Distinct prion-like strains of amyloid beta implicated in phenotypic diversity of Alzheimer’s disease

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\textbf{ABSTRACT.} Vast evidence on human prions demonstrates that variable disease phenotypes, rates of propagation, and targeting of distinct brain structures are determined by unique conformers (strains) of pathogenic prion protein (PrP\textsuperscript{Sc}). Recent progress in the development of advanced biophysical tools that inventory structural characteristics of amyloid beta (A\textsubscript{\beta}) in the brain cortex of phenotypically diverse Alzheimer’s disease (AD) patients, revealed unique spectrum of oligomeric particles in the cortex of rapidly progressive cases, implicating these structures in variable rates of propagation in the brain, and in distinct disease manifestation. Since only \textasciitilde30\% of phenotypic diversity of AD can be explained by polymorphisms in risk genes, these and transgenic bioassay data argue that structurally distinct A\textsubscript{\beta} particles play a major role in the diverse pathogenesis of AD, and may behave as distinct prion-like strains encoding diverse phenotypes. From these observations and our growing understanding of prions, there is a critical need for new strain-specific diagnostic strategies for misfolded proteins causing these elusive disorders. Since targeted drug therapy can induce mutation and evolution of prions into new strains, effective treatments of AD will require drugs that enhance clearance of pathogenic conformers, reduce the precursor protein, or inhibit the conversion of precursors into prion-like states.

\textbf{KEYWORDS.} Amyloid beta, Alzheimer’s disease, human prion strains, neurodegeneration
RAPIDLY PROGRESSIVE ALZHEIMER’S DISEASE (RPAD)

Rapidly progressive dementia (RPD) is a group of conditions that are characterized by an accelerated disease course. Although there is variability in definition, most studies consider dementia to be of rapid progression if severe dementia or death occurs within 2 years of symptom onset. The differential diagnosis of RPD is broad and includes neurodegenerative, autoimmune, infectious, metabolic, toxic, neoplastic, endocrine, and vascular etiologies. In-depth diagnostic testing is typically required to obtain the proper diagnosis, and in turn, directs the further management of the patient. Diagnostic studies typically include blood tests, brain magnetic resonance imaging (MRI), lumbar puncture, and full body computed tomography (CT) scans.

Alzheimer’s disease (AD) is usually not considered a RPD. While the clinical course varies widely, most cases of AD have a survival time of around 10 years from onset of symptoms to death. Initial symptoms typically include problems with short-term memory and word finding difficulties. These symptoms get progressively worse until they begin to affect daily functioning. Later in the disease course, patients also develop apraxia and agnosia. Neurological impairment, mostly due to severe apraxia, is typically a late stage feature of AD. Diagnosis is usually based on the above clinical symptoms of AD, lack of other possible etiologies, and the presence of biomarkers that are suggestive of AD (e.g., hippocampal volumes on brain MRI, amyloid and fludeoxyglucose positron emission tomography, and amyloid beta (Aβ) and p-Tau levels in the cerebrospinal fluid).

An atypical phenotype of AD that often leads to diagnostic confusion can be one of rapid progression (rpAD). Generally, rpAD is defined as a case of AD with a decrease in the Mini-Mental State Examination score of 6 or more points per year. Depending on how rpAD is defined, its estimated frequency is approximately 10-30% of all AD cases. Some studies have not detected any differences in age at onset, but 2 studies have demonstrated a statistically significant younger age at onset in rpAD cases (60 and 68 y of age respectively). Survival time of rpAD is typically a couple of years and there is an overrepresentation of females in rpAD samples, which is generally observed in AD samples as a whole.

Interestingly, the largest risk gene for AD seems to play little to no role in rpAD. The apolipoprotein E gene (APOE) e4 allele significantly increases the risk of developing AD in a dose response relationship and it also decreases the age of onset. Despite the large risk that APOE e4 contributes to the development of AD, it is vastly underrepresented in rpAD cases. Some studies have also suggested that methionine homozygosity at codon 129 of the prion protein gene (PRNP) may also contribute to rapid progression in AD, a phenomenon also observed in prion disease.

The clinical features of rpAD differ from what is typically observed in AD. The neuropsychological profile of rpAD is characterized by frontal lobe impairment early in the disease course. Individuals with rpAD demonstrate early executive dysfunction and language impairment. Psychiatric symptoms of apathy and psychosis are also associated with rpAD, although the directional relationship of this association is unclear. The most striking difference observed in rpAD is the early involvement of gross neurological symptoms. Neurological symptoms observed early in the course of rpAD include motor impairment, extrapyramidal symptoms, myoclonus, and gait disturbance. These symptoms typically are not observed in AD until the patient has reached the severe stage. Myoclonus is the most commonly described neurological symptom in rpAD samples (75%) aside from dementia.

The rapid progression and early neurological impairment observed in rpAD often mimics the clinical course of prion disease. From a clinical prospective, rpAD most resembles Creutzfeldt-Jakob disease (CJD) as opposed to AD. This often leads to diagnostic confusion that can be further exacerbated by CSF biomarker findings. Like CJD, rpAD can have elevated 14-3-3 protein and tau levels in the CSF, as both are surrogate markers of neuronal injury. One important differentiator is that p-Tau is
elevated in rpAD, whereas it is normal in CJD. Because most rpAD studies have been conducted through prion disease surveillance centers, one must consider that this may introduce a selection bias, hence capturing a subset of rpAD that closely resembles CJD. Although this clinical phenotype of rpAD obviously exists, it is less clear if there are other clinical phenotypes of rpAD that have yet to be elucidated.

rpAD is a very important clinical entity that deserves further study. Endophenotypes, the concept of identifying clinical phenotypes associated with underlying genotypes, can be helpful for exposing mechanisms of disease. Unlike most cases of AD, rpAD has a unique clinical, genetic, and CSF biomarker profile. Despite these differences, the underlying neuropathology is relatively the same, suggesting that something is specifically mediating biological features of the illness and possibly the risk of developing the illness (e.g., structural differences in a-beta). Identifying this powerful mediator can lead to the further understanding of AD and other neurodegenerative conditions, and possibly identify novel treatment targets.

**NEUROPATHOLOGY OF RAPIDLY PROGRESSIVE AD**

Although the structures that we now recognize as senile plaques had been described in the brains of dementia patients prior to the turn of the 20th century, it was Alzheimer’s observation of neurofibrillary tangles in association with such plaques that gave rise to the dementing illness that now bears his name. The seminal 1991 Braak and Braak study of autopsy brains from demented and non-demented individuals found that the distribution and density of amyloid deposits was of limited significance in staging the neuropathology of AD, while neurofibrillary tangles demonstrated a characteristic distribution pattern amenable to staging and clinical pathological correlation. Utilizing this staging system, prospective studies validated the assertions that limited neurofibrillary degeneration involving the entorhinal cortex was compatible with many years of normal cognition, while similar degeneration involving the limbic system usually manifested as mild cognitive impairment, and neocortical neurofibrillary degeneration was incompatible with normal cognition. The Consortium to Establish a Registry of AD (CERAD) utilized semi quantitative analysis of cortical senile plaque density (as demonstrated by silver-based histochemistry) to support or refute the diagnosis of Alzheimer’s disease in demented patients, and these 2 systems were later combined to form the basis of the National Institute on Aging – Reagan Institute criteria for Alzheimer’s disease neuropathological diagnosis.

Subsequently, new high-affinity antibodies directed against the amyloid precursor protein and $A\beta$ provided a much higher sensitivity in the detection of cerebral amyloid deposits, revealing so-called diffuse amyloid plaques that are frequently seen in cognitively normal aged patients (designated originally as "pathological aging"). These antibodies enabled Thal and colleagues to elucidate a staging system for amyloid deposition within the central nervous system: immunoreactive amyloid is encountered first within the neocortex, followed by the limbic allocortex, then the deep gray nuclei, and finally the posterior fossa structures. Onset of cognitive symptomatology correlated with the involvement of the deep gray nuclei, which was subsequently demonstrated to predict neurofibrillary degeneration within the medial temporal lobe structures. Development of the ability to image this immunoreactive amyloid in living patients resulted in both an incremental and monumental shift in the diagnosis and conceptualization of AD: Thal staging of immunoreactive amyloid was incorporated into the current guidelines for neuropathological diagnosis of AD, and the concept of pathological aging was replaced by a new category of preclinical AD. The current National Institute of Aging – Alzheimer’s Association guidelines for neuropathological diagnosis of AD also includes assessments of other well-established comorbidities that may contribute to cognitive decline. Taken together, the complex and incompletely understood interplay between $A\beta$
and hyper phosphorylated Tau proteins remain at the center of the AD pathogenesis.

Importantly, the recent National Institute of Aging – Alzheimer’s Association guidelines for neuropathological diagnosis of AD incorporate assessments for non-Alzheimer’s pathological features known to contribute to cognitive decline in the elderly. These include Lewy body/alpha-synuclein deposits, vascular parenchymal injury, and hippocampal sclerosis (especially when accompanied by TDP-43 pathology). Therefore, we carefully excluded cases exhibiting any of these comorbid pathologies, confining our further analyses to patients manifesting only Alzheimer’s type pathological changes.

Using the Institutes of Aging - Alzheimer’s Association guidelines, our recent comparative study of the neuropathology of classical slowly progressive AD (spAD) and rpAD suggested a trend toward more cases with less severe pathology in the rpAD group, but the difference was not statistically significant due to considerable inter individual variability. We also found no differentiating patterns in the morphology of NFTs and amyloid plaques, or their distribution in different anatomical areas, and no alpha-synuclein deposits or TDP-43 proteinopathy that could explain the difference in progression rate by comorbid pathology. Additionally, the diffuse and glial deposits of Aβ occurred inconsistently in both rpAD and spAD cases, and if present, constituted a very small proportion of the total Aβ deposition. Cumulatively, the detailed comparative neuropathology doesn’t explain the differences in progression rate and clinical phenotypes, and the data suggested that other factors have to be responsible. However, it will be important to complement the classical neuropathology and limited, antibody-dependent number of protein targets with proteomic analysis using laser capture micro dissected neurons, amyloid plaques, and neurofibrillary tangles from formalin-fixed, paraffin-embedded (FFPE) Alzheimer’s disease brain tissue. This powerful new technique is able to provide accurate and unbiased data and has great potential for future high resolution localized proteomics using very small amounts of archived FFPE tissues.

**PRION STRAIN PARADIGM OF PHENOTYPIC DIVERSITY IN ALZHEIMER’S DISEASE**

The causal mutations in the amyloid precursor protein gene (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) genes, which have been identified in early-onset forms, and protective polymorphism in the APP gene, established the central role of Aβ and it’s processing in AD. A major determinant in the risk of late-onset AD is the polymorphism of the APOE, in which a single e4 allele increases the risk by a factor of 4, and 2 e4 alleles increases the risk by a factor of 13. Additional polymorphisms in several recently added genes may also moderately increase the risk of disease, but these genes can explain only 30% of phenotypic variance of AD and the rate of progression of late onset AD remains unexplained.

Many lines of evidence from human prion diseases indicate that different structural organization of prions (strains) can propagate different phenotypes of disease, and target with variable speeds distinct brain structures. Since the classical neuropathology, comorbidity, or known genetic risk factors didn’t explain the rapid clinical decline in rpAD, we decided to test the prion paradigm using novel biophysical techniques derived from conformation-dependent immunoassay (CDI) and conformational stability assay (CSA). These methods allow us to compare different conformational structures formed by the same protein or peptide, and if the structures have the same amino acid sequence, then the difference in the domain display and the susceptibility to denaturation (stability) is a reliable indicator of a different structural organization in brain tissue. These techniques were extensively validated for tracking distinct human and animal prion strains, and they are used in prion laboratories worldwide. Using labeled antibodies for monitoring the conformation allows for the comparison of the protein structures directly in the brain tissue or cell cultures, over a concentration range of >4 orders of magnitude and with sensitivity in picogram range and, as a result, these methods yield highly reproducible data.
The recent data accumulated at the National Prion Disease Pathology Surveillance (NPDPSC) with these techniques and rapid sedimentation velocity separation by a high speed centrifugation in sucrose gradient, provide evidence of at least 3 discrete subpopulations of brain Aβ42 conformers with varying structures in AD.4 Despite the extensive conformational variability of Aβ42 particles, a distinct pattern emerged: significantly higher levels of less stable conformers in rapidly progressive cases.4 In contrast to the more abundant and very stable conformers at high concentrations of denaturant [≥7M Guanidine hydrochloride (Gdn HCl)], the generally lower stability of Aβ42 structures at intermediate concentration of denaturant present in rpAD suggests that they have unique structural organization. Taken together, the extraordinary structural diversity of brain Aβ42 in rpAD far exceeds the structural heterogeneity of human prions.4,38,39

To explain the discrepancy between amyloid load and the onset of clinical symptoms in AD, several groups posit a toxic subspecies of Aβ assemblies.40,41 However, whether these toxic oligomers observed in vitro and in transgenic models of AD exist in the brains of AD patients and what role they play in the AD pathogenesis generate, due to technical challenges, an ongoing debate.42,43 Our recent sedimentation velocity experiments performed under non denaturing conditions, revealed evidence of a broad spectrum of Aβ42 particles in the AD brain, with 3 particle populations composed of 30, 100, and >3000 monomers.4 These native particles of Aβ42 feature a distinct structural organization and surprisingly, Aβ40 did not form similar particles and did not participate in the formation of the major Aβ42 particles. The rapidly progressive AD cases accumulated fewer 30-mers and more 100mers, and more exposed N- and C-terminal domains suggest differences in the structural organization of the monomeric Aβ42 building block, or the way the monomers are assembled – amyloid particle packing.44,45

These recent data, obtained with a tandem of advanced biophysical techniques, convincingly demonstrate that rapidly progressive malignant forms of AD are coupled to different polymorphisms in the APOE gene and Aβ42 with distinct conformational characteristics.4 Thus far, the findings argue for the paradigm that emerged in investigations of human prion diseases, where the synergy between variable conformational characteristics of the pathogenic prion protein and polymorphisms in the PRNP generates vastly different disease phenotypes.30,31,46

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The recent progress in the investigation of the cellular biology of Parkinson’s disease (PD), frontotemporal dementia (FTD), multiple system atrophy (MSA), and amyotrophic lateral sclerosis (ALS) suggests that the prion-like aggregates generated from α-synuclein, tau, and superoxide dismutase may accelerate the pathogenesis in transgenic mice disease models.47-51 Although these seminal findings are exciting and prove, in principle, the prion-like mechanisms of brain propagation, and in some cases prion-like strains, whether such strains exist in the brains of patients with PD, FTD, MSA, and ALS and are responsible for the phenotypic variability still remains to be established. The recent preliminary study of growth hormone recipients has been interpreted as evidence that some aspects of AD pathology are transmissible from human to human but to draw a definite conclusion will require more cases with matched controls complemented by bioassays.52 Such bioassay studies will require isolation, cloning, and propagation of distinct strains of Aβ in susceptible transgenics, and tracking with new biophysical methods able to differentiate distinct structures formed by the same protein. Determining polymorphisms in new genes that may be contributing to the rapidly progressive AD phenotype will require prospective analysis of detailed endophenotypic characteristics with advanced genetic techniques. The highest priority is to establish detailed characteristics of different conformational subsets of brain Aβ42 using advanced tools such as a solid state nuclear magnetic resonance (SS NMR).53
Even though all the genetic evidence points to the altered Aβ processing as a triggering pathogenetic step in AD, investigating the conformational structure of brain Tau protein is critical for deciphering the role of their interaction in the variable phenotypes of AD. The early data obtained on Aβ in AD, with distinctly different phenotypes and rate of progression, validate this approach and represent the first step for a systematic investigation of the genetics and molecular pathology of Aβ and tau in patients, which should lead to the identification of biological factors responsible for the variable progression rates of AD. These findings will be crucial in developing new diagnostic and therapeutic targets for AD, for molecular probes targeting disease causing proteins, and for individualized therapeutic approaches.54 Disappointing therapeutic trials targeting amyloid deposits in AD triggered a reexamination of the pathogenetic assumptions that lead to their development, and exposed a critical need for new therapeutic targets and earlier diagnostic detection of the disease.55 Although these failures likely had multiple reasons, investigations of prion adaptation and evolution imply that misfolded proteins, including those causing AD, may mutate, and thus gain resistance or even dependence on the compound that targeted them.30,56,57 Transgenic mice expressing target proteins and inoculated with cloned isolates of Aβ or tau should provide a more relevant model for AD in the search for therapeutics designed for delaying or slowing down the progression of the disease.

**ABBREVIATIONS**

| Abbreviation | Description |
|--------------|-------------|
| Aβ42         | human amyloid beta with amino acid sequence 1-42 |
| Aβ40         | human amyloid beta with amino acid sequence 1-40 |
| AD           | Alzheimer’s disease |
| ALS          | amyotrophic lateral sclerosis |
| APOE         | apolipoprotein E gene with e2, e3, or e4 allelic polymorphisms |
| APP          | amyloid precursor protein gene |
| CDI          | conformation-dependent immunoassay |
| CID          | Creutzfeldt-Jakob disease |
| FTD          | fronto-temporal dementia |
| HX MS        | hydrogen/deuterium exchange followed by mass spectroscopy |
| MAPT         | microtubule associated protein tau |
| MSA          | multiple system atrophy |
| SSNMR        | solid state nuclear magnetic resonance |
| PD           | Parkinson’s disease |
| PRNP         | prion protein gene |
| PrPC         | normal or cellular prion protein |
| PrPSc        | misfolded pathogenic prion protein |
| PSEN1, 2     | presenilin 1 and presenilin 2 genes |
| rpAD         | rapidly progressive Alzheimer’s disease |

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

No potential conflicts of interest were disclosed.

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