# An Introduction to *Prion Diseases*

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An expanding set of scientific investigations indicate that prions are likely to cause many different neurodegenerative diseases (NDs) and possibly some other disorders as well, including type 2 diabetes (Prusiner 2014; Mukherjee et al. 2015; Mukherjee and Soto 2017; Westermark et al. 2017). As the aging population in the developed world increases, the incidence of NDs will continue to grow. Age is clearly the greatest risk factor for these diseases. In the case of Alzheimer's disease (AD), the number of individuals with AD aged 65 and older is projected to increase from 5.2 million to ~14 million in the United States alone by 2050 (Alzheimer's Association 2016). This near tripling of the prevalence of AD will likely change our nation and other nations in the developed world (Hebert et al. 2013; James et al. 2014). Currently, ~$200 billion is spent annually to care for AD patients in the United States; this amount also includes lost wages from patients and caregivers (Hurd et al. 2013). Each year, there are 500,000 new cases of AD, and a similar number of individuals with AD die annually.

The studies summarized in this volume focus on the individual diseases caused by prions, including AD, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and others (Table 1). Nearly every facet of the various NDs is described and analyzed in this volume. Each of the prion proteins is discussed in terms of its underlying protein that becomes a prion. Moreover, each prion protein is described along with the structural changes it undergoes as it is modified during the formation of nascent prions.

Currently, there are no effective drugs for the treatment of NDs. From 2002 to 2012, for example, 413 clinical trials for AD were conducted on 244 unique molecules. During this period, the failure rate was a disappointing 99.6%. The only successful FDA approval was for memantine, an MDMA (3,4-methylenedioxymethamphetamine) receptor antagonist that provides only symptomatic treatment for moderate to severe AD patients (Cummings et al. 2014; Langreth and Koons 2016). Such a record argues that the underlying premises are likely to be incorrect. Additionally, the blood–brain barrier complicates the discovery of effective therapeutics.

The incidence of most NDs increases with advancing age, and an expanding collection of evidence argues that various NDs are caused by prions (Prusiner 2012). Prions arise when normal proteins acquire an alternative conformation that becomes self-propagating. The most well-studied mammalian prions are composed of $PrP^{Sc}$ proteins that cause Creutzfeldt–Jakob disease (CJD) in humans, scrapie in sheep, chronic wasting disease (CWD) in deer and elk, and bovine spongiform encephalopathy (BSE), or mad cow disease, in cattle (Prusiner et al. 2004; Wells and Wilesmith 2004). Conformational variants of $PrP^{Sc}$ create different regional patterns of PrP accumulation and thus

**Table 1.** Neurodegenerative diseases caused by prions

| Neurodegenerative diseases (NDs) | Causative prion proteins | Percent inherited (%) |
|---|---|---|
| Creutzfeldt–Jakob disease (CJD) | $PrP^{Sc}$ | 10–15 |
| Gerstmann–Sträussler–Scheinker (GSS) | | 90 |
| Fatal insomnia (FI) | | 90 |
| Bovine spongiform encephalopathy (BSE) | | <1 |
| Scrapie | | <1 |
| Chronic wasting disease (CWD) | | <1 |
| Alzheimer's disease (AD) | Aβ→tau | 1–3 |
| Multiple system atrophy (MSA) | α-synuclein | <1 |
| Parkinson's disease (PD) | | 10–15 |
| Dementia with Lewy bodies (DLB) | | |
| Frontotemporal dementia (FTD) | tau, TDP-43, FUS, C9orf72, PGRN | 15–25 |
| Progressive supranuclear palsy (PSP) | | |
| Pick's disease (PiD) | | |
| Argyrophilic grain disease (AGD) | | |
| Corticobasal degeneration (CBD) | | |
| Chronic traumatic encephalopathy (CTE), also called posttraumatic FTD | | |
| Amyotrophic lateral sclerosis (ALS) | SOD1, TDP-43, FUS, C9orf72 | 15–25 |
| Huntington's disease (HD) | huntingtin | 100 |

produce different disease phenotypes (Bruce et al. 1989; Hecker et al. 1992). Ten to twenty percent of CJD patients have a familial form of the disease caused by mutations in the *PRNP* gene, which encodes $PrP^{C}$ (Kong et al. 2004). Prions composed of $PrP^{Sc}$ are formed from $PrP^{C}$ by a posttranslational process that results in a profound change in conformation. In some CJD patients, $PrP^{Sc}$ and smaller fragments are found in amyloid plaques. Although the number of prions identified in mammals and fungi continues to expand, the existence of prions in other phylogeny remains undetermined.

In addition to the PrP diseases, which include CJD, kuru, Gerstmann–Sträussler–Scheinker (GSS) disease, and fatal insomnia (FI) (Bockman et al. 1985; Brown et al. 1986; Medori et al. 1992; DeArmond et al. 2004; Will et al. 2004), unequivocal evidence for α-synuclein prions has been recently obtained through studies of multiple system atrophy (MSA) (Prusiner et al. 2015; Woerman et al. 2015). In our studies in transgenic (Tg) mice, two brain samples from deceased MSA patients were homogenized and inoculated intracerebrally into Tg(α-synuclein$^{*}$A53T$^{+/-}$)M83 mice. After ~120 d, the mice developed signs of neurological dysfunction and died within a few days (Watts et al. 2013). Moreover, 12 additional cases of MSA were obtained from three different continents, and all caused central nervous system (CNS) dysfunction ~120 days postinoculation (dpi) (Prusiner et al. 2015). In all of the 14 initial cases and an additional five cases of MSA, α-synuclein prion infection was demonstrated in the Tg mice.

Following our initial transmission studies of MSA prions into TgM83$^{+/-}$ mice, we developed a cell-based assay using a mutant α-synuclein fragment fused to yellow fluorescent protein (YFP) (Woerman et al. 2015). All the prion-positive MSA samples transmitted to both TgM83$^{+/-}$ mice and human embryonic kidney (HEK) cells expressing mutant α-synuclein–YFP. Our cultured HEK cell assay was based on earlier studies by Marc Diamond and

colleagues, who developed tau–YFP and α-synuclein–YFP fusion protein bioassays (Kfoury et al. 2012; Sanders et al. 2014; Holmes and Diamond 2016).

MSA is clearly caused by the transformation of the α-synuclein protein into a prion (Watts et al. 2013; Prusiner et al. 2015; Woerman et al. 2017). In contrast to PD, MSA tissues intracerebrally inoculated into Tg mice expressing mutant human α-synuclein (A53T) cause disease ~120 dpi. In MSA, α-synuclein amyloid fibrils coalesce into glial cytoplasmic inclusions (GCIs) (Spillantini et al. 1998a; Tu et al. 1998; Wakabayashi et al. 1998). In contrast to MSA, α-synuclein amyloid fibrils in PD combine to form Lewy bodies (Spillantini et al. 1998b) and do not transmit disease to Tg(α-synuclein*A53T) mice.

Many investigators have studied the transmissibility of AD, beginning with the work of Carleton Gajdusek and Joseph Gibbs. These studies followed earlier success with the transmissibility of kuru and CJD to chimpanzees and monkeys (Gajdusek et al. 1966; Gibbs et al. 1968). Although two cases of familial AD (fAD) were reported to be transmissible to intracerebrally inoculated monkeys, the results could not be reproduced (Goudsmit et al. 1980; Godec et al. 1991). Similar studies were later performed using marmosets, which were analyzed using anti-Aβ antibodies to measure AD transmission (Baker et al. 1994; Ridley et al. 2006). This approach provided convincing evidence for the transmission of Aβ prions, but the prolonged incubation periods and the expense of housing marmosets limited the number of studies that could be performed.

A more facile experimental system of studying AD prions was developed by Mathias Jucker, Matthias Staufenbiel, and Lary Walker using Tg mice expressing mutant amyloid precursor protein (APP) (Meyer-Luehmann et al. 2006; Walker et al. 2016). Subsequently, they demonstrated transmission of AD to Tg(APP23) mice via intraperitoneal inoculation (Eisele et al. 2010, 2014). Following those studies, we inoculated bigenic mice co-expressing mutant APP under control of the *Thy 1.2* promoter and luciferase under control of the glial fibrillary acidic protein (GFAP) promoter with synthetic mutant Aβ peptides polymerized into amyloid fibrils (Watts et al. 2011; Stöhr et al. 2012). These fibrils induced the formation of Aβ prions in the brains of the bigenic mice. Depending on the APP mutation, the sites of γ-secretase cleavage were modified, resulting in different amyloid fibril deposits (Stöhr et al. 2014; Watts et al. 2014).

Different mechanisms appear responsible for neuronal dysfunction, and at least half a dozen different proteins can lead to cerebral amyloid deposition. PrP amyloid plaques are frequently found in the brains of patients with GSS and kuru, as well as in the brains of animals with BSE and CWD. PrP prions polymerize into fibrils that are sequestered within plaques in these diseases. However, the formation of PrP amyloid plaques seems likely to represent a process that is protective rather than detrimental. Similar arguments can be made for Aβ amyloid plaques, where the sequestration of the Aβ peptide is not toxic (Lannfelt et al. 1994; Selkoe 2011). Many older people with Aβ amyloid plaques detected by positron emission tomography (PET) using Pittsburgh compound B do not exhibit dementia (Oh et al. 2016). Although unproven, it seems likely that at least one conformer of the Aβ peptide becomes toxic when it stimulates the tau protein to form prions that polymerize into amyloid filaments and coalesce into neurofibrillary tangles (NFTs) (Hof et al. 1992; Bierer et al. 1995; Giannakopoulos et al. 2003). In contrast to Aβ amyloid plaques, NFTs or smaller multimers of tau prions cause brain dysfunction. Many studies of the tauopathies have shown unequivocally that tau proteins, presumably tau prions, feature in CNS deterioration (Arriagada et al. 1992a,b; Hof et al. 1992; Bierer et al. 1995; Giannakopoulos et al. 2003; Goedert 2015; Clavaguera et al. 2016; Holmes and Diamond 2016; Kriegel et al. 2017).

