Proteopathic Seed Amplification Assays for Neurodegenerative Disorders

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KEYWORDS

• Seed • PMCA • RT-QuIC • Prion • Synuclein • Tau • β-Amyloid • Biomarkers

KEY POINTS

- Prion-like, self-propagating protein aggregates can cause, and serve as biomarkers for, multiple neurodegenerative diseases.
- The seeded polymerization growth mechanism of pathologic protein aggregates has been exploited to develop ultrasensitive assays for many of these biomarkers.
- Some of these types of assays (eg, prion and α-synuclein real-time quaking-induced conversion assays), can provide highly sensitive and specific diagnoses of prion diseases and α-synucleinopathies when applied to patients' cerebrospinal fluid or nasal brushings.
- Multiple ultrasensitive seed amplification assays have also been developed for many disease-associated types of tau and Aβ aggregates in biospecimens, but these assays are in earlier stages of diagnostic evaluation.
- The development of a broad panel of proteopathic seed amplification biomarker assays should facilitate the diagnosis of neurodegenerative diseases as well as the execution of clinical trials of potential therapeutics.

INTRODUCTION

In order to function properly, proteins typically must fold and assume defined native three-dimensional structures. However, disruption of native protein folding may allow abnormal aggregation into self-propagating assemblies (seeds) that can grow by recruiting, and misfolding, additional monomers. Such protein aggregates can be more toxic[1,2] or more infectious[3] when small (oligomeric), but continued growth can result in the accumulation of highly ordered amyloid fibrils, and bundles thereof, that comprise the pathologic protein deposits that often characterize protein misfolding diseases.[4] Protein quality control (proteostasis) mechanisms usually limit the accumulation of abnormally folded and aggregated proteins.[5] However, with aging,

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pathogenic mutations in specific proteins, inoculation of preformed seeds, or perhaps other factors, protein quality control mechanisms can be overwhelmed, allowing protein aggregation to spiral out of control. The accumulation of protein aggregates may, or may not, have devastating consequences for the host.[6]

Nervous tissue, with its lack of neuronal turnover, is particularly vulnerable to the effects of pathologic protein aggregation. The aggregation of specific proteins is known to feature prominently in the pathogenesis of many neurodegenerative diseases, including Alzheimer disease (AD), Parkinson disease (PD), and prion diseases.[1] In genetic forms of these diseases, mutated proteins seem to be more prone to aggregation, leading to the appearance of neurodegenerative disorders in earlier stages of life. Considering the relative inability of neurons to regenerate, accurate diagnosis in the early stages of these diseases should facilitate effective treatment while neuronal damage is not yet too extensive and/or irreversible. In this context, proteopathic seed amplification assays have provided means of detecting minute amounts (attograms to femtograms) of self-propagating protein aggregates in diverse biological specimens by exploiting their inherent seeded polymerization growth mechanisms.[7–15]

The assembly of proteins into amyloid fibrils is akin to one-dimensional crystallization.[16] As with other crystallizations, the de novo, or spontaneous, formation of seeds in a solution of monomers usually takes much longer than the growth of preexisting seeds. In proteopathic seed amplification assays, a biospecimen is incubated in the presence of a vast stoichiometric excess of soluble protein monomers under appropriate conditions. If the specimen contains seeds, the assembly of monomers into amyloid fibrils occurs more rapidly than the de novo assembly that eventually occurs in the absence of seeds. Thus, seeds can be detected by comparing lag phases, or the reaction times required to detect amyloid that is newly formed from the monomeric substrate molecules. This use of substrate is analogous to the substrate in an enzymatic reaction rather than to a structure onto which something builds. Because the substrate molecules are much more abundant than the seeds in a biospecimen, their conversion into amyloid fibrils can, in effect, provide billion-fold, or even trillion-fold amplifications of the seed.[17,18] Such amplification can enable ultrasensitive detection of even a few seed particles in a test sample. This article highlights many of these types of assays, with an emphasis on their applications to human proteopathies.

