Frontiers in Neurodegenerative Disorders

May 3, 2017

190 Kapoor Hall

Links to text books

- Basic and Clinical Pharmacology

http://accesspharmacy.mhmedical.com.gate.lib.buffalo.edu/book.aspx?bookid=388

Very little on neurodegenerative disease outside PD

- Goodman and Gilman's The Pharmacological Basis of Therapeutics

http://accesspharmacy.mhmedical.com.gate.lib.buffalo.edu/book.aspx?bookid=374

Chapter 22 – Alzheimer's, ALS, Huntington's

Neurodegenerative Diseases

- **Alzheimer’s disease**
- Parkinson's disease
- Huntington’s disease
- Amyotrophic Lateral Sclerosis (ALS)
- Others:
  - Frontotemporal dementia with Parkinsonism
  - Prion diseases
  - Multiple sclerosis

Common features?

1. Loss of neurons and neurological function
2. Inherited (rare & typically early onset) and sporadic forms (more common, unclear cause)
3. More prevalent in older populations – problem is exacerbated as global population ages
4. Common pathological observations are the intracellular and extracellular protein aggregation and deposition of insoluble proteins.
5. Mitochondrial dysfunction?

Concepts

- Functional reserve
  - Threshold below which clinical signs appear
- Selective vulnerability
  - Genetic mutation in all cells causes dysfunction and death in subset of neurons
    - PD – SNpc DA neurons
    - HD – medium spiny neurons in neostriatum
    - ALS – upper and lower motor neurons
    - AD – cholinergic neurons
- Common mechanisms?
  - Misfolding & protein accumulation
    - PD – alpha-synuclein
    - AD – beta-amyloid and tau
    - HD – huntingtin
    - ALS – SOD1 and TDP-43
  - Excitotoxicity/NMDA

Selective vulnerability

- Specific neurodegeneration – specialized function of subsets of neurons
- Transmitter usage (dopamine)
- Receptors
- Biochemical specialization

Precise mechanisms largely unclear

Alzheimer's disease

- Prevalence
  - USA: 5.5 million patients, with 350,000 new patients / yr

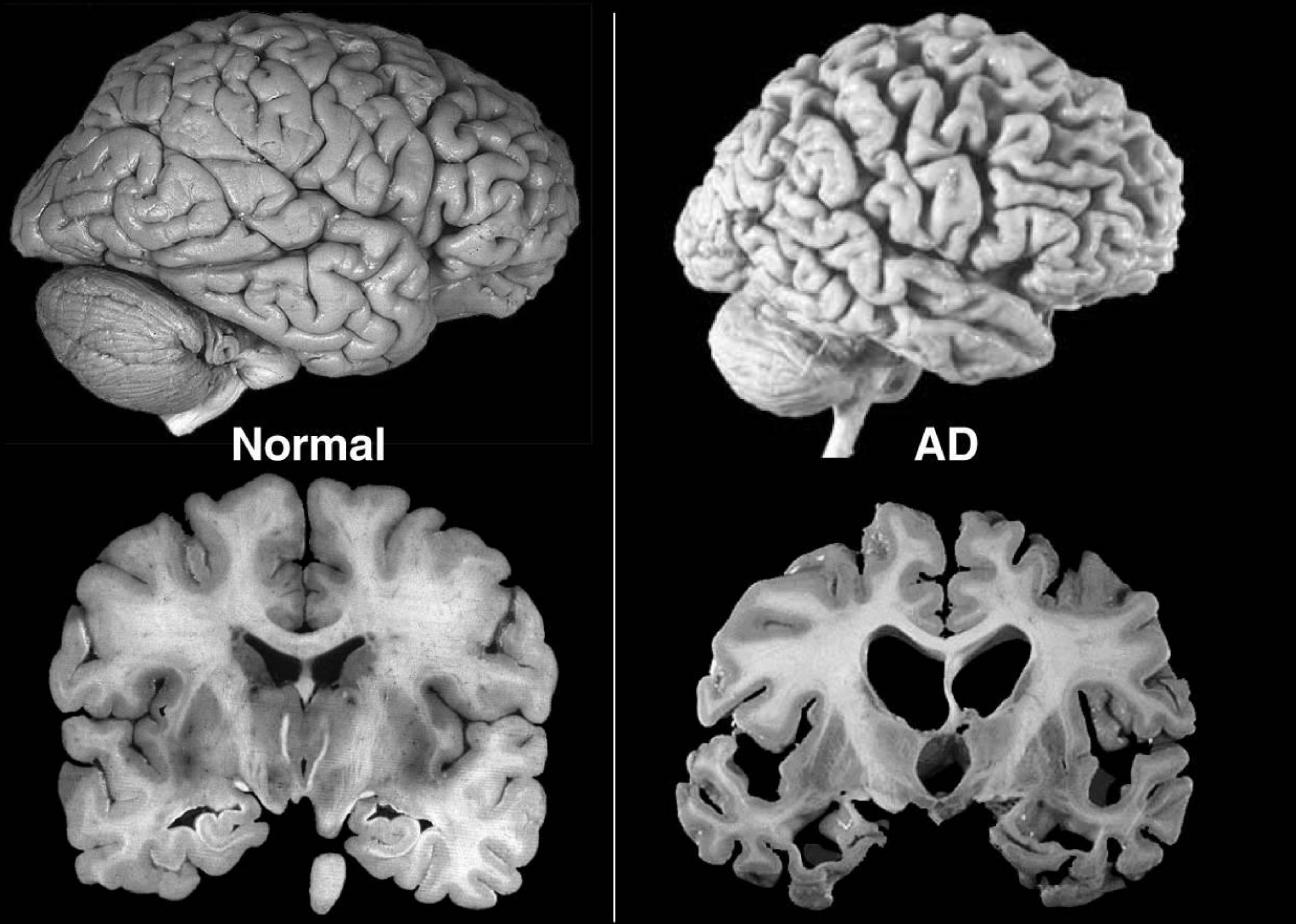
  - Under 65 yr age, rare but increases with age
  - > 85 yr age, between 10-30% of population!
  - Most common cause of dementia (50-56%)

- Common genetic causes (1-2% familial)
  - Amyloid precursor protein (APP)
  - Presenilin 1 (PSEN1) – *11% of genetic cases*
  - Presenilin 2 (PSEN2)
  - APOE4, an important risk factor

Clinical overview

- Medial temporal lobe – entorhinal cortex and hippocampus
- Anterograde episodic memory loss: repeated questions, misplaced items, missed appointments, and forgotten details of daily life.
  (termed mild-cognitive impairment)
- AD diagnosis requires dementia
- MCI progresses to AD at rate of 10% / yr
- Imaging is used to exclude other diagnosis
- Death: 3-9 years after diagnosis
- Definitive AD only possible post-mortem

Brain Atrophy in Advanced Alzheimer's Disease



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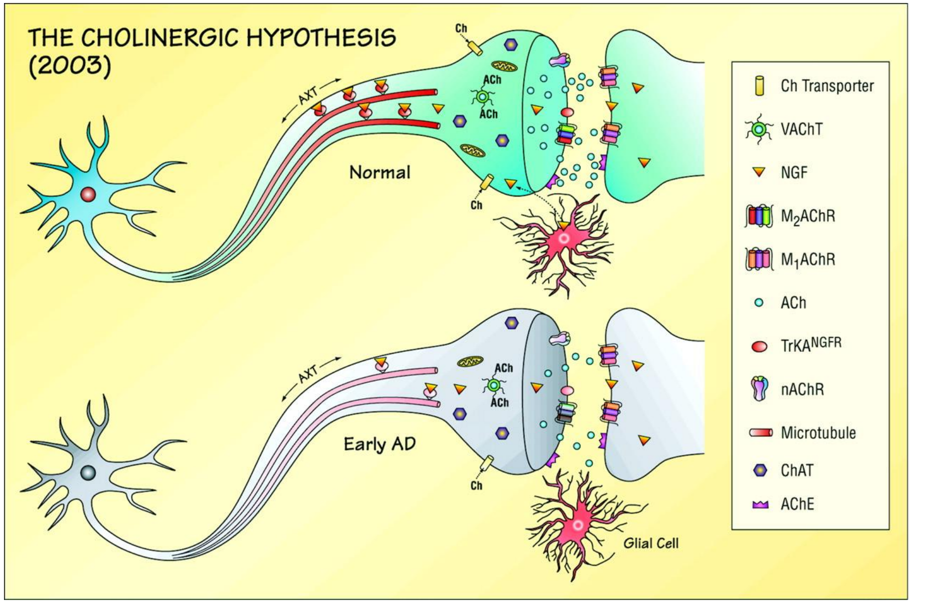
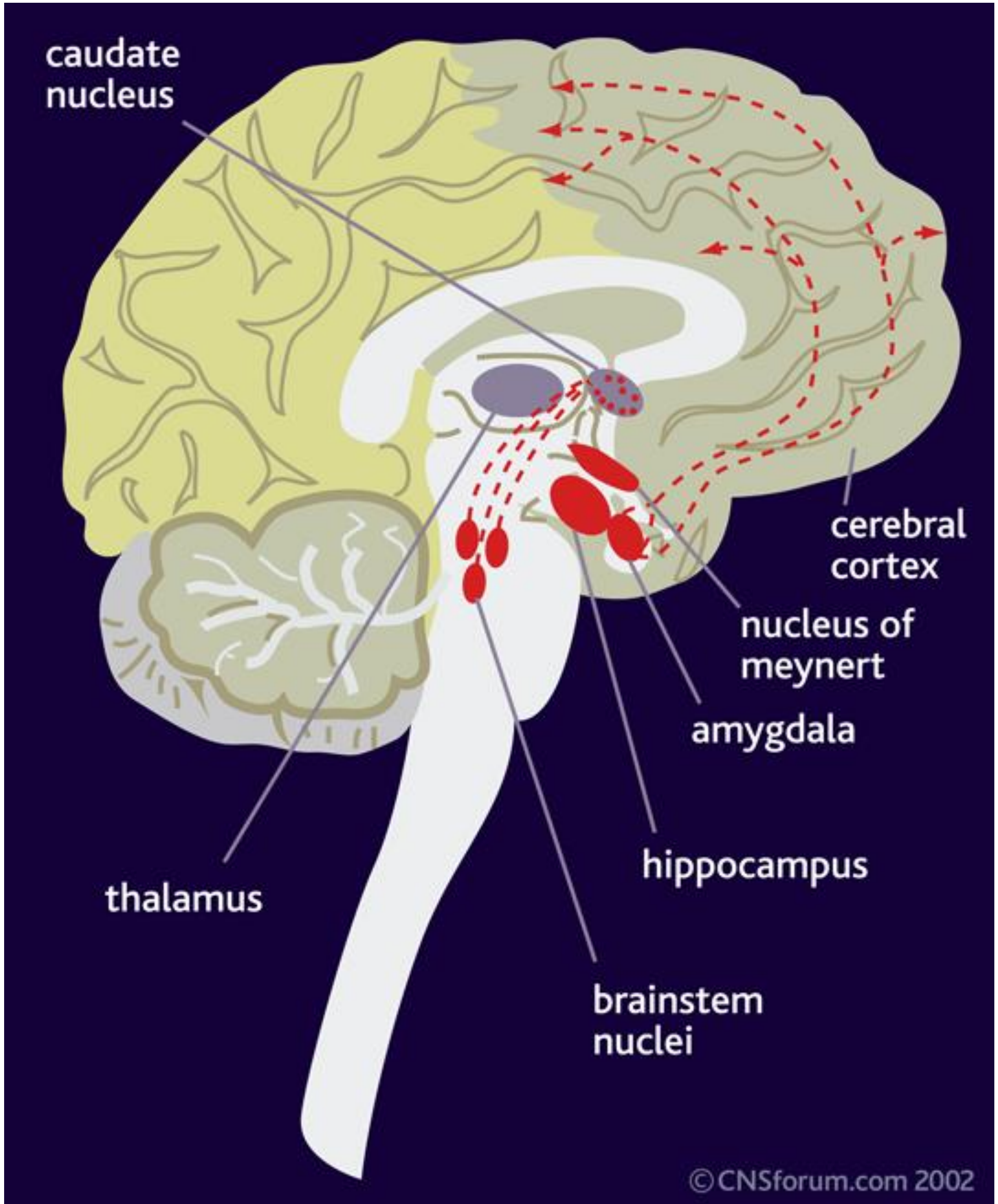
Neurochemistry of AD
the *cholinergic hypothesis*

- Profound deficiency of acetylcholine (ACh)
- Atrophy of subcortical cholinergic neurons
  - Basal forebrain cholinergic neurons
  - Noradrenergic neurons in locus ceruleus
  - 5-HT neurons in raphe
- Cholinergic antagonists can induce similar 'confused' state to that observed in AD patients




caudate nucleus
cerebral cortex
nucleus of meynert
amygdala
hippocampus
thalamus
brainstem nuclei
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Approved Alzheimer's drugs – NONE MODIFY THE DISEASE

- AChE inhibitors
  - Donepezil
  - Rivastigmine
  - Galantamine
  - Tacrine (rarely used due to adverse side-effect profile)
- Non-competitive NMDAR antagonist
  - Memantine, acts on Mg^{2+} site to prevent excessive activation
- Combination of donepezil and memantine statistically significant benefit but of marginal effect size
- Treatment of behavioral systems in AD
  - Atypical antipsychotics, mood stabilizers, antidepressants

	Donepezil	Rivastigmine	Galantamine	Tacrine[a]
Brand name	ARICEPT	EXELON, generic	RAZADYNE, generic	COGNEX
Enzymes inhibited[b]	AChE	AChE, BuChE	AChE	AChE, BuChE
Mechanism	Noncompetitive	Noncompetitive	Competitive	Noncompetitive
Typical maintenance dose[c]	10 mg once daily	9.5 mg/24h (transdermal) 3-6 mg twice daily (oral)	8-12 mg twice daily (immediate-release) 16-24 mg/day (extended-release)	20 mg, four times daily
FDA-approved indications	Mild–severe AD	Mild–moderate AD, Mild–moderate PDD[d]	Mild–moderate AD	Mild–moderate AD
Metabolism[e]	CYP2D6, CYP3A4	Esterases	CYP2D6, CYP3A4	CYP1A2

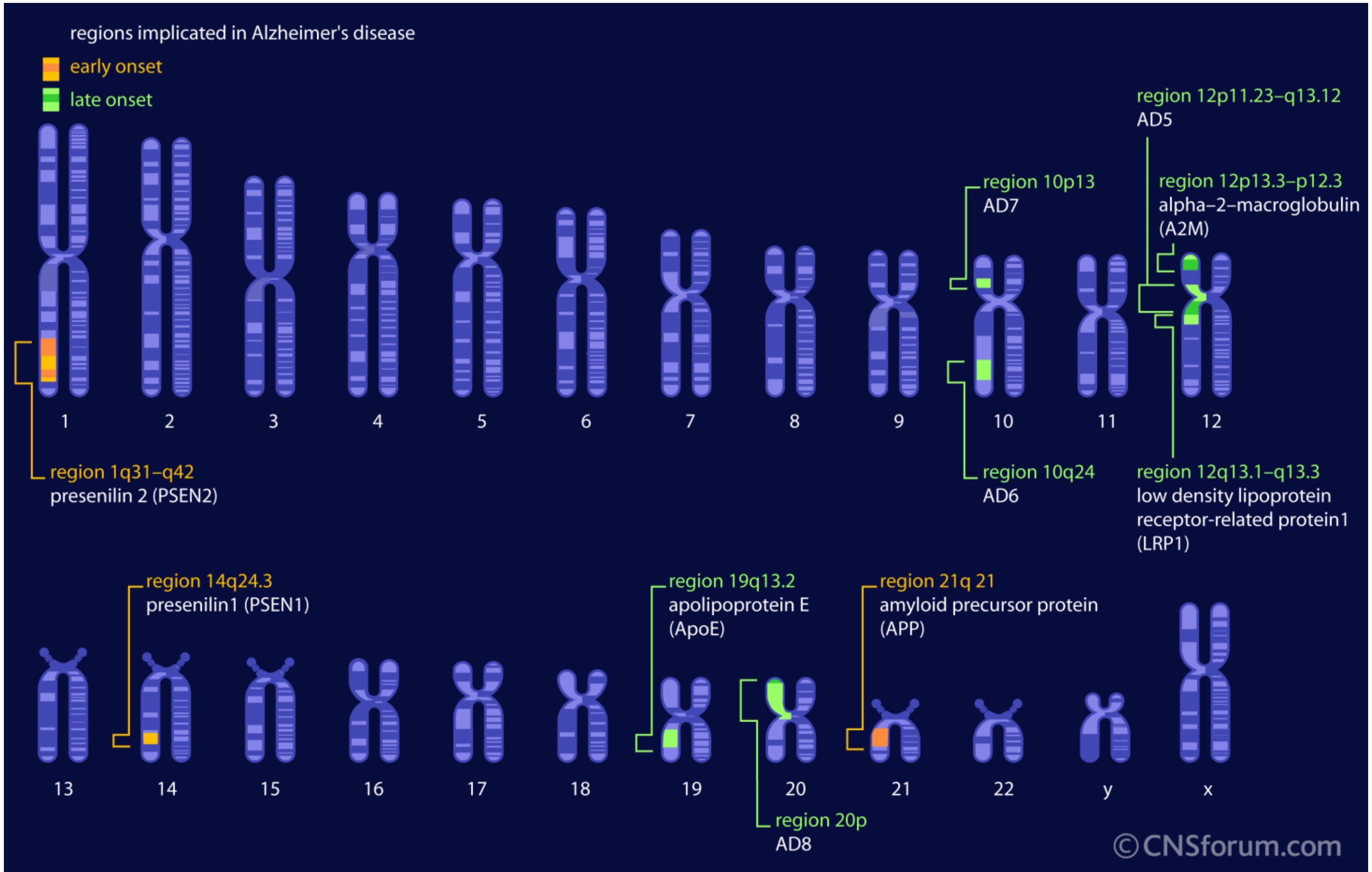
Why do AChE inhibitors fail?

- Many other neuronal systems affected
  - Glutamatergic, 5-HT, neuropeptides
  - Cortical and hippocampal atrophy in addition to cholinergic degeneration

- Disease is far more complex!

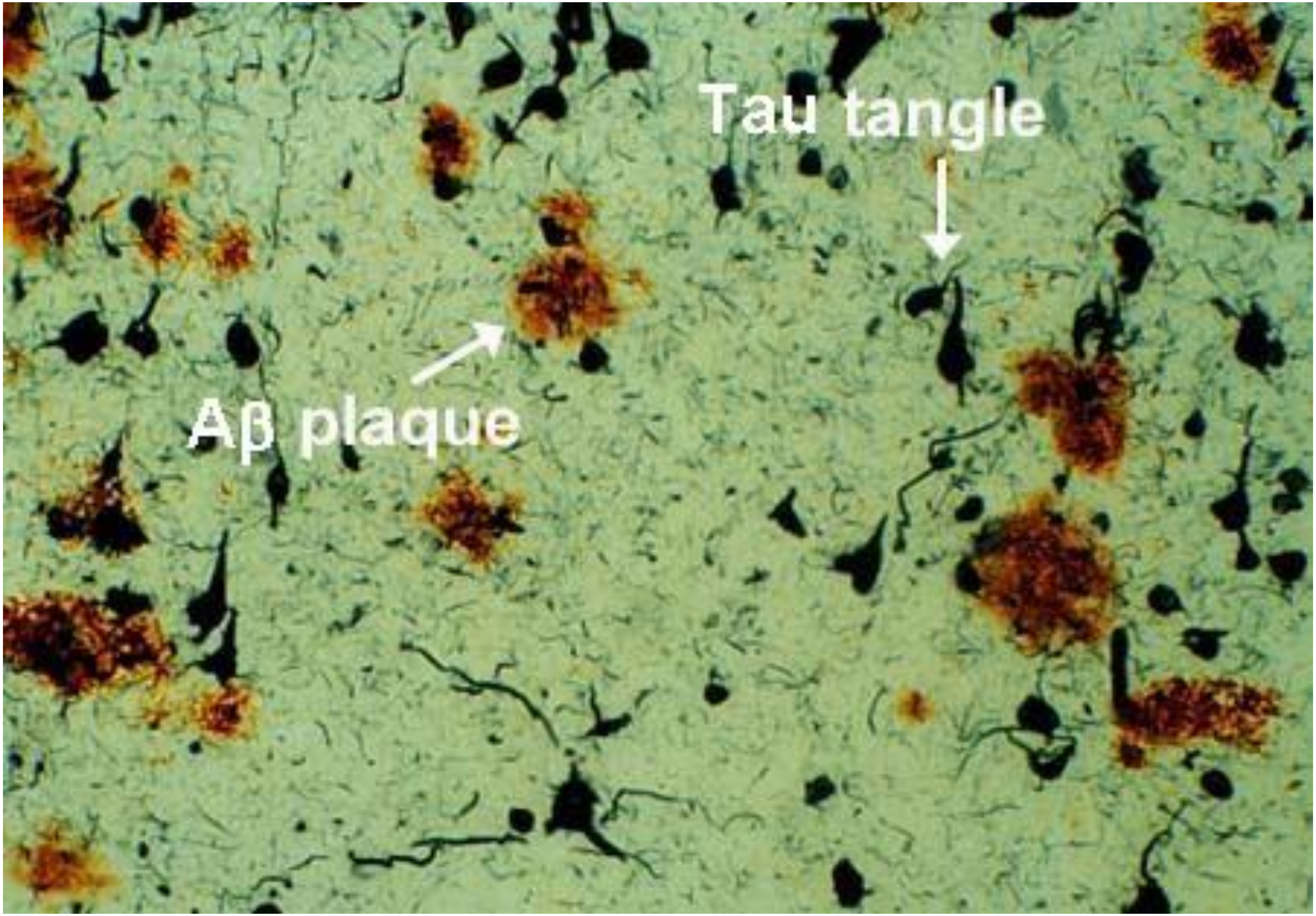
Familial AD

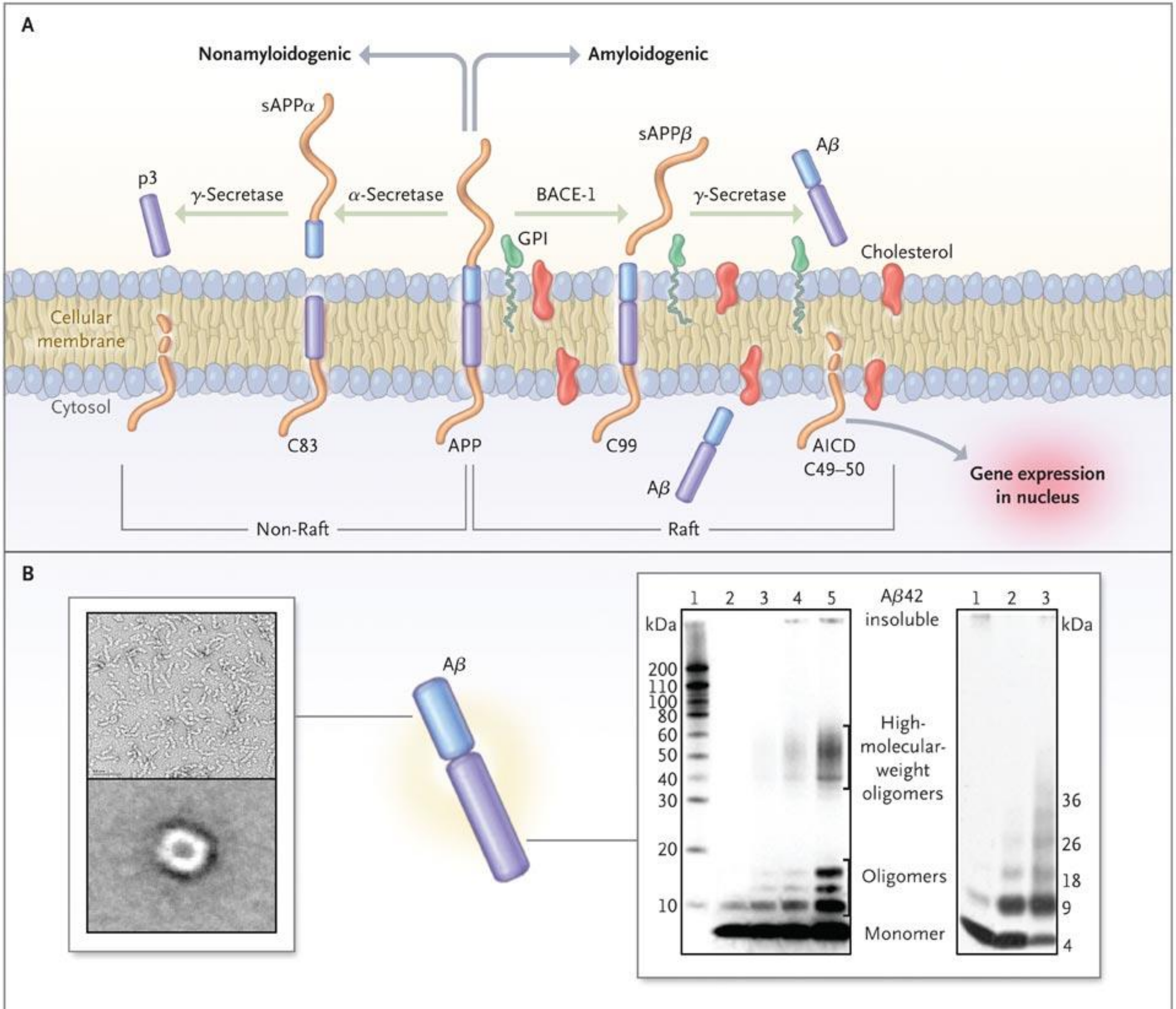
- Mutations in APP -> early onset AD
    - Mouse models show plaques not tangles
- Trisomy 21 – very early onset dementia like AD with massive AD-like pathology
    - Mouse models of APP over-production do not result in AD-like pathology
- Presenilin 1 & 2 – causes over-production of Aβ



Pathological hallmarks of AD

- Cortical wasting – widening of sulci and loss of tissue
- Senile plaques - Beta-amyloid (Aβ) accumulation
  - Soluble Aβ is highly neurotoxic
- Neurofibrillary tangles
  - Comprised of hyper-phosphorylated Tau
  - Appears to be a consequence of Aβ accumulation
- Characteristic pattern of changes
  - Early – temporal lobe in entorhinal cortex
  - Hippocampus
  - Later – other cortical areas
  - Consistent with idea of spread along known connections


Tau tangle
Aβ plaque

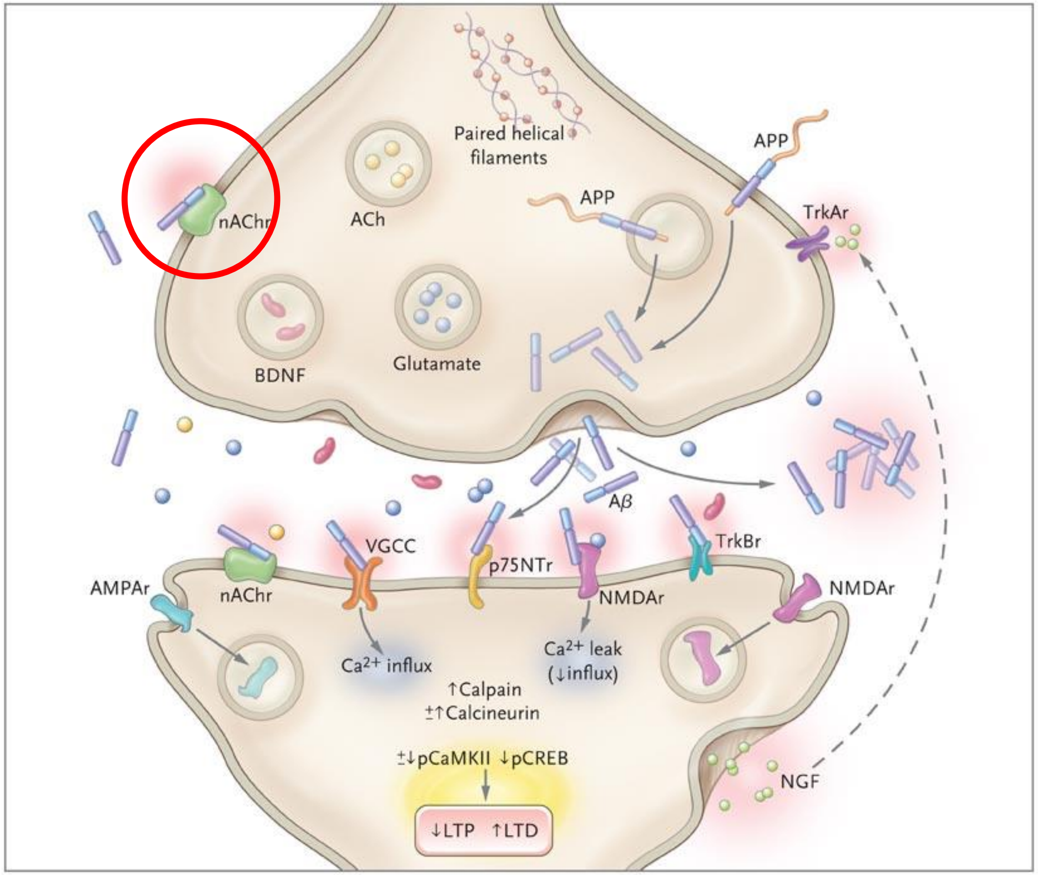


Querfurth H, LaFerla F. N Engl J Med 2010;362:329-344

Aβ & Amyloid Hypothesis

- Produced by abnormal processing of APP
  - BACE
  - Gamma-secretase (4 subunit protein, contains PSEN1/2)
- Aβ is 36-43 amino acid fragment of APP
- Accumulates, oligomerizes & forms insoluble plaques
- $A\beta_{42}$ is <u>directly</u> toxic to synapses
- Extracellular Aβ can lead to excitotoxic cell death by mediating glutamate release

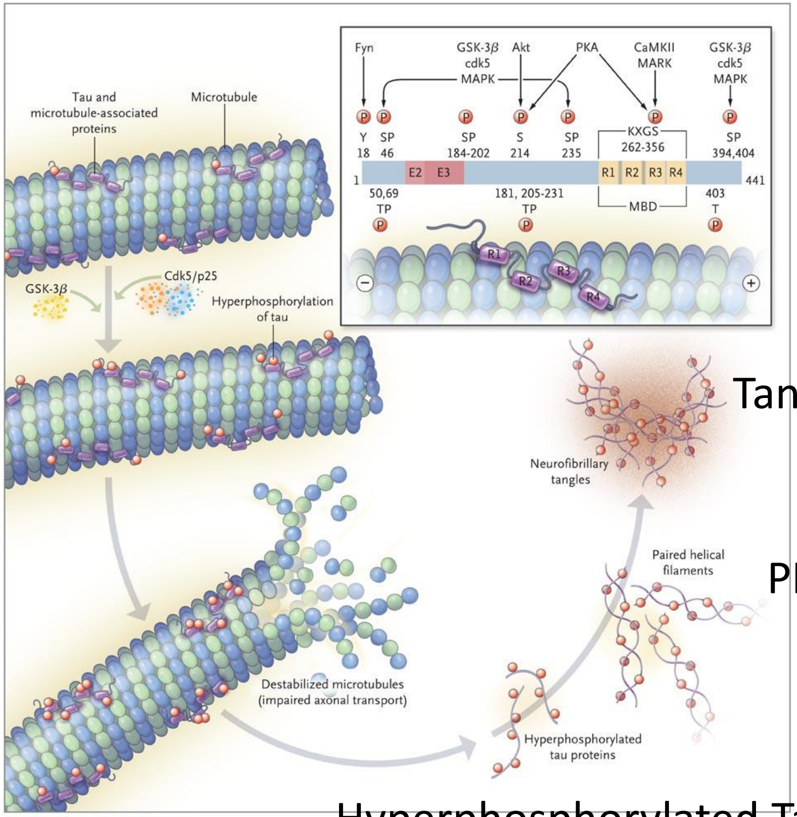
Synaptic dysfunction caused by Aβ



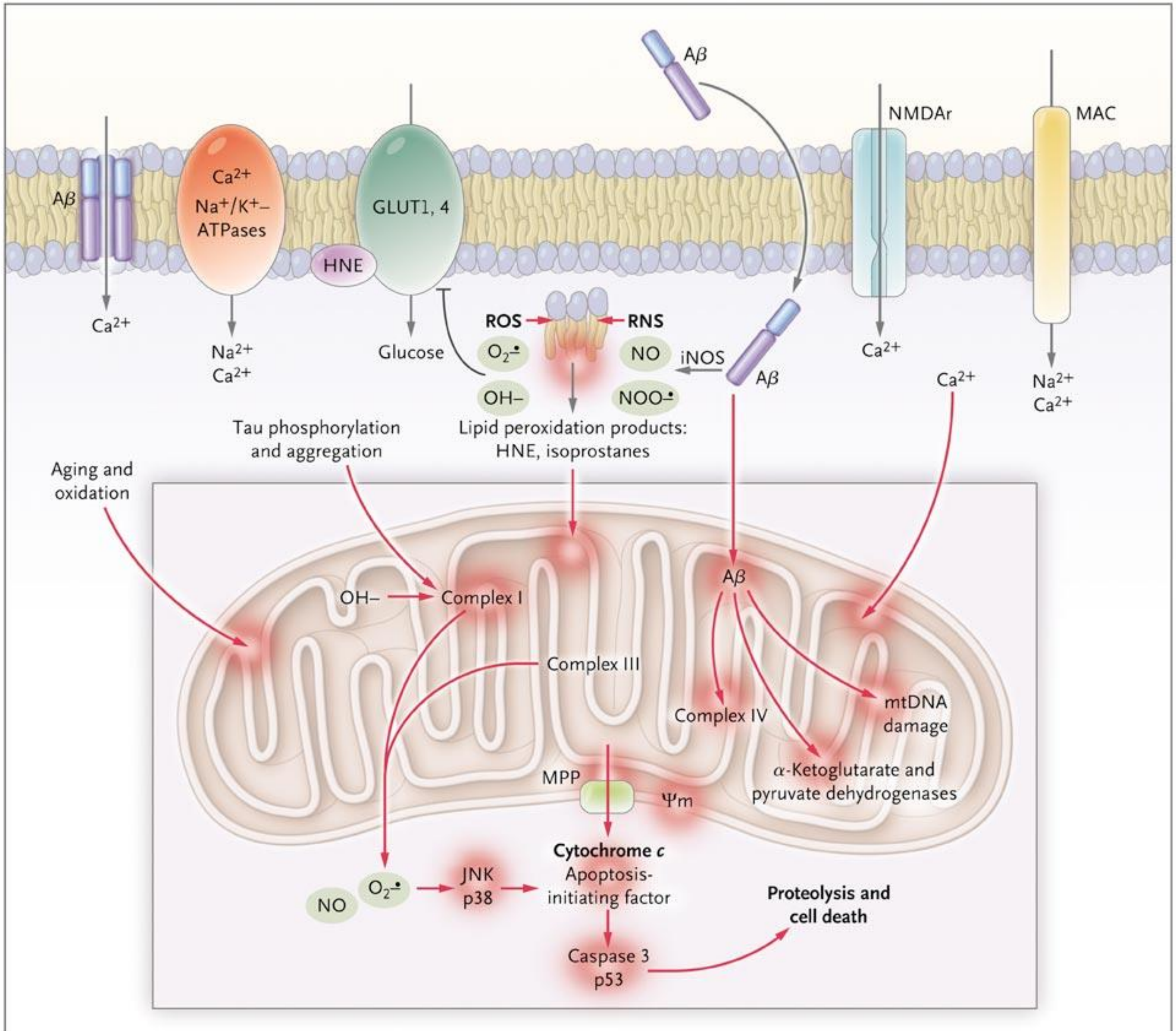
Querfurth H, LaFerla F. N Engl J Med 2010;362:329-344

Tau & Tangles

- Abnormally phosphorylated <u>tau</u>
- Insoluble and associates to formed paired-helical filaments
- Tau is a microtubule associated protein binds to and stabilizes microtubules (MTs)
- Phosphorylation of tau reduces binding
- Likely secondary to Aβ



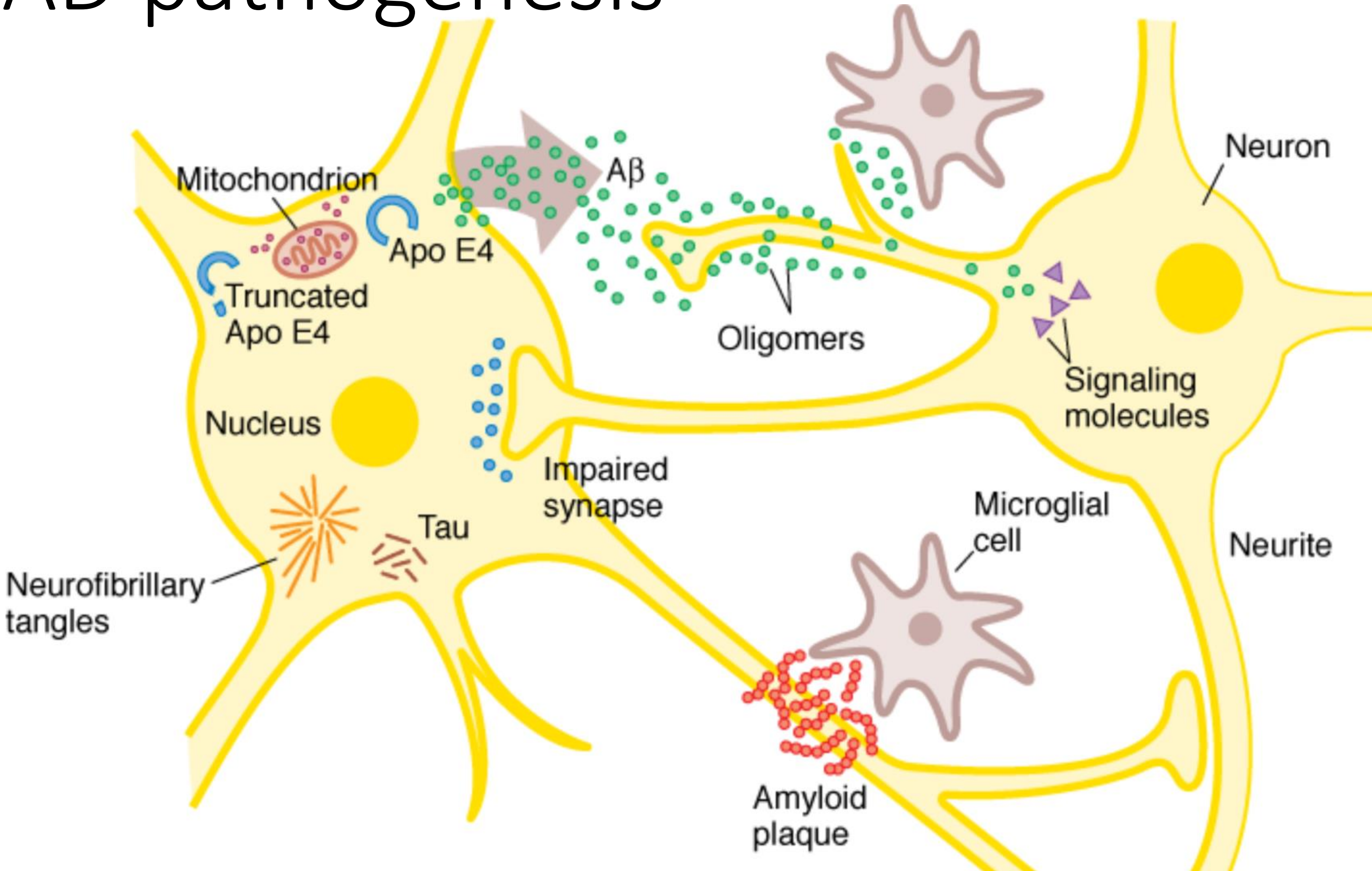
Oxidative stress and mitochondrial failure



New drugs

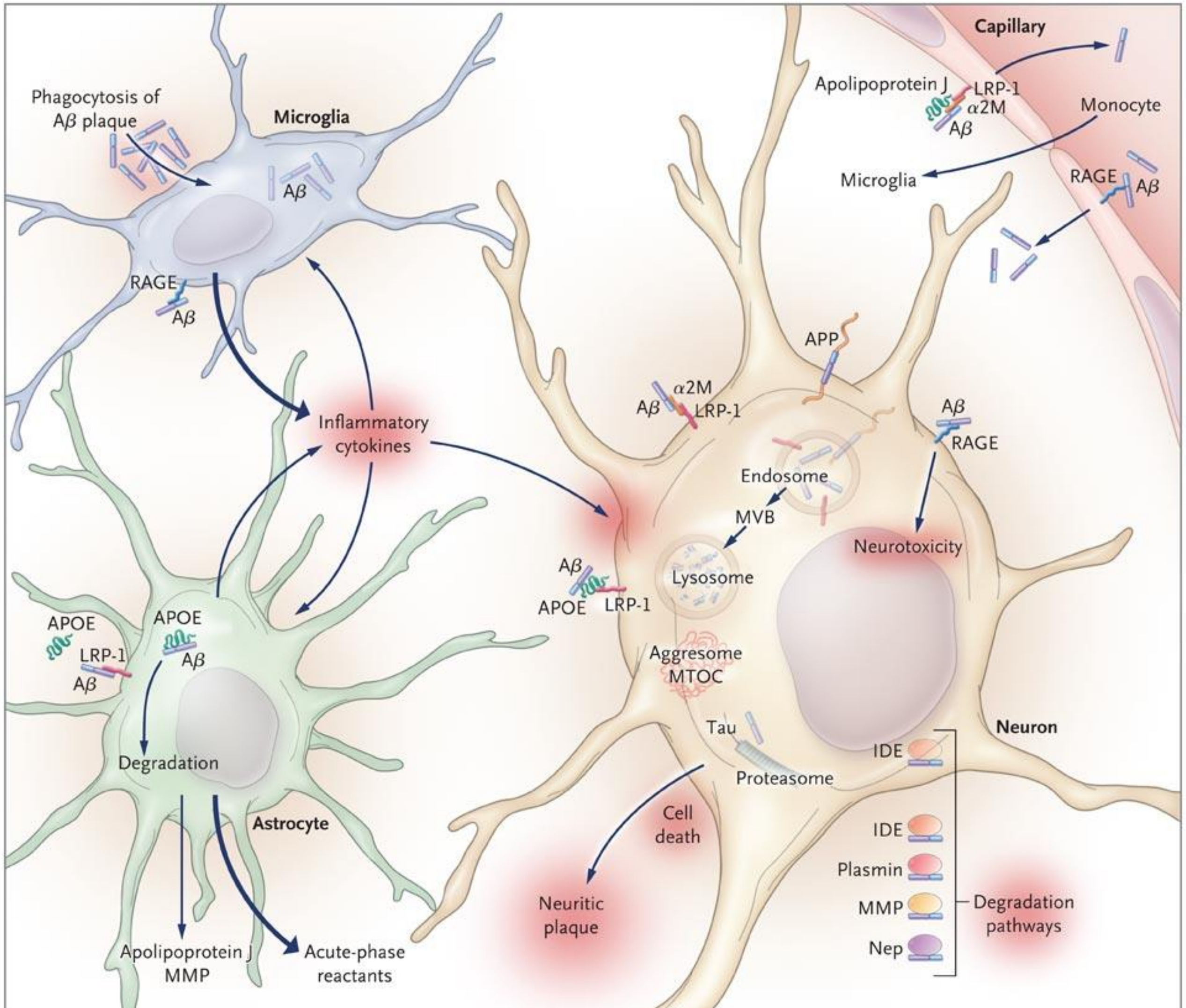
- Targeted at beta-amyloid
  - LY450139 – γ-secretase inhibitor (Phase 3 – failed in 2010)
  - Vaccines to elicit immune response against Aβ
    - AM-1792 – Phase 2a: stopped due to brain inflammation adverse events.
    - ACC-001 – Phase 2a. Better safety profile. Phase 3: ineffective.
  - Antibody against Aβ. Bapineuzumab. Also failed Phase 3.
- Neuroprotection
  - AL-108 – intranasal peptide – Phase 2a promising (2009).
  - PBT2 – Phase 2a promising (2010) – metal chaperone reduce free divalent metal ions that result in ROS damage
  - Etanercept (Enbrel) – RA drug, small Phase 2 promising (2008)
- Tau
  - Methythionium chloride – methylene blue, reduces tau oxidation and aggregation (TRx023 - ongoing Phase III trials)

AD pathogenesis

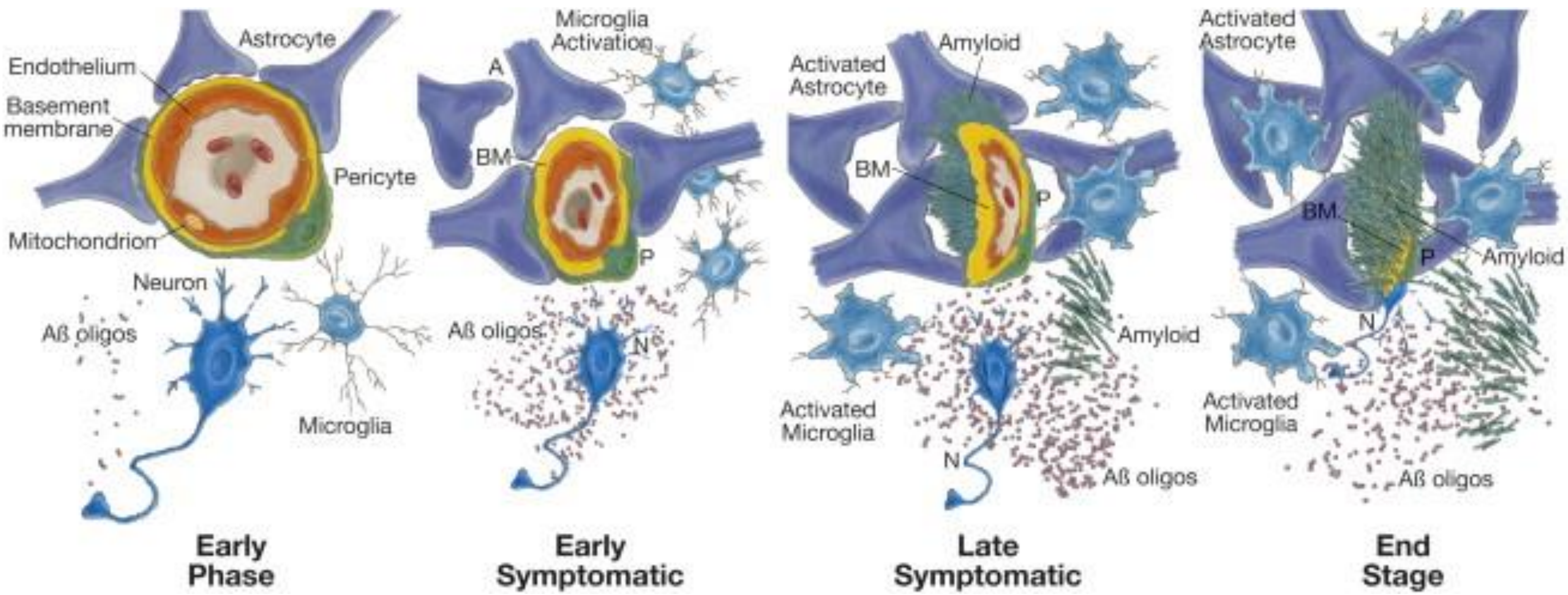


Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition*: www.accessmedicine.com

More complex interactions of Aβ with neural environment



Does impaired clearance of Aβ lead to neurovascular dysfunction?



YES! 60-90% patients have ischemic vascular disease, with high incidence of major infarctions. Many vascular dementia's have pathological changes akin to AD.

Aβ is toxic to endothelial and small muscle components of neurovascular compartment.

Future

- 1772 trials in ClinicalTrials.gov
- 523 open trials
- 54 Phase III
  - Amyloid targeting vaccine, 2nd generation (CAD106, CNP520)
  - BACE1 inhibitors (LY3314814, JNJ-54861911)
  - TTP448 (antagonist of RAGE, interacts with Aβ)
  - Encenicline (α7-nAChR agonist)

Neurodegenerative Diseases

- Alzheimer’s disease
- Parkinson's disease
- **Amyotrophic Lateral Sclerosis (ALS)**
- Huntington’s disease
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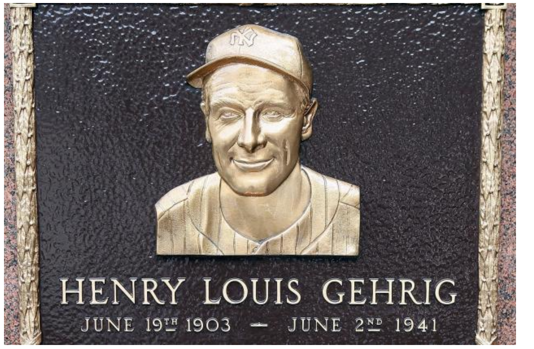
Amyotrophic Lateral Sclerosis



- Motor neuron disease (Lue Gehrig's disease)
- 5-10% cases have known genetic cause (i.e. familial)
- Rapidly fatal (1-5 years)

- Loss of upper (cortical) and lower (spinal) motor neurons

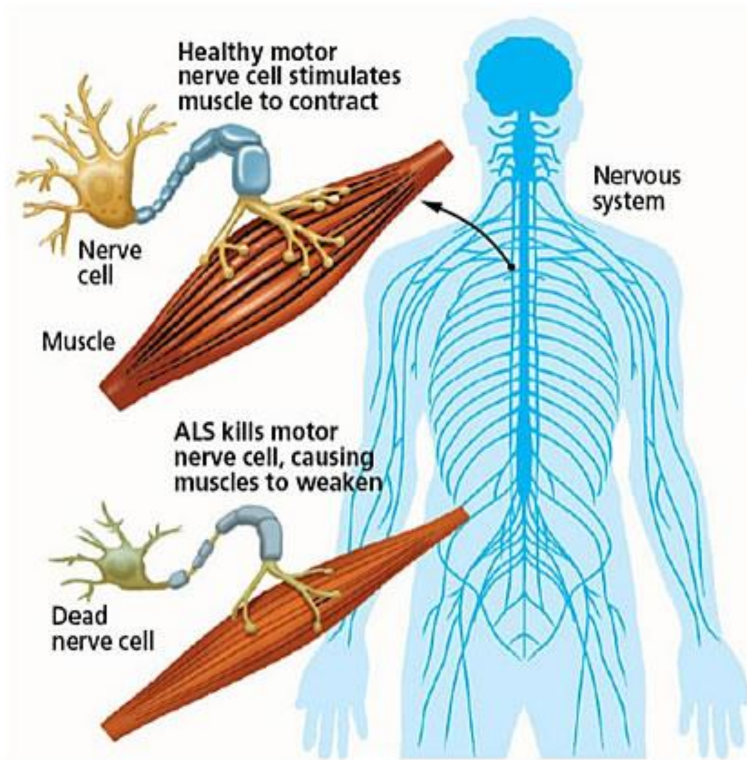
Epidemiology and genetics

- Prevalence
  - Much rarer than AD and PD
  - Age of onset between 40-60 yrs
  - Men > Women
  - USA: 6000-8000 people, 530 new cases / yr
  - 4.7 cases per 100,000
  - Lifetime risk is 1 in 1000

- Genetic risk (90% are sporadic)
  - Superoxide dismutase (SOD1) – 15-20% of genetic cases
  - TAR DNA binding protein (TDP-43)
  - FUS/TLS
    - Both bind DNA/RNA regulating transcription

Clinical overview

- Rapid progressive weakness, muscle atrophy and fasciculations (twitch), spasticity (stiffness), dysarthria (speech), dysphagia (eating), respiratory compromise.
- Sensory (non-motor) function is spared
- ALS usually is progressive and fatal. Most patients die of respiratory compromise and pneumonia after 2-3 years.

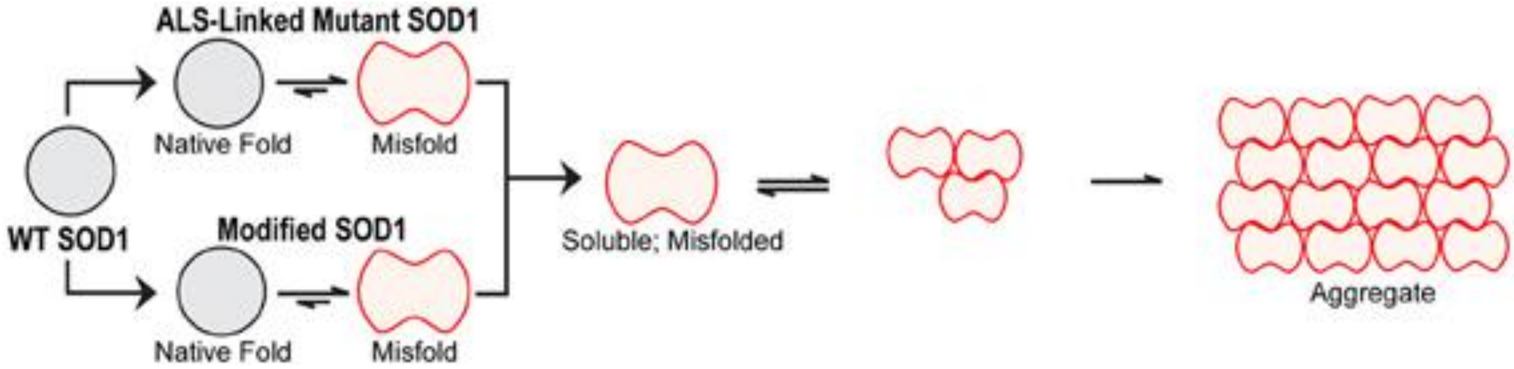


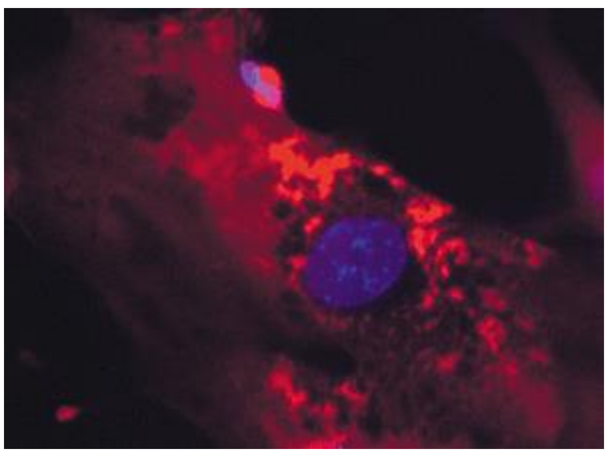
Pathological mechanisms?

<u>SOD1 mutations in ALS identified in 1993</u>

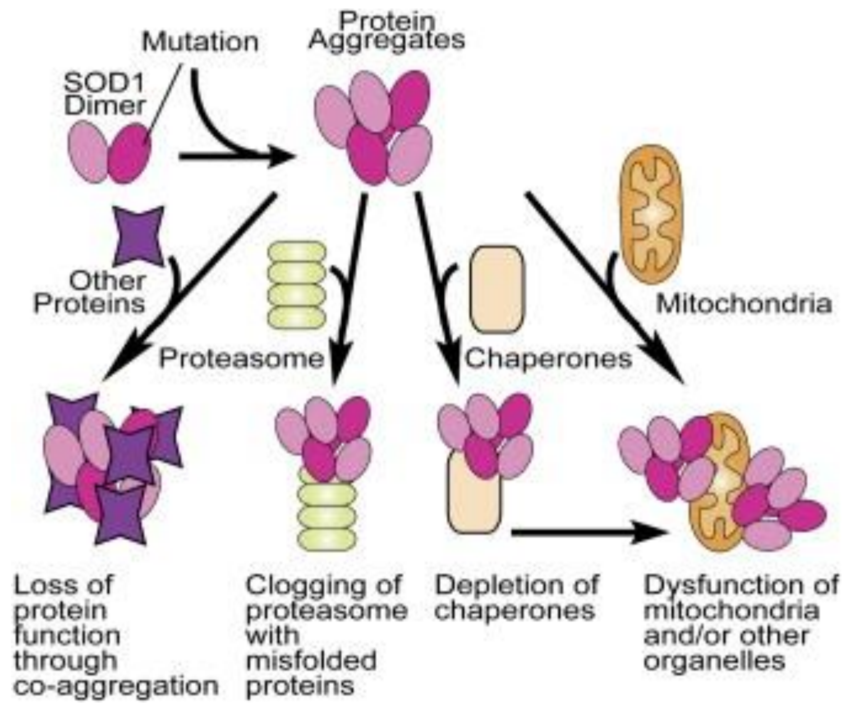
- <u>Oxidative hypothesis model</u>
  - SOD1 involved in converting superoxide radicals
  - Some SOD mutations can result in reduce function leading to oxidative stress
  - Others do not effect enzyme function
  - SOD1 null mice do not develop ALS-like pathology
  - Suggests a toxic ‘gain of function’

