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INVITED REVIEW

Risk of Transmissibility From Neurodegenerative Disease-Associated Proteins: Experimental Knowns and Unknowns

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Abstract

Recent studies in animal models demonstrate that certain misfolded proteins associated with neurodegenerative diseases can support templated misfolding of cognate native proteins, to propagate across neural systems, and to therefore have some of the properties of classical prion diseases like Creutzfeldt-Jakob disease. The National Institute of Aging convened a meeting to discuss the implications of these observations for research priorities. A summary of the discussion is presented here, with a focus on limitations of current knowledge, highlighting areas that appear to require further investigation in order to guide scientific practice while minimizing potential exposure or risk in the laboratory setting. The committee concluded that, based on all currently available data, although neurodegenerative disease-associated aggregates of several different non-prion proteins can be propagated from humans to experimental animals, there is currently insufficient evidence to suggest more than a negligible risk, if any, of a direct infectious etiology for the human neurodegenerative disorders defined in part by these proteins. Given the importance of this question, the potential for noninvasive human transmission of proteopathic disorders is deserving of further investigation.

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INTRODUCTION

Recent data raise the possibility that classical neurodegenerative diseases share characteristics with prion diseases (1–13). If so, this would be of scientific interest, have potential importance for therapeutic approaches, and, in the extreme case, have public health considerations. A group was convened by the National Institute of Aging to discuss the evidence regarding this hypothesis and the types of remaining data needed to ascertain research priorities toward understanding this phenomenon better.

The characteristics of "prion-like behavior" include (1) evidence for protein-based templated misfolding, leading to amplification of a misfolded species and the formation of *proteopathic seeds* (this nomenclature has been adopted in accord with a similar conference held among European experts and will be used here as it remains agnostic to disease classification [14]); (2) capacity for "transmissibility" between individuals of the same species and (less commonly) across species, with species barriers often present; (3) generally the existence of polymorphic "strains" defined as having different conformers that lead to differences in neuropathological and clinical phenotypes; (4) the ability to "propagate" or spread along with neural systems which may translate into clinical progression of disease; and (5) striking stability of pathologic aggregates, often with marked protease resistance. We briefly review these issues for the proteopathic seeds that have been described for amyloid-β (Aβ) and tau, associated with Alzheimer disease (AD), other tauopathies, and for α-synuclein, associated with Parkinson disease (PD), Lewy body disease (LBD), and multiple system atrophy (MSA). Given the overlapping features of proteopathic seeds between prion diseases (Creutzfeldt-Jakob disease, fatal familial insomnia, etc.) and other neurodegenerative protein aggregation disorders, it is reasonable to consider whether research biohazard and clinical safety measures need to be aligned across some or all of these diseases. There is experimental evidence that each of these misfolded proteins in neurodegenerative diseases can behave as proteopathic seeds, but it is critical to understand whether the "potency" of any of these proteopathic seeds is sufficient to pose a risk in laboratory, clinical, or public health contexts. For these reasons, it is essential to address questions about the mechanistic biology of protein aggregation, cell-to-cell transmission and then, by extension, potential broader consequences for investigators, patients, and healthcare providers. We close by focusing on a series of recommendations regarding research risk, experimental data needed for guidance, and comment on the adequacy of "universal precautions" for safeguarding both patients and providers.

AMYLOID BETA

Amyloid beta (Aβ) is a small amphipathic peptide that accumulates as amyloid plaques in the neuropil in AD. In vitro, it readily fibrillizes, and in vivo, in mouse experiments, plaques can be seen to nucleate and grow quite quickly (15). Aβ fibrils are polymorphic in appearance and biochemical characteristics, with various lengths and morphological characteristics. Advanced biophysical examination of Aβ derived from brain tissue of patients with AD shows polymorphic structures (16). There is a typical distribution of plaques in AD brains, with brain areas that are connected among the "default network" typically affected early; in animal models, there has also been some demonstration of "propagation" along neural systems (17, 18) suggesting some axonal transport of seeds that ultimately lead to extracellular deposits.

Injection of Aβ seeds derived from human AD into a mouse leads to the development of plaques in mice (if they express a human amyloid precursor protein sequence), suggesting to some extent a "species barrier" given the differences in sequence between human and mouse Aβ. Recent data suggest that only minute quantities of the "seed" are necessary and that the seed can be introduced directly into the CNS, or peripherally (even intraperitoneally [19]) with consequent development of plaques in the brain months later in vulnerable mice (20). Even wires soaked in Aβ containing brain homogenate can initiate and propagate amyloid plaques after intracerebral inoculation in susceptible mice (21).

The clearest evidence for potential transmission to humans comes from observations that some cohorts of individuals exposed to cadaveric-derived human growth hormone demonstrated subsequent emergence of amyloid deposition at unexpectedly younger ages (22). Although this finding was consistent with that in another cohort of growth hormone recipients, no deaths attributable to AD were found in a long-term mortality study of the cohort (23, 24). Additionally, there are suggestive data from recipients of dura mater allografts (25–28), as well as in individuals who underwent neurosurgical procedures as children or young adults, developed vascular wall deposition of Aβ in the form of cerebral amyloid angiopathy (CAA) and hemorrhage several decades later (8, 29, 30). However, these cases are exceedingly rare, and there is a counter-argument that the "trauma" associated with graft placement has been put forward as a cause of amyloid deposition (31). Neither cadaveric-derived human growth hormone nor dura matter allografts are still manufactured for clinical use, which limits any potential for future exposure risk. Ongoing surveillance of exposed individuals remains important.

TAU

Strong data also suggest that tau becomes misfolded in neurodegenerative diseases including progressive supranuclear palsy (PSP), some forms of frontotemporal dementia, chronic traumatic encephalopathy, and, most commonly, AD (31–37). The consequences of misfolding in each of these diseases are the accumulation of intracytoplasmic aggregates that can be propagated to cells and to animals (38–41). Tau aggregates across these disorders are distinctly misfolded and composed of distinct protein variants such that the distinctive lesions differ in their biochemistry (e.g. isoforms of tau and patterns of hyperphosphorylation), cells affected, the morphology at the light microscopy, electron microscopy (EM), and, recently, cryo-EM appearance. Thus, tau adopts different

stable conformations, consistent with the notion of "strains" as may be seen in genetic and phenotypic variation, but also arguably with the concept of genetic and phenotypic diversity, such as seen with different environmental stimuli (39, 42, 43).

Since tau is expressed predominantly in neurons, rather than glial cells, the observation of tau aggregates in astrocytes and oligodendrocyte has been used to support the concept that release of (likely misfolded) tau from neurons (or oligodendroglia [40]) and results in uptake into other cells. The release of tau from neurons as a naturally occurring phenomenon is unequivocally supported by the presence of tau in the cerebrospinal fluid (CSF). A substantial amount of experimental data in cell and mouse models shows that neuron-to-neuron transmission of tau is also possible, and, in experimental systems, can occur on the scale of days to weeks, perhaps even faster. Strong experimental data in cells and mice also suggest that a particular type of misfolded tau can "instruct" wild-type tau molecules to adopt that conformation and engage in templated misfolding (2, 7, 10, 44–47). Unlike classical prion disease, there does not appear to be a large amount of sequence specificity in this regard: human P301L tau and wild-type mouse tau coexist in tau aggregates in transgenic mice, and multiple different isoforms of tau coaggregate in paired helical filaments in AD while in other disorders, such as PSP, tangles contain only a single isoform. A substantial amount of data from cell and mouse models indicates neuron-to-neuron transmission of tau aggregates, and, in experimental systems, this can occur on the scale of days to weeks.

In conclusion, current data suggest that inoculation of misfolded tau derived from human brain tissue into the brain of an experimental animal (generally one that is overexpressing human tau, often in mutant forms) can lead to a tauopathy-like picture within months of inoculation, and that this material remains bioactive over generations of mice. Current data in experimental systems suggest that (misfolded) human tau can be taken up by neurons, is amplified by "corrupting" endogenous human or mouse tau through templated misfolding and can be transmitted across neural systems to spread throughout the brain. No current data are available regarding transmissibility of tau in humans.

α-SYNUCLEIN

Like tau, α-synuclein, a natively unfolded protein, becomes misfolded and aggregates in cell inclusions and neurites in neurodegenerative diseases including PD, LBD, MSA, and, not uncommonly, in selected brain regions, in AD. α-Synuclein inclusions may be primarily neuronal as in PD and LBD, but in MSA, oligodendroglial inclusions are prominent. The Braak hypothesis suggests that the anatomical distribution of lesions in PD are linked by connections, perhaps from the gut, supporting a role for propagation of α-synuclein across neural systems (48). More direct evidence for α-synuclein propagation between neurons comes from the striking observation of a few Lewy bodies in grafted fetal neurons in the substantia nigra of Parkinson patients, years after the transplant (49, 50). α-Synuclein proteopathic seeds have been implicated in MSA (51–55). Like tau, the specific molecular misfolding that occurs leads to aggregates that differ in the cells affected, the morphological shape of aggregates at the light microscopy, EM, and, recently, cryo-EM levels where fibril polymorphs are observed (56).

