MULTIPLE SYSTEM ATROPHY
UPDATE ON A TRIPARTITE DISEASE
A PUZZLING OUTLIER IN NDD?
A STRANGE FIRST COUSIN TO PD?

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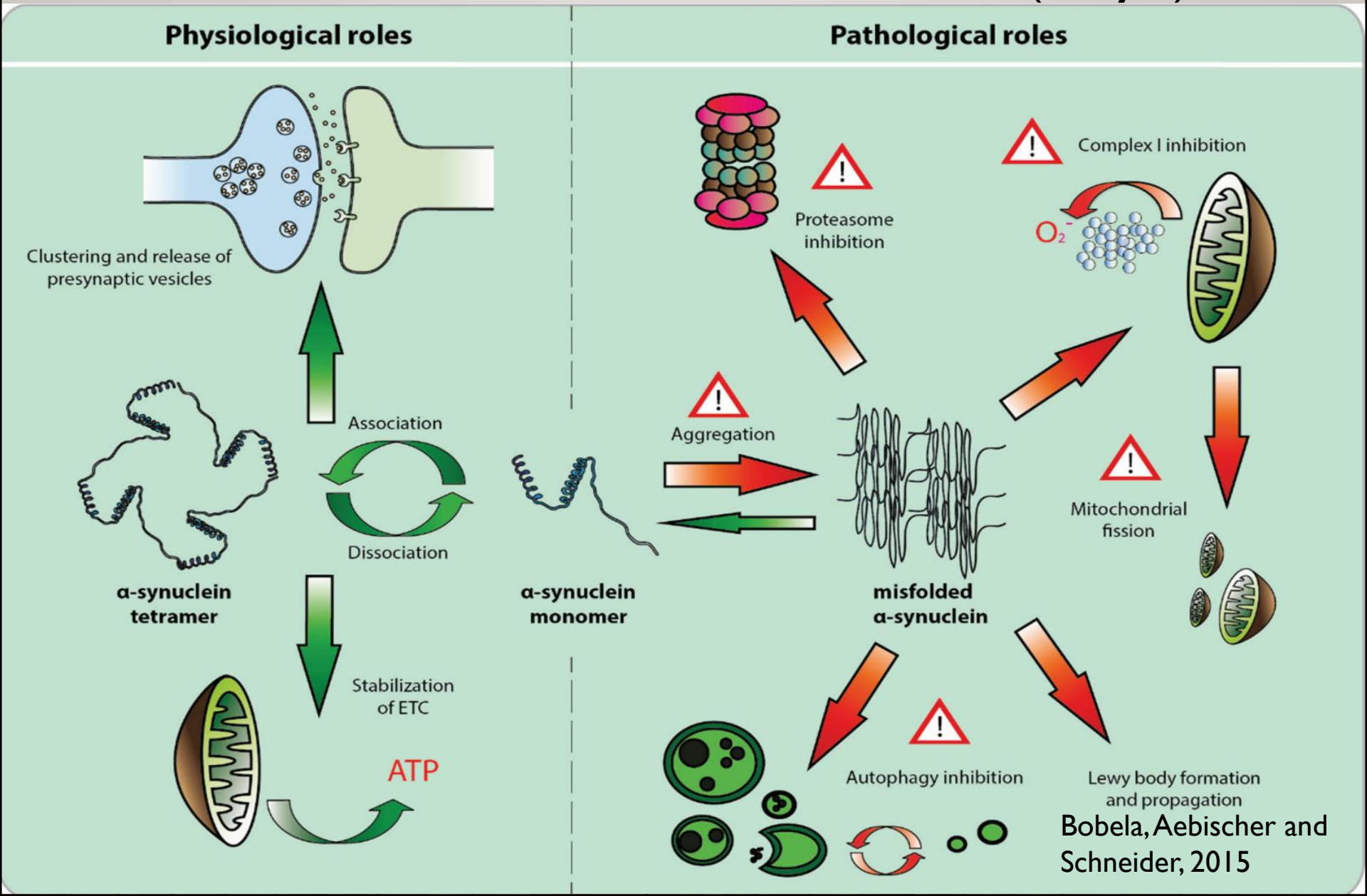
2 HISTORY OF CONCEPTS AROUND MULTIPLE SYSTEM ATROPHY

- Historically, three clinicopathological entities assumed to be discrete are now termed MSA: ***1) olivopontocerebellar atrophy*** (patients w/predominant ataxia/other cerebellar features), ***2) striatonigral degeneration*** (patients w/parkinsonism) and ***3) Shy–Drager syndrome*** (patients w/orthostatic hypotension often with other autonomic dysfunction.)
- ***Graham & Oppenheimer (1969***) proposed unifying notion of MSA as a superordinate umbrella concept for these disorders, supported by (***Papp 1989***) pathological demonstration of ***pathognomonic glial (oligodendrocyte) cytoplasmic inclusions (GCIs)*** in all MSA.
- Integration of several clinical phenotypes under single umbrella presaged events @ ***FTD/ALS.***
- ***MSA-P patients are often mis-diagnosed as PD***, w/this sometimes corrected in vivo – poor response to levodopa→ questions @ presumption of PD, along w/severe dysautonomia.
- Neuronal cytoplasmic inclusions (***NCIs***), neuronal nuclear inclusions (***NNIs***), dystrophic neurites ***(DNs)*** also seen, but appearing at much lower frequencies than ***GCIs***. Contrasts w/predominance of neuronal inclusions in other NDD (w/inclusions in other CNS cells seen).

3 BASICS OF MSA (PART II)

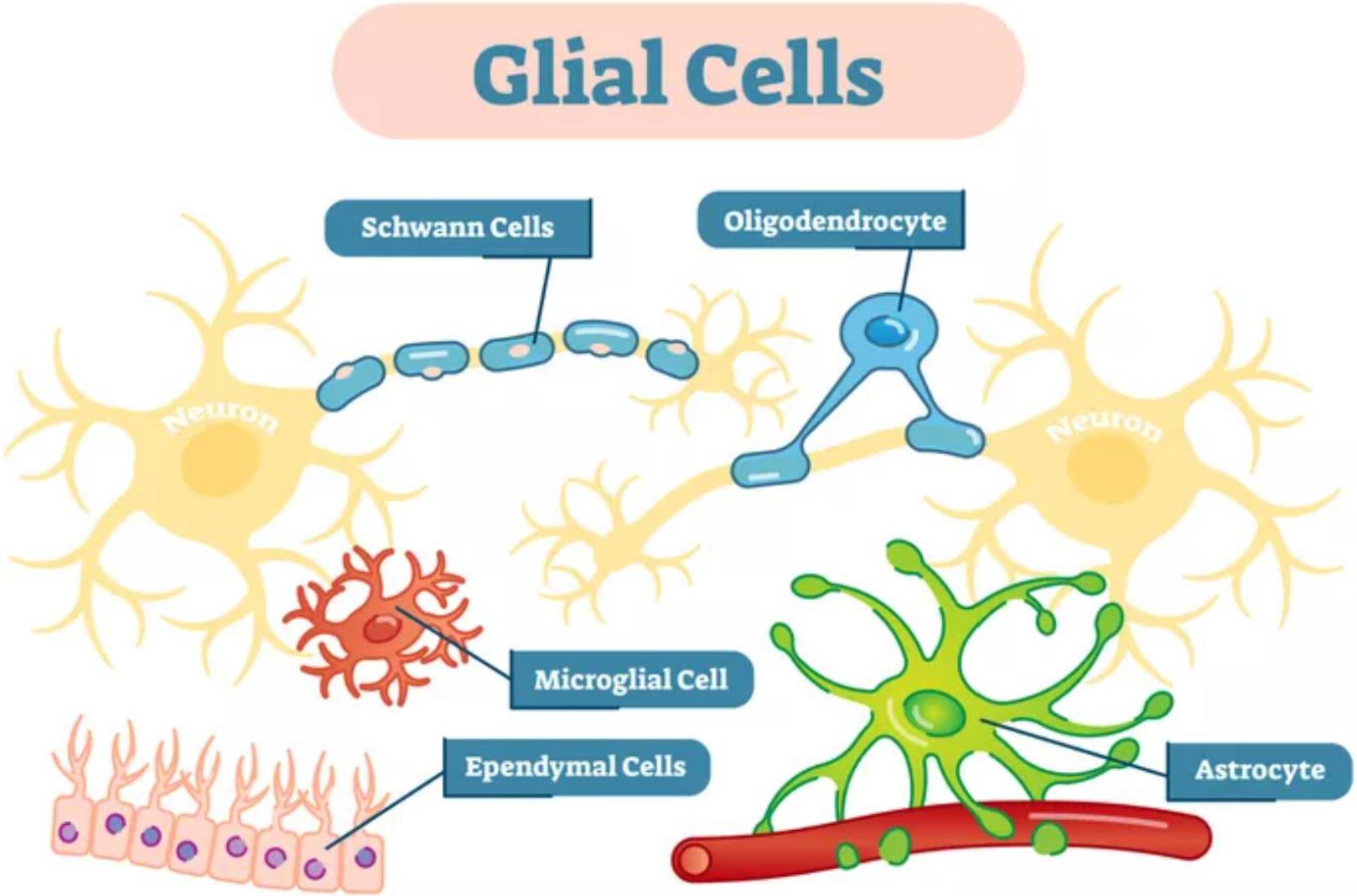
- Subsequent pathology studies showed that misfolded & aggregated p-α-synuclein was main component of these inclusions. ***MSA, Parkinson's disease (PD) and mixed-pathology diffuse Lewy body disease (DLB) (often w/comorbid plaques & tangles), are now all grouped as synucleinopathies. Why α-synuclein found primarily in oligodendrocytes ('GCI'), not nearly as much in neurons is unclear/central to MSA (more on this later).***
- ***MSA is typically the most aggressive synucleinopathy, possibly excepting DLBD***, and is typically characterized by rapid progression leading to severe disability within 5–6 years and death within ±10 yrs of first symptoms, ***on average***. However, there is significant variability on rate of progression, not well understood or modeled (true of all NDD).
- In contrast to PD, less effective therapies are available to treat the symptoms of MSA (3 Rx with some impact plus some sx focused Rx); development of disease-modifying interventions is pressing unmet need in MSA (no truly disease modifying Rx has been validated in any NDD).
- Relatively rare, thus a partially orphaned disease from standpoint of research. 1.6 cases/ 100,000 but 3-4/100,000 over 50. ***10-25 fold lower incidence compared to PD***. No gender bias in risk, unlike in PD. Median age of symptom onset is in 50's, but earlier onset in 4th decade of life (young-onset MSA) and later onset in 7th or 8th decades of life (late-onset MSA) shown in autopsy-proven MSA. In Western countries, MSA-P predominates, 66–82% of MSA patients. MSA-C is more common in Eastern countries, 67% of MSA patients.

4 PATHOLOGICAL & PHYSIOLOGICAL ROLES OF ALPHA SYNUCLEIN 140 AMINO ACID (α-syn)



Bobela, Aebischer and Schneider, 2015

5 THE COMMUNITIES OF GLIA –VIEW OF NDD AS JUST NEURON-CENTRIC DISORDERS IS DEAD



GENES AND OTHER RISK FACTORS FOR MSA – A BIG VOID IN THE SCIENCE?

- Weak evidence for any consistent genetic/familial disease, very different from PD or even AD. Is MSA anomalous? Or do we need more work to define linkages?
  - Has low inheritability, but w/***rare*** familial cases, with regional phenotype variation, Eastern cohorts pathway gene CoQ10 COQ2 (OR=3), w/MSA-C but not MSA-P.
  - Inconsistent findings @ role for mutations in SNCA or MAPT (as in PD/FTD), outside of SYNC copy number variations/DNA mosaicism. MITO linkages in COQ2 variant?
  - Evidence for *overlap vulnerability* around C9orf72 (FTD linkage), GBA/LRRK2 mutations (PD link?), and inflammatory bowel disease. Polygenes w/global vulnerability to NDD?
  - SNP in several candidate genes, FBXO47, ELOVL7, EDN1, MAPT. Still weak evidence.
- Unlike PD, weak evidence @ roles for pesticide/other neurotoxin exposure.
- Possible protective role for NSAIDs, but data is not definitive (like AD?)
- Weak evidence base regarding ***sleep, diet/microbiome, exercise & social support***. Probably relevant, given primary role in aging and all diseases of aging.
- Uncertain Tx role or efficacy, but ***attention to these things never hurts, may take gas off of the neurodegenerative fire,*** even if not yet proven to do so.

TRIPARTITE DIAGNOSIS OF MSA AND ITS MAJOR PHENOTYPES

Not that easy to diagnose MSA, or separate it from PD and other imitators.

Several important caveats, patterns and clues to help clinicians do proper differential diagnosis, but we need cheap and not intrusive biomarkers.

Neuropathologically established MSA:

- Post-mortem detection of widespread glial cytoplasmic inclusions
- Neurodegeneration of the striatonigral or olivopontocerebellar regions

Clinically established MSA:

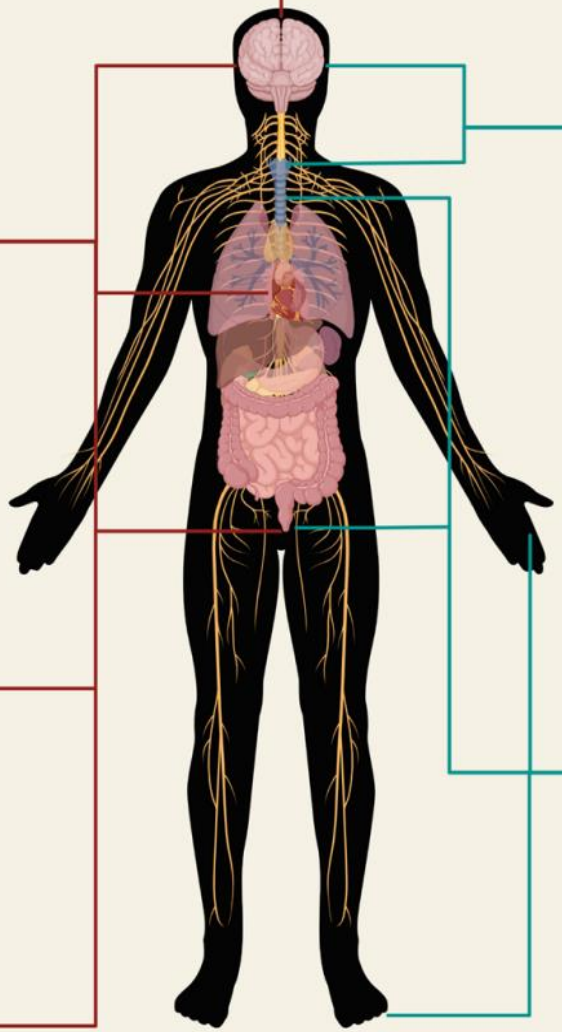
- Sporadic, progressive adult onset disease (>30 years)
- At least one feature of autonomic dysfunction: unexplained voiding difficulties, unexplained urinary urge incontinence, neurogenic orthostatic hypotension
- At least one feature: Parkinsonism with poor Levodopa response, Cerebellar syndrome
- At least two supportive clinical features
- At least one brain magnetic resonance imaging marker
- No exclusion criteria
- Disease subtype based on predominant motor syndrome and brain magnetic resonance imaging markers

Clinically probable MSA:

- Sporadic, progressive adult onset disease (>30 years)
- At least one feature of autonomic dysfunction: unexplained voiding difficulties, unexplained urinary urge incontinence, neurogenic orthostatic hypotension
- At least one feature: Parkinsonism, Cerebellar syndrome
- At least one supportive clinical feature

Possible prodromal MSA:

- Sporadic, progressive adult onset disease (>30 years)
- At least one clinical non-motor feature: Rapid eye movement sleep behavior disorder, neurogenic orthostatic hypotension, urogenital failure
- At least one clinical motor feature: subtle parkinsonian or cerebellar signs



Supportive motor features:

- Withing 3 years of motor onset:
  - *Rapid progression*
  - *Moderate to severe postural instability*
  - *Severe speech impairment*
  - *Severe dysphagia*
- Craniocervical dystonia induced or exacerabted by levodopa in the absence of limb dyskinesia
- Unexplained Babinski sign
- Jerky myoclonic postural or kinetic tremor
- Postural deformities

Supportive non-motor features:

- Stridor
- Inspiratory sighs
- Cold discolored hands and feet
- Erectile dysfunction below the age of 60
- Pathologic laughter or crying

Criteria for a categorical diagnosis of Multiple System Atrophy as set out by the Movement Disorder Society

COMPLEX SYMPTOMATOLOGY OF MSA 15M

- *Cerebellar dysfunction:*
- Gait and limb ataxia/↓↓coordination
- Nystagmus (involuntary oscillatory eye movements w/slow & fast components)
- Ataxic dysarthria ('sloppy', irregular speech like someone intoxicated).
- *Other Motor Issues:*
- Orofacial and/or cervical/axial dystonia (involuntary muscle contractions)
- Babinski sign, postural deformities, falls
- *Other Non-motor Symptoms:*
- Stridor (noisy/high-pitched breathing), inspiratory sighs, cold/discolored hands & feet, constipation, REM sleep BD (prodromal), emotional disinhibition, cognitive impairment (overlap w/PD) w/an executive/WM/attentional fxn phenotype

- *Autonomic failure:*
- Neurogenic orthostatic hypotension (NOH) w/postural dizziness, syncope
- Supine hypertension
- Urinary urgency, frequency, w/urge incontinence & urinary retention
- Sexual dysfunction incl erectile dysfxn
- *Parkinsonianism:*
- Limb bradykinesia (speed & amplitude), plus rigidity, w/↓response to levodopa
- Jerky myoclonic postural or kinetic tremor, rarely classic rest tremor of PD
- Small-stepped gait and flexed posture
- Hypomimia (reduced facial expressions)
- Severe dysphonia (volume, pitch & articulation of voice) vs. hypophonia of PD
- Severe dysphagia (difficulty swallowing)

Table 1.

Supporting and nonsupporting features of multiple system atrophy

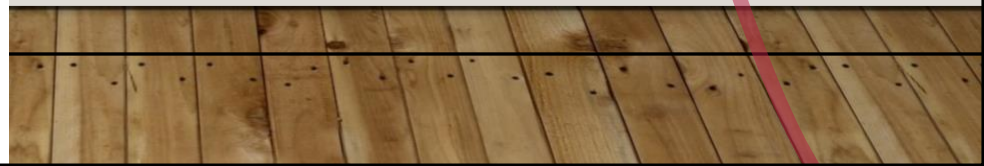
Orofacial dystonia
Disproportionate antecollis
Camptocormia and/or Pisa syndrome
Contractures of hands or feet
Inspiratory sighs
Severe dysphonia
Severe dysarthria
New or increased snoring
Cold hands and feet
Pathologic laughter or crying
Jerky, myoclonic postural/action tremor
Nonsupporting features [10]
Classic pill-rolling rest tremor
Clinically significant neuropathy
Hallucination not induced by drug
Onset after age 75 years
Family history of ataxia or parkinsonism
Dementia (on DSM-IV)
White matter lesions suggesting multiple sclerosis

‘axial dystonia’

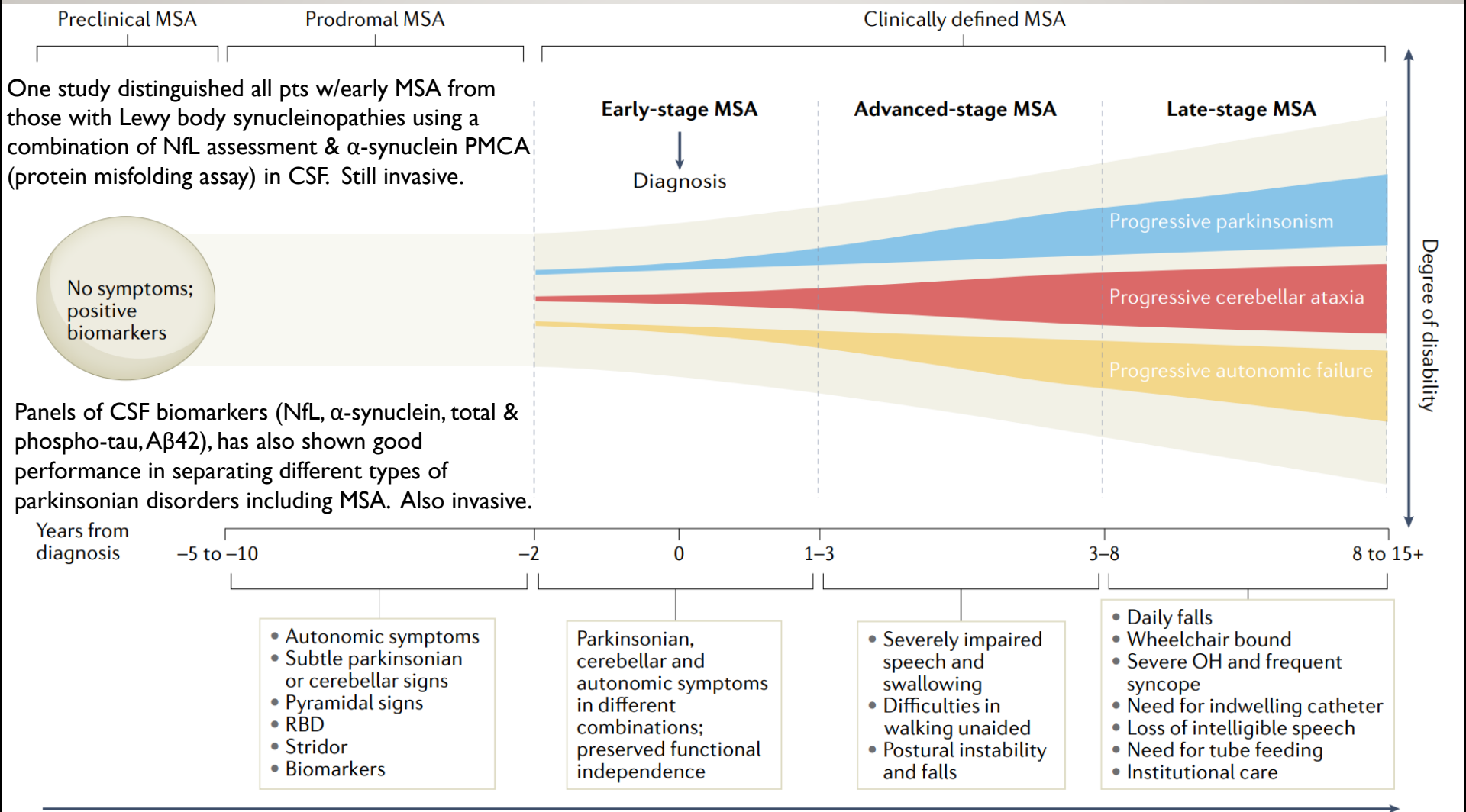
RED FLAGS AGAINST PD THAT SUPPORT MSA AND ALSO AGAINST MSA

Gibbons C, Wang N, Freeman R et al. Cutaneous α-Synuclein Signatures in Patients With Multiple System Atrophy and Parkinson Disease. Neurology. 2023

Patients w/PD showed reduced peripheral nerve fiber densities compared with patients with MSA ($p <$ 0.05, all fiber types). All patients with MSA and 51/54 with PD had evidence of phosphorylated α-synuclein in at least one skin biopsy. No phosphorylated α-synuclein was detected in controls. ***Patients with MSA had greater phosphorylated α-synuclein deposition ($p < 0.0001$) & more widespread peripheral distribution ($p < 0.0001$) than patients with PD.*** These results provided >90% sensitivity and specificity in distinguishing between the 2 disorders.

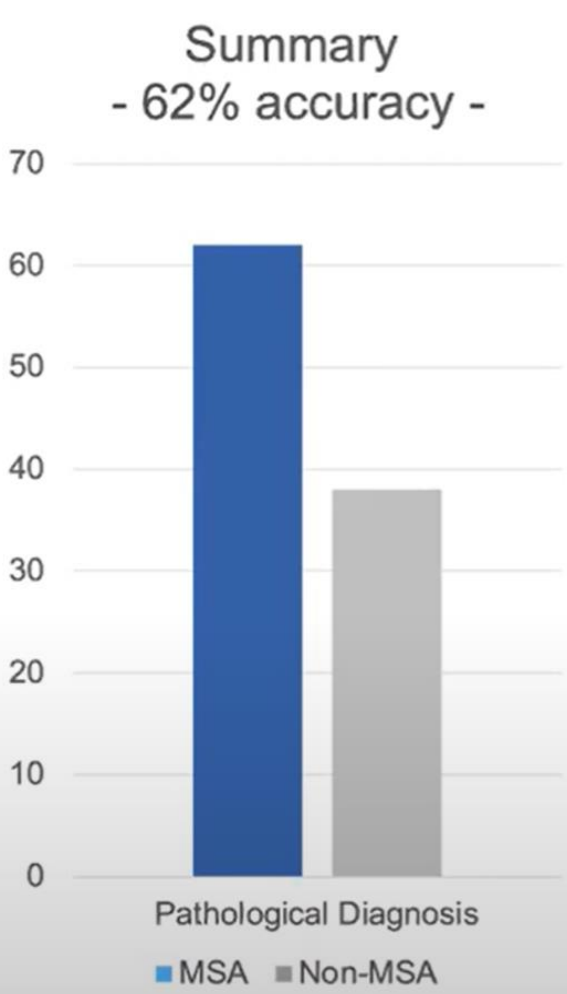


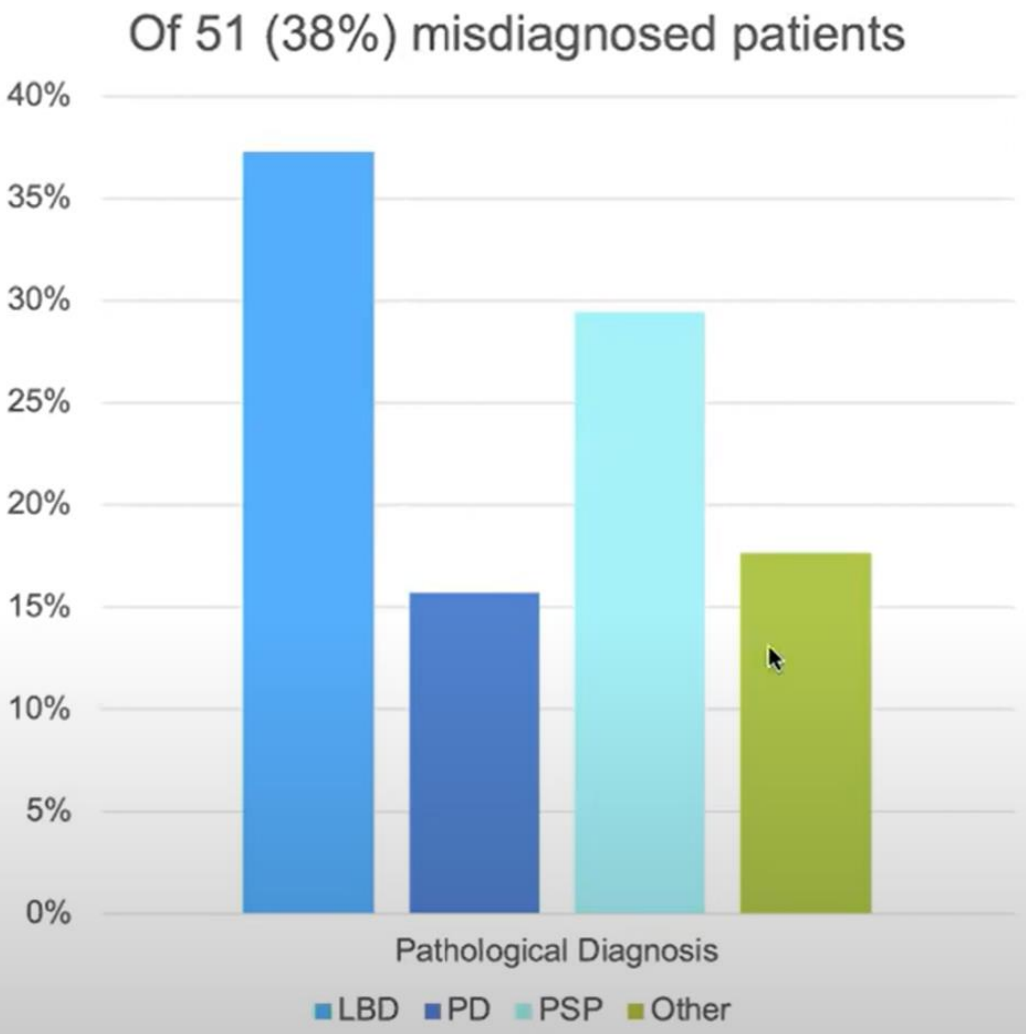
Preclinical MSA: earliest asymptomatic state of disease in which neurobiological changes detected with biomarkers. Reliable/validated biomarkers for preclinical MSA are lacking. ***Prodromal MSA:*** clinical features show symptomatic spectrum of MSA, but severity & specificity does not meet thresholds for clinical diagnosis. ***Clinically defined MSA*** shows presence of core clinical features & results of diagnostic testing. Progression from early-stage to late-stage MSA shows an average of 10–15 years from symptom onset. Duration of preclinical and prodromal phases of MSA is less clear, but estimated to last for up to 10 years or longer, (REM sleep BD, and dysautonomia common)



(Poewe et al, 2022 NRDP)

Of 134 clinically diagnosed MSA patients



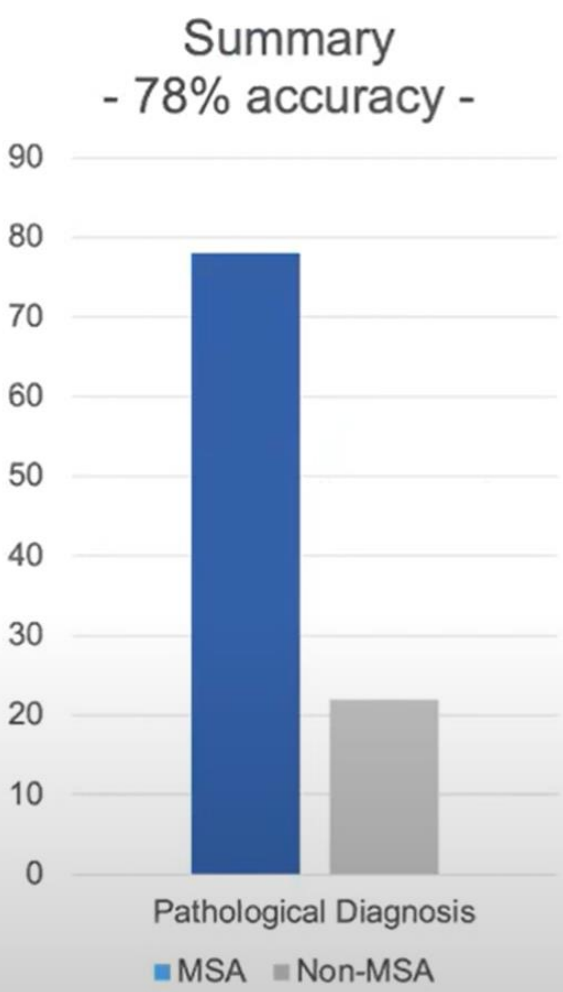


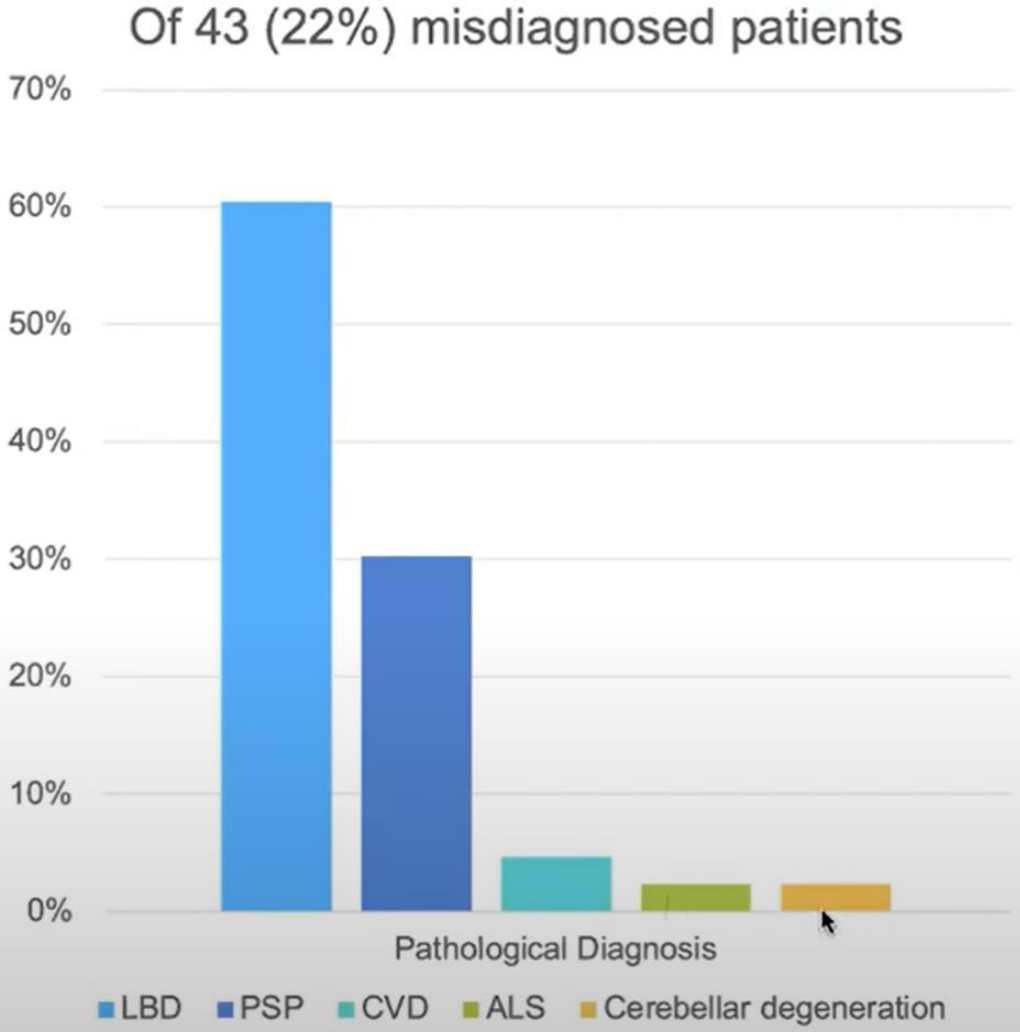
Koga et al. Neurology 2015; 85:404

WHEN DLB, PD, & PSP MASQUERADE AS MSA-P

Features	MSA	DLB	PD	PSP	Others	*p* Value
Demographic features						
Number of patients	83	19	8	15	9	
Male, % (n)	60 (50/83)	81 (16/19)	75 (6/8)	60 (9/15)	44 (4/9)	0.12
Patients with clinical records, % (n)	95 (79/83)	93 (18/19)	88 (7/8)	93 (14/15)	78 (7/9)	0.97
Quality of clinical records, median (25th, 75th percentile)	3 (3, 4)	4 (3, 4)	4 (3, 4)	3 (1, 4)	3 (3, 4)	0.73
Age at onset, mean ± SD	57 ± 9	63 ± 10	68 ± 8[a]	66 ± 11[a]	59 ± 4	<0.001
Age at death, mean ± SD	65 ± 8	72 ± 9[a]	77 ± 8[b]	74 ± 9[b]	68 ± 3	<0.001
Symptoms duration, mean ± SD	8.4 ± 3.7	9.0 ± 3.8	9.4 ± 5.5	8.1 ± 3	8.7 ± 4.1	0.95
FH of parkinsonism, % (n)	11 (9/79)	22 (4/18)	0 (0/7)	7 (1/14)	43 (3/7)	0.38
FH of dementia, % (n)	13 (10/79)	22 (4/18)	14 (1/7)	21 (3/14)	0 (0/7)	0.68
Pathologic features						
Brain weight, g, mean ± SD	1,219 ± 142	1,245 ± 167	1,192 ± 96	1,175 ± 236	1,144 ± 209	0.47
Braak NFT stage, median (25th, 75th percentile)	I (0, II)	III (I, III)[a]	II (II, III)[a]	II (I, III)	I (I, II)	0.001
Thal Aβ phase, median (25th, 75th percentile)	0 (0, 1)	2 (1, 3)[a]	2 (0, 2)	0 (0, 3)	0 (0, 1)	0.008
Lewy-related pathology, % (n)	8 (7/83)	100 (19/19)	100 (8/8)	0 (0/14)	22 (2/9)	—
Brainstem subtype, % (n)	86 (6/7)	0 (0/19)	100 (8/8)	—	22 (2/9)	—
Transitional subtype, % (n)	14 (1/7)	63 (12/19)	0 (0/8)	—	0 (0/9)	—
Diffuse subtype, % (n)	0 (0/7)	37 (7/19)	0 (0/8)	—	0 (0/9)	—
Variant of MSA, % (n)						
MSA-SND	31 (26/83)	—	—	—	—	—
MSA-OPCA	19 (16/83)	—	—	—	—	—
MSA-SND/OPCA	49 (41/83)	—	—	—	—	—

Of 203 clinically diagnosed MSA patients





Miki et al. Brain 2019; 142:2813

DIAGNOSTIC CRITERIA FOR "CLINICALLY ESTABLISHED" & "CLINICALLY PROBABLE" MULTIPLE SYSTEM ATROPHY

DIVISION INTO CLINICALLY ESTABLISHED MSA-P OR MSA-C

	Clinically established MSA	Clinically probable MSA
Core clinical features	1. Autonomic dysfunction defined as (at least one is required) ○ Unexplained voiding difficulties with post-void urinary residual volume ≥100 mL ○ Unexplained urinary urge incontinence ○ Neurogenic OH (≥20/10 mmHg blood pressure drop) within 3 minutes of standing or head-up tilt test and at least one of 1. Poorly L-dopa-responsive parkinsonism 2. Cerebellar syndrome (at least two of gait ataxia, limb ataxia, cerebellar dysarthria, or oculomotor features)	At least two of: 1. Autonomic dysfunction defined as (at least one is required): ○ Unexplained voiding difficulties with post-void urinary residual volume ○ Unexplained urinary urge incontinence ○ Neurogenic OH (≥20/10 mmHg blood pressure drop) within 10 minutes of standing or head-up tilt test 2. Parkinsonism 3. Cerebellar syndrome (at least one of gait ataxia, limb ataxia, cerebellar dysarthria, or oculomotor features)
Supportive clinical (motor or non-motor) features	At least two	At least one[a]
MRI marker	At least one	Not required
Exclusion criteria	Absence	Absence

Supportive clinical features

Supportive motor features	Rapid progression within 3 years of motor onset	**Supportive non-motor features**	Stridor
	Moderate to severe postural instability within 3 years of motor onset		Inspiratory sighs
	Craniocervical dystonia induced or exacerbated by L-dopa in the absence of limb dyskinesia		Cold discolored hands and feet
	Severe speech impairment within 3 years of motor onset		Erectile dysfunction (below age of 60 years for clinically probable MSA)
	Severe dysphagia within 3 years of motor onset		Pathologic laughter or crying
	Unexplained Babinski sign		
	Jerky myoclonic postural or kinetic tremor		
	Postural deformities		

MRI markers of clinically established MSA

Each affected brain region as evidenced by either atrophy or increased diffusivity counts as one MRI marker.

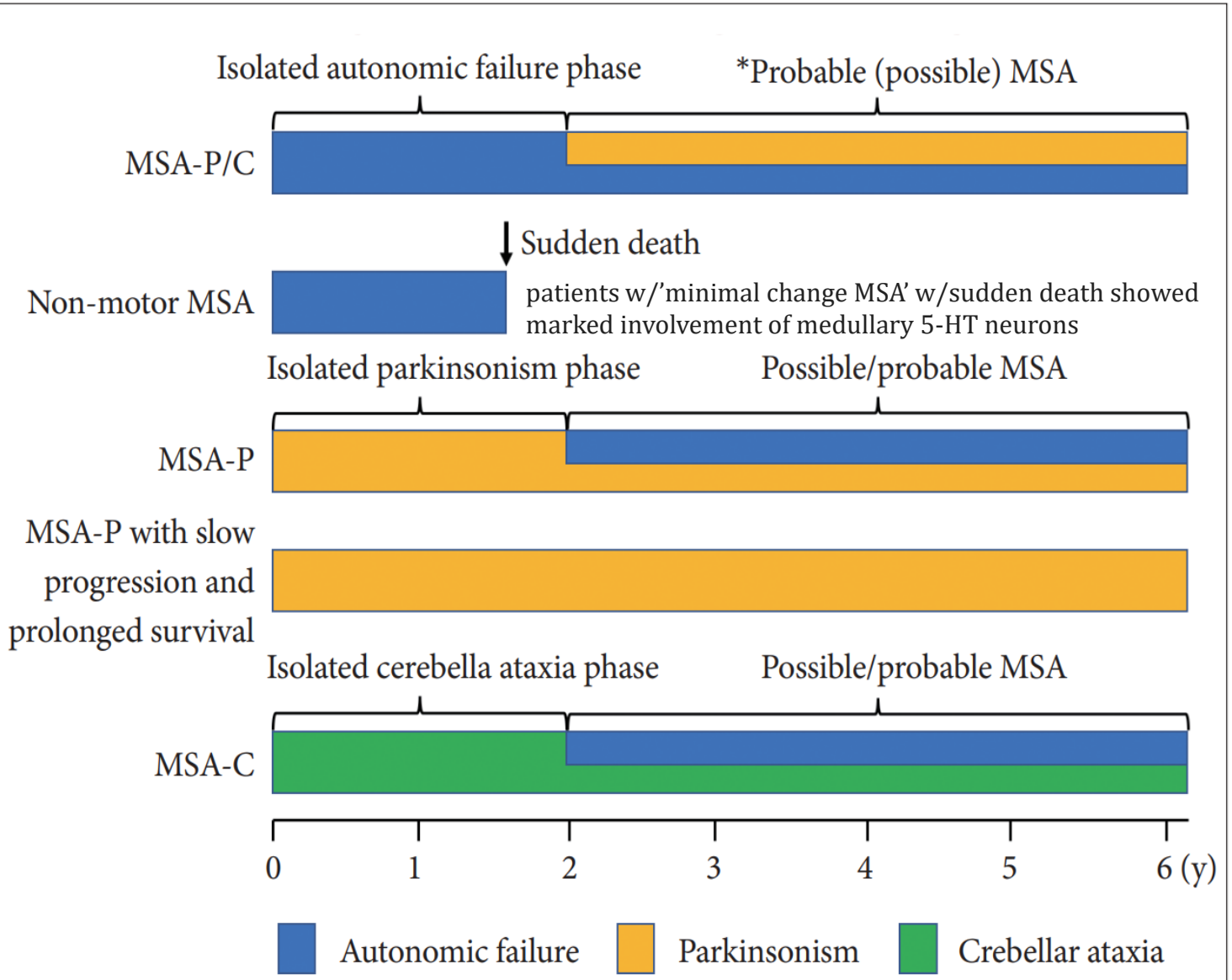
For MSA-P Increased diffusivity of putamen on DWI in MRI

- Atrophy of:
  - Putamen (and signal decrease on iron-sensitive sequences)
  - Middle cerebellar peduncle
  - pons
  - Cerebellum
- "Hot cross bun" sign

For MSA-C Increased diffusivity of putamen & MCP on DWI in MRI

- Atrophy of:
  - Putamen (and signal decrease on iron-sensitive sequences)
  - Infratentorial structures (pons and middle cerebellar peduncle)
- "Hot cross bun" sign

17 'ISOLATED SYNDROMES' SERIOUSLY CLOUD & COMPLICATE EARLY DIFFERENTIAL DIAGNOSIS



'Isolated' MSA-P & MSA-C presentations take years for dysautonomia to be manifest. Average time for dual sxns →Dx is ~***2 yrs***. Signif. minority take ***4 yrs***. Idiopathic late onset cerebellar ataxia (ILOCA) is any slowly progressive, adult-onset ataxia; MSA-C, spino-cerebellar ataxias (SCAs), fragile X-ataxia syndrome, Friedreich's ataxia, autosomal recessive cerebellar ataxia type 1, & other genetic disorders.

Gilman et al. reported that median time from isolated cerebellar ataxia (ILOCA phenotype) to combined cerebellar ataxia & autonomic failure (MSA) was 4.5 years.

NATURAL HISTORY OF MSA

"Compared with PD, MSA has a much more aggressive course of disease and leads to severe disability and death typically within ~10 years from symptom onset. Around 30% require walking aids after an average of 3 years following diagnosis, up to 60% are wheelchair-bound 5 years after dx.

A European natural history study of patients with moderately advanced MSA, with an average disease duration from symptom onset of ~5 years, found that, by this time, 23% of patients reported daily falls, 3% had developed severe dysphagia requiring PEG tube placement for feeding, 7% had lost speech intelligibility owing to severe dysarthria, and a further 10–20% reached these milestones after only one additional year of follow-up, showing its rapidity.

A clinicopathological series of 84 patients with MSA found that, by the time of the last visit, 54% of patients were wheelchair-dependent, 41% had unintelligible speech and 26% required a urinary catheter, with average disease duration of 5–7 years from symptom onset before reaching any of these milestones"
Poewe et al, NRDP

MSA mimics	Consider if...
Parkinson's disease	Significant and sustained response to L-dopa (>30% improvement in UPDRS-III), anosmia, delayed onset of autonomic symptoms
Dementia with Lewy bodies[60]	Fluctuating consciousness and cognitive change with hallucination (particularly visual), not secondary to L-dopa treatment
Progressive supranuclear palsy[63]	Vertical saccade slowing, supranuclear gaze palsy Frontalis overactivity and retropulsion Frontal cognitive change, non-fluent variant primary progressive aphasia
Corticobasal degeneration[64]	Asymmetrical limb dystonia, alien limb
Vascular Parkinsonism[65]	Onset >75 years, vascular risk factors, dementia
Normal pressure hydrocephalus	Cognitive impairment, lack of upper limb signs
Neurogenetic conditions	
Spinocerebellar ataxia types 1,2,3,6,7,12,17[66]	May have a positive family history, but deterioration likely slow
CANVAS[67]	Chronic dry cough, positive head impulse test, peripheral neuropathy
C9ORF72 expansion[68]	Positive family history, lower motor neurone signs (eg, fasciculation, wasting)
Friedreich's ataxia[66]	Peripheral neuropathy, pes cavus, loss of lower limb reflexes cardiomyopathy, type two diabetes mellitus
Fragile X tremor–ataxia syndrome[66]	X-linked inheritance, peripheral neuropathy, behavioural disorders with executive dysfunction

Currently, no reliable biomarkers exist for MSA. 10% of clinically diagnosed PD pts have MSA, up to 7% clinically diagnosed MSA patients life have PD. 30% of pts with sporadic adult-onset cerebellar ataxia have MSA

CANVAS, cerebellar ataxia, neuropathy, vestibular areflexia syndrome; MSA, multiple system atrophy; UPDRS-III, Unified Parkinson's Disease Rating Scale part III.

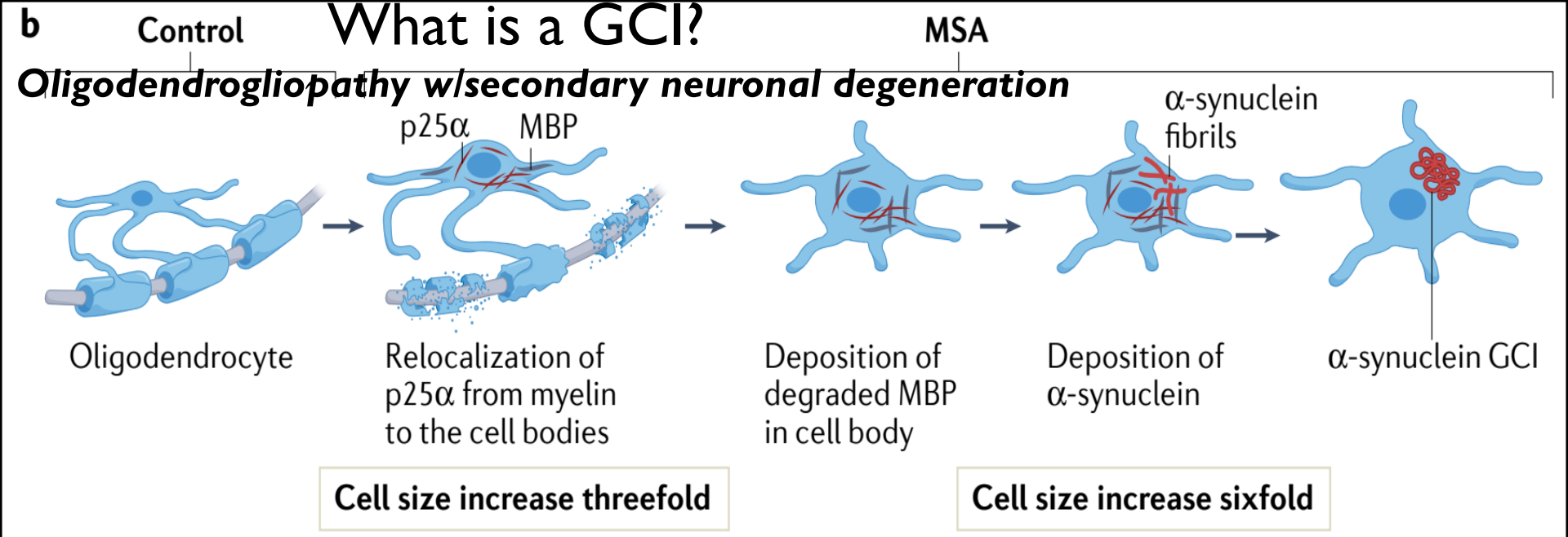
NEUROPATHOLOGY – HISTOPATHOLOGY AND PATHOGENIC MECHANISMS

Overlap with other synucleinopathies. Prion-like spread of protein aggregates ('seeding') in animal models. Why α-syn aggregates end up mainly in oligodendrocytes is still unclear but recent study may have key insight.

BASIC NEUROPATHOLOGY OF MSA

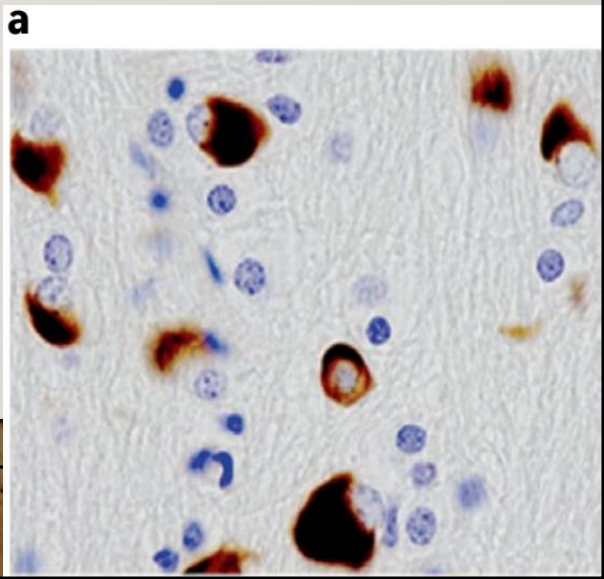
- GCI are silver-positive (aka fibrillar) & ubiquitinated, suggesting that autophagy /proteasome overloaded and/or failing. Density of these ***GCIs*** correlates with regional network atrophy, suggesting that neurodegeneration is tied to ***GCI***.
- Demyelinating lesions invariably contain ***GCIs***, also found in BG, cortex, brainstem, cerebellar and motor regions of cortex w/out formal demylination.
- There are nonfibrillar aggregates of ***α-syn*** (oligomers) in neurons, in overlapping distribution with ***GCI***, along with ***intranuclear inclusions of fibrillar α-syn*** in both glia OLD and neurons, but neuronal inclusions ***only in regions of GCIs***.
- Morphological changes in oligodendrocytes detectable early, along with changes in a microtubule-associated protein ***p25a,*** and ***MBP*** (myelin basic protein).
- Highly relevant that ***regional distribution of gliosis falls in α-syn-overloaded white matter***, also correlating heavily with degree of neurodegeneration.

What is a GCI?



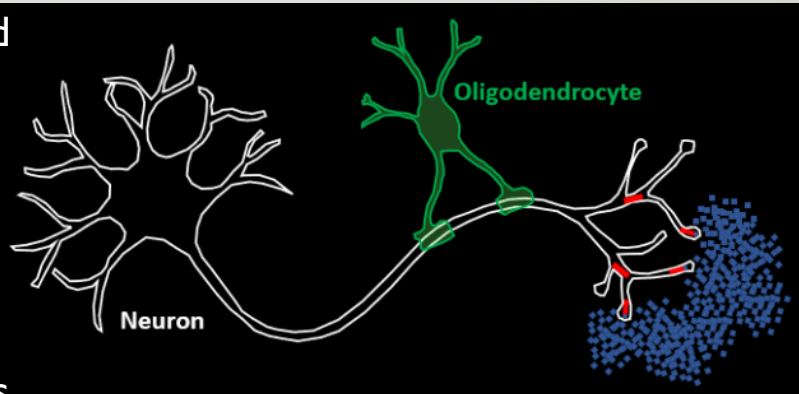
b) The oligodendroglial protein p25α relocates to the cytoplasm causing cellular enlargement. Degraded myelin basic protein (MBP), a major protein of myelin, is also deposited in cytoplasm, indicating breakdown in myelin. α-Synuclein starts to accumulate in the enlarged cell cytoplasm and fibrilizes to form a GCI.

a) phosphorylated α-synuclein-immunoreactive glial cytoplasmic inclusions (GCIs, brown) in white matter oligodendrocytes in pons of 49-year-old woman w/ parkinsonian MSA subtype (MSA-P) for 7 years.



23 MECHANISM FOR CGI IN MSA – PRUNING OF LEWY NEURITES BY OL → Incorporation of α-syn aggregates. Model with the best explanation for locus of α-syn in MSA. Answers to puzzling paradox about MSA?

Graphic of pruning/engulfment of diseased axonal segments ('Lewy neurites') by oligodendrocytes resulting in CGIs w/α-syn. This AM consistent with a basic problem in NDD, namely, that cellular failures in one cell type generates a cascade of compensations in other cell types that also fail. Which INFLAM signals generate this OL behavior? Unknown!



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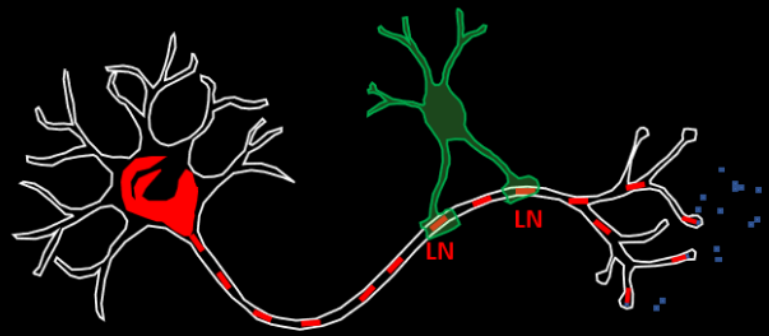

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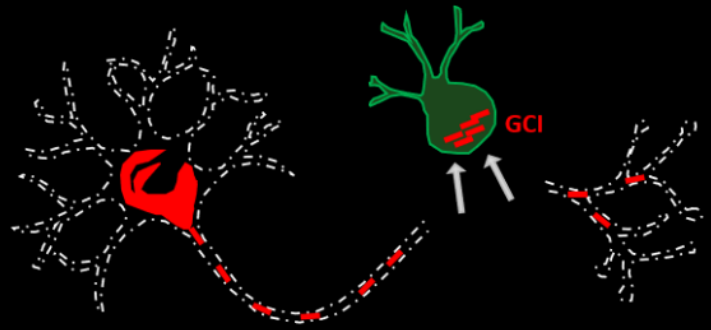


t = 6 months

DeNuccio et al, Biomolecules 2023, 13, 269.

NEUROPATHOLOGICAL MECHANISMS

- ***Like all NDD, neuropathological processes remain partially opaque.*** Pathology outside of neuronal cell body is part of overwhelming evidence that NDD must be modelled in multiple types of CNS cell populations, not just neurons (glial change AD, astrocyte α-syn PD!)
- Classic views are that protein aggregates spread prion-like, seeding progressive spread of folded protein/oligomers/fibrils as central engine. Inhibiting impact of α-syn (in various assembly states?) on autophagy and paradoxical effects of proteases are relatively neglected.
- Several studies have shown that α-syn aggregates can transmit from neuron to neuron (Desplats et al., 2009; Lee et al., 2012b), neuron to astroglial/oligodendroglial cells (Lee et al., 2010; Reyes et al., 2014), oligodendroglial to astroglial cells (Valera et al., 2014), leading to neuronal dysfunction, apoptosis, & neuroinflammation. In contrast to PD, astrocytes themselves do not contain α-synuclein aggregates.
- Interactions between proteinopathy & other factors (↓Fe homeostasis, MITO dysfxn, ↓↓myelination/other trophic support, neuroinflammation) remain to be fully plotted.
- Poor understanding of pathological and neurodegenerative sequence(s) and feedback.

A-SYNUCLEIN STRAINS, OTHER PROTEINS AGGREGATED IN NEURODEGENERATION

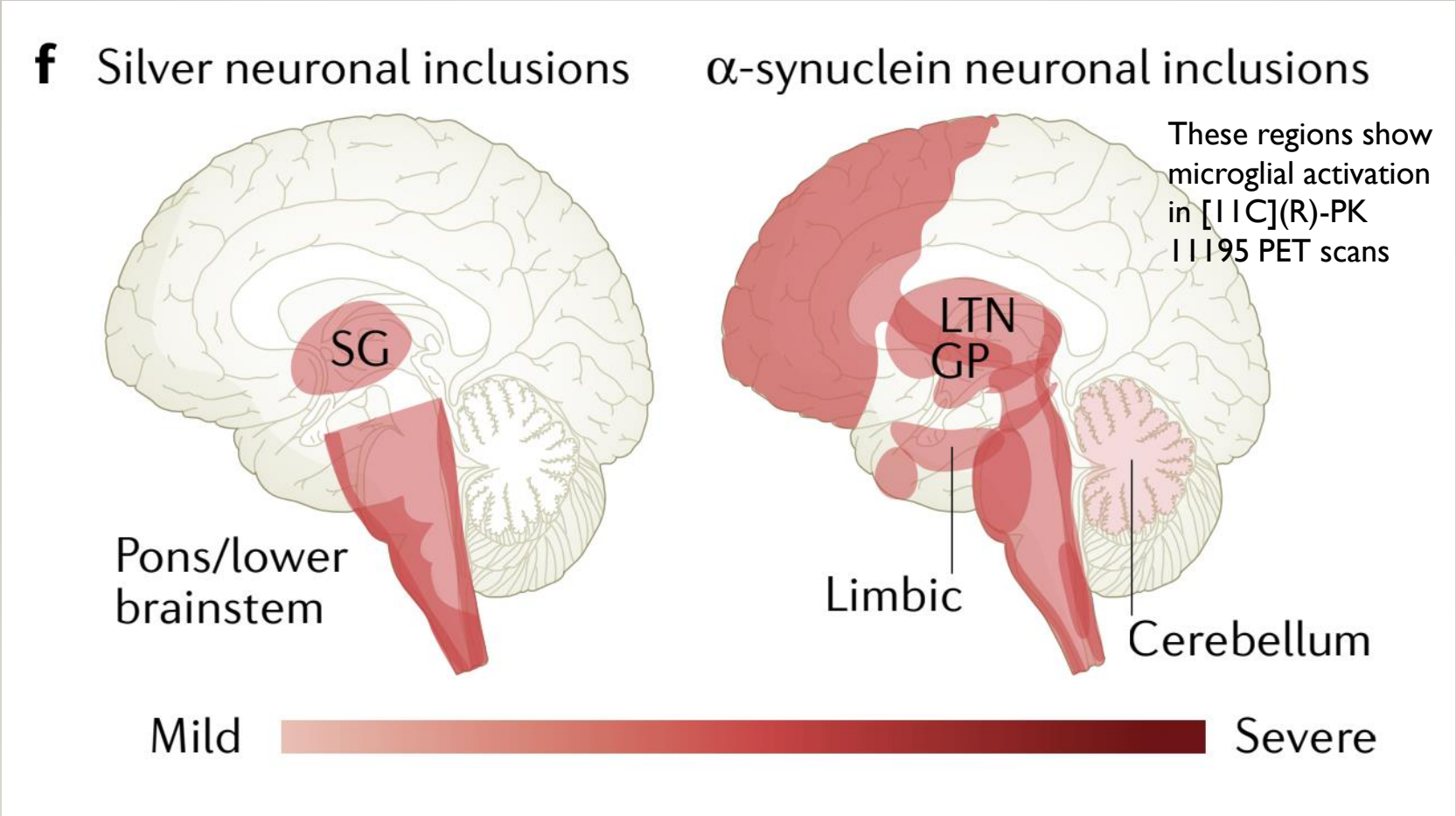
- Disease-specific polymers/fibrils of ***α-syn*** in GCIs of MSA are different from Lewy bodies in PD (Requires high tech protein assays). These different 'strains' of ***α-syn*** correlate with somewhat different locations for ***α-syn*** proteinopathy across these different NDD. Why locations/strains vary across synucleinopathies is mysterious.
- ***Mechanisms leading to aggregates of α-syn in oligodendrocytes are uncertain***. Although OL may be where most of pathologically aggregated ***α-syn*** ends up, strong evidence that neurons are likely primary source. ***OL may phagocytose/prune axons with α-syn-loaded dystrophic neurites →α-syn in GCIs***.
- Changes in MBP and p25α (another oligodendroglial protein) processing occur early in MSA, along with disordering of its removal via exosomes. P25a appears to combine with ***α-syn*** to form pathological aggregates. Role for iron dys-homeostasis?
- The mislocation of a protein p25a into cytoplasm may be factor in aggregation of ***α-syn***, which is then dumped into extracellular space, as a prion-like seed (tau in AD?). This may be taken up by neurons, then becoming dystrophic or Lewy-like neurites.

PATTERNS IN NEUROPATHOLOGY WITH STAGING BETWEEN MSA-P AND MSA-C

- Three grades of severity across both types of movement disorders.
- MSA-P
  - Degeneration beginning with 1) substantia nigra ('minimal change MSA'), then progressing to 2) involvement of putamen, then 3) caudate & GP.
- MSA-C
  - Degeneration beginning with 1) mild loss of Purkinje cells with 'myelin pallor' in cerebellum (often w/degeneration in SN), progressing to 2) degeneration in pons and inferior olive, progressing to 3) atrophic change in cerebellar vermis & hemispheres.
- *Neurofilament light chain correlates w/clinical disease severity*, progression and prognosis in MSA. Combined with clinical and imaging analysis, NFL can inform patient stratification, and could be reliable biomarker of treatment response in future MSA trials of putative disease-modifying agents (Chelban et al, 2022, *Brain*).

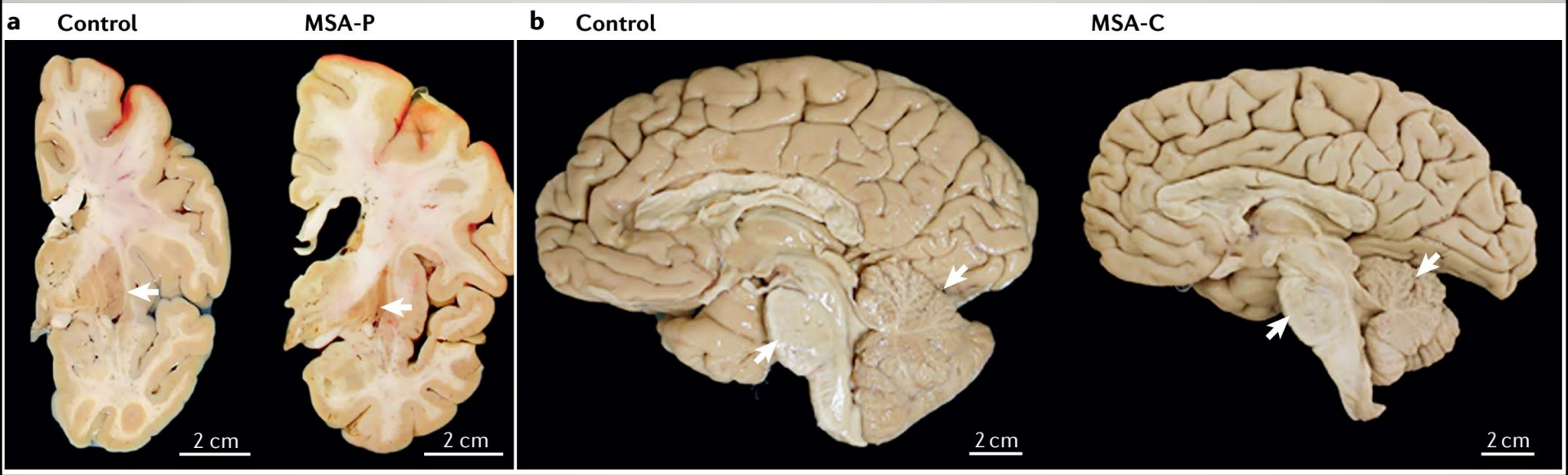
Clinical Subtype	Main Motor Feature	Pathological Distribution
MSA-P	Parkinsonism	Nigrostriatal atrophy
MSA-C	Limb and gait ataxia	Olivopontocerebellar atrophy
Mixed subtype	Both motor features	Combined atrophy
MSA-autonomic failure	No motor features	Central autonomic brain regions
MSA-dementia	No motor features	Frontotemporal plus combined atrophy

27 TWO VISUALIZATIONS OF PATHOLOGY



Distribution of silver-positive ***fibrillar*** neuronal inclusions (***f left***) mainly in basal ganglia, pons & lower brainstem, where neuronal cell loss is severe. By contrast, distribution of α-synuclein-immunoreactive neuronal inclusions is more extensive (neocortex, limbic cortices & cerebellum) w/these additional regions w/out major neuronal loss. GP, globus pallidus; LTN, lateral thalamic nuclei, SG, supragenual nucleus (neurons embedded in the pontine central gray) from Poewe et al,

28 REGIONAL ATROPHY IN PUTAMEN (MSA-P) & PONTINE-CEREBELLAR REGIONS IN MSA-C

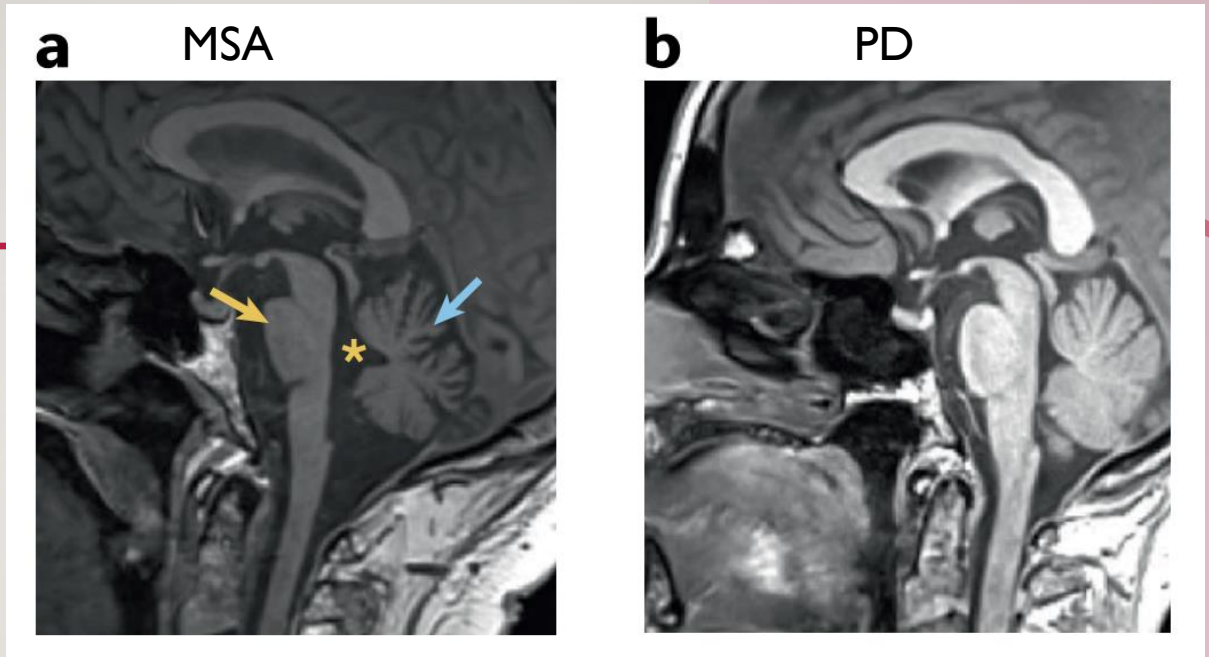


a Coronal brain sections through posterior putamen (arrowhead) of a 79-year-old asymptomatic man (control) and a 75-year-old woman who had had parkinsonian MSA subtype (MSA-P) for 7 years. Note the obvious atrophy & discoloration in posterior putamen (arrowhead) in patient with MSA-P.

b | Midsagittal brain images in the 79-year-old asymptomatic man (control) and a 74-year-old woman who had had cerebellar MSA subtype (MSA-C) for 7 years. Note the obvious atrophy in the pons and cerebellum (arrowheads) in the patient with MSA-C.

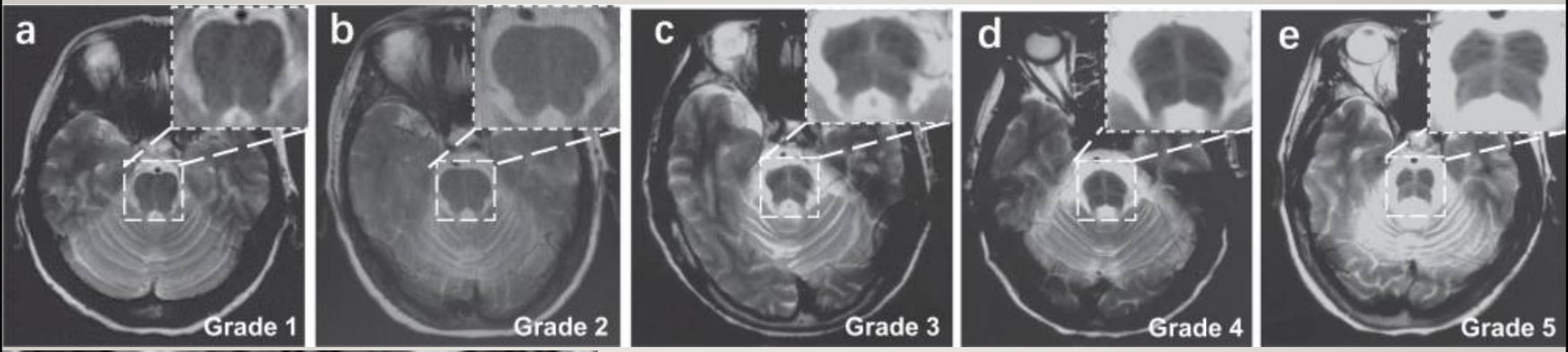
NEUROIMAGING IN MSA

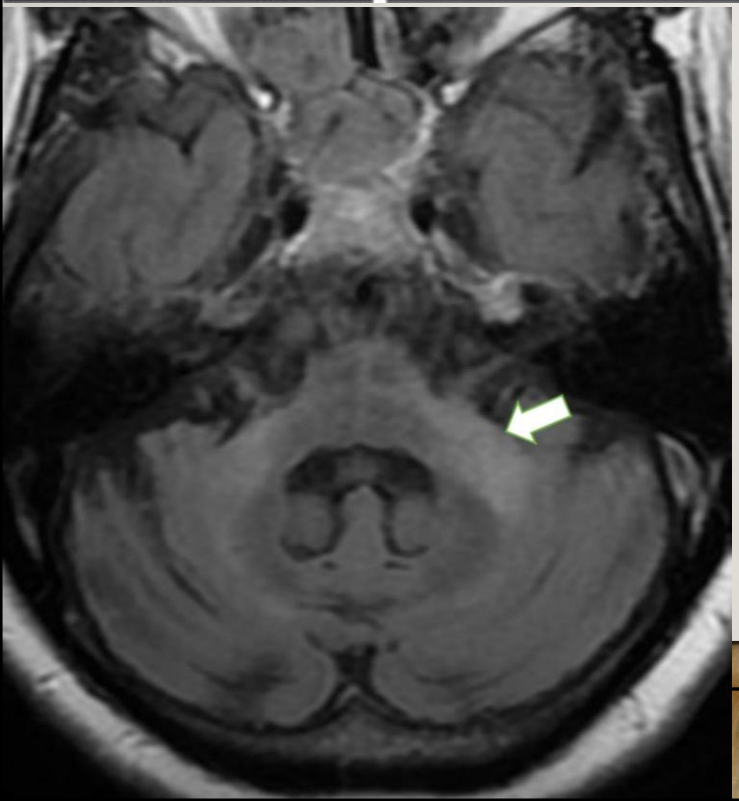
Neuroimaging: widespread white matter microstructure abnormalities w increased mean diffusivity in BG, olivoponto-cerebellar system, pyramidal tract & cortical regions. Also putaminal atrophy and/or hypointensity on T2-weighted sequences, atrophy of infra-tentorial structures (pons, mid cerebellar peduncle (MCP), medulla oblongata, cerebellum)



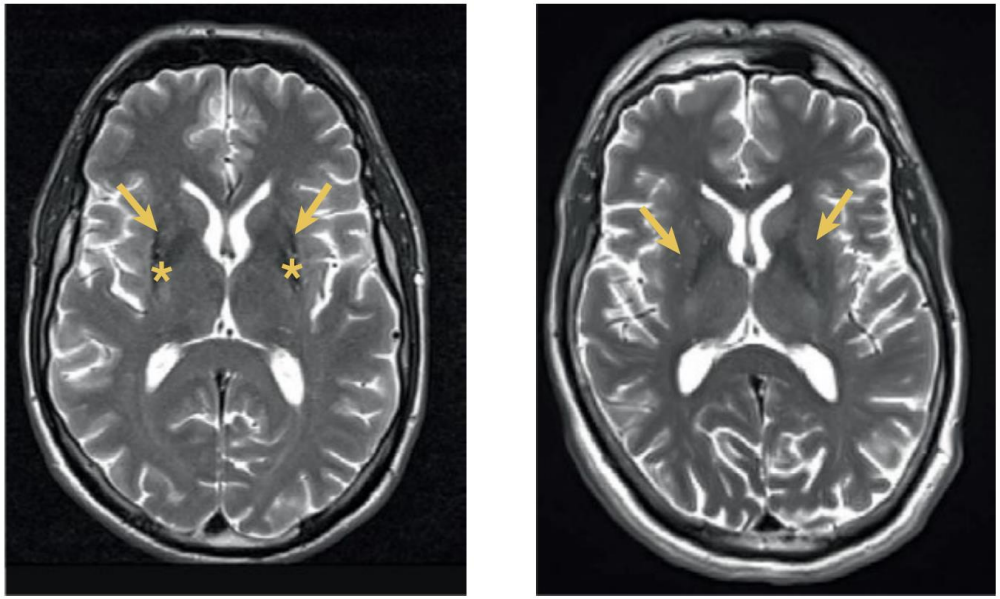
a) showing pontine (yellow arrow) and cerebellar atrophy with enlarged fissures and interfolial spaces of the cerebellum (blue arrow) and dilation of the fourth ventricle (yellow asterisk), compared with **b)** patient with Parkinson disease (PD) (part b) w/out 'infratentorial atrophy

NEUROIMAGING FINDINGS IN MSA-C – "THE HOT CROSSED BUN SIGN"



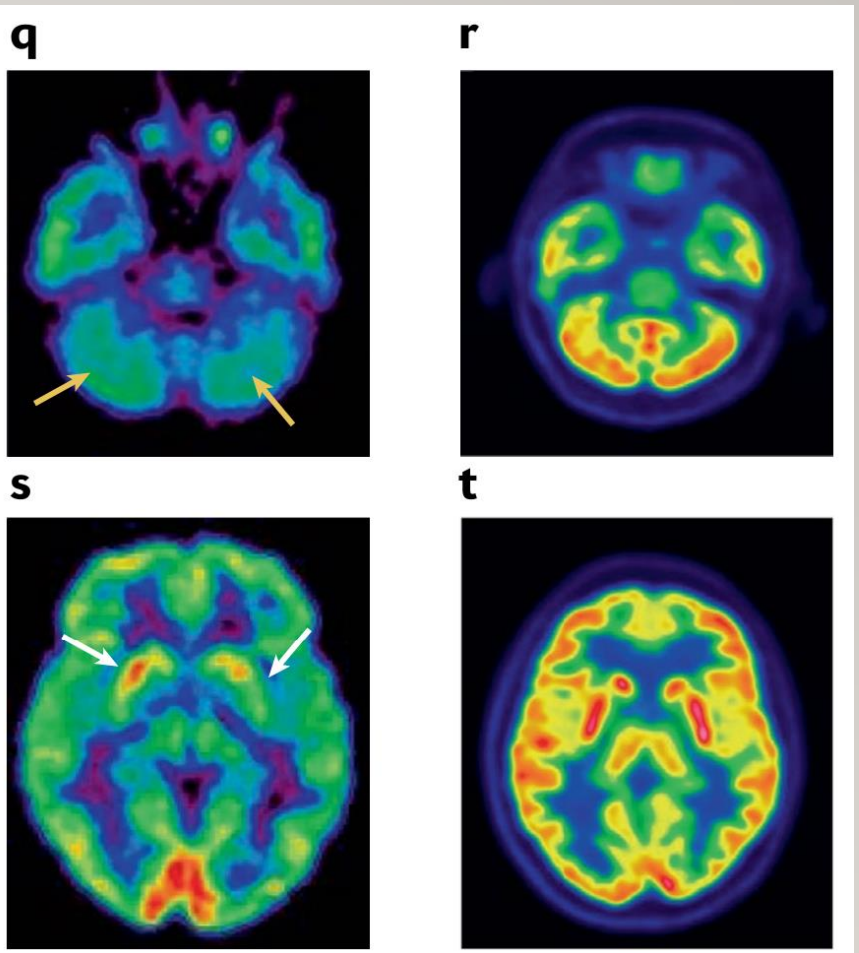


Severity of HCBs have linear correlation with SARA scores (ataxia scale) in MSA-C. Statistically, an increase in HCBs grade predicts an increased likelihood of disability/disease severity in MSA for those with MSA-C subtype. ***HCB grade correlates w/atrophy of pons and MCP Middle Cerebellar Ped***. SCA 2, SCA 3, SCA 7, SCA 8 also show HCB sign. Hyperintensity in T2 in MCP is seen in fragile X tremor/ataxia syndrome, Wilson's disease, liver cirrhosis, MS, stroke, etc. SCA 1/2/3/6/17, dentatorubral-pallidoluysian atrophy (CAG repeat DO) was confirmed in 22 of 302 (***7.3%***) of clinically diagnosed MSA cases. ***Consider MSA <u>and</u> SCAs when patients show a combination of an HCB sign and T2 hyperintense MCP.***



T2-weighted MR images **(g, h)** in MSA pt **(g)** showing bilateral putamen atrophy (yellow arrows)/hypointensity (yellow asterisk), and in a control patient with PD **(h)** w/normal-appearing putamen (yellow arrow).

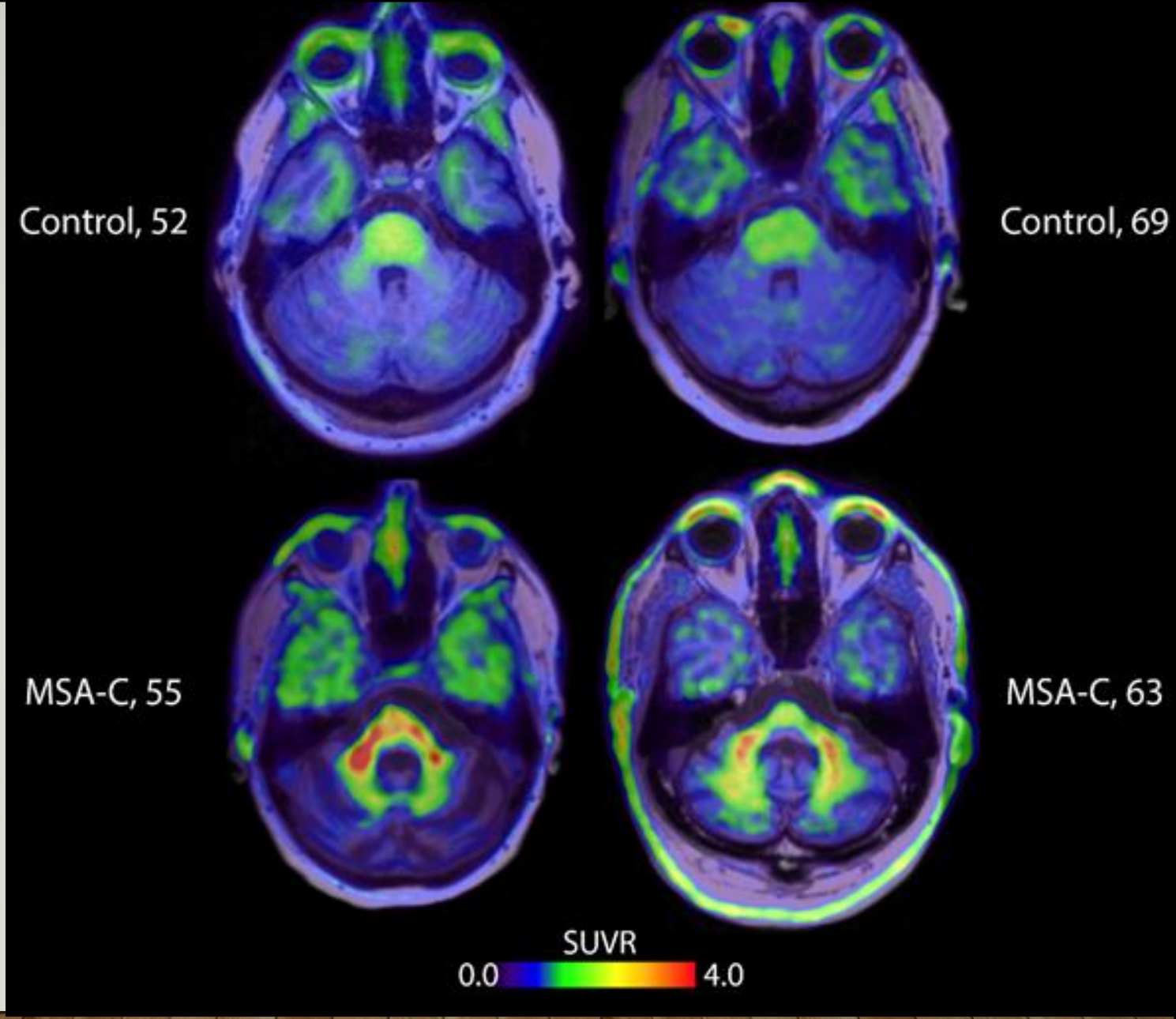
Computerized volumetric T1-weighted MRI has shown MSA-specific patterns of volume loss involving putamen, cerebellum & MCP offering high diagnostic accuracy versus PD or PSP. Major aide to differential diagnosis. DTI MRI shows putamen/MCP changes in MSA-P/C.



18F-FDG PET in MSA pt (**q**/**s**) shows cerebellar (**q**, yellow arrows) & putamen (**s**, white arrows) hypometabolism, w/control (**r** & **t**) showing normal cerebellar (r) & putaminal (t) uptake (Poewe et al, 2022).

PET ligand ACI-12589

distinguished MSA from other a-synucleinopathies & healthy age matched volunteers



NEURODEGENERATION: CORRELATES, DRIVERS? LINEARITY VS. RECURSION? (42M)

Animal models only partially duplicate human clinical disease or require artificial manipulations of genes.

Like all other NDD, 'hard proteinopathy' models dominate but have failed badly as heuristics for treatment in other NDD (esp. AD but also PD and FTD). Why?

'Hard' versus 'soft' proteinopathy arguments? Linear dominoes vs. loops/feedback? ***We must humbly confess that neurodegeneration is still in many ways mysterious***. Many players.

MSA, like all other NDD, appears to be staged, with a relatively silent preclinical phase characterized by GCIs involving several other proteins, then α-syn.

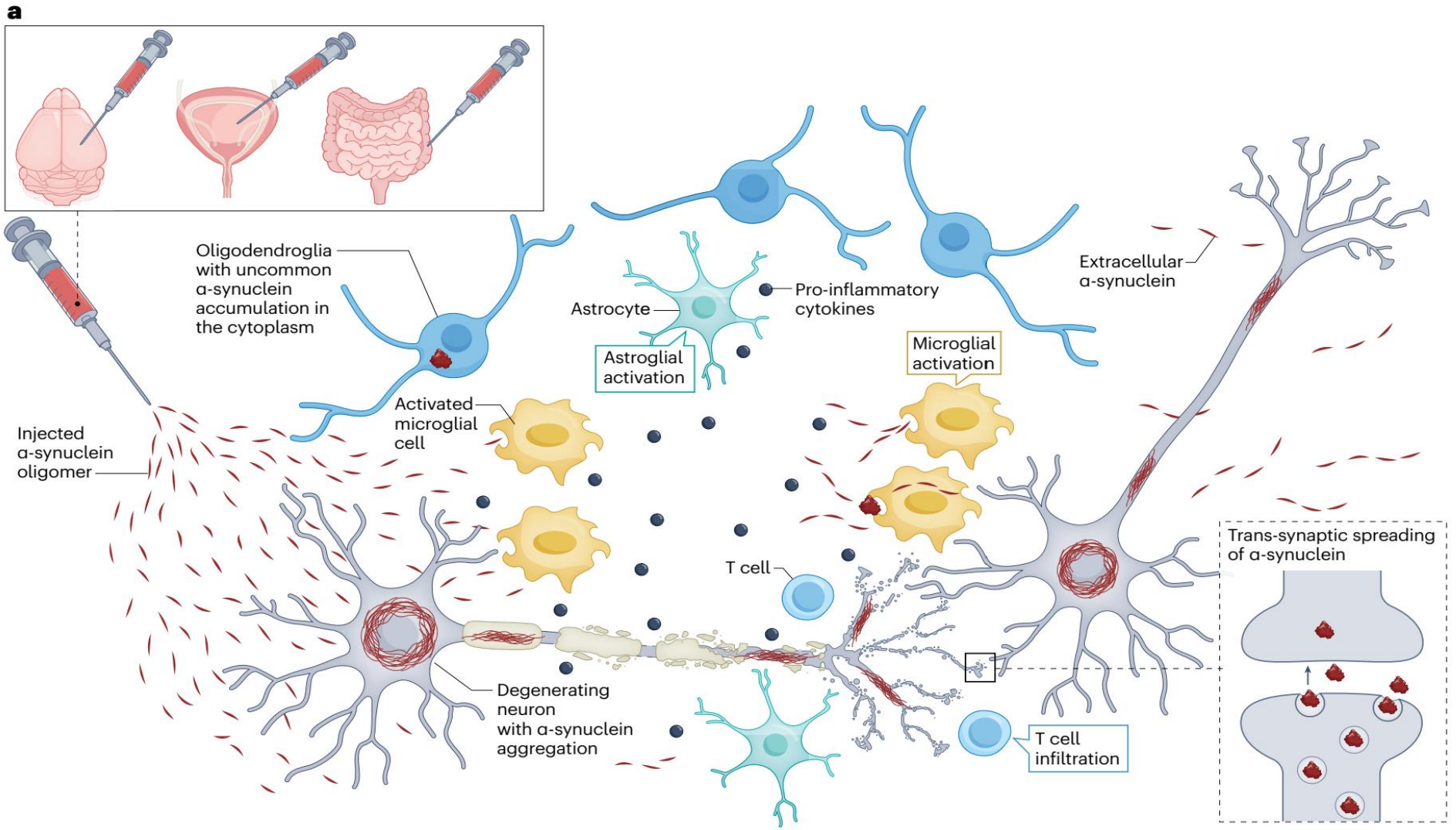
But what causes or starts the OL proteinopathy? Still mysterious. *A pre-preclinical stage*?

Failure of proteostasis is complex, and overabundance of toxic forms hides question of shortages in normal protein 'moieties'. Also, all deposition proteins appear challenging for brain autophagy systems as possible common denominator?

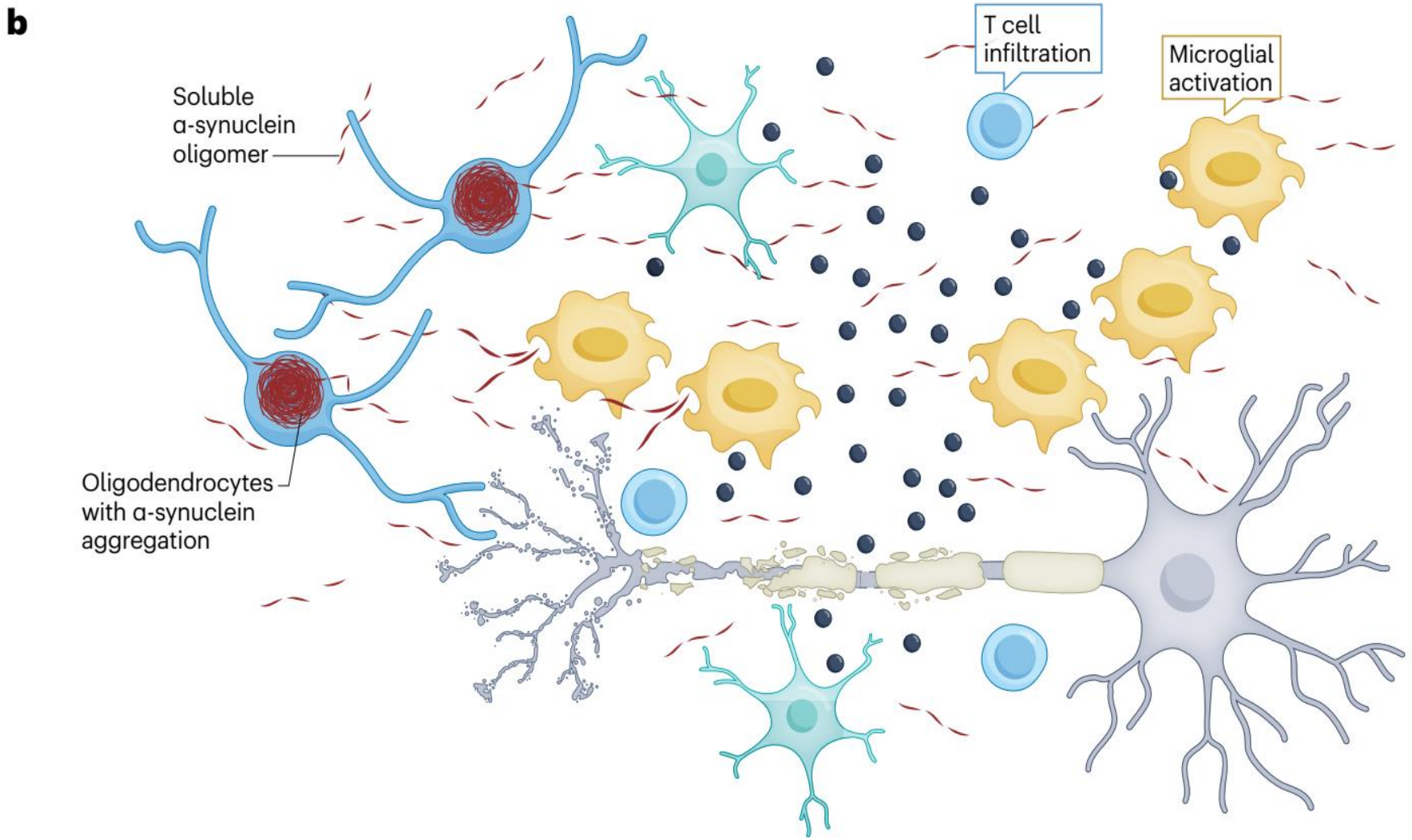
34 MORE MYSTERIES/CLUES @ NEURODEGENERATION

- Transcriptome studies: differential overexpression of SNCA as well as genes in neuroinflammation, autophagy & mitochondrial pathways. ***Classic triad in NDD***.
- The mRNA level of SNCA (α-synuclein) in adult mouse cortex is **12:5:1** for ***neurons:OPCs:oligodendrocytes***, vs. ratio of **3:1** for ***neurons:oligodendrocytes*** in adult human C, suggesting α-syn upregulation in OPC early in development.
- α-synuclein aggregates from CNS of patients w/MSA significantly more potent in rat inoculation models in seeding neuronal ***(but not oligodendrocyte!)*** α-syn pathology, w/spreading of pathology & neuroinflammation, than using α-synuclein aggregates from CNS of PD pts.
- ***Mysterious in 2 ways: 1) why are MSA proteins more prion-like than PD proteins? ; 2) why do these not re-create the OL locus of pathology?***
- Suggests need for an OL-centric model of proteinopathy. GCI not seen in OPCs.

Propagation animal models of MSA: Local injection (CNS, GI tract, bladder) of fibrils of recombinant or patient-derived α-synuclein: ***prominent neuronal α-syn aggregates***, which propagate (predominantly trans-synaptically) to other neurons. ***But α-synuclein aggregates in oligodendrocytes are rare***. Gliosis and neuroinflammatory responses accompany and may advance neurodegeneration, despite glial phagocytosis of α-syn. Similar to PD/AD.

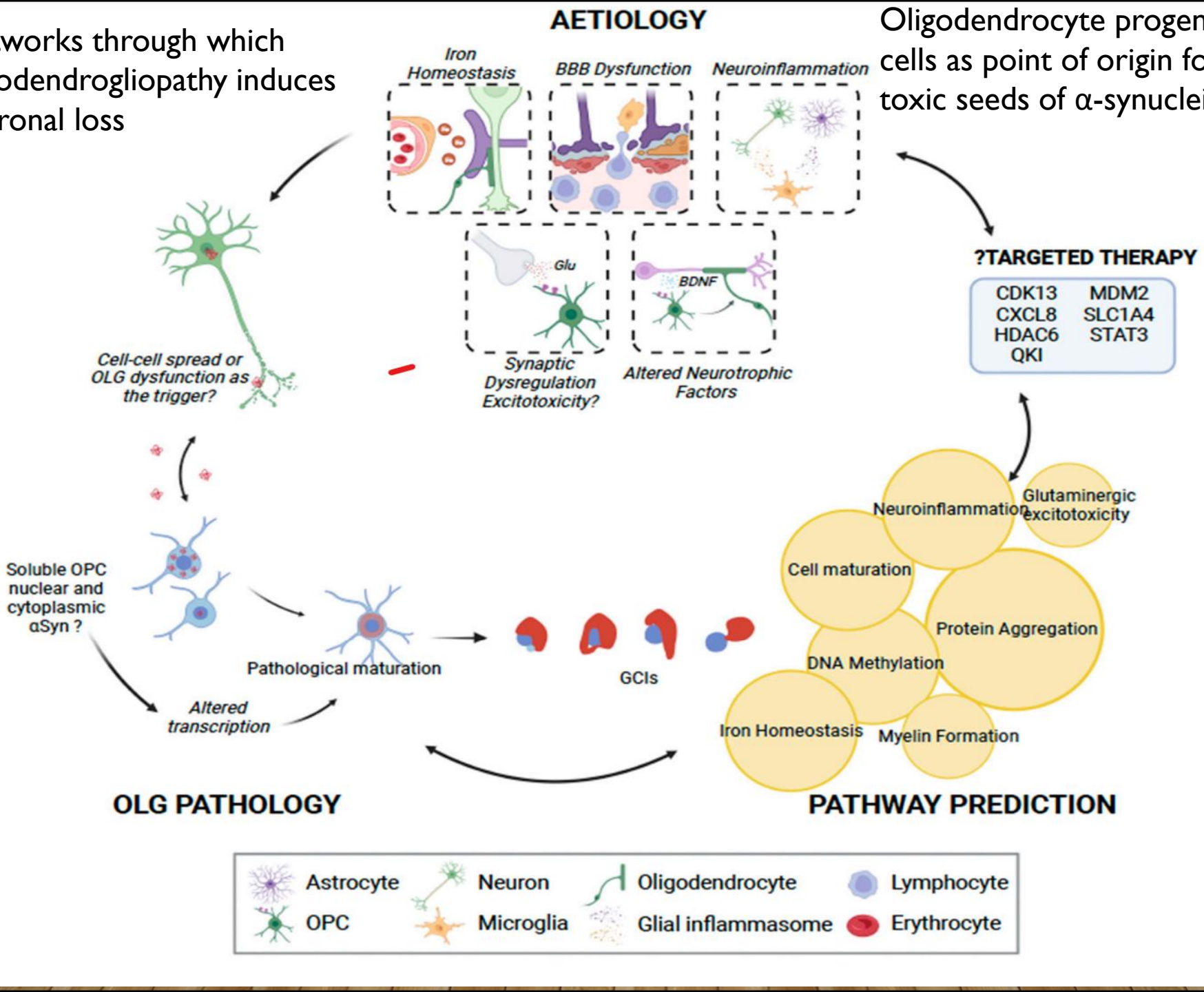


Animal models with targeted OL overexpression of α-syn in OL: show glial cytoplasmic inclusion-like α-synuclein aggregates. GCI pathology w/spreading of soluble α-syn oligomers → ↑neuroinflammation with activation of microglia/astroglia as well as oligodendroglial dysfunction. This leads to progressive regional neurodegeneration, but none of the neuron-to-oligodendroglia spreading of α-syn believed to take place in human MSA in seen in these models.



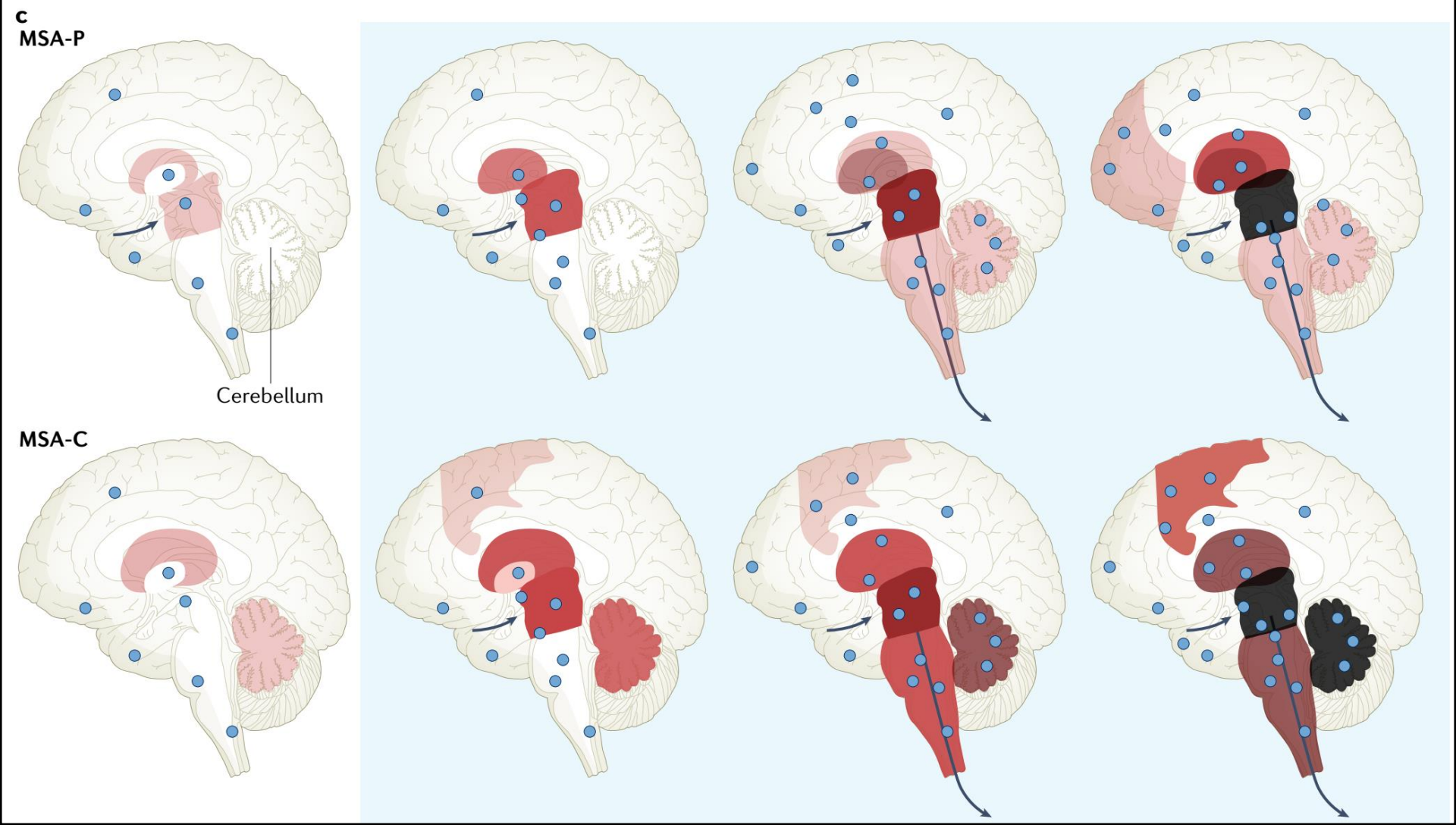
Networks through which oligodendrogliopathy induces neuronal loss

Oligodendrocyte progenitor cells as point of origin for toxic seeds of α-synuclein?



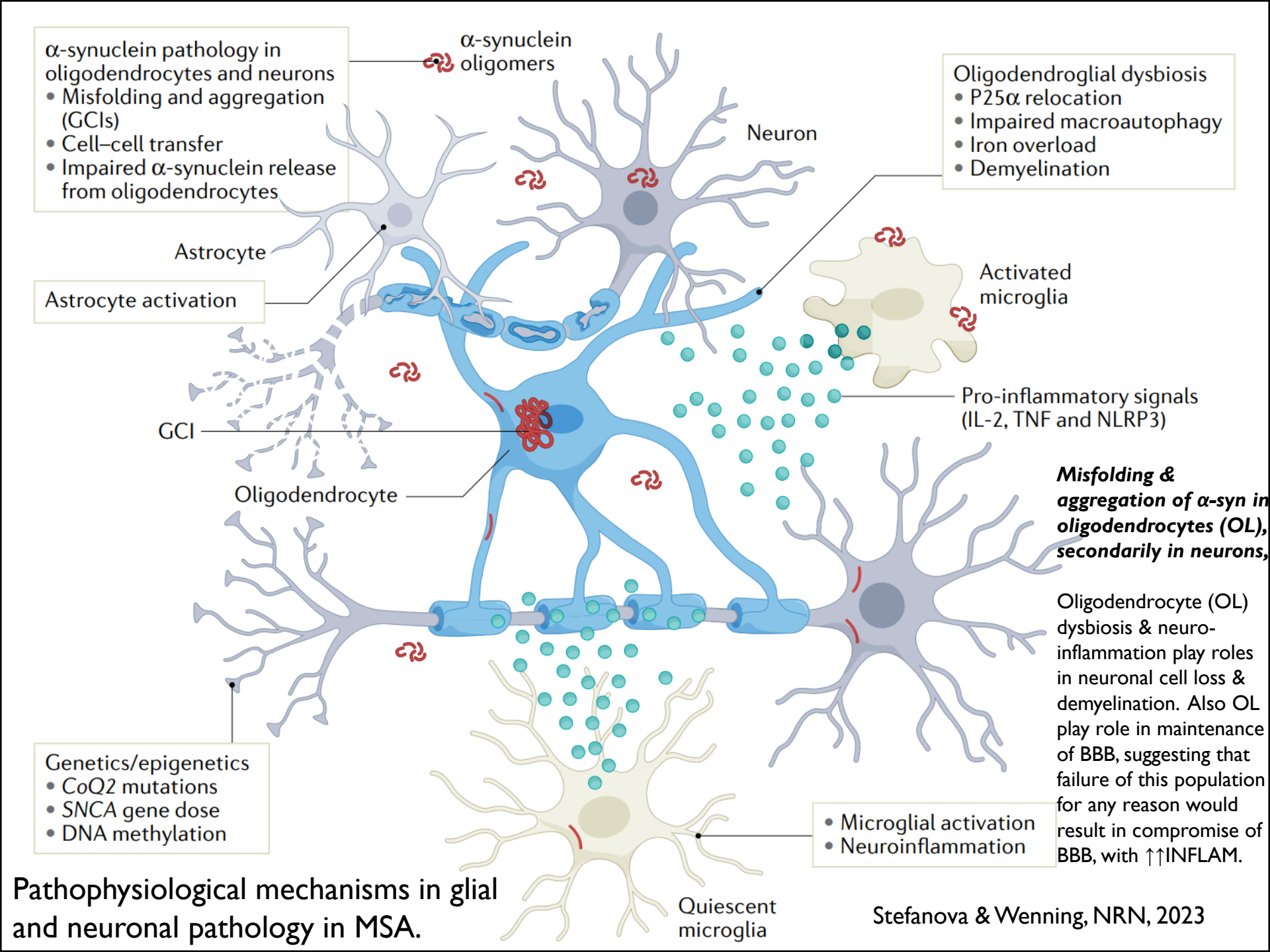
38 Progression (black arrows) of glial cytoplasmic inclusion pathology (blue circles) through the brain in MSA-P & MSA-C.

In MSA-P, pathology is concentrated in the midbrain and basal ganglia whereas in MSA-C, pathology is concentrated in the midbrain, cerebellum, brainstem and then the basal ganglia, with greater infiltration into the motor cortices with time.



MYSTERIES OF/CLUES @ NEURODEGENERATION

- Iron homeostasis disturbed, w/critical role for oligodendrocytes. Fe crucial for myelination in development, in iron-enriched brain regions such as striatum, SN, cerebellar nuclei – all vulnerable in MSA, showing ↑↑ ferritin and Fe deposition. May play role in disordering/aggregating proteins at earliest stages of ND.
- Like in all other NDD, autophagy failing, and is altered in OPCs exposed to α-syn aggregates, and in iPSC-derived DA neurons from MSA patients. Similar to what is seen in AD/PD, pilling up of non-degraded autophagosomes in OLDC.
- Increasing expression of transcription factor EB (master regulator of ATG) in oligodendrocytes showed neuroprotection in transgenic MSA mouse model (from overexpression of human wild-type α-synuclein in oligodendrocytes).
- Abundant OPCs also seen in other demyelinating diseases (MS), average onset between 20 and 40 years, when proliferative potential of OPCs is greater, may therefore suggest a key role for OPCs initiating pathology. Are OPCs dysfunctional? But α-synuclein aggregation not yet observed in OPCs, so no basis yet?



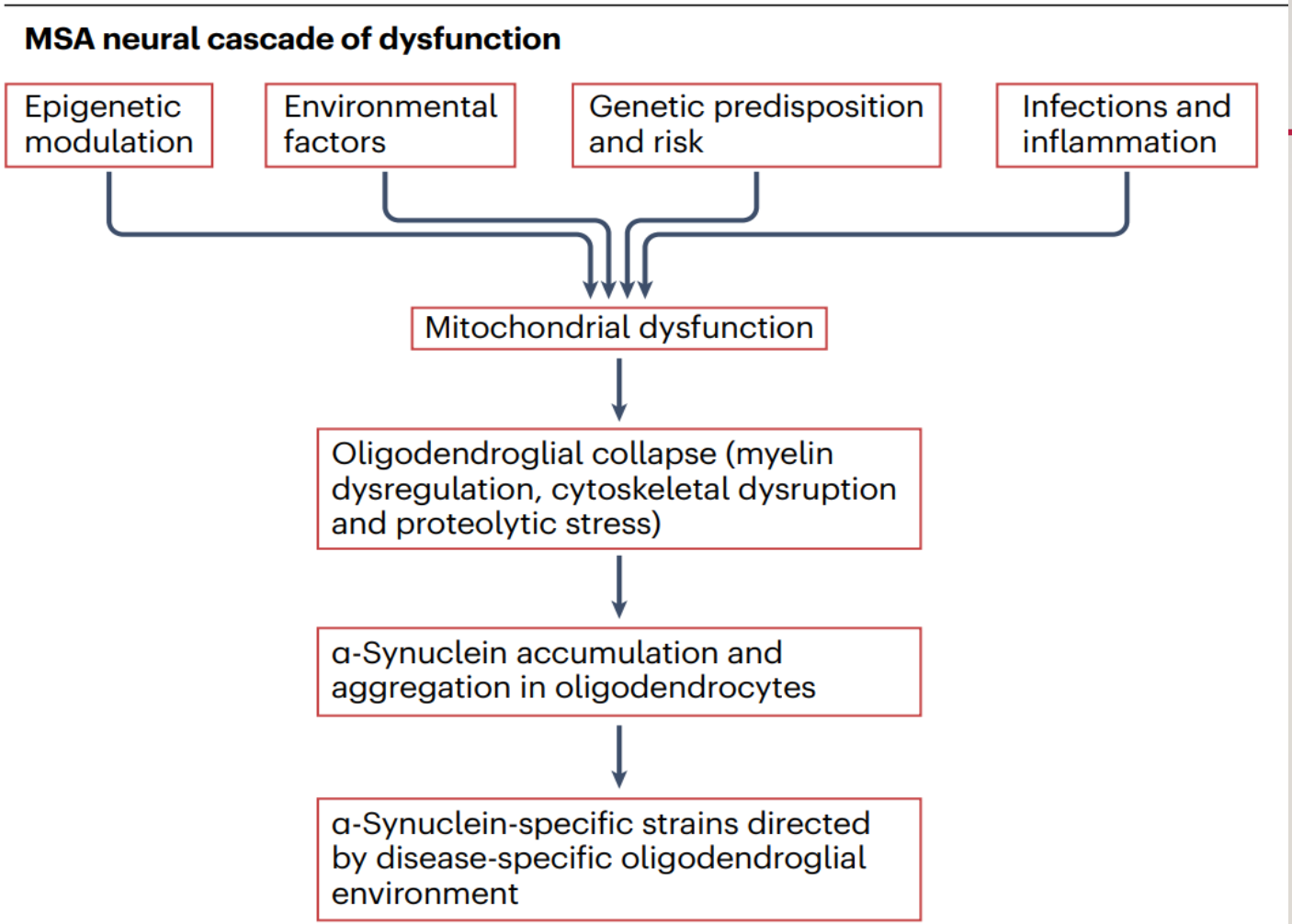
Misfolding & aggregation of α-syn in oligodendrocytes (OL), secondarily in neurons,

Oligodendrocyte (OL) dysbiosis & neuro-inflammation play roles in neuronal cell loss & demyelination. Also OL play role in maintenance of BBB, suggesting that failure of this population for any reason would result in compromise of BBB, with ↑↑INFLAM.

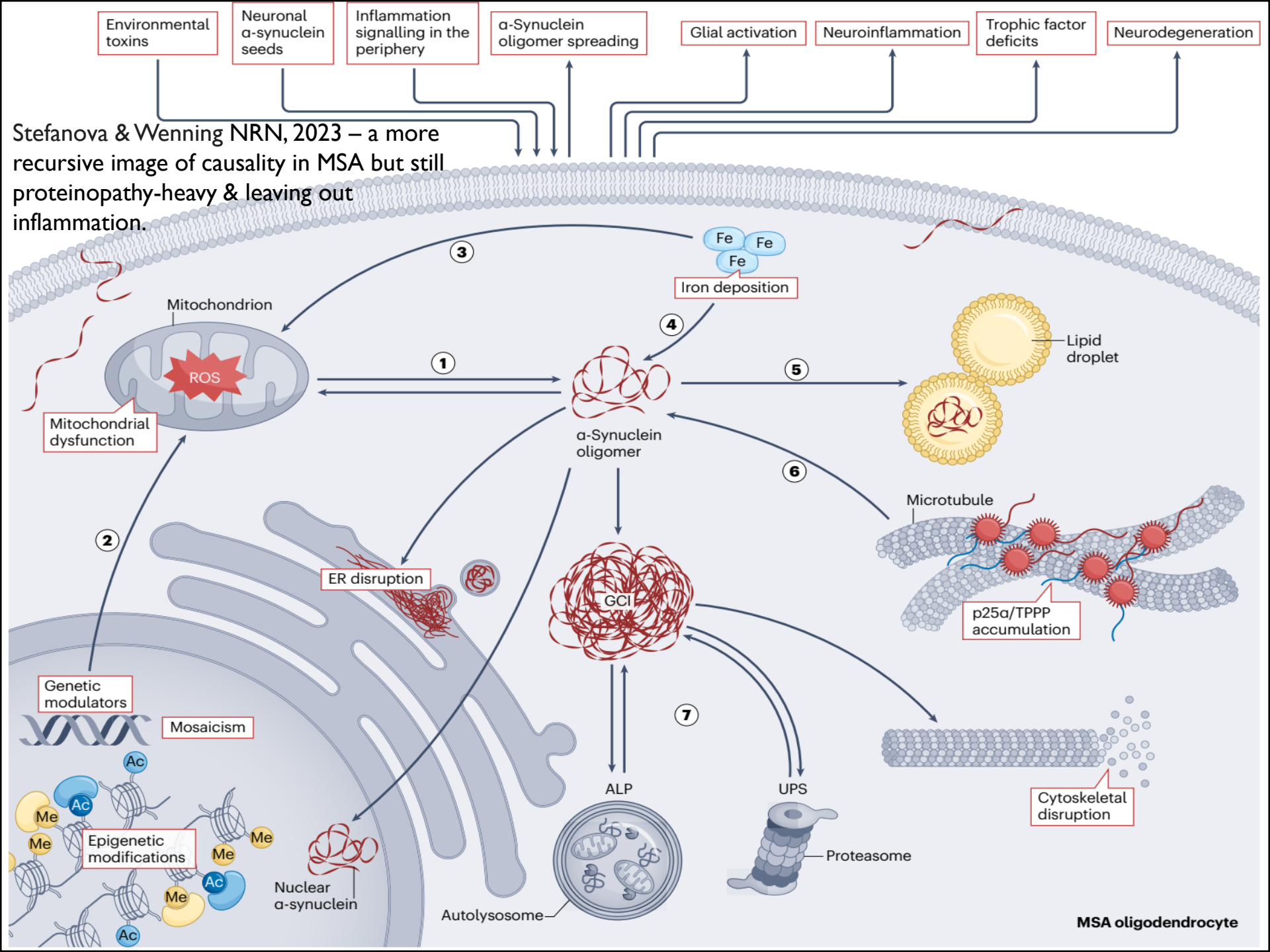
Pathophysiological mechanisms in glial and neuronal pathology in MSA.

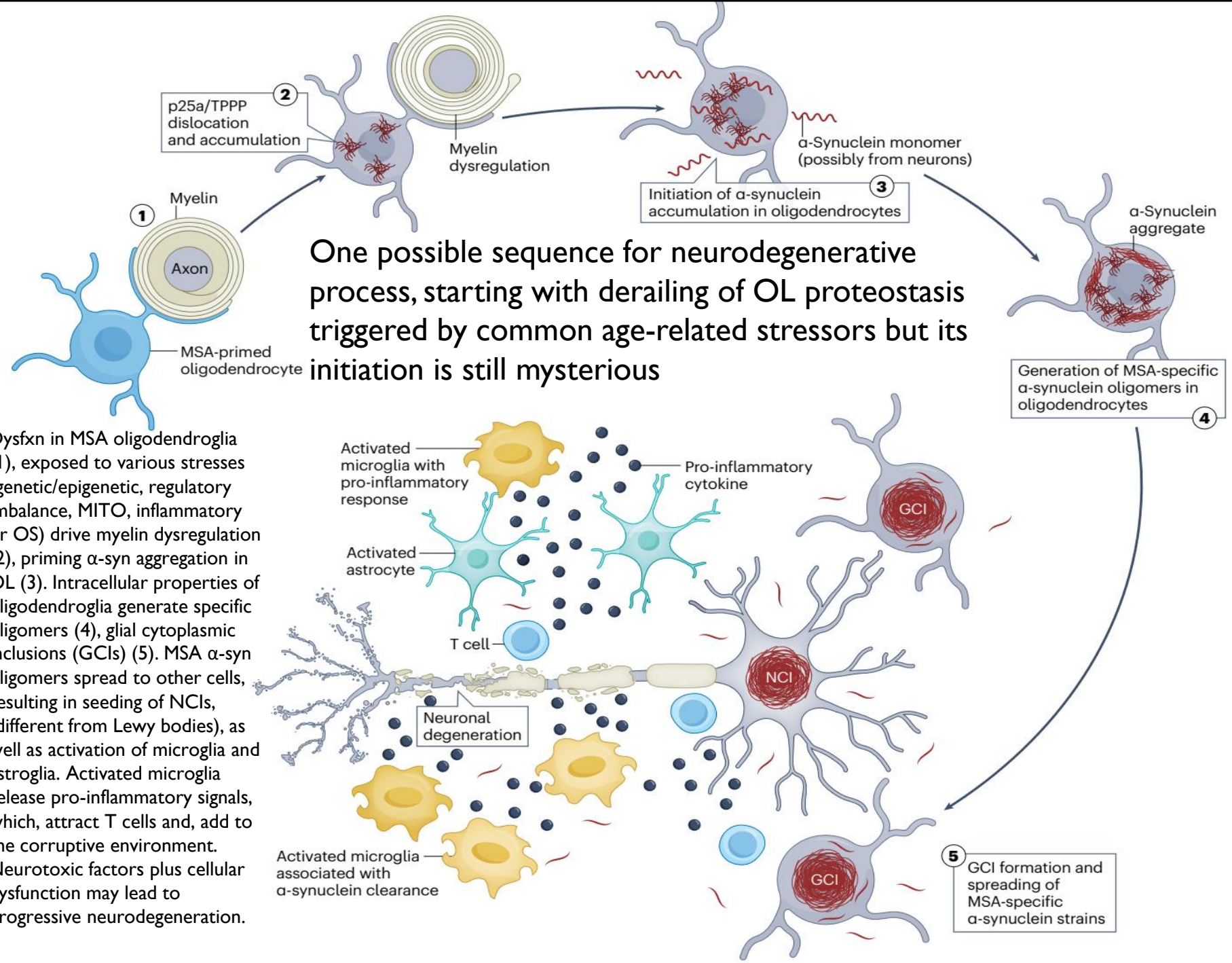
Stefanova & Wenning, NRN, 2023

EXAMPLE OF PUTATIVE NEURODEGENERATIVE CASCADE. BUT MANY OTHER POSSIBLE CASCADES/RELATIONSHIPS BETWEEN CELLULAR FACTORS CAN'T BE EXCLUDED



Stefanova & Wenning, 2023. Not clear that there is strong data showing that MITO dysfxn is primary. Clearly sequential organization in MSA neurodegeneration is not unequivocally established. Dominoes are appealing model for NDD (esp. popular in AD) but biology is recursive and not linear.

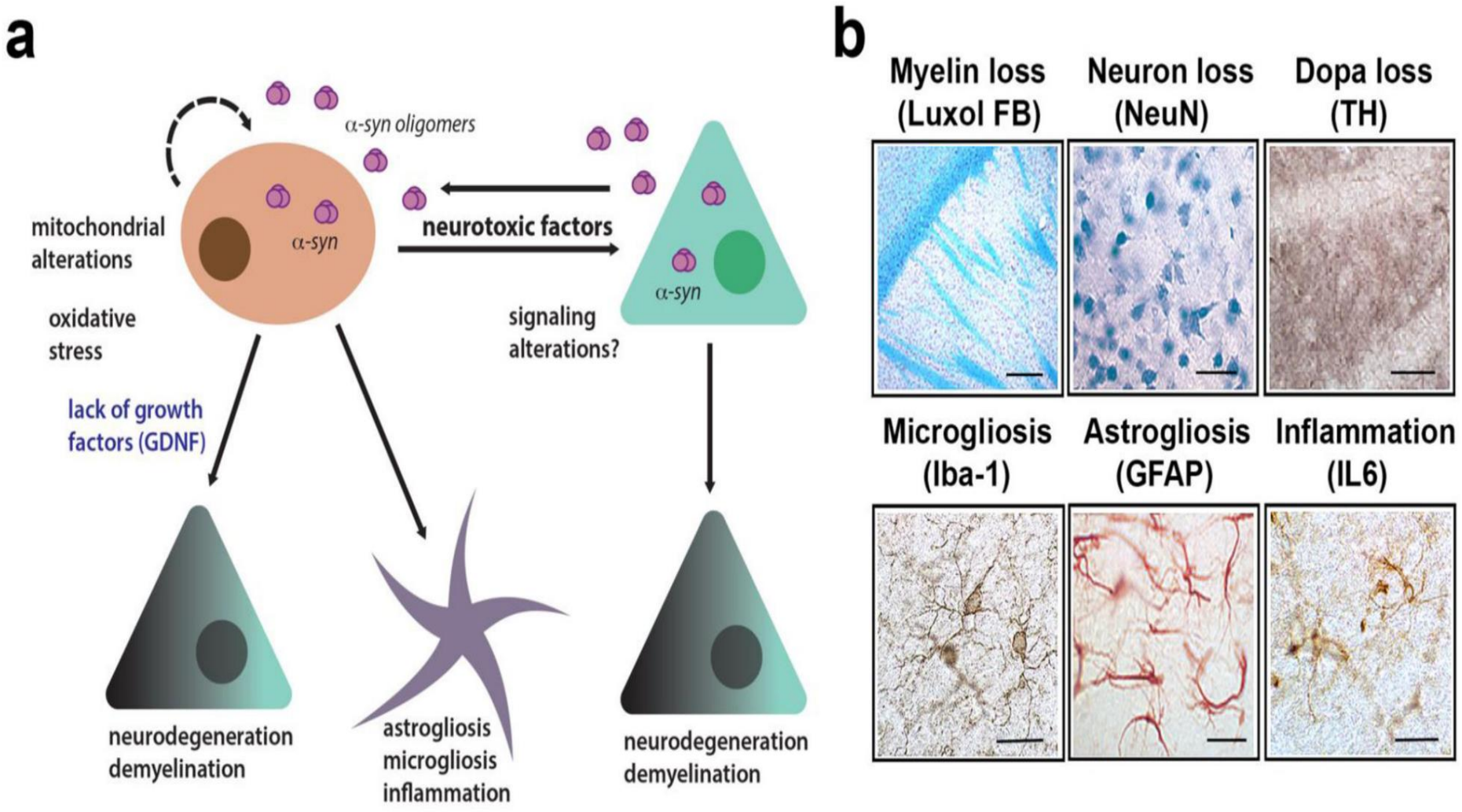

Environmental toxins
Neuronal α-synuclein seeds
Inflammation signalling in the periphery
α-Synuclein oligomer spreading
Glial activation
Neuroinflammation
Trophic factor deficits
Neurodegeneration
Stefanova & Wenning NRN, 2023 – a more recursive image of causality in MSA but still proteinopathy-heavy & leaving out inflammation.
Fe
Fe
Fe
Iron deposition
Mitochondrion
ROS
Mitochondrial dysfunction
α-Synuclein oligomer
Lipid droplet
Microtubule
p25α/TPPP accumulation
ER disruption
GCI
Genetic modulators
Mosaicism
Epigenetic modifications
Ac
Me
Nuclear α-synuclein
Autolysosome
ALP
UPS
Proteasome
Cytoskeletal disruption
MSA oligodendrocyte



One possible sequence for neurodegenerative process, starting with derailing of OL proteostasis triggered by common age-related stressors but its initiation is still mysterious

Dysfxn in MSA oligodendroglia (1), exposed to various stresses (genetic/epigenetic, regulatory imbalance, MITO, inflammatory or OS) drive myelin dysregulation (2), priming α-syn aggregation in OL (3). Intracellular properties of oligodendroglia generate specific oligomers (4), glial cytoplasmic inclusions (GCIs) (5). MSA α-syn oligomers spread to other cells, resulting in seeding of NCIs, (different from Lewy bodies), as well as activation of microglia and astroglia. Activated microglia release pro-inflammatory signals, which, attract T cells and, add to the corruptive environment. Neurotoxic factors plus cellular dysfunction may lead to progressive neurodegeneration.

ANOTHER GRAPHIC OF PUTATIVE MULTIPLE RELATIONSHIPS IN NEURODEGENERATION



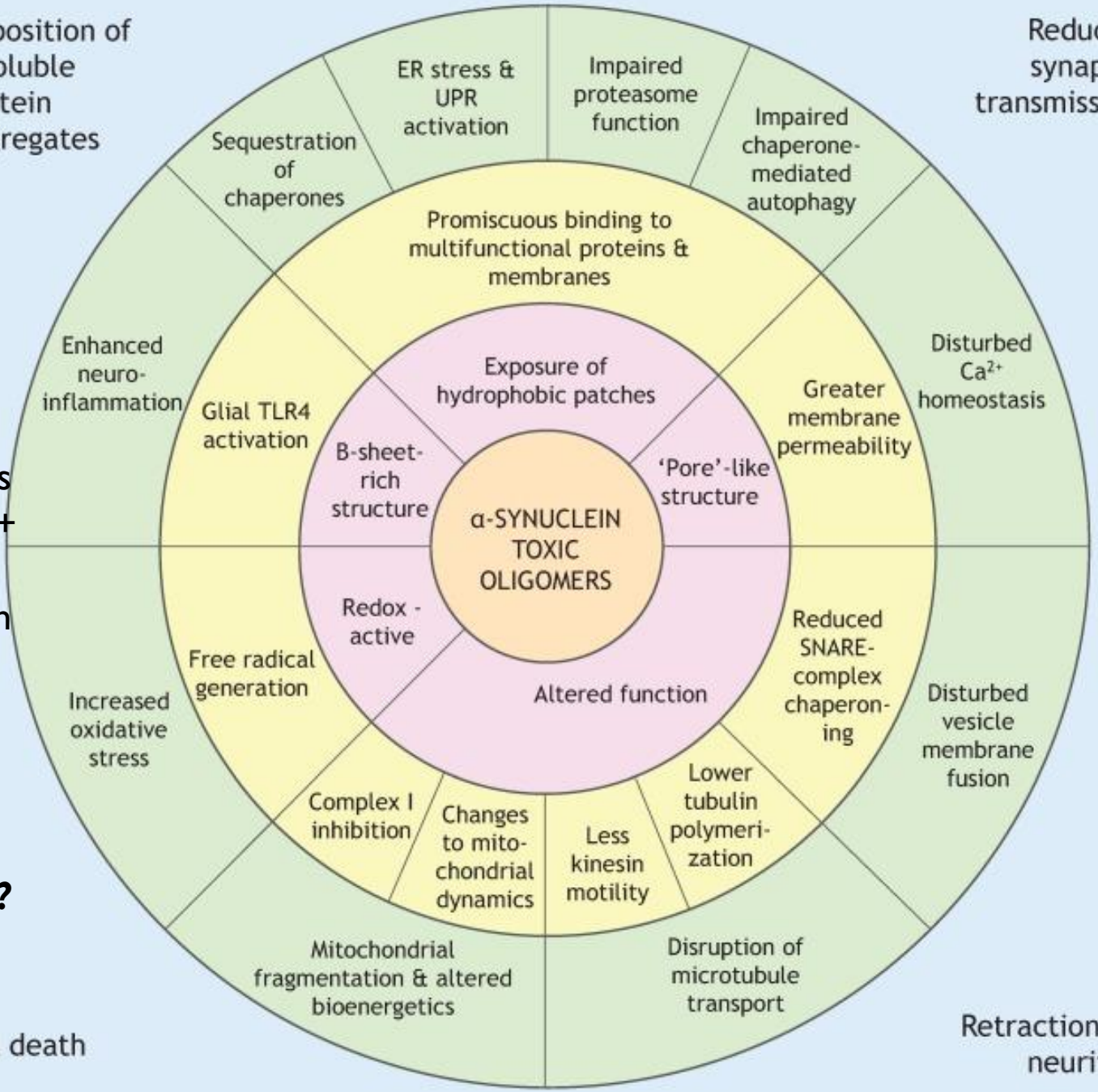
Facets of α-syn proteinopathy:

1) innate immune activation, w/oxidative stress 2) impaired autophagy, 3) ER stress →UPR failure, 4) Ca++ homeostasis, MITO & mitophagy, synaptic fxn are all disturbed.

Complex footprint driving cellular failure.

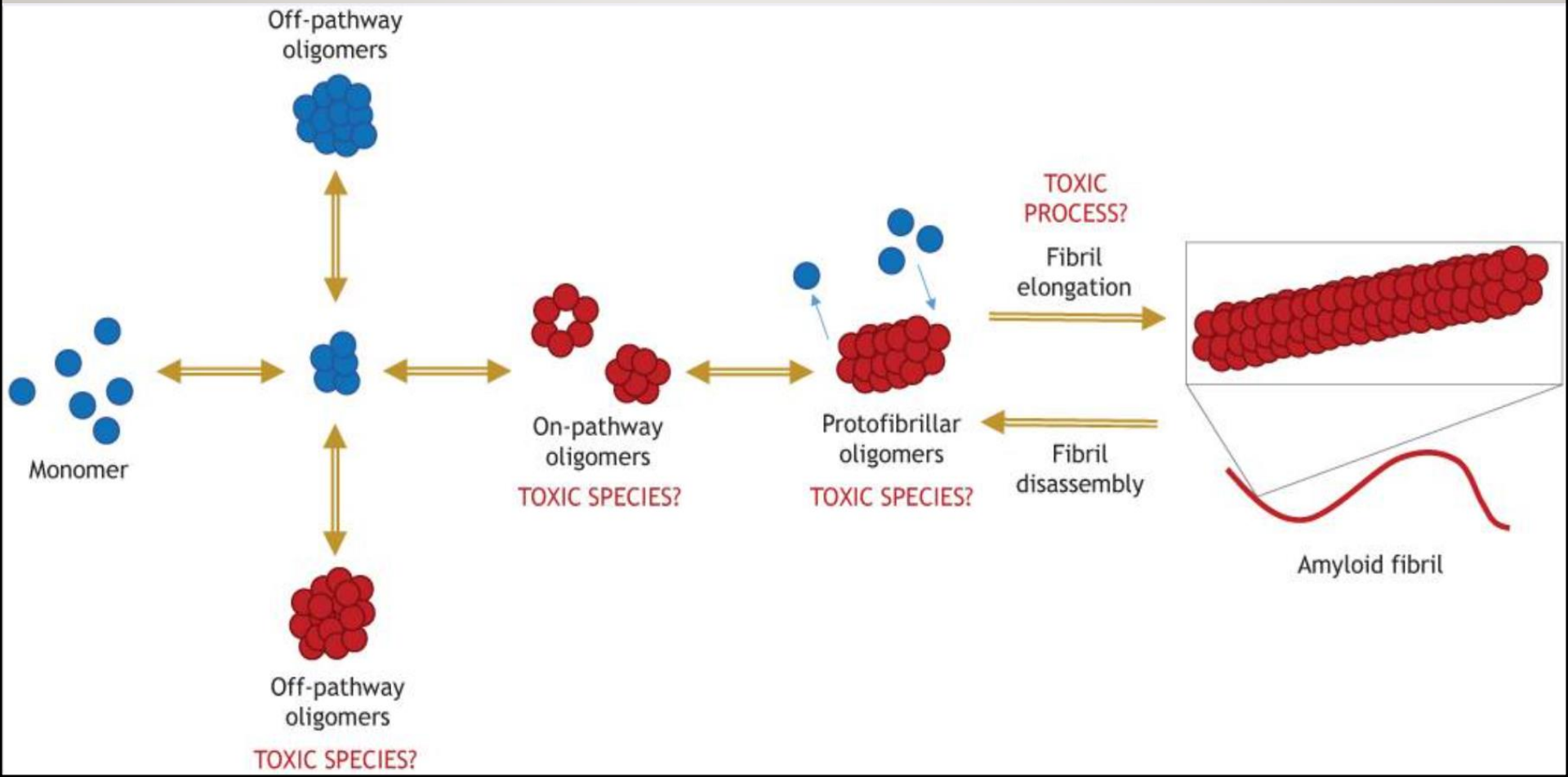
But what causes the α-syn proteinopathy?

Roberts & Brown, 2015, MDPI

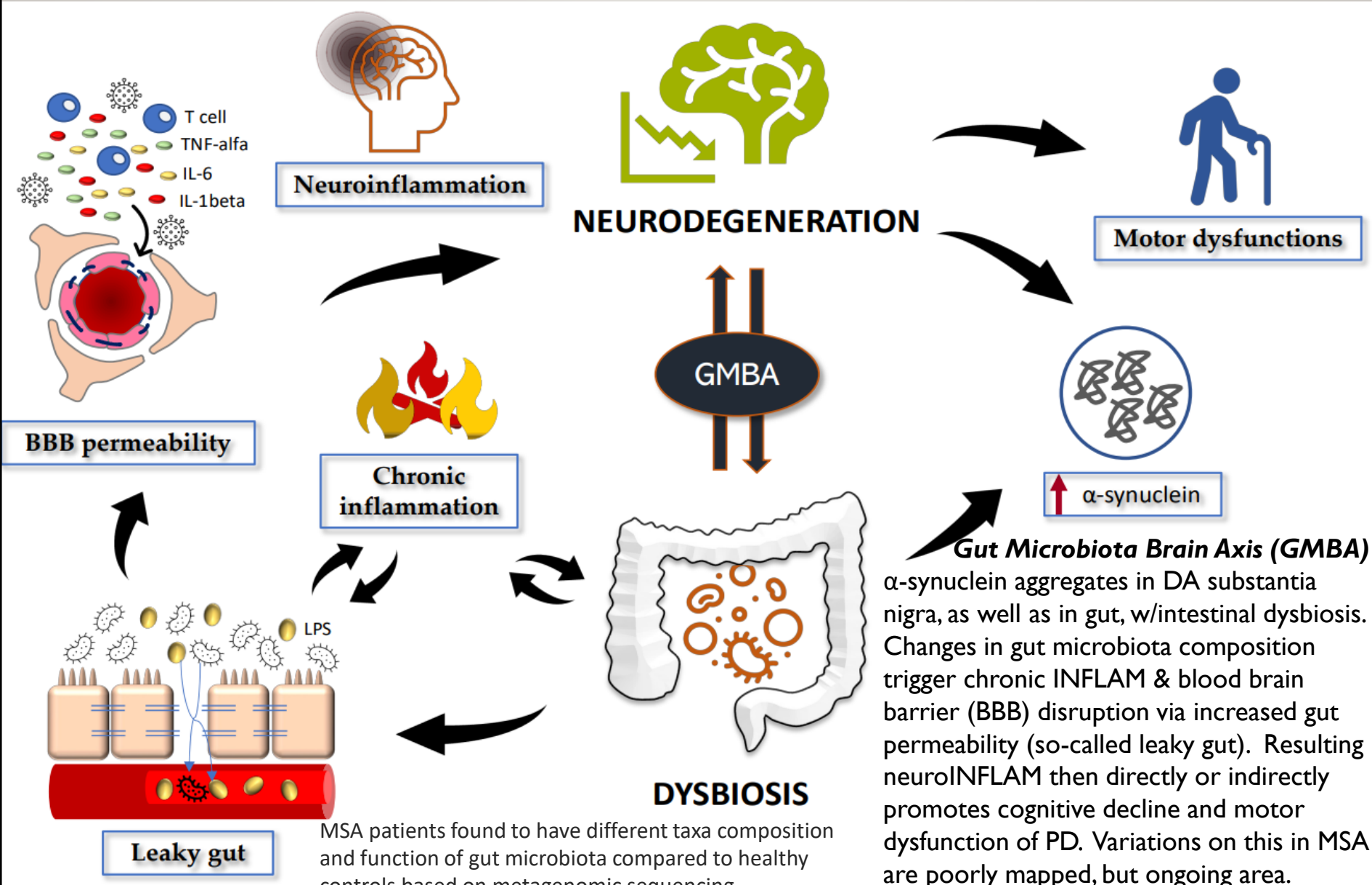


GRAPHICAL DEPICTION OF POSSIBLE α-SYNUCLEIN AGGREGATION PATHWAYS

Toxic α-synuclein oligomers in relation to the pathway of amyloid fibril formation. Toxic oligomers as reported by different studies as being "on-pathway" or "off-pathway" to amyloid fibril formation. Covalent bonding by oxidative modifications may be involved in stabilizing toxic "off-pathway" oligomers. Non-toxic oligomers that are "off-pathway" are stabilized by pharmacological inhibitors of fibril formation (eg EGCG). Toxicity of oligomers thought related to their high levels of ***β-sheet secondary structure***. Additionally, hypothesized that protofibril/fibril elongation may be toxic. Blue circles- Little/no β-structure; Red circles- High β-structure. ***Glymphatic clearance limited to oligomers.***



47 EVIDENCE OF ROLE FOR DYSBIOSIS IN PROTEIN-OPATHY & NEURODEGENERATION (VARESI ET AL 2022)



MSA patients found to have different taxa composition and function of gut microbiota compared to healthy controls based on metagenomic sequencing.

Gut Microbiota Brain Axis (GMBA)

α-synuclein aggregates in DA substantia nigra, as well as in gut, w/intestinal dysbiosis. Changes in gut microbiota composition trigger chronic INFLAM & blood brain barrier (BBB) disruption via increased gut permeability (so-called leaky gut). Resulting neuroINFLAM then directly or indirectly promotes cognitive decline and motor dysfunction of PD. Variations on this in MSA are poorly mapped, but ongoing area.

TREATMENT

Treatment approaches with familiar targets – targeting protein aggregates/aggregation, MITO fxn, inflammation, neurotrophins. It has not been possible to replicate benefits of preclinical therapeutic approaches in any randomized controlled human trials. Why not???

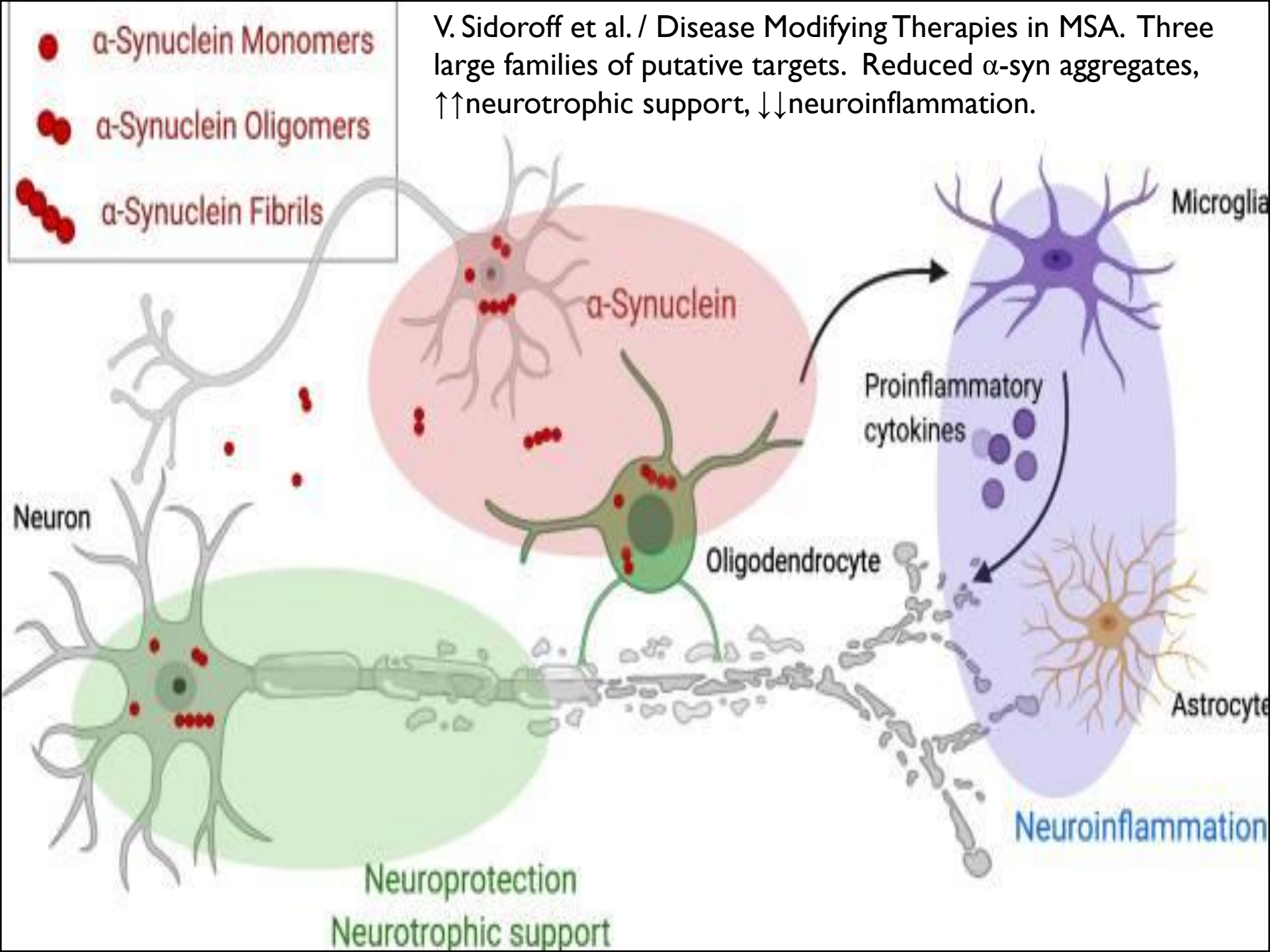
WEIN (what else is new?) in NDD? What are we missing mechanistically, and as targets?

Little attention so far to CRM except rapamycin (failed). No other CRMs tested.

Deeply frustrating for patients and families, but the news is really not much better around other NDD for which there are approved treatments. No truly major DM Rx yet.

Variety of variably effective symptomatic approaches for parkinsonian and autonomic issues – we will not cover those. Symptomatic treatments covered in detail elsewhere (see refs list, esp. the first 3 references).

V. Sidoroff et al. / Disease Modifying Therapies in MSA. Three large families of putative targets. Reduced α-syn aggregates, ↑↑neurotrophic support, ↓↓neuroinflammation.



APPROACHES TO DIS MOD TREATMENT AND NEUROPROTECTION

- Immunotherapies to block toxic propagation of α-syn species, also to reduce various forms of α-syn accumulation. This approach has notably failed in all other NDD once in clinical stages, and has only phase I results in MSA (2022). Safety worries (immune attack on WM?), but all Rx in Phase I were tolerable. Too early to say if effective?
- Stimulating α-syn/protein degradation pathways, by promoting autophagy. Phase II randomized controlled study assessing efficacy of oral mTOR inhibitor rapamycin was prematurely terminated because of futility. Nilotinib (tyrosine kinase inhibitor→ ↑autophagy) failed to show disease modifying effect in mouse models. $LiCO_3$ phase II terminated due to SE. Too early to say, given lack of CRM trials but targets past ATG.
- Reducing neuroinflammation induced by α-syn/other factors may lessen MSA pathology; reducing overactivation of glial cells and production of pro-inflammatory cytokines.
- Flip side of ↓INFLAM: targeting trophic support & neuroplasticity, esp. GDNF & BDNF.

Trials targeting α-synuclein, other than some positive safety results, v. disappointing

Mode of action	Substance	Phase	Design	Primary outcome	Results	Comments
Immunotherapy	PD01A/PD03A	Phase I	RCT	Safety & tolerability	Safe & well-tolerated	PD01A: significant immunoresponse against α-synuclein
	Lu AF82422	Phase I	RCT	Safety & tolerability	Safe & well-tolerated	In healthy controls and PD patients
Antisense oligonucleotides	BIIB101	Phase I	RCT	Safety & tolerability	–	Ongoing in MSA patients
Aggregation inhibitors	EGCG	Phase III	RCT	UMSARS part II score	Negative	Exploratory analyses suggested reduced striatal volume loss
	NPT200-11A	Phase I	RCT	Safety & tolerability	Results pending	In healthy volunteers
	Anle138b	Phase Ib	RCT	Safety & tolerability	–	Ongoing in mild to moderate PD
	ATH-434	Phase I	RCT	Safety & tolerability	Safe & well-tolerated	In healthy volunteers
	CLR01	Preclinical				Molecular tweezer
	NPT088	Preclinical				Fusion protein
	Synuclein-D	Preclinical				Small molecule
	IkT-148009	Preclinical				Small molecule
	Kallikrein-6	Preclinical				Neurosin
Degradation enhancers	Rifampicin	Phase III	RCT	UMSARS part I score	Early termination	Futility criteria were met
	Rapamycin	Phase II	RCT	UMSARS total score	Early termination	Futility criteria were met
	Lithium	Phase II	RCT	Number of SAE & nSAE	Early termination	Severe adverse events

RCT, randomized-controlled trial; UMSARS, United Multiple System Atrophy Rating Scale; SAE, serious adverse event; nSAE, non-serious adverse event; PD, Parkinson's disease; MSA, multiple system atrophy.

V. Sidoroff et al. 2022 Disease Modifying Therapies in MSA

Target	Substance	Phase	Design	Primary outcome	Results	Comments
Inhibition of neuro-inflammation	**IVIG**	Phase II	OL	Number of AEs	Positive	Motor improvement (small sample size, short treatment period)
Inhibition of microglial activity	**Minocycline**	Phase II	RCT	UMSARS part II score	Negative	No motor improvement
Oxidative stress reduction	**Verdiperstat**	Phase III	RCT	Modified UMSARS total score	Negative	Failed in terms of primary and key secondary endpoints

IVIG, intravenous immunoglobulin; RCT, randomized-controlled trial; OL, open label trial; UMSARS, Unified Multiple System Atrophy

Clinical trials targeting Neuroprotection and neurotrophic support

Target	Substance	Phase	Design	Primary outcome	Results	Comments
FAF-1	KM-819	Phase I	RCT	Safety & tolerability	Safe & well tolerated	–
Lipidomic neurotoxicity	YTX-7739	Phase Ib	–	Safety & tolerability	–	Ongoing
IGF1 pathway	Intranasal insulin	Phase II	RCT	Verbal fluency total score	–	Motor improvement (only 1 MSA patient)
IGF1 pathway	Exendin-4	Phase II	OL	UMSARS part I & II score	–	Ongoing
Mitochondrial dysfunction	Coenzyme Q10	Phase II	RCT	UMSARS part II score	–	Ongoing
Neuronal/glial proliferation	Growth Hormone	Phase II	RCT	Safety & tolerability	Safe & well tolerated	Trend for less worsening of UMSARS [152]
Immuno-modulation, neuro-protection	MSCs	Phase II	RCT	UMSARS part II score	Positive	Only in MSA-C, imaging not done in all patients [156]
Mitochondrial dysfunction	Rasagiline	Phase II	RCT	UMSARS part I & II score	Negative	No motor improvement [160]
Neurotrophic support	Fluoxetine	Phase II	RCT	UMSARS part I & II score	Negative	No motor improvement [163]
Reduced excitotoxicity	Riluzole	Phase III	RCT	UPDRS part II & III	Negative	No motor improvement or survival rates [137]
NMDA-modulator	Tllsh2910	Phase III	RCT	SARA score	–	Ongoing

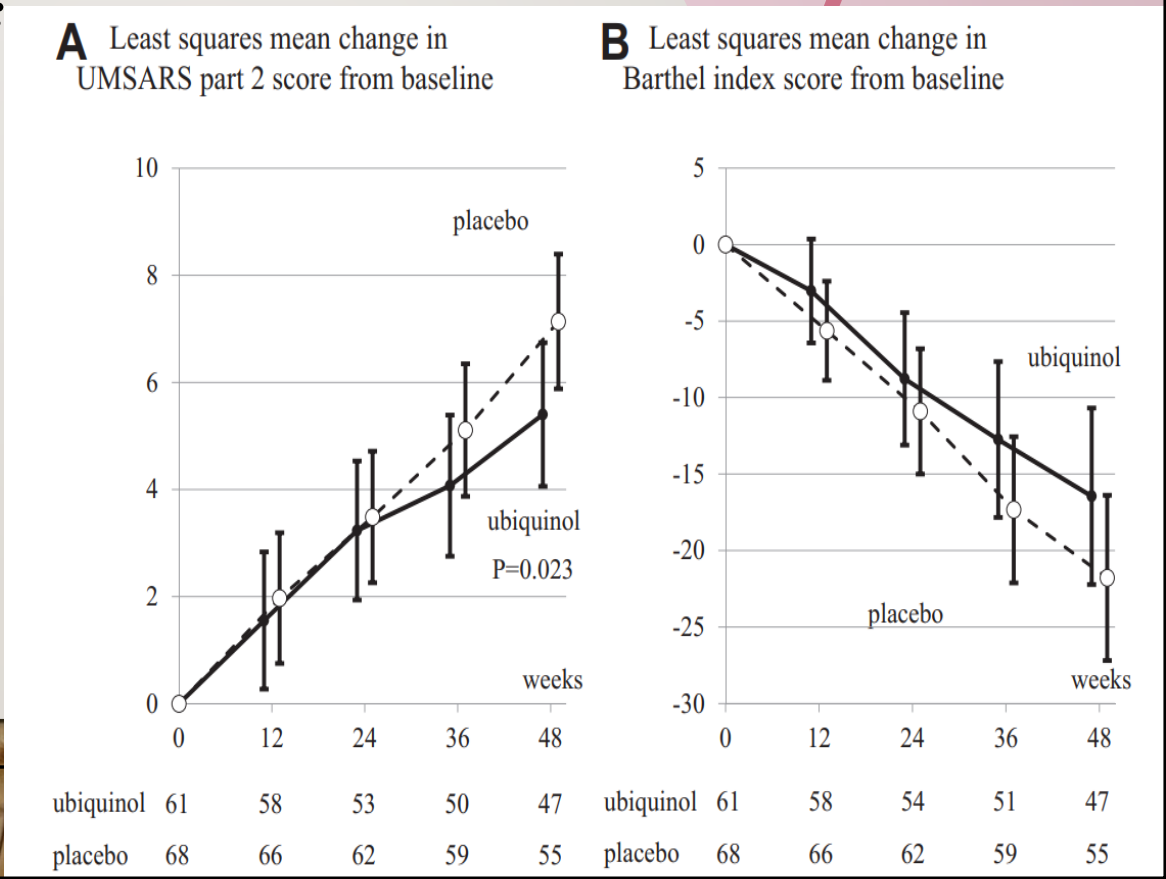
IVIG, intravenous immunoglobulin; RCT, randomized-controlled trial; OL, open label trial; UPDRS, Unified Parkinson's Disease Rating Scale; SARA, Scale for the assessment and rating of ataxia; UMSARS, Unified Multiple System Atrophy Rating Scale; MSA, multiple system atrophy.

Sidoroff et al. 2022 Disease Modifying Therapies in MSA

53 OTHER CUTTING (CRUMBLING?) EDGE RX

- Intra-arterial administration of autologous mesenchymal stem cells demonstrated modest benefit; evaluation of intrathecal administration underway (Moretti, 2019).
- Benefits of 1.5g CoQ10 statistically significant (eClinicalMedicine 2023;59: 101920).
- Benefits of other CRMs (AMPK/PPARγ/GLP1 agonists, adiponectin agonists-osmotin ***not tested***. ***These are big opportunities!*** Exercise not tested (catch-22?).
- ***INFLAM/malnutrition targets:***

  High neutrophil count, red-cell distribution width, C-reactive protein, ESR, ↓↓HGB, total protein, albumin, creatinine, predict higher mortality in MSA. Suggests concerted focus on these as targets might be disease modifying? (npjPD (2022) 8:141). ***What about multiple targets?***



OVERVIEW OF ISSUES IN NDD – MSA AND BEYOND
WHERE ARE WE AT, IN THE CLINICAL SCIENCE OF NEURODEGENERATION?

Sobering facts with many possible interpretations?

Treatment biases – the usual approaches are tried over and over despite a growing poor track record (AD→PD→FTD/ALS).

What are we missing? It could be substantial!

Perhaps we have forgotten that all NDD are ***diseases of aging***? How do we get the science of aging more into the calculus of research into neurodegenerative disorders?

We must prioritize the true precedent science of neurodegeneration which is largely absent in the current biopharma attempts at treatment.

WHY HAVE CR MIMETICS BEEN GIVEN SHORT END OF THE STICK IN RANDOMIZED CLINICAL TRIALS IN NEURODEGENERATIVE DISORDERS?

- Siloed knowledge and ignorance about CRM may be primary factor, w/secondary factor being few attractive/fashionable and on-patent agents? Pigeonholed as drugs to treat type II diabetes or obesity. Exploding interest in GLP1 class . . . Even in NYT!!
- Science of CR (calorie restriction) and its mimetics is neglected in medical school – rarely appreciated that ***CRM have broadest footprint of neuroprotective effects***.
- CRM are only class of compounds shown to intercept virtually all age-related (and pathology-related) cellular derailments in NDD: ***disinhibited INFLAM (esp. innate), ↓neurotrophins, oxidative stress, MITO dysfxn, proteostatic/autophagy failure.***
- Only autophagy effects appreciated as CRM targets, and often only testing with rapalogs (as classical mTOR inhibitor), although now some consistent attention to GLP-agonist class of DMII drugs (on patent so garnering more biopharma interest).
- These cellular phenotypes of neurodegeneration be differentially relevant at different stages of illness, but CRM and other therapies probably have biggest therapeutic opportunity early on, to preserve quality of life.

A VIEW OF NEURODEGENERATION FROM 10,000 FT
– THERE'S GOOD NEWS AND THERE IS BAD NEWS! ***('GN'…AND 'BN'!)***

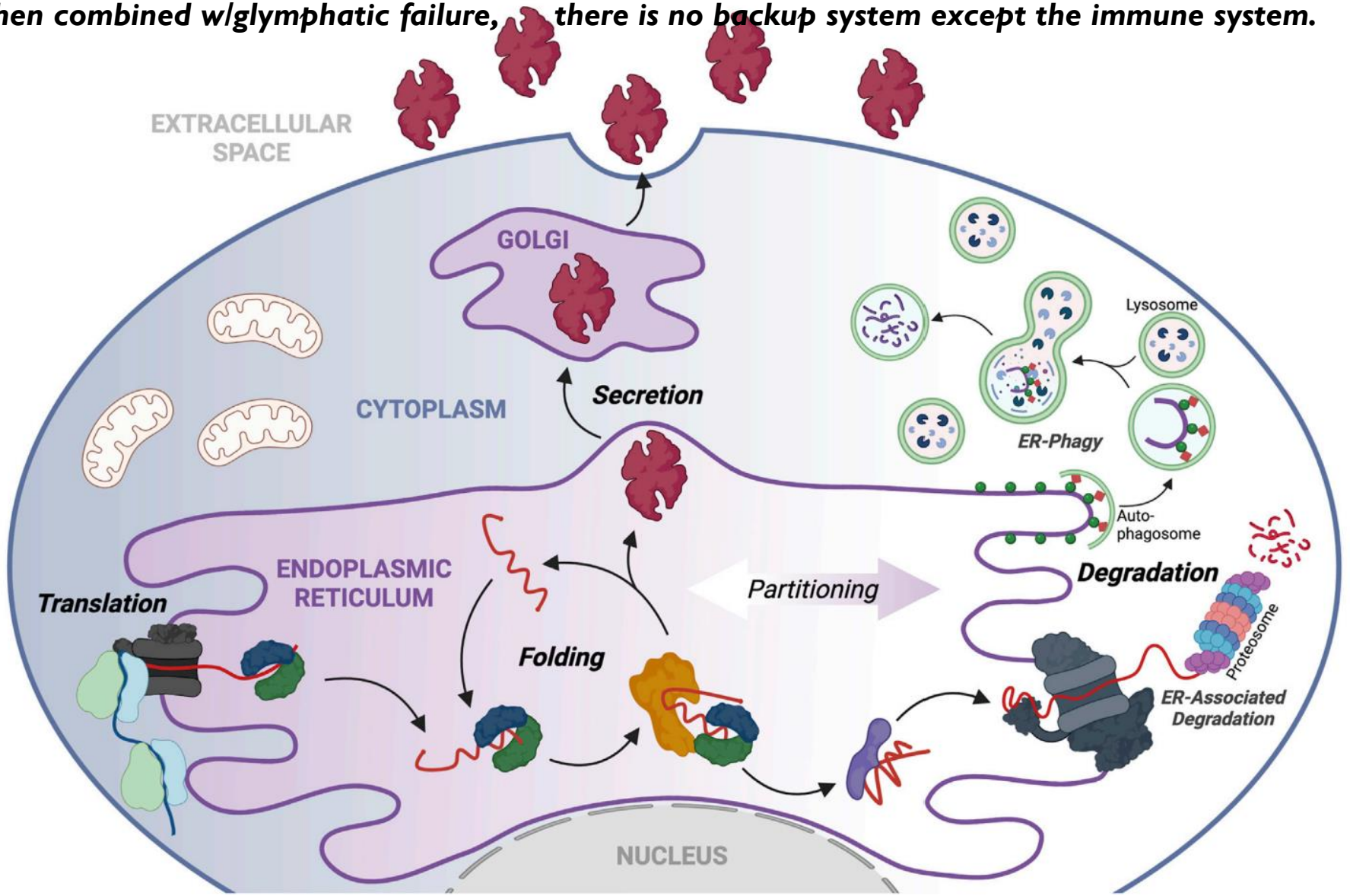
- The ***good news*** is we're making steady if slow progress. We've identified a lot of operative cellular phenotypes: ***failing autophagy/proteostasis, ↑INFLAM, ↓MITO, ↓neurotrophins/neuroplasticity, w/overpromotion of apoptosis as common features in NDD.*** This is maybe the ***best good news***. Yea!
- The ***bad news*** is we still don't understand what's underpinning this neurodegenerative process, and our lack of understanding may be ***fundamental and not superficial***.
- Modeling of ND has generally been linear: failing proteostasis/toxic protein seeding as primary drivers for later neuroinflammatory and pro-apoptotic events. But this looks like a ***spreading cascade failure*** rather than a successful compensation? GN or BN?
- Results from many animal models across many NDD emphasizing above concepts have ***failed to translate into disease modifying Rx*** for clinical pts. This failure rate isn't occasional, isn't 50%, not 80%, isn't even 90%, it's actually > ***99.7+% failure rate***.
- As a result of all this brilliant but failing work, ***there is no disease modifying therapy for any neurodegenerative disorder.*** This much basic science fails? ***Why?***
- ***It fails because it is applied or technical science, but where the precedent basic science is lacking. What is that?***

57 OUR TREATMENT FAILURE IN NDD – A FEW MAJOR PIECES OF A POSSIBLE MULTIFACTORIAL EXPLANATION

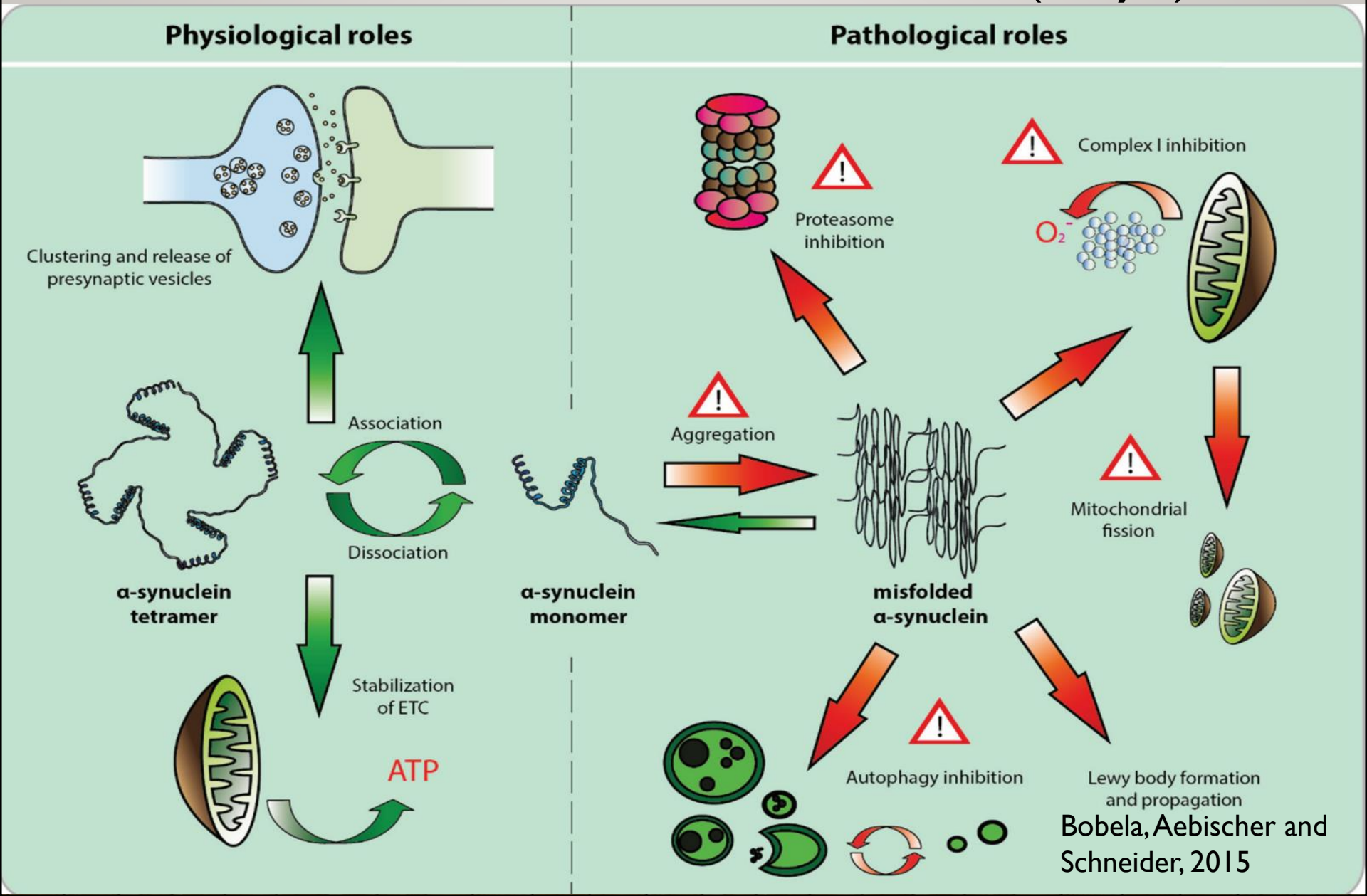
1. ***Relationships/causality between factors complex***, w/many loops/interactions. ***Not dominoes***. *Why do we continue to model biology as linear when we know it is chaotic?*
2. ***We still have no clearly defined 'ramp' into any of these disorders***. Amyloid, tau, α-syn or any protein aggregate as an proximal explanation is a ***explanatory failure,*** or a pseudo-explanation that begs questions. Like modeling home run from 3rd base?
3. ***Proteins in NDD as 'perfect Trojan horses'*** – ↑↑in aging but also ↓↓autophagy, MITO dysfxn, w/prion-like effects, suggesting deep intrinsic vulnerability that may be hard to reverse? Evolution 'doesn't care much' about aging, leaves 'holes' in system (entropy).
4. ***Failure of proteostasis*** more complex/multidimensional than just forms of toxic protein.
5. ***AD*** as paradigm case: ***age as primary risk factor*** suggests aging-related processes driving the ramp into NDD; failing proteostasis is, after all, a core cellular phenotype of aging.
6. ***Interventions may be <u>way</u> too late*** – ***<u>neurodegeneration accelerates</u>*** as it progresses. ***Why?*** A) positive feedback loops between factors, including backstop glymphatic failure? B) later recruitment of neuro-predatory inflammation is a 'killer' process? C) progressive recruitment of apoptosis (***MITO/INFLAM/ER stress*** as the known prime movers in apoptosis).
7. ***Are cellular changes just dimensions to a systemic cellular failure, starting regionally but then becoming global***? What are the front line organelles in aging, what happens to their interaction? MITO, ER, proteosome/lysosome/autophagy, chromatin/epigenetic Δ, all interactive. ***Perhaps the cellular failure in NDD rests in the failing partnerships of these systems!***

These are the familiar cellular organelles involved in protein quality control, & that fail in neurodegenerative disorders. Cells use these exocytosis pathways to mitigate the failure of UPR/autophagy (ATG), 2 protein quality control guardians. NIMBY/exocytosis *solves this cell's problem but messes up the neighborhood*. NIMBY approach may index that the deep logic of multicellular cooperation has failed? Is this a watershed event?

When combined w/glymphatic failure, there is no backup system except the immune system.

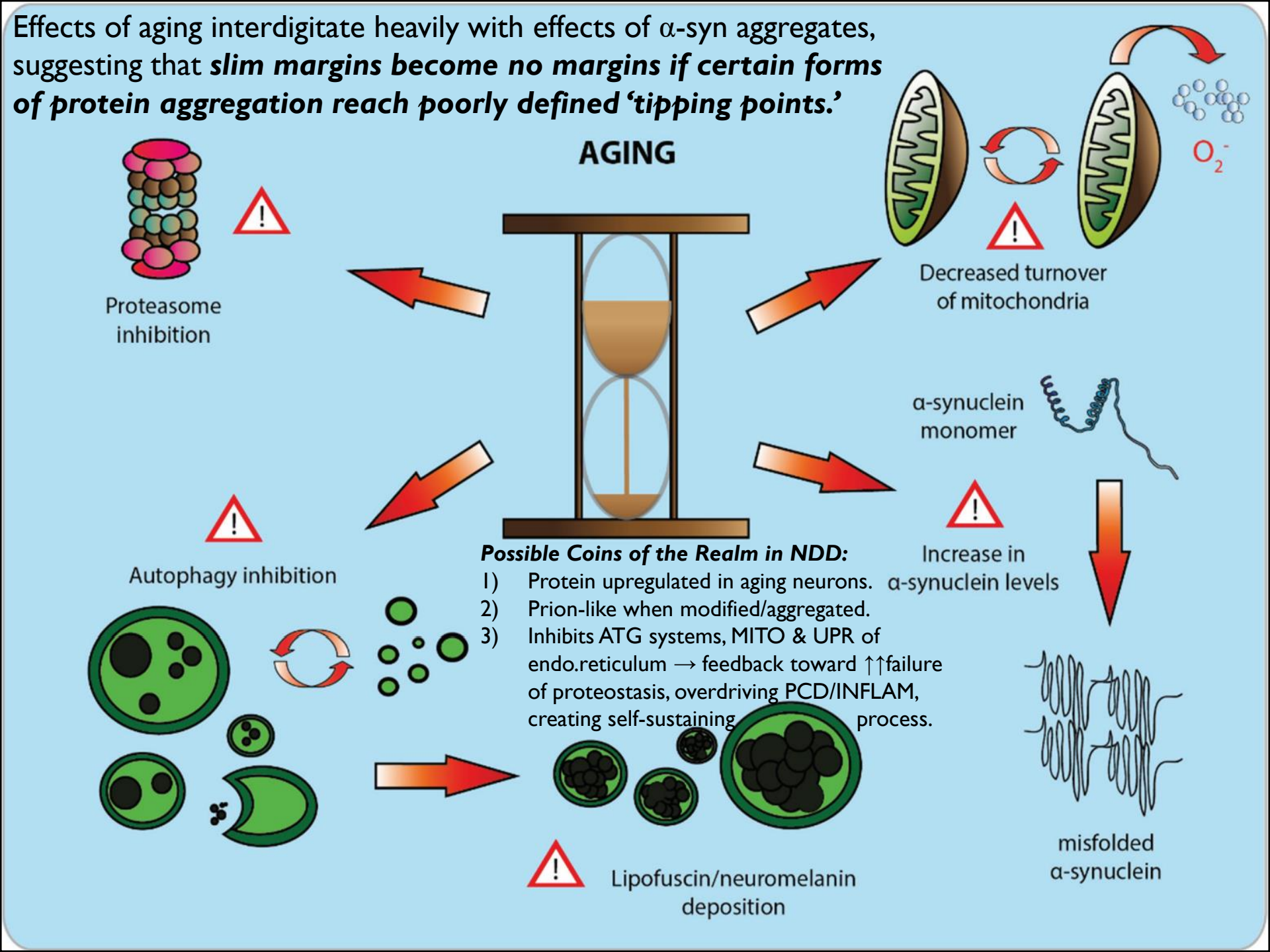


PATHOLOGICAL & PHYSIOLOGICAL ROLES OF ALPHA SYNUCLEIN 140 AMINO ACID (α-syn)



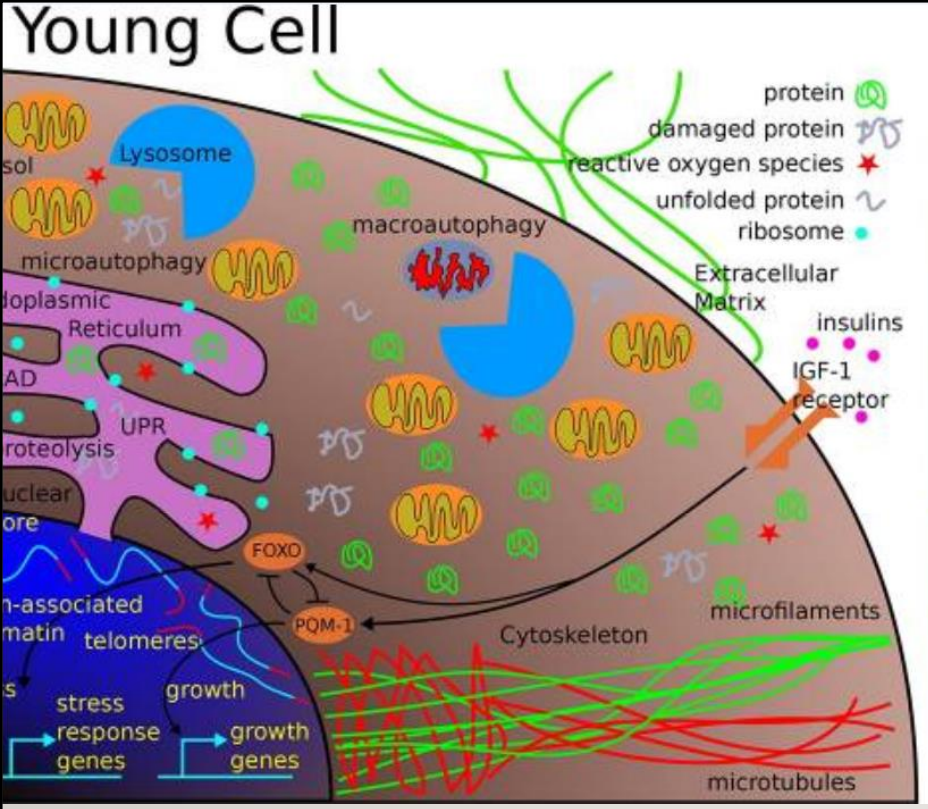
Bobela, Aebischer and Schneider, 2015

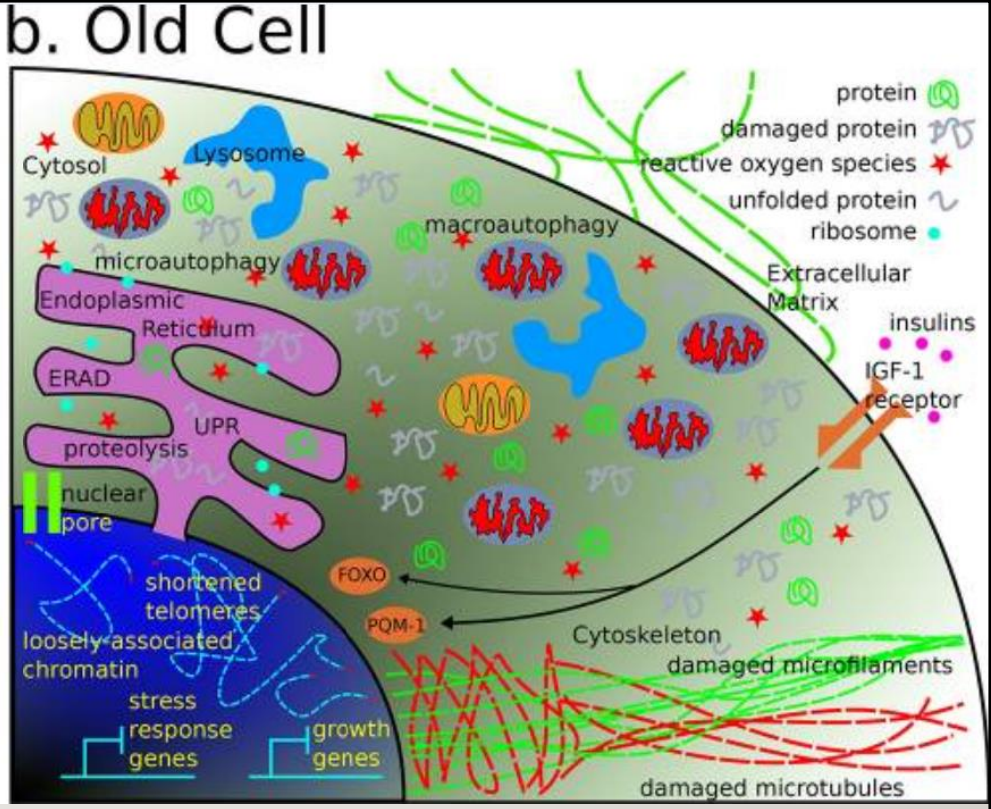
Effects of aging interdigitate heavily with effects of α-syn aggregates, suggesting that ***slim margins become no margins if certain forms of protein aggregation reach poorly defined 'tipping points.'***

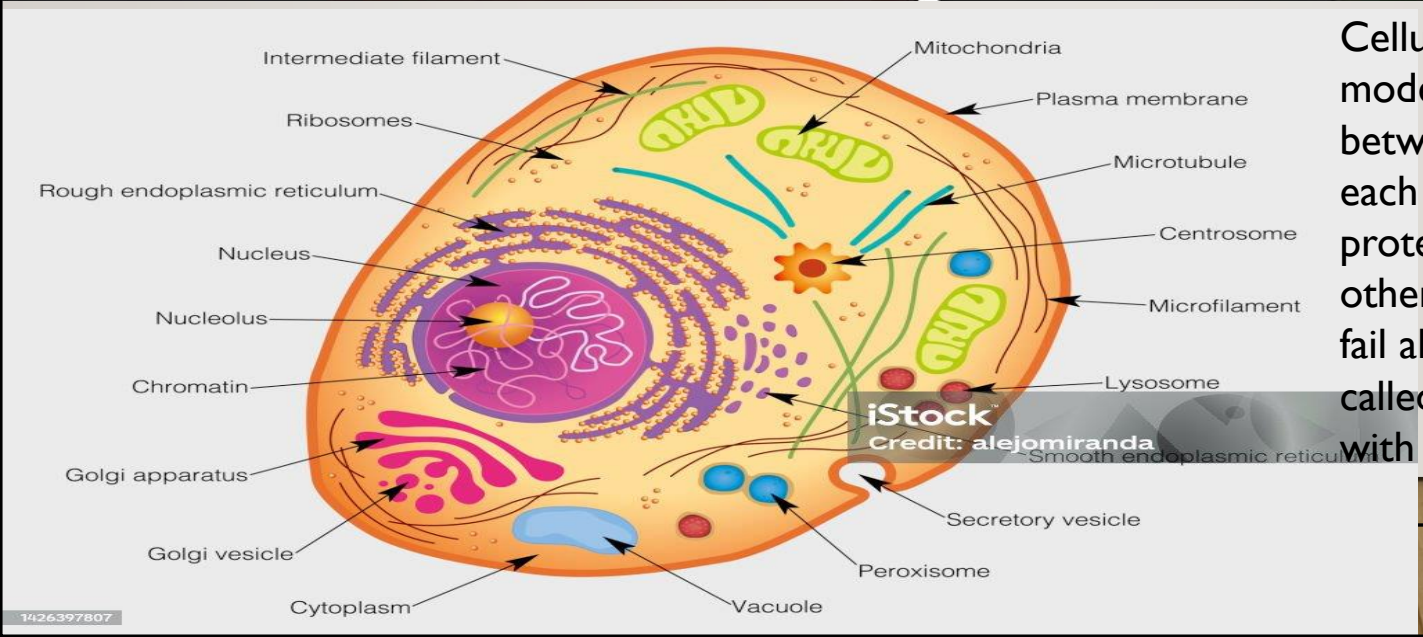


Possible Coins of the Realm in NDD:

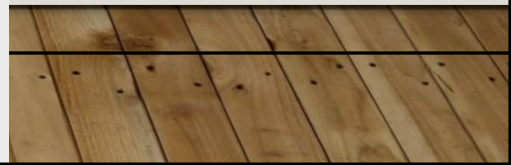
1) Protein upregulated in aging neurons.
2) Prion-like when modified/aggregated.
3) Inhibits ATG systems, MITO & UPR of endo.reticulum → feedback toward ↑↑failure of proteostasis, overdriving PCD/INFLAM, creating self-sustaining process.



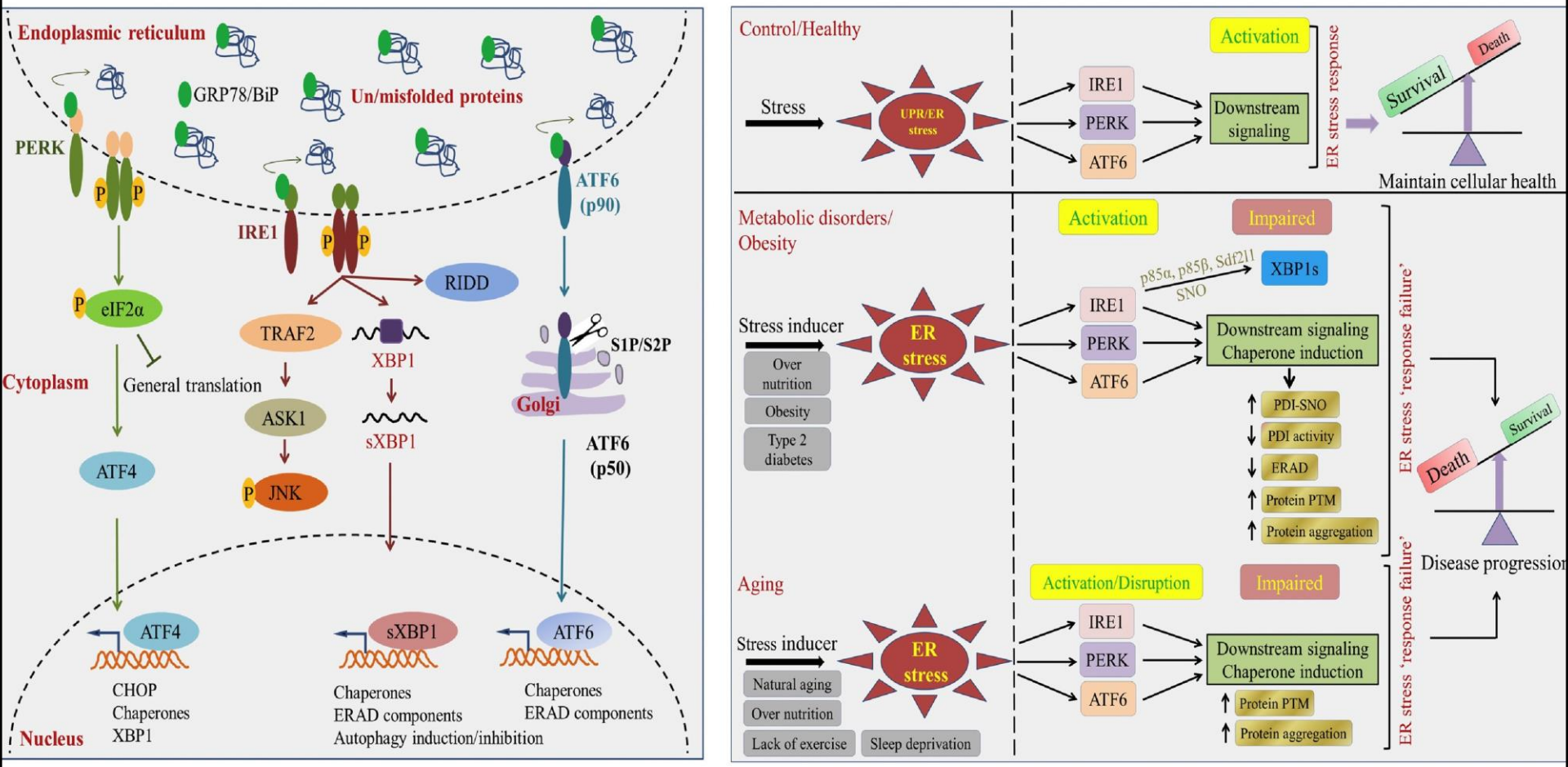




Cellular homeostasis increasingly modelled in terms of relationship between many cellular organelles, each doing a critical job. Failure o proteostasis can't be 'covered' by other systems, causing those to fail also, with immune systems called in to clean up the mess, with progressively poorer results.



ER stress response failure in disease. Acute ER stress activates the UPR, which can restore cellular function by ↓↓protein translation, ↑↑degradation, or ↑↑ molecular chaperones to increase survival/decrease apoptosis. Upon UPR activation, 3 UPR sensors, IRE1, PERK, and ATF6, are activated. These arms of UPR further activate downstream signaling, collectively termed the ER stress response. The acute or mild ER stress increases cell survival by inducing an adaptive response, also known as ER hormesis, that maintains cellular health. During severe ER stress in metabolic diseases, activated UPR elements may not trigger downstream signaling, which is termed ***ER stress response failure*** reducing cell survival and inducing cell death, leading to disease progression. Impaired downstream signaling of UPR elements reduces expression of molecular chaperone activity. The aberrant post-translational modification (PTM) of proteins may disrupt ERAD function and thus increase protein aggregation. Similarly, aging is associated w/impaired UPR and declining chaperone activities, followed by apoptosis. ***Once proteins escape these two stages of containment UPR & ↑autophagy, with prion-like proteins, and glymphatic clearance also fails, a cascade of cellular failure may accelerate towards tipping point, otherwise known as a neurodegenerative disorder. But this suggests that critical dynamics antecedent to PP,***



NEURODEGENERATION MODELS MORE INFORMED BY SCIENCE OF AGING

- ***Aging of cellular organelles implicated in NDD*** – MITO, ER, & ATG systems, all declining in aging, while proteins constituting challenges to ATG/MITO/ER may be upregulated as aging compensation. The collapse of "slim margins" → no margins.
- Intersection between these determines type and onset of NDD, potentially exacerbated by both genes and environs/lifestyle.
- Entropy finds these holes or weak spots in the system, as evolution doesn't have selection process to deal with this – aging is the problem evolution forgot?
- Once prion-like forms of proteins able to escape containment, proteinopathy spreads to cells & systems where proteostasis hadn't failed. ***Tipping point early***.
- Once predatory forms of INFLAM are activated by both proteinopathic and other pro-inflammatory signals, downhill course is "baked in" and accelerated.
- Once ER stress, MITO failure and high levels of TNF-α are common features of network environment, programmed cell death is baked in and accelerated.
- ***Therapies will have to "back out" the potentiation of both INFLAM & PCD, while restoring/propping up failing proteostasis as widely as possible.***
- ***But how to do this is very poorly plotted.*** We need more science of the aging at cellular/organelle level. *CRMs should be tried both singly and in combination.*

64 REFERENCES ON MULTIPLE SYSTEM ATROPHY

- Poewe, W., Stankovic, I., Halliday, G. *et al.* Multiple system atrophy. *Nat Rev Dis Primers* **8**, 56 (2022). https://doi.org/10.1038/s41572-022-00382-6
- Stefanova, N., Wenning, G.K. Multiple system atrophy: at the crossroads of cellular, molecular and genetic mechanisms. *Nat Rev Neurosci* **24**, 334–346 (2023). https://doi.org/10.1038/s41583-023-00697-7.
- Goh YY, Saunders E, Pavey S, Rushton E, Quinn N, et al (2023) Multiple system atrophy. Practical Neurology, 23(3), 208–221. https://doi.org/10.1136/pn-2020-002797.
- Sidoroff V, Bower P, Stefanova N, Fanciulli A, Stankovic I, Poewe et al. (2022). Disease-Modifying Therapies for MSA: Where Are We in 2022?. Journal of Parkinson's Disease, 12(5), 1369–1387. https://doi.org/10.3233/JPD-223183,
- Wenning, G. K., Stankovic, I., Vignatelli, L., Fanciulli, A., Calandra-Buonaura, G., et al (2022). The Movement Disorder Society Criteria for the Diagnosis of Multiple System Atrophy. *Movement Disorders : Official Journal of the Movement Disorder Society*, *37*(6), 1131–1148. https://doi.org/10.1002/mds.29005.
- Stankovic, I., Fanciulli, A., Kostic, V. S., Krismer, F., Meissner, W. G., Palma, J. A., Panicker, J. N., Seppi, K., Wenning, G. K, et al(2021). Laboratory-Supported Multiple System Atrophy beyond Autonomic Function Testing and Imaging: A Systematic Review by the MoDiMSA Study Group. *Movement Disorders Clinical Practice*, 8(3), 322–340. https://doi.org/10.1002/mdc3.13158.
- Chelban, V., Nikram, E., Perez-Soriano, A., Wilke, C., Foubert-Samier, A., Vijiaratnam, N., Guo, T., Jabbari, E., Olufodun, S., Gonzalez, M., et al (2022). Neurofilament light levels predict clinical progression and death in multiple system atrophy. Brain : a journal of neurology, 145(12), 4398–4408. https://doi.org/10.1093/brain/awac253.