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Aβ prions and the pathobiology of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disease in humans and will pose a considerable challenge to healthcare systems in the coming years. Aggregation of the β-amyloid (Aβ) peptide within the brain is thought to be an initiating event in AD pathogenesis. Many recent studies in transgenic mice have provided evidence that Aβ aggregates become self-propagating during disease leading to a cascade of protein aggregation in the brain, which may underlie the progressive nature of AD. The ability to self-propagate and the existence of distinct "strains" reveals that Aβ aggregates exhibit many properties indistinguishable from those of prions composed of the PrP prion protein. Here, we review the evidence that Aβ can become a prion during disease and discuss how Aβ prions may be important for understanding the pathobiology of AD.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in humans and is most frequently diagnosed in individuals 65 years of age or older. The incidence of AD varies with age: ~1 in 9 people age 65 or older will develop AD, whereas ~1 in 3 people age 85 or older will get the disease (Hebert et al. 2013). Early-onset cases of AD, which occur in individuals younger than age 65, are comparatively rare and include familial cases caused by mutations in genes linked to the production of the β-amyloid (Aβ) peptide. The predominant clinical symptoms of AD are cognitive deficits and behavioral changes. AD patients typically present initially with difficulties in remembering recent occurrences, but with intact memories of older events. The earliest, pre-dementia stages of the disease are often classified clinically as mild cognitive impairment, which describes the presence of memory problems greater than those expected due to normal aging. As the disease progresses, additional symptoms become apparent, which may include problems with language, confusion, mood swings, long-term memory loss, and withdrawal from society. Eventually, AD patients become completely dependent on caregiver assistance. There are currently no cures or effective treatments for AD. Although available treatments may temporarily improve behavioral problems, they do not address the root cause of the disease or its progression.

AD is a progressive neurodegenerative disease of aging. The brains of AD patients exhibit a remarkable loss of synapses and neurons, which results in an overall shrinkage of the brain, particularly in the temporal and parietal lobes as well as in the frontal cortex. At the microscopic level, two principal pathologies are observed: amyloid (senile) plaques and neurofibrillary tangles (NFTs). The dense, extracellular amyloid

plaques are composed of aggregated Aβ peptide, whereas the intracellular NFTs are made up of paired helical filaments of hyperphosphorylated tau protein. Aβ is generated by the sequential endoproteolytic cleavage of the amyloid precursor protein (APP), a Type-1 transmembrane protein that exists as three different isoforms with lengths of 695, 751, and 770 amino acids. In the amyloidogenic processing pathway, APP is cleaved by two different proteases: β-secretase and γ-secretase (Fig. 1). β-secretase first cleaves the extracellular juxtamembrane region of APP to leave a membrane-embedded stump termed C99. The transmembrane portion of C99 is then cleaved by γ-secretase, a multiprotein complex that includes the enzymatically active presenilin proteins (De Strooper et al. 2012), to liberate the Aβ peptide. Cleavage of C99 by γ-secretase is heterogeneous in nature and results in the generation of Aβ peptides varying in length from 37 to 43 amino acids (Benilova et al. 2012). If APP is first cleaved by α-secretase instead of β-secretase, the generation of Aβ is prevented.

The amyloid cascade hypothesis, which is the most widely accepted theory for the molecular sequence of events in AD, postulates that the initiating event in AD is the aggregation and subsequent deposition of Aβ peptide in the brain (Hardy et al. 2002). This results in the hyperphosphorylation and polymerization of tau into NFTs and, ultimately, degeneration of neurons. This hypothesis is supported by the following observations: (1) mutations in the gene encoding APP cause early-onset forms of AD and increase the production of Aβ or enhance its aggregation potential (Chartier-Harlin et al. 1991; Citron et al. 1992); (2) duplication of the APP coding locus results in early-onset AD (Rovelet-Lecrux et al. 2006); (3) in Down's syndrome patients, an extra copy of chromosome 21, which contains the *APP* gene, invariably causes AD-like

pathological changes in the brain (Wisniewski et al. 1985); (4) mutations in the *APP* gene that decrease Aβ production are protective against AD (Jonsson et al. 2012); and (5) mutations in the genes encoding the presenilin proteins increase the relative abundance of pro-amyloidogenic Aβ isoforms and cause early-onset AD (Sherrington et al. 1995). Thus, increased Aβ production and deposition in the brain is sufficient to cause AD, suggesting that Aβ aggregation is central to AD pathogenesis. In contrast, mutations in the gene encoding tau result in the production of NFTs in the brain (Hutton et al. 1998; Spillantini et al. 1998), but not amyloid plaques. Such patients develop a tauopathy termed FTDP-17 that is clinically and pathologically distinct from AD, revealing that tau mutations are insufficient to elicit the full neuropathological spectrum observed in AD.

MOUSE MODELS OF ALZHEIMER'S DISEASE

Transgenic (Tg) mice that overexpress mutant human APP containing one or more mutations that cause early-onset, familial forms of AD develop Aβ-containing amyloid plaques in their brains with aging (Morrissette et al. 2009). These mice, referred to hereafter as Tg(AD) mice, thus recapitulate one of the key neuropathological hallmarks of AD. The kinetics of Aβ deposition in Tg(AD) mice is governed by the relative level of APP overexpression as well as the specific mutations present. For example, Tg2576 mice expressing human APP containing the Swedish mutation (K670N/M671L) (Citron et al. 1992; Mullan et al. 1992), which causes enhanced cleavage of APP by β-secretase (Haass et al. 1995) and, consequently, increased Aβ production, develop Aβ plaques in their brains beginning around 12 months of age (Hsiao et al. 1996). In contrast, TgCRND8 mice expressing a mutant APP allele containing the Swedish

mutation and the Indiana mutation (V717F) (Murrell et al. 1991), which increases the relative abundance of the more aggregation-prone Aβ42 isoform (Suzuki et al. 1994), develop amyloid plaques at around 3–4 months of age (Chishti et al. 2001).

Some lines of Tg(AD) mice exhibit age-dependent memory impairment, mimicking the cognitive deficits observed in AD patients, and exhibit some tau hyperphosphorylation (reviewed in (Morrissette et al. 2009)). Moreover, the cerebrospinal fluid of Tg(AD) mice contains increased levels of tau and decreased levels of Aβ42 peptide, mirroring the biomarker signature of individuals with AD (Maia et al. 2013). These data support the translational utility of Tg(AD) mice. Indeed, Tg(AD) mice have been used to demonstrate that reducing levels of Aβ aggregates in the brain, either by vaccination or passive immunotherapy, can rescue cognitive deficits (Schenk et al. 1999; Bard et al. 2000; Janus et al. 2000). However, current Tg(AD) mice do not constitute an ideal animal model of AD because, unlike AD patients, Tg(AD) mice do not develop NFTs in their brains in response to Aβ aggregation and do not display frank neuronal loss (Jucker 2010).

Tg(AD) mice do not exhibit overt progressive clinical signs of neurological illness with aging, despite the presence of abundant Aβ plaques in their brains. Thus, the only way of assessing the kinetics of Aβ deposition in the brain is to perform postmortem analysis of fixed tissue using neuropathological techniques. This is not ideal for several reasons. First, this greatly increases the number of animals needed for a study because separate cohorts are required for each time point under investigation. Second, it necessitates “guessing” when Aβ deposition is expected to occur in the brain. Finally, it hinders the development of therapeutics because of the lack of easily and rapidly

quantifiable measures of disease. One solution to this problem is the use of bioluminescence imaging (BLI) to monitor the kinetics of Aβ deposition in living mice. Tg mice that express firefly luciferase (luc) under the control of the glial fibrillary acidic protein (Gfap) promoter (Zhu et al. 2004) emit increased levels of light from their brains in response to stimuli that cause astrocytic gliosis and a concomitant upregulation of GFAP protein in astrocytes. Because GFAP-positive astrocytes frequently decorate the perimeters of Aβ amyloid deposits in the brain, we hypothesized that Tg(*Gfap*-luc) mice would be useful for tracking the kinetics of Aβ deposition in vivo. Indeed, bigenic mice expressing a mutant APP transgene and the *Gfap*-luc reporter exhibited age-dependent increases in the brain bioluminescence signal (Fig. 2), providing a quantitative assessment of the kinetics of Aβ deposition in living mice (Watts et al. 2011).

Two recently described Tg(AD) models have provided significant advances to the field. Knock-in mice in which the mouse APP gene was modified so that it contains a humanized Aβ sequence and the Swedish and Iberian (I716F) (Guardia-Laguarta et al. 2010) mutations develop typical age-dependent Aβ pathology and memory deficits (Saito et al. 2014). These mice are noteworthy in that APP is expressed at physiological levels and with correct spatiotemporal patterning. A promising Tg(AD) rat model has also been developed recently. TgF344-AD rats, which express human APP containing the Swedish mutation and a mutant presenilin 1 transgene, develop Aβ pathology and cognitive deficits and some associated tau pathology and neuronal loss (Cohen et al. 2013).

EVIDENCE FOR THE EXISTENCE OF Aβ PRIONS IN AD

The pioneering work of Gajdusek, Gibbs, and Alpers revealed that Creutzfeldt–Jakob Disease (CJD) and kuru could be transmitted to nonhuman primates, demonstrating for the first time that these human spongiform encephalopathies are transmissible illnesses (Gajdusek et al. 1966; Gibbs et al. 1968). It is now known that prions composed of misfolded and aggregated prion protein (PrP) are the infectious agent in these diseases (Colby et al. 2011). PrP prions were originally defined as proteinaceous infectious particles that cause rare, transmissible neurodegenerative diseases such as scrapie and CJD (Prusiner 1982) but are now more generally defined as alternative protein conformations that exhibit self-propagation (Prusiner 2012). There are two key features of prions: (1) the existence of at least two distinct stable conformational states of a protein in the absence of any posttranslational modifications, one of which exhibits a propensity for forming multimeric structures and/or aggregates; and (2) the multimeric state is self-propagating, meaning that it can catalyze the conversion of the “normal,” monomeric conformation into an additional copy of the prion conformation.

Evidence that Aβ becomes a prion during AD pathogenesis is growing. In AD patients, the pattern of Aβ deposition in the brain is not random in nature. Instead, Aβ deposition follows a stereotypical progression through five distinct phases. Phases 1 through 3 are associated with Aβ deposits occurring in nondemented individuals, whereas phases 3 to 5 are associated with AD patients (Thal et al. 2002). In phase 1, Aβ deposits are exclusively found in the neocortex and then spread to the hippocampus in phase 2. In phase 3, Aβ deposits can additionally be found in the amygdala, thalamus, and striatum. In phase 4, certain areas of the brainstem and the substantia

nigra become laden with Aβ deposits, and then in phase 5, Aβ deposits can also be found in additional brainstem nuclei and the cerebellum. This progressive, nonrandom spreading of cerebral Aβ deposition is reminiscent of the spread of PrP prions throughout the brain by templated conformational conversion. Unlike in AD, amyloid plaques are only present in the brain in ~10% of CJD cases (DeArmond et al. 2004). However, upon purification, PrP prions aggregate to form rod-like structures that exhibit all of the properties of amyloid, including Congo red birefringence (Prusiner et al. 1983). The molecular and pathological similarities between AD and the PrP prion diseases led to speculation that prions may feature in the pathogenesis of AD, and that, like CJD and kuru, AD may be transmissible to nonhuman primates (Prusiner 1984).

Attempts to transmit AD to monkeys have produced discordant results (Table 1). Gajdusek and colleagues inoculated 52 different cases of sporadic and familial AD into a variety of primate species and found that only 2 of the cases transmitted disease (Goudsmit et al. 1980). However, the resultant pathology in the monkeys inoculated with these 2 cases did not resemble AD; instead, it was identical to that observed in monkeys inoculated with CJD and kuru. Moreover, new preparations of inocula from these 2 cases failed to transmit disease, suggesting that contamination or other laboratory errors are a more likely explanation for the initial positive transmissions. Similar issues may explain the inability to reproduce an initial finding that buffy coat fractions from the blood of AD patients induce a spongiform encephalopathy in inoculated hamsters (Manuelidis et al. 1988; Godec et al. 1994).

Later, Ridley, Baker, and colleagues demonstrated that marmosets inoculated with brain homogenate from an AD patient develop some Aβ-containing senile plaques

and cerebral amyloid angiopathy in their brains 6–7 years postinjection (Baker et al. 1993; Baker et al. 1994). A total of 24 out of 27 marmosets injected with brain homogenate containing Aβ aggregates exhibited modest numbers of Aβ deposits in their brains after incubation periods of up to ~8 years (Ridley et al. 2006). All monkeys that survived >3.5 years postinoculation exhibited Aβ deposits. Only 5 of 29 uninjected marmosets aged >10 years and none of the uninjected marmosets aged 5 to 10 years exhibited spontaneous Aβ deposits in their brains. These studies also found that brain homogenate from non-AD patients, synthetic Aβ aggregates, and CSF from AD patients were poor inducers of Aβ deposition in marmosets. Importantly, no clinical signs of AD or NFTs were observed in any of the Aβ-positive inoculated marmosets, suggesting that although cerebral β-amyloidosis can be initiated or accelerated by inoculation of pre-formed Aβ aggregates, the full clinicopathologic spectrum of AD cannot be recapitulated by inoculation, at least within the timeframe studied.

USING TRANSGENIC MICE TO STUDY Aβ PRIONS

The most convincing experimental evidence for the existence of self-propagating Aβ aggregates (or simply, Aβ prions) in AD has come from in vivo seeding studies in Tg(AD) mice. In many lines of Tg(AD) mice, such as the widely used Tg2576 and APP23 (Sturchler-Pierrat et al. 1997) models, spontaneous Aβ deposits are not observed until the animals are older, providing a window during which the induction of Aβ deposition by inoculation of exogenous Aβ aggregates can be assessed. In a typical in vivo seeding experiment, young (2–3 months old) Tg mice (that have not yet developed deposits) are intracerebrally injected with brain extract containing abundant Aβ aggregates, and then the induction of Aβ deposition is examined following an

incubation period of 3–5 months. Two inoculation techniques have been used to introduce exogenous Aβ aggregates into the brain of Tg(AD) mice: stereotaxic and nonstereotaxic (“standard”) injections. Stereotaxic inoculations commonly involve the injection of 2–3 μL of brain extract directly into the hippocampus and overlying cortex using a Hamilton syringe. Although this technique is very time consuming, it has the advantage that Aβ aggregates can be introduced into very defined brain regions (Eisele et al. 2009). The standard inoculation procedure involves the inoculation of ~30 μL of brain extract into the right cerebral hemisphere using a standard syringe and a 27-gauge needle (Stöhr et al. 2012). This technique is less precise (roughly injecting the inoculum into the thalamus and the overlying hippocampal and cortical regions) but is much more rapid.

In 2000, Lary Walker and colleagues demonstrated that intracerebral infusion of brain extracts from AD patients into Tg2576 mice induced small amounts of cerebral Aβ deposition following an incubation period of 5 months (Kane et al. 2000). No Aβ deposition was observed in mice injected with brain extract from a young individual or in age-matched uninjected animals, and only minor amounts of induced Aβ deposition were seen in mice inoculated with brain extract from an aged patient without AD. Later, Walker, working with Mathias Jucker and colleagues, revealed that Aβ aggregates present in the brain extracts were necessary for the induction of Aβ deposition following intracerebral inoculation (Meyer-Luehmann et al. 2006). Stereotactic inoculation of APP23 mice into the hippocampus and overlying cortex with brain extract from aged Tg(AD) mice resulted in the induction of Aβ deposition after an incubation period of 4 months. The induction of cerebral Aβ deposition could be blocked or significantly

attenuated by either (1) immunodepleting the brain extracts of Aβ aggregates prior to inoculation; (2) passively immunizing the mice with anti-Aβ antibodies; or (3) treating the brain extract with formic acid prior to inoculation. Depletion of Aβ aggregates from brain extracts using a small molecule also suppresses seeding activity (Duran-Aniotz et al. 2014). These experiments indicated that, like PrP prions, brain-derived Aβ aggregates are self-propagating. However, no induced Aβ deposition was observed in mice inoculated with a variety of synthetic Aβ aggregate preparations, implying that not all Aβ aggregates are capable of self-propagation (Meyer-Luehmann et al. 2006).

