The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics

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Abstract | The amyloid cascade hypothesis, which posits that the deposition of the amyloid-β peptide in the brain is a central event in Alzheimer's disease pathology, has dominated research for the past twenty years. Several therapeutics that were purported to reduce amyloid-β production or aggregation have failed in Phase III clinical testing, and many others are in various stages of development. Therefore, it is timely to review the science underpinning the amyloid cascade hypothesis, consider what type of clinical trials will constitute a valid test of this hypothesis and explore whether amyloid-β-directed therapeutics will provide the medicines that are urgently needed by society for treating this devastating disease.

Amyloid-β
Amyloid-β peptides result from sequential cleavage of the amyloid precursor protein by β-cleaving amyloid precursor protein enzyme (BACE) and γ-secretase. These peptides vary in length, with Aβ40 (the 40-amino acid form of the peptide) being predominant.

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doi:10.1038/nrd3505

The amyloid cascade hypothesis for Alzheimer's disease (AD) (FIG. 1) has been very influential in the research conducted in academia and the pharmaceutical industry. This hypothesis synthesizes histopathological and genetic information, and posits that the deposition of the amyloid-β peptide in the brain parenchyma initiates a sequence of events that ultimately lead to AD dementia.

However, all of the amyloid-β-centric approaches that reached Phase III clinical trials have failed; tramiprosate, tarenflurbil and semagacestat have now been discontinued. The preclinical data and the clinical biomarker data for tramiprosate and tarenflurbil were not strong[1], but the failure of semagacestat — a well-characterized γ-secretase inhibitor (GSI) — was of particular note. Semagacestat was being evaluated in two Phase III trials, the Interrupting Alzheimer's Dementia by Evaluating Treatment of Amyloid Pathology (IDENTITY) trial (ClinicalTrials.gov identifier: NCT00594568) and the IDENTITY-2 trial (ClinicalTrials.gov identifier: NTC00762411). This compound reduced amyloid-β deposition in the PDAPP transgenic mouse (which overexpresses a mutated human amyloid precursor protein (APP)) after 5 months of dosing[2] and also showed a statistically significant reduction in newly synthesized amyloid-β in human volunteers using stable isotope-linked kinetic (SILK) technology[3]. In fact, GSIs such as semagacestat have a very complex mode of action. This class of compounds is able to inhibit the production of amyloid-β at high concentrations of the substrate and compound, but stimulates γ-secretase at lower concentrations of substrate and compound[4].

Interestingly, an interim analysis from the Phase III trials demonstrated that patients who were treated with semagacestat displayed an increased deterioration in cognition and activities of daily living compared to placebo-treated controls. It remains to be determined whether this compound-mediated worsening of the disease is reversible or not, as this will indicate whether the compound accelerated the disease process or had a temporary effect on disease symptoms. It should also be recognized that γ-secretase has a range of substrates, including Notch[5], and thus it is not possible to determine whether the adverse effects of semagacestat on cognition are related to the inhibition of the processing of proteins other than APP.

This failure has led some in the field to question the role of amyloid-β and amyloid deposition in AD (reviewed in REFS 6,7), although in our opinion it is premature to accept the null hypothesis. However, two key questions remain unanswered: by how much should amyloid-β production be lowered, or amyloid-β clearance facilitated, to mediate a therapeutic disease-modifying effect? And at what stage of the disease process would an amyloid-β-directed therapeutic approach be likely to show clinical efficacy?

In this Review, we attempt to place these crucial questions into the context of what is known and what might be inferred from preclinical and clinical studies. We consider afresh the science underpinning the amyloid cascade hypothesis and we review the 'brain amyloid-β economy', amyloid-β deposition and the stability of amyloid plaques. We describe the relationships between amyloid plaques,

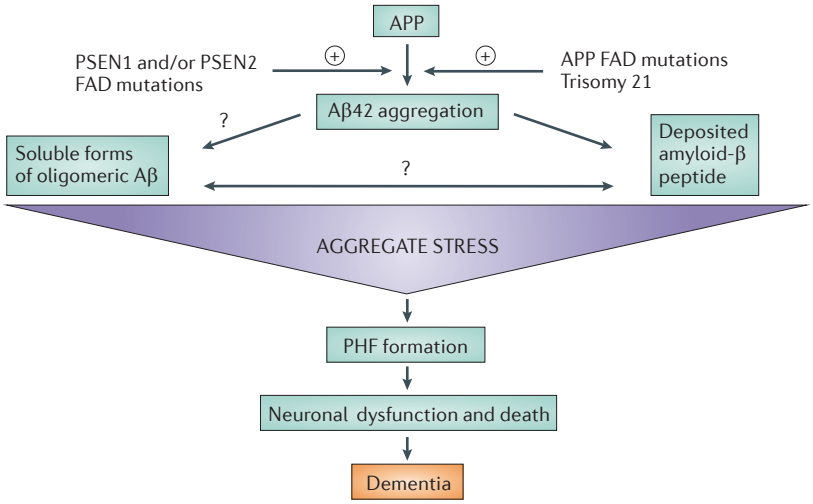

Figure 1 | **The amyloid cascade hypothesis.** The amyloid cascade hypothesis posits that the deposition of the amyloid-β peptide in the brain parenchyma is a crucial step that ultimately leads to Alzheimer's disease. Autosomal dominant mutations that cause early onset familial Alzheimer's disease (FAD) occur in three genes: presenilin 1 (*PSEN1*), *PSEN2* and amyloid precursor protein (*APP*). This hypothesis has been modified over the years as it has become clear that the correlation between dementia or other cognitive alterations and amyloid-β accumulation in the brain in the form of amyloid plaques is not linear, neither in humans[66] nor in mice[143]. The concept of amyloid-β-derived diffusible ligands[113] or soluble toxic oligomers[144,145] has been proposed to account for the neurotoxicity of the amyloid-β peptide. These intermediary forms lie somewhere between free, soluble amyloid-β monomers and insoluble amyloid fibrils, but the exact molecular composition of these oligomers remains elusive. Toxic, soluble amyloid-β in different forms has been isolated from transfected Chinese hamster ovary cells[145], transgenic mouse brains[114], the human brain[115] or it has been reconstituted *in vitro* under various experimental conditions[113,146,147]. The amyloid cascade hypothesis now suggests that synaptotoxicity and neurotoxicity may be mediated by such soluble forms of multimeric amyloid-β peptide species. The dynamic nature of these species and the poorly defined mechanism (or mechanisms) of toxicity make this topic particularly controversial in the field. Given this uncertainty, we prefer to use the term 'aggregate stress' to describe the potential mechanisms that may lead to amyloid-β aggregation, the formation of paired helical filaments (PHFs) of tau aggregates and, ultimately, result in neuronal loss. Aβ42, the 42-amino acid form of amyloid-β.

neuronal loss and dementia. Finally, we explore what we can learn from AD caused by familial autosomal dominant mutations, in particular the distinction between age of onset and duration of disease, and what might be inferred with respect to therapeutic intervention. As we discuss, the answers to these various questions have considerable implications for the design of clinical trials that purport to test the amyloid cascade hypothesis.

The amyloid cascade hypothesis

It has been widely recognized that AD represents a daunting, worldwide challenge for society and health-care providers (BOX 1). Patients with dementia require resource-intensive care, and the cost of care for a patient with AD is approximately US$57,000 per year in the United States[8]. Age is a major risk factor for succumbing to AD. With the predicted demographic shift to increasingly elderly populations, it is estimated that well over 100 million patients will develop AD by 2050 (REF. 9). Unless effective therapies are developed that can delay the onset or deflect the course of the disease, the toll of AD on society and health-care systems will be unbearable.

The pathognomonic signs of AD were first described by Alois Alzheimer in 1907 (REF. 10). The plaques and neurofibrillary tangles that he identified after histological examination of brains of patients with AD were subsequently shown to be composed largely of amyloid-β peptide and hyperphosphorylated tau, respectively. The discovery that AD could be inherited in an autosomal dominant fashion[11] was a seminal event in AD research. The mutation that was described was in the gene coding for APP, the holoprotein from which the amyloid-β peptide is excised via sequential scission by the β-APP cleaving enzyme (BACE)[12–16] and γ-secretase[17,18]. These observations led to the articulation of the amyloid cascade hypothesis[19–22] (FIG. 1).

This hypothesis was further supported by the discovery that AD could also be caused by autosomal dominant mutations in presenilin 1 (PSEN1)[23] and PSEN2 (REFS 24,25), which are both homologous proteins that can form the catalytic active site of γ-secretase. The apolipoprotein E (*APOