COMMENTARY

Breakthroughs in antemortem diagnosis of neurodegenerative diseases

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The World Health Organization forecasts that within 2 decades neurodegenerative disorders will eclipse cancer to become the foremost cause of death in the developed world after cardiovascular disease. Accurate detection of pathological processes goes hand in hand with the goals of treatment and prevention and, in light of their protracted but worsening clinical progression, the earlier a diagnosis can be made the better. However, the challenge underlying accurate detection of neurodegenerative diseases during their clinical phase is that specific biomarkers are not present at high enough concentrations for routine detection in accessible specimens. Consequently, it has only been possible to definitively diagnose these conditions by examination of brain pathology after death. The paper by Metrick et al. (1) in PNAS addresses the issue of improved antemortem biomarker detection for a spectrum of neurological disorders, using assays which capitalize on prionic amplification of misfolded, pathogenic proteins.

Protein Misfolding and Prions in Neurodegenerative Diseases

During neurodegenerative diseases biochemical changes in particular proteins lead to their misfolding and accumulation in specific neuronal populations. Alzheimer's disease (AD) is characterized by accumulation of extracellular plaques enriched with amyloid beta (Aβ) peptide derived from the amyloid precursor protein and intracellular neurofibrillary tangles composed of hyperphosphorylated microtubule-associated protein tau. Of the 6 tau versions, 3 have 3 microtubule-binding domains (3R isoforms) while the others contain 4 (4R isoforms). The involvement of tau in frontotemporal dementia with parkinsonism-17, Pick disease (PiD), progressive supranuclear palsy, argyrophilic grain disease, and corticobasal degeneration results in the blanket term of tauopathies to describe these disorders. While tau deposits in PiD are composed predominantly of 3R isoforms, roughly equivalent amounts of 3R and 4R isoforms are deposited in AD. Other tauopathies accumulate predominantly 4R isoforms. Abnormal cytoplasmic accumulation of a normally soluble and unfolded protein called α-synuclein is the hallmark of diseases referred to as synucleinopathies. Neuronal deposition of α-synuclein aggregates in Lewy bodies occurs in Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Yet another protein—the prion protein (PrP)—is central to a group of interrelated disorders commonly referred to as prion diseases.

Since the concept underlying the diagnostic approach taken by Metrick et al. (1) derives from studies of prions, it is worth reviewing what we have learned about PrP and the applicability of these findings to other proteopathic diseases. The prion disorders are transmissible neurodegenerative diseases affecting animals and humans. The most common human form is Creutzfeldt–Jakob disease (CJD) which occurs most frequently as a sporadic, rapidly progressive condition of older individuals. The discovery that CJD and kuru—an epidemic spread by cannibalism among tribal peoples in Papua New Guinea—were transmissible and that they shared neuropathological features with scrapie, a similarly infectious disorder of sheep and goats, led to their grouping in a category called transmissible spongiform encephalopathies (TSEs). Additional TSE epidemics were subsequently identified, including bovine spongiform encephalopathy (BSE), also known as "mad cow disease," and chronic wasting disease (CWD), a contagious and rapidly spreading disease of deer, moose, elk, and other cervids. Transmission of BSE to humans as variant CJD (2), most likely by consumption of prion-contaminated foodstuffs, epitomizes the potential for interspecies prion transmission. Pioneering studies by Prusiner et al. (3) demonstrated that prions, the causative agents of TSEs, were infectious proteins devoid of DNA or RNA. According to the prion hypothesis the infectious particle is composed of PrP^{Sc}, a conformationally altered pathogenic version of a host-encoded protein, PrP^{C}. Agent replication occurs by a process in which PrP^{Sc} interacts with and templates its abnormal β-sheet conformation on PrP^{C} which is largely α-helical (4). The ensuing PrP^{Sc} is then

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capable of transforming additional PrP^C, the result being exponential prion accumulation.

Prionic Amplification of Proteopathic Seeds Facilitates Neurodegenerative Disease Diagnosis