Though earlier efforts failed to demonstrate any seeding of cerebral Aβ deposition following peripheral or systemic application of Aβ seeds (Eisele et al. 2009), examining later time points following intraperitoneal inoculation of APP23 mice with Tg(AD) brain extract revealed that cerebral Aβ deposition can be initiated by peripheral inoculation, suggesting that Aβ prions, like PrP prions, are neuroinvasive (Eisele et al. 2010). This is not an artifact of ectopic APP expression in the periphery due to the use of the Thy-1 promoter to drive mutant APP expression in APP23 mice because cerebral Aβ deposition can also be induced by peripheral Aβ inoculation in R1.40 Tg mice, which express mutant APP under the control of its endogenous promoter (Eisele et al. 2014). Similarly, cerebral Aβ deposition can also be induced by intracerebral inoculation of R1.40 mice with Aβ-containing brain extract (Hamaguchi et al. 2012), arguing that cerebral Aβ induction cannot be explained as an artifact arising from improper patterns of APP expression. Although the majority of studies have investigated the induction of cerebral Aβ deposition using Tg(AD) mice or rats (Rosen et al. 2012) that express mutant APP, it is also possible to induce Aβ deposition in Tg mice that overexpress wild-

type human APP and do not develop spontaneous cerebral Aβ deposition within their normal lifespan. Intracerebral inoculation of HuAPPwt mice with AD patient brain extract resulted in the induction of Aβ pathology at ~10 months postinoculation (Morales et al. 2012). It is not yet known whether Aβ induction can be induced in non-Tg mice or rats.

The specific species of Aβ responsible for inducing cerebral Aβ deposition are not currently known. Aβ aggregates purified from the brains of Tg(AD) mice are potent inducers of Aβ deposition in the brain (Fig. 2) (Stöhr et al. 2012), suggesting that Aβ aggregates themselves are the infectious prion species. This was confirmed by demonstrating that Aβ aggregates composed exclusively of synthetic Aβ peptides can also initiate cerebral Aβ deposition in APP23 mice (Fig. 2) (Stöhr et al. 2012; Stöhr et al. 2014). Treatment of Aβ prion preparations with proteinase K (PK) did not abolish their infectivity (Langer et al. 2011; Stöhr et al. 2012), implying that self-propagating Aβ aggregates are densely packed and likely large in size. This is supported by the observation that formaldehyde treatment does not inactivate Aβ prion activity present in brain extracts (Fritschi et al. 2014a) and the remarkable finding that Aβ prions can persist for up to 6 months postinoculation in Aβ-inoculated APP knockout mice (Ye et al. 2015a). However, soluble Aβ species present in Tg(AD) or AD patient brain extracts that are sensitive to PK digestion can also induce cerebral Aβ deposition in APP23 mice (Langer et al. 2011; Fritschi et al. 2014b), suggesting that multiple distinct Aβ prion strains are capable of exhibiting self-propagation.

Like PrP prions, Aβ prions are capable of inducing cerebral Aβ deposition even when present at low levels in the inoculum. For example, induced Aβ deposition was still apparent in Tg2576 mice when injected with 10^5- to 10^6-fold dilutions of aged Tg2576

brain extract (Morales et al. 2015). Moreover, brain extracts from patients with mild cognitive impairment or nondemented individuals with AD neuropathology were also capable of inducing cerebral Aβ deposition in Tg(AD) mice (Duran-Aniotz et al. 2013). Induced Aβ prions also appear to “spread” away from the original site of inoculation (Eisele et al. 2009; Ye et al. 2015b), providing a potential molecular explanation for the progression of Aβ pathology observed in AD patients (Thal et al. 2002).

STRAINS OF Aβ PRIONS

In the PrP prion diseases, distinct strains of prions can be classified according to their incubation periods upon inoculation into rodents, the resultant clinical signs of disease and neuropathological features, and their biochemical characteristics, such as their relative resistance to protease digestion and their stability upon exposure to chemical denaturants (Collinge et al. 2007). A myriad of evidence argues that prion strain-specific properties are encoded within unique conformations of PrP aggregates (Bessen et al. 1994; Telling et al. 1996). AD is a clinically and pathologically heterogeneous disorder, with variability present in the age of onset, the rate of cognitive decline, and the morphology and neuroanatomical location of Aβ pathology (i.e., parenchymal versus vascular). It is conceivable that some of this heterogeneity may arise due to the presence of distinct “strains” of Aβ prions in the brain.

