MEDICAL HYPOTHESIS

Revisiting protein aggregation as pathogenic in sporadic Parkinson and Alzheimer diseases

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Abstract

The gold standard for a definitive diagnosis of Parkinson disease (PD) is the pathologic finding of aggregated α-synuclein into Lewy bodies and for Alzheimer disease (AD) aggregated amyloid into plaques and hyperphosphorylated tau into tangles. Implicit in this clinicopathologic-based nosology is the assumption that pathologic protein aggregation at autopsy reflects pathogenesis at disease onset. While these aggregates may in exceptional cases be on a causal pathway in humans (e.g., aggregated α-synuclein in *SNCA* gene multiplication or aggregated β-amyloid in *APP* mutations), their near universality at postmortem in sporadic PD and AD suggests they may alternatively represent common outcomes from upstream mechanisms or compensatory responses to cellular stress in order to delay cell death. These 3 conceptual frameworks of protein aggregation (pathogenic, epiphenomenon, protective) are difficult to resolve because of the inability to probe brain tissue in real time. Whereas animal models, in which neither PD nor AD occur in natural states, consistently support a pathogenic role of protein aggregation, indirect evidence from human studies does not. We hypothesize that (1) current biomarkers of protein aggregates may be relevant to common pathology but not to subgroup pathogenesis and (2) disease-modifying treatments targeting oligomers or fibrils might be futile or deleterious because these proteins are epiphenomena or protective in the human brain under molecular stress. Future precision medicine efforts for molecular targeting of neurodegenerative diseases may require analyses not anchored on current clinicopathologic criteria but instead on biological signals generated from large deeply phenotyped aging populations or from smaller but well-defined genetic–molecular cohorts.

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Glossary

Aβ = β-amyloid; **AD** = Alzheimer disease; **DLB** = dementia with Lewy bodies; **PD** = Parkinson disease.

A cognitive dissonance in research on biomarkers and disease-modifying treatments for Parkinson disease (PD) and Alzheimer disease (AD) is the dual acceptance of 2 opposite tenets: that their clinical heterogeneity reflects several diseases subsumed within each and that we are on the verge of finding the set of perfect biomarkers that will explain their collective progression and response to therapy.[1] Recent review articles on biomarkers and precision medicine start with the standard disclaimer that a major challenge is the existence of many diseases included under PD and AD (e.g., "…trying to make one drug work for all PD patients … is wrong because (1) PD is not a single disease, and (2) no 2 individuals have the same biological makeup"[2]), only to revert to traditional form by reviewing or proposing analyses of a large set of clinical and biological data collected on cohorts of clinically diagnosed individuals to overcome heterogeneity.[3] Enormous financial and logistical resources have been devoted to protein-based biomarkers and anti-β-amyloid (Aβ) treatments with little return on investment. Therefore, it is imperative to review the disease framework on which biomarker development and the design of disease-modifying therapies are anchored.

Protein aggregation as causal of a single disease: Bradford Hill assessment

Mutations in and multiplications of α-synuclein- and Aβ-related genes cause certain forms of PD and AD in affected families with these genetic abnormalities.[4,5] Overexpression of these proteins coupled to excessive aggregations has been clearly shown to cause neuronal dysfunction and death in numerous models.[6,7]

To examine the causality of α-synuclein/Aβ/tau aggregation in human sporadic PD/AD (i.e., without the point mutations or gene multiplication in the families where protein aggregation is assumed to be directly causal), we utilized the Bradford Hill criteria for causality assessment.[8,9] These are a set of 9 criteria developed by Sir Austin Bradford Hill to provide epidemiologic evidence of a causal relationship between an apparent cause and an observed effect. We tested the current disease model under which α-synuclein and Aβ/tau aggregations are thought to be causal to PD and AD, respectively, by compiling all the published evidence from studies on humans available and categorizing it according to each of the criteria.

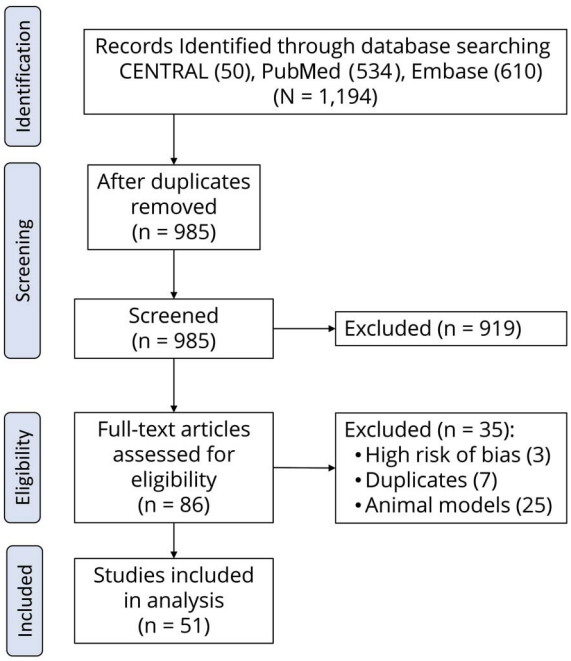
Search strategy and selection criteria

We conducted a search in MEDLINE and PubMed for articles published until June 6, 2018, using the search terms "protein aggregation," "alpha synuclein," "oligomers," "fibrils," "amyloid," "senile plaque," "phospho-tau," "Lewy body," "Parkinson disease," "Alzheimer disease," "biomarker," and "pathology." We also searched references and ClinicalTrials.gov for relevant studies. No language restrictions were applied. The final reference list was generated on the basis of relevance to the topics covered in this Hypothesis article.

We conducted a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[10] Eligible epidemiologic, molecular (e.g., neuroinflammation, mitochondrial dysfunction, oxidative stress, ubiquitin-proteasome system dysfunction, calcium signaling dysregulation, autophagy dysfunction, synaptic dysfunction, cholesterol metabolism alteration),[11,12] pathologic, autopsy, imaging, and interventional studies on α-synuclein, Aβ, and tau were included. We excluded animal models and vascular dementia/parkinsonism studies. Electronic search of articles published up to January 2018 was conducted using the Cochrane Central Register of Controlled Trials (CENTRAL), EMBASE, PubMed, and references from relevant articles. Search strategy included free text and Medical Subject Headings (MeSH) terms (table e-1, doi.org/10.5061/dryad.g1nq02r). No restrictions were applied to sex, language, or sample size. Titles and abstracts of all studies identified were screened for inclusion and exclusion criteria. Full-text studies of eligible articles were obtained to confirm eligibility and discard duplicates. A data collection form was used to extract variables of interest for the selected studies. Given the heterogeneity of study designs, risk of bias of individual studies was appraised utilizing the National Heart, Lung, and Blood Institute tools,[13] following the Cochrane handbook recommendations.[14] Studies with high risk of bias were excluded from further analysis. Remaining studies underwent data extraction and Bradford Hill criteria–based classification. These procedures were conducted independently by 2 authors and disagreements were settled by consensus among authors.

Out of the 1,194 records derived from the initial search strategy, 54 studies[15–24,e1–e41] met full search criteria (figure 1). Three studies[e42–e44] were classified as having high risk of bias and were excluded from further analysis. Agreement was met between evaluators in all cases. A total of 2 and 4 of the 9 Bradford Hill criteria supported causality for PD and AD, respectively (tables 1 and e-2).[8,9] Altogether, human studies of protein aggregation in AD and PD do not meet a minimum set of Bradford Hill's criteria to support a causal role. Although the absence of support for causality in the literature of protein aggregation in human studies does not establish absence of causality,[25] these findings encourage the examination of alternative pathogenic hypotheses in PD and AD.

Figure 1 Flow chart of study selection



CENTRAL = Central Register of Controlled Trials.

Current model of disease and challenges

Ongoing work on biomarker development and disease-modifying therapies is partly built on a model of protein misfolding and subsequent aggregation as an initial process in a cascade of subsequent, serial events held as causal to a single disease. In PD, it is proposed that α-synuclein monomers assemble into oligomers, which in turn give rise to α-synuclein fibrils, the earliest disease-causing abnormalities, and the major protein contribution of Lewy bodies and Lewy neurites (figure 2, upper panel).[26] It is surmised that α-synuclein aggregation leads to neuronal dysfunction, loss of connectivity, and neuronal death. In AD, it is proposed that the amyloid precursor protein cleavage product and hyperphosphorylated tau protein aggregate into extracellular plaques and intracellular neurofibrillary tangles, respectively.[12] These plaques and tangles may then elicit a variety of secondary metabolic and molecular changes, which in turn lead to cellular dysfunction that (1) perpetuates the formation of additional pathogenic protein aggregates or (2) directly accelerates or magnifies the effect on cell death through these molecular changes ("kindling" phenomenon)[11] (figure 2, lower panel). Nevertheless, 3 main arguments challenge these models.

First challenge: Uncertain temporality and biological gradient

Unlike other fields in medicine, brain tissue is not readily accessible for diagnostic or research purposes. Instead, research endeavors have relied on specimens obtained from 2 sources: (1) extracranial sources (e.g., biopsies of skin, salivary gland, gut; samples of blood and CSF) collected during life and (2) the brain itself, harvested postmortem, many years after events of interest have taken place. Even pathology studies that include samples from patients with different disease severity or "stages" are inherently cross-sectional. In an attempt to assess the temporality of pathologic events, AD biomarker-to-autopsy validation studies have yielded extraordinary progress, with in vivo CSF sampling and PET imaging of Aβ/tau now accepted as valid surrogates for protein brain deposition.[27] These advances have led to 2 observations in AD: that Aβ and tau, sequentially or concurrently, may be placed at the start of a chain of events before the onset of cognitive dysfunction, at what we assume to be time zero (see "Temporality" criteria in tables 1 and e-2)[15–17]; and that the co-presence of Aβ and tau leads to greater cognitive decline than either alone (see "Biological gradient" criteria in tables 1 and e-2 [doi.org/10.5061/dryad.g1nq02r]).[15,16] There is no corresponding neuroimaging or CSF biomarker counterpart for idiopathic PD. While this in vivo visualization has been assumed to represent time zero, it is also plausible that disease pathogenesis begins before the aggregation of proteins becomes detectable, rendering them as intermediate rather than initiating events in the pathway to cell death.

Second challenge: Incidental pathology, copathology, and nonspecific pathology

Without the ability to serially examine living brain tissue for potentially dynamic components in cellular proteostatic mechanisms, diseases are classified as proteinopathies (e.g., synucleinopathy) based on protein aggregates as end products. For synucleinopathies, for instance, this is the case in PD, multiple system atrophy, and dementia with Lewy bodies (DLB), as well as nonparkinsonian (peripheral) synucleinopathies, such as pure autonomic failure, and atypical synucleinopathies, including selected neurodegenerations with brain iron accumulation such as mitochondrial membrane protein-associated neurodegeneration,[28] the parkinsonian lysosomal disorders Kufor-Rakeb syndrome, Gaucher disease, and Chediak-Higashi syndrome, and the nonparkinsonian Sanfilippo syndrome.[29,30] The α-synuclein ubiquity permeates into AD: Lewy body pathology accumulates in over 50% of autopsy-proven AD brains[31] and, conversely, 77% of autopsy-proven PD dementia or DLB cases exhibit AD pathology.[32] Separately, the classic AD proteins Aβ and tau-positive neurofibrillary tangles have been respectively reported in 13% and 48% of sporadic Creutzfeldt-Jakob disease cases.[24] Given their widespread appearance in a variety of phenotypically diverse diseases, pathologies of α-synuclein and Aβ/tau may not be pathogenic (see "Specificity" criteria in tables 1 and e-2 [doi.org/10.5061/dryad.g1nq02r]).

The term "incidental pathology" has been applied to the finding of protein aggregation on postmortem studies of neurologically normal individuals. In the framework of α-synuclein- and Aβ/tau-based proteins as AD and PD "pathology," it is often assumed that, when found on autopsy, these might represent prodromal individuals, on the road to clinical disease had they

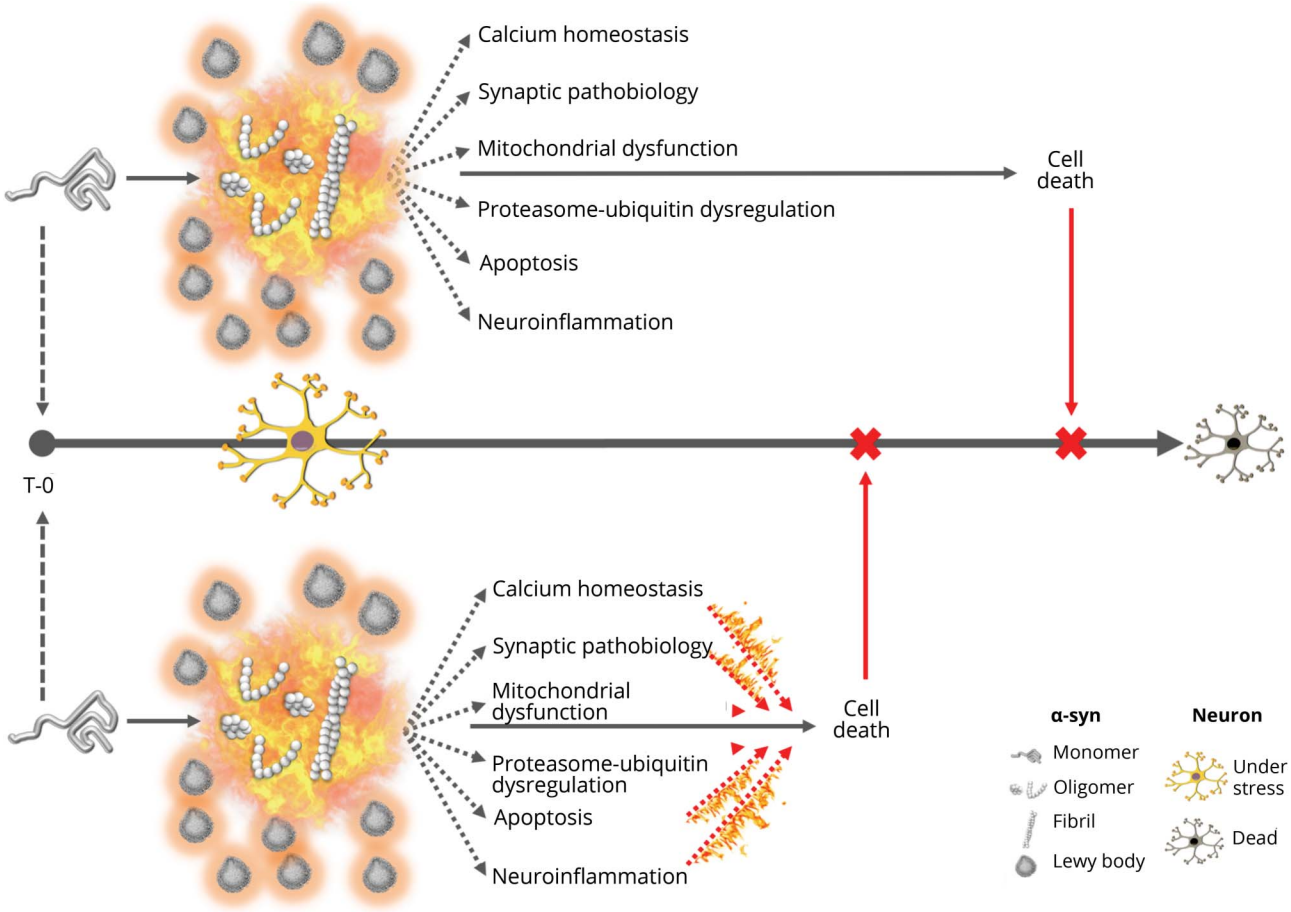
Table 1 Bradford Hill criteria applied to human studies of α-synuclein (α-syn) and β-amyloid (Aβ)/tau aggregation in Parkinson disease (PD) and Alzheimer disease (AD)

	Criteria definition	PD	AD
Strength	α-Syn/Aβ/tau aggregation significantly correlates with PD/AD using appropriate methodology and accounting for confounding factors	Supporting: 3[e1–e3]	Supporting: 10[e4–e13]
		Opposing: 0	Opposing: 0
		Conclusion: Support	Conclusion: Support
Consistency (replication)	Multiple studies using several locations, populations, and methods show a consistent correlation between α-syn/Aβ/tau aggregates with PD/AD	Supporting: 3[e1–e3]	Supporting: 10[e4–e13]
		Opposing: 0	Opposing: 0
		Conclusion: Support	Conclusion: Support
Temporality	Cause preceding the effect: time interval between α-syn/Aβ/tau aggregation ascertained in vivo before symptom development in PD/AD	Supporting: No studies	Supporting: 3[15–17]
		Opposing: No studies	Opposing: 0
		Conclusion: No support	Conclusion: Support
Experiment	Interventions targeted against α-syn/Aβ/tau aggregates decrease PD/AD incidence, severity, or rate of progression	Supporting: 0	Supporting: 0
		Opposing: 0	Opposing: 7[e14,18–23]
		Conclusion: No support	Conclusion: No support
Biological gradient	Dose-response curve between α-syn/Aβ/tau aggregates concentration and PD/AD incidence, severity, or rate of progression	Supporting: 0	Supporting: 5[e15–e18,17]
		Opposing: 2[e1,e2]	Opposing: 0
		Conclusion: No support	Conclusion: Support
Plausibility	α-Syn/Aβ/tau aggregates affect molecular pathways associated with PD/AD	Supporting: 2[e19,e20]	Supporting: 5[e22–e26]
		Opposing: 1[e21]	Opposing: 4[e27–e30]
		Conclusion: Uncertain	Conclusion: Equivocal
Analogy	If α-syn/Aβ/tau aggregates cause PD/AD, similar protein aggregations could also cause PD/AD	A pathologic definition of PD precludes this analysis to avoid circular reasoning	A pathologic definition of AD precludes this analysis to avoid circular reasoning
Specificity	If α-syn/Aβ/tau aggregates cause PD/AD, these proteins should not be present in diseases other than PD/AD or healthy controls	Supporting: 0	Supporting: 0
		Opposing: 10[e31,e32,e34–e41]	Opposing: 10[24,e33–e41]
		Conclusion: No support	Conclusion: No support
Coherence	Cause–effect should be consistent across all available evidence, without contradictions or discrepancies	Supporting: 0	Supporting: 0
		Opposing: 13[e1,e2,e21,e31,e32,e34–e41]	Opposing: 21[e14,18–24,e27–e30,e33–e41]
		Conclusion: No support	Conclusion: No support