PRION SEED AMPLIFICATION ASSAYS

The most highly developed proteopathic seed amplification assays are those for prions, with current assays allowing highly accurate molecular diagnoses of prion diseases in living patients.[15,19–21] The ability of infectious prions to induce the conversion of natively folded prion protein (PrP^{C}) into a prion-like protease-resistant form (PrP^{res}) in a cell-free system was first demonstrated in 1994.[22] This experimental system revealed prion strain and sequence specificities of this prion-seeded conversion reaction,[23–27] but did not support the continuous prion propagation that would be required for an amplification assay.

Protein Misfolding Cyclic Amplification

Several years later Soto and coworkers[7] developed a highly sensitive PrP^{Sc} (infectious scrapie prion protein [PrP]) amplification method termed protein misfolding cyclic amplification (PMCA).[7] In this assay, attogram amounts of PrP^{Sc} present in a tissue sample can be amplified to detectable levels by its incubation with an excess of noninfected brain homogenate, which provides the PrP^{C} monomers needed for the

polymerization process. By interleaving incubation and sonication steps, the newly synthesized misfolded aggregate is intermittently broken, providing more seeds, amplifying PrP^{res} and prion infectivity exponentially. The final product is then subjected to a proteinase K (PK) treatment and proteinase-resistant fragments are revealed by Western blotting with an anti-PrP antibody. PrP^{Sc} can be amplified from a 10^{-12} dilution of infected hamster brain homogenate containing ~26 PrP^{Sc} molecules.[17] A key feature of PMCA is that it faithfully replicates the PrP^{Sc} structure such that the amplified products are fully infectious.[28,29] Thus, PMCA has been invaluable as an in vitro experimental system for studying prion propagation. In contrast, infectious prion amplification can be a disadvantage for routine diagnostic applications when it would be preferable not to generate large amounts of infectivity. Notwithstanding that practical concern, PMCA has clear diagnostic potential because prions have been amplified from blood samples of experimentally infected animals at late[30] and early[31] stages of the disease, as well as in urine samples from animals[32] and humans with variant Creutzfeldt-Jakob disease (CJD).[33]

To circumvent the need to use brain homogenates as a source of PrP^{C} substrate, Atarashi and colleagues[8] developed PMCA reaction conditions that allowed the use of bacterially expressed recombinant PrP^{C} as the reaction substrate. This assay was called rPrP PMCA and allowed the detection of attogram-range amounts of PrP^{Sc} in 2 to 3 days compared with the 2 to 3 weeks required for conventional PMCA assays at that time. A subsequent rPrP PMCA permutation called quaking-induced conversion (QuIC) allowed the substitution of more reproducible shaking for sonication.[12]

Amyloid Seeding Assay

Meanwhile, Colby and colleagues[9] also developed a highly sensitive assay for PrP^{Sc} using recombinant PrP^{C} as a substrate, called the amyloid seeding assay (ASA). The ASA, which was also shaken rather than sonicated, had the major practical advantages of having a multiwell plate–based assay format and a direct fluorescence readout. The basic principle was that PrP^{Sc} seeds in a sample could induce the formation of recombinant PrP amyloid fibrils, which were then detected with the amyloid-sensitive dye thioflavin T (ThT). Frequent fluorescence measurements while the reactions progressed in a shaking fluorescence plate reader allowed convenient measurement of the relative lag phases of reactions seeded with prion-infected (eg, sporadic CJD [sCJD]) versus negative control brain homogenates. However, in contrast with most RT-QuIC assays, a phosphotungstate-precipitation step was needed in order to purify the seeds before its use in the ASA.

Real-Time Quaking-Induced Conversion

By combining various aspects of the QuIC and ASA assays and further optimizations, Atarashi and colleagues[10] developed the first real-time (RT) QuIC assays (**Fig. 1**), which provided easier distinction of prion-infected and uninfected biospecimens such as cerebrospinal fluid (CSF),[10,11] often without the need of doing a preclearing of the sample. Many laboratories have since contributed to the development of assays for most known prions of mammals (eg, Ref.[34–36] and references therein). The analytical sensitivities of these assays often reach down in the low femtogram to attogram range and typically meet or markedly exceed the sensitivities of animal bioassays for prions. RT-QuIC assays have been adapted to diverse tissues and biological fluids, including brain, skin, olfactory mucosa, blood, CSF, saliva, urine, and feces.[18,33,37–45] Faster and more sensitive second-generation RT-QuIC assays for human prions allow nearly 100% accurate antemortem diagnosis of sCJD when CSF, nasal brushings, or both are tested.[21,41,46] Analysis of CSF specimens alone provide 92% to 96% overall