The first evidence for conformational heterogeneity of Aβ aggregates was observed in Aβ fibrils generated from synthetic Aβ using either quiescent or agitated conditions (Petkova et al. 2005). The two types of fibrils exhibited different morphologies when examined under the electron microscope and distinct solid-state NMR spectra. Moreover, the distinct morphologies were maintained upon serial seeding, indicating

that these unique structural states are self-propagating. Variations in the morphology of fibrils formed from synthetic Aβ have also been obtained by varying the buffer conditions and/or the formation temperature (Meinhardt et al. 2009; Kodali et al. 2010). At the molecular level, structural polymorphism may arise due to differences in symmetry and the conformations of Aβ residues not involved in the core parallel in-register β-sheet structure (Paravastu et al. 2008). Interestingly, Aβ fibrils formed from synthetic Aβ can also exhibit strain-like behavior in vivo. Synthetic Aβ42 polymerized in the presence of 0.1% (wt/vol) sodium dodecyl sulfate (SDS) resulted in shorter fibrils than when Aβ42 was aggregated in the absence of SDS (Stöhr et al. 2014). When inoculated into APP23 mice, the Aβ42 fibrils generated in the absence of SDS induced a greater number of Aβ plaques, and these plaques contained a higher ratio of Aβ42/Aβ40 peptides than the plaques in mice inoculated with Aβ42 fibrils produced in the presence of SDS.

Distinct strains (or “morphotypes”) of Aβ prions have also been observed between two different lines of Tg(AD) mice, APP23 and APPPS1 (Radde et al. 2006). Using luminescent conjugated polymers (Sigurdson et al. 2007), it was shown that the Aβ deposits present in the brains of the two Tg(AD) lines emit distinct fluorescent spectra, which is suggestive of conformationally unique aggregates (Heilbronner et al. 2013). When APP23 Aβ aggregates were inoculated into APP23 mice, the induced Aβ prions were spectrally similar to those present in aged APP23 mice. In contrast, when APPPS1 Aβ prions were inoculated into APP23 mice, the induced Aβ aggregates produced spectra similar to those present in aged APPPS1 mice, indicating that the brain extracts from aged APP23 and APPPS1 mice contain distinct strains of self-propagating Aβ pathogens.

By seeding synthetic Aβ with fibrils isolated from an AD patient's brain, it was revealed that the Aβ aggregates present in AD patients are structurally distinct from those formed spontaneously from synthetic Aβ (Paravastu et al. 2009). Using a similar approach, it has recently been determined that the Aβ aggregates isolated from two different sporadic AD patients produce seeded Aβ aggregates with unique structural properties, arguing that distinct strains of Aβ aggregates may exist among AD patients (Lu et al. 2013). Indeed, the Aβ42 aggregates in AD patients with a more rapidly progressive version of the disease are conformationally distinct from those in AD patients with a stereotypical disease course, as judged by a conformation-dependent immunoassay (Cohen et al. 2015). The existence of distinct Aβ strains among AD patients has also been demonstrated using brain extracts from two patients with early-onset familial versions of the disease. Using a conformational stability assay that measures the relative resistance of Aβ aggregates to chemical denaturation, it was shown that the Aβ aggregates from an AD patient with the Swedish mutation in APP can be distinguished from those in an AD patient with the Arctic mutation (E693G) (Nilsberth et al. 2001) in APP (Watts et al. 2014). When brain extracts from these two patients were inoculated into APP23 mice, distinct vascular Aβ pathologies were observed (Fig. 3), and these differences were maintained upon second passage in APP23 mice. These results reveal that, like PrP prions, strain-specific properties of Aβ aggregates are serially transmissible in mice.

CONCLUDING REMARKS

It is becoming increasingly clear that Aβ aggregates exhibit many similarities to prions composed of PrP (Table 2), making it more difficult to refute the notion that Aβ can

become a prion during AD. However, there is currently minimal evidence to suggest that Aβ aggregates can be transmitted from human to human. Many individuals that received human cadaveric growth hormone injections ultimately died of CJD because the pituitary extracts were contaminated with PrP prions (Brown et al. 2000). Although no increased incidence of AD has been observed in surviving individuals who received growth hormone injections (Irwin et al. 2013), it has recently been shown that the brains of growth hormone recipients who died of CJD contain much higher levels of Aβ deposition than would be expected (Jaunmuktane et al. 2015). One possible interpretation is that Aβ seeds were present in the growth hormone extracts, which initiated cerebral Aβ deposition following peripheral injection. Increased amounts of Aβ deposition have also been observed in patients that developed iatrogenic CJD following dura mater grafts (Frontzek et al. 2016). Notably, tau deposition was not observed in either of these instances, potentially indicating an early stage of AD pathogenesis. Although iatrogenic AD transmission is only one potential explanation for these observations, sterilization methods for surgical tools may need to be reconsidered, especially since Aβ seeds remain active once bound to stainless steel (Eisele et al. 2009).

Although AD is unlikely to be transmissible under physiological circumstances, the realization that Aβ aggregates can become prions during disease has many potential implications. First, the existence of self-propagating Aβ aggregates may provide a molecular explanation for why AD is a progressive disorder. Spreading of Aβ prions along neuroanatomical pathways in conjunction with a potential stimulation of tau prion formation may cause a progressive worsening of disease symptoms. Moreover,

like CJD, ~90% of AD cases are sporadic, suggesting that the initiating event may be the rare, age-related stochastic formation of a self-propagating Aβ aggregate seed. In early-onset genetic AD, the necessity for Aβ prion formation may explain why disease most commonly manifests in the fifth or sixth decade of life, despite the fact that elevated levels of pathogenic Aβ are produced from birth. Second, the formation of self-propagating Aβ species may represent an early event in disease progression that can be targeted pharmacologically. Third, the existence of distinct strains of Aβ prions poses a considerable challenge for the development of Aβ-directed therapeutics for AD. Heterogeneity in the conformation of Aβ aggregates may partially explain the disappointing results obtained thus far in clinical trials of Aβ monoclonal antibodies (Delrieu et al. 2012) because antibody efficacy may be highly strain-specific. Although studies of the biology of Aβ prions are still in their infancy, they hold great promise for understanding basic disease mechanisms and for the development of effective therapeutics for AD.

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FIGURE LEGENDS

Figure 1. Generation of Aβ peptide via endoproteolytic cleavage of APP. The mature form of the longest isoform of human APP (following removal of an N-terminal signal peptide) spans residues 18-770. In the amyloidogenic pathway, cleavage of the APP extracellular domain by β-secretase produces a secreted fragment termed sAPPβ and a membrane-embedded C-terminal fragment called C99. Cleavage of C99 by γ-secretase produces the APP intracellular domain (AICD) and the Aβ peptide. Cleavage of APP by γ-secretase is heterogeneous, resulting in the generation of Aβ peptides that vary in length between 37 and 43 residues.

Figure 2. Monitoring spontaneous and induced Aβ deposition in bigenic mice using in vivo bioluminescence imaging (BLI). The brain BLI signal in bigenic Tg(APP23:*Gfap*-luc) mice (*red*) exhibits an age-dependent increase, which is indicative of cerebral Aβ deposition, compared with Tg mice expressing just the *Gfap*-luc reporter (*blue*). Intracerebral inoculation of Tg(APP23:*Gfap*-luc) mice with either Aβ aggregates purified from the brain of an aged APP23 mouse (*black*) or Aβ aggregates formed from synthetic Aβ(1-40) peptide (*green*) accelerate the onset of the brain BLI signal increase compared with uninoculated mice, indicating that both inocula contain Aβ prions. This figure has been adapted with permission from Stöhr J, et al. 2012. Purified and synthetic Alzheimer's amyloid beta (Aβ) prions. *Proc Natl Acad Sci* **109:** 11025–11030.

Figure 3. Arctic and Swedish AD brain extracts induce distinct pathologies in APP23 mice. (*A*) Intracerebral inoculation of APP23 mice with brain extract from an AD patient with the Swedish APP mutation, which generates wild-type Aβ, or brain

extract from an AD patient with the Arctic APP mutation, which generates E22G-mutant Aβ. At 11 months postinoculation, induced Aβ cerebral amyloid angiopathy is present in the thalamus (4G8 immunostaining). Whereas mice inoculated with Swedish AD extract exhibited a thin layer of Aβ deposition surrounding blood vessels (*black arrows*), many of the blood vessels in mice inoculated with Arctic AD extract were surrounded by thick, "furry" Aβ deposits (*red arrows*). (*B*) Similar morphological differences in Aβ cerebral amyloid angiopathy were observed at 11 months postinoculation of APP23 mice with APP23-passaged Swedish and Arctic AD extract, indicating that Aβ strain-specified properties are serially transmissible. Scale bars represent 100 μm. This figure has been adapted with permission from Watts JC, et al. 2014. Serial propagation of distinct strains of Aβ prions from Alzheimer's disease patients. *Proc Natl Acad Sci* **111:** 10323–10328.

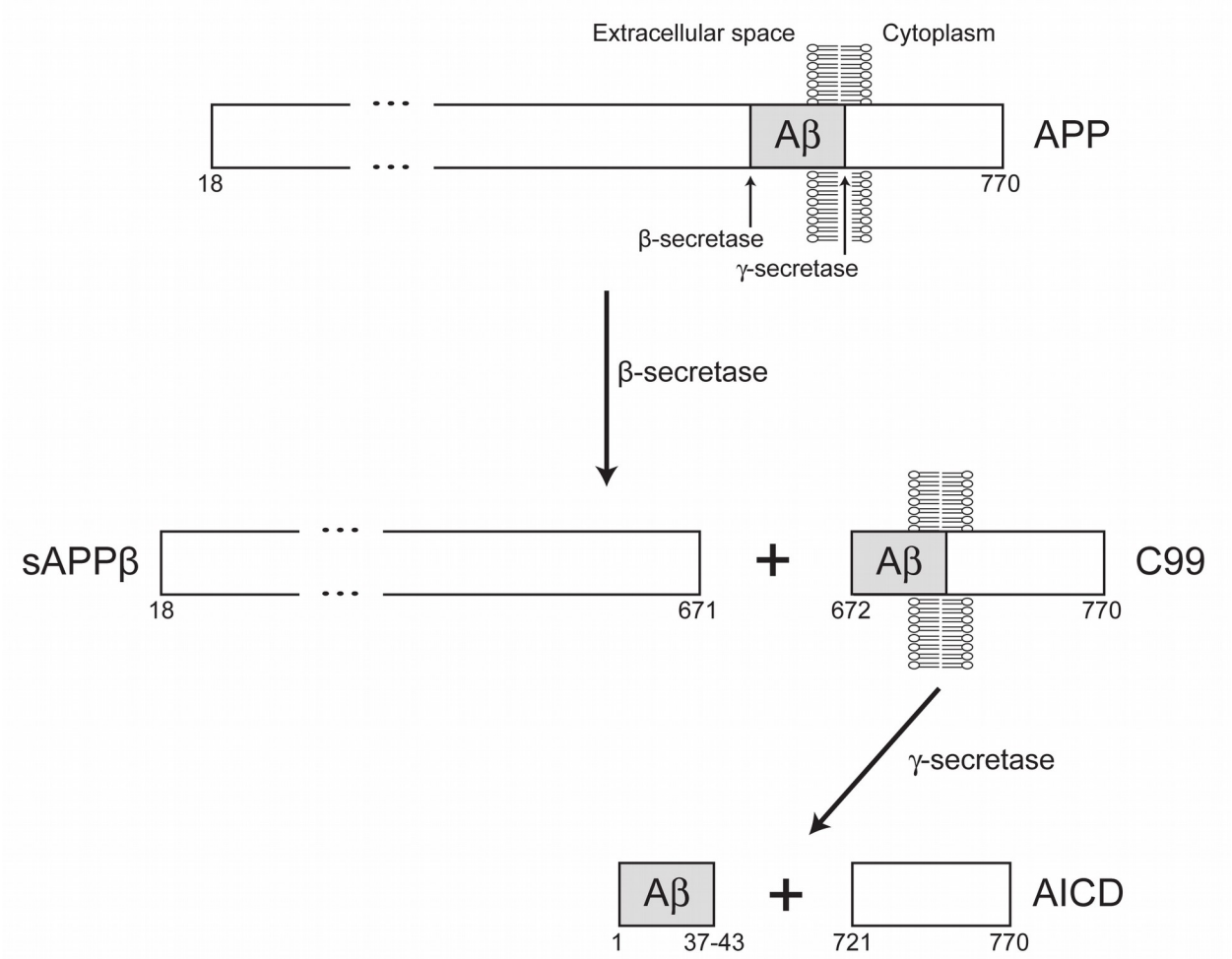
Extracellular space
Cytoplasm
Aβ
APP
18
770
β-secretase
γ-secretase
β-secretase
sAPPβ
18
671
+
Aβ
672
770
C99
γ-secretase
Aβ
1
37-43
+
721
770
AICD

Figure 1

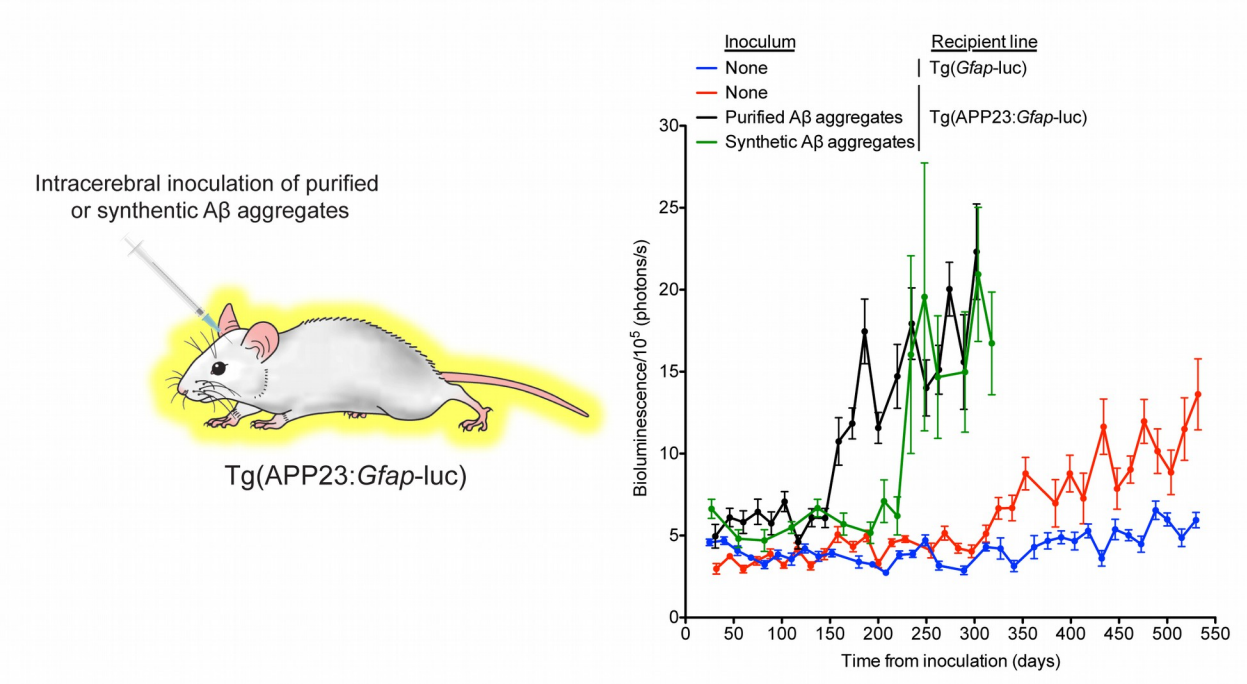
Intracerebral inoculation of purified
or synthentic Aβ aggregates
Tg(APP23:*Gfap*-luc)
Inoculum
None
None
Purified Aβ aggregates
Synthetic Aβ aggregates
Recipient line
Tg(*Gfap*-luc)
Tg(APP23:*Gfap*-luc)
Bioluminescence/10^5 (photons/s)
0
5
10
15
20
25
30
0
50
100
150
200
250
300
350
400
450
500
550
Time from inoculation (days)

Figure 2

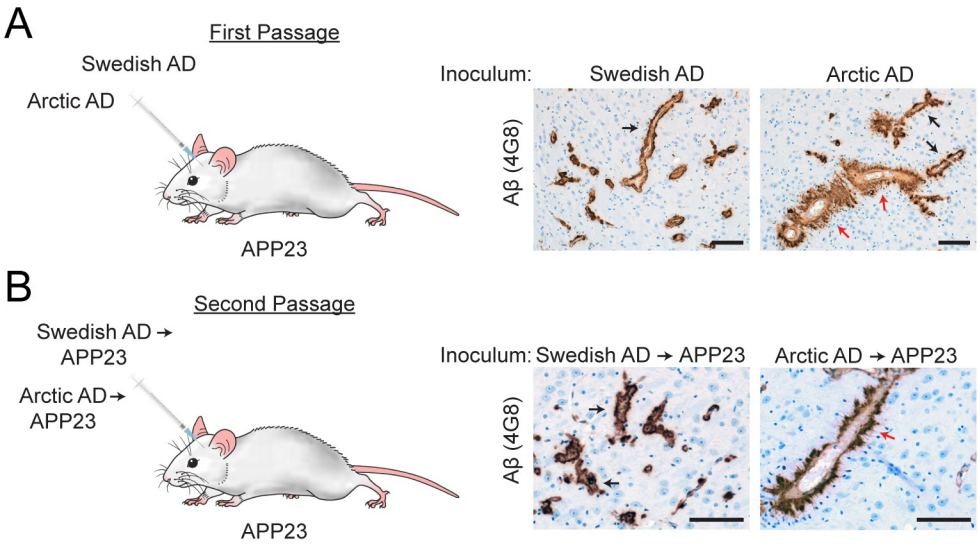
A
First Passage
Swedish AD
Arctic AD
APP23
Inoculum:
Swedish AD
Arctic AD
Aβ (4G8)
B
Second Passage
Swedish AD → APP23
Arctic AD → APP23
APP23
Inoculum: Swedish AD → APP23
Arctic AD → APP23
Aβ (4G8)

Figure 3

Table 1. Attempts to transmit Alzheimer's disease to primates.

Number of cases inoculated	Host primate(s)	Incubation period examined	Findings	Reference(s)
34 (sporadic AD)	Chimpanzees, capuchins, squirrel monkeys, spider monkeys, African green monkey, stumptail monkeys	13 cases examined for >50 months; 21 cases examined for <50 months	No clinical signs of neurological disease in any of the inoculated animals; Aβ pathology analysis not performed	(Goudsmit et al. 1980)
18 (familial AD)	Chimpanzees, capuchins, mangabeys, squirrel monkeys, spider monkeys, African green monkey, stumptail monkeys, rhesus monkeys, cynomolgus monkeys	4 cases examined for >50 months; 14 cases examined for <50 months	16/18 cases produced no clinical signs of neurological disease in the inoculated animals; Aβ pathology analysis not performed; 2/18 cases produced a spongiform encephalopathy at 23-40 months postinoculation[1]	(Goudsmit et al. 1980)
3 (sporadic AD)	Marmosets	Up to 95 months	No clinical signs of neurological disease in any of the inoculated animals; 8/9 inoculated animals exhibited Aβ pathology (plaques and cerebral amyloid angiopathy)	(Baker et al. 1994; Ridley et al. 2006)
1 (familial AD)	Marmosets	Up to 72 months	No clinical signs of neurological disease in any of the inoculated animals; 4/5 inoculated animals exhibited Aβ pathology (plaques and cerebral amyloid angiopathy)	(Ridley et al. 2006)

[1]These two positive transmissions were not reproducible.

Table 2. Similarities and differences between PrP and Aβ prions.

Characteristic	PrP prions	Aβ prions	Reference(s) for Aβ
Induction of protein aggregation in Tg mice expressing mutant precursor protein	Yes	Yes	(Kane et al. 2000; Meyer-Luehmann et al. 2006)
Induction of protein aggregation in Tg mice expressing wild-type precursor protein	Yes	Yes	(Morales et al. 2012)
Induction of protein aggregation in non-Tg mice	Yes	No	(Meyer-Luehmann et al. 2006)
Induction of protein aggregation in primates	Yes	Yes	(Ridley et al. 2006)
Induction of a lethal disease in mice	Yes	No	(Stöhr et al. 2012)
Induction of neuronal loss in mice	Yes	No	(Ye et al. 2015b)
Induction of astrocytic gliosis in mice	Yes	Yes	(Watts et al. 2011)
Progressive spreading of protein aggregation	Yes	Yes	
Neuroinvasion following peripheral inoculation	Yes	Yes	(Eisele et al. 2014)
Horizontal or iatrogenic transmission	Yes	Minimal evidence	(Frontzek et al. 2016)
Zoonotic transmission	Yes	No	
Serially transmissible	Yes	Yes	(Watts et al. 2014)
Existence of distinct strains	Yes	Yes	(Stöhr et al. 2014)
Titratable levels of infectivity	Yes	Yes	(Morales et al. 2015)
Resistance to digestion with proteinase K	Yes	Yes	(Stöhr et al. 2012)
Resistance to formaldehyde inactivation	Yes	Yes	(Fritschi et al. 2014a)
Adherence to stainless steel wires	Yes	Yes	(Eisele et al. 2009)