Although early work by Caughey and coworkers (5) provided important proof of concept that PrP conversion could be replicated in cell-free systems, the excessive amounts of PrP^{Sc} required to convert PrP^C after prolonged periods of incubation precluded the use of this approach as a diagnostic tool. Using a technique referred to as protein misfolding cyclic amplification (PMCA), Soto and coworkers (6) discovered that much smaller amounts of PrP^{Sc} were capable of converting PrP^C derived from normal brains in a cell-free system. In PMCA, disruption of growing PrP^{Sc} oligomers by sonication generates multiple smaller templates for continued recruitment and conversion of PrP^C. Using a simplified approach referred to as quaking-induced conversion (QuIC), Caughey and coworkers (7) used bacterially expressed recombinant PrP (rPrP) as conversion substrate and replaced sonication with alternating cycles of incubation and shaking to facilitate fibril fragmentation and reseeding. In this way minute starting amounts of prion seeds could be amplified to a detectable level. In a further development, the amyloid seeding assay (ASA) used the fluorescent dye thioflavin T (ThT) to detect seeded polymerization of rPrP (8). By incorporating aspects of the ASA and QuIC, Caughey and coworkers (9) developed real-time QuIC (RT-QuIC) in which the ability of prions to seed conversion of the rPrP template could be monitored in real time by incorporation of ThT into growing amyloid fibrils. Since biospecimens containing as little as attogram amounts of prion seeds can be detected after amplification by many orders of magnitude, RT-QuIC has become a preferred method for prion diagnosis in a variety of veterinary and medical settings (10). Several RT-QuIC assays have been developed for prion detection in accessible patient specimens including cerebral spinal fluid, nasal brushings, and skin (11–14).

Whereas the process of interactive conformational templating of normally folded host protein by a pathological counterpart was originally considered an exclusive property of PrP, increasing evidence linked this canonical mechanism to proteins involved in the pathogenesis of additional neurodegenerative diseases (15). Evidence of AD transmission to marmosets (16) was confirmed and extended by investigators employing transgenic mouse models of AD inoculated with either patient brain homogenates or synthetic amyloid-β peptides (17, 18). Similar prion-like transmission was demonstrated for tau and α-synuclein (19, 20). This mechanistic overlap became the guiding principle behind the development of RT-QuIC assays to detect proteopathic seeds in other protein misfolding disorders including the synucleinopathies PD and DLB and for tau in AD and PiD (21–24).

In PNAS, Metrick et al. (1) extend and refine studies of seeded amplification for the specific diagnosis of different neurodegenerative diseases. Their focus was to systematically investigate the effects of modulating the ionic composition of RT-QuIC assays on protein polymerization caused by different disease-associated seeds. The Hofmeister series refers to a relative ordering of strongly and weakly hydrated salts with respect to their ability to precipitate proteins out of solution. Metrick et al. (1) discovered that the effects of 15 different Hofmeister salts on the sensitivity, fidelity, speed, and specificity of protein aggregation depended on whether the RT-QuIC assays were designed to amplify tau, α-synuclein, or PrP disease-associated seeds. Hofmeister salts were found to have pronounced and distinct effects on the ability to differentiate between rapid seeded propagation of amyloids and the spontaneous aggregation of substrate protein in RT-QuIC assays for tau, α-synuclein, or PrP. While weakly hydrated salts showed optimal effects on kinetics of fibril formation in AD- and prion-seeded reactions, seeding of a different form of tau by PiD brain homogenate was fastest with more strongly hydrated anions, and the α-synuclein RT-QuIC assay using seeds from patients with DLB was affected by yet other Hofmeister salts. Metrick et al. (1) also detected pronounced Hofmeister effects on the sensitivity of RT-QuIC assays. During the process of testing 4R tauopathy brains they observed gains in sensitivity of up to a million-fold using sodium citrate compared to sodium chloride which had been used in previous assay conditions. In contrast, no gains in seeding activity were seen for seeding assays from 3R or 3R/4R tauopathy cases.

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Metrick et al. (1) also found that Hofmeister effects impacted amplification and detection in the context of diagnostically available specimens. While seed detection in brain homogenate using the AD tau RT-QuIC assay was enhanced by weakly hydrated anions, the trend was reversed in human blood plasma where the effects of inhibitors were overcome by strongly hydrated salts. Sodium iodide increased the analytical sensitivity of CJD prion seed detection by 6- to 32-fold in nasal brushings as well as in brain homogenate and improved detection of prions in homogenates of ear biopsies from deer with CWD. This promising development raises the possibility that accessible ear punch biopsies might be used for antemortem monitoring of this uncontrolled prion disease of wild animals.

In summary, a rapidly emerging picture supports the self-seeding ability of proteopathic aggregates as a proven means to diagnose a spectrum of human neurodegenerative diseases. Enhancement of assay sensitivities by manipulating the ionic environment of seeded polymerization using weakly or strongly hydrated anions not only provides opportunities for earlier detection using diagnostically available tissues, but also delivers an added layer of control to distinguish the etiologies of these disorders which will be a decisive factor for tailoring therapeutic approaches. Improved diagnostic capabilities are also critical in the management of novel prion diseases which are emerging in free-ranging animals and livestock and ultimately for limiting any future zoonotic risks posed by these prions.

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