Adapted from references 8, 9, and 25. Individual characteristics of supporting and opposing studies are supplied in table e-2 (doi.org/10.5061/dryad.g1nq02r).

lived long enough. However, supra-surviving individuals (the over-90 or oldest-old) exhibit high frequency of AD pathology without dementia (~50%)[33,34] and PD pathology without parkinsonism (~25%).[35] Notably, no cognitive tests among the oldest-old without dementia can predict AD neuropathology within 3 years before death.[36] In fact, neuropathology studies in the oldest-old have either shown no correlation between premortem cognitive function and AD neuropathology[37] or even a paradoxical association between normal cognition and increased AD neuropathology.[38] The 90+ Study (183 brain autopsies from individuals who died at or after the age of 90 years) yielded robust support for an inverse relationship between AD pathology and clinical dementia, even beyond the simple observation that intermediate to high AD pathology clusters similarly in people with (23%) and without dementia (28%).[39] Further analysis of the data suggests a protective effect of AD pathology. Compared to non-AD pathology (microinfarcts and white matter disease; dementia, n = 19; no dementia, n = 6), AD pathology (dementia, n = 23; no dementia, n = 24) was associated with a significantly reduced risk of dementia (odds ratio 0.3; 95% confidence interval 0.1–0.9; $p = 0.03$; analysis not in the original publication).[39] Therefore, it is plausible that these individuals may have lived well beyond a normal lifespan without neurologic symptoms because of, not despite, AD pathology. Under this framework, Aβ and tau aggregation may have served as a mechanism to compensate for active disease-causing biological abnormalities (while minimizing the presumably toxic soluble fibrils required

Figure 2 Current model of protein aggregation in Parkinson disease (single disease model)



Abnormal soluble oligomers and fibrils of α-synuclein are directly pathogenic (upper panel). Alternatively or complementarily, secondary molecular changes created after protein aggregation combine with oligomers and fibrils to hasten cell death (lower panel). T-0 = time zero; α-syn = α-synuclein.

to form them), with clinical disease appearing after this mechanism is overwhelmed (see "Specificity" criteria in tables 1 e-2 [doi.org/10.5061/dryad.g1nq02r]).

Third challenge: Experimental failure in anti-amyloid therapies

Anti-amyloid therapies that target insoluble Aβ forms (e.g., AN1792, gantenerumab, bapineuzumab), soluble forms (e.g., solanezumab), or prevent its formation (e.g., semagacestat, γ-secretase inhibitor) have failed to produce cognitive benefits in AD.[18–23] Some agents have been associated with adverse events (e.g., AN1792, aseptic meningoencephalitis[18]; gantenerumab and bapineuzumab, Aβ-related abnormalities[19,20]; semagacestat, skin cancer and infections),[21] possibly due to immunologic mechanisms (e.g., AN1792, T-cell response)[18] or impaired protein processing (e.g., semagacestat, Notch protein)[21] in selected cases. Although unsuccessful or insufficient Aβ load reduction may account for clinical futility, an issue that may be definitively answered after the ongoing phase III aducanumab trial results are reported (ClinicalTrials.gov Identifier: NCT02477800), one can argue that protein aggregation is not the culprit for cell death (see "Experiment" criteria in tables 1 and e-2 [doi.org/10.5061/dryad.g1nq02r]).

Alternative models of disease

If α-synuclein and Aβ/tau protein aggregation are not in a direct causal pathway in PD and AD, they may still contribute to neurodegeneration, or be an epiphenomenon, or guard against toxic soluble forms of the proteins by sequestering them as innocuous insoluble forms. Each of these 3 hypothetical models may be influenced by lifelong exposure to genetic and environmental factors that might contribute to increased susceptibility, exhaustion of adaptive responses, and, ultimately, cell dysfunction and death. We use α-synuclein to illustrate these alternative models (figure 3), but they apply similarly to Aβ and tau protein aggregation.

Alternative model 1: Protein aggregation as accelerator of pathology of multiple diseases

Aside from disorders where a genetic mutation causes initial accumulation of α-synuclein (e.g., missense mutations and multiplication of *SNCA*)[4] or of Aβ (e.g., triplication of *APP*

in Down syndrome or amyloid precursor protein [*APP*] gene mutations and duplication),[5] in which these protein aggregates are truly pathogenic, it is plausible that accumulation of α-synuclein or of Aβ/tau enhances other primary pathogenic molecular mechanisms of neurodegeneration in sporadic forms of PD and AD (figure 3A). Putatively, a prion-like propagation of α-synuclein fibrils between adjacent cells[29] falls into this category, without requiring that oligomers and fibrils act as the initial pathogenic event. This model accepts heterogeneous upstream pathogenic events, but relies on soluble proteins (α-synuclein, Aβ) misfolding and aggregating, as a convergent driver of cellular death. In this model, misfolded variants of α-synuclein and Aβ are suitable for biomarker development and therapeutic targeting.

Alternative model 2: Protein aggregation as epiphenomena of multiple diseases

According to this model, a range of molecular abnormalities lead to cells being unable to adequately degrade misfolded proteins, which aggregate into α-synuclein fibrils in Lewy bodies (figure 3B; or from misfolded amyloid precursor protein into misfolded oligomers, then fibrils, then plaques in AD). These protein aggregates become byproducts (common denominators or epiphenomena) of several pathogenic pathways, and themselves exert no pathogenic or protective role. According to this model, different underlying molecular abnormalities remain pathogenic of different molecular diseases; their relationship to one another rests only in the type of protein they cannot properly recycle.

Alternative model 3: Protein aggregation as protective mechanism of multiple diseases

In this model, molecular abnormalities affect the cellular machinery that normally degrades proteins. These misfolded proteins are sequestered into Lewy bodies as a mechanism to promote maintenance of neuronal and synaptic function despite coexistent and actively disrupting molecular abnormalities (figure 3C). Each molecular abnormality is pathogenic to a specific disease, which shares with others only the type of protein actively sequestered as a protective mechanism. Such protective sequestration of proteins could allow neurons to function for decades before becoming overwhelmed, delaying the progression of neurologic disease. In this model, targeting soluble oligomers or fibrils (e.g., using anti-aggregant therapies or possibly passive or active immunotherapy) might potentially be deleterious if the toxicity of any of the protein species is outweighed by their protective role in the human brain under molecular stress.

Implications for biomarkers and clinical trials

Hypothesis 1: Biomarkers of protein aggregates are relevant to common pathology but not to subgroup pathogenesis

Based on the challenges discussed above, abnormal protein aggregates (pathology) may represent downstream events, unhelpful to classify PD and AD with pathogenic relevance. Thus far, biomarker development efforts have been based on disease models of clinico-pathologic convergence. One such convergence biomarker is [^{123}I]-FP-CIT SPECT imaging for striatal dopamine deficiency. It is positive in virtually everyone with PD. Because biomarker discovery has been based on clinically defined cohorts (e.g., tremor-dominant PD vs postural instability gait disorder type), significant results within and between cohorts have substantial overlap.[40]

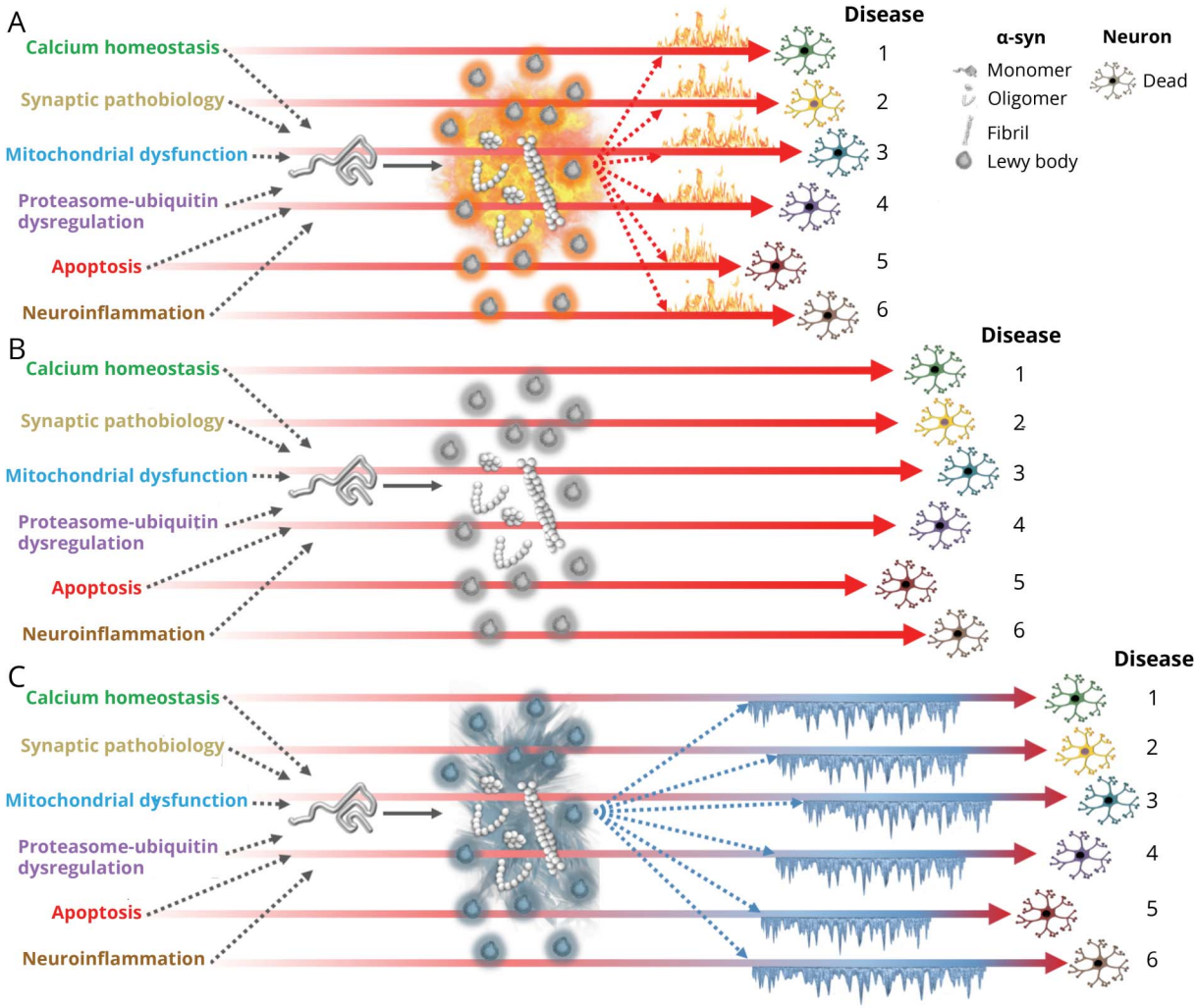
Hypothesis 2: Disease-modifying treatments targeting oligomers or fibrils might be futile or deleterious because these proteins are epiphenomena or protective in the human brain under molecular stress

Targeting these proteins across sporadic or otherwise non-genetic disease forms is unlikely to slow progression of these disorders, no matter how early the treatments are implemented (i.e., prodromal stage), unless protein aggregation acts as an accelerator of pathology, as suggested in alternative model 1. The corollary is that applying molecular therapies aimed at achieving neuroprotection to clinically (pathologically) defined, but not molecularly subtyped, populations will remain unlikely to succeed. The history of medicine has repeatedly shown that single (untreatable) clinical diseases are often replaced by several (treatable) molecular diseases. As a consequence, treatments designed to target specific mechanisms work best in certain disease subtypes, but not in others.

Relinquishing reductionism (the clinically heterogeneous but single-disease concepts for AD and PD) will require accepting that sophisticated data analyses of large cohorts will not be capable of identifying molecular disease subtypes, as the analyses cannot overcome the fundamental flaw of existent datasets: cohorts invariably assembled based on strict clinical criteria. Big data may not easily be converted into good data (for the purposes of assisting precision medicine) if the data are based on binary definitions of AD vs control and PD vs control. Important insights may be derived, on a smaller scale, from studying rare genetic forms of disease (e.g., PD-LRRK2, PD-GBA, AD-PS1), for which targeted therapeutic efforts may be most likely to succeed—in these subtypes. On a wider scale, molecularly targetable neurodegenerative diseases may be best identified from population-based studies applying non-hypothesis-based analytic approaches to biospecimens from large cohorts of aging people, carefully characterized across a spectrum of brain functions and deficits. Thus, aging cohorts ought to include atypical clinical presentations and other, ostensibly unrelated neurologic disorders beyond the current criteria for PD and AD. The analyses will need to be anchored on biological signals, not on clinical phenotypes or clinical criteria, and account for genetic and lifelong biochemical and environmental factors. Molecular subtypes of disease thus identified may or may not fall into standard clinical classifications.

The first proven neuroprotective therapy might only work in fewer than 5% of everyone currently subsumed as "AD" or

Figure 3 Alternative models of protein aggregation in Parkinson disease (multiple disease model)



Abnormal soluble oligomers and fibrils of α-synuclein (α-syn), while not directly pathogenic, act as accelerators of neurodegeneration ("fueling the fire") due to early pathogenic molecular abnormalities, each representing molecularly distinct diseases (A, model 1). Alternatively, abnormal soluble oligomers and fibrils of α-synuclein aggregate into Lewy bodies as byproducts of earlier pathogenic molecular mechanisms, without directly affecting the neurodegenerative process brought on by each molecular disease (B, model 2). Finally, α-synuclein aggregates into Lewy bodies as a mechanism to protect the neuron from toxic protein species or from the biological dysfunction that may have generated the formation of toxic species, "cooling" progression of cell degeneration under biological stress (C, model 3). Note that each molecularly defined disease has a different time to death; collectively, time to neuronal death is longest in diseases corresponding to model C.

"PD" (e.g., GZ/SAR402671 in early-stage PD-GBA [ClinicalTrials.gov Identifier: NCT02906020]) or in specific at-risk populations (e.g., solanezumab or gantenerumab for healthy individuals with an autosomal dominant AD-causing mutation [Dominantly Inherited Alzheimer Network Trial; ClinicalTrials.gov Identifier: NCT01760005]). Such disease-modifying treatment in neurodegenerative disease will help usher in the biomarker-driven disease subtyping strategy long adopted by oncology and other fields of medicine.

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Luca Marsili, MD, PhD	University of Cincinnati	Author	Literature search, review and critique of the manuscript
Anthony E. Lang, MD, FRCPC	University of Toronto, Canada	Author	Review and critique of the manuscript
David K. Simon, MD, PhD	Harvard Medical School, Boston, MA	Author	Review and critique of the manuscript
Aristide Merola, MD, PhD	University of Cincinnati, OH	Author	Review and critique of the manuscript
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Francesca Morgante, MD, PhD	University of Messina, Italy, and St George's University of London, UK	Author	Review and critique of the manuscript
Rodolfo Savica, MD, MSc	Mayo Clinic, Minneapolis, MN	Author	Review and critique of the manuscript
J. Timothy Greenamyre, MD, PhD	University of Pittsburgh, PA	Author	Review and critique of the manuscript
Francesca Cambi, MD, PhD	University of Pittsburgh, PA	Author	Review and critique of the manuscript
Tritia R. Yamasaki, MD, PhD	University of Kentucky, Lexington	Author	Review and critique of the manuscript
Caroline M. Tanner, MD, PhD	University of California–San Francisco	Author	Review and critique of the manuscript
Ziv Gan-Or, MD, PhD	McGill University, Montreal, Canada	Author	Review and critique of the manuscript
Irene Litvan, MD	UC San Diego, CA	Author	Review and critique of the manuscript

Appendix *(continued)*

Name	Location	Role	Contribution
Ignacio F. Mata, PhD	University of Washington, Seattle	Author	Review and critique of the manuscript
Cyrus P. Zabetian, MD, MS	University of Washington, Seattle	Author	Review and critique of the manuscript
Patrik Brundin MD, PhD	Van Andel Research Institute, Grand Rapids, MI	Author	Review and critique of the manuscript
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