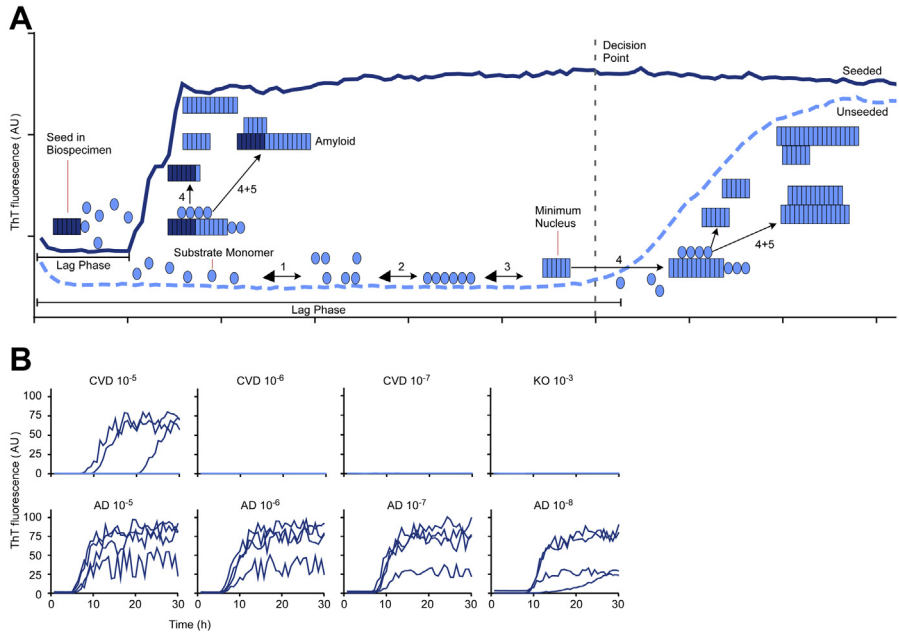

Fig. 1. Seeded polymerization in real-time quaking-induced conversion (RT-QuIC) reactions. (*A*) Reactions are set up in multiwell plates by diluting a biospecimen into reaction mixture containing a vast stoichiometric excess of an appropriate protein monomer (substrate) for the type of disease-associated seed being detected. The reaction also contains the amyloid-sensitive fluorescent dye, thioflavin T (ThT). When seeds are present in the biospecimen (*left*), they immediately start to grow (4) by recruiting new monomers. Elongated fibrils can promote secondary nucleation (5); that is, the production of new seeding surfaces either by fragmentation or by providing lateral surfaces that can facilitate the ordering and conformational conversion of monomers into additional fibrils. Secondary nucleation contributes to the exponential growth of the new amyloid that enhances ThT fluorescence (*dark blue trace*). The lag phase in a seeded reaction represents the time it takes for the seeded amyloids to accumulate to levels that are detectable with ThT. Eventually, the reaction plateaus when all of the available monomer is converted to amyloid. In the absence of seeds in a negative control biospecimen (*light blue trace*), spontaneous nucleation (1–3) may occur, but only after a prolonged lag phase during which the kinetically unfavorable process of forming minimal stable nuclei (in this case a 6-mer) occurs.[16] A key to developing an effective assay is finding substrates and assay conditions giving the greatest fold separation between the lag phases of seeded versus unseeded reactions (eg, see Ref.[79]). (*B*) Representative primary Alzheimer disease (AD) tau RT-QuIC data comparing seeding by serial 10-fold dilutions of human familial AD (age 44 years) and cerebrovascular disease (CVD; age 53 years) brain tissue with reference to a 10^0 dilution being solid brain tissue. Tau knockout (KO) mouse brain served as a completely tau-free negative control. Traces from quadruplicate wells for each brain dilution are shown. AD brain could be diluted at least 10^5-fold and 10^3-fold further than the KO and CVD brains, respectively, and still seed positive reactions. Although the CVD brain was not recorded as having tau pathology by immunohistochemistry, the typically higher sensitivity of RT-QuIC assays likely allowed detection of tau aggregates that were less than the detection limit of immunohistochemistry. AU, arbitrary units.

diagnostic sensitivity (the percentage of patients with sCJD giving positive assays) and nearly 100% specificity (the percentage of patients with nonprion disease giving negative assays) in multiple independent studies by different research groups.[21,40,41,46,47] Before the availability of these assays, definite diagnoses of CJD required biochemical or immunohistologic analysis of brain tissue, which is usually only available postmortem. According to the US Centers for Disease Control and Prevention (CDC), a positive RT-QuIC result in combination with neuropsychiatric symptoms provides a probable diagnosis of CJD, although immunodetection of PrP^{res} is still needed for a definite diagnosis.[48] Similar diagnostic criteria are used by the UK National CJD Research and Surveillance Unit.[49] Recent demonstrations of the detection of sCJD prions in the skin[43] and multiple components of the eyes[50] of all tested patients with sCJD have raised the possibility that these tissues might also be useful diagnostically, as well as being potentially biohazardous sources of prion infectivity. Studies in prion-infected rodents have shown that prion seeding activity can be detected in skin far in advance of the onset of overt clinical signs of prion disease.[38] From a practical perspective, concerns have been raised about whether the massively amplified products of sCJD-seeded RT-QuIC assay products are infectious, and therefore biohazardous. However, inoculation of the products of sCJD MM1-seeded RT-QuIC reactions into transgenic mice overexpressing human PrP^{C} showed that they were at least 10^{10}-fold less infectious per unit protein than the sCJD prions that seeded its aggregation.[51] Detailed protocols for performing prion RT-QuIC assays have been reported elsewhere.[20,52]

Scrapie Cell Assay

Klohn and colleagues[53] described a prototypic cellular seeding assay that exploits the ability of prions to infect cells and induce the de novo accumulation of PrP^{Sc}. Briefly, neuroblastoma-derived cell lines are incubated with dilutions of prion-infected brain homogenates from rodents, and the proportion of cells accumulating PrP^{Sc} (detected by an enzyme-linked immune absorbent spot assay after 3 in vitro passages over ~2 weeks total) were found to correlate with the prion concentration in the original sample. The standard scrapie cell assay has been automated[54,55] and is as sensitive as the mouse prion bioassay but is faster and less expensive, with the advantage of no use of animals. There have been many revealing applications of the scrapie cell assay (eg, Refs.[55–57]); however, so far the assay has been limited to the measurement of rodent-adapted prion strains. To date, no scrapie cell assay has been adapted for the detection of human prion strains.

SEED AMPLIFICATION ASSAYS FOR SYNUCLEINOPATHIES

α-Synuclein Real-Time Quaking-Induced Conversion and Protein Misfolding Cyclic Amplification

To provide molecular diagnoses of PD and dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), several laboratories have now developed RT-QuIC–like assays for pathologic forms of α-synuclein (αSyn^{D}), called αSyn RT-QuIC[58–63] or αSyn-PMCA.[64] These assays can have unprecedented sensitivity for αSyn^{D} down into the low femtogram range and detect up to 10^{8}-fold dilutions of patients' brain tissue. When applied to CSF specimens collected from living patients, the diagnostic sensitivities for PD and DLB have ranged from 88% to 96% and specificities from 82% to 100%.[58,60,63–65] Blinded comparisons of the performance of 2 of these assays on a large set of patients with PD and healthy controls revealed a high degree of concordance in diagnostic performance.[65] Although the earlier assays took 5 to

13 days, newer permutations take 1 to 2 days[60] or 3 days[63] with comparable diagnostic performance and the ability to detect αSyn^D seeds in CSF collected early in the clinical course of PD. Moreover, a recent study by Rossi and colleagues extensively validated a rapid αSyn RT-QuIC assay for Lewy body-associated α-synucleinopathies, e.g. Parkinson's disease and dementia with Lewy bodies.[66] Importantly, this study also detected αSyn^D seeds in 18/18 isolated REM sleep behavior disorder and 26/28 pure autonomic failure cases prior to the appearance of parkinsonism or cognitive decline. Similarly rapid assays have been reported using postmortem submandibular gland tissue from PD and incidental Lewy body disease decedents, giving 100% sensitivity and 94% specificity for synucleinopathy.[62] αSyn^D seeding activity can often be detected in nasal brushings from patients with synucleinopathy.[67] It is notable that studies have also reported evidence of strain-like differences between types of synucleinopathy-associated seeds.[60,61,68]

Although the multiple initial analyses of αSyn RT-QuIC and related assays have been encouraging, more extensive studies will be required to fully understand their diagnostic and prognostic utilities and the extent to which αSyn^D seeding activities in accessible biospecimens might vary over time in individual patients. Such information will likely be important in understanding the extent to which the monitoring of αSyn^D seeding activities might be helpful in setting up and assessing the progress of clinical trials of treatments designed to reduce αSyn^D burden in the brain.

Human Embryonic Kidney Cell Bioassay for Multiple System Atrophy

Woerman and colleagues[69,70] expressed yellow fluorescent protein–tagged αSyn in human embryonic kidney (HEK) cells and found that phosphotungstic acid–precipitated extracts of brain homogenates from MSA decedents could be assayed on these cells 4 days after exposure by quantitating cells with fluorescent aggregates. The assay had an analytical sensitivity of 70 pg/mL, and revealed differences in regional distribution of αSyn seeding activity between patients with MSA. Interestingly, this assay was not sensitive to the αSyn seeds of PD cases.[69,70] The results provided evidence for the MSA and PD being distinct αSyn^D prion strains.

HANdai Amyloid Burst Inducer Assay

Recently, an ultrasonication-based assay was described that uses the HANdai Amyloid Burst Inducer (HANABI) system to amplify αSyn^D.[71] Analogous to what is done in classic PMCA, the investigators applied ultrasonication to break up αSyn^D oligomers in order to increase the nuclei from which the polymerization starts. The addition of αSyn preformed fibrils in artificial CSF enhanced the reaction speed in a dose-dependent fashion. Although ultrasonication provided faster reactions, it also increased the unseeded polymerization in negative control reactions. The analysis of human CSF from patients with PD and controls showed overlapping reaction kinetics, complicating the diagnostic sensitivity and specificity of this assay.

SEED AMPLIFICATION ASSAYS FOR TAUOPATHIES

Multiple neurodegenerative diseases involve the pathologic accumulation of tau filaments, including AD, chronic traumatic encephalopathy (CTE), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), frontotemporal dementia and parkinsonism 17 microtubule-associated protein tau (FTDP 17 *MAPT*), argyrophilic grain disease (AGD), and Pick disease (PiD). In adult humans, the alternative splicing of the *MAPT* gene gives rise to 6 tau isoforms. These isoforms differ from each other by the presence or absence of inserts near the N terminus and by the number of

microtubule binding domains near the C terminus; that is, 3 (3R) or 4 (4R) isoforms. The tau pathologies of different diseases are characterized by the preferential deposition of 3R, 4R, or both 3R and 4R tau isoforms. For example, patients with AD and CTE accumulate roughly equivalent amounts of both 3R and 4R isoforms because all the isoforms contain the residues comprising the amyloid cores of the paired helical and straight tau filaments of these diseases.[72,73] Patients with PiD accumulate mainly 3R isoforms,[74,75] consistent with their compatibility with the distinct amyloid core of PiD filaments.[76] In contrast, patients with PSP, CBD, and FTDP 17 *MAPT* preferentially accumulate 4R isoforms, which, in the case of CBD, can again be rationalized by the core structure of the CBD-associated filaments.[77]

Tau Real-Time Quaking-Induced Conversion Assays

Initial studies by Margittai and colleagues showed that, in sonicated, multiwell plate–based reactions, synthetic fibrils formed with different 3R and 4R tau constructs had distinct seeding activities[78] and also that AD brain homogenates could seed the fibrillization of tau constructs faster than control homogenates.[79] Since then, ultrasensitive and selective tau RT-QuIC assays have been developed for disease-associated 3R, 4R, and 3R + 4R tau aggregates, and some subclasses thereof.[6,80–83] Tau RT-QuIC performance can be improved (ie, higher sensitivity can be achieved without compromising specificity) by systematic comparisons of conditions such as salts of the Hofmeister series.[82] These tau RT-QuIC assays can detect seeds in 10^8-fold to 10^{10}-fold dilutions of brain tissue, with orders of magnitude of selectivity for the types of tauopathy for which they were optimized. The optimization of each of these assays involved comparisons of various recombinant tau substrate constructs, cofactors, and other reaction components and conditions. Use of a 3R tau fragment (K19CFh) as a substrate first allowed the development of the 3R tau RT-QuIC assay, which specifically detects PiD seeds in brain tissue and postmortem CSF samples.[80] To detect AD and CTE tau filaments, a substrate (τ306) spanning the entire amyloid core of the associated filaments was then included in the reaction to allow the amplification of the seeds.[6] Seeds of multiple diseases with 4R tauopathy can be detected with the recently developed 4R Tau RT-QuIC.[81] This assay is the first tau RT-QuIC assay to allow detection of tau seeds in antemortem, as well as postmortem, CSF specimens. In addition, the authors more recently used another tau substrate (K12CFh) to develop a single tau RT-QuIC assay for the detection and discrimination of tau seeds of AD and PiD.[83] This assay simplifies the testing for these types of tau seeds and indicates that they differ in conformational templating activity. As with the prion and αSyn RT-QuIC assays, the ability to detect tau seeds in accessible diagnostic specimens will be important for antemortem diagnostic utility of tau RT-QuIC assays. However, at present, further research is necessary to better establish any such utility.

Another valuable feature of the 4R RT-QuIC assay is its ability to differentiate 3 classes of 4R tau seeds based on characteristics of the fibrillar reaction products, namely their relative enhancement of ThT fluorescence, and their β-sheet conformations as assessed by Fourier transform infrared (FTIR) spectroscopy: class A is represented by FTDP-17 with P301L *MAPT* mutation; class B includes the FTDP-17 with N279K mutation and CBD; and class C includes PSP.[81]

In applying tau RT-QuIC assays to biological specimens, it is important to bear in mind that various types of tau aggregates and seeding activities can be found at lower levels (usually by orders of magnitude) in brain tissue, at least of individuals without primary tauopathies.[6,80–83] Thus, the diagnostic specificities of these ultrasensitive assays when applied to biospecimens may depend on quantitative, as well as qualitative, differences in tau seeding activities.

Biosensor Cell Assays

A highly sensitive and specific biosensor cell seeding assay for tau seeds has also been developed[84,85] by exploiting the ability of tau aggregates to penetrate cells and induce the fibrillization of soluble endogenous tau.[86] Briefly, when engineered HEK293T cell lines expressing 3R or 4R tau constructs fused to fluorescent proteins were exposed to exogenous proteopathic tau, these protein aggregates penetrated the cells and seeded the polymerization of intracellular tau.[84] The intracellular protein aggregation was detected by fluorescence resonance energy transfer (FRET), which was measured by fluorescence microscopy or flow cytometry. The exposure of the cells to increasing amounts of recombinant tau fibrils resulted in the gain of FRET signal in a dose-dependent fashion. Moreover, the incubation of HEK293T cells expressing fluorescent 3R or 4R tau constructs, or both, with specific tauopathy brain lysates (eg, from AD, CTE, PSP, CBD, AGD) triggered the formation of tau inclusions, whereas no tau aggregation was induced by negative controls. This finding showed that the polymerization was promoted by aggregated tau and not by other protein aggregates. Comparison of tau seeds from multiple sources showed them to behave like different strains by inducing characteristic intracellular inclusions that could be faithfully propagated through many in vitro passages and then on inoculation into transgenic mice.[87] To compare the sensitivity of this technique with histology (the gold standard postmortem method to diagnose AD[88]), a time-course analysis in a tauopathy mouse model was performed. Tau seeding activity preceded histopathologic detection by more than 4 weeks. Although these experimental systems have revealed much about the prion-like propagation and strain-dependent seeding capacity of tau aggregates, the practicality of these cellular models for routine clinical diagnostic purposes in clinical practice may be limited by the need for tissue cultures and either immunostaining or flow cytometry.

β-AMYLOID SEED AGGREGATION ASSAYS

Kinetic Aggregation Assay

In addition to the aforementioned neurofibrillary tangles of tau, amyloid plaques of the β-amyloid (Aβ) peptide are major lesions found in brains from patients with AD.[89] To detect Aβ aggregates, Kelly and colleagues developed a seeded polymerization-based assay.[90] In this kinetic aggregation assay, Aβ aggregates from mammalian cell culture media, *Caenorhabditis elegans* lysates, and AD mouse brain homogenates seeded the fibrillization of monomeric $A\beta_{1\text{-}40}$ peptide, in a cell-free reaction containing ThT to monitor fibril formation over time. The amount of Aβ amyloid fibrils in the sample was proportional to the half-time of the growth phase (t50). To prove the specificity of this assay, experiments with αSyn aggregates were also conducted and differences in the kinetic reactions were observed, with reactions seeded with 4.3-μg/mL $A\beta_{1\text{-}40}$ fibrils showing a t50 of ~5 hours, whereas 14.5-μg/mL α-synuclein fibrils yielded a ~9-hour t50. The investigators stated that this selectivity is needed to allow the quantification of Aβ amyloid fibrils in tissues that can potentially contain other amyloid seeds; however, human biological specimens from patients with different neurodegenerative disorders were not assessed by this assay in order to prove its specificity.

β-Amyloid Protein Misfolding Cyclic Amplification

Continuing along these lines, Soto and colleagues developed an assay called Aβ-PMCA, which detects misfolded Aβ oligomers down to as little as 3 fmol in a multiwell plate–based assay with ThT readout as in RT-QuIC assays.[91] Application of Aβ-PMCA to CSF specimens from patients with and without AD provided

discrimination between the 2 sets with 90% sensitivity and 92% specificity. Because it is well known that cognitively normal or other patients without AD can have some Aβ deposits in their brain tissue, the specificity of the Aβ-PMCA presumably depends on disease-dependent differences in concentration of Aβ seeds in the CSF. Seed concentration is typically inversely correlated with lag time in seeded protein polymerization reactions, which likely explains the shorter lag phases that were observed with CSF samples from patients with AD. This assay promises to provide a useful complement to the AD tau RT-QuIC assay described earlier in measuring the key pathologic protein aggregates of AD.

HUNTINGTON DISEASE SEED AMPLIFICATION ASSAY

The applicability of an ASA of postmortem brain extracts from human patients with Huntington diseases, and transgenic mice models thereof, was reported by Gupta and coworkers.[92] Misfolded huntingtin partially purified from the diseased brains induced amyloid formation of a largely polyglutamine substrate more rapidly than extracts from negative control brains. Although this assay is inhibited by components of crude brain tissue, hence the need for partial purification of the seeds from brain, it serves as a prototypic test for pathologic forms of huntingtin.

SUMMARY

Continuing work by many laboratories is yielding a growing panel of ultrasensitive assays for the various misfolded self-propagating protein aggregates that cause many neurodegenerative diseases. These assays provide important tools for fundamental research into the pathogenesis of these diseases. Moreover, the ability to detect miniscule amounts of such proteopathic aggregates as biomarkers in accessible specimens is allowing more accurate molecular diagnoses of diseases that can otherwise be difficult to discriminate, especially early in pathogenesis when appropriately targeted treatments are more likely to be successful. Importantly, given the frequency with which patients with neurodegenerative disease have more than 1 type of aggregated protein in their central nervous systems, the high sensitivity of these assays allows detection of both primary and secondary proteopathies. Such testing should also facilitate the development of new therapies by allowing clearer identification of cases and controls for clinical trials, as well as the measurement of specific protein seeds as key etiologic biomarkers over the course of treatment.

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DISCLOSURE

B. Caughey is an inventor on patents or patent applications relating to prion, αSyn, and tau RT-QuIC assays. N.C. Ferreira has nothing to disclose.

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