- <u>Aggregation?</u>





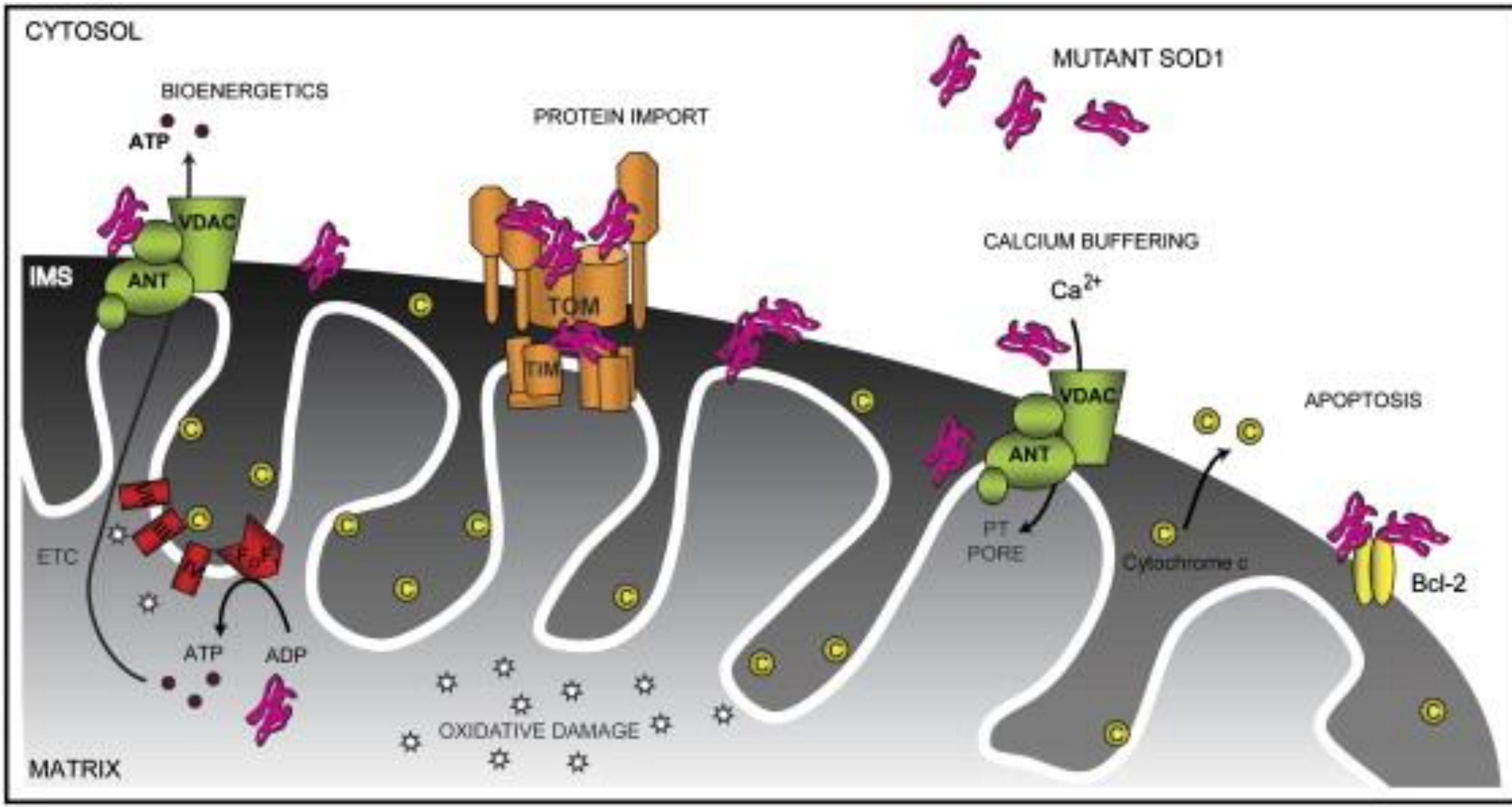
SOD1 mutant aggregates



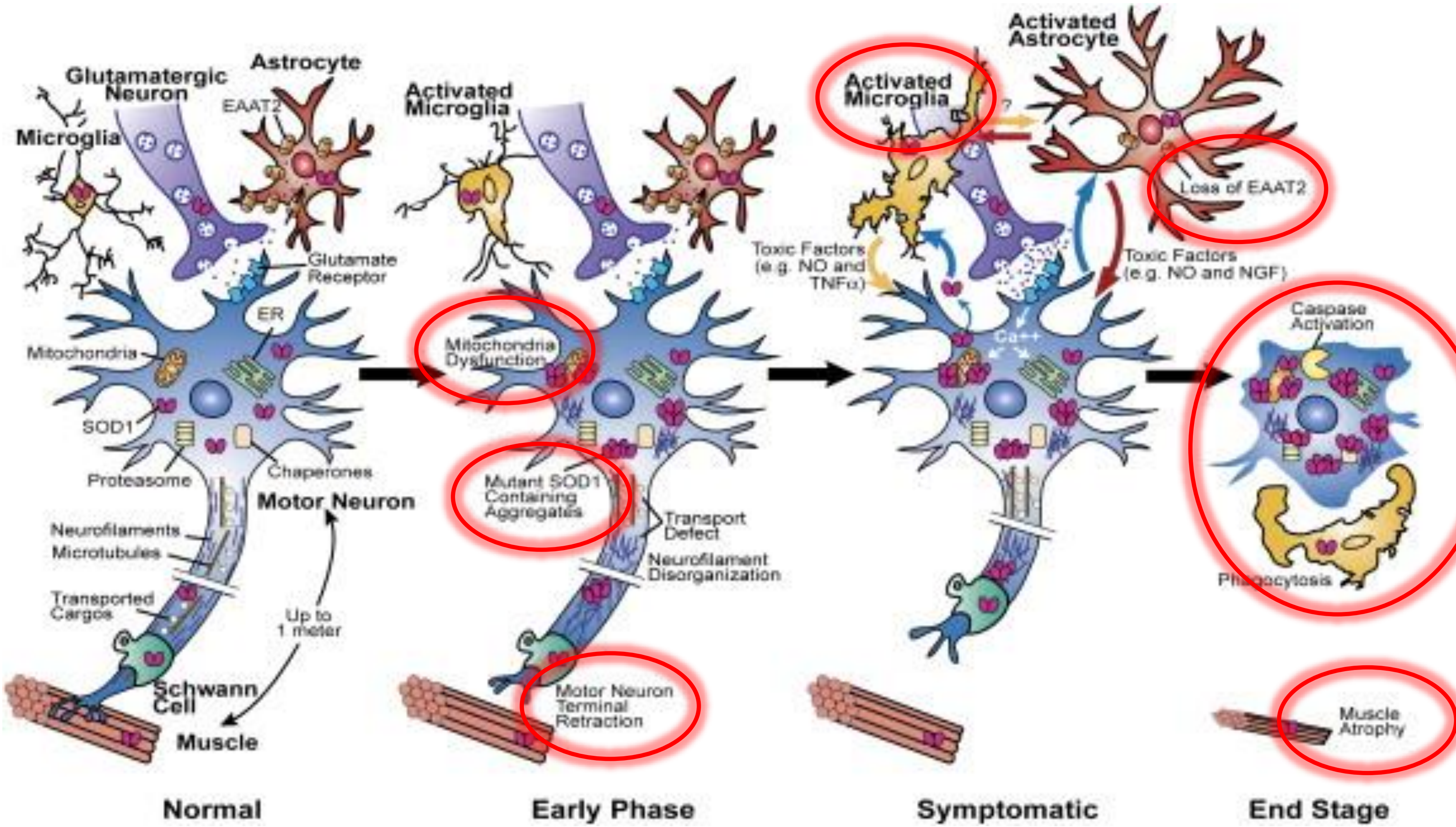
Why do SOD1 aggregates kill motor neurons?

- SOD1 misfolding is detected by the *unfolded protein response* (UPR), a cellular stress response.
- This should activate a cellular stress pathway in an attempt to restore homeostasis
  1. Increases proteolysis via proteasome (increase clean-up of misfolded protein)
  2. Increases chaperone expression (improve folding)
  3. Reduces protein translation (reduce burden)
- High threshold for this stress response in motor neurons may contribute to *selective vulnerability*
- Failure of the UPR can result in stimulation of apoptosis

Mitochondrial dysfunction as a proximal cause of MN death?



Evolution of MN injury in ALS



Treatment of ALS

- ONLY Riluzole approved for treatment

Mechanism of action is poorly characterized, effects include:

1. Inhibition of glutamate release
2. Blockade of post-synaptic NMDA and kainate receptors
3. Inhibition of Na_v channels.
4. GPCR target?

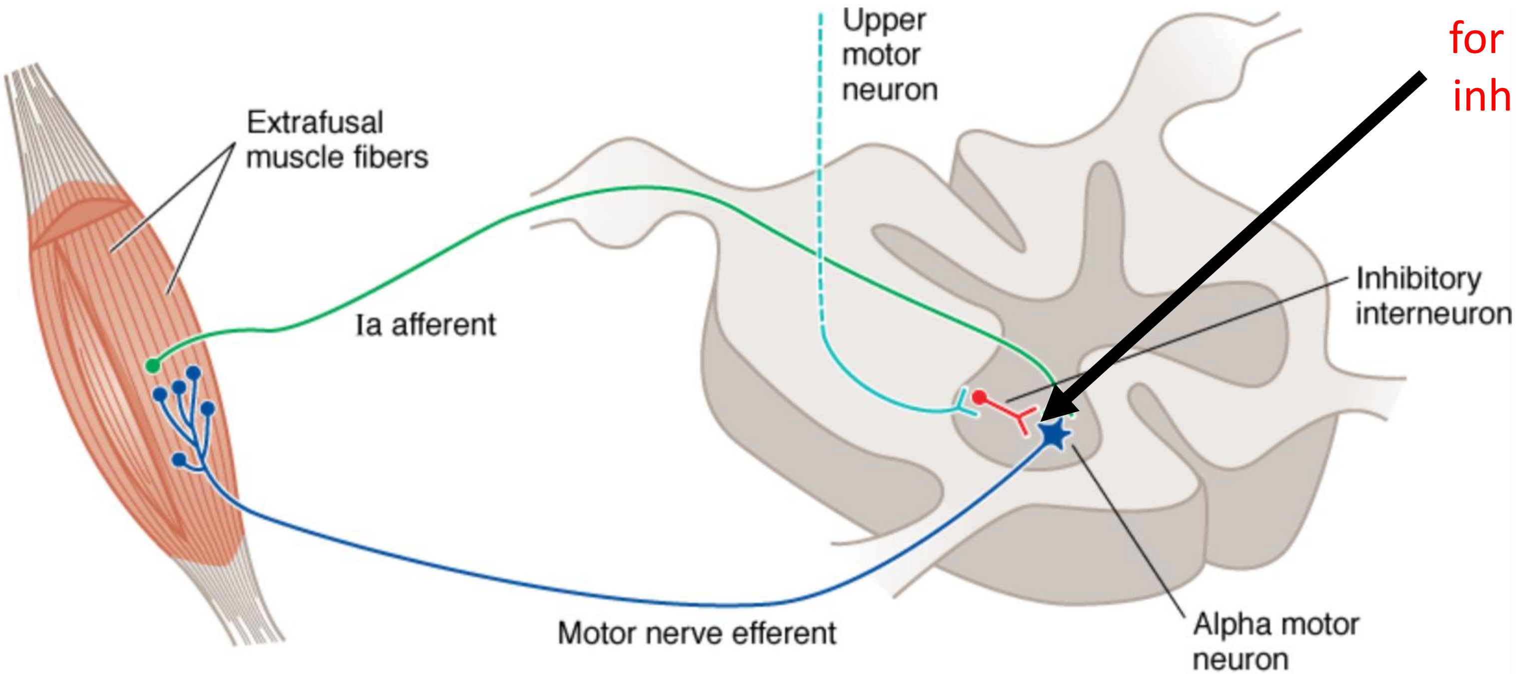
Modest effect on survival and well tolerated. Milestone but unclear how this will lead to future advances in drug therapy.

Spasticity – Balcofen ($GABA_B$) and diazepam ($GABA_A$)

Dysphagia – Amitriptyline (TCA) – anticholinergic to prevent excess saliva production

Loss of upper MN input leads to spasticity

GABA agonists substitutes for loss of inhibitory input



Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition*: www.accessmedicine.com

Current trials on ClinicalTrials.gov

- 38 trials Phase III – 5 open
  - Olanzapine
    - Aimed at treating appetite loss
  - Tirasemtiv (Cytokimetics)
    - Skeletal muscle troponin activator – increases sensitivity to Ca^{2+} thereby allowing fewer MN fibers to elicit larger effects, i.e. may delay loss of muscle function
  - Masitinib (AB1010) – anti-cancer – inhibits RTKs
    - Reduce inflammation

Neurodegenerative Diseases

- Alzheimer’s disease
- Parkinson's disease
- Amyotrophic Lateral Sclerosis (ALS)
- **Huntington’s disease**
- Others:
  - Frontotemporal dementia with Parkinsonism
  - Prion diseases
  - Multiple sclerosis

Huntington's disease

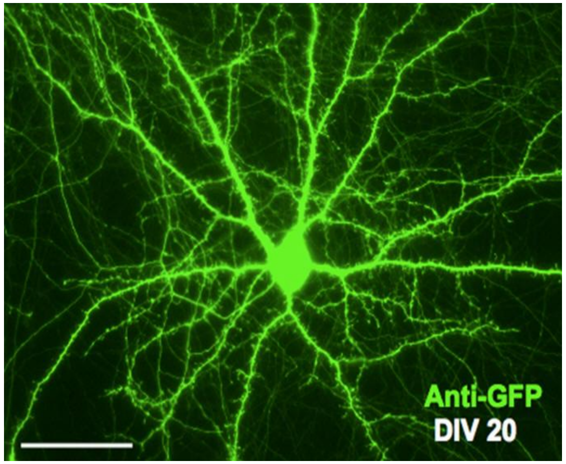
- Incidence 4-10 / 100,000 new cases per year
- Genetically determined autosomal dominant
- Near 100% dominance with prominent anticipation
  - i.e. subsequent generations suffer from earlier onset
- Trinucleotide repeat disease (CAG_n) in the huntington gene (Chr 4)
  - Normal = 9-34 triplets (median: 19)
  - HD = 40-100+

