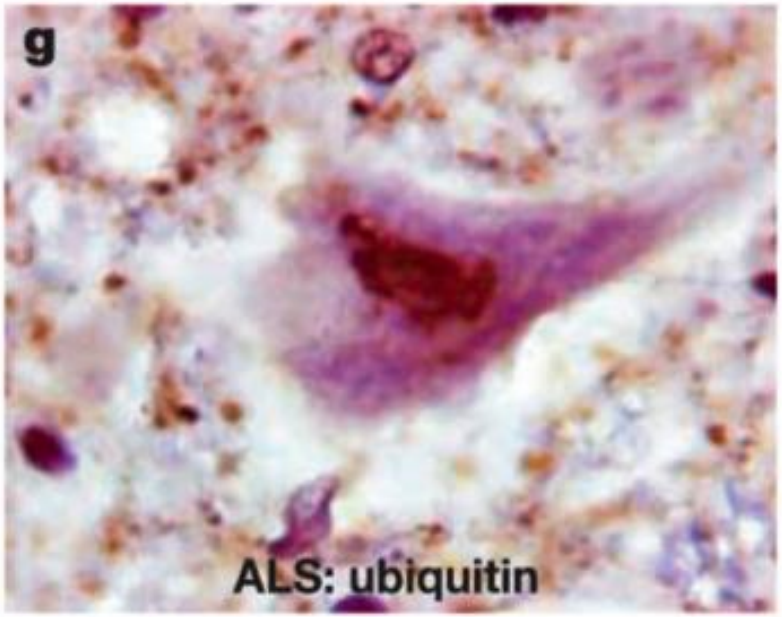
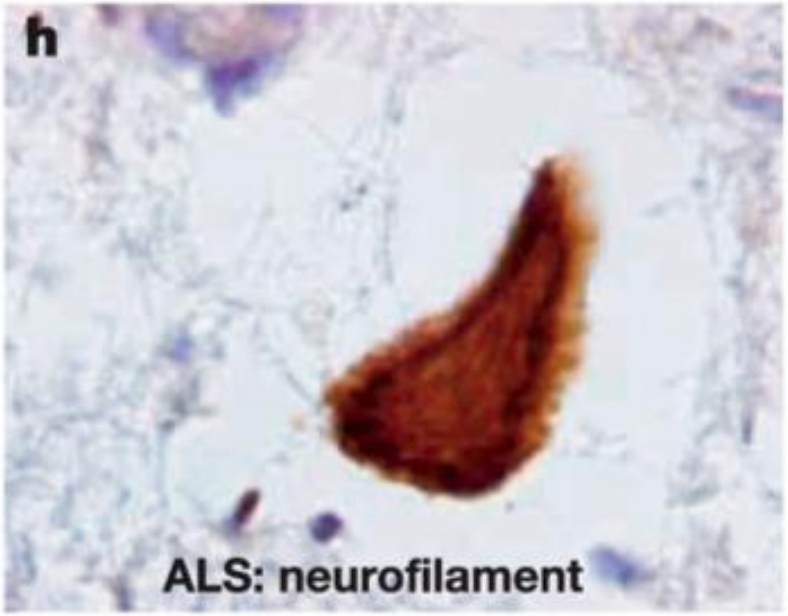


Fig. 1. Both Alzheimer's and prion diseases are characterized by the deposition of pathological proteins in the brain, often in the form of plaques. The brown color is indicative of immunostained cortical deposits of the Aβ peptide and of the PrP^{Sc} protein in brains of patients suffering from Alzheimer's disease (**A**) and Creutzfeldt-Jakob disease (**B**), respectively. Scale bar: 20 μm.

Agregati pri ALS

ALS: progresivna fatalna bolezen, ki jo povzročijo degeneracija spodnjih motoričnih nevronov v lateralnem rogu hrbtenjače in zgornjih motoričnih nevronov iz korteksa. Netopne citoplazemske inkluzije so značilne za možgane ALS pacientov. Inkluzije so označene tudi z ubikvitinom.





Ross et al., Nat Med 2004

Zaključek:

- Za večino NDB je značilno znotraj- ali zunaj-celično kopičenje netopnih molekul.
- Ni znano, če je to vzrok ali posledica bolezni.
- Predpostavljajo, da naj bi bile prve molekule najbolj toksične, ker imajo nenormalne interakcije z drugimi proteini.
- Proteini z β strukturami, ki tvorijo netopne agregate, naj bi bili navzgor od kaskade dogodkov, ki vodi v nevrodegeneracijo (vse bolezni imajo skupne faktorje in da imajo povečano izražanje proteinov z β strukturami.

Box 2 | **Terminology of aggregation**

Part of the difficulty in the field has been the use of vague or conflicting terminology. We propose the following definitions for terms commonly used in the field.

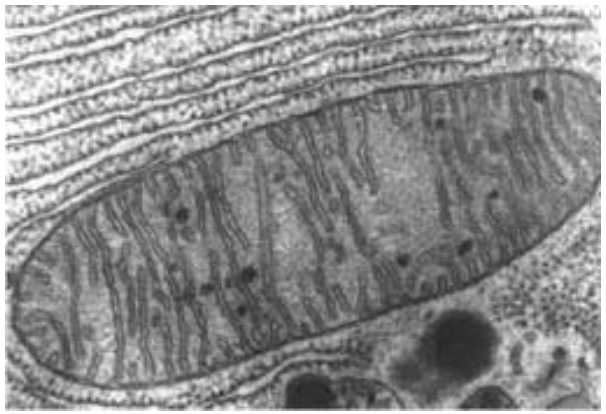
- Aggregation: Any abnormal association of misfolded proteins (or parts of proteins). Aggregation is a process that begins with the abnormal association of as few as two molecules and that has the potential to form larger structures that are visible by microscopy. This definition is therefore a morphological one. Small aggregates can be soluble under many conditions, but large aggregates are insoluble under physiological conditions. At the biochemical level, aggregation can be any abnormal association of misfolded proteins (or parts of proteins). This definition is important, as biochemical aggregates might not be visible using conventional microscopic techniques.
- Aggregation intermediate: A putative metastable molecular species that may be 'on' or 'off' the pathway to fibril formation and that forms large aggregates and inclusions.
- Aggresome: A cytoplasmic inclusion body located in the perinuclear region near the centriole that results from the collection of small aggregates from other parts of the cell through active, microtubule-based transport.
- Amyloid: Insoluble fibrillar aggregates composed of amyloid fibrils, which can be seen using electron microscopy (EM) or highlighted by birefringent staining using Congo Red.
- Amyloid fibril: A thermodynamically stable, structurally organized, highly insoluble, filamentous protein aggregate. The amyloid fibril is composed of repeating units of β-sheets aligned perpendicular to the fibre axis, with a distinctive X-ray fibre diffraction pattern ('cross β') that is similar to crystalline silk and consistent with high β-sheet content.
- Amorphous aggregate: protein aggregates without amyloid fibrils, often with a granular appearance when viewed by EM.
- Annular protofibril (or annular oligomeric aggregate). An annular species that has been suggested to form a pathogenic pore in biological membranes.
- Inclusion body: A large accumulation of aggregated material in a cell, visible by light microscopy, often well demarcated from other cellular constituents. It can contain amyloid fibrils and amorphous aggregates, as well as other material. Classic examples are Lewy bodies of Parkinson's disease and intranuclear inclusions of Huntington's disease.
- Microaggregate: A small accumulation of aggregated protein, detected by EM or other specialized microscopic technique. It can consist of any of the species of aggregates defined here.
- Oligomeric aggregate: Small (approximately 5–10 nm) assembly with a globular appearance, probably comprised of about 3–50 monomers, which might represent an aggregation intermediate.
- Protofibril: Soluble, short fibril-shaped aggregated structure, usually thinner or shorter than a mature fibril, which might represent an aggregation intermediate.
- Protofilament: A proposed single strand of a mature, mutistranded amyloid fibril.

Ross et al., Nat Rev MCB 2005

Disfunkcija mitohondrijev in oksidativni stres

Disfunkcija mitohondrijev

Normalni



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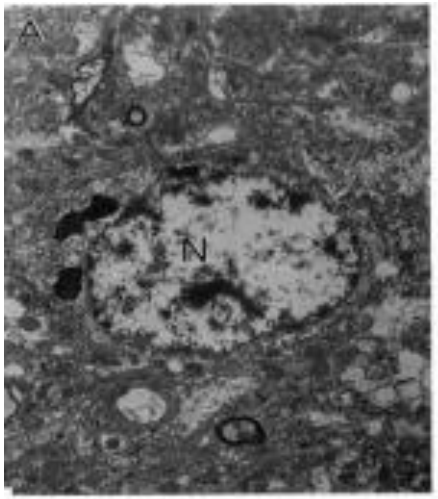
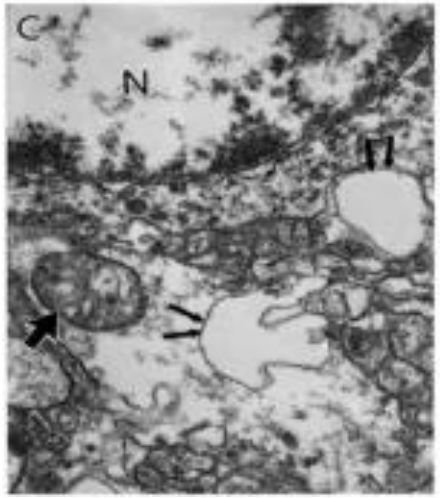
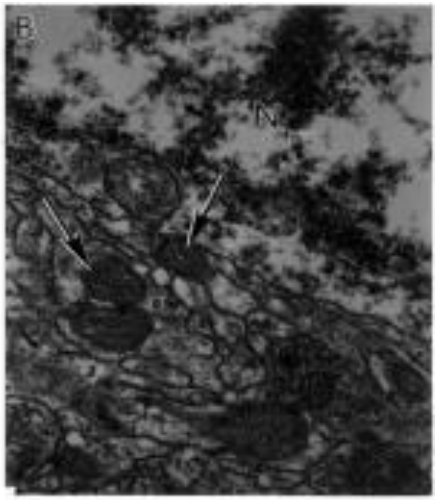
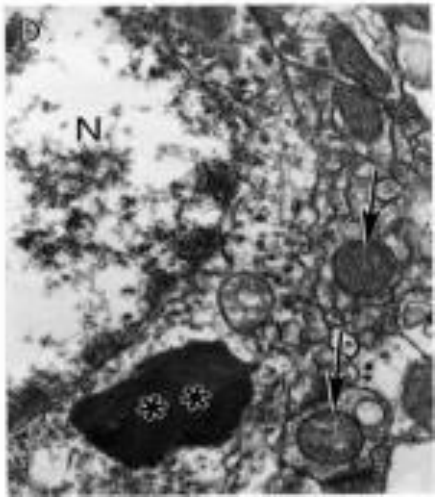
Poškodovani





Hipoksični





Pharmaceuticals 2010, 3(1), 158-187

Dinamika mitohondrijev in nevrodegeneracija

Fiziološka delitev in zlitje

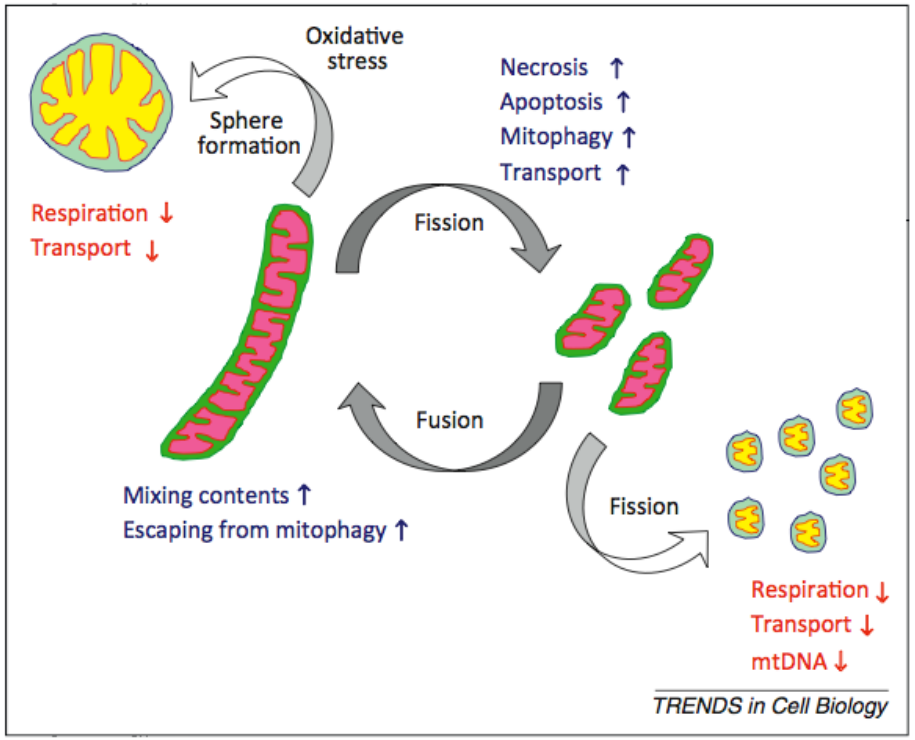


Figure 1. Cellular and physiological roles of mitochondrial dynamics. Mitochondria divide and fuse, maintaining their short tubular structures. Mitochondrial fission facilitates cell death, mitophagy, and organelle transport, whereas fusion allows content mixing and can block mitophagy. Overly fused mitochondria accumulate oxidative damage and further transform their shape into large spheres. Such large round mitochondria are associated with impaired respiration and defective in organelle transport. Excessive fragmentation can generate mitochondria that lack mtDNA, leading to impaired respiration.

Uravnoteženje ali bolezen

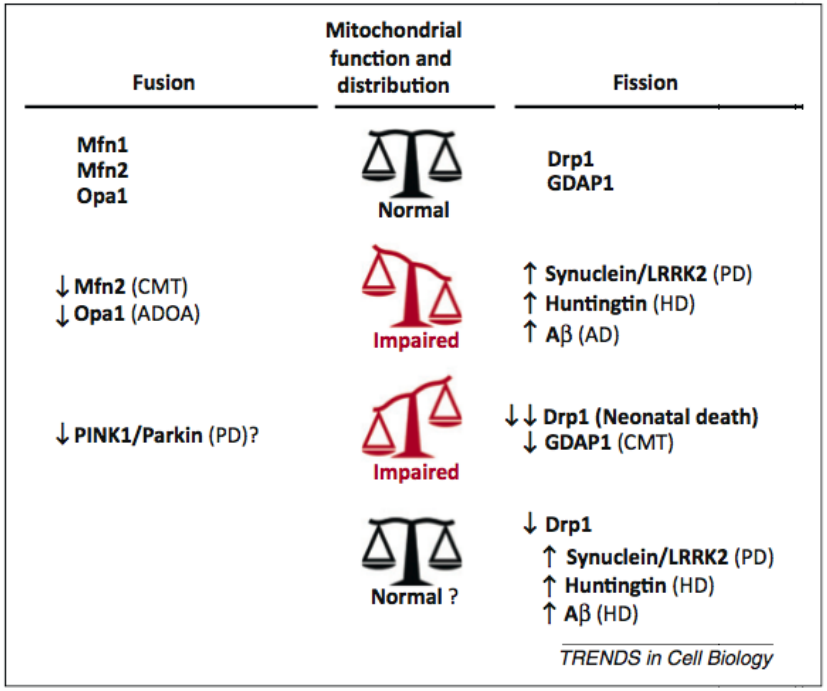


Figure 2. Disruption of the balance between mitochondrial fusion and fission in neurodegenerative disease. Mitochondrial morphology is normally regulated by the balance between mitochondrial fusion proteins (e.g., Mfn1, Mfn2, and Opa1) and fission proteins (including Drp1 and GDAP1). Disruption of either fusion or fission proteins can result in neurologic disease. Mutation and/or overexpression of a variety of proteins implicated in neurodegenerative diseases, including PD, HD, and AD, also disrupt mitochondrial dynamics, and normalizing the fusion–fission balance may have therapeutic value. The bottom row shows one strategy in which a normal level of fission is restored by decreasing Drp1, thus compensating for increased fission by disease proteins. It remains unknown if normalizing mitochondrial morphology will restore function. Abbreviations: AD, Alzheimer's disease; ADOA, autosomal dominant optic atrophy; CMT, Charcot–Marie–Tooth neuropathy; HD, Huntington's disease; PD, Parkinson's disease.

Itoh et al., Trends in Cell Biology 2012

Med sintezo molekul vode nastanejo številne reaktivne kisikove spojine (Reactive Oxygen Species -ROS)

Poškodbe mitohondrijev zaradi staranja ali disfunkcije proteinov vodijo v izhajanje reaktvnih molekul iz mitohondrijev v citosol skozi kanale (ki so odvisni od napetosti).

$O_2^{-\cdot}$ Superoksidni ion and e^-

Ta fenomen poveča oksidativni stres in vodi v nastanek drugih ROS, ki so toksični za celico.

Zakaj je oksidativni stres toksičen?

Zato ker oksidira:

1. proteine
2. lipide
3. kateholamine (adrenalin, noradrenalin, dopamin)
4. DNA

Izgubi se njihova funkcija, kar vodi v nepravilno celično aktivnost in posledično smrt.

Antioksidacijski proteini in encimi

Superoxide Dismutase SOD: spremeni superoksid $O_2^{-\cdot}$ v vodikov peroksid H_2O_2

-SOD1 (CuZnSOD): večinoma je lokaliziran v citosolu, jedru in medmembransem prostoru mitohondrijev. Ima visok nivo izražanja v možganih in hrbtenjači. Več kot 100 mutacij je povezanih z ALS.

-SOD2 (MnSOD): potreben za mitohondrijsko celovitost. Ce se nitrozilira, SOD2 izgubi funkcijo.

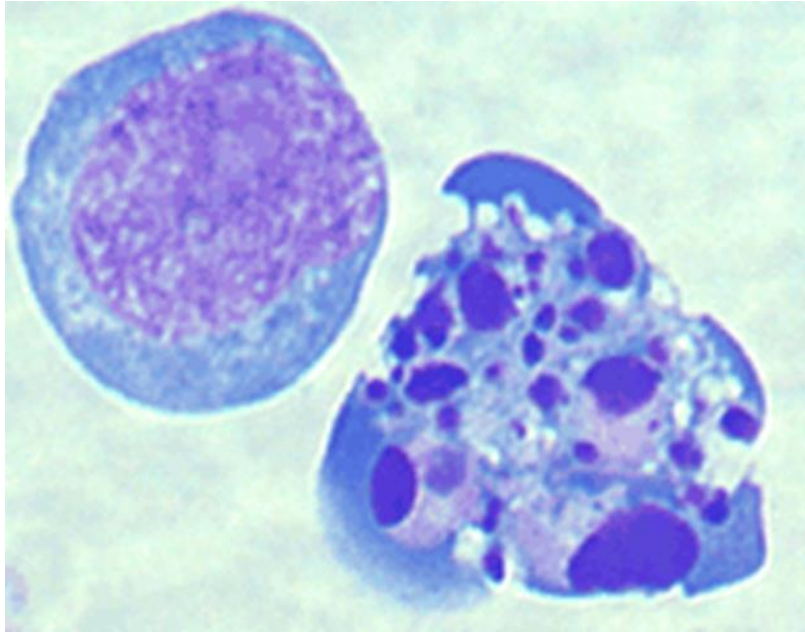
-SOD3: se nizko izraža v možganih.

Apoptoza

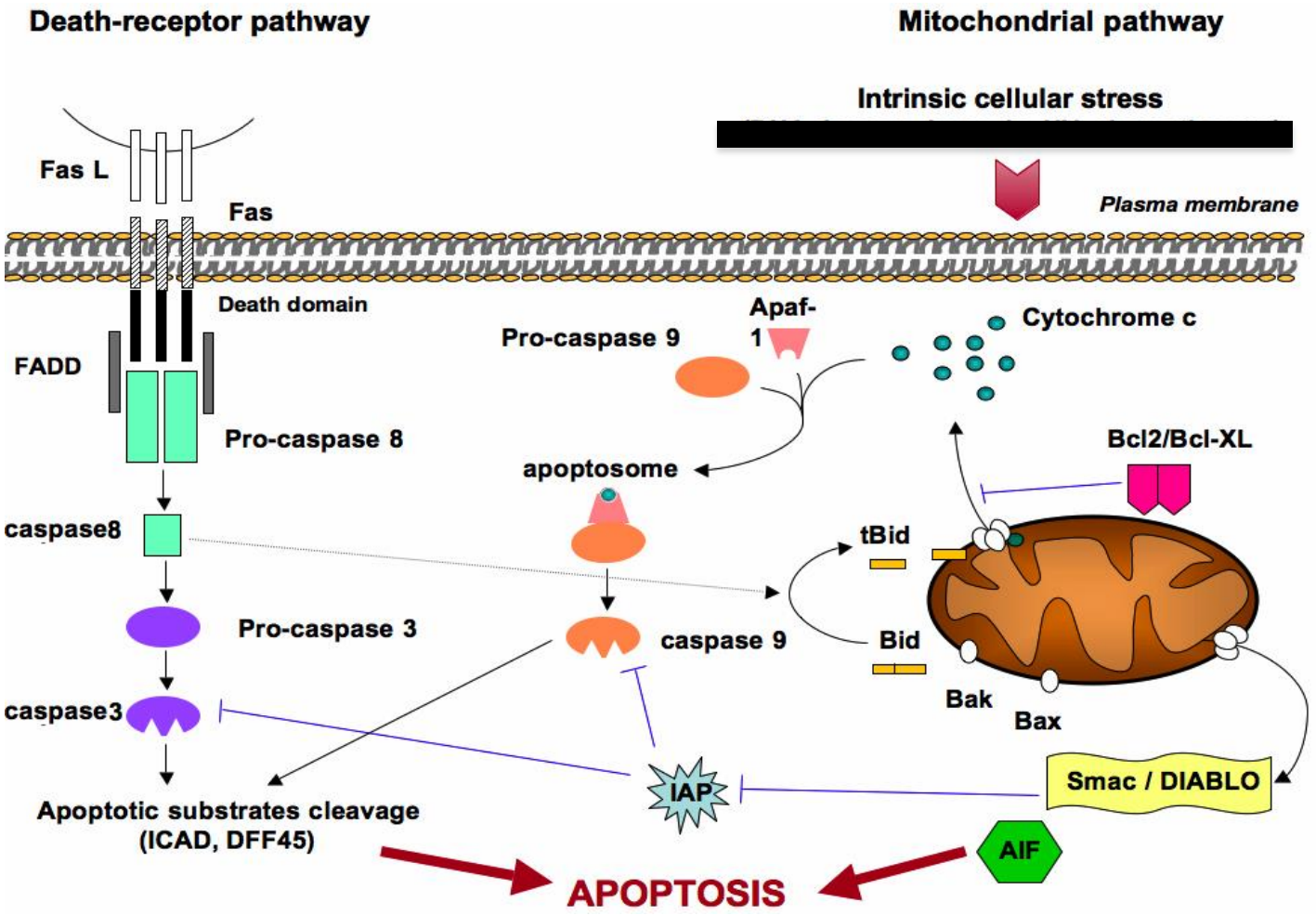
Apoptoza: mehanizem programirane celične smrti

Koraki, ki vodijo v apoptozo:

1. Kondenzacija jedrnega kromatina.
2. Citosolni organeli se skoncentrirajo (zbijejo), celična membrana se izviha (blebbing).
3. Zmanjša se volumen celice.
4. Spremeni se celična membrana.
5. Fagocitoza.



Apoptoza – 2 poti



Endogena pot je povezana z oksidativnim stresom.

Eksogena pot apoptoze je povezana s Huntingtonovo boleznijo, kapjo, Parkinsonovo boleznijo (ne pa z AB).

http://www.bioscience.org/

Razgradnja proteinov

UPP in lizosomalne poti razgradnje proteinov

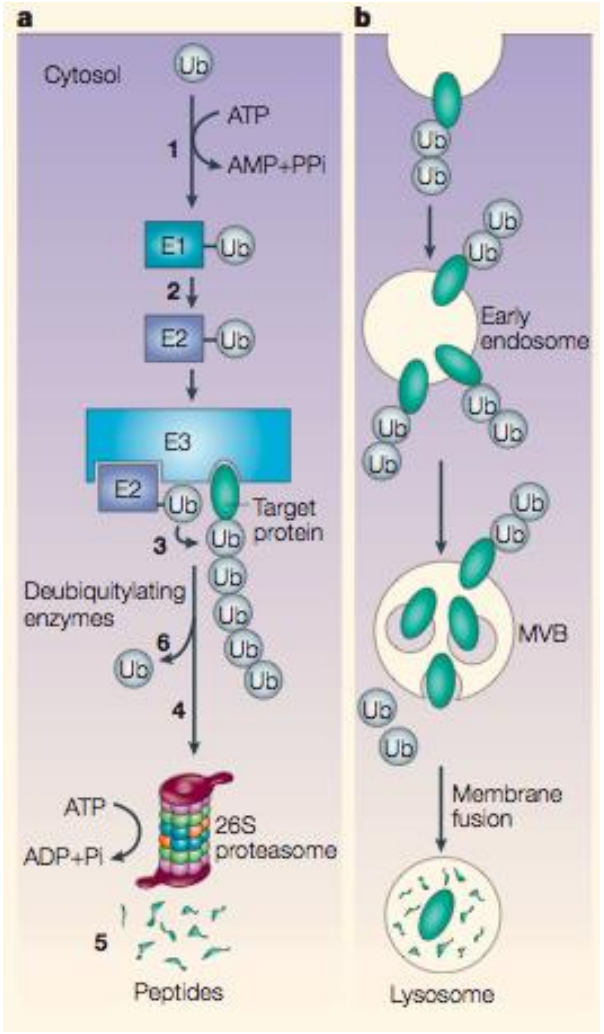


Figure 2 | **Some of the various functions of modification by ubiquitin and ubiquitin-like proteins.** Proteasome-dependent degradation of cellular proteins (**a**). Ubiquitin is activated by the ubiquitin-activating enzyme (E1; step 1), and its transfer to a ubiquitin carrier protein (ubiquitin-conjugating enzyme; E2; step 2) then follows. The E2 enzyme and the protein substrate both bind specifically to a particular ubiquitin-protein ligase (E3), and the activated ubiquitin moiety is then transferred to the protein substrate (step 3). The successive conjugation of ubiquitin moieties generates a polyubiquitin chain that functions as a signal to target the protein substrate to the 26S proteasome for degradation (step 4). The substrate is degraded to short peptides (step 5), and reusable ubiquitin is released by deubiquitylating enzymes (step 6). Mono- or oligoubiquitylation (**b**) targets membrane proteins for degradation in the lysosome.

Mehanizmi avtofagije

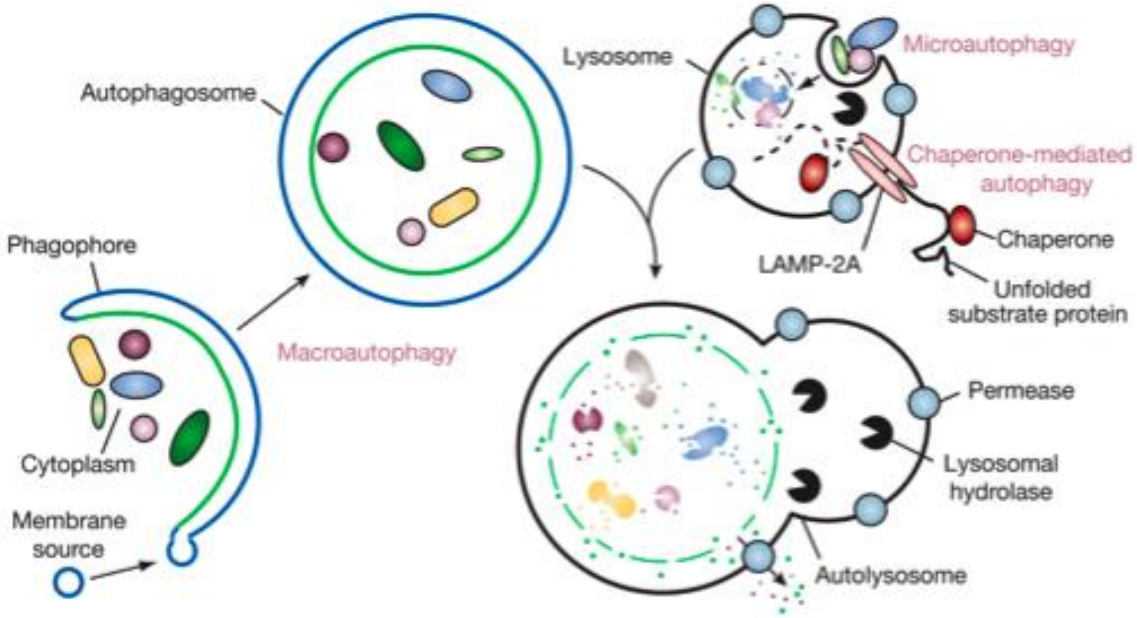
Makroavtofagija: sistem za razgradnjo celih regij citosola, vključno z organeli.

Pomembno pri nevrodegeneraciji, ker uniči nefunkcionalne organele kot je mitohondrij. V razgradnjo vodi velike agregate, ki lahko zmotijo celične funkcije.

Od šaperonov odvisna avtofagija (CMA or Chaperone Mediated Authophagy): vrsta avtofagije, kjer se odstranijo izbrani topni proteini. Napake v razgradnji napačno zvitih proteinov so povezane s CMA.

Mikroautofagija: slabo karakterizirana pri sesalcih.

Vrste avtofagije



Microautophagy refers to the sequestration of cytosolic components directly by lysosomes through invaginations in their limiting membrane. The function of this process in higher eukaryotes is not known, whereas microautophagy-like processes in fungi are involved in selective organelle degradation. In the case of macroautophagy, the cargoes are sequestered within a unique double-membrane cytosolic vesicle, an autophagosome. Sequestration can be either nonspecific, involving the engulfment of bulk cytoplasm, or selective, targeting specific cargoes such as organelles or invasive microbes. The autophagosome is formed by expansion of the phagophore, but the origin of the membrane is unknown. Fusion of the autophagosome with an endosome (not shown) or a lysosome provides hydrolases. Lysis of the autophagosome inner membrane and breakdown of the contents occurs in the autolysosome, and the resulting macromolecules are released back into the cytosol through membrane permeases. CMA involves direct translocation of unfolded substrate proteins across the lysosome membrane through the action of a cytosolic and lysosomal chaperone hsc70, and the integral membrane receptor LAMP-2A (lysosome-associated membrane protein type 2A).

Nature. 2008 February 28; 451(7182): 1069–1075

Vpliv avtofagije na človeške bolezni

Neurodegeneration

Pro: Basal autophagy is a homeostatic process that prevents intracellular proteins from accumulating to toxic levels.

Con: Inefficient lysosomal clearance results in intracellular accumulation of autophagosomes, which may process the amyloid precursor protein into toxic forms.

Myopathies

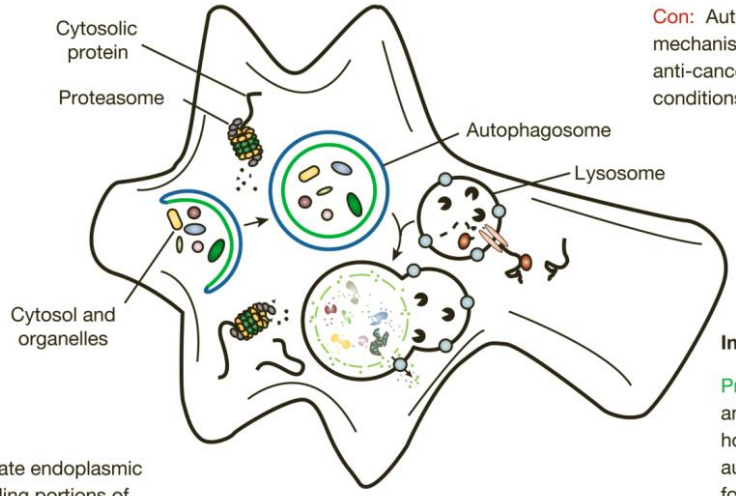
Pro: Autophagy prevents aggregate-prone protein accumulation that leads to physiological dysfunction.

Con: Autophagy may contribute to muscle wasting and defective autophagosome clearance may interfere with cellular function.

Cancer

Pro: Autophagy acts in tumour suppression by removing damaged organelles and possibly growth factors, and reduces chromosome instability.

Con: Autophagy acts as a cytoprotective mechanism that helps cancer cells resist anti-cancer treatments and survive in conditions of low nutrient supply.



Ageing

Pro: Autophagy removes damaged organelles and can limit production of reactive oxygen species.

Liver disease

Pro: Autophagy can alleviate endoplasmic reticulum stress by degrading portions of the organelle containing misfolded proteins.

Con: Excessive autophagy may cause liver damage.

Heart disease

Pro: Autophagy may be protective during ischaemia and pressure overload.

Con: Autophagy is harmful during reperfusion.

Infection and immunity

Pro: Intracellular bacteria, viruses and protozoans are removed from host removed from host cells by autophagy, and antigens are processed for MHC class II presentation. Autophagy may prevent auto-immune and inflammatory diseases.

Con: Some microbes have evolved to subvert autophagy to establish a replicative niche.

Degradation, in particular through autophagy and the proteasome, is important in cellular physiology. Autophagy can act as a cytoprotective mechanism to prevent various diseases, and dysfunctional autophagy leads to pathology. In some cases, however, autophagy can be deleterious; for example, some microbes subvert autophagy for replication, and the cytoprotective action can allow cancer cells to resist anti-cancer treatments.

Nature. 2008 February 28; 451(7182): 1069–1075

Proteostaza: regulacija homeostaze proteinov v celici

Homeostaza: lastnost sistema, da regulira notranje okolje in vzdržuje stabilne, konstantne pogoje.

Proteostaza: kontrola koncentracije, oblike, interakcij, lokacije posameznega proteina s prilagajanjem biologije celice, najpogosteje z mehanizmi transkripcije in regulacije.

Proteostaza – homeostaza proteinov

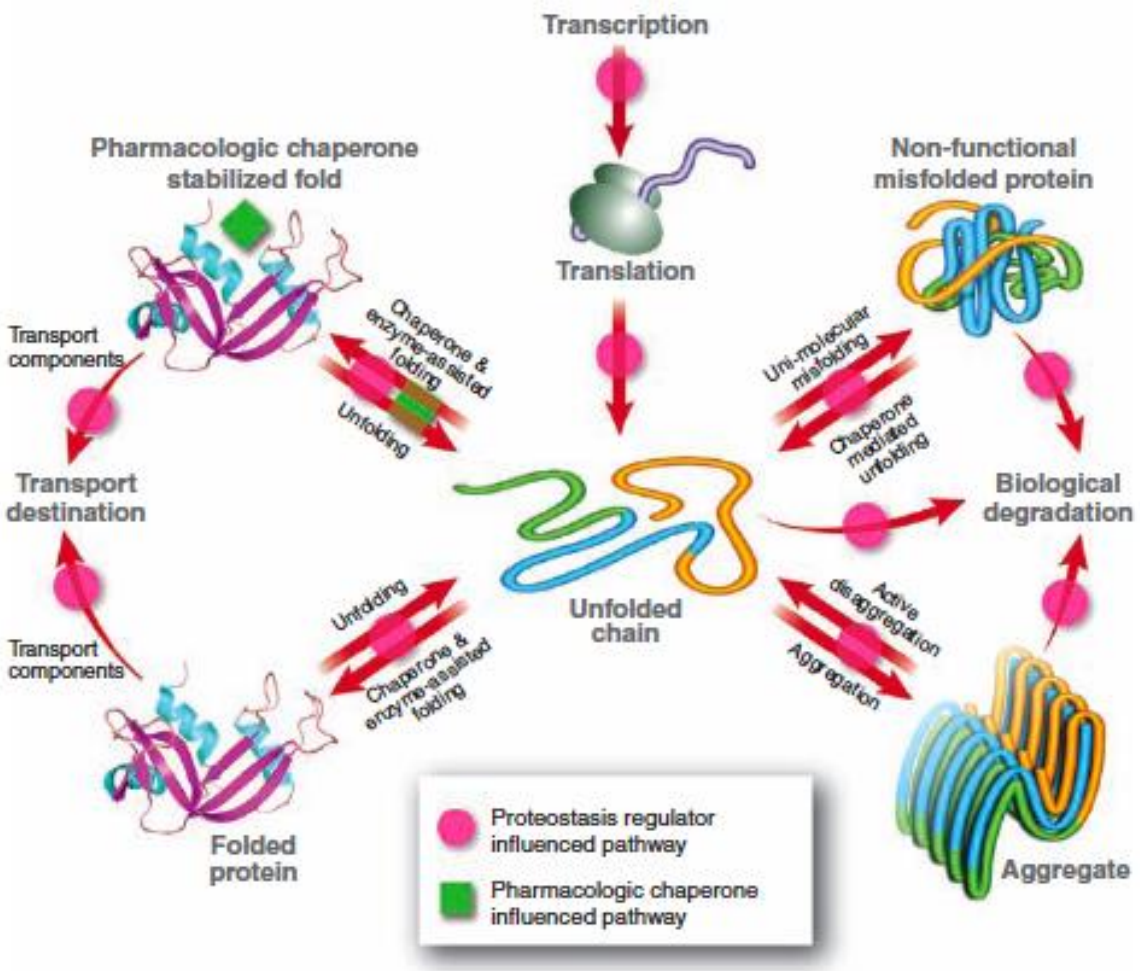


Fig. 1. A proteostasis network comprising pathways represented by the red arrows. Imbalances in proteostasis often lead to disease and, therefore, proteostasis regulators (magenta circles) that manipulate the proteostasis pathways/network can restore protein homeostasis and ameliorate both loss- and gain-of-function diseases. A finite population of the folded conformational ensemble is required for pharmacological chaperones (green squares) to enhance folding and trafficking, through a mechanism distinct from the innate biological pathways influenced by proteostasis regulators (ribonuclease A is shown; Protein Data Bank ID, 2BLP).

Vloga šaperonov pri vzdrževnju pravilne proteostaze

- Inhibicija tvorbe večjih agregatov.

- Favoriziranje razgradnje proteionov/avtofagije.

- Regulacija insulinske signalne poti.

Funkcije šaperonov

- Prepoznajo nastajajoči protein.
- Med translacijo sodelujejo pri:

a) Zorenju intermediatov zvijanja.

b) Sestavljanju multimernih kompleksov.

c) Transport organel.

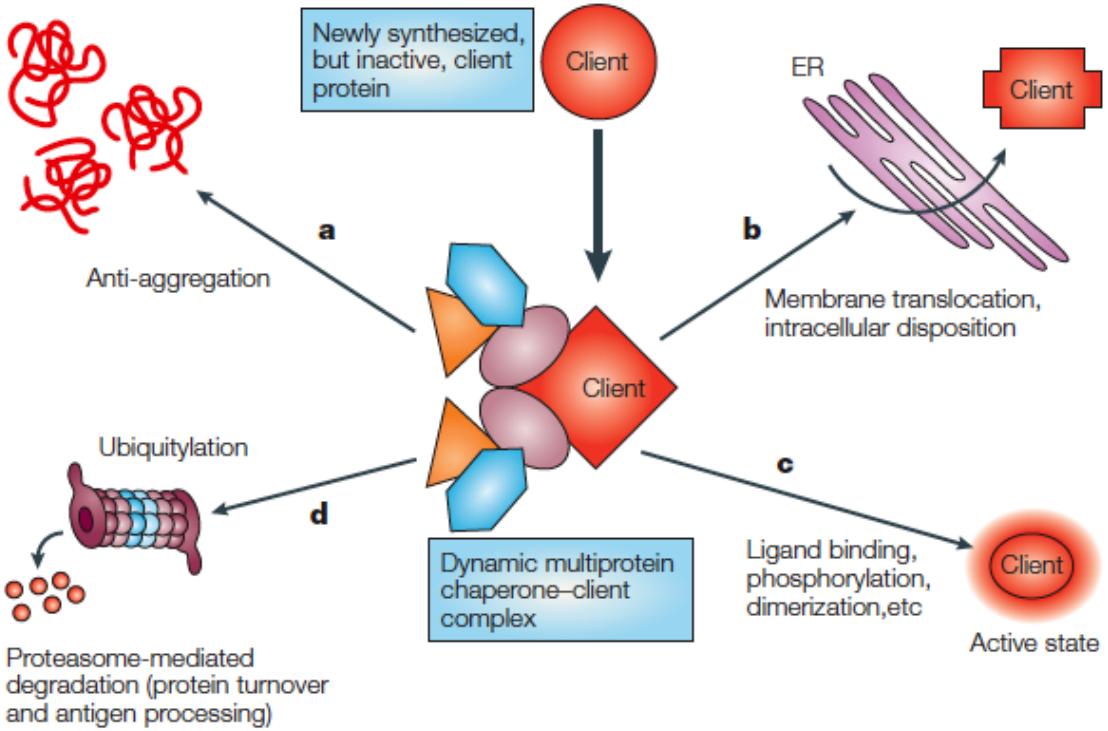


Figure 1 | **Participation of molecular chaperones in regulating many aspects of post-translational protein homeostasis.** Newly synthesized, conformationally labile client proteins associate with multi-protein complexes that contain various chaperones, co-chaperones and accessory molecules (different coloured shapes). The particular components of a complex vary according to the client and also help specify the function of a particular complex. Dynamic association of a client with chaperone complexes can prevent its aggregation (**a**) and assist in its intracellular trafficking, especially its translocation across membranous structures such as the endoplasmic reticulum (ER) (**b**). For many clients involved in signal transduction pathways, association with the chaperone machinery maintains the protein in a meta-stable state that allows it to be activated by specific stimuli such as ligand binding, phosphorylation or assembly into multisubunit signalling complexes (**c**). In the absence of appropriate stimuli, chaperone complexes can target the client for degradation through the ubiquitin–proteasome pathway, thereby regulating its steady-state cellular level (**d**).

Šaperoni sodelujejo pri nevrodegeneraciji

Table 1 | **Important components of the HSP90 chaperone machinery**

Protein family	Classification	Function
HSP90	Chaperone	Supports meta-stable protein conformations, especially in signal transducers
HSP70	Chaperone	Helps fold nascent polypeptide chains; participates in assembly of multiprotein complexes
HSP40	Co-chaperone	Stimulates HSP70 ATPase activity
HIP, HOP	Adapters	Mediate interaction of HSP90 and HSP70
CDC37/p50	Co-chaperone	Modulates interactions with kinases
AHA1	Co-chaperone	Stimulates HSP90 ATPase activity
p23	Co-chaperone	Stabilizes HSP90 association with clients
Immunophilin	Prolylisomerase	Modulates interactions with hormone receptors

AHA1, activator of HSP90 ATPase homologue 1; CDC37, cell division cycle 37 homologue; HIP, HSP70-interacting protein; HOP, HSP70/HSP90-organizing protein; HSP, heat-shock protein.

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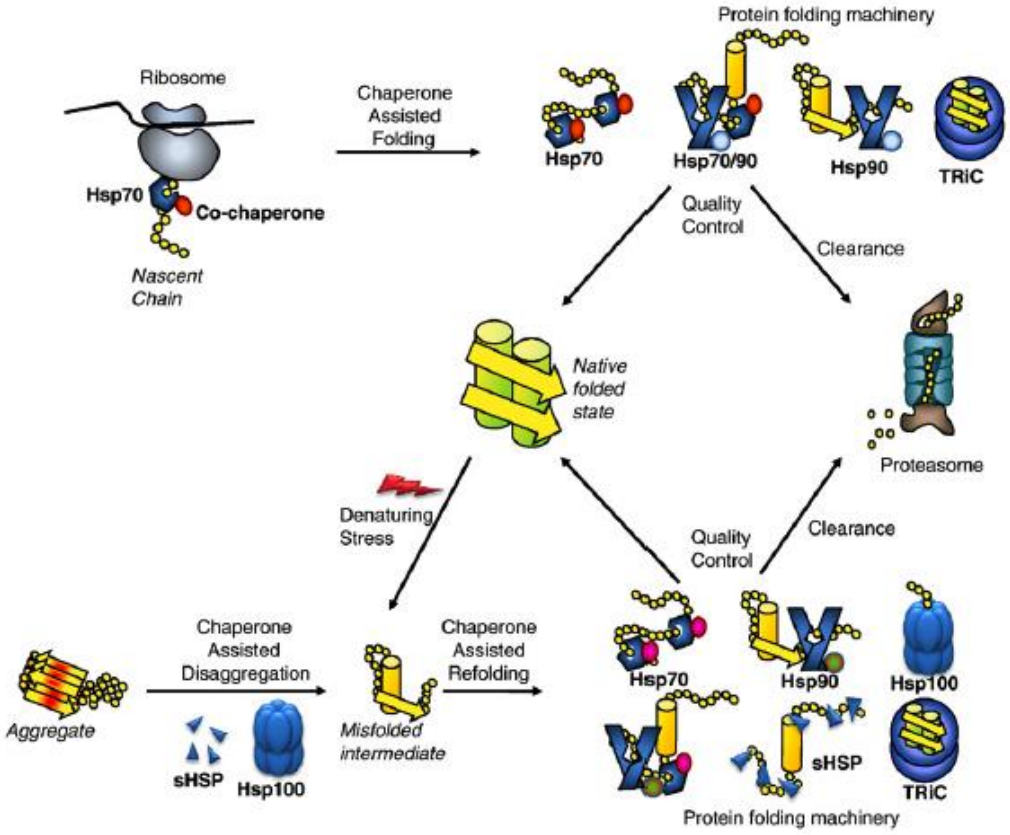


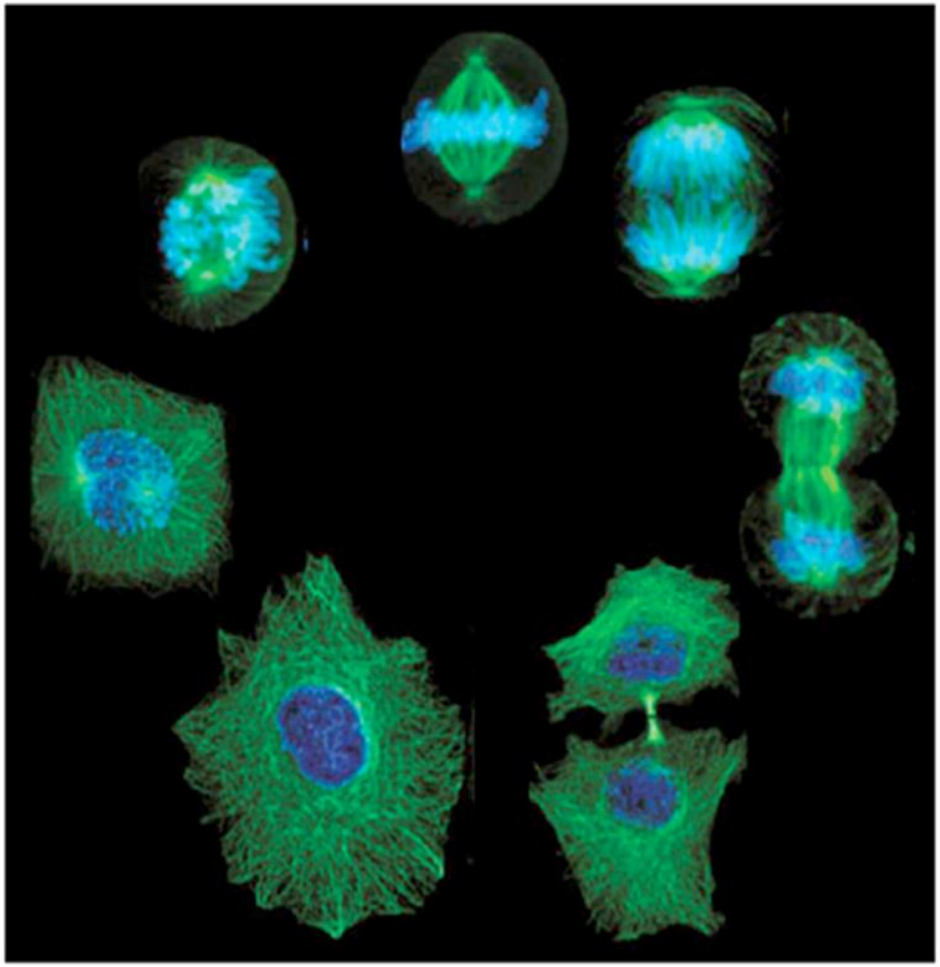
C. Voisine et al / Neurobiology of Disease 40 (2010) 12–20

Šaperoni, ki vplivajo na napačno zvijanje

Table 2
Chaperones that influence protein misfolding.