Since, under physiological conditions, α-synuclein is thought to be expressed predominantly in neurons, and not expressed in glial cells, the observation of α-synuclein aggregates in glia suggests release of (likely misfolded) α-synuclein from neurons and uptake into other cells, suggesting at least local propagation. A similar conclusion is drawn from the presence of α-synuclein aggregates in the engrafted fetal neurons placed for potential treatment of PD, a few years after grafting (49, 50). Since multiple different aggregated forms of α-synuclein exist in different diseases, α-synuclein adopts different stable conformations, again consistent with the idea of "strains" as was discussed above for tau. A substantial amount of experimental data in cell and mouse models show that neuron to neuron transmission of α-synuclein is also possible, and, in experimental systems, can occur on a fairly rapid time scale (days) (57–60).

Artificial recombinant α-synuclein fibrils clearly can propagate after being injected into experimental animals (61–64). α-Synuclein derived from human neuropathological conditions appears to be even more potent, and to some extent replicate the disease phenotype of the brain that the α-synuclein was isolated from, with differential patterns of propagation and inclusions of α-synuclein derived from PD or MSA (see, e.g. [65]). Unlike classical prion disease, there do not appear to be strong species barriers for α-synuclein, with human α-synuclein fibrils causing α-synuclein aggregation in wild-type mice.

Thus, strong data suggest that misfolded human α-synuclein can be taken up by neurons and glial cells, which is amplified by "corrupting" endogenous α-synuclein through templated misfolding and be transmitted across neural systems to spread throughout the brain. However, direct evidence in humans suggesting propagation across cells is limited to the studies of the occurrence of Lewy bodies in fetal neurons in transplants, and the reasoning that oligodendroglial inclusions are likely derived from neuronal α-synuclein; however, there is currently no direct confirmation of human transmission. Moreover, the idea that PD itself reflects intrinsic vulnerability of neural systems, rather than a prion-like spread, has been articulated (66–68) and provides a counter-point to a strict "spreading" hypothesis; this argument can in general be applied to α-synuclein, tau, and Aβ.

RESEARCH ISSUES (TISSUE SPECIMENS AND BIO FLUIDS; CELL CULTURE MODELS; ANIMAL MODELS), AND COMPARISON TO PROCEDURES FOR PRION DISEASES

For established prion diseases, there are distinct and greater requirements for handling for animal models, cell culture systems, tissue (both human and animal), and biological fluids in Biosafety level 2 (BL2) facilities (e.g. https://www.phe.gov/s3/BioriskManagement/biosafety/Pages/Biosafety-Levels.aspx; https://www.cdc.gov/labs/pdf/CDC-Biosafety-MicrobiologicalBiomedicalLaboratories-2009-P.PDF), similar to many other circumstances, although decontamination of

surfaces and instruments requires special chemicals. Some institutions may require higher levels of biosafety, especially in human research settings. Currently, no comparable guidelines are in place for neurodegenerative disease tissue specimens, biofluids, cell culture, animal model, or biochemical studies beyond good laboratory practices, and, for human-derived specimens, "universal precautions." While we might argue that being "overly safe" presents minor disadvantages, were requirements comparable to prion disease be put in place for other neurodegenerative disease systems, there could be notable operational and financial burden which might constitute substantial barriers to advancement in the field. Therefore, such requirements should be recommended only with due consideration about whether current procedures are adequate to provide protection. For example, simple formaldehyde fixation does not inactivate proteopathic seeds (69–71), whereas hypochlorous acid appears to be more effective (72). Similarly, there are distinct and more elaborate processes for handling of biospecimens and surgical as well as other instruments (endoscopes, etc.) from individuals with possible prion disease. Again, extending similar precautions to individuals with other neurodegenerative diseases could represent a barrier to care and treatment, particularly given the far greater incidence of diseases, such as AD and PD in the aging population along with the longer prodromal and preclinical phases (years to decades) for many of these diseases when compared with prion diseases. Thus, it is important to address the question as to whether current universal precautions and decontamination/cleaning methods are adequate to address any transmissibility risk in neurodegenerative diseases. Finally, returning to the research arena, the laboratory handling, distribution, and potential exposure to human tissue, and other biospecimens, would need to be addressed in the same manner.

APPLICATION TO PUBLIC HEALTH, RISK TO HEALTH PROFESSIONALS, AND INFORMATION NEEDED FOR PUBLIC POLICY DECISIONS

Importantly, there are little or no clinical data to suggest "infectivity" or "transmissibility" of tau, α-synuclein, or Aβ, but direct examination in which research focuses on this point is largely missing. For example, there is no known increased risk of these disorders among those healthcare professionals who might be expected to have increased relative exposure, such as neuropathologists or neurosurgeons, but the data are largely absent rather than negative. Similarly, no epidemiological data suggest that partners or close contacts of affected individuals are at any higher risk than the general public for developing their partner's disease. It was shown that in a series of cadaveric human growth hormone (hGH) recipients who died of iatrogenic Creutzfeldt-Jakob disease, none died with Alzheimer or Parkinson neuropathologic changes (24). In contrast, in other series of cadaveric hGH recipients who developed iatrogenic Creutzfeldt-Jakob disease, some cases did develop cerebral amyloid angiopathy (22, 23, 73, 74), with one series suggesting that about half of the cases developed at least amyloid deposits around cerebral blood vessels. Thus, the development of amyloid lesions, and the full picture of AD, may well be dissociated. Together, these data suggest that the transmissibility of the AD-related proteopathic seeds, at least in the context of these clinical studies, is less than Creutzfeldt-Jakob prions. Thus, while no clear data currently suggest a public health risk to individuals or physicians, this issue has not been deeply explored.

There are a series of critical questions to be answered about the transmission process that would need to be answered to more definitively assess risk:

- infectivity (titer in tissue, biological fluids; infectious unit);
- stability (time, temperature, freezing/fixation);
- inactivation (fixation, be contaminants);
- anatomic distribution of infectivity (brain versus CSF versus blood versus other tissues); and
- horizontal transmission/environmental exposure.

Issues that require understanding derived from biological experiments include greater insight into the molecular identity of the proteopathic seeds:

- What is the half-life of seeds introduced into animals or cultured cells?
- Can peripheral exposure, under any circumstances (for example with disruption of the blood-brain barrier), lead to CNS access with subsequent spread of proteopathic seeds?
- Do standard neuropathologic handling methods (fixation, tissue processing, etc.) block bioactivity (69, 75, 76)? Initial data suggest, for example, that formaldehyde may not be sufficient for inactivation of proteopathic seeds (53, 69–71).
- What are the stability characteristics of proteopathic seeds?
- How long might proteopathic seeds persist in a host (77), which would require assessment of cumulative risk rather than single exposure risk?

When assessing these characteristics of proteopathic seeds from human tissue, animal and cell culture models, and synthetic material, it is important to ensure that biological assays are used for assessment of potency rather than just their biophysical properties. In addition to the cell-based and animal transmissibility models noted above, distinct cell-free systems that use protein conformational changes as measures of seeding (e.g. RT-QuIC) promise both an ability to discriminate distinct conformational patterns (65); increased sensitivity has become available in recent years (10, 72, 78–87). Optimal assessment of integrity of proteopathic seeds would employ multiple methods, as methods may differ in their sensitivity and it is not possible to determine *a priori* how such sensitivity in a given assay corresponds to exposure risk.

Such information would be critical to more fully assess potential risk in laboratory settings, and determine if further measures beyond "universal precautions" and common sense used with all human autopsy material to prevent exposure to infectious or toxic materials might be warranted in neurodegenerative disease-focused laboratories or if distinct animal handling requirements were needed for models of neurodegenerative diseases. Issues around the potential broader clinical and public health concerns raised by proteopathic seeds have recently been addressed by a European group, which made a series of proposals based on observations regarding

transmissibility of Aβ from experimental and clinical settings (14). It remains too early to suggest that comparable approaches should be followed in the setting of other proteins that demonstrate comparable biologic potential, although it is possible that further experimental evidence will guide similar levels of caution. It also remains a task for the scientific community to pursue further studies designed to address the outstanding questions raised above. Information gained from such studies will help more definitively settle the questions of whether neurodegenerative diseases can, under any circumstances, be communicated via proteopathic seeds person to person, in either research or clinical settings.

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