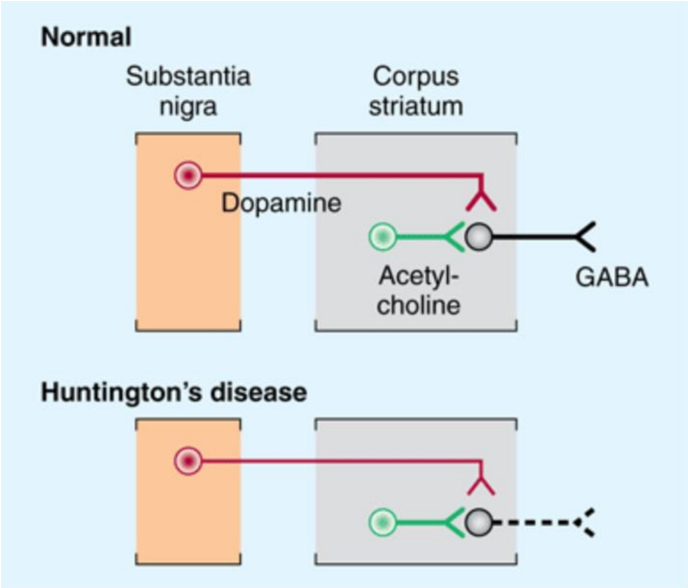
Clinical overview

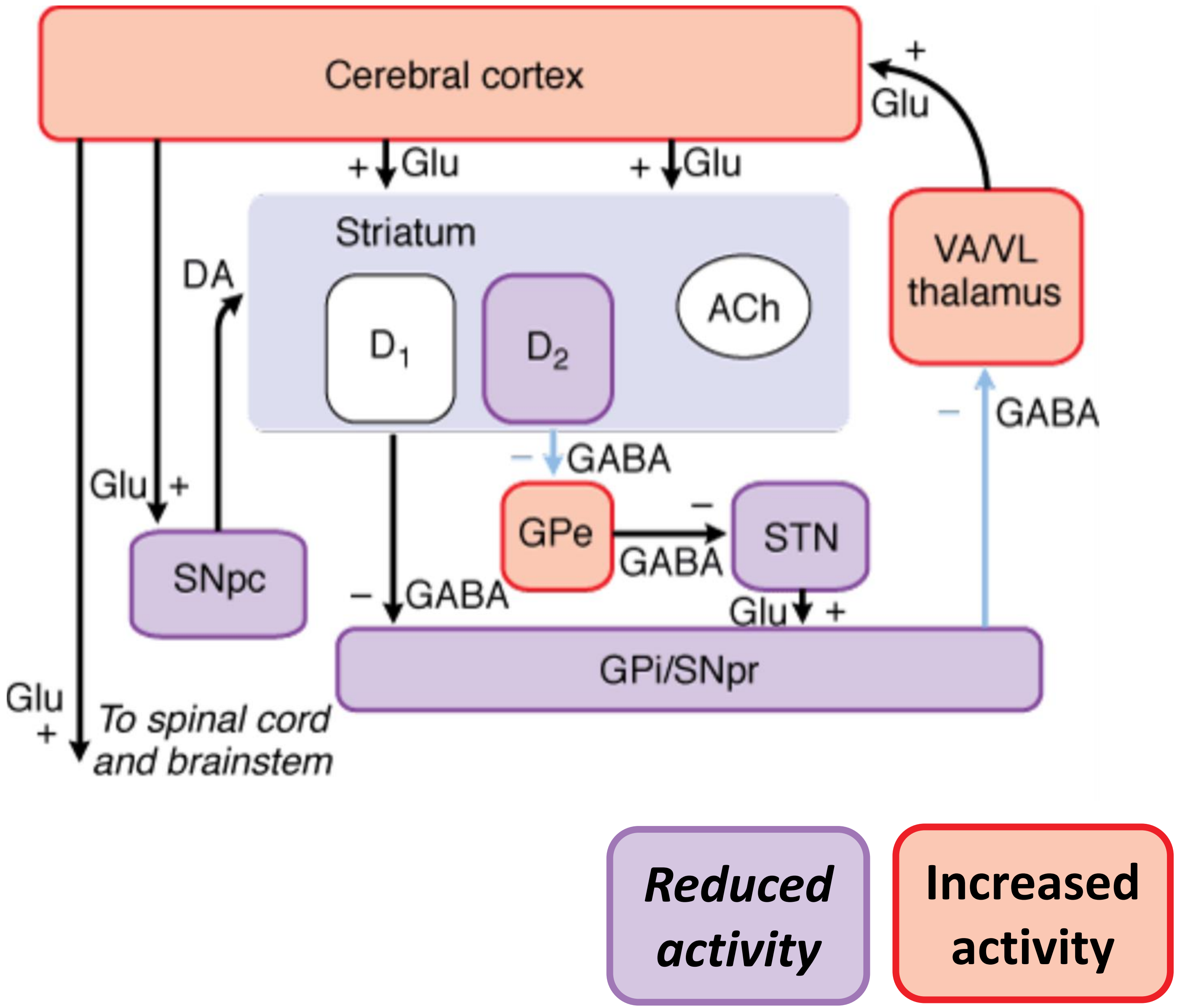
- Gradual onset of motor incoordination and cognitive decline
- Huntington’s chorea – movement disorder – brief jerk-like movements
- Fine-motor coordination and impairment of rapid eye movements are early symptoms
- Psychiatric symptoms (confusion, amnesia, psychosis) and cognitive dysfunction (dementia)
- Disease is fatal, typically over 15-20 years.

Basal Ganglia Pathology

- Massive loss of medium spiny neurons in neostriatum (up to 95%)
  - GABAergic inhibitory interneurons that receive input from SNpC DA neurons
  - Affects innervation of GPi and SNpr (indirect) before Gpe (direct pathway)






Cerebral cortex
+
Glu
+ Glu
+ Glu
Striatum
DA
D1
D2
ACh
VA/VL
thalamus
– GABA
– GABA
Glu +
GPe
–
STN
GABA
SNpc
– GABA
Glu +
GPi/SNpr
Glu
+
To spinal cord
and brainstem
Reduced
activity
Increased
activity

NONE MODIFY THE DISEASE

	Drug	Class	Main adverse effects and treatment notes
Chorea			
Myoclonus, c… spasticity			…withdrawal
Myoclonus			…naemia, liver
	Levetiracetam	Anticonvulsant	Gastrointestinal disturbance, rash, mood changes, myalgia
Rigidity (particularly associated with juvenile Huntington's disease or young adult-onset parkinsonian phenotype)	Levodopa	Amino acid precursor of dopamine	Gastrointestinal disturbance, postural hypotension, insomnia, agitation, psychiatric symptoms, increased chorea
Rigidity, spasticity	Baclofen, tizanidine	Skeletal muscle relaxants	Sedation, drowsiness, confusion, gastrointestinal disturbances, hypotension
Bruxism, dystonia	Botulinum toxin	Inhibits acetycholine release at neuromuscular junction to cause muscle paralysis	Could paralyse nearby muscles
Psychosis, irritability	Olanzapine	Atypical neuroleptics	Sedation, parkinsonism, tardive dyskinesia, and neuroleptic malignant syndrome, but less risk of these than with older neuroleptics, raised triglycerides, weight gain from increased appetite, which could be beneficial (in relation to the weight loss seen in Huntington's disease). Caution should be exercised in patients with diabetes, and blood glucose should be monitored. Might rarely cause prolonged QT interval. Useful if patient also has agitation, irritability, and anxiety
	Quetiapine	Atypical neuroleptics	As above for olanzapine, but less metabolic syndrome
Psychosis, chorea, irritability	Risperidone	Atypical neuroleptics	As above for olanzapine, but less effect on increasing appetite
	Sulpiride	Older neuroleptics	Agitation, dystonia, akathisia, sedation, hypotension, dry mouth, constipation
	Haloperidol	Older neuroleptics	Sedation, more parkinsonism than atypical neuroleptics, dystonia, akathisia, hypotension, constipation, dry mouth, weight gain, tardive dyskinesia, higher risk of neuroleptic malignant syndrome than atypical neuroleptics
Treatment-resistant psychosis	Clozapine	Atypical neuroleptics	As for other neuroleptics, plus agranulocytosis, myocarditis, and cardiomyopathy. Needs blood monitoring
Psychosis with prominent negative symptoms	Aripiprazole	Atypical neuroleptics	Parkinsonism, akathisia, drowsiness, gastrointestinal disturbance, tremor, blurred vision
Depression, anxiety, obsessive compulsive behaviour, irritability	Citalopram	SSRI	Gastrointestinal disturbance, hypersensitivity reactions, drowsiness, syndrome of inappropriate antidiuresis, postural hypotension
	Fluoxetine	SSRI	As for citalopram, sleep disturbances
	Paroxetine	SSRI	As for other SSRIs, raised cholesterol
	Sertraline	SSRI	As for other SSRIs
	Mirtazapine	Presynaptic α2-antagonist, increases central noradrenaline and serotonin activity	Weight gain, oedema, sedation, headache, dizziness, tremor. Useful for sedation when insomnia is a problem
	Venlafaxine	Serotonin and noradrenaline reuptake inhibitor	Hypertension, gastrointestinal disturbance, hypersensitivity reactions, drowsiness, agitation, syndrome of inappropriate antidiuresis, palpitations
Altered sleep-wake cycle	Zopiclone, zolpidem	Hypnotics	Drowsiness, confusion, memory disturbance, gastrointestinal disturbance
Mania or hypomania	Sodium valproate	Anticonvulsants	As above for myoclonus
	Carbamazepine	Anticonvulsants	Hypersensitivity reactions, drowsiness, blood dyscrasia, hepatitis, hyponatraemia, dizziness, gastrointestinal disturbance
	Lithium	Mood stabiliser	Renal insufficiency, hypothyroidism, and tremor, with a narrow therapeutic window, and overdose can cause delirium and renal failure

SSRI=selective serotonin reuptake inhibitor. Adapted from ref 10, with permission of BMJ Publishing Group.

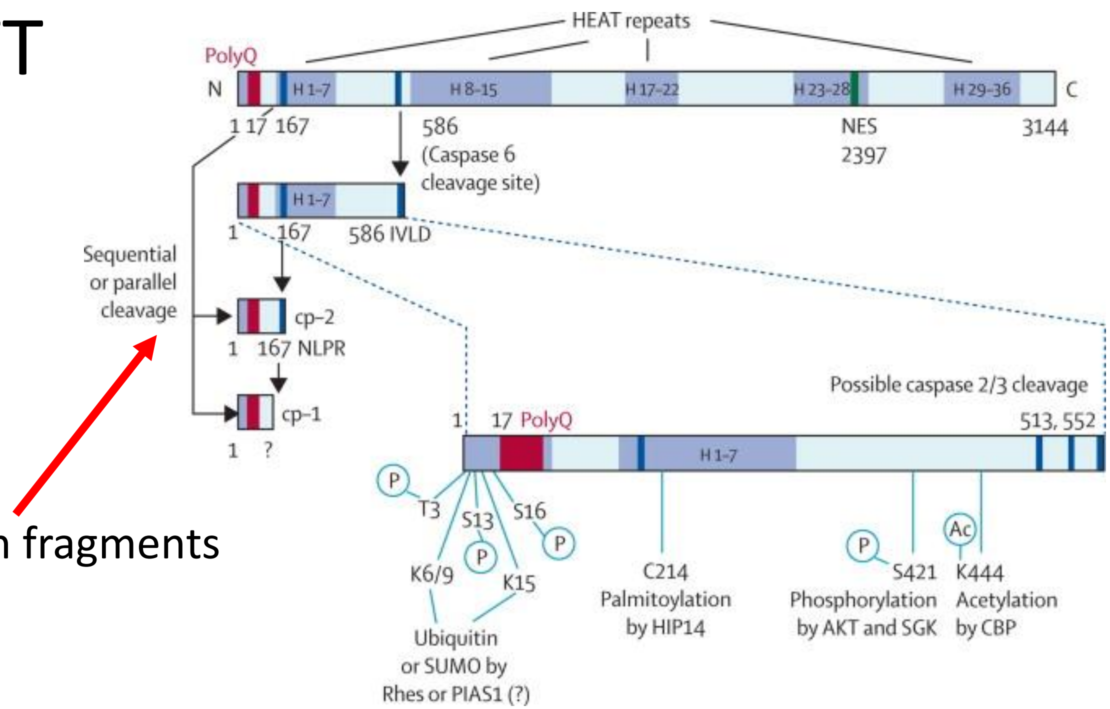
Table: **Symptomatic drug treatment for Huntington's disease**

Pathological mechanisms – *two hypotheses*

- Excitotoxicity
  - Animal model – infusion of excitotoxin into striatum can produce similar motor symptoms and loss of MSNs
  - MSNs receive large excitatory input from neocortex
- Mitochondrial dysfunction
  - Ultrastructural evidence
  - PET – reduced glucose and O_2 metabolism
  - Mitochondrial toxins into striatum cause similar pattern of MSN loss with preservation of other interneurons
    - Which can be blocked by removal of cortical input or NMDA antagonists
- Combination?

HD is a monogenic autosomal dominant disease

- One gene – when mutated – causes disease
- Identified in 1993 as Huntingtin (HTT)
- Mutation characterized as a CAG triplet repeat in HTT

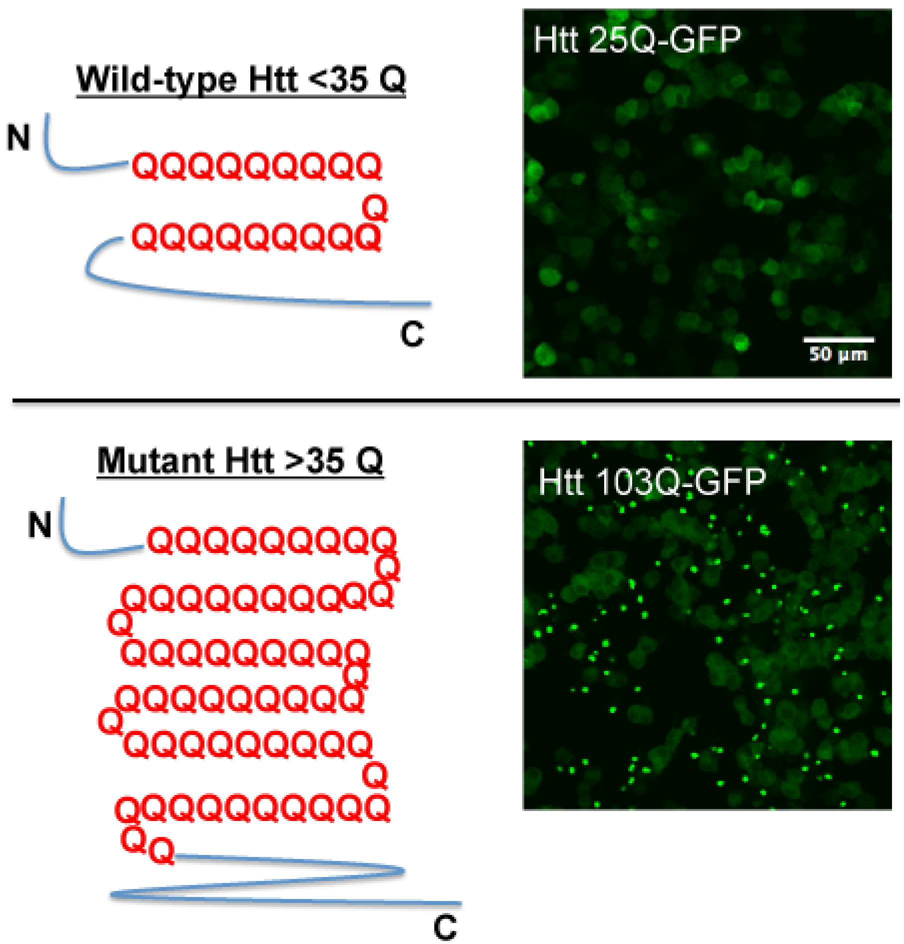


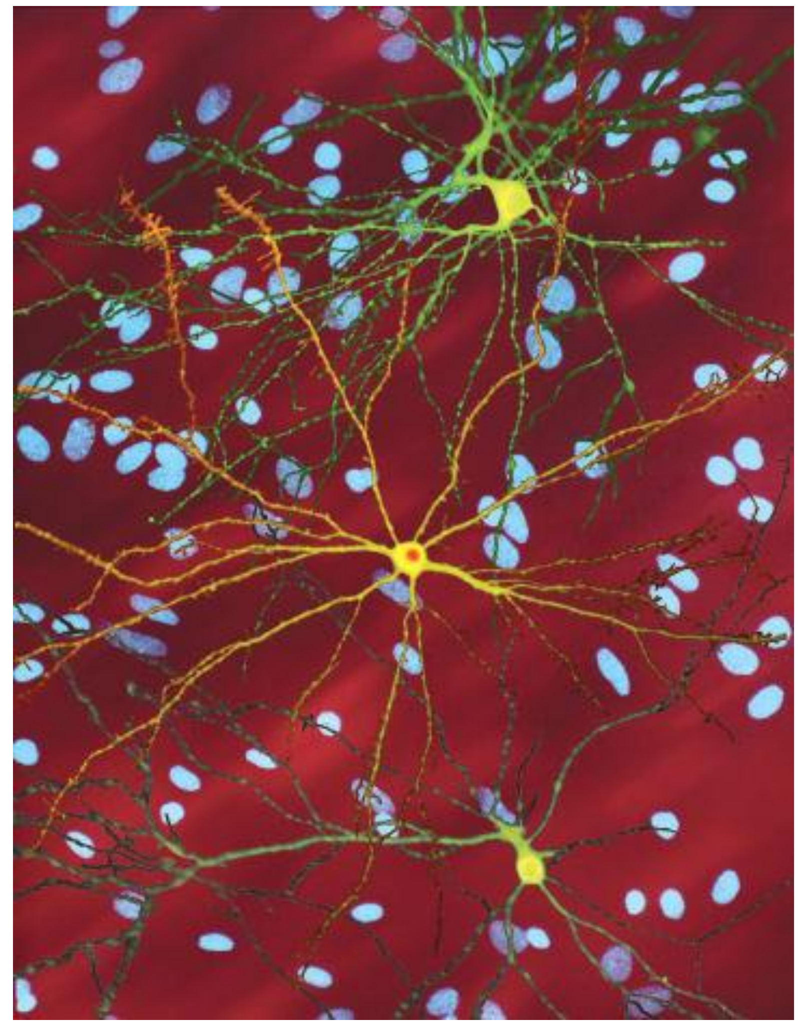
Toxic N-term fragments

Key features of HD pathogenesis

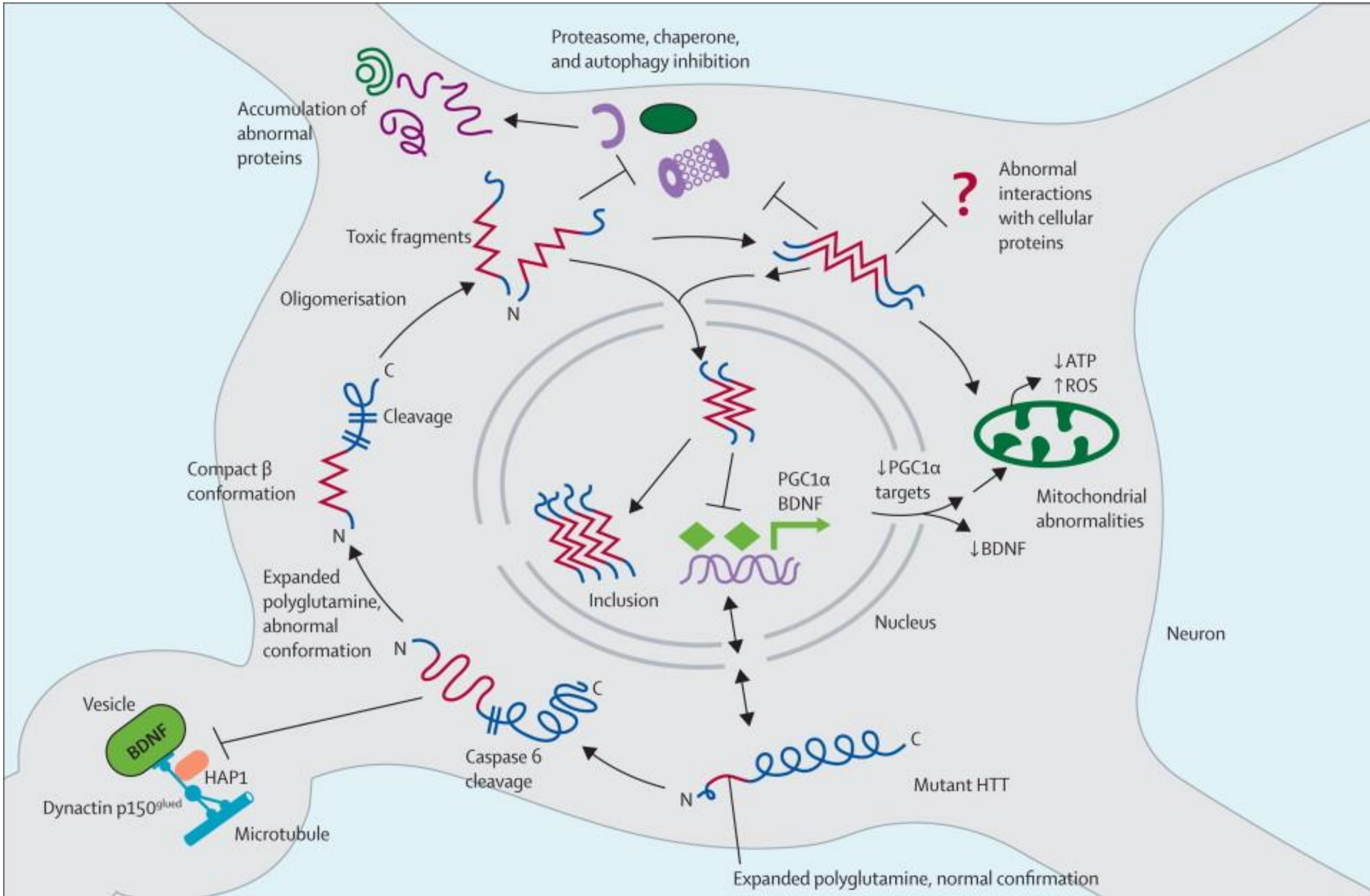
1. Mutant HTT misfolds
2. Unfolded protein response is impaired
3. Mutant HTT is truncated and fragments are highly toxic
4. Post-translational modifications of HTT influence toxicity
5. Nuclear translocation of HTT contributes to toxicity
6. Cellular metabolism is impaired

HTT inclusion in medium spiny neurons

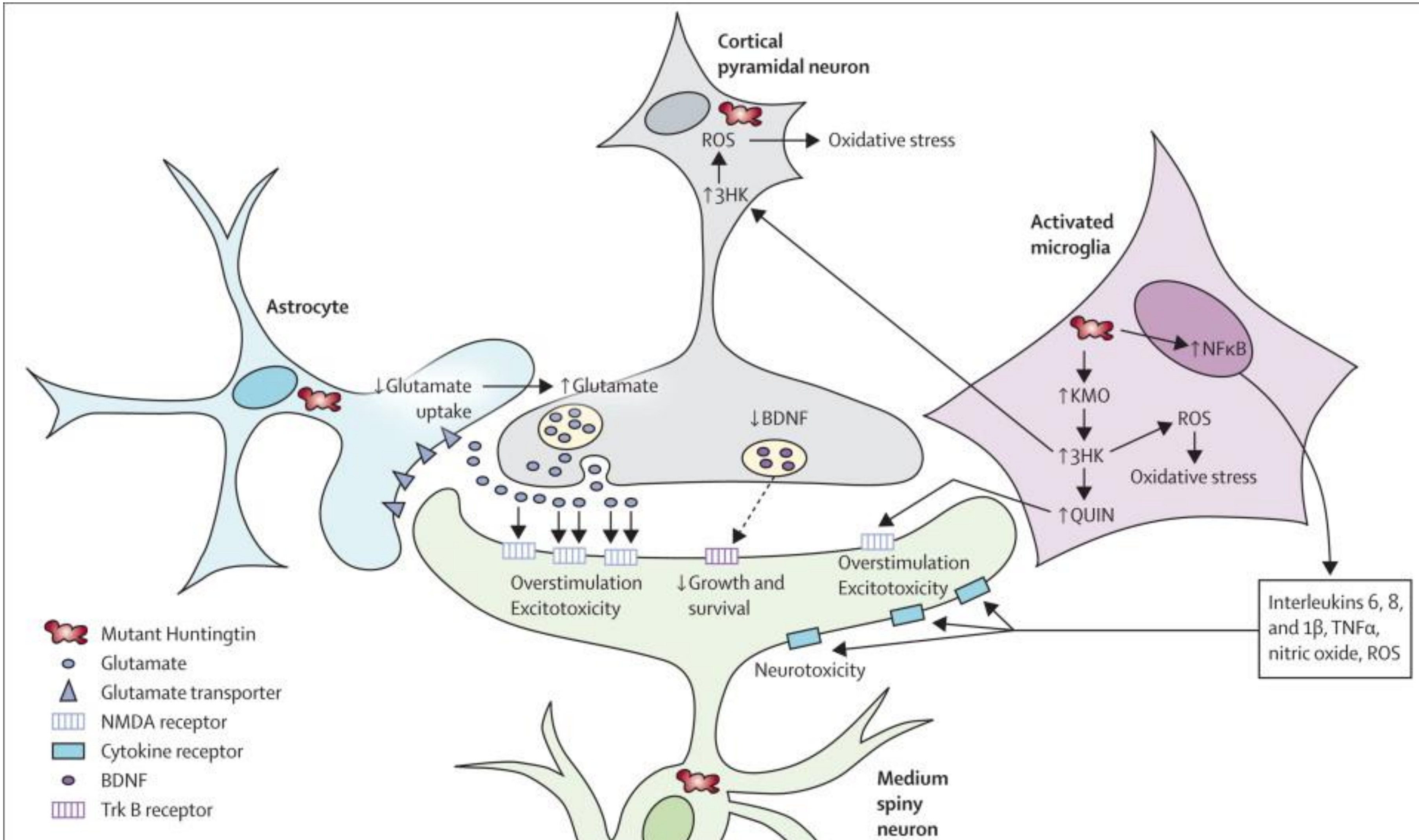




Intracellular pathogenesis in HD



Cell interactions and intercellular pathogenesis

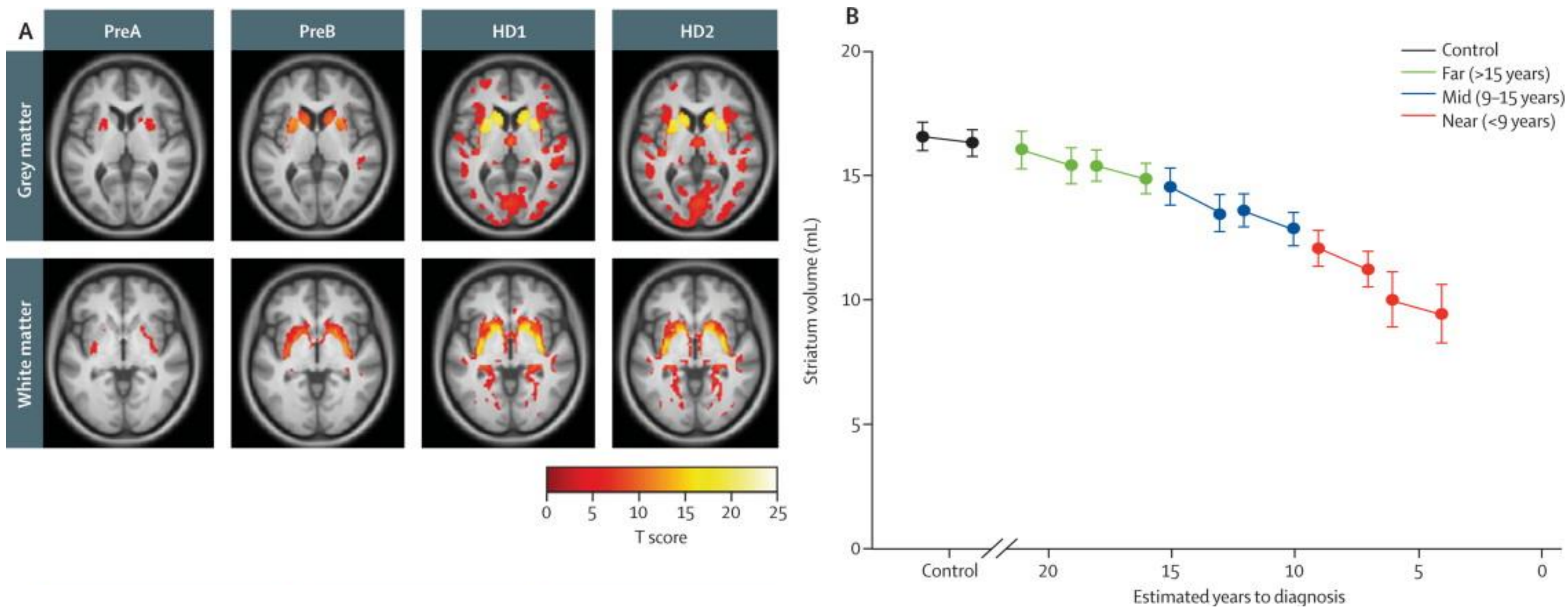


Several new HD targets -> Clinical Trials

- No drug has proven efficacious
- HD progresses slowly, and disease is heterogeneous
- Variability in assessment of disease progression
  - Therefore trial design is difficult

- Focus on novel biomarkers – including imaging
- Observational rather than interventional trials

MRI identifies prodromal disease and may be used to identify HD patients for improved intervention

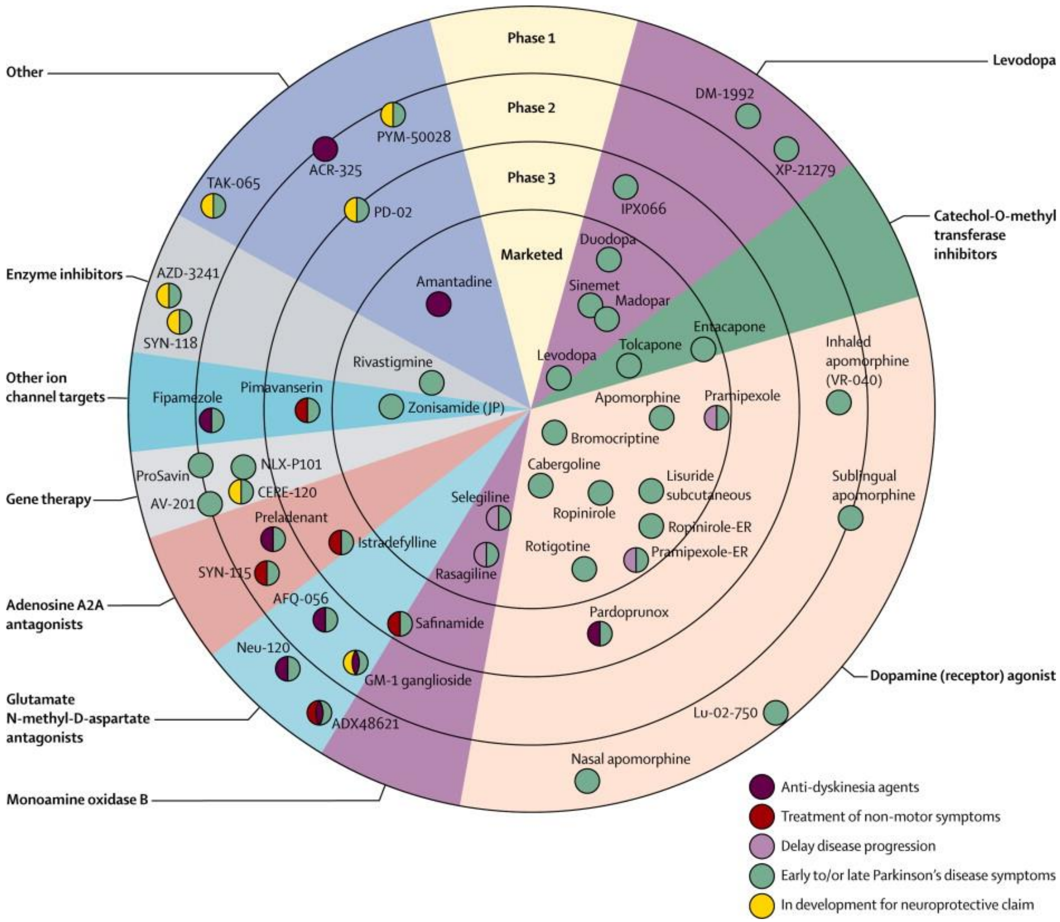


Other triplet repeat disease

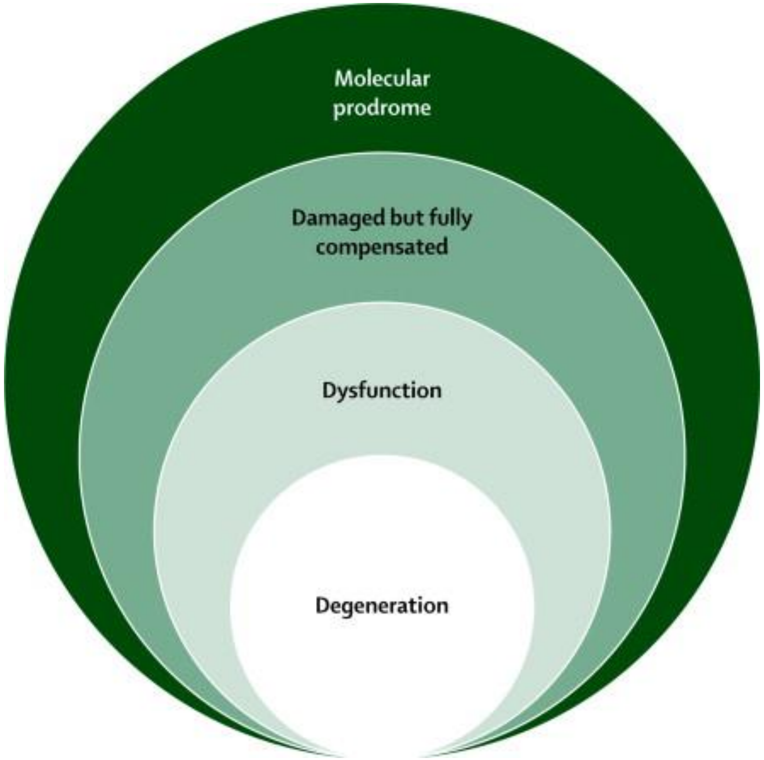
- HD part of a family of triplet repeat diseases characterized by polymorphic triplet repeats
- Show ‘anticipation’ – earlier onset in subsequent generations
- Can be in coding and non-coding regions, in coding regions they encode poly amino acid repeats

Huntington’s Disease	CAG
Fragile X Disease	CGG
Myotonic Dystrophy	CTG
Spinocerebellar atrophy (type 1)	CAG
Spinal and bulbar muscular atrophy (Kennedy’s disease)	CAG

Existing and potential treatments in PD

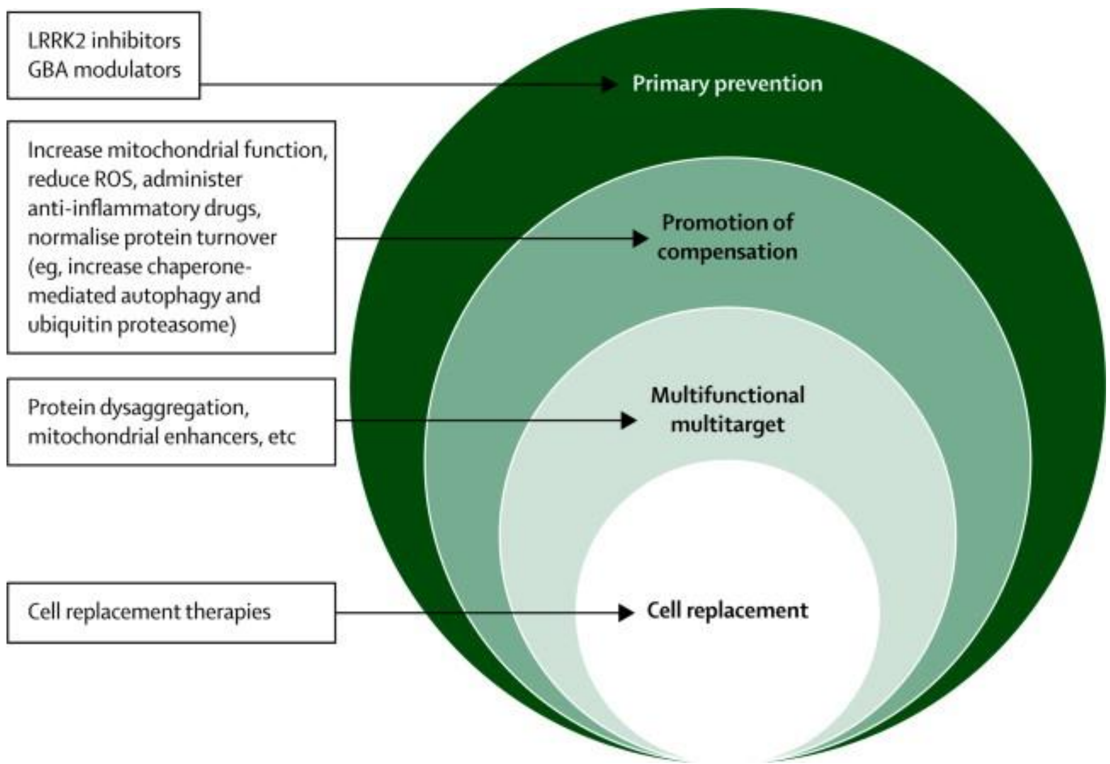


Evolution of neurodegenerative diseases

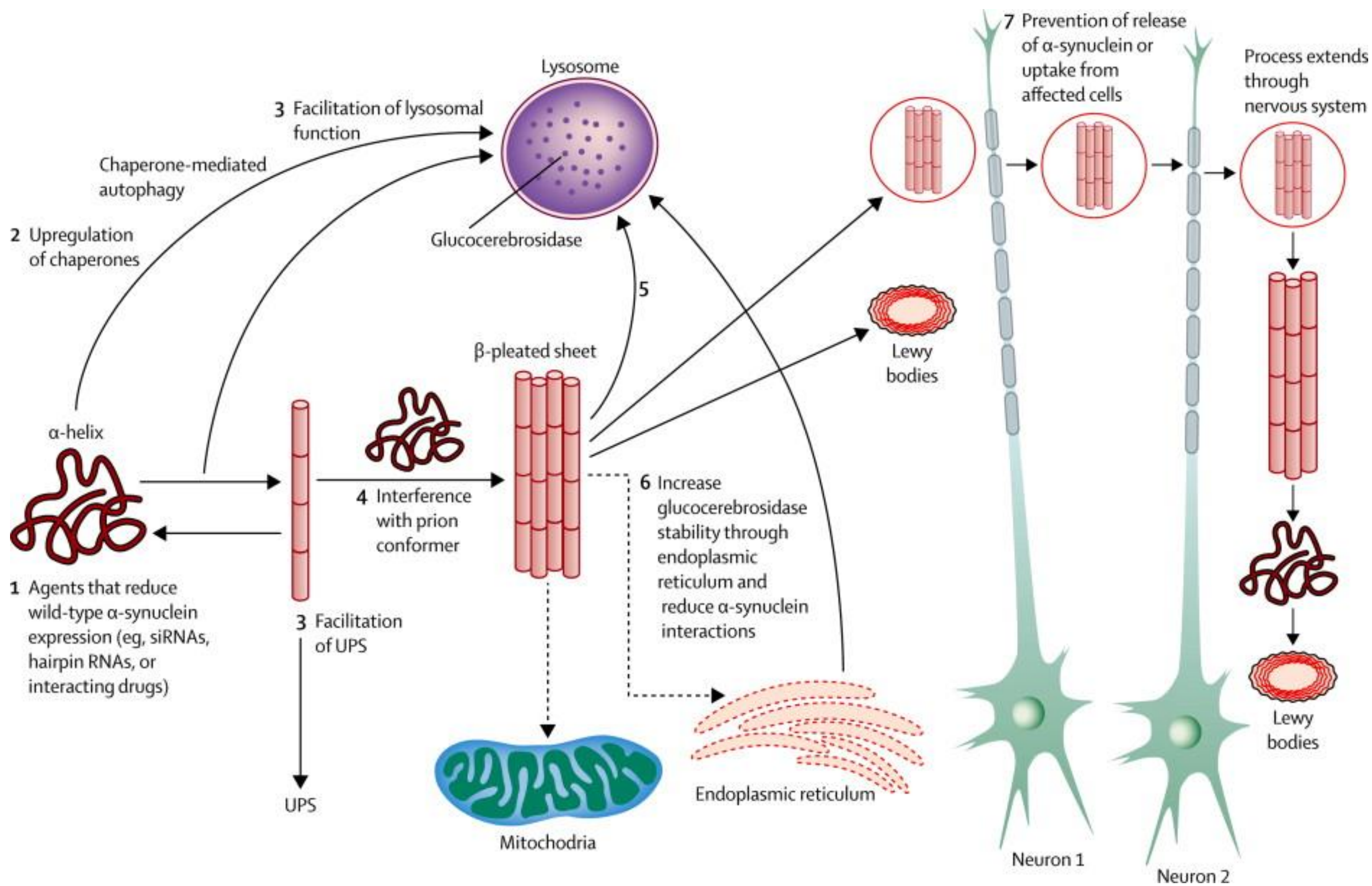


Therapeutic strategies

Examples in PD



Strategies to prevent protein misfolding, aggregation and UPR stress



Examples in PD

Gene and cell therapy for neurodegenerative disease

- Cell replacement?
  - PD – fetal mDA neurons, iPSCs, etc
  - HD – MSNs
  - ALS – MNs
  - AD – unclear, too challenging
- Gene therapy?
  - Viral vectors – likely AAV-based
  - GDNF, BDNF, IGF, etc.

Cellular Transplantation

- Cellular source and preparation
- Placement of cells
  - Original site of damage (requires projection though tissue)
  - Target site (will not receive appropriate inputs)
- Immunologic rejection
  - CNS privileged site but immunosuppression will be necessary for allogenic or xenografts
- Function

Transplantation in PD

- 6-OHDA model – apomorphine induce rotations due to R supersensitivity
- Inject DA neuron-containing graft
- Assess rotational behavior

- Early clinical trials (Sweden, 1987) used chromaffin cells – failure
- Human fetal midbrain grafts
  - PET confirmation of DA release
  - 12 months to reach peak
- Stem cells: hES & iPSCs
- Directed reprogramming: PMY516 recitation