## PATHOGENIC MAMMALIAN PRIONS

Following the experimental transmission of kuru and CJD to apes and monkeys by Gajdusek and Gibbs, the search intensified for what was believed to be a slow-acting virus causing the analogous disease, scrapie, in sheep and goats

(Hadlow 1959; Gajdusek et al. 1966; Gibbs et al. 1968; Gajdusek 1977). As preparations from the brains of scrapie-infected hamsters were enriched for infectivity, evidence for an essential protein emerged, but no similar data for a nucleic acid could be generated. To the consternation of many, I introduced the term "prion" to distinguish from viruses the proteinaceous infectious particles causing scrapie and CJD (Prusiner 1982, 2014). Despite numerous attempts to demonstrate a scrapie-specific nucleic acid, none could be found, and as such, those investigations were eventually abandoned (Safar et al. 2005).

Soon after the introduction of the prion concept, a protein with a molecular weight of 27–30 kDa was found in purified fractions containing high levels of scrapie infectivity (Bolton et al. 1982; Prusiner et al. 1982a; McKinley et al. 1983). This protein was designated prion protein 27–30 (or PrP 27–30). Scrapie infectivity and PrP 27–30 were found at the top of sucrose gradients, indicating that some of the infectivity was likely to be very small and in agreement with the ionizing-radiation target size, whereas the majority of the infectivity sedimented to the bottom (Alper et al. 1966; Prusiner et al. 1978, 1980; Bellinger-Kawahara et al. 1988). The rod-shaped structures at the bottom of the gradients were shown to be composed of PrP 27–30 and to stain with Congo red dye, establishing that these large aggregates were amyloid (Prusiner et al. 1983). This discovery suggested that amyloid deposits in other disorders such as AD and PD might be composed of causative proteins (i.e., prions) (Prusiner 1984).

## PLAQUES, TANGLES, AND INCLUSIONS

Beginning in the mid-1980s, the proteins in plaques, tangles, and intracellular bodies in the brains of patients who died of neurodegeneration were identified through purification or immunostaining (Glenner and Wong 1984b; Brion et al. 1985; Grundke-Iqbal et al. 1986; Kosik et al. 1986; Wood et al. 1986; Spillantini et al. 1997). Each of these proteins was found to aggregate into fibrils under some conditions and to form amyloids (Fig. 1). The amyloid fibrils in AD were found to contain the Aβ peptide (Glenner and Wong 1984a; Masters et al. 1985), which is cut from the larger APP. The tangles present in many NDs were found to contain the tau protein. Additionally, the GCIs present in MSA were shown to contain α-synuclein. These same proteins were also identified when genetic studies demonstrated that specific mutant genes cause familial forms of neurodegeneration (Hsiao et al. 1989; Goate et al. 1991; Polymeropoulos et al. 1997; Hutton et al. 1998). Subsequently, expression of these mutant genes in Tg mice was shown to recapitulate many aspects of the human illnesses (for review, see Prusiner 2001).

Despite the similarities between various NDs and CJD, many investigators still prefer to think of these disorders as unrelated because Gajdusek, Gibbs, and their collaborators were unable to transmit AD, PD, and several other NDs to apes and monkeys (Goudsmit et al. 1980; Godec et al. 1991). These initial attempts to demonstrate transmissibility of AD and PD were made long before the isolation of Aβ peptide and α-synuclein, and, therefore, the respective causative proteins could not be used as biomarkers. Later, Ridley, Baker, and colleagues demonstrated transmission of neurodegeneration to marmosets, as indicated by Aβ cerebral amyloidosis, following intracerebral injections of AD brain homogenates (Baker et al. 1994; Ridley et al. 2006). The incubation times in those studies exceeded 3.5 yr, making independent confirmation of such experiments impractical. Later, Tg mouse studies would supplant the use of nonhuman primates.

## INHERITED NEURODEGENERATIVE DISEASES

The recognition that 5%–10% of CJD cases occur in families suggested that heredity might play a role in the pathogenesis of the disorder (Table 1). Subsequently, transmission of familial CJD (fCJD) and GSS to apes and monkeys was reported, but the multiple explanations for this finding involved slow viral illnesses in families (Roos et al. 1973; Masters et al. 1981). It was first suggested that the putative CJD virus might be passed from mother to offspring during childbirth as was later shown for human immu-

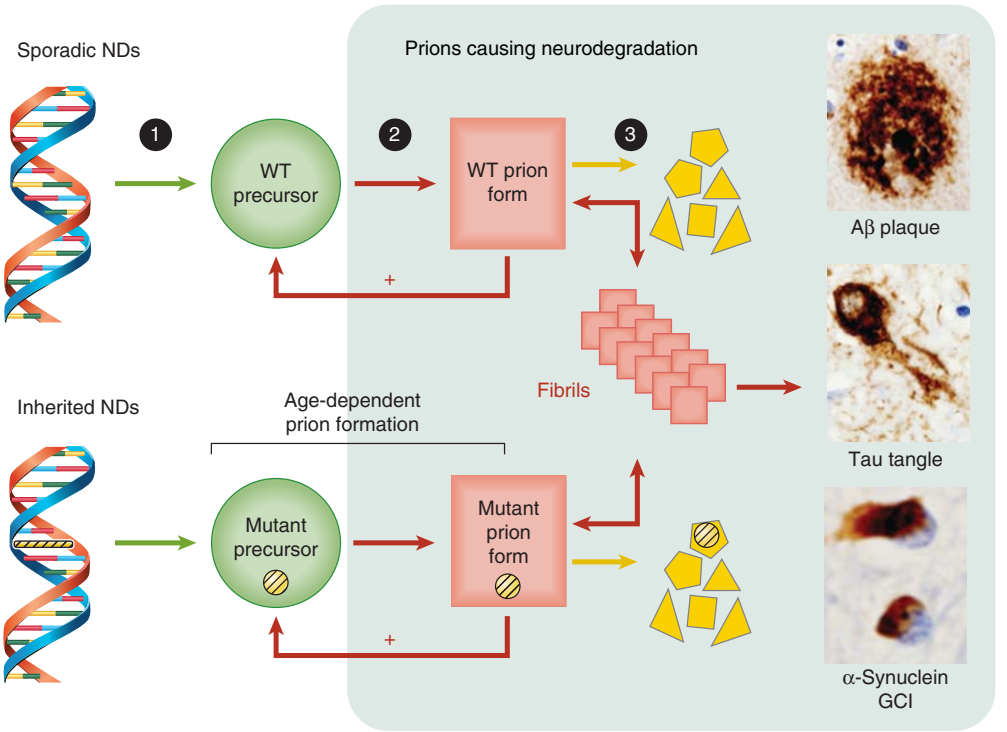


**Figure 1.** Neurodegeneration caused by prions. In sporadic neurodegenerative diseases (NDs), wild-type (WT) prions multiply through self-propagating cycles of posttranslational modification, during which the precursor protein (green circle) is converted into the prion form (red square), which is generally high in β-sheet content. Pathogenic prions are most toxic as oligomers and less toxic after polymerization into amyloid fibrils. Depending on the protein, the fibrils coalesce into Aβ amyloid plaques in Alzheimer's disease (AD), neurofibrillary tangles in AD and Pick's disease, or glial cytoplasmic inclusions (GCIs) in multiple system atrophy. Drug targets for the development of therapeutics include (1) lowering the precursor protein, (2) inhibiting prion formation, and (3) enhancing prion clearance. Late-onset heritable neurodegeneration argues for two discrete events: the synthesis of mutant precursor protein (*bottom* green circle) and the age-dependent formation of mutant prions (*bottom* red square). The highlighted yellow bar with diagonal lines in the DNA structure represents mutation of a base pair within an exon, and the small yellow circles with diagonal lines signify the corresponding mutant amino acid substitution. Green arrows represent a normal process. Yellow arrows indicate clearance of proteolytic prion fragments (yellow polygons), and red plus signs represent the cyclical process of prion formation. Red arrows indicate the pathogenic process in which wild-type or mutant prions act as a template to convert the precursor protein into a prion. (Images on *far right* reproduced from Dugger et al. 2017 with permission from Cold Spring Harbor Laboratory Press, © 2017. Remaining portion of figure is reproduced with permission of *Annual Review of Genetics*, Volume 47, © 2013 by Annual Reviews, http://www.anualreviews.org.)

nodeficiency virus (HIV). Second, it was proposed that the CJD virus might be passed to offspring in utero or during childhood when parents and children live in close proximity. A third possibility was that a chromosomal gene conferred susceptibility to a ubiquitous CJD virus. All three explanations proved incorrect.