Disease protein	Tissue expression pattern	Chaperones identified	Reference
$A\beta_{42}$	Body wall muscles	HSF1 HSP70 (*hsp-1, hsp-3*) sHSPs (*hsp-16.11, hsp-16.48, hsp-16.2*) Co-chaperone-TPR (*R05F9.10*)	Fonte et al. (2002)
Polyglutamine tract	Body wall muscles	HSF1 HSP70 (*hsp-1, hsp-6*) HSP40 (*rme-8*) TRiC (*cct-1, cct-2, cct-4, cct-5, cct-6, cct-7*) Co-chaperone-TPR (*C50F2.3, M03F8.3*)	Nollen et al. (2004)
α-Synuclein	Body wall muscles	*HSP70 (hsp-70)* *HSP90 (R151.7)* *Co-chaperone-TPR (hip-1)*	van Ham et al. (2008) and Roodveldt et al. (2009)
Superoxide dismutase	Pan-neuronal	HSF1 HSP40 (*dnj-19*) sHSPs (*F08H9.4*) TRiC (*cct-4, cct-5*) Co-chaperone-nucleotide exchange (*C30C11.4, stc-1*)	Wang et al. (2009)
Tau	Pan-neuronal	HSF1 HSP70 (*hsp-1*) Co-chaperone-FKBP (*fkb-6*) Co-chaperone-TPR (*T09B4.10*)	Kraemer et al. (2006)

Vstop nevronov v celični cikel



Dokazi za zvezo med celičnim ciklom in nevronsko smrtjo

- KO modeli

- Analize možganov

- Pomanjkanje NGF: smrt s povečanimi markerji celičnega cikla

- Zdravila, ki blokirajo celični cikel, preprečijo celično smrt zaradi pomanjkanja NGF

- Poveča se izražanje proteinov celičnega cikla

    v možganih pacientov z nevrodegenerativnimi boleznimi (AB)

    v občutljivih nevronih

Hipoteza dveh zadetkov (The two hit hypothesis):

nenormalen vstop v celični cikel naredi nevrone ranljive za sekundarni toksični napad

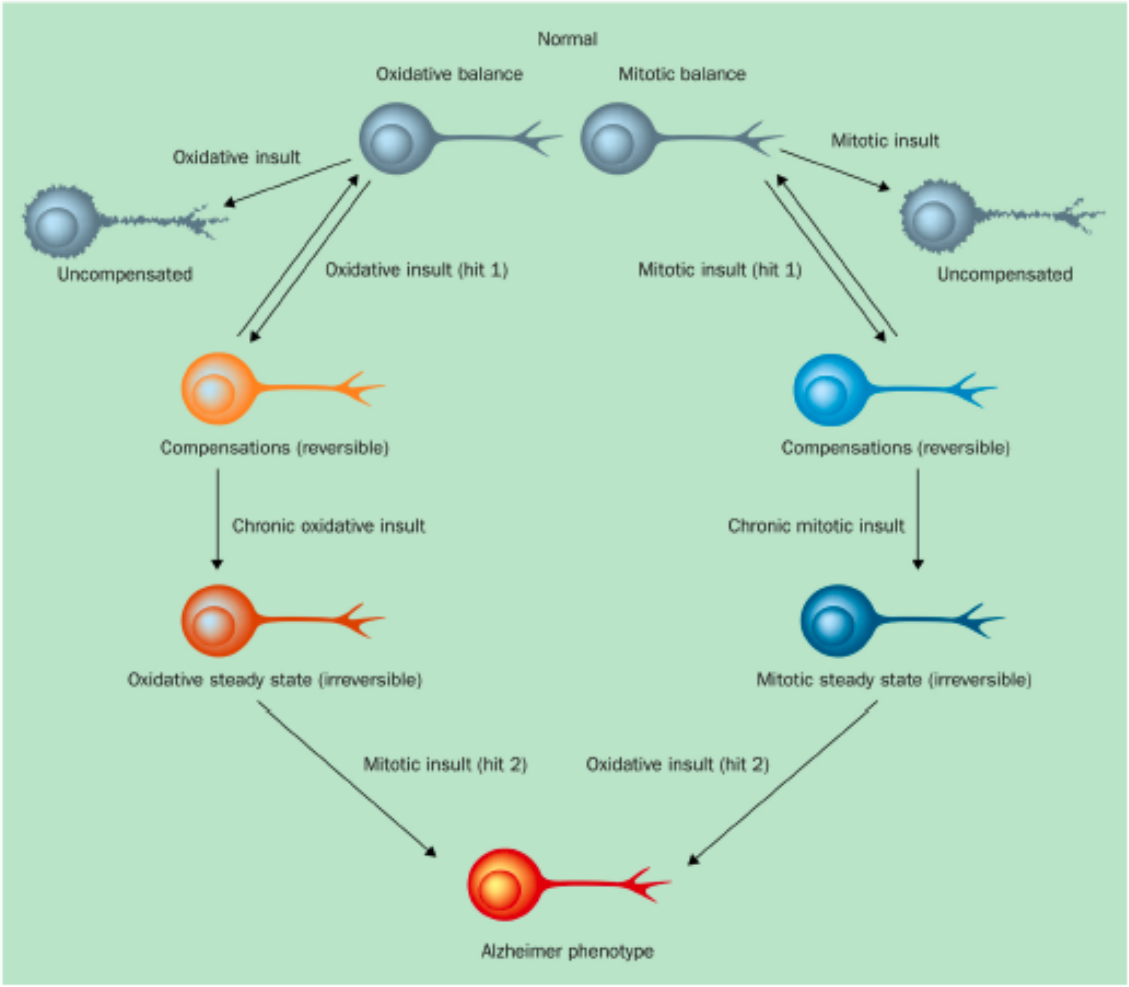


Figure 2. The two-hit hypothesis
An initial insult, whether oxidative or mitotic, that is chronic and above threshold limits leads to a new steady state (either oxidative steady state or mitotic steady state). It is in this new steady state when neurons are vulnerable to the subsequent second hit, which causes the Alzheimer disease phenotype. Reprinted from Ref. [60], with permission from Elsevier © 2004.

Expert Rev Mol Med. 2010 Jun 29;12:e19. Review.

Kopičenje proteinov celičnega cikla pri AB

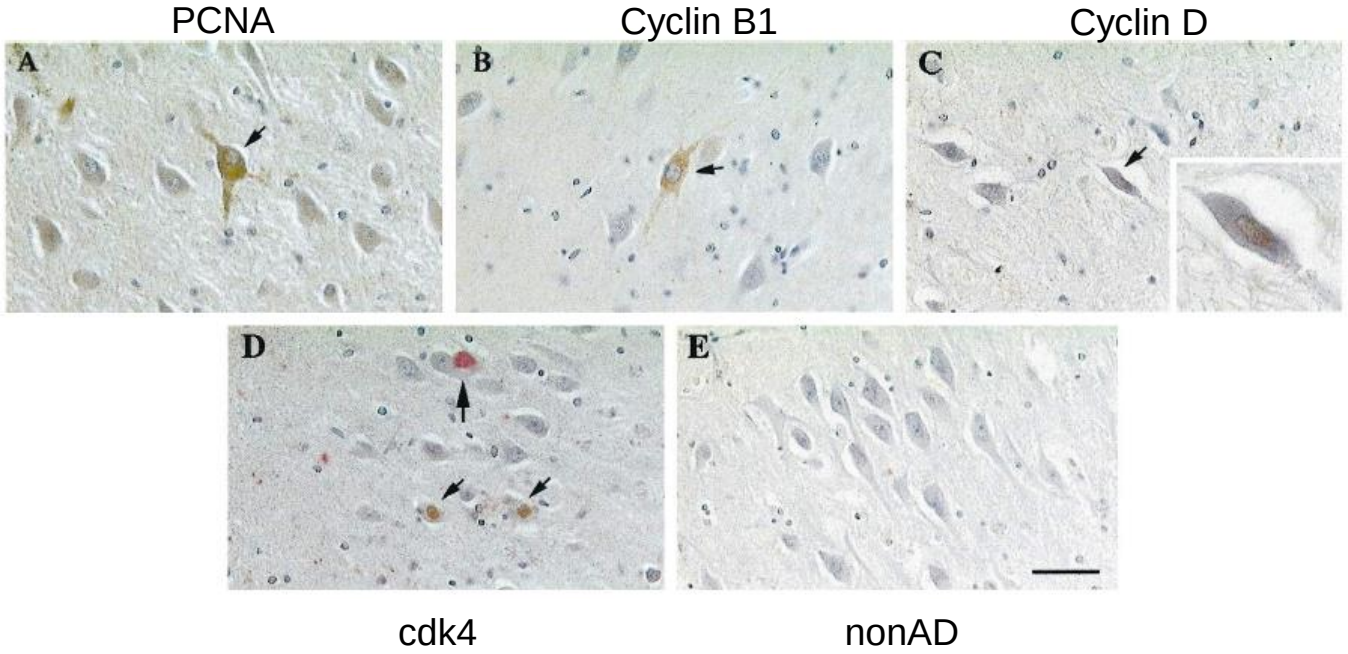
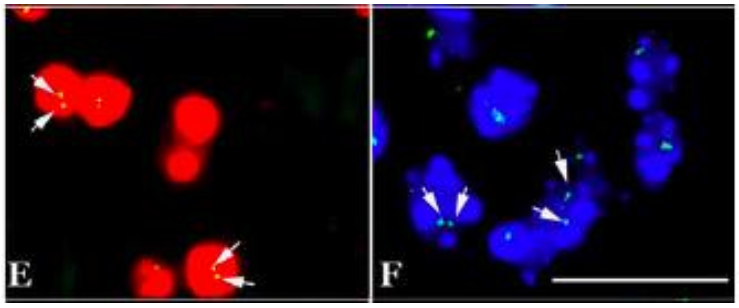


Figure 1. **Top.** AD hippocampus stained for cell cycle markers PCNA (*A*), cyclin B1 (*B*), cyclin D (*C*), and cdk4 (*D*). *E*, Similar field in a nondemented, age-matched control brain stained for PCNA. The *small arrows* indicate cells positive for the respective cell cycle marker. In *D*, the *larger arrow* points to a TG3-positive neuron that is cdk4-negative. Scale bars: 50 μm; *C, inset*, 20 μm.

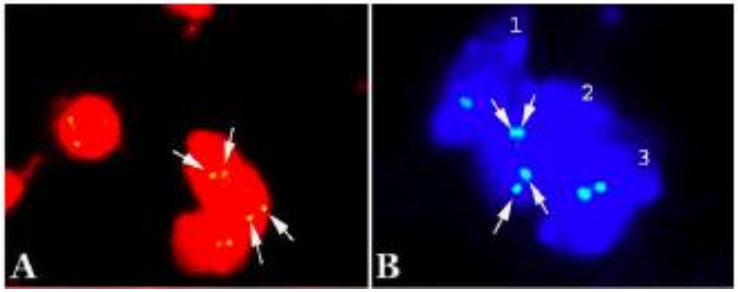
DNA sinteza in duplikacija centromer pri modelih AB

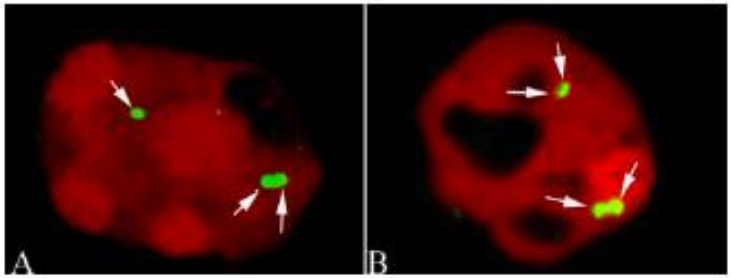
Cortex Hippocampus

WT



APPTgR1.40





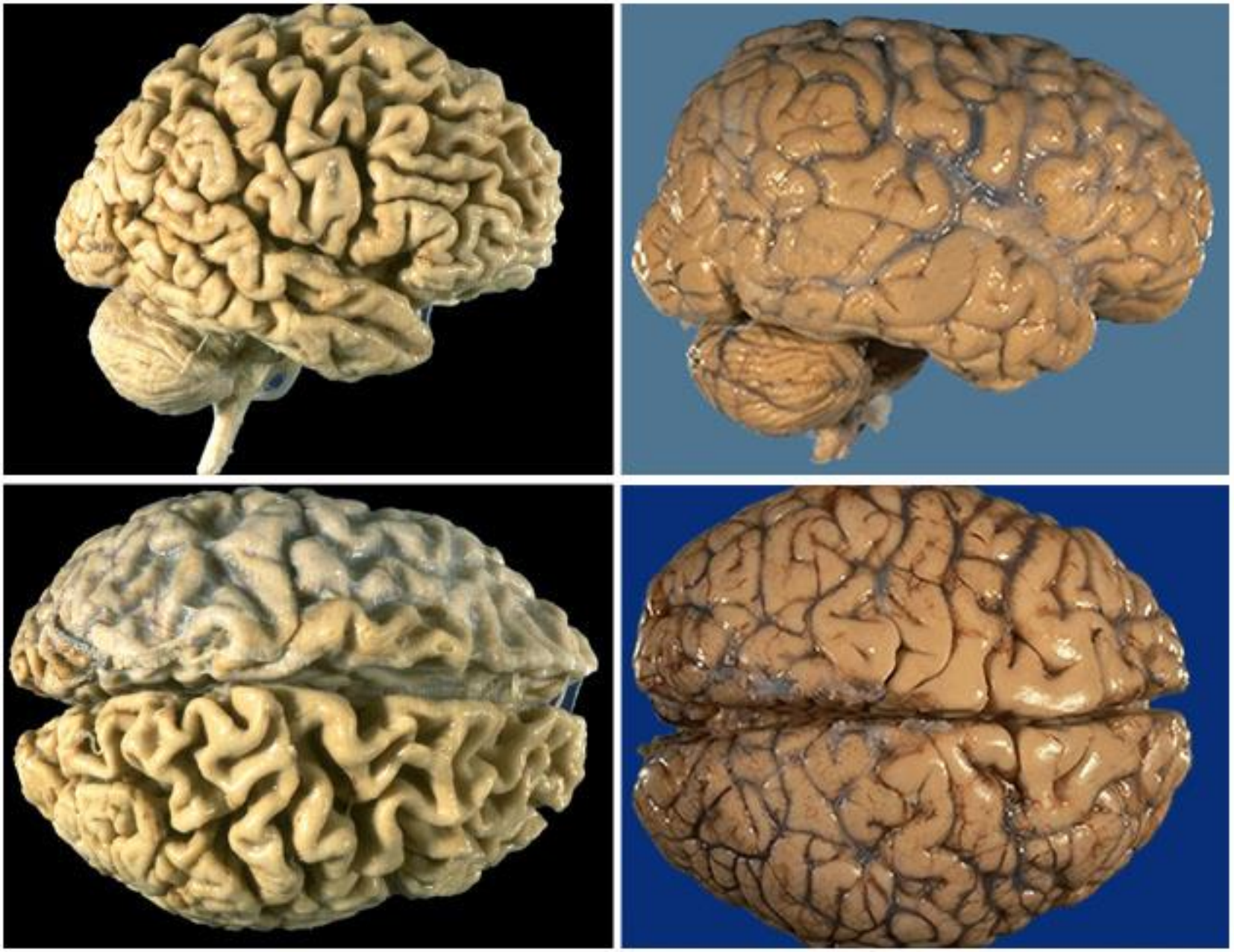
ALZHEIMERJEVA BOLEZEN

AB statistika

- Približno 12 milionov ljudi ima AB.
- Številka se bo potrojila do leta 2050 zaradi staranja baby-boom generacije.
- Prevalenca je približno 7% za ljudi nad 65 let. Verjetnost za bolezen se podvoji na vsakih 5 let po 65.

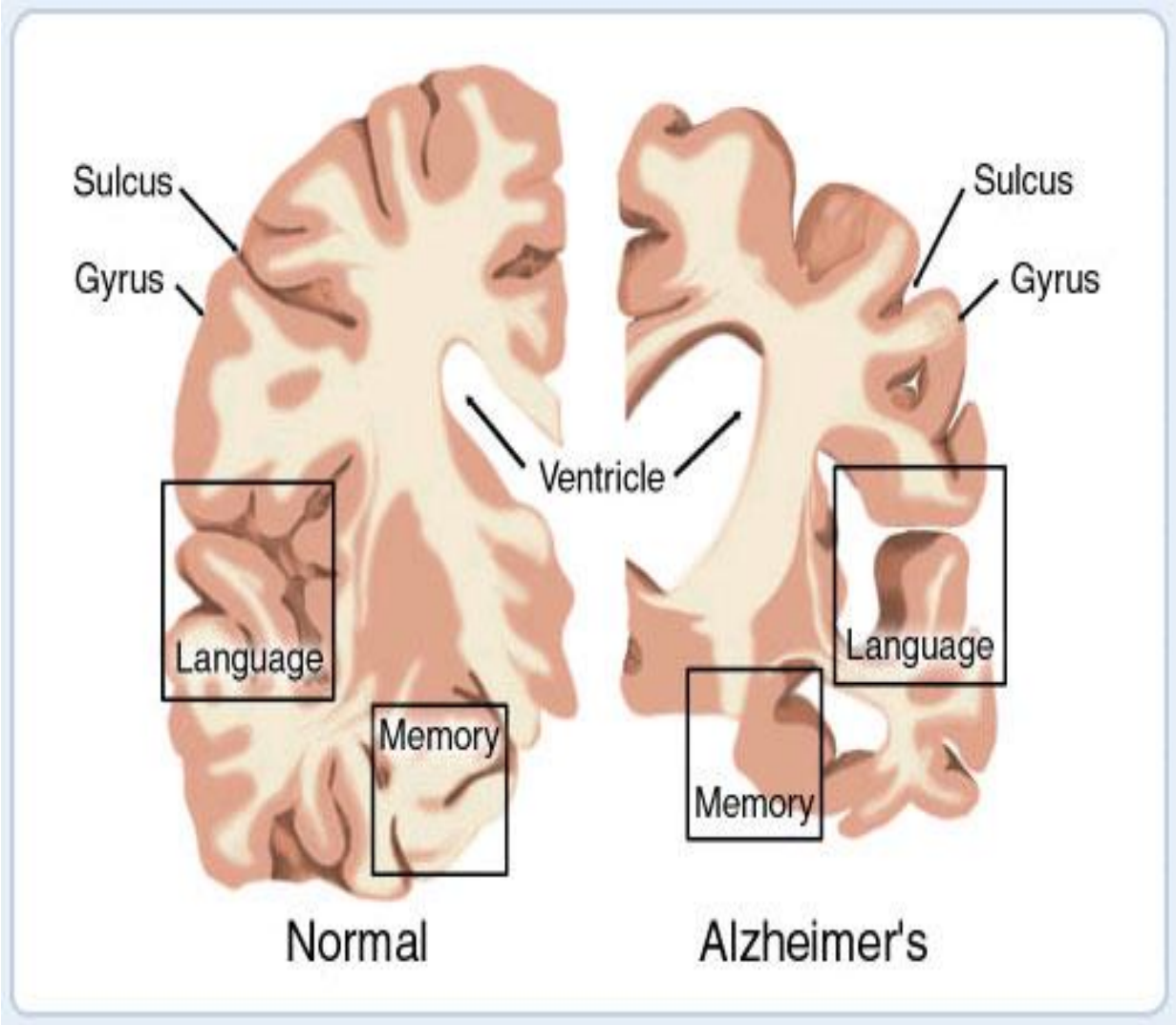
Možgani bolnika z AB

Normalni možgani



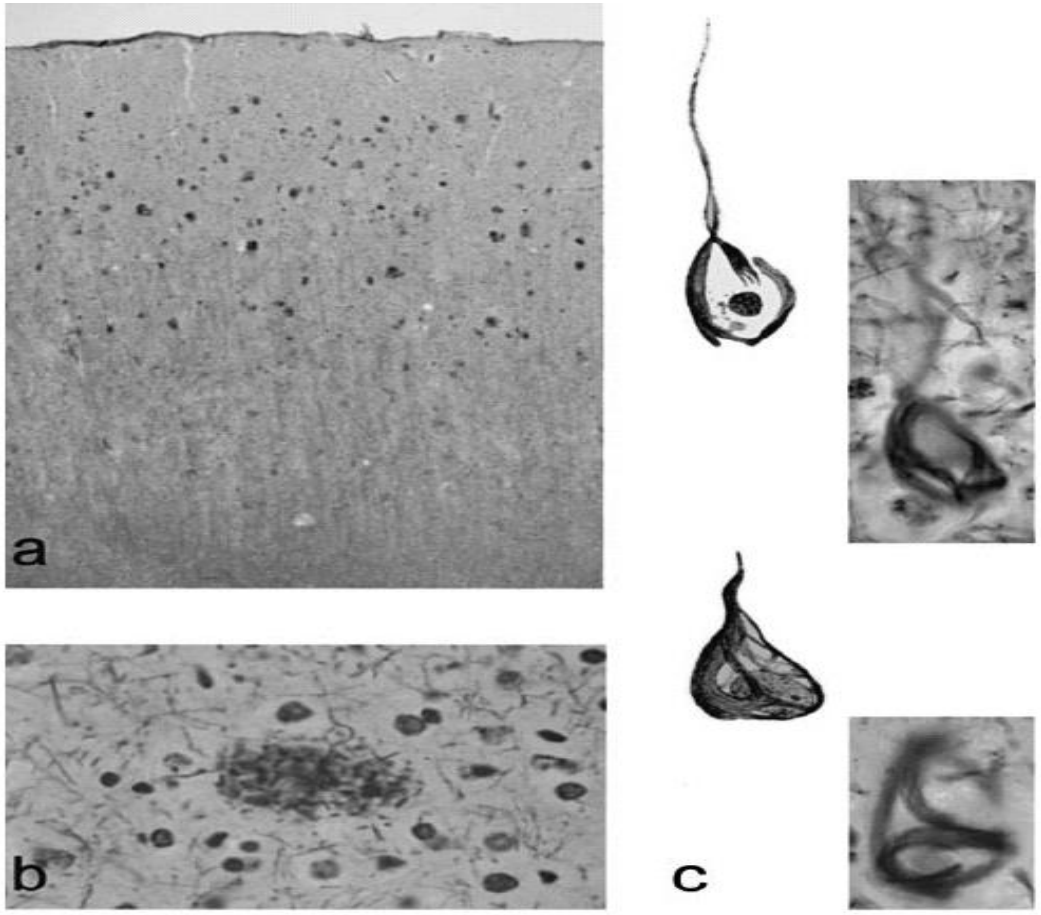
Alzheimerjeva bolezen

- Največje spremembe v skorji velikih možganov.
- Povečani ventrikli.
- Senilni plaki.
- Nevrofibrilarne pentlje v bolnih nevronih.
- Zmanjšanje nevrotransmiterjev – poškodbe v medcelični komunikaciji.
- Zahbtna in neusmiljeno napredujoča bolezen.



Plaki

Pentlje



Sedem zgodnjih znakov AB

Ameriški Nacionalni Institut za Staranje (The National Institute on Aging) je objavil sedem zgodnjih znakov možnega nastopa AB:

1. Večkratno ponavljanje istega vprašanja.
2. Dobesedno ponavljanje istih zgodb.
3. Pozabljanje rutinskih aktivnosti, npr. kuhanje ali igranje kart.
4. Izguba sposobnosti vodenja ključnih aktivnosti kot plačevanje računov ali vodenje lastnih financ.
5. Izgubljanje v znanem okolju ali pozabljanje mesta, kjer se nahajajo stvari v hiši.
6. Pomanjkljivosti pri lastni higieni in ne priznavanje tega.
7. Pričakovanje, da se drugi odločijo ali odgovorijo na zadeve, ki so jih prej reševali sami.

Potek AB

1. Stopnja - 2-4 leta

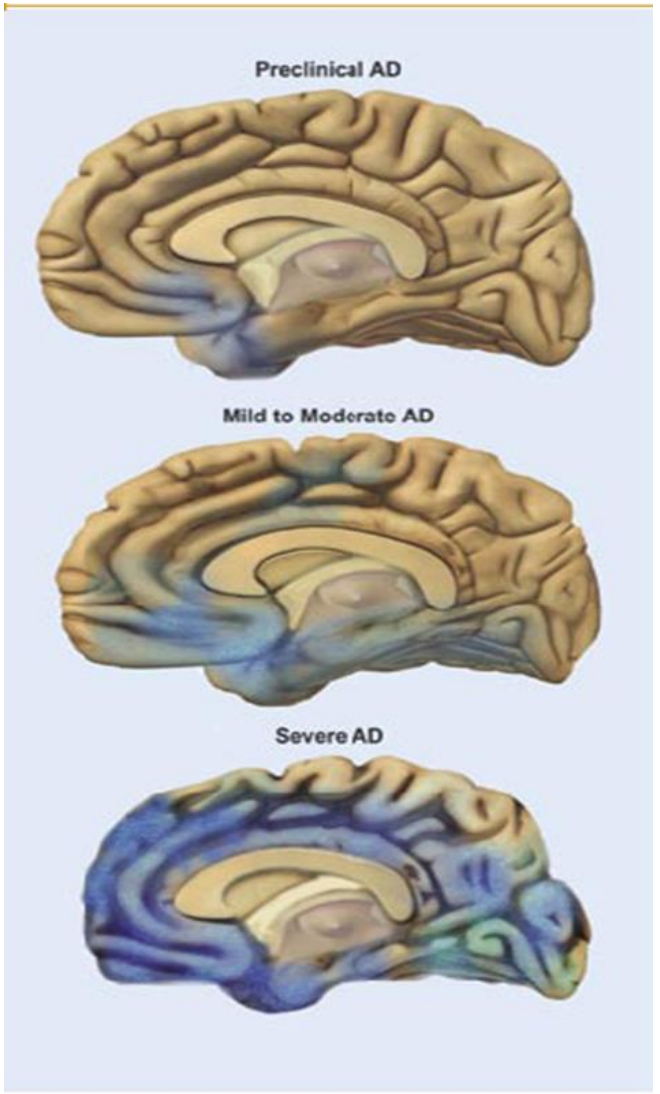
Izguba spomina – predvsem kratkoročnega.
Rahle spremembe v osebnosti.

2. Stopnja - 2-5 let

Padec umskih sposobnosti – jezikovnih, reševanje problemov.
Depresija, zmedenost, tavanje.

3. Stopnja – 1-2 leti

Apatija, izguba zanimanja za hrano.
Inkontinenca (urina in blata).
Ne prepoznavanje sorodnikov ali prijateljev.
Lahko tudi epileptični napadi.



Klasifikcija AB

(1) Dedna AB (FAD) in sporadična AB (SAD):

- Ni popolnega konsenza.
- Pri FAD je pogost vsaj še eden sorodnik prve stopnje.
- včasih 2 sorodnika druge stopnje.

(2) Zgodnji ali pozni začetek:

- Zgodnji začetek je pred 65 letom.
- Zgodnji začetek korelira s FAD.
- LOAD (late onset AD) – AB s poznim začetkom.

Preboj v razumevanju genetike AB

(1) Downov sindrom

- Starejši bolniki imajo AB patologijo v možganih.
- Običajno ne kažejo znakov AB

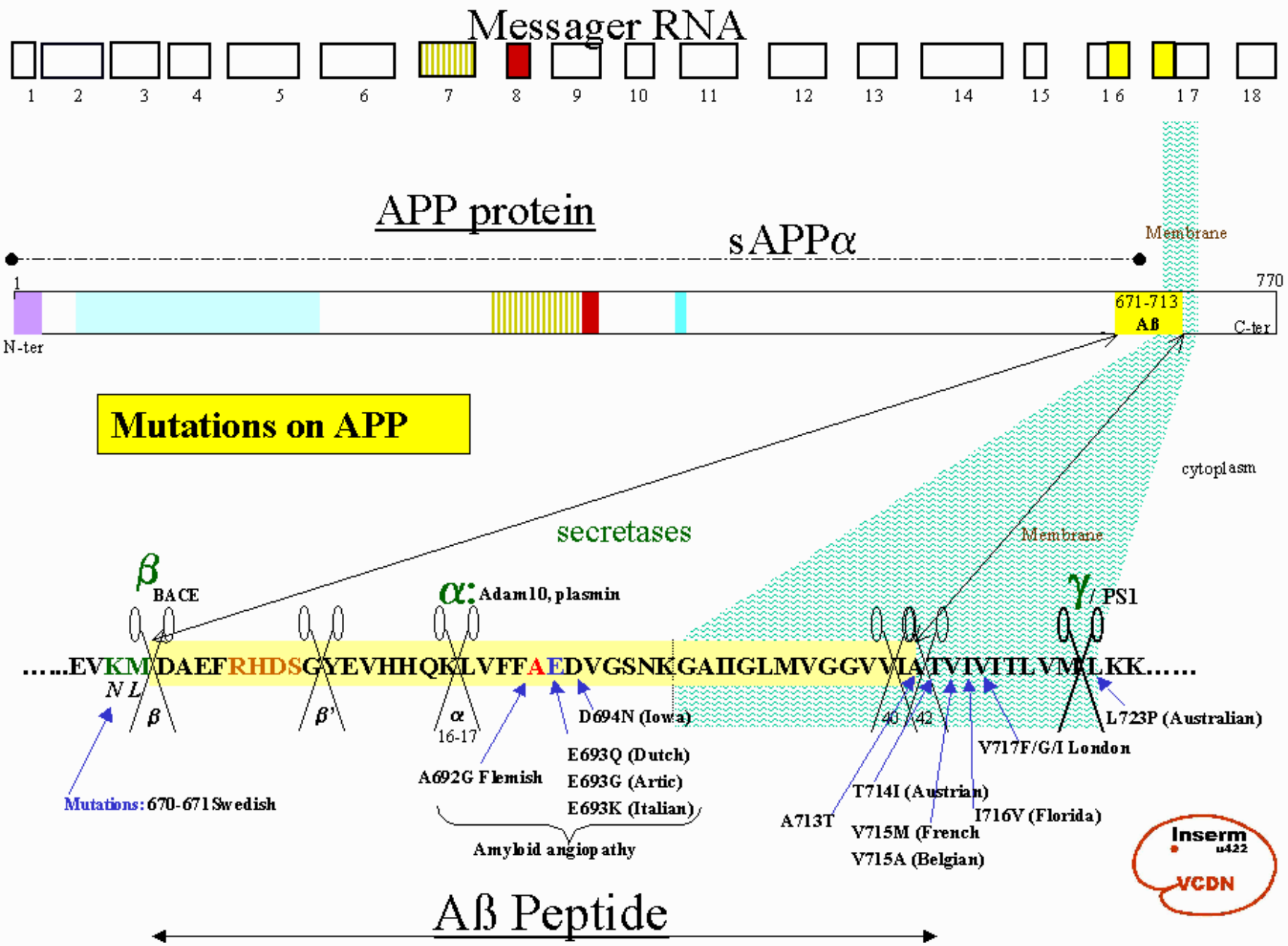
(2) Pedigreji s dominantnim prenosom

- največja povezava s C 21

AB, tip 1

- Več mutacij na genu za APP na C21.
- Najbolj pogosta je Val717Iso.
- Tvori se patološki Aβ fragment.
- 15%-20% FAD z zgodnjim začetkom
- Avtosomno dominatna mutacija.

http://ghr.nlm.nih.gov/condition=alzheimerdisease


Messager RNA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
APP protein
sAPPα
Membrane
1
770
671-713
Aß
C-ter
N-ter
Mutations on APP
cytoplasm
secretases
Membrane
β
BACE
α: Adam10, plasmin
γ / PS1
……EVKMDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITLVMLKK……
N L
β
β'
α
16-17
40
42
D694N (Iowa)
L723P (Australian)
V717F/G/I London
A692G Flemish
E693Q (Dutch)
E693G (Artic)
E693K (Italian)
Amyloid angiopathy
Mutations: 670-671 Swedish
A713T
T714I (Austrian)
V715M (French
V715A (Belgian)
I716V (Florida)
Inserm u422
VCDN
Aß Peptide

AB, tip 3

- Mutacije (> 130) v genu presenilin1 na C14.
- Pri večini mutacij pride do zamenjave a.k.
- Prekomerno se tvori patološki Aβ fragment.
- 30% - 70% FAD z zgodnjim začetkom.
- Avtosomno dominatna mutacija.

AB, tip 4

- Mutacije v genu presenilin2 na C1.
- 2 mutaciji: Asn141Iso in Met239Val
- Pride do čezmerne tvorbe patološkega Aβ fragmenta.
- <5% FAD z zgodnjim začetkom (na svetu samo nekaj družin).
- Avtosomno dominatna mutacija.

AB, tip 2

• Epsilon 4 (ε4 ali E4) alela Apolipoprotein E (ApoE) gena na C19, ki poveča verjetnost razvoja AB.

• Epsilon 2 (ε2 ali E2) alela, ki zmanjša verjetnost razvoja AB.

• ApoE je VLDL, ki prenaša holesterol.

• Mehanizem ni popolnoma znan. ApoE naj bi sodeloval pri proteolitski razgradnji Aβ. ApoE-ε4 ni tako učinkovit ter s tem poveča verjetnost razvoja AB. Verjetnost razvoja AB do leta 75 se pri nosilcih 2 E4 alel poveča za 10-30 krat.

• Večina bolnikov je dednih z poznim začetkom.

Dve glavni hipotezi AB
beta amiloidni protein (BAP) ali tau

1. **BAPtisti**: kopičenje Aβ-42 fragmenta (42 ak fragment APP) vodi do tvorbe amiloidnih plakov, ki ubijejo nevrone.

2. **TAUisti**: Pride do patološke fosforilacije tau proteina, ki jih naredi „lepljive“ in vodi do razpada mikrotubulov. Poškoduje se aksonski transport in nastopi smrti nevronov.

Amiloidna hipoteza

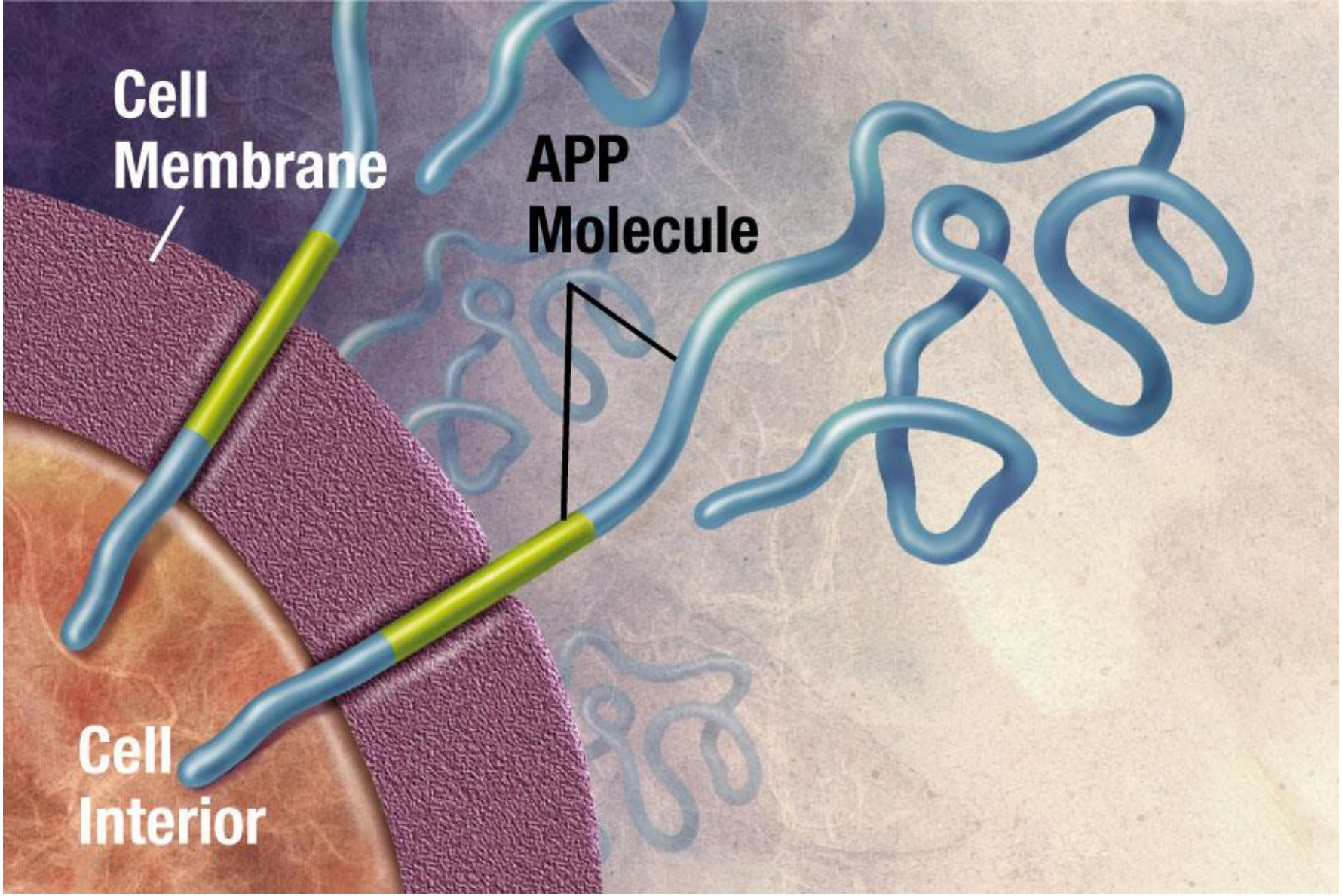
1. Sekretaze razgradijo amiloidni prekurzoski protein (APP).

2. Netopni fragment APP Aβ-42 se kopiči izven celice.

3. Netopnost ali lepljivost Aβ-42 pomaga drugim proteinom (kot je apoE), da se združijo v plake.

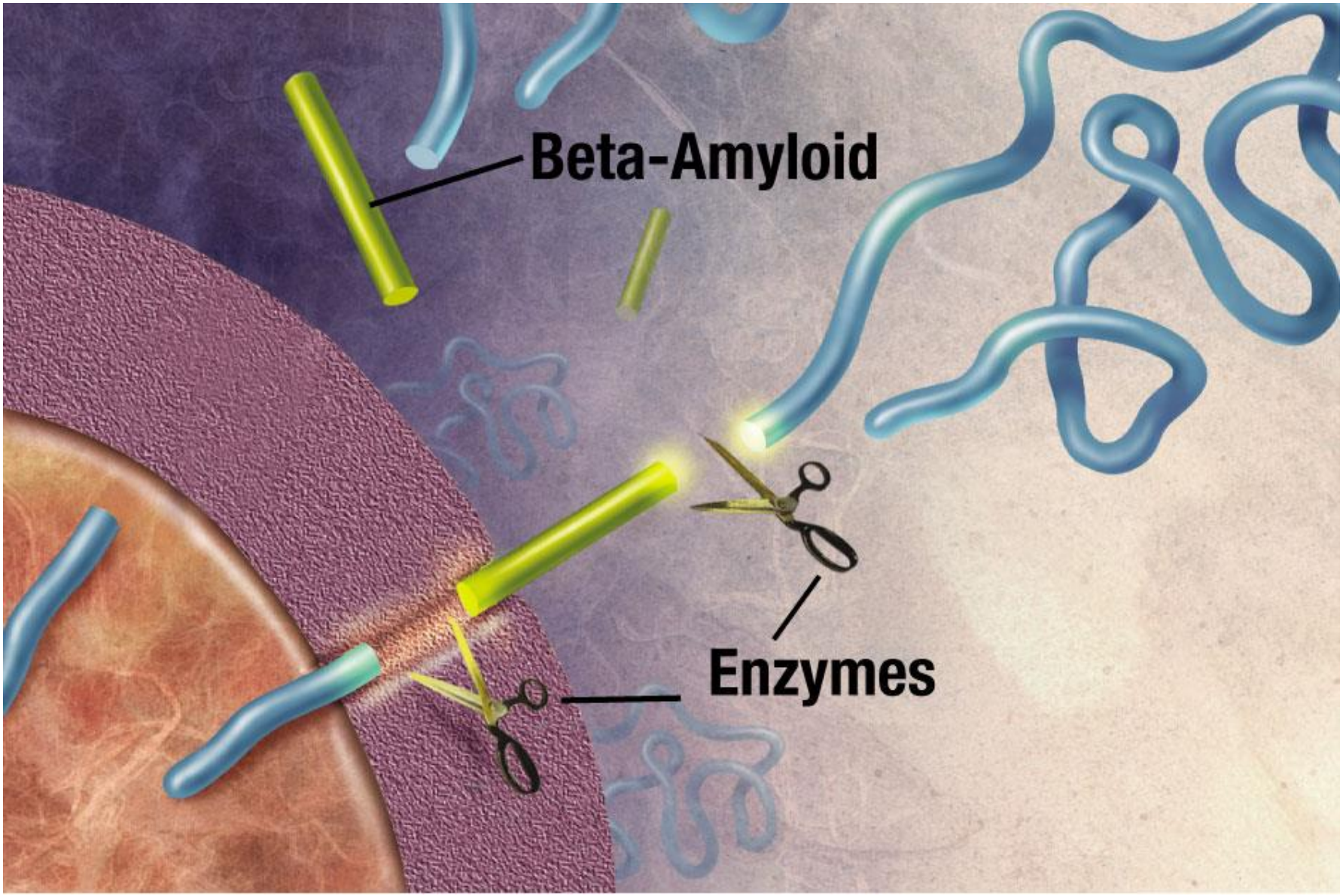
4. Plaki (ali migracija Aβ-42 izven celice) povzročijo smrt nevronov.

5. Gena PSEN1 & PSEN2 sta podenoti γ sekretaze.



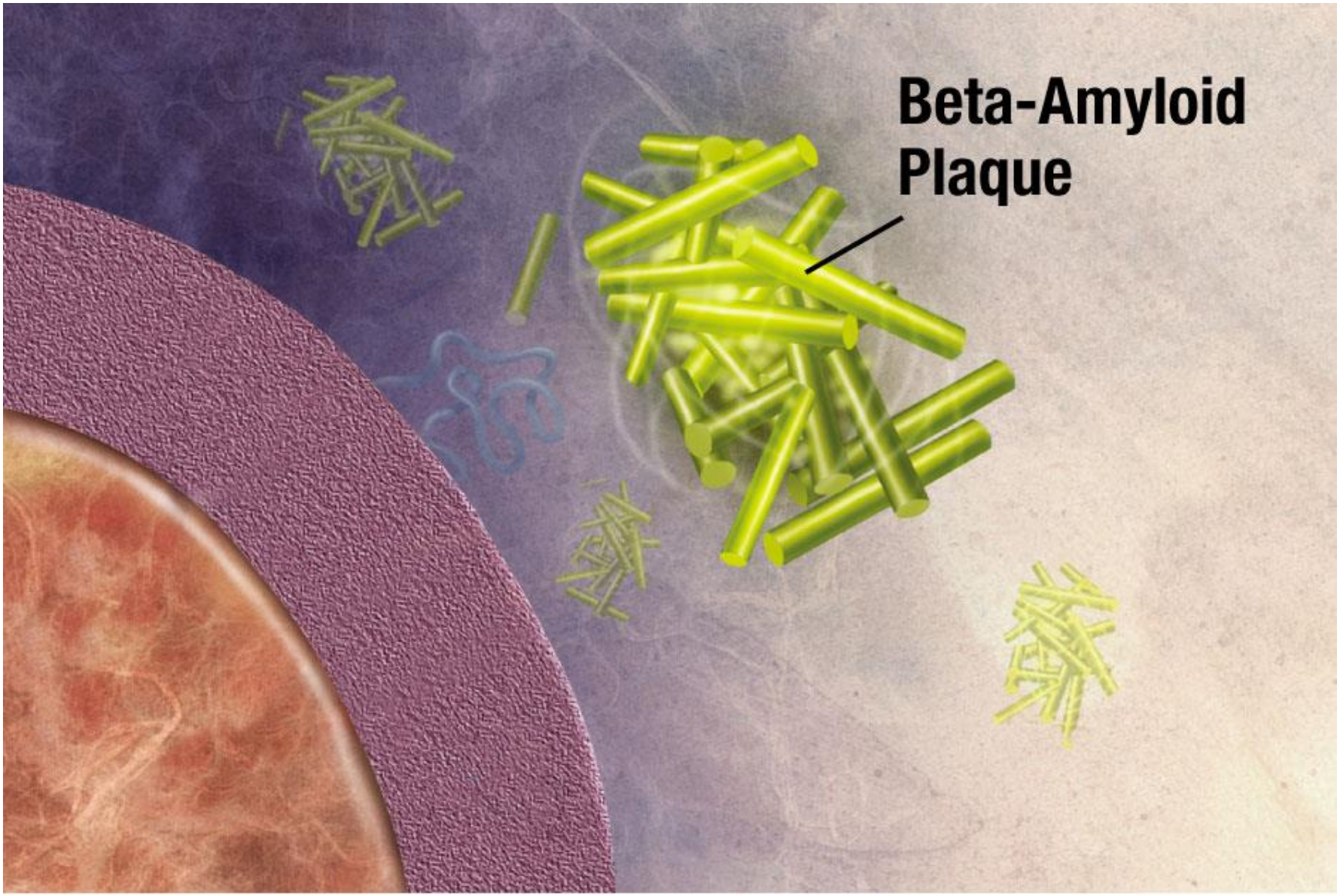
APP je membranski protein, ki je zasidran v membrani in sega v zunajcelični prostor. Naj bi imel pomen pri rasti, preživetju in popravljanju nevronov.

www.niapublications.org/pubs/unraveling/01.htm



Encimi cepijo APP v fragmente. Za AB je najbolj pomemben fragment b-amiloid (beta-amyloid) ali Ab.

www.niapublications.org/pubs/unraveling/01.htm



Ab je „lepljiv“ in skupaj z drugimi proteini tvori senilne plake v možganih bolnikov.

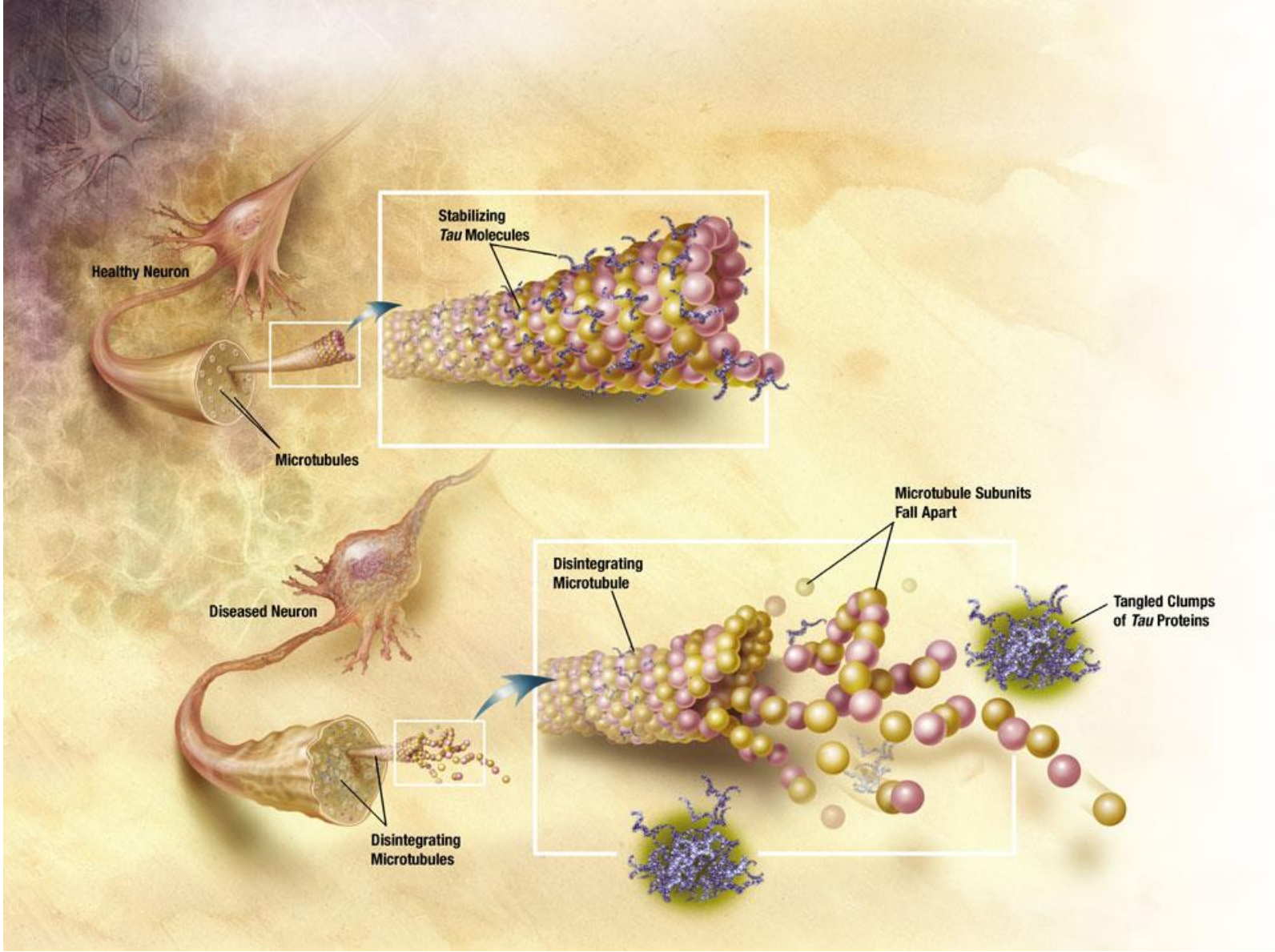
www.niapublications.org/pubs/unraveling/01.htm

Tau hipoteza

1. Tau protein stabilizira mikrotubule. Mikrotubule so pomembne pri aksonalnem transportu.

2. Kopičenje fosfatov na tau proteinu povzroči parjenje filamentov, ti pa v nastanek nevrofibrilarnih pentelj.

3. Poškodovan transport po aksonu je verjetno vzrok za celično smrt.



Mikrotubule so kot železniške tračnice, po katerih se prenašajo nutrienti in druge molekule. Tau-proteini delujejo kot železniški pragovi, ki stabilizirajo strukturo mikrotubulov. Pri AB se tau proteini zavozlajo in destabilizirajo strukturo mikrotubulov. Izguba aksonskega transporta vodi v smrt celice.

Teorija AB: vključuje več dejavnikov in več poti

- Kopičenje proteinov: → plaki & pentlje
- Vnetje: neregulirana aktivacija glije
- Porazdelitev lipidov v membrani vpliva na cepitev APP v membrani.

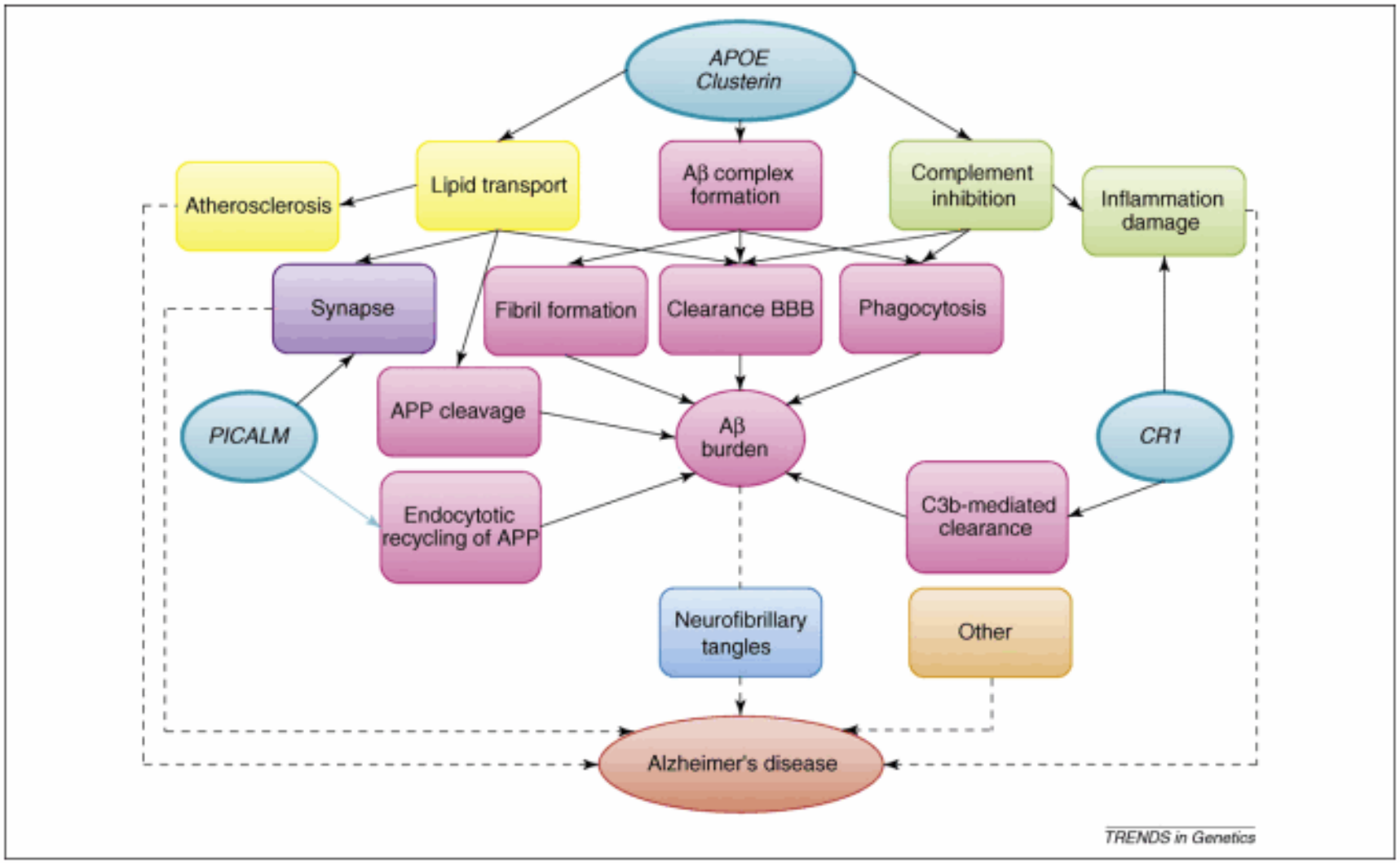


Figure 1. Linking the genes to the pathophysiology of AD. An overview of how *APOE*, *CLU*, *PICALM* and *CR1* are implicated in AD susceptibility. The information based on current experimental or observational evidence is depicted by solid black arrows, with hypotheses shown by blue arrows. Several pathophysiological pathways thought to contribute to disease (Aβ (in pink), neurofibrillary tangles (blue), chronic inflammation (green), atherosclerosis (yellow), loss of physiological function at the synapse (purple) and others (orange)) are indicated by interrupted arrows. Note that neurofibrillary tangles are not necessarily downstream of Aβ deposition. Abbreviation: BBB, blood–brain barrier.

Sleegers et al. Trends in Genetics 2010

Geni, ki so kandidati za AB:

- Precejšnje število kandidatov.
- http://Alzgene.org

Box 2. Mouse models of Alzheimer's disease

This is not an exhaustive list of all AD transgenic models available but serves to highlight those models that are either in widespread use or have significantly advanced our understanding of AD pathogenesis. There are also many invertebrate models both in *Drosophila melanogaster* and *Caenorhabditis elegans* that have proved invaluable to AD research.

PDAPP: First mutant APP transgenic model with robust plaque pathology [2]. Mice express a human *APP* cDNA with the Indiana mutation (APP_{V717F}). Plaque pathology begins between 6–9 months in hemizygous PDAPP mice. There is synapse loss but no overt cell loss and no NFT pathology is observed. This model has been used widely in vaccination therapy strategies.

Tg2576: Mice express mutant APP_{SWE} under control of the hamster prion promoter [3]. Plaque pathology is observed from 9 months of age. These mice have cognitive deficits but no cell loss or NFT pathology. It is one of the most widely used transgenic models.

APP23: Mice express mutant APP_{SWE} under control of the Thy1 promoter. Prominent cerebrovascular amyloid, amyloid deposits are observed from 6 months of age and some hippocampal neuronal loss is associated with amyloid plaque formation [39–41].

TgCRND8: Mice express multiple APP mutations (Swedish plus Indiana). Cognitive deficits coincide with rapid extracellular plaque development at ~3 months of age. The cognitive deficits can be reversed by Aβ vaccination therapy [29].

$PSEN1_{M146V}$ or $PSEN1_{M146L}$ (lines 6.2 and 8.9, respectively): These models were the first demonstration *in vivo* that mutant *PSEN1* selectively elevates Aβ42. No overt plaque pathology is observed [7].

PSAPP (Tg2576×$PSEN1_{M146L}$ [9], PSEN1-A246E+APP_{SWE} [8]): Bigenic transgenic mice, addition of the mutant *PSEN1* transgene markedly accelerated amyloid pathology compared with singly transgenic mutant *APP* mice, demonstrating that the PSEN1-driven elevation of Aβ42 enhances plaque pathology.

APP_{Dutch}: Mice express *APP* with the Dutch mutation that causes hereditary cerebral hemorrhage with amyloidosis–Dutch type in humans. APP_{Dutch} mice develop severe congophilic amyloid angiopathy [18]. The addition of a mutant *PSEN1* transgene redistributes the amyloid pathology to the parenchyma indicating differing roles for Aβ40and Aβ42 in vascular and parenchymal amyloid pathology.

BRI-Aβ40 and BRI-Aβ42: Mice express individual Aβ isoforms without *APP* over-expression [16]. Only mice expressing Aβ42 develop senile plaques and CAA, whereas BRI-Aβ40 mice do not develop plaques, suggesting that Aβ42 is essential for plaque formation.

JNPL3: Mice express 4R0N *MAPT* with the P301L mutation [53]. This is the first transgenic model, with marked tangle pathology and cell loss, demonstrating that MAPT alone can cause cellular damage and loss. JNPL3 mice develop motor impairments with age owing to severe pathology and motor neuron loss in the spinal cord.

Tau_{P301S}: Transgenic mice expressing the shortest isoform of 4R *MAPT* with the P301S mutation [51]. Homozygous mice develop severe paraparesis at 5–6 months of age with widespread neurofibrillary pathology in the brain and spinal cord and neuronal loss in the spinal cord.

Tau_{V337M}: Low level synthesis of 4R MAPT with the V337M mutation (1/10 endogenous mouse MAPT) driven by the promoter of platelet-derived growth factor (PDGF) [75]. The development of neurofibrillary pathology in these mice suggests the nature of the MAPT rather than absolute MAPT intracellular concentrations drives pathology.

Tau_{R406W}: Mice expressing 4R human MAPT with the R406W mutation under control of the CAMKII promoter [76]. Mice develop MAPT inclusions in the forebrain from 18 months of age and have impaired associative memory.

rTg4510: Inducible *MAPT* transgenic mice using the TET-off system [50,77]. Abnormal MAPT pathology occurs from one month of age. Mice have progressive NFT pathology and severe cell loss. Cognitive deficits are evident from 2.5 months of age. Turning off the transgene improves cognitive performance but NFT pathology worsens.

Htau: Transgenic mice expressing human genomic MAPT only (mouse MAPT knocked-out) [78]. htau mice accumulate hyperphosphorylated MAPT from 6 months and develop Thio-S-positive NFT by the time they are 15 months old.

TAPP (Tg2576×JNPL3): Increased MAPT forebrain pathology in TAPP mice compared with JNPL3 suggesting mutant APP and/or Aβ can affect downstream MAPT pathology [63].

3×TgAD: Triple transgenic model expressing mutant APP_{SWE}, $MAPT_{P301L}$ on a $PSEN1_{M146V}$ 'knock-in' background (PSEN1-KI) [68,79]. Mice develop plaques from 6 months and MAPT pathology from the time they are 12 months old, strengthening the hypothesis that APP or Aβ can directly influence neurofibrillary pathology.

McGowan et al., Trend in Genetics 2006

Zdravljenje Alzheimerjeve bolezni

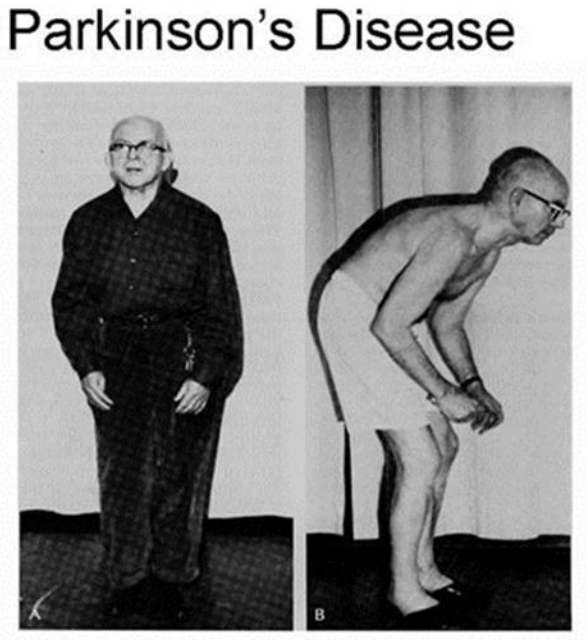
Začetna in srednja faza AB

Holinesterase inhibitorji naj bi upočasnili ali preprečili poslabšanje simpromov za nekaj časa. Pomagali naj bi pri kontroli obnašanja

- **Razadyne®** (galantamine oz. Reminyl®)
- **Exelon®** (rivastigmine)
- **Aricept®** (donepezil)

Pozne faze AB

- **Namenda®** (memantine), N-metil D-aspartat (NMDA) antagonist.
- Zdravilo upočasni nekatere simptome.
- Omogoči bolnikom, da ohranijo vsakodnevne funkcije malo dalj časa.



PARKINSONOVA BOLEZEN

Parkinsonova bolezen

- Prvi jo je opisal James Parkinson v delu “Essay on Shaking Palsy” (Paralysis-agitans; 1817).
- **PB je nevrodegenerativna bolozen, karakterizirana s poškodovanimi dopaminergičnimi nevroni v Substantia Nigra pars compact (SNpc) in s kopičenjem intracelularnih fibrilarnih agregatov – Lewyjeva telesca.**
- PD je druga najpogostejša NDB za AB, ki se pojavi s staranjem.
- 95% primerov je sporadičnih, samo 5% je dednih.
- Zaradi vpliva toksinov iz okolja imajo razvite, industrijske države več bolnikov s PB v primerjavi z manj industrijsko razvitimi državami. Razlika med državami lahko obstaja zaradi drugačnega načina diagnosticiranja, zdravljenja in spremljanja bolnikov.
- Povprečno se bolezen pojavi po 60. letu (sporadična PB) in prizadene 1 % populacije pri teh letih.

Epidemiologija

- Povprečna starost ob začetku ~ 60 let
  - < 20 let: juvenilna PB (ponavadi dedna)
  - Med 20 - 40 let: PB z zgodnjim začetkom
- M : Ž = 3 : 2
- Prevalenca: ~ 200 - 300 / 100.000
- Incidenca: ~ 10 - 20 / 100.000
- Prevalenca in incidenca naraščata s starostjo
- Slovenija: > 4000 bolnikov

Simptomi PB

-Tresenje v mirovanju (tresenje okončin, ki se poveča, ko so pri miru)

-Bradikinesia (težave pri začenjanju gibov, upočasnjeno izvajanje)

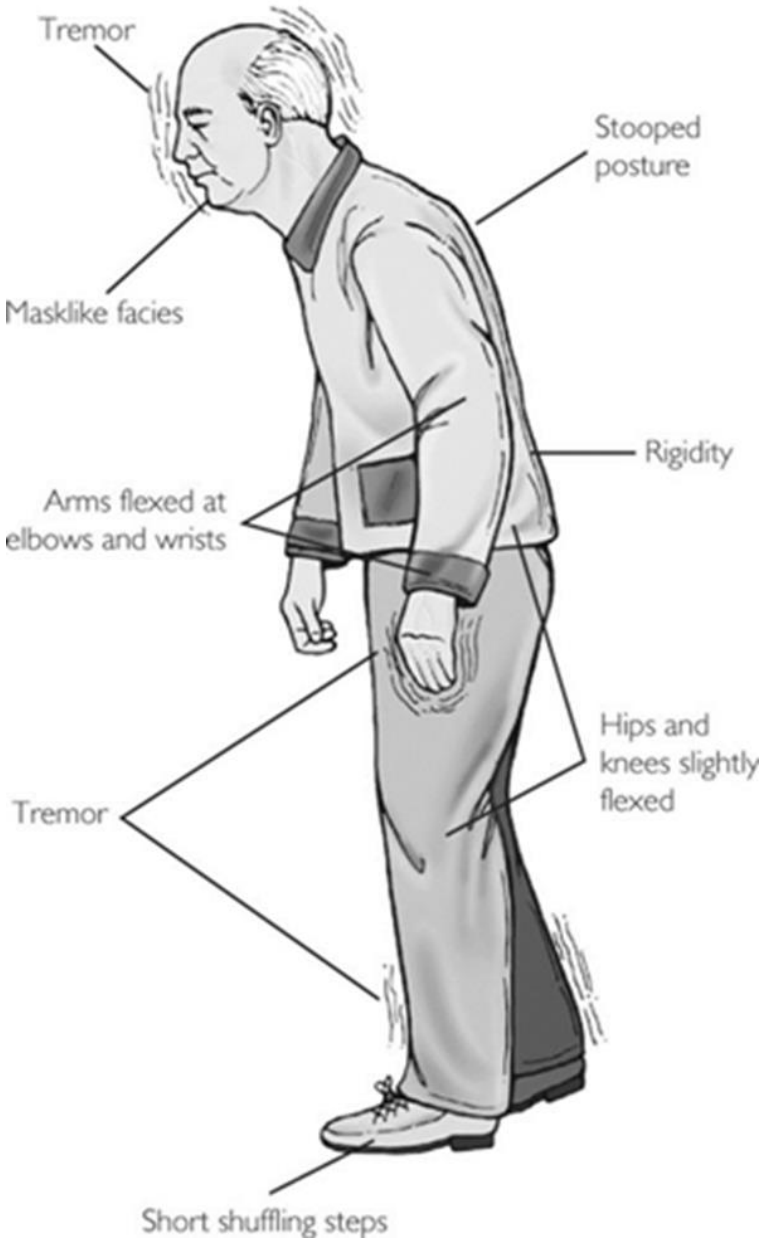
-Rigidnost (povečan mišični tonus, povečan odpor na pasivno premikanje okončin)

-Spremenjena hoja (kratki, podrsavajoči koraki), ki jo spremljajo motnje ravnotežja in sključena drža.

-Spremembe govora (monoton in tih).

-Brezizrazen obraz (obrazne mišice, ki so pomembne za izraz, se ne premikajo). Pogosto takšen izraz obraza zamenjamo za depresijo.

PB se predpostavi, ko pacijent kaže vsaj dva našteta simptoma.

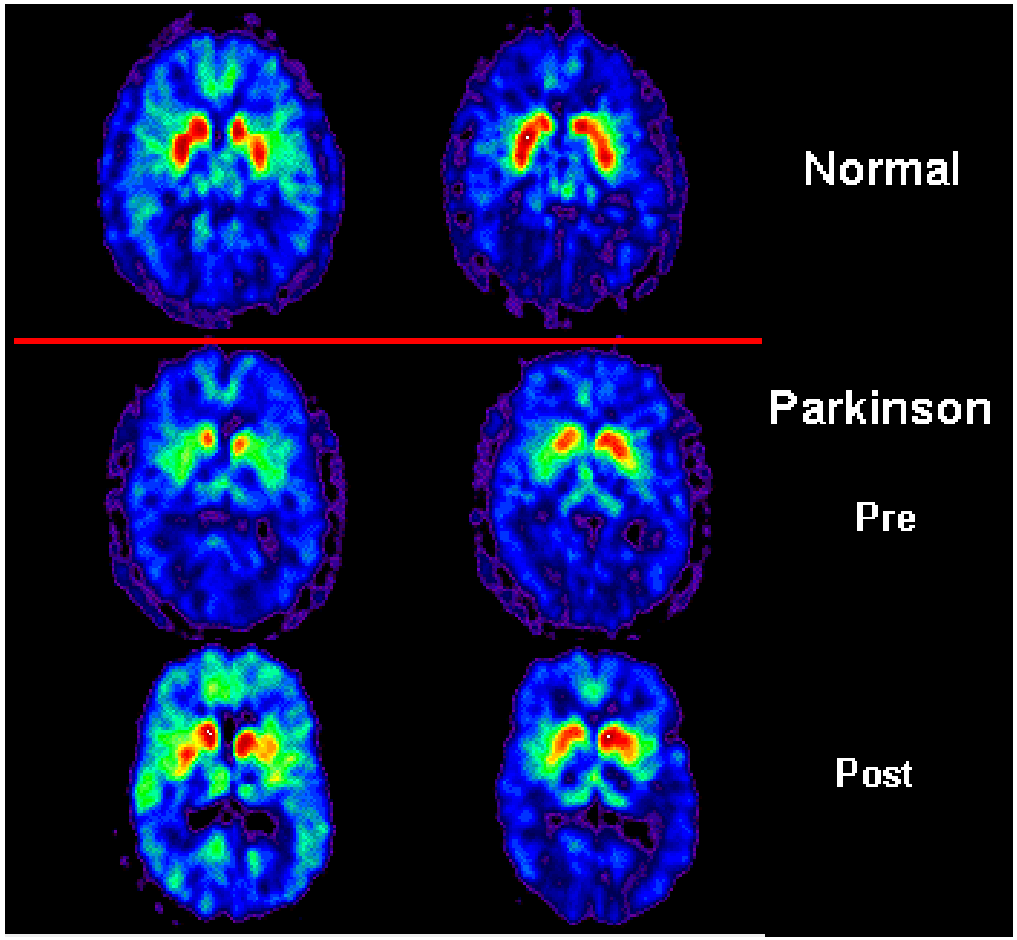


Nemotorične motnje

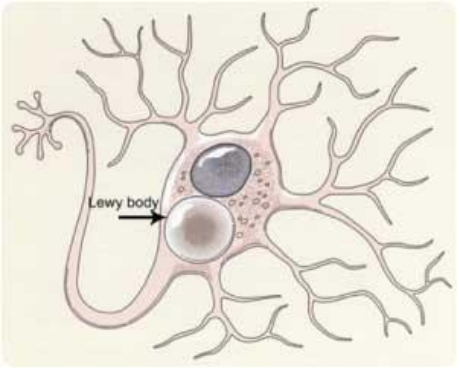
- Bolečina
- Motnje spanja
- Kognitivne / psihiatrične motnje
  - Depresija (~ 50 %)
  - Anksioznost (~ 40 %)
  - Halucinacije
  - Demenca (30 - 70 %)
    - motnje delovnega spomina in izvršitvenih funkcij

Diagnoza PB

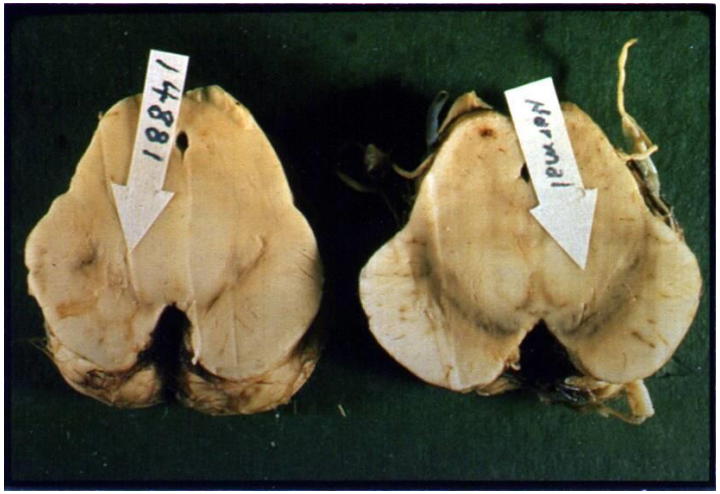
- -V zgodnjih fazah razvoja PB je klinična diagnoza precej negotova. Zanesljivih bioloških označevalcev ni.
- Odzivnost na L-DOPA je zanesljiv indikator PB.
- Slikanje s PET.
- Zanesljiva diagnoza samo post-mortem.



Patologija PB

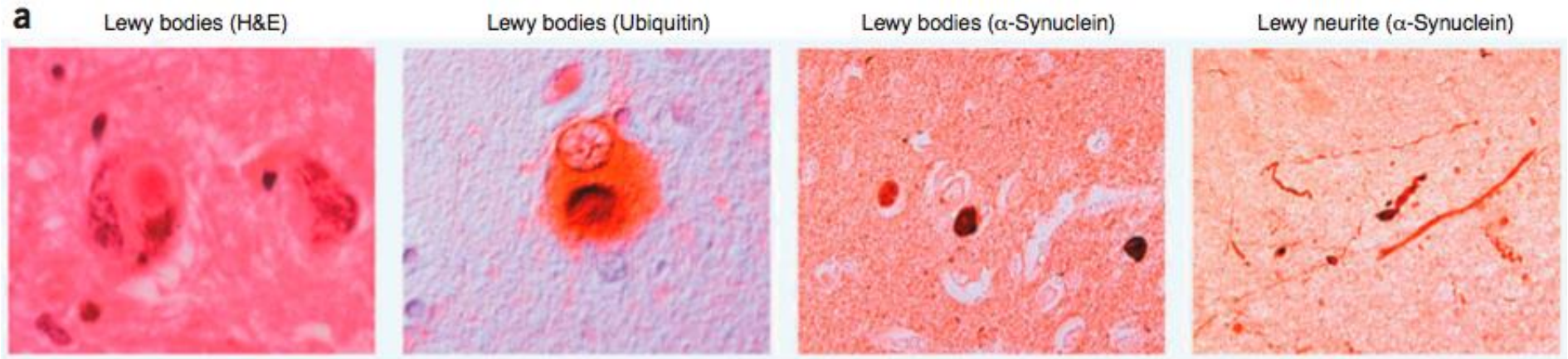


1. Izguba nigrostriatalnih dopaminergičnih nevronov.
2. Prisotnost proteinskih citoplazemskih inkluzij – Lewyjevih telesc.
3. Vzorec izgube celic je podoben vzorcu nivoja izražanja transporterja DA (DAT).
4. Ob pojavu simptomov je Substantia Nigra Pars Compacta (SNpc) že ~60% poškodovana.
5. Več poškodb nastane na živčnih končičih, kar pomeni, da so končiči dopaminergičnih nevronov primarna tarča za nevrodegeneracijo.



Lewyeva telesca

1. Znotrajcelični agregati niso značilni samo za PB, ampak tudi za nekatere druge NDB (Npr. demenca z Lewyevimi telesci).
2. Sferični eozinofilni citoplazemski proteinski agregati, ki so premera 7-20 nm in vsebujejo: **a-sinuklein, parkin, ubikvitin** in **nevrofilamente**

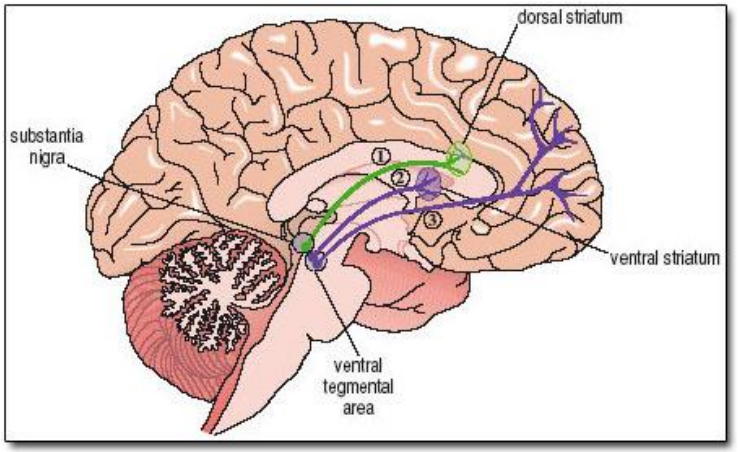


Bossy-Wetzel et al., Nat Med 2004

3. **Mehanizem toksičnosti še ni znan.** Agregati lahko direktno poškodujejo celico tako, da naredijo napako v znotrajceličnem transportu ali s sekvestracijo topnih proteinov, ki so ključnega pomena za celico.
4. Inkluzije lahko nastanejo zaradi procesa nabiranja topnih napačno zvitih proteinov v citosolu.

Patogeneza PB: stadiji po Braaku kažejo na progresivno sinukleinopatijo

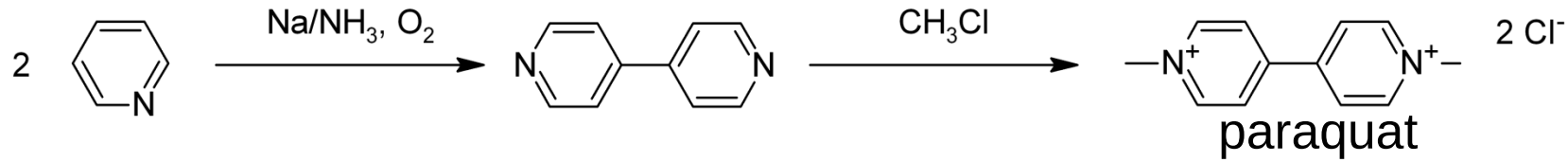
- LB patologija se širi posteriorno →anteriorno
- Stadij 1-2: LB v meduli in olfaktornem bulbusu (predvsem simptomatično)
- Stadij 3-4: LB v SNpc, locus coeruleus, holinergični bazalni sprednji možgani (simptomi PB se opazijo, ko je zgubljenih >80% nevronov SNpc).
- Stadij 5-6: LB tudi v sprednjih možganih (demenca)



Faktorji rizičnosti za PB

Okoljski faktorij: neposredna korelacija!

1) Izpostavljenost toksinom in pesticidom (MPTP, rotenon-insekticid, pesticid, paraquat-herbicid); z oporečnega sadja in zelenjave ali kontaminirane vode. Verjetost za PB se znatno poveča pri ljudeh, ki so delali več kot 10 let na plantažah. Inhibira se mitohondrijski kompleks I in posledično nastanejo poškodbe mitohondrijev.
2) Varjenje in izpostavljenost težkim kovinam (cink, amalgam, baker, svinec, železo, mangan,…), ki povzročajo oksidativni stres in proizvodnjo ROS.



Osebne navade

Dovoljene droge

Tobak:

Med kadilci zmanjšan rizik za PB. Nikotin mogoče ščiti nevrone s:

- s spodbujanjem sproščanja dopamina

- z delovanjem kot antioksidant

- z vplivanjem na delovanje MAOB

Kava:

Pomembno zmanjšanje rizika med ljudmi, ki pijejo kavo. Možno je, da kafein ščiti nevrone z inhibicijo adenozinskih A2 receptorjev, ki zmanjšuje vnetja in vzdržuje delovanje dopamiskih D2 receptorjev.

Alkohol:

Alkohol lahko vzdržuje sporščanje dopamina, vendar še ni jasno, če dejansko pomaga pri PB.

Prehrambeni dodatki

Antioksidanti:

Vitamini C in E nevtralizirajo proste radikale in s tem zmanjšajo dovzetnost za PB. Visoke količine vitamina E znatno zmanjšajo rizik za PB.

Maščobe in maščobne kisline

Dovzetnost za PB se poveča z mastno prehrano. Poveča se peroksidacija maščob in posledično tvorbo ROS.

Polinenasičene m.k. (**omega-3 and omega-6** esencielne m.k., še posebaj arahidonska in linolenska kislina) pomagajo pri zmanjšanju dovzetnosti za PB.

Železo

V SNpc bolnikov se kopiči železo, ki je lahko povezano s tvorbo prostih radikalov.

Etiologija PB

Genetski

a-sinuklein

Parkin

PINK1

LRRK2

UCHL-1

DJ-1

Sporadični

Okoljski toksini:

MPTP

Paraquat

Rotenon

Kokain

Endogeni toksini:

ROS

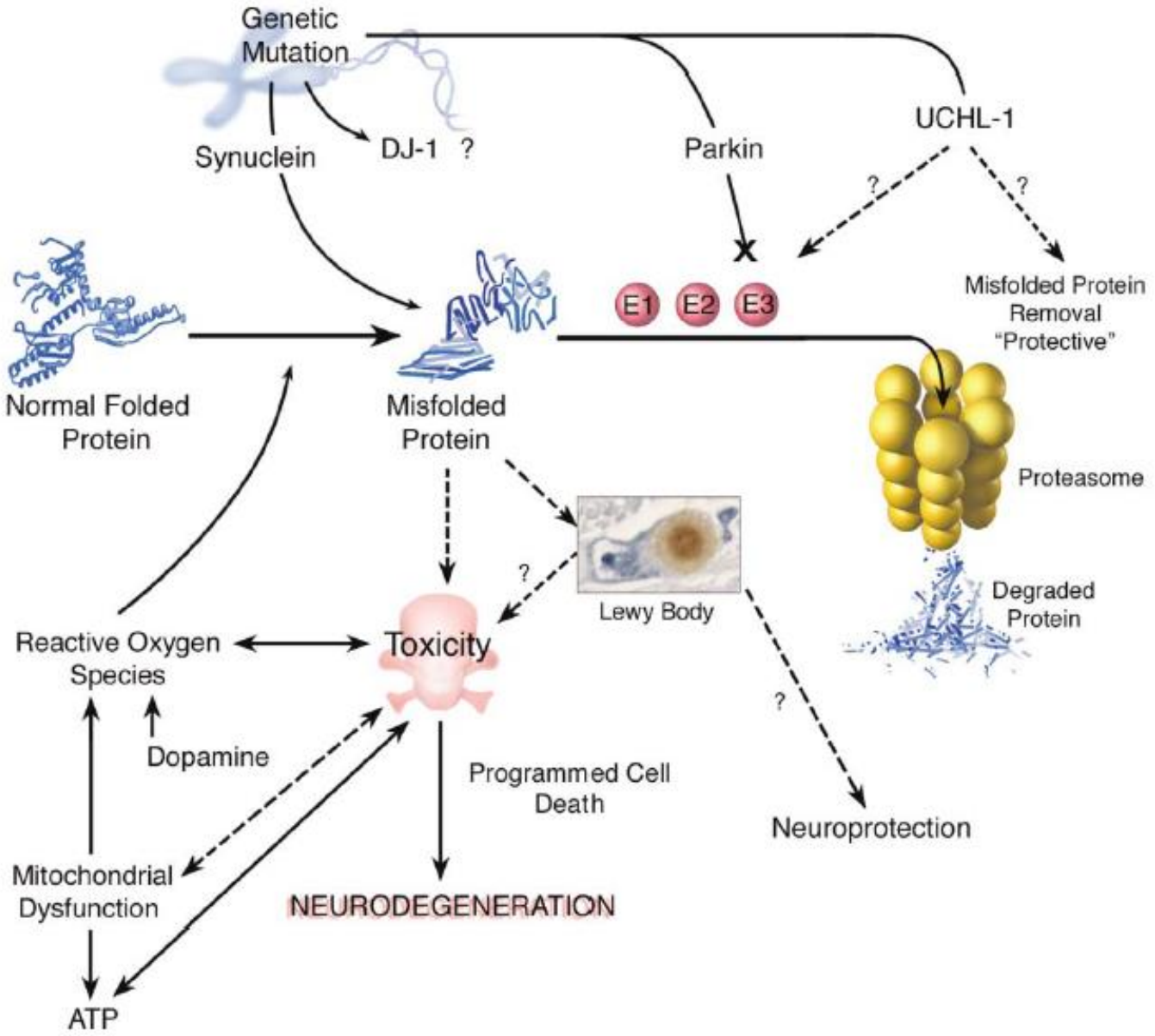
Mutacije pri PB: vloga in začetek bolezni

Table 1 | **Parkinson's disease-associated genes**

Locus	Gene	Inheritance	Function	Phenotype
*PARK1/4	α-Synuclein	Autosomal dominant	Involved in synaptic vesicle formation	Age of onset: 30–60 years Lewy bodies: ++
PARK2	Parkin	Autosomal recessive	An E3 ligase	Age of onset: ~30 years ‡Lewy bodies: –
PARK6	Phosphatase and tensin homologue (PTEN)-induced kinase 1 (*PINK1*)	Autosomal recessive	A mitochondrial kinase	Age of onset: 30–50 years Lewy bodies: ?
PARK7	Parkinson's disease (autosomal recessive, early onset) 7 (*DJ1*)	Autosomal recessive	Involved in oxidative stress response	Age of onset: 20–40 years Lewy bodies: ?
PARK8	Leucine-rich repeat kinase 2 (*LRRK2*)	Autosomal dominant	A protein kinase	Age of onset: 40–60 years Lewy bodies: + variable pathology
Unmapped	HtrA serine peptidase 2 (*HTRA2*, also known as *OMI*)	Autosomal dominant? Predisposition	A serine protease and/or involved in stress response	Age of onset: 44–70 years Lewy bodies: ?

*PARK1 and 4 share an entry because they have been shown to be caused by the same gene. ‡There has been one reported case of a parkin-positive patient with Lewy bodies. ++ Fulminant Lewy body pathology. + Lewy bodies present.

Okoljski in genetski dejavniki pri PB



Dauer et al., Neuron 2003

Zdravljenje PB

- Dopaminergčna terapija ima občuten vpliv na simptome PB.
- Kiruški posegi – selektivne lezije ali stimulatorne terapije vplivajo na simptome PB.
- Genske terapije, matične celice, v razvoju…
- Ni terapije, ki bi preprečila propad nevronov.

L-DOPA: olajša sintezo dopamina. Za enkrat je najboljše zdravilo za PB in “standard”, s katerim se primerjajo vsa druga zdravila. Uprablja se tudi pri diagnozi (odzivnost na L-DOPA).

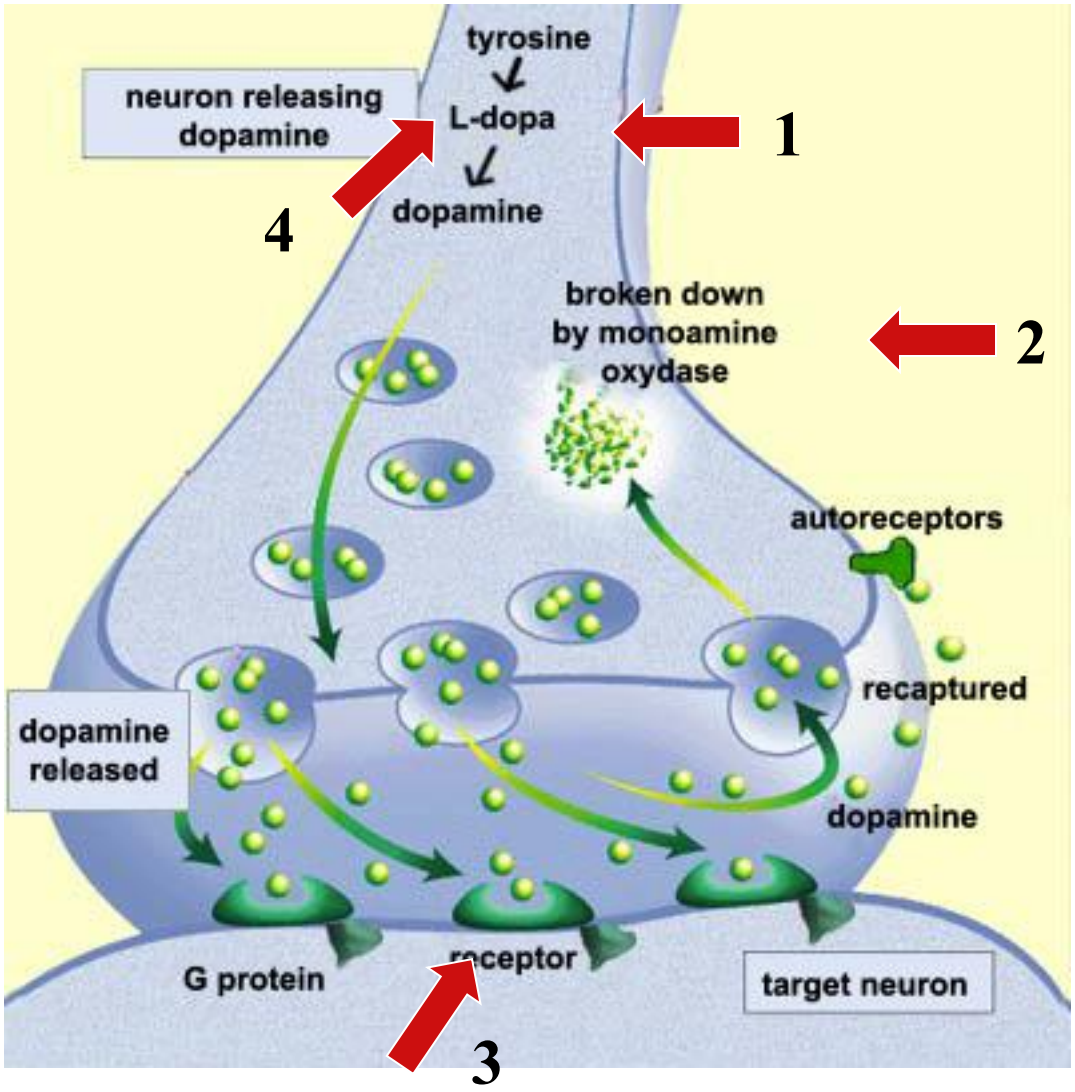
Uporablja se skupaj z zdravilom Carbidopa, ki zmanjša procesirnje L-DOPA (v dopamin) v krvi. Na ta način več L-DOPA pride do možganov in so zato potrebne manjše doze. Po daljšem času jemanja L-DOPA nima več učinka.

Inhibitorji MAOB (monoamin oksigenaza): zmanjšajo razgradnjo dopamina.
Selegiline - Uporablja se v vseh fazah bolezni, ker poveča učinek L-DOPA.

Agonisti dopaminskih receptorjev: strukturno so podobni dopaminu in stimulirajo dopaminske receptorje. Povečajo signalizacijo pri dopaminergičnih nevronih. **(Bromocriptine, Pergolide, apomorfin hidroklorid, Ropinirole).**

Inhibitorji COMT : Entacapone, Tolcapone. Inhibirjao aktivnost COMT (catechol O-methyltransferase), ki razgradi L-DOPA z metilacijo ene hidroksilne skupine na kateholnem jedru molekule. Uporablja se skupaj z L-DOPA.

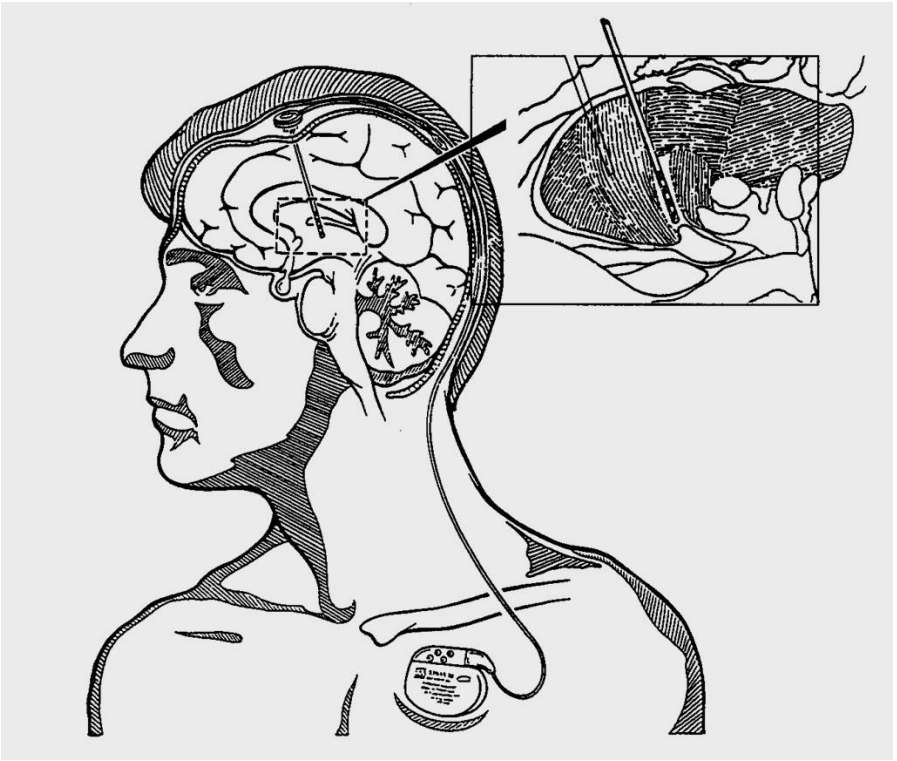
Sinteza dopamina in terapija



1) L-DOPA
2) Inhibitorji MAOB
3) Agonisti dopaminskih receptrojev
4) Inhibitorji COMT

Kirurško zdravljenje

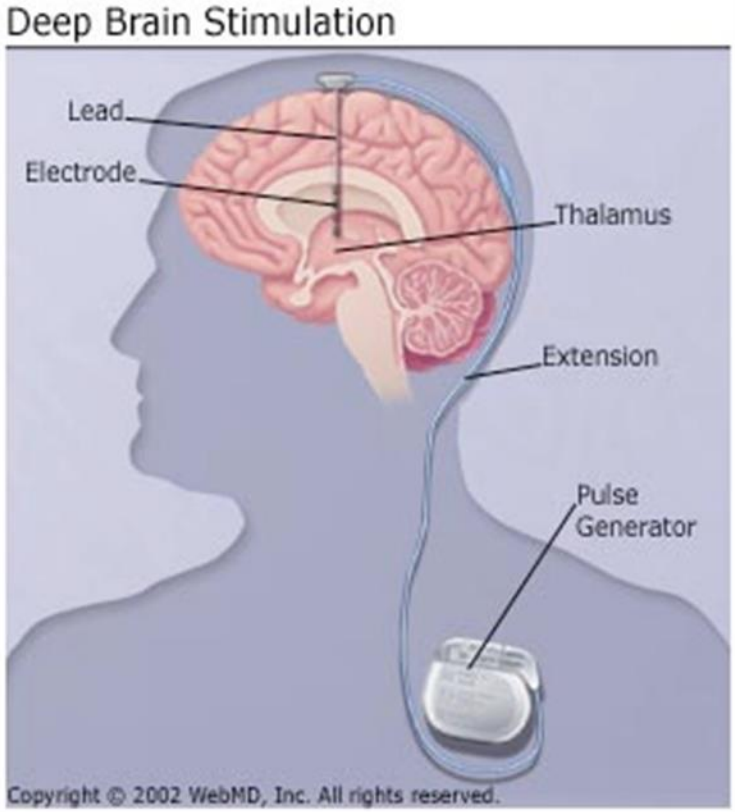
- Stereotaktična
  - Lezije v možganih
  - Spodbujanje (*deep brain stimulation* = *DBS*)
  - Talamus, notranji del globus palidusa, subtalamično jedro
- Transplantacija matičnih celic
  - Trenutno ni priporočena



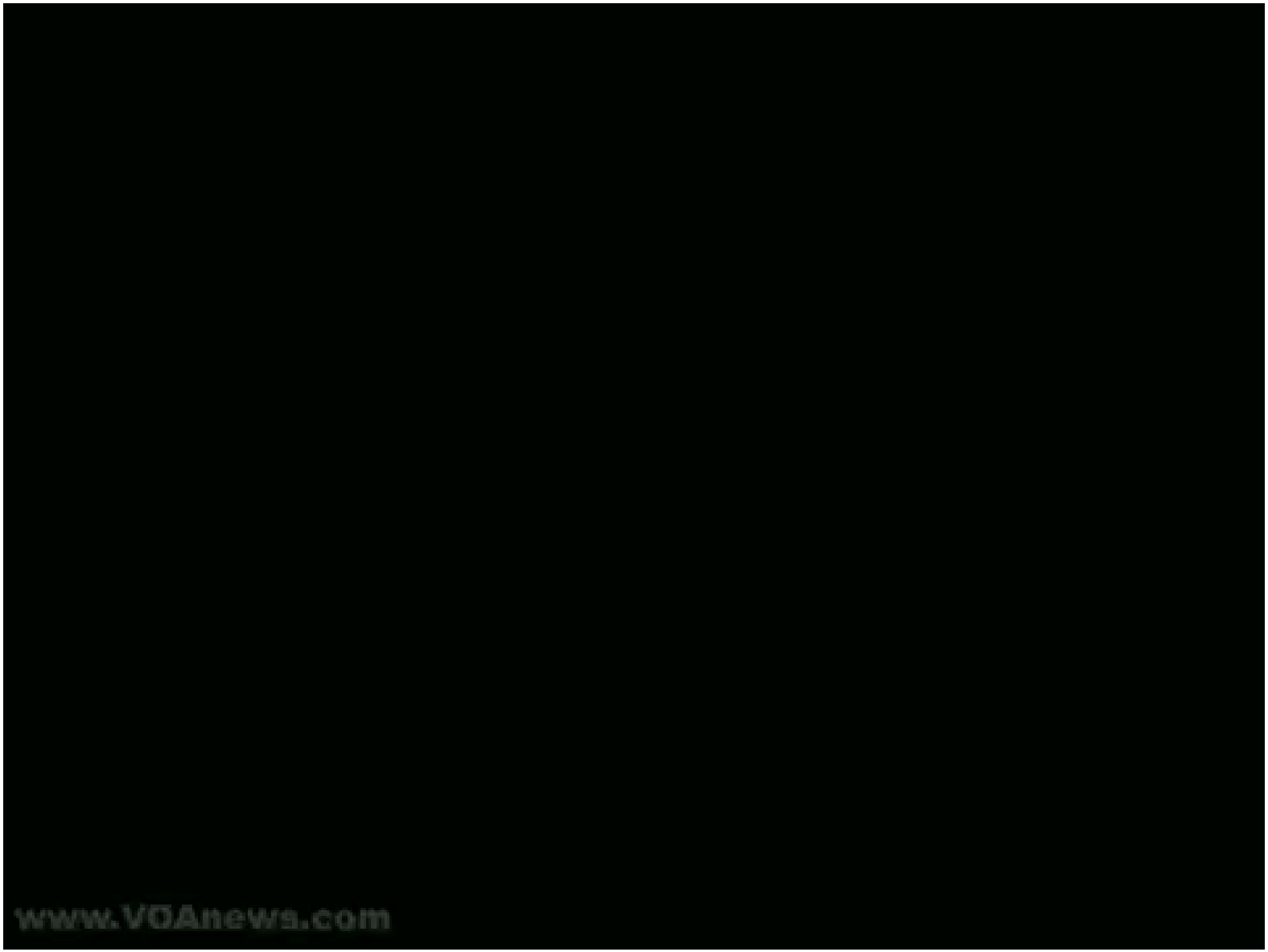
Spodbujanje možganov (Deep Brain Stimulation (DBS))

Kirurška procedura, ki se uporablja pri blaženju simptomov številnih hudih nevroloških obolenj. Pogosto se uporablja pri PB za zmanjšanje tremorja, rigidnosti, počasnih gibov in težave s hojo.

Pri DBS se uporablja nevrostimulator (podoben spodbujevalcu srca), ki z električno stimulacijo spodbuja del možganov, ki uravnavajo gibanje in pri tem blokirajo abnormalne živčne signale, ki povzročajo tresenje pri PB.



Film DBS parkinson



- http://www.youtube.com/watch?v=WYDoHmg9ECI

Modeli PD

6-OHDA

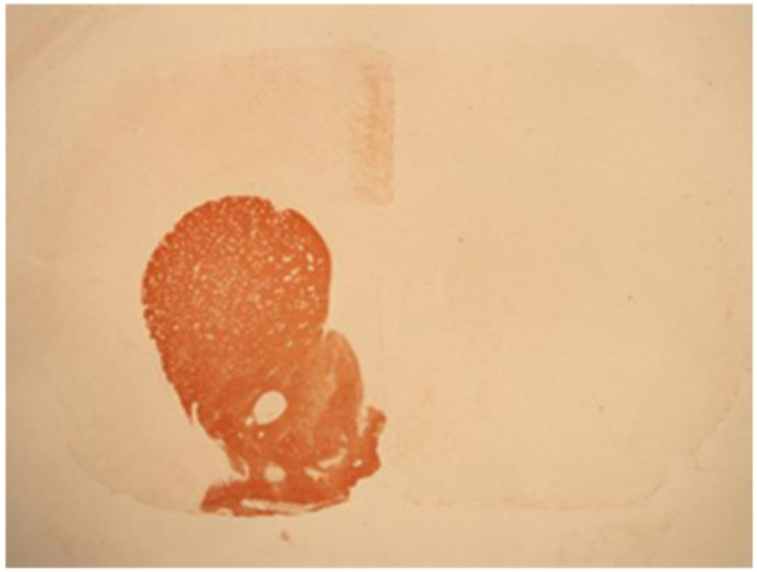


FIGURE 1: Photomicrograph of a 6-OHDA lesioned rat striatum immunostained for tyrosine hydroxylase (TH). Densities of TH-immunoreactivity striatal fibers are clearly reduced after the 6-OHDA injection (right side) as compared to the densities of striatal TH-immunoreactivity fibers in control rat (left side).

MPTP

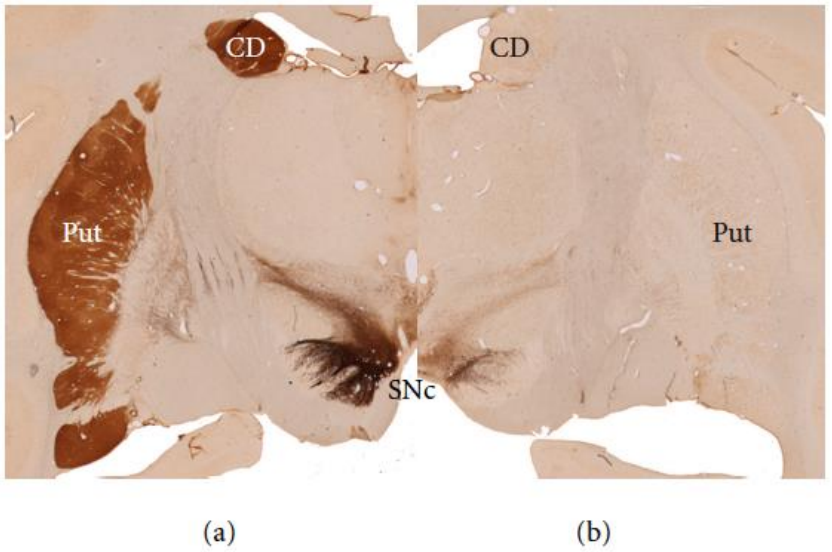


FIGURE 2: Photomicrographs of nonhuman primate immunostained for tyrosine hydroxylase (TH). Dopaminergic neurons located in the substantia nigra compacta (SNc) project to the caudate (CD) and putamen (PUT). Note the markedly reduced TH immunoreactivity in the substantia nigra and striatum (CD and PUT) in the MPTP-treated monkey (b) compared to control (a).

Journal of Biomedicine and Biotechnology
Volume 2012, Article ID 845618, 10 pages
doi:10.1155/2012/845618

Živalski modeli PB

Table 1

Model	Behavioral symptoms	Nigrostriatal damage	Synuclein aggregation/Lewy body formation	Uses of the model	Disadvantages
6-OHDA	Rotational behavior after unilateral injection	Loss of DA innervation at injection site (striatum)	No inclusions	Screen therapies that may improve PD symptoms. Study mechanisms of cell death	Requires intracerebral injection, very little synuclein involvement.
MPTP	Motor impairments in primates Less obvious motor impairments in acute rodent models	Loss of DA neurons dependent on dosing regimen, reaching 95% in acute high-dose conditions. Reduced DA levels in striatum concurrent with midbrain DA neuron loss	Inclusions not prevalent. Few cases of synuclein aggregation in nonhuman primates, as well as increased synuclein immunoreactivity in rodents.	Screen therapies that may improve PD symptoms. Study mechanisms of cell death	Nonprogressive model of cell death. Inclusiones are rare.
Rotenone	Reports of decreased motor activity in rodents	Loss of DA neurons accompanied by reduced DA innervation in striatum	Synuclein aggregation in DA neurons.	Test neuroprotective compounds	Substantial morbidity and mortality. Labor and time intensive.
Paraquat	No clear motor deficits	Decreased striatal TH immunoreactivity	No inclusions present, but increased synuclein immunoreactivity in DA neurons of the SN	Test neuroprotective strategies	Not extensively tested. Effects in other neurotransmitter systems.
α-synuclein	Severe motor deficits in the A53T model, less in the A30P model	Generally no DA neuron degeneration observed	Synuclein aggregation found in DA neurons, generally restricted to A53T model	Study the role of synuclein aggregation in PD, as well as the normal role of synuclein	Generally no DA neuron death observed with synuclein models
LRRK2	Few behavioral deficits seen in Drosophila mutation models	No effect on DA development or maintenance in knockouts, minimal levels of degeneration in mutation models	Generally not observed	Study the role of LRRK2 mutations related to PD	General lack of degeneration and general lack of synuclein aggregation.

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doi:10.1155/2012/845618

HUNTINGTONOVA BOLEZEN

Huntingtonova bolezen

- Znana tudi kot huntingtonova horea.
- Horea - nehoteni, hitri, odsekani, neredni, brezciljni gibi, ki se stalno spreminjajo in so najizrazitejši na distalnih delih udov in na obrazu.
- Je hiperkinetsko oboljenje, ki prizadene tri sisteme:
  - Gibanje
  - osebnost, razpoloženje, vedenje, čustva
  - kognicijo
- začne se kot:
  - motnja gibanja (60 %)
  - psihiatrična simptomatika (15 %)
  - kombinacija obojega (25 %)
- avtosomno-dominantna, visoko penetrantna, nevrodegenerativna, kronična, progresivna
- prevalenca: 4-10/ 100.000
- Slovenija: 120 bolnikov
- starost: 30-50 let
- smrt: 10-20 let po nastanku težav

THE

MEDICAL AND SURGICAL REPORTER.

No. 789.] PHILADELPHIA, APRIL 13, 1872. [Vol. XXVI.—No. 15.

Original Department.

Communications.

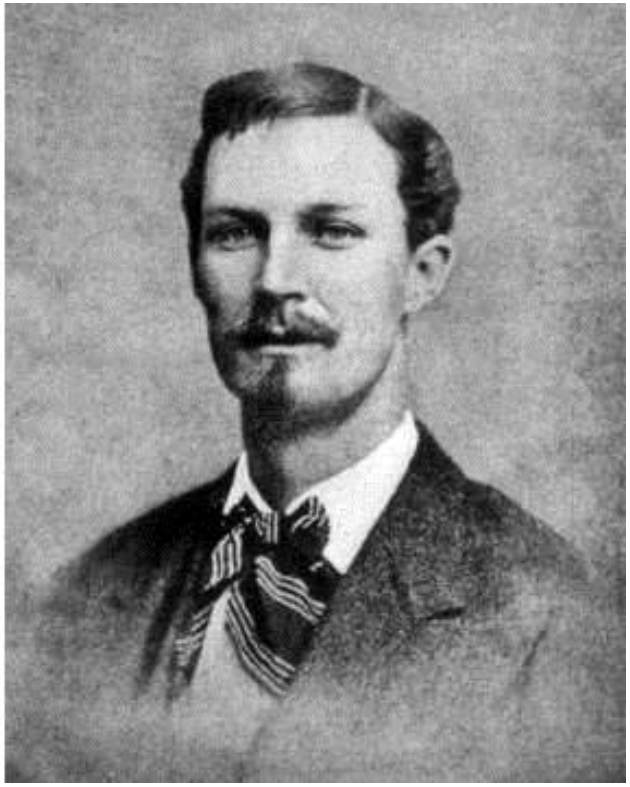
ON CHOREA.

By George Huntington, M. D.,
Of Pomeroy, Ohio.

Essay read before the Meigs and Mason Academy of Medicine at Middleport, Ohio, February 15, 1872

Chorea is essentially a disease of the nervous system. The name "chorea" is given to the disease on account of the *dancing* propensities of those who are affected by it, and it is a very appropriate designation. The disease, as it is commonly seen, is by no means a dangerous or serious affection, however distressing it may be to the one suffering from it, or to his friends. Its most marked and characteristic symptoms are manifested in the muscles of voluntary motion. The upper extremities may be the first affected, or both simultaneously. All the voluntary muscles are liable to be affected, those of the face rarely being exempted.

If the patient attempt to protrude the tongue it is accomplished with a great deal of difficulty and uncertainty. The hands are kept rolling—first the palms upward, and then the backs. The shoulders are shrugged, and the feet and legs kept in perpetual motion; the toes are turned in, and then everted; one foot is thrown across the other, and then suddenly withdrawn, and, in short, every conceivable attitude and expression is assumed, and so varied and irregular are the motions gone through with, that a complete description of



George Huntington (1850-1916)

J. C. Lund:

Chorea Sti Viti i Sætersdalen. Uddrag af Distriktslæge J. C. Lunds Medicinalberetning for 1860.

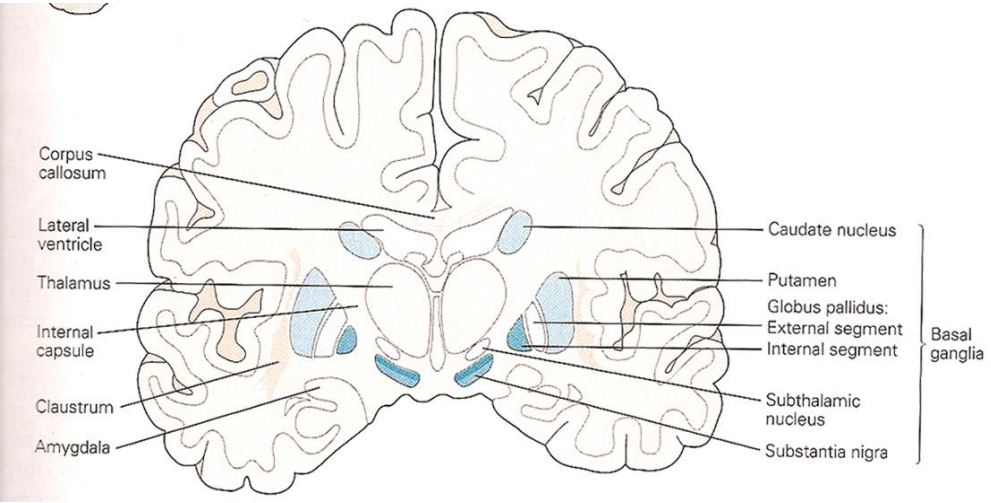
Beretning om Sundhedstilstanden m.m. i Norge i **1860**, pp. 137-138.

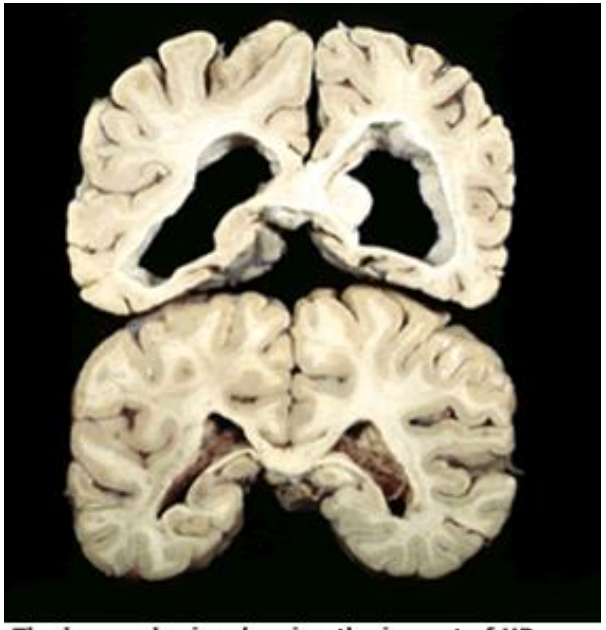
Ni prevedeno v angleščino do leta 1959.

PATOGENEZA

- mutacija gena za protein huntingtin na 4. kromosomu (l.1993)
- nestabilne ponovitve CAG tripletov
- poliglutaminska bolezen (polyQ)
- nestabilna mutacija, anticipacija
- več CAG-ov, prej zboliš (npr. >80, pred 10.letom)
- mutirani huntingtin je bolj odporen na proteolitično razgradnjo
- produkti neuspešne proteolize se kopičijo v citoplazmi in jedru, motijo celično funkcijo
- nevrodegeneracija, izguba nevronov, glioza
  - regionalna selektivnost: kaudatus, putamen, korteks, subkortikalna belina
  - sprva prizadeta indirektna kortiko-striato-palido-talamo-kortikalna pot, ki povzroči inhibicijo talamo-kortikalne aktivnosti in hiperkinezo
  - kasneje prizadeta tudi direktna pot, hiperkineza preide v akinezo (parkinsonizem)
- HB nastane zaradi selektivne degeneracije GABAergičnih nevronov v striatumu.

Propad možganov pri HB





HB

Normalno

The human brain, showing the impact of HD on brain structure in the basal ganglia region of a person with HD (top) and a normal brain (bottom).

http://kobiljak.msu.edu

Starost bolnikov, ko se HB pojavi:

HB je prava dominantna bolezen, ker so homozigotni prenašalci enako prizadeti kot heterozigotni.

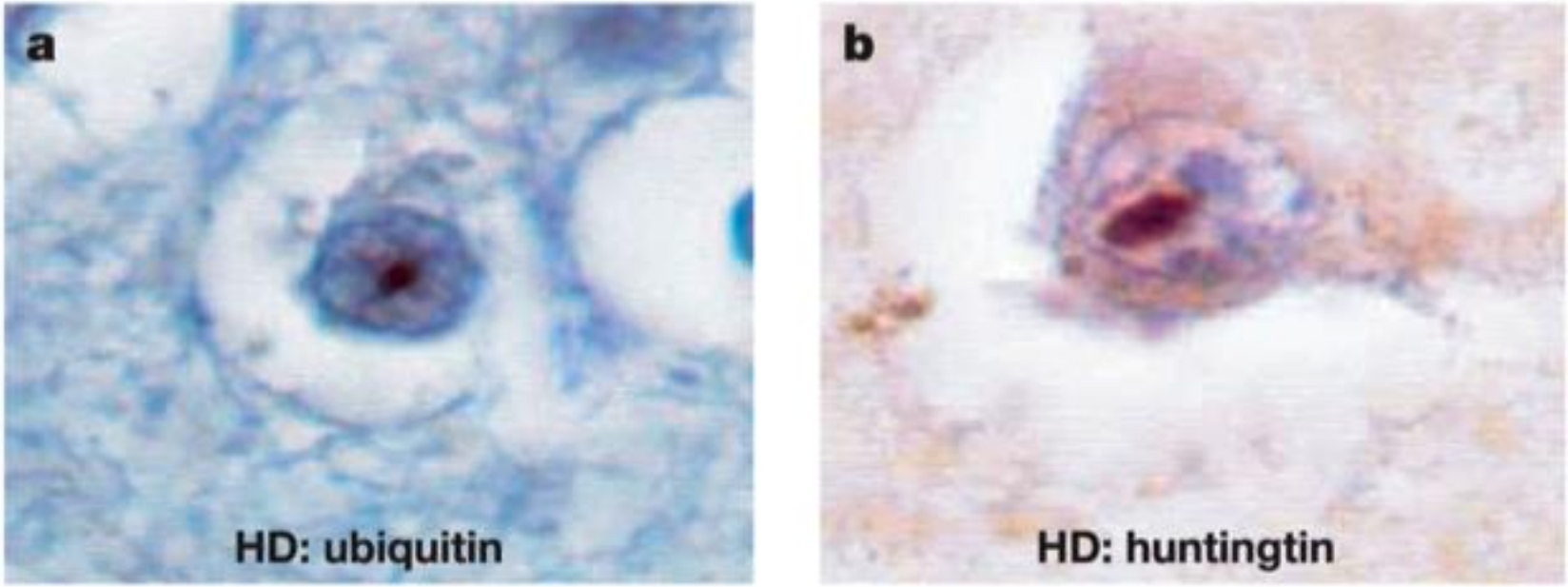
Pri normalni posamezniki je število ponovitev <36. Pri bolnikih s HB, ki imajo 46 ponovitev, pa se bolezen pojavi med 25. in 52. letom.

Mnogo faktorjev vpliva na začetek bolezni:

- Nepravilno delovanje mitohondrijev
- Apoptoza
- Izguba nevrotrofičnih faktorjev
- Nevronska ekscitotoksičnost

Agregati pri HB

Huntingtonova bolezen: je progresivna NDB, karakterizirana s CAG ponovitvami, ki nosijo zapis za poly Q motiv v N-koncu proteina **huntingtina**. Nastop bolezni obratno korelira s številom CAG ponovitev. Huntingtin tvori inkluzijska telesca.

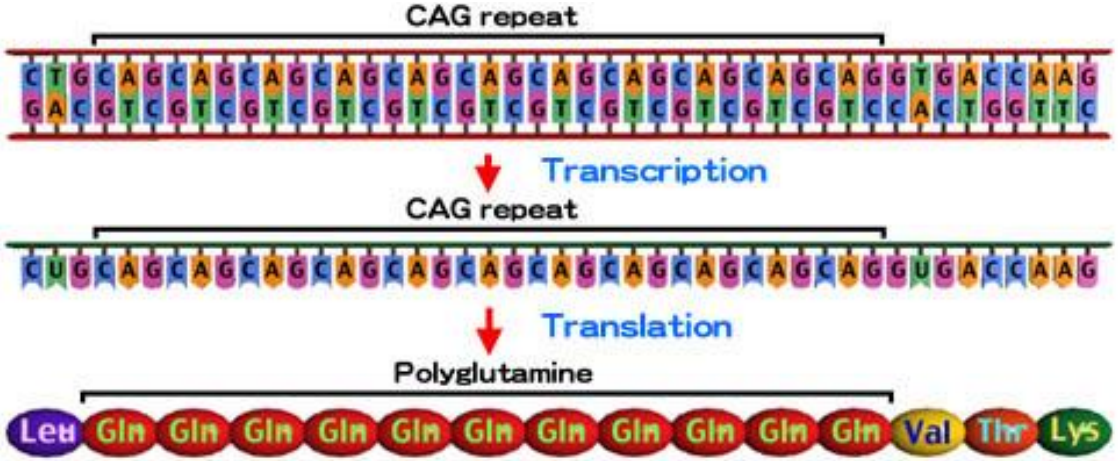


Ross et al., Nat Med 2004

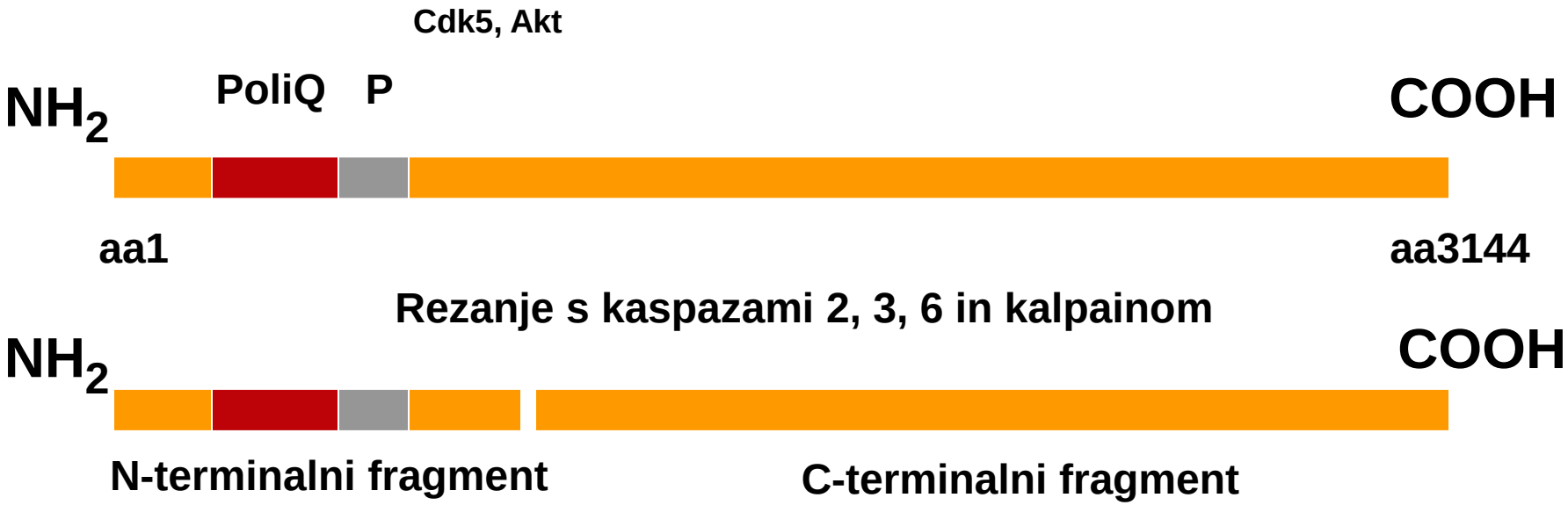
Huntingtin

Človeški gen za huntingtin nosi zapis za velik citosolni protein s 3144 ak in velikostjo 348 kDa.

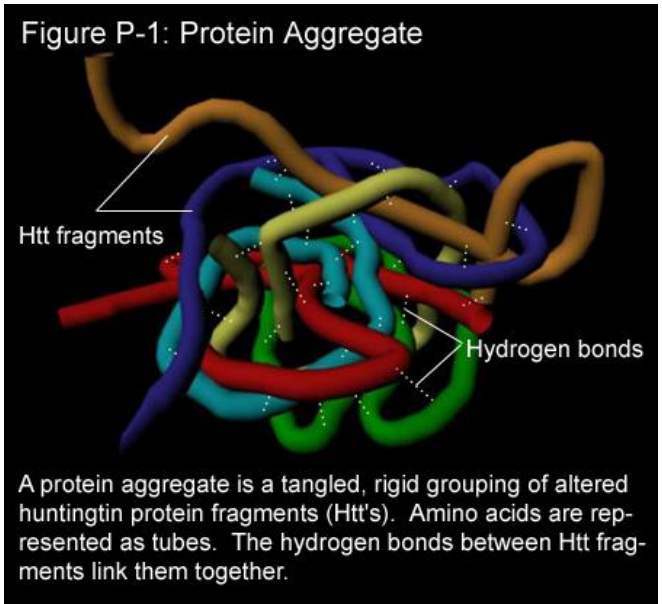
Normalno je v proteinu PoliQ<36, pri Bolnikih s HB pa >36.



Procesiranje PoliQ mutiranega huntingtina



N-terminalni htt-fragment tvori agregate



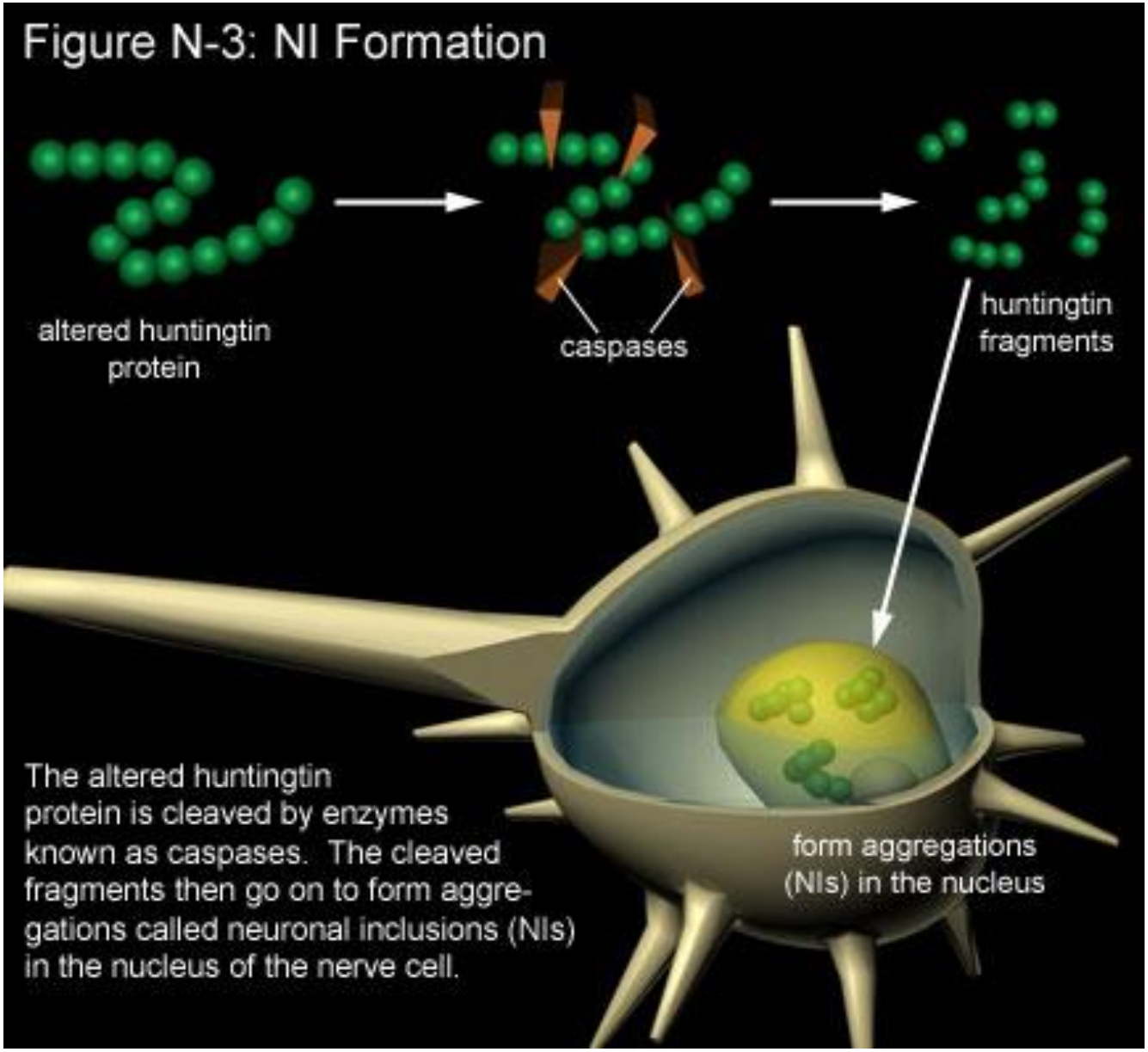
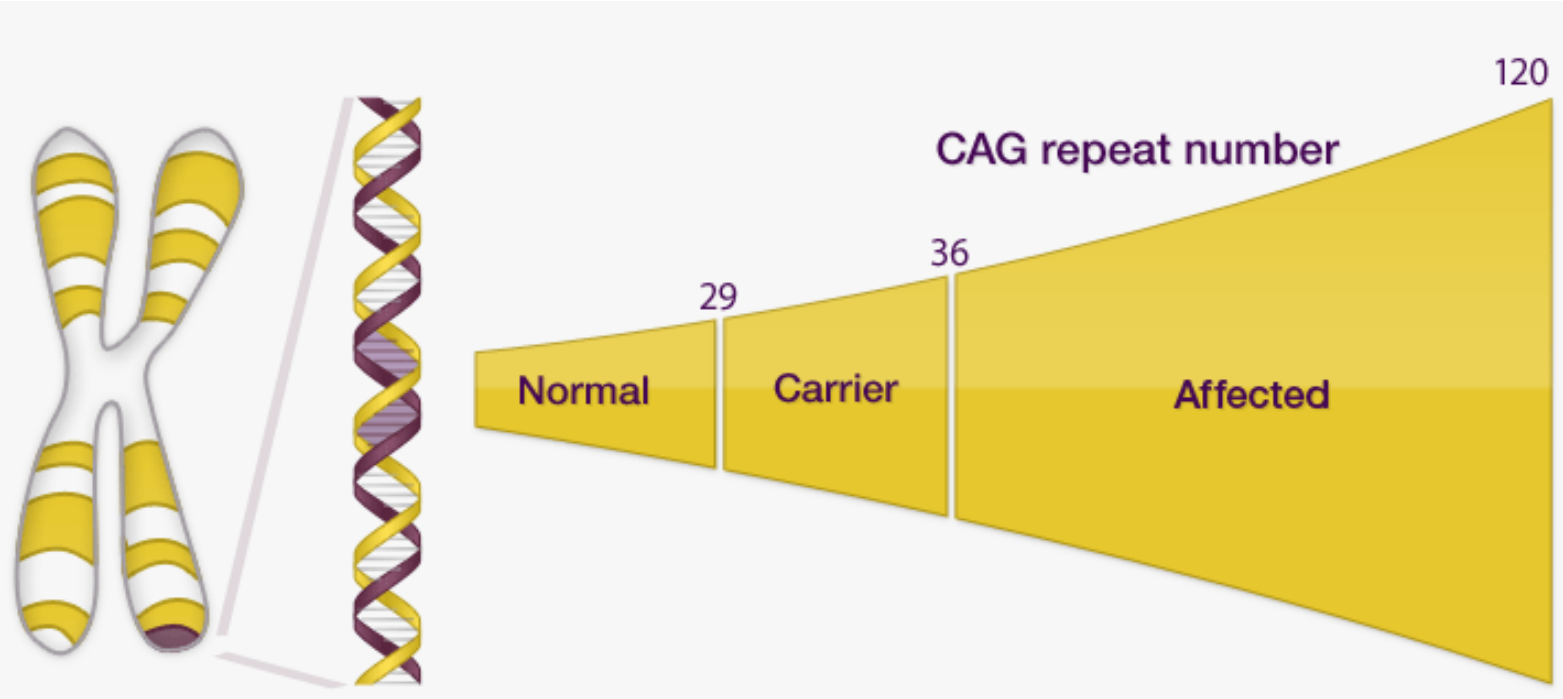


Figure N-3: NI Formation
altered huntingtin protein
caspases
huntingtin fragments
The altered huntingtin protein is cleaved by enzymes known as caspases. The cleaved fragments then go on to form aggregations called neuronal inclusions (NIs) in the nucleus of the nerve cell.
form aggregations (NIs) in the nucleus


120
CAG repeat number
36
29
Normal
Carrier
Affected

Pri HB so agregati prisotni v prizadetih delih možganov, a ni nujno, da so v degeneriranih nevronih.

Ali pri HB agregati ščitijo pred nevrodegeneracijo? Ali so htt fragmenti najbolj toksične molekule?

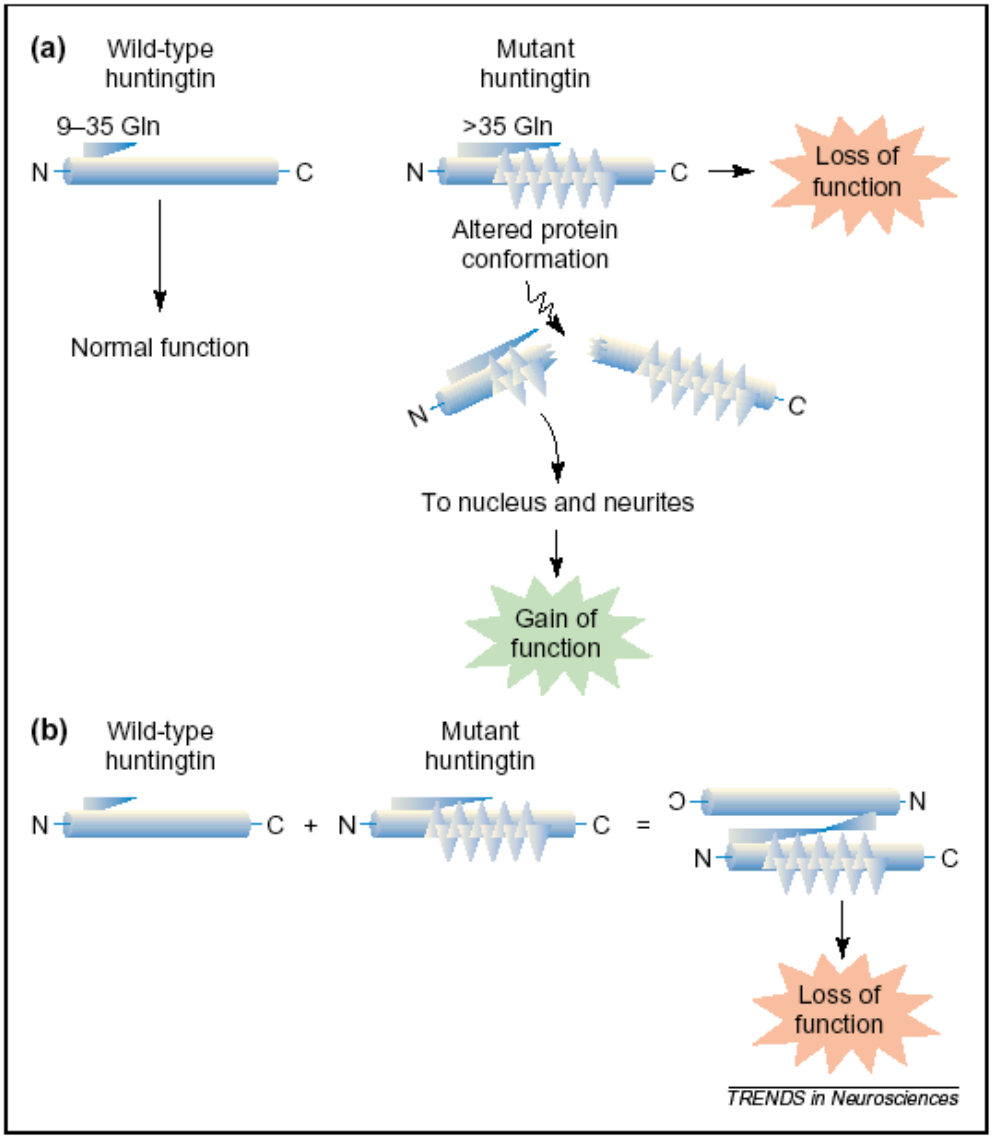
HB: izguba funkcije huntingtina in toksičnost mutiranega huntingtina

- Hetreozigotni pacienti so enako prizadeti kot homozigotni: nativni huntinghtin ne ‚ublaži' negativnega efekta mutiranega (poliQ) huntingtina.

- Mutiran huntingtin v netopne agregate (inkluzije) poveže nativni protein.

- Nevroni z večjimi inkluzijami so manj prizadeti kot tisti s razpršenimi htt fragmenti (lahko vsebujejo fragmente, ki niso agregirani v inkluzijah).

Potencialni mehanizem celične smrti pri HB



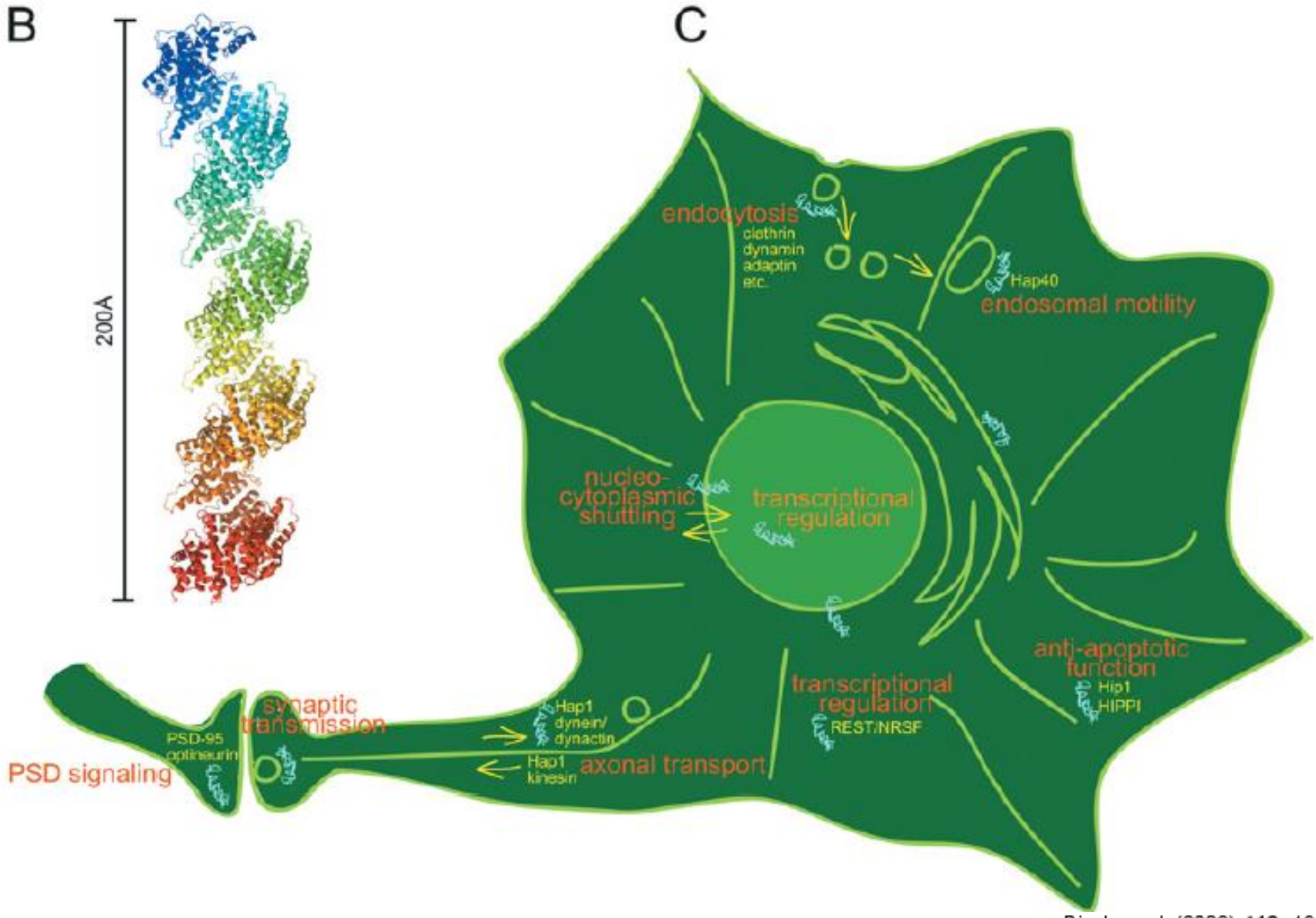
Fiziološka vloga huntingtina

- Huntingtin je potreben za razvoj organizma. KO miške umrejo 7,5 dni po rojstvu. Heterozigoti doživijo odraslo dobo.

- Huntingtin se veže na različne proteine, npr. proteine citoskeleta in proteine, povezane z membranskimi vezikli, kar nakazuje njegovo vlogo pri znotrajceličnem transportu, recikliranju membrane in retrogradnem hitrem aksonalnem transportu.

- PoliQ ponovitve, ki jim sledi poliP domena so povezane s transkripcijsko regulacijo proteinov. **Število CAG ponovitev je obratno povezano s transkripcijsko aktivnostjo.** Ko se huntingtin veže na proteine za regulacijo transkripcije, sodeluje pri njihovem prenašanju po citosolu in jedru.

Fiziološka vloga nativnega huntingtina



Biochem. J. (2008) **412**, 191–209

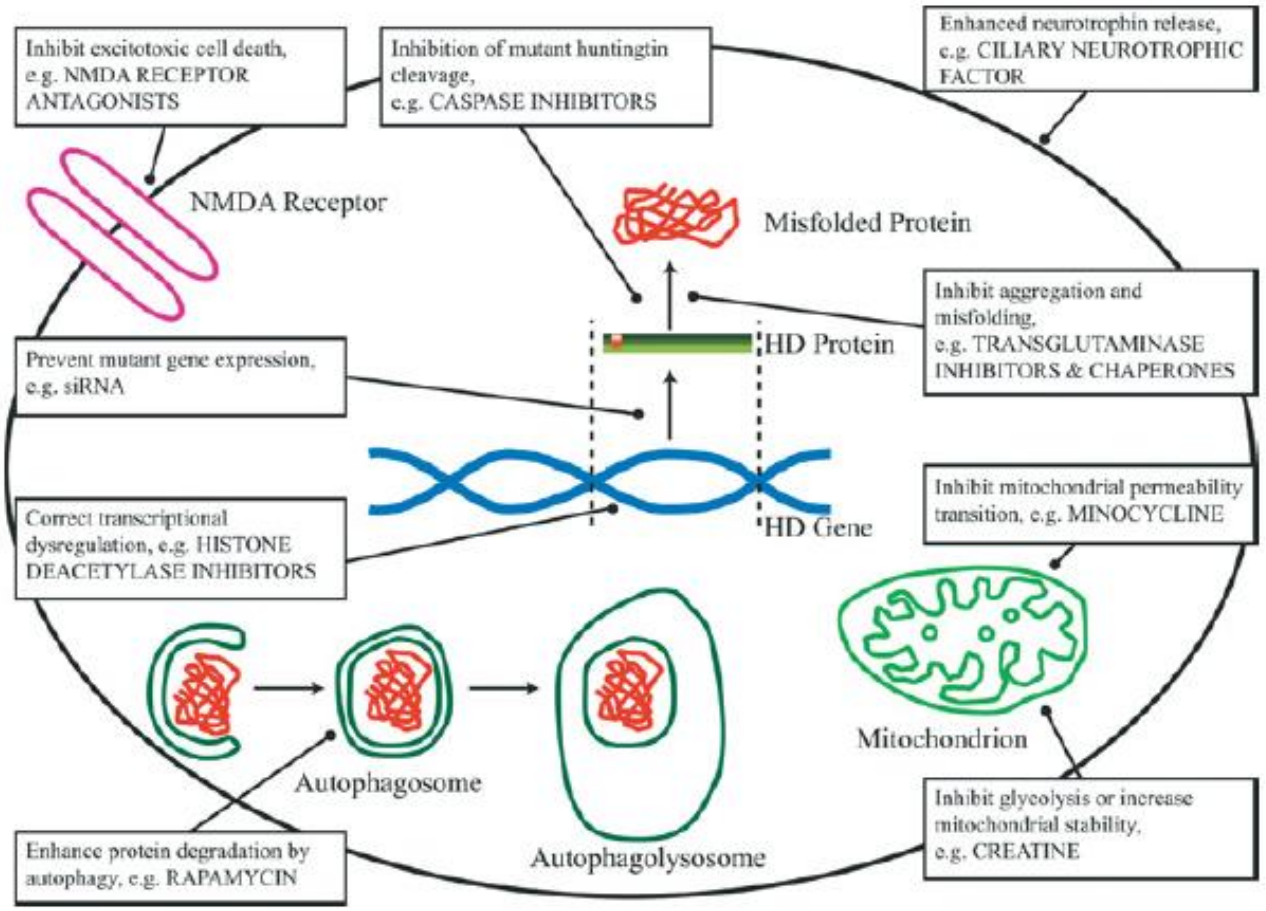
Terapija pri HB

- Presimptomatsko zdravljenje se začne po potrditvi HB z genetskim testom.
- Simptomatsko zdravljene proti depresiji, psihozi in horei:

  - Tetrabenazine – odstrani DA iz centralnih nevronov.

- siRNA proti mutiranem htt
- Inhibitorji kaspaz (vendar daljša uporaba poveča rizik za razvoj raka).
- Minociklin (antibiotik, ki je že v uporabi) je zdaj v kliničnih preiskavah za HD. Verjetno prepreči apapotozo zaradi inhibicije kaspaz in permeabilnosti mitohondijev. Verjetno sodeluje še pri drugih neznanih mehanizmih.
- Pospešitev odstranjevanja mutiranega Htt (npr. z avtofagijo).
- NMDA receptor antagonisti blokirajo z glutamatom inducirano eksocitotoksičnost.
- Induciranje fiziološke funkcije Htt.

Potencialne terapije za HB



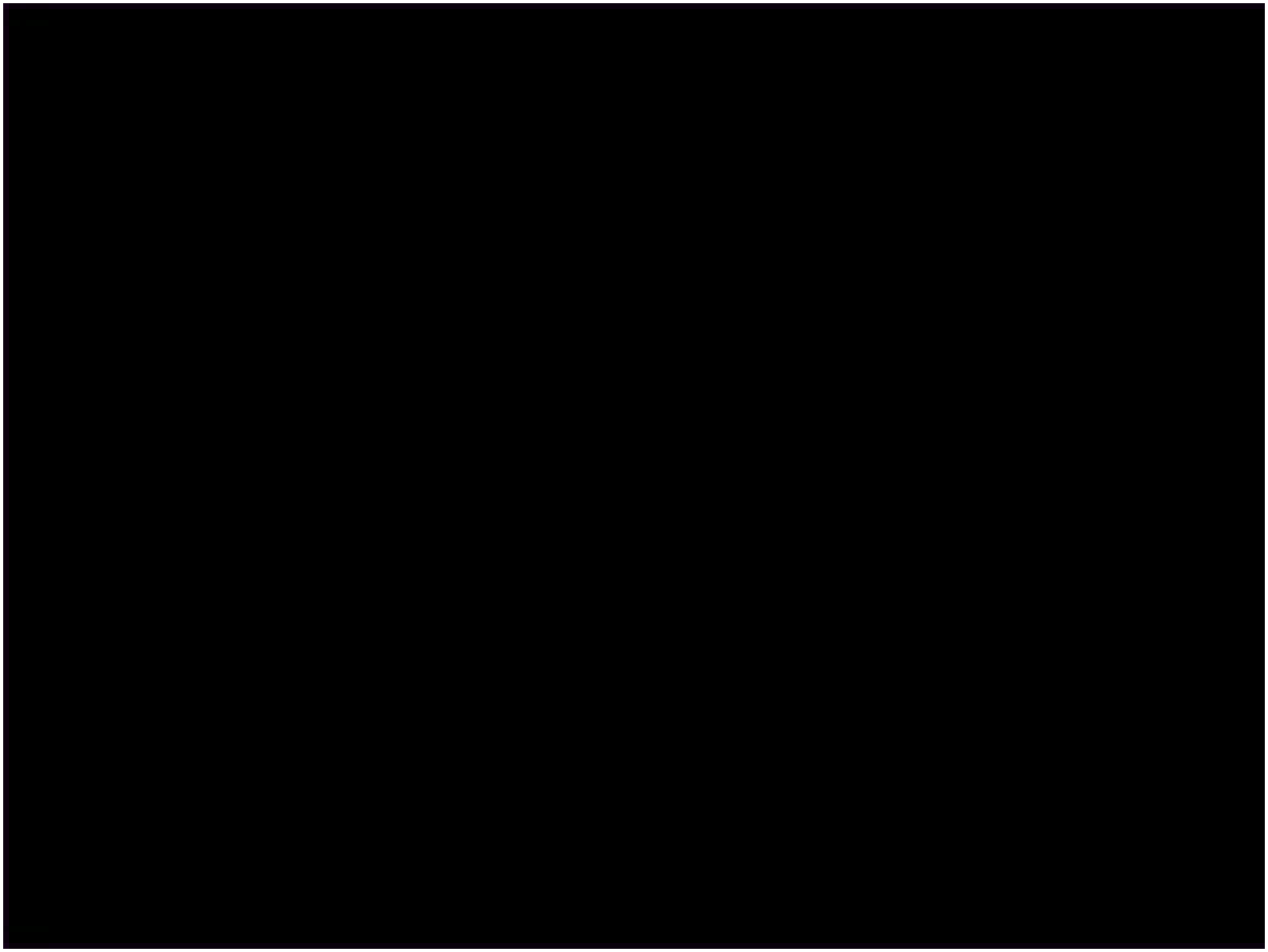
HD toxicity may be ameliorated by direct modification of the mutant gene or protein. Strategies which seek to achieve this include repression of mutant gene expression, inhibition of aggregation or misfolding, inhibition of the cleavage of the protein to form toxic fragments and increased clearance of the mutant protein by up-regulating autophagy. Alternative strategies depend on mitigating the deleterious effects of the mutant protein by stabilizing mitochondria or correcting transcriptional dysregulation. More general neuroprotective strategies which may be important include attempts to decrease excitotoxic cell death or enhance neurotrophin release. See the text for more details.

Biochem. J. (2008) **412**, 191–209

Vedenjski testi

- Vodni labirint
- Rotirajoča palica
- Ravnotežje na palici
- Test novosti

Vodni labirint



- http://www.jove.com/video/2920/morris-water-maze-test-for-learning-memory-deficits-alzheimers

Vodni labirint

Rotirajoča palica (rotarod)

Ravnotežje na palci

Test novosti