In GSS patients, the identification of a mutation followed by the demonstration of genetic linkage was first reported for the P102L mutation in the PrP gene (Hsiao et al. 1989). Subsequently, four more mutations in the PrP gene were linked to fCJD, FI, and GSS with NFTs (Dlouhy et al. 1992; Medori et al. 1992; Poulter et al. 1992; Gabizon et al. 1993). More than 50 different mutations in the PrP gene have been identified, of which 35 are point mutations and the remainder are octapeptide expansions or

deletions (see Kong et al. 2004; Mead 2006; van der Kamp and Daggett 2009). Virtually all cases of GSS and FI appear to be caused by germline mutations in the PrP gene. Some mutations seem to have arisen de novo; whether any of these mutations in PrP are responsible for the development of the PrP prion diseases remains to be established.

For years, the late-onset presentation of fCJD and GSS presented a conundrum. Despite mutations in PrP being expressed early in life, PrP prions do not cause disease for decades. In studies of fCJD caused by the E200K mutation, actuarial analyses showed that if carriers lived long enough, they would all develop CJD (i.e., there was complete penetrance) (Chapman et al. 1994; Spudich et al. 1995).

Analogous to discoveries from GSS and fCJD cases, mutant proteins causing other inherited NDs were determined to be the same as those found in disease-specific amyloid deposits within intracellular inclusions, such as NFTs or Lewy bodies, or outside of cells as plaques (for review, see Prusiner 2001). These parallel findings offered support to the early proposition that more common diseases such as AD and PD might be caused by prions (Prusiner 1984).

## LATE-ONSET NEURODEGENERATION

Despite the fact that most mutant proteins that cause familial NDs are expressed early in embryogenesis, signs of neurological dysfunction are generally delayed for decades. This finding argues that some event occurs with aging that renders a disease-specific protein pathogenic. Many explanations have been offered to explain the late onset of familial NDs (Box 1), including age-dependent mitochondrial DNA mutations; oxidative modifications of DNA and proteins; proteasome malfunction; diminished innate immunity; exogenous toxins such as alcohol and drugs; concomitant conditions such as atherosclerosis; somatic mutations; chaperone malfunction; haploinsufficiency; RNA-DNA differences; expanded repeat segments in proteins and noncoding regions of DNA; and post-infectious syndromes. However, little evidence supports most of these proposed mechanisms. The formation of prions from their respective proteins provides a plausible explanation for the late-onset presentations of many different NDs and is consistent with the observation that aging appears to be the most significant risk factor. These proteins must first refold into prions and then accumulate in sufficient numbers for an infection to become self-sustaining (Prusiner 2012). Presumably, the number of prions that are generated by this self-propagating process has to reach a threshold before an infection can continue unabated (Prusiner 1989). Under these conditions, prion propagation becomes uncontrolled, eventually resulting in CNS dysfunction.

One fascinating insight into the control of the timing of disease onset arises from studies of approximately 35 fCJD families with expanded PrP octarepeat regions (Croes et al. 2004; Stevens et al. 2009). Wild-type $PrP^{C}$ contains four octarepeats. One to four additional octarepeats cause late-onset fCJD with a mean age of 62 yr; five to eight additional octarepeats cause early-onset fCJD or GSS with a mean age of 32 yr. In other words, the fifth additional octarepeat decreases the age of disease onset by three decades. This profound transition in pathogenesis was found to correlate with a shift in $Cu^{2+}$ binding to PrP: a high $Cu^{2+}$ occupancy state shifted to a low state (Stevens et al. 2009). Titrations showed that one $Cu^{2+}$ ion binds to each histidine in the high occupancy state; each of the four octarepeats contains a single histidine. In the low occupancy state, a single $Cu^{2+}$ ion coordinates with four histidines. The mechanism by which $Cu^{2+}$ ions that are bound to mutant PrP with octarepeat expansions participate in the formation of $PrP^{Sc}$ remains unknown. Whatever the process that controls the formation of prions in the inherited PrP prion diseases may prove relevant to other age-dependent NDs.

## SPORADIC NEURODEGENERATIVE DISEASES

In the absence of a mutation or an environmental cause such as an infection or a toxin, NDs are generally referred to as sporadic. Such illnesses are thought to arise stochastically. Although

**BOX 1. Some possible explanations for late-onset neurodegeneration**

1. Mitochondrial DNA mutations (Coskun et al. 2010; Morais and De Strooper 2010)
2. Oxidative modifications of DNA, lipids, or proteins (Larsson 2010; Johri and Beal 2012)
3. Impaired autophagy (Olanow and McNaught 2006)
4. Altered apoptosis (Yuan and Yankner 2000)
5. Posttranslational chemical modification (Yuan and Yankner 2000)
6. Modified innate immunity (Tracey 2009)
7. Accumulation of exogenous toxins such as heavy metals, alcohol, drugs, and hormones (Chen et al. 2011)
8. Concomitant conditions such as atherosclerosis (Korczyn et al. 2012)
9. RNA-DNA differences (Li et al. 2011)
10. Chaperone malfunction (Macario and Conway de Macario 2005)
11. Somatic mutations (Larsson 2010)
12. Altered regulation of transcription (Bithell et al. 2009)
13. Haploinsufficiency (Deutschbauer et al. 2005; Rademakers et al. 2007)
14. Postinfectious syndromes of the central nervous system including late polio, subacute sclerosing leukoencephalitis, postencephalitic Parkinson's disease, and Lyme disease (Greenfield and Matthews 1954; Zilber et al. 1983; Jubelt 2004)
15. Modifier genes like apolipoprotein E and LRRK2 (Castellano et al. 2011; Corti et al. 2011)
16. Polyglutamine expansions (Wheeler et al. 2002; Lee et al. 2012)
17. Dipeptide repeat proteins (Mori et al. 2013)
18. $Cu^{2+}$ binding to expanded PrP octarepeat region (Stevens et al. 2009)
19. Prion formation and accumulation (Prusiner 1989; Olanow and Prusiner 2009)

10%–20% of NDs are inherited, >80% are sporadic. This relationship has been found for CJD, AD, PD, the tauopathies, and ALS but not for Huntington's disease (HD), which is always inherited (Huntington's Disease Collaborative Research Group 1993; Jimenez-Sanchez et al. 2016; Pearce and Kopito 2017). Notably, inheritance of the *e4* allele of apolipoprotein E is the only well-established genetic risk factor for sporadic AD (Mahley et al. 2006; Harold et al. 2009). Presumably, many disease-causing proteins can form prions, but most prions are cleared before they begin to multiply in sufficient numbers to sustain infection, which involves spread to adjacent cells and eventually to other regions of the brain. One possible mechanism to explain sporadic NDs involves precursor proteins that are transformed into disease-causing prions through a stochastic process, which most of the time probably represents a dead-end route in which small numbers of prions are cleared via protein degradation pathways.

## PrP PRIONS

Synthetic PrP prions have provided indisputable evidence that the disease-causing agent of CJD and other PrP prion diseases is composed solely of protein (Legname et al. 2004; Colby et al. 2009; Makarava et al. 2010; Wang et al. 2010; Ghaemmaghami et al. 2013). The first prospect of a synthetic $PrP^{Sc}$ prion arose when we produced a synthetic PrP peptide of 55 amino acids containing the P101L mutation (mouse numbering), which causes GSS in hu-

mans; the peptide was made by coupling individual amino acids together as was later done for the synthetic Aβ peptide (Kaneko et al. 2000). After we combined the PrP peptide with acetonitrile, which caused it to adopt a β-sheet-rich structure, the peptide was injected into a Tg mouse model of GSS (Hsiao et al. 1990, 1994; Tremblay et al. 2004). Approximately a year later, the inoculated mice became ill, whereas the uninoculated mice remained healthy for another 6 mo. Some investigators have argued that the subsequent illness in the uninoculated controls showed that the peptide was not a prion but was merely an accelerator of disease; such arguments persisted for more than a decade (Nazor et al. 2005). Importantly, we were able to show by serial passage that the brains of the inoculated mice contained a transmissible entity (i.e., a prion) (Tremblay et al. 2004).

Influenced by studies of Sup35 in yeast (Sparrer et al. 2000), we purified recombinant mouse PrP(89–230) made in bacteria, assembled the PrP(89–230) into amyloid fibrils (Baskakov et al. 2002), and injected the fibrils into Tg mice expressing the same N-terminally truncated PrP. When the mice remained healthy for >250 d, we concluded that the experiment was negative (Baskakov et al. 2002). Unintentionally, the experiment was not terminated, and ~600 dpi, the mice showed signs of neurological dysfunction. Moreover, upon neuropathological explanation, their brains showed the hallmarks of prion disease (Legname et al. 2004). The prion strain found in these mice was more resistant to denaturation at higher concentrations of guanidine hydrochloride than is generally seen with naturally occurring prion strains (Legname et al. 2005).

When we examined the stability of a half-dozen prion isolates in mice, including one of the synthetic prions, we found that the more stable strains, which resisted denaturation, produced longer incubation times, and the less stable strains yielded shorter incubation times (Legname et al. 2006). On the basis of this finding, we produced synthetic prions under conditions that created PrP preparations that were highly resistant to denaturation, those that were quite susceptible, and those that were intermediate. The stable preparations produced long incubation times, whereas the more labile ones yielded much shorter incubation times (Colby et al. 2009; Ghaemmaghami et al. 2013). Although these investigations confirmed and extended our earlier work, they were expensive because the incubation times exceeded 500 d on first passage. More extensive analyses in cell culture and Tg mice have demonstrated the evolution of prions toward strains causing shorter incubation times and harboring an unglycosylated, protease-resistant $PrP^{Sc}$ fragment (PrP 27–30) of 21 kDa (Li et al. 2010; Ghaemmaghami et al. 2011, 2013).

## Aβ PRIONS

In a series of incisive experiments, Mathias Jucker and colleagues transmitted Aβ prions to weanling Tg(APP23) mice expressing mutant human APP. Much of their work was performed with brain homogenates from aged Tg(APP23) mice that spontaneously develop Aβ amyloid plaques. Brain homogenates prepared from aged Tg(APP23) mice were inoculated into the brains of young Tg(APP23) mice. The inoculation dramatically accelerated the deposition of nascent Aβ amyloid; in addition, immunoabsorption of Aβ in the inoculum prevented the accelerated deposition of Aβ (Meyer-Luehmann et al. 2006; Walker et al. 2016). These investigators also reported transmission of Aβ prions after intraperitoneal injections (Eisele et al. 2010). More recently, Claudio Soto and colleagues, as well as Jucker and coworkers, reported that brain homogenates from AD patients could also transmit disease to Tg mice expressing wild-type human APP and Tg rats expressing mutant APP, neither of which develops cerebral Aβ deposits spontaneously (Morales et al. 2012; Rosen et al. 2012).

Using bigenic mice expressing mutant APP and luciferase under the control of the murine *Thy 1.2* and *Gfap* promoters, respectively, my colleagues and I found that brain homogenates from aged Tg(APP23) mice transmitted Aβ prions, as reflected by increased bioluminescence and Aβ deposits (Watts et al. 2011; Watts and Prusiner 2017). Subsequently, we found that

fractions highly enriched for Aβ produced an increase in bioluminescence after ~5 mo, which was ~6 wk faster than that seen with crude brain homogenate. Most importantly, synthetic Aβ peptides became prions during polymerization into amyloid, establishing that some hypothetical contaminate was not responsible for the change in bioluminescence (Stöhr et al. 2012). Serial passage of different Aβ prion strains isolated from human AD strains bred true upon repeated passage, as did synthetic Aβ peptides polymerized into amyloid fibrils (Stöhr et al. 2014; Watts et al. 2014).

Remarkably, Heiko Braak not only described the spread of Aβ amyloid plaques but also showed the concurrent deposition of NFTs, composed of the tau protein, in the brains of deceased AD patients (Braak et al. 1996; Braak and Del Tredici 2011, 2016). Recent studies have traced the spread of tau prions using functional magnetic resonance imaging (fMRI) intrinsic connectivity analysis (Zhou et al. 2012). Similar spreading was described much earlier for scrapie prions in ovines and rodents (Fraser and Dickinson 1968; Hadlow et al. 1974; Kimberlin and Walker 1979; Fraser 1982; Scott et al. 1992; Taraboulos et al. 1992; Tatzelt et al. 1999).

### Familial Alzheimer's Disease

Soon after the identification of mutant PrP genes, missense mutations in the APP gene were discovered in fAD followed by mutations in the presenilin 1 and 2 genes (*PSEN1* and *PSEN2*), which encode the catalytic subunits of γ-secretase; these mutations result in elevated levels of wild-type Aβ peptide (Table 1) (Goate et al. 1991; Schellenberg et al. 1992; St George-Hyslop et al. 1992; St George-Hyslop 1999; Hardy and Selkoe 2002; Schellenberg and Montine 2012). In addition, more than a dozen mutations within the Aβ peptide have been discovered (Walsh and Selkoe 2007; Benilova et al. 2012), most of which also cause fAD. All of the mutations causing fAD, which account for 10%–20% of AD cases, are autosomal dominant and produce one or two amino acid substitutions.

In contrast to APP mutations causing fAD, the A673T mutation in APP identified in a genome-wide study of Icelandic people seems to protect against late-onset AD (Jonsson et al. 2012). This missense mutation is located at the β-secretase cleavage site and presumably inhibits the production of the Aβ peptide. Diminished wild-type Aβ levels would seem likely to reduce the chances of sustained Aβ prion formation leading to AD.

Although no authentic animal models of AD exist, much has been learned from mice (and more recently Tg rats) (Hsiao et al. 1996; Cohen et al. 2013) that express mutant human APP transgenes. The initial mouse models of fAD used Tg mice expressing human APP with mutations that increased the level of wild-type Aβ (Games et al. 1995); these mice exhibited numerous amyloid plaques filled with fibrils composed of the Aβ peptide, as well as astrocytic gliosis and behavioral changes. Mice were subsequently constructed expressing mutant human APP, presenilin, and tau to build a more authentic model of AD; these mice were designated triple transgenics, or 3·Tg-AD mice (Oddo et al. 2003). Such Tg models have been used to assess the therapeutic efficacy of antibodies for the clearance of the Aβ peptide (DeMattos et al. 2012). Unfortunately, attempts to treat AD patients with anti-Aβ antibodies have yet to produce an FDA-approved therapy (Selkoe 2011).

### Unifying Hypotheses for Alzheimer's Disease

AD is a poorly understood disorder: besides being the most common ND by far, two different prions appear to feature simultaneously in its pathogenesis. Although plaques and tangles result from both Aβ and tau forming prions, the dynamic process of prion formation is crucial to understanding the pathogenesis of this common malady. Prion proteins undergo profound conformational changes that are the basis of their pathogenicity: accumulation of these conformationally modified proteins produces neuronal dysfunction resulting in neurological deficits.

Molecular genetic studies have established the primacy of prion formation in AD pathogenesis. Mutations in the APP gene produce inherited AD. The mutant Aβ exhibits an alter-

native structure resulting in prion formation. To defend against Aβ prion propagation, Aβ prions assemble into amyloid fibrils. In some cases, the mutation occurs within the Aβ peptide resulting in the deposition of mutant Aβ amyloid plaques. Once mutant Aβ prions begin to accumulate, their formation becomes autocatalytic. As the Aβ fibrils coalesce, they form plaques, which are one of the hallmarks of AD.

In other cases of inherited AD, the Aβ peptide has the wild-type sequence, as do the Aβ amyloid plaques. In some cases, like the Swedish mutation, the elevated level of wild-type Aβ rises, which appears to be sufficient to induce Aβ prion formation. As expected, wild-type Aβ amyloid plaques accumulate. As described by Braak and coworkers, the Aβ prions spread from one neuronal pathway to another as AD progresses (Braak and Del Tredici 2016). This movement along one neural pathway to another is pathognomonic of the infectious aspects of prion diseases. Most prion diseases spread from cell to cell but not from one person to another. Some investigators have chosen to narrow the description of prion diseases to those that involve spread from one organism to another, such as with kuru via ritualistic cannibalism or iatrogenic CJD spread to young people treated for short stature with cadaveric growth hormone.

Because mutations in the tau gene (*MAPT*) do not produce AD but do cause inherited tauopathies, it seems likely that Aβ prions are capable of stimulating the conversion of tau into prions (Fig. 2). The foregoing scheme suggests that AD is a "double prion disease," for which there is an expanding body of support underpinning this hypothesis. Curiously, not all Aβ peptides stimulate tau proteins to form prions. Nearly half of older people who have been studied using PET imaging have been found to have numerous Aβ plaques but no intellectual deficits (Oh et al. 2016). At autopsy, these cognitively normal older people do not have any evidence of tau prions or NFTs; most importantly, these older people are not demented at the time of death.

## TAU PRIONS

The tauopathies reside at an interface between psychiatry and neurology. These disorders often present as psychiatric illnesses in patients over the age of 40. Often psychiatrists see patients with frontotemporal dementia (FTD) for several years before they refer the patients to neurologists with an incorrect diagnosis of AD. Tau lesions in the frontal lobes can produce behavioral disinhibition, apathy, inappropriate social interactions, depression, and insomnia, as well as reduced executive function (Rabinovici and Miller 2010). Later, semantic dementia, drug abuse, alcoholism, and sometimes suicide are seen. As behavioral and language problems worsen, these inherited and sporadic neurological disorders are frequently classified clinically

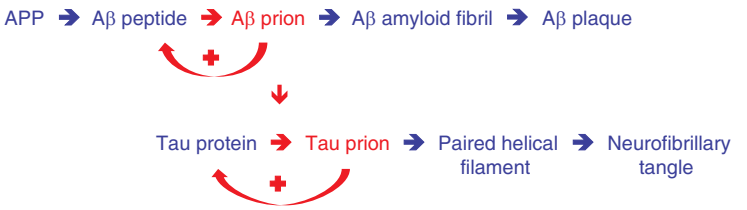


**Figure 2.** Unifying hypothesis for Alzheimer's disease (AD). Both Aβ and tau form prions resulting in neuronal dysfunction. Molecular genetic studies established the primacy of these two proteins in AD. Mutations in the APP gene, from which Aβ is cleaved, demonstrate that Aβ initiates the process. The mutant Aβ exhibits a modified structure that leads to prion formation. Once Aβ prions begin to accumulate, their formation becomes autocatalytic. In defense of Aβ prion propagation, Aβ prions assemble into amyloid fibrils. As the Aβ fibrils coalesce, they form plaques, which are one of the hallmarks of AD. Because mutations in the tau gene do not produce AD but rather primary tauopathies, it seems likely that Aβ prions stimulate tau prions to form. The foregoing scheme suggests that AD is a double prion disease, for which there is an expanding body of findings to support this hypothesis.

as behavioral variants of FTD (bvFTD). Different phenotypes of tauopathy, including progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and Pick's disease (PiD), may result from isoform and conformational variants of tau prions, producing lesions in different neuronal circuits (Seeley et al. 2009; Woerman et al. 2016).

In an important series of studies over the past four decades, athletes who have played contact sports, including boxers as well as football and hockey players, were found to have symptoms similar to those in FTD patients. Moreover, numerous NFTs were found postmortem in the frontal lobes of these athletes (Corsellis et al. 1973), some of whom committed suicide. The first football player identified with a tauopathy was Pittsburgh Steeler Mike Webster, who played professional football for 16 seasons and died at age 50 (Omalu et al. 2005). Subsequently, 50 other football players at the high school, college, or professional level who committed suicide were identified with tauopathies (McKee et al. 2013; Kriegel et al. 2017). Recently, a 27-year-old Marine who suffered multiple episodes of traumatic brain injury (TBI) during explosions of roadside bombs in the Iraq conflict was found to suffer from a tauopathy. Following the diagnosis of posttraumatic stress disorder (PTSD), he was honorably discharged, divorced, and became alcoholic. Eight months after his discharge, he committed suicide; at autopsy, numerous NFTs were found in his frontal lobes (Omalu et al. 2011). Many similar cases of military veterans with the symptoms of PTSD have been reported (McKee et al. 2013).

U.S. Army studies show that multiple episodes of mild TBIs from exposure to blast from improvised explosive devices increase the risk for PTSD (Hoge et al. 2008). The latest estimates suggest that approximately 400,000 of the two million military personnel who have served in the Iraq or Afghanistan conflicts will develop PTSD. How many of these individuals will develop a posttraumatic tauopathy (or chronic traumatic encephalopathy [CTE]) caused by tau prions is unknown. Although concussive forces presumably stimulate tau prion formation in the human brain, the mechanism by which this occurs remains unknown (Goldstein et al. 2012).

Aggregates formed from truncated recombinant human tau composed of residues 242–364 (K18 fragment) in the presence of arachidonic acid were shown to enter C17 cells and seed the polymerization of endogenous tau (Frost et al. 2009). These studies were extended using HEK293 cells expressing full-length wild-type human tau (2N4R) as well as truncated and mutant human tau (P301S) (Guo and Lee 2011; Kfoury et al. 2012; Holmes and Diamond 2016). Michel Goedert, Markus Tolnay, and colleagues described the transmission of mutant tau (P301S) prions produced in Tg mice to recipient mice expressing wild-type human tau (Clavaguera et al. 2009, 2013, 2014, 2016). After ~6 mo, the inoculated Tg mice showed wild-type tau aggregates that had spread from the site of inoculation to neighboring regions. Synthetic tau fibrils were formed from full-length tau (2N4R) with the P301S mutation and from the truncated K18 fragment with the P301L mutation expressed in *Escherichia coli*. They were purified and polymerized in the presence of heparin. The fibrils were inoculated intracerebrally into young Tg(*MAPT**P301S) mice overexpressing mutant human tau (Iba et al. 2013). These inoculations produced NFT-like inclusions that propagated from injected sites to connected brain regions in a time-dependent manner. Interestingly, injection of the tau fibrils into either the hippocampus or the striatum, along with the overlaying cortex, produced a distinct pattern of spreading. Unlike tau pathology that spontaneously develops in older Tg(*MAPT**P301S) mice, the tau deposits resembled NFTs as indicated by their acetylation, thioflavin S (ThioS) positivity, and resistance to limited proteinase K digestion.

Recently, it was shown that tau prions from the brains of deceased AD and CTE patients could be transmitted to HEK cells expressing tau–YFP fusion proteins (Woerman et al. 2016). Brain extracts from deceased individuals with PiD, a disease characterized by aggregates of three-repeat (3R) tau isoforms, infected HEK293T cells expressing a fragment of mutant 3R tau–YFP. Extracts from argyrophilic grain

disease (AGD), CBD, and PSP patients, which contain four-repeat (4R) tau aggregates, infected HEK293 cells expressing a fragment of the 4R isoform of mutant tau. These studies demonstrated that tau prion propagation in HEK cells requires matching isoforms between prion and substrate. Interestingly, tau aggregates in AD and CTE patient samples contain both 3R and 4R isoforms, but they were unable to robustly infect the 3R or 4R fragment-expressing cells that were susceptible to infection with isoform-specific tauopathies. However, HEK293T cells expressing both 3R and 4R tau isoforms supported tau prion propagation when infected with AD or CTE brain extracts. Additionally, 3R/4R-samples containing AD and CTE prions successfully infected HEK293T cells expressing a higher level of mutant 4R tau. Most importantly, tau prions in AD and CTE brain extracts could be measured in the cell lines developed in these studies (Woerman et al. 2016).

As with Aβ amyloid plaques in AD, NFTs also spread along defined neuroanatomical pathways (Braak and Braak 1995; Braak and Del Tredici 2016). Presumably, tau prions spread transsynaptically as they move from one neuron to another. Recent Tg mouse models expressing human tau in the entorhinal cortex show spread along neuroanatomically defined pathways to hippocampal pyramidal neurons, especially in CA1 and dentate gyrus granule cells (Bolmont et al. 2007; Clavaguera et al. 2009; de Calignon et al. 2012; Liu et al. 2012; Polymenidou and Cleveland 2012).

## Inherited Tauopathies

Familial forms of the FTDs result from mutations in the *MAPT* gene, which encodes the tau protein; these disorders include PSP, PiD, frontotemporal lobar degeneration (FTLD), CBD, and AGD (Table 1). FTLD with parkinsonism has been linked to mutations in the *MAPT* gene on chromosome 17 (and is often referred to as FTLDP-17). Individuals with the disorder present with behavioral and personality changes, cognitive impairment, and signs of motor dysfunction. In addition to the gene encoding tau, mutations in other genes encoding TDP-43, FUS, progranulin (PGRN), and C9orf72 have been linked to familial forms of the FTDs. As with CJD and AD, familial FTDs account for 15%–25% of FTD cases (Table 1). Most FTD cases appear to be sporadic, seem to occur spontaneously, and are likely to be caused by tau prions or other prions formed from TDP-43 or FUS. Both PGRN and C9orf72 are thought to cause disease through haploinsufficiency (Rademakers et al. 2007; Mori et al. 2013). Interestingly, the expanded hexanucleotide repeat upstream of the C9orf72 coding region appears to encode several dipeptides that are translated into proteins, which deposit as neuronal cytoplasmic inclusions, suggesting an alternative pathogenic mechanism (Ash et al. 2013; Mori et al. 2013; Guo and Shorter 2016; Gendron and Petrucelli 2017; Gijselinck et al. 2017; Mackenzie and Neumann 2017; Nonaka and Hasegawa 2017).

In the FTDs caused by tau prions, NFTs are the neuropathological hallmark. The discovery of NFTs composed of tau fibrils in many CNS disorders led to the conclusion that tangles were nonspecific, unimportant histopathological entities. However, the discovery of mutations in the tau gene in patients suffering from familial FTDs brought remarkable clarity to a once rather Byzantine area of neurology (Hong et al. 1998; Hutton et al. 1998; Rademakers et al. 2012). As with $PrP^{Sc}$, conformational variants of tau prions, or strains, create different regional patterns of tau accumulation in the brain. These distinct patterns of tau prion accumulation produce different disease phenotypes.

The primary tauopathies encompass both the familial and sporadic forms of the FTDs, in which pathologic deposits of tau accumulate as either NFTs or collections of fibrils within neurons. In contrast, the secondary tauopathies, the most common of which is AD, have many causes. Importantly, ablation of the *MAPT* gene encoding tau ameliorated disease in AD mouse models expressing mutant APP (Roberson et al. 2007). Other disorders in which tau-laden NFTs accumulate include viral illnesses such as rabies, subacute sclerosing panencephalitis, and postencephalitic PD; inherited disorders such as Niemann–Pick disease and

myotonic dystrophy; and a disease of unknown etiology called Guam ALS-PD with dementia. In addition, the F198S point mutation and an octarepeat expansion in PrP have been reported to cause GSS and fCJD with NFTs, respectively (Hsiao et al. 1992; Kumar et al. 2011).

## SYNUCLEIN PRIONS

In the late 1990s, fetal brain cells from the substantia nigra of aborted fetuses were transplanted into patients with advanced PD to mitigate the loss of dopaminergic neurons. A decade later, Lewy bodies were found in the grafted fetal brain cells when the PD patients died (Kordower et al. 2008; Li et al. 2008). The surface of a Lewy body is covered with fibrils composed of β-sheet-rich α-synuclein proteins, and the normal form of α-synuclein seems to be either unstructured or high in α-helical structure. However, like many other prion precursor proteins, α-synuclein can adopt a β-sheet-rich conformation. Although unproven, it seems likely that β-sheet-rich α-synuclein prions crossed from the PD patient's own neurons into the grafted cells, inducing a change in the structure of α-synuclein (Olanow and Prusiner 2009). Once established, this process became self-propagating, as is the case for all pathogenic prions. This scenario is consistent with the findings of Braak and colleagues, who have mapped the spread of aggregated α-synuclein (called Lewy neurites) from the gut into the brainstem and throughout the cerebral hemispheres (Braak et al. 1996, 2003; Polymenidou and Cleveland 2012).

### Inherited Parkinson's Disease

Genetic linkage of the A53T mutation in the α-synuclein gene (Polymeropoulos et al. 1997; Nussbaum 2017) led to the identification of α-synuclein in Lewy bodies, which are found in both inherited and sporadic forms of PD (Spillantini et al. 1997; Tofaris et al. 2016). Duplication and triplication of the α-synuclein gene have also been reported in PD patients (Hardy et al. 2006). Additionally, the G2019S variant in the leucine-rich repeat kinase 2 (LRRK2) protein imposes an increased risk for sporadic late-onset PD (Healy et al. 2008). Although mutations in other proteins have been identified as the cause of other forms of inherited PD, the relevance, if any, of these polypeptides to sporadic PD is unclear.

In studies by Giasson and colleagues, Tg(Hu-αSyn*A53T)M83$^{+/-}$ mice expressing mutant human α-synuclein (A53T) were found to exhibit neurological disease at ∼400 d of age (Giasson et al. 2002). When brains from these ill mice were homogenized and injected into weanlings, the recipient mice became ill at ∼200 d of age (Luk et al. 2012b; Mougenot et al. 2012). Recombinant α-synuclein was purified and induced to form fibrillar aggregates in vitro. These α-synuclein aggregates were found to undergo self-propagation in cultured cells, in Tg mice expressing mutant human α-synuclein (A53T), and in non-Tg mice inoculated with aggregates of recombinant wild-type α-synuclein. These studies demonstrated the self-propagation of an altered conformation of α-synuclein and, as such, its transformation into a prion (Volpicelli-Daley et al. 2011; Luk et al. 2012a,b).

### MSA Prions

MSA is an ND that is often characterized as a "Parkinson's plus" condition. In addition to parkinsonian features, MSA patients may show autonomic nervous system dysfunction including orthostatic hypotension, incontinence, temperature dysregulation, indigestion, and erectile dysfunction. MSA has an incidence of three new cases per 100,000 individuals per year (Bower et al. 1997). Typically, MSA patients die within 5–10 yr after diagnosis.

In contrast to α-synuclein aggregates in PD (Lewy bodies and Lewy neurites found in neurons throughout the neuraxis), collections of α-synuclein, GCIs, are found in oligodendrocytes in MSA (Graham and Oppenheimer 1969; Spillantini et al. 1998a). Initially, it was thought that α-synuclein was not expressed in glial cells (Solano et al. 2000; Ozawa et al. 2001; Miller et al. 2005); however, recent findings propose that α-synuclein is expressed in oligodendrocytes, albeit at lower levels than in neurons

(Asi et al. 2014; Djelloul et al. 2015). Alternatively, it has been suggested that α-synuclein is secreted from neurons and taken up by neighboring oligodendrocytes to form GCIs (Reyes et al. 2014). In experimental studies, both recombinant α-synuclein and conditioned media from neurons overexpressing α-synuclein were taken up by immortalized oligodendrocyte cell lines and primary rat oligodendrocytes (Kisos et al. 2012). In other studies, clathrin-dependent internalization of recombinant α-synuclein after incubation for 24 h with the KG1C oligodendrocyte cell line was reported (Konno et al. 2012). The resulting α-synuclein aggregates were ThioS-positive, ubiquitinated, and immunoreactive for the phosphorylated α-synuclein antibody (pSer129), which is commonly used for the pathological confirmation of synucleinopathy postmortem (Rey et al. 2016). These findings demonstrate the ability of oligodendrocytes to take up and accumulate α-synuclein in structures similar to GCIs.

The unanticipated results of a study in 2013 showed that two cases of MSA transmitted CNS dysfunction to TgM83$^{+/-}$ mice expressing mutant human α-synuclein*A53T protein (Watts et al. 2013; Woerman et al. 2017). In that initial report, brain homogenates prepared from two cases of MSA were intracerebrally injected into TgM83$^{+/-}$ mice, which developed progressive CNS dysfunction after ∼120 d. The brains of the Tg mice exhibited extensive phosphorylated α-synuclein deposits in the cytoplasm and axons of neurons.

To determine whether the transmissions of the two MSA cases were anomalous, we inoculated TgM83$^{+/-}$ mice with another 12 cases from three different continents: the United Kingdom, Australia, and the United States (Prusiner et al. 2015). The 12 homogenates prepared from the MSA patient samples produced an experimental synucleinopathy with the same features as those described above for the initial two transmissions. Using multiple brain regions from some of the MSA cases, a total of 19 homogenates from 14 MSA cases produced CNS dysfunction in TgM83$^{+/-}$ mice and infected HEK cells expressing α-syn140*A53T fused to YFP, resulting in cytoplasmic aggregates of the fusion protein that were measured by confocal fluorescence microscopy (Prusiner et al. 2015; Woerman et al. 2015). From these transmission studies in both TgM83$^{+/-}$ mice and cultured cells, we concluded that MSA is a transmissible human ND caused by α-synuclein prions.

The discovery that α-synuclein, a presynaptic protein composed of 140 amino acids, is the major constituent of Lewy bodies and GCIs led to many studies designed to elucidate the molecular mechanisms responsible for the pathogenesis of PD and MSA. Cellular studies examined α-synuclein aggregation and spreading from one neuron to another using overexpression of the protein (Desplats et al. 2009). Additional studies used synthetic α-synuclein fibrils to inoculate cultured cells and mice (Luk et al. 2009; Volpicelli-Daley et al. 2011; Kisos et al. 2012; Konno et al. 2012).

In 2009, Desplats and colleagues used SH-SY5Y cells differentiated toward dopaminergic neurons to study the propagation of α-synuclein in vitro (Desplats et al. 2009). Using a co-culture approach, these investigators overexpressed myc-tagged α-synuclein in one group of cells (the donor group) while fluorescently tagging the second group with Qtracker (the acceptor group). Importantly, the acceptor cells did not overexpress α-synuclein. Within 24 h of co-culturing the two cell lines, α-synuclein aggregates were detected in the Qtracker-labeled acceptor cells, demonstrating cell-to-cell propagation of α-synuclein. The aggregates in the acceptor cells were ubiquitinated and positive for ThioS staining, similar to GCIs in MSA patients. In a similar co-culture approach, Hansen and colleagues developed both HEK and SH-SY5Y neuroblastoma cell lines expressing α-synuclein fused to either DsRed or green fluorescent protein (GFP) (Hansen et al. 2011). When the two α-synuclein fusion proteins were expressed in co-culture, regardless of the cell type used, GFP-positive α-synuclein was found to have migrated into cells expressing α-synuclein fused to DsRed, and vice versa. These findings could be interpreted to support the hypothesis that α-synuclein spreads from one cell to another and causes CNS dysfunction.

The hypothesis that α-synuclein prions can spread from one cell to another was supported by studies using HEK cells that overexpressed wild-type α-synuclein that had been aggregated into fibrils (designated as preformed fibrils or PFFs) (Luk et al. 2009). Myc-tagged PFFs were used to infect HEK cells, and α-synuclein aggregates were detected 48 h later. These aggregates were hyperphosphorylated, detergent-insoluble, and ubiquitinated, similar to aggregates isolated from human samples. Interestingly, co-staining for myc and phosphorylated α-synuclein revealed that the exogenous PFFs formed the core of the aggregates, whereas endogenous α-synuclein formed the exterior. α-Synuclein PFFs could also induce endogenous α-synuclein aggregation in primary neuronal cultures (Volpicelli-Daley et al. 2011). After 4 d of incubation in the cultured HEK cells, α-synuclein aggregates were seen in the neurites, and their spread to the soma of the neurons was apparent by day 10. Interestingly, hippocampal neurons grown in microfluidic chambers and infected with PFFs demonstrated retrograde spreading of α-synuclein aggregates, starting in the neurites and moving up to the soma, as well as anterograde propagation from the soma down to the neurites.

In light of earlier work measuring tau prions using tau–YFP fusion proteins (Kfoury et al. 2012; Sanders et al. 2014; Holmes and Diamond 2016), we developed a similar assay for α-synuclein prions. Our initial α-synuclein constructs were based on the aggregation-promoting regions of the protein (Burré et al. 2012, 2017). Based on systematic mutagenesis studies, we found that cells expressing mutated α-synuclein (α-syn140*A53T–YFP) were more susceptible to infection with α-synuclein fibrils compared with cells expressing wild-type α-synuclein (α-syn140–YFP), although neither cell line exhibited spontaneous aggregate formation (Woerman et al. 2015, 2017). After isolating protein aggregates from six MSA patient samples using sodium phosphotungstic acid (PTA) (Lee et al. 2005), we incubated the brain extracts with the α-syn140*A53T–YFP cells for 4 d and found that all six samples induced α-synuclein–YFP accumulation, as defined by the appearance of intensely fluorescing foci within the cells. This discovery was specific to MSA; none of the 17 control or three PD patient samples tested infected the α-syn140*A53T–YFP cells, demonstrating selectivity for α-synuclein prions from MSA patient samples.

Importantly, we also tested the ability of MSA prions to serially propagate in cultured cells. Lysate harvested from both stable clones and uninfected α-syn140*A53T–YFP cells was incubated with naïve HEK293T cells for 3 d. Both clones robustly infected the cells, whereas lysate from the uninfected cells had no effect. Serial propagation or templating of protein misfolding is a hallmark of prion diseases. Significantly, the ability to continuously propagate a prion strain in vitro provides an opportunity to rapidly investigate the disease process and potentially identify compounds that interfere with disease progression.

## α-Synuclein Prions in Mammals

α-Synuclein prions can be propagated like PrP prions in vivo, as shown by inoculation of α-synuclein fibrils into wild-type animals. Studies by Luk et al. showed that a single stereotactic injection of α-synuclein PFFs into the striatum results in phosphorylated α-synuclein neuropathology 180 dpi; however, similar results were not obtained when PFFs were inoculated into α-synuclein knockout mice (*Snca*$^{-/-}$) (Luk et al. 2012a). Importantly, this indicates that PD-like pathology seen in the wild-type animals specifically arises from the propagation of α-synuclein prions.

Masuda-Suzukake et al. (2013) demonstrated the first transmission of misfolded α-synuclein using protein isolated from a human synucleinopathy case (Masuda-Suzukake et al. 2013; Hasegawa et al. 2016). After inoculating insoluble mouse or human recombinant α-synuclein fibrils into the substantia nigra (SN) of wild-type mice, the authors found α-synuclein deposition 15 mo later. Following the fibril inoculations (which induced neuropathology in ∼94% of the mice), the investigators injected the SN of wild-type mice with sarkosyl-insoluble extracts prepared from patients with demen-

tia with Lewy bodies (DLB). Ipsilateral α-synuclein pathology was apparent in half the inoculated mice (and only 7% of the mice showed spreading to the contralateral hemisphere within 15 mo).

The following year, Recasens et al. (2014) isolated Lewy bodies from the SNs of three deceased PD patients and inoculated the aggregated protein adjacent to the SNs in wild-type mice. The inoculations produced a considerable decrease in dopaminergic fibers and an increase in phosphorylated α-synuclein aggregates in the striatum and SN 17 mo postinjection. Moreover, the authors inoculated the same PD patient samples into the striatum and SN of macaque monkeys and found a reduction in dopaminergic neurons by ∼40% and ∼15%, respectively, after 12 mo. The dopaminergic neuron loss and concomitant phosphorylated α-synuclein deposition argues that the human synucleinopathies can be transmitted to both rodents and primates.

The discovery that both α-synuclein PFFs and human Lewy body samples induced α-synuclein neuropathology in wild-type animals supports the argument that α-synuclein misfolds into a prion. However, these models were hindered by the lack of neurological deficits seen in synucleinopathy patients. Giasson et al. (2002) developed a Tg mouse model expressing human α-synuclein with the A53T mutation expressed under the mouse prion protein (*Prnp*) promoter. The A53T mutation causes familial PD. At ∼1 yr of age on average, homozygous mice ($M83^{+/+}$) spontaneously developed motor deficits in addition to the presence of substantial α-synuclein pathology in the spinal cord, brainstem, and cerebellum. Mougenot et al. (2012) inoculated young asymptomatic $M83^{+/+}$ mice with brain homogenate from aged (12 to 18 mo) homozygous mice showing motor deficits, decreasing the onset of disease from <1 yr to >6.5 mo. In contrast, when the authors inoculated mice with brain homogenate from 2-mo-old asymptomatic homozygous animals, the mice did not develop motor deficits for ∼1 yr. Similarly, Luk and colleagues performed stereotactic inoculations of brain homogenate from symptomatic $M83^{+/+}$ mice into the striatum and overlying cortex of young $M83^{+/+}$ mice. The inoculated animals developed progressive motor signs in addition to an increase in phosphorylated α-synuclein, ubiquitin, and ThioS immunostaining in the brain (Luk et al. 2012b). To confirm that these findings resulted from the transmission of α-synuclein prions, the authors inoculated the mice with PFFs prepared from recombinant human α-synuclein. $M83^{+/+}$ mice showed corresponding motor deficits and neuropathological findings, demonstrating that acceleration of the observed disease arises from transmission of a spontaneous synucleinopathy that develops in these mice.

In our studies, hemizygous $M83^{+/-}$ mice did not develop spontaneous disease. However, hemizygous animals did develop motor deficits and pathological α-synuclein aggregates after they were inoculated with aged $M83^{+/+}$ homozygous mice brain homogenate (Watts et al. 2013). We also inoculated $M83^{+/-}$ mice with brain homogenate prepared from two MSA patient samples from the Parkinson's UK Brain Bank. Following inoculation, the mice developed neurological symptoms at ∼125 dpi. Both MSA samples induced robust α-synuclein pathology in the hindbrain and in some areas of the mesencephalon (Watts et al. 2013). Sarkosyl-insoluble fractions from MSA samples also induced cerebral deposition of phosphorylated α-synuclein 6–9 mo after intracerebral inoculation of Tg mice expressing wild-type human α-synuclein (Bernis et al. 2015). However, in these mice, which do not express mouse α-synuclein but do overexpress human α-synuclein under the control of its endogenous promoter, no signs of neurological illness were observed following inoculation with the MSA samples.

In additional studies, we demonstrated that aggregated protein alone transmits neurological disease. To demonstrate this finding, we digested brain homogenate from an MSA patient with sarkosyl, as well as benzonase, to degrade the nucleic acids. We then precipitated the remaining protein aggregates with PTA (Woerman et al. 2015). Next, we inoculated $M83^{+/-}$ mice with the protein pellet and found that the mice developed neurological dysfunction with similar α-synuclein deposits in the brain. Im-

portantly, this finding indicates that the misfolded protein is responsible for disease transmission. Moreover, inoculating $M83^{+/-}$ mice with PD patient brain homogenate did not transmit neurological dysfunction. This suggests that MSA and PD arise from unique conformations of misfolded α-synuclein (i.e., different prion strains) (Prusiner et al. 2015).

## INHERITED ALS AND SOD1 PRIONS

Studies of the progressive spread of motor neuron lesions along the neuraxis suggest an orderly and active process in ALS, also known as Lou Gehrig's disease (Ravits and La Spada 2009). More than 150 different mutations in the gene encoding SOD1 have been found to cause familial forms of ALS (Valentine et al. 2005). Aggregates of mutant human SOD1(H46R) protein have been used to initiate its self-propagation in cultured cells, which can continue indefinitely; as such, mutant SOD1(H46R) forms prions (Münch et al. 2011). In another study, two human SOD1 constructs harboring either the G127X or G85R mutation induced wild-type SOD1 to misfold and aggregate in human neural cell lines (Grad et al. 2011).

In addition to mutant SOD1, mutations in two RNA-binding proteins, TDP-43 and FUS, have been identified in patients with familial ALS. Involved in RNA metabolism, TDP-43 and FUS form aggregates in neurons in some cases of ALS and FTD (Udan and Baloh 2011; Polymenidou and Cleveland 2012). Recent evidence indicates that both TDP-43 and FUS contain fungal prion domains rich in glutamine and asparagine residues, and in the case of TDP-43, this domain contains a substantial number of disease-causing mutations (Guo and Shorter 2016; March et al. 2016; Ghasemi and Brown 2017; Mackenzie and Neumann 2017; Nonaka and Hasegawa 2017; Polymenidou and Cleveland 2017).

Although the most extensively studied cause of familial ALS has been mutations in the SOD1 gene, mutations in FUS and TDP-43 have taken on unexpected importance in deciphering the pathogenesis of inherited ALS. Single amino acid substitution mutations have been found in the low-complexity (LC) domains of TDP-43, FUS, and several hnRNP proteins (Bentmann et al. 2013; Kim et al. 2013). LC domains have been implicated in the formation of membrane-less compartments and in yeast prion proteins, which have the ability to interconvert into fibers similar to a liquid state (Patel et al. 2015). FUS acts physiologically to form dynamic liquid compartments that contain assemblies of RNAs and proteins termed RNA granules. Mutant FUS and TDP-43 proteins increase the likelihood that aggregation within RNA granules can lead to familial ALS and, presumably, other age-related diseases (Lim et al. 2016; Polymenidou and Cleveland 2017).

## HUNTINGTIN PRIONS

Unlike the aforementioned NDs, HD is always inherited. The wild-type huntingtin protein harbors an N-terminal region of approximately 35 glutamine residues. An additional 5–20 glutamines, resulting from mutations in the huntingtin gene, are found in most patients with HD, but many more have also been recorded (Lee et al. 2012). The length of the polyglutamine expansion is inversely proportional to the age of onset of HD.

Expanded polyglutamine repeats in a fragment of the huntingtin protein show spontaneous aggregation that self-propagates in cultured cells; it seems increasingly likely that huntingtin prions form through stress granules via the polyglutamine expansion, which is a low complexity sequence (Ren et al. 2009; Lim et al. 2016; March et al. 2016; Protter and Parker 2016; Kato and McKnight 2017; Pearce and Kopito 2017). The idea of huntingtin prions is attractive because it would explain why people with five to 10 additional glutamines do not become ill until they are 40–50 yr of age, even though the mutant protein is produced beginning in embryogenesis.

## CONCLUSIONS

The convergence of studies on these common neurodegenerative maladies has been remarkable (Table 1). Although many mysteries are

now explicable within the framework of the prion concept, the science of prions is still in its infancy. Many more unanticipated discoveries seem likely to emerge from future studies in the field.

## Evidence That Prions Cause Many Different NDs

Over the past few years, there has been a persuasive accumulation of new experimental data arguing that AD, PD, and the tauopathies are caused by prions. This advance in our understanding of the pathogenesis of these illnesses will undoubtedly lead to new diagnostics and therapeutics. Notably, studies of NDs represent a curious mixture of advancements and disappointments. In the case of AD, the discovery of the Aβ peptide in cerebrovascular amyloids and plaques as well as the tau protein in tangles remains central to our understanding of the disease's pathogenesis. However, the current lack of any therapy that halts or even slows a single ND is a colossal tragedy.

Studies of PrP prions are likely to be helpful in developing improved models and bioassays for Aβ, tau, and α-synuclein prions. Studies using bigenic mice expressing mutant human APP and luciferase under control of the *Gfap* promoter (Watts et al. 2011; Stöhr et al. 2012) are based on earlier experiments using bigenic mice expressing luciferase and many different PrP transgenes (Tamgüney et al. 2009; Watts et al. 2012). The strategy for using mutant transgenes overexpressed in cells and mice involves increasing the likelihood that a particular protein will adopt a β-sheet-rich conformation that becomes self-propagating. In bigenic mice expressing mutant APP, the use of bioluminescence is proving to be an invaluable tool because such mice do not develop overt clinical signs of neurological dysfunction.

Although no cultured cell systems for propagating Aβ prions have been developed, some excellent systems for propagating tau and α-synuclein prions are now available. Tau prions have been formed using tau fragments and the full-length protein in vitro by exposure to arachidonic acid or heparin. Full-length tau or tau fragments are then introduced into cells where they recruit wild-type and/or mutant tau to become prions. Similar experimental protocols have been adopted for studies of α-synuclein prions.

Many aspects of NDs can be explained by prions. The relentless and continuous spread of prions in the CNS provides a plausible explanation for the uninterrupted progression of the NDs. Prions also provide a unifying mechanism by which late-onset neurodegeneration can be understood. Although germline mutations are present from the early stages of embryogenesis in familial cases of AD, PD, and the tauopathies, a second event is needed to explain the late onset of these disorders. This event is likely the generation of enough prions to create their self-sustaining replication.

Among the NDs, the primary tauopathies offer a remarkably wide spectrum of illnesses with respect to clinical presentations and progression. These diseases include PSP, PiD, CBD, FTLD, and AGD. Using fMRI to map connectivity networks, the progression of these different tauopathies can be defined in vivo. The most plausible explanations for these clinically distinct tauopathies seem to be different strains of tau prions with distinct neurotropisms.

## Some Arguments against Prions Causing Many NDs

In contrast to CJD and kuru, there are no examples of person-to-person transmissions for AD, PD, and the tauopathies; on the basis of the lack of transmission, some investigators argue that prions do not cause these disorders (Irwin et al. 2013). As additional evidence against a tau prion etiology, they also cite the inability of patient brain extracts to transmit these disorders to wild-type animals. However, a recent study by these same investigators has forced a reinterpretation of these findings since tau extracts from the brains of AD patients were found to be transmissible to wild-type mice (Guo et al. 2016). Notably, α-synuclein prions have also been transmitted to wild-type mice (Luk et al. 2012a; Masuda-Suzukake et al. 2013).

Choosing narrow criteria for defining prion diseases seems rather artificial and unhelpful in

advancing our knowledge of the NDs. Moreover, this constricted definition ignores much of the information that has accumulated about prions over the past three decades. Although some prions replicate more rapidly and accumulate outside of cells, others do not. Fungal prions are not secreted into the media bathing yeast cells; rather, they remain intracellular and pass from mother to daughter cells as the yeast cells multiply (Chien et al. 2004; Wickner et al. 2007). Passage of yeast prions from an infected cell to an uninfected cell requires cytoduction, in which cytoplasmic mixing of the two fungal cells occurs. As such, fungal prions may be a better model for understanding the properties of prions causing many of the NDs.

It is not surprising that cultured cells and Tg mice that overexpress mutant transgenes are needed to demonstrate the transmission of some prions causing NDs. Elevated transgene expression generally shortens the incubation times in Tg mice infected with prions, but there are occasional exceptions (Prusiner et al. 1990; Tamgüney et al. 2006; Giles et al. 2012). Human point mutations in the transgenes appear to facilitate transmission of Aβ, tau, and α-synuclein prions, not unlike the incidence of the NDs. As noted above, the penetrance of familial forms of the NDs approaches 100%, whereas the prevalence of the sporadic forms of these disorders in individuals vary from one per million for CJD to one per 60 for AD in the United States. Using transgene-encoded mutant proteins demands that the inoculated mice develop detectable neurodegeneration well before spontaneous disease in uninoculated controls is evident.

Although some investigators persist in using such terms as "transmissible proteins," "protein strains," and "amyloid assemblies," there is no difference between these terms and infectious proteins (i.e., prions). The term "prion" was coined to distinguish between proteinaceous infectious particles and small biologically active particles that possess a nucleic core, such as viruses and viroids. The proliferation of alternative nomenclature (Box 2) makes the scientific literature more obscure than is necessary and in some cases may even slow progress in the field.

**BOX 2. Alternative terms for "prion"**

1. Prion-like protein aggregates
2. Transmissible proteins
3. Templated proteins
4. Prionoids
5. Proteopathic seeds
6. Misfolded proteins
7. Self-propagating strains
8. Protein strains
9. Protein pathogens
10. Quasi-prions
11. Pseudo-prions
12. Amyloid assemblies
13. Proteinaceous nucleating particles
14. Protein assemblages
15. Propagons

Certainly, the urgency in advancing our knowledge of prions in order to develop effective therapeutics is enormous by any measure.

## Implications of Prion Etiologies for Patient Care

The discovery that prions are not confined to a small group of disorders in which PrP prions accumulate has important implications for the development of informative molecular diagnostics and effective therapeutics. Early diagnosis will likely require molecular reporters, such as PET ligands, to identify prions long before symptoms appear. Such PET ligands will need to distinguish the normal protein precursors from the prion forms, whether the proteins are PrP, Aβ, tau, or α-synuclein. Although there is considerable interest in using the levels of Aβ and tau in cerebrospinal fluid for the diagnosis of AD, changes in the levels of these proteins are generally small and may prove problematic in monitoring responses to therapeutic interventions. Additionally, meaningful treatments are likely to require combinations of drugs that diminish the precursor protein, interfere with the

conversion of precursors into prions, and/or enhance the clearance of prions.

Some investigators have expressed concern that fear among medical staff regarding the transmissibility of prions will result in AD patients receiving less care than they need, particularly those who are incontinent and/or cannot control saliva (Hardy and Revesz 2012). Although a recent study argues that there is no evidence for the person-to-person transmission of Aβ, tau, or α-synuclein prions (Irwin et al. 2013), it may prove wise to institute some minimal precautions in the care of AD and PD patients that resemble those followed in cases of CJD and HIV until definitive investigations can be performed. The history of CJD and AIDS is replete with regrets, and earlier vigilance may have prevented many deaths. It would be a mistake to deny a prion etiology for these common illnesses under the guise of facilitating patient care.

Equally important is the issue of prion contamination of biologics prepared from patients with AD, PD, and the tauopathies. The contamination of cadaveric human growth hormone preparations with CJD prions is a distressing chapter in the development of therapeutics for children with short stature (Mills et al. 2004). In the case of variant CJD, four people with the disease, contracted from eating tainted beef prepared from "mad" cows, unknowingly transmitted variant CJD prions to recipients of blood transfusions (Hewitt et al. 2006; Health Protection Agency 2009; Peden et al. 2010). Dura mater grafts and cornea transplants have also transmitted CJD prions (Shimizu et al. 1999). Moreover, inadequately sterilized surgical instruments and implanted electrodes have transmitted sporadic CJD prions (Bernoulli et al. 1977). These prions have been shown to bind tightly to stainless steel wires and retain infectivity (Zobeley et al. 1999; Giles et al. 2008, 2017). Aβ prions have also been shown to bind to steel wires and induce Aβ deposition in the brains of Tg mice (Eisele et al. 2009).

The risk of transmitting Aβ, tau, α-synuclein, or even SOD1 prions through biologics, organ grafts, or inadequately sterilized surgical instruments has not been well studied because the transmissibility of these proteins has only recently been understood, and bioassays are only now beginning to be developed (Sanders et al. 2014; Woerman et al. 2015, 2016). Endpoint titrations, incubation times, and bioluminescence imaging have been used to measure PrP prion titers in fractions prepared from prion-infected mammals (Eklund et al. 1963; Hunter et al. 1969; Prusiner et al. 1982b; Tamgüney et al. 2009). Overexpression of wild-type PrP transgenes has generally been found to decrease incubation time, as measured from inoculation to a sustained increase in bioluminescence signal or to the onset of progressive neurological dysfunction (Prusiner et al. 1990; Browning et al. 2004; Tamgüney et al. 2006; Watts et al. 2012, 2013; Prusiner et al. 2015), although there are exceptions (Giles et al. 2012). Purified recombinant tau and α-synuclein have been induced to aggregate in vitro, used to initiate prion replication in cultured cells, and used to measure prion titers (Woerman et al. 2015, 2016). Although Tg mice have been used to detect the presence of Aβ, tau, and α-synuclein prions in mouse and human brains, quantitative assays need to be established. Developing accurate methods to measure prions is important in assessing the risk of biologics and organ transplants prepared from or donated by patients with neurodegeneration. Moreover, determining the number of such prions that may contaminate surgical instruments and prosthetics, including chronically implanted electrodes, is imperative. Of particular concern may be the increasing number of patients with PD who undergo neurosurgical procedures for the implantation of electrodes for deep brain stimulation (Okun 2012). It remains uncertain whether more stringent guidelines for such procedures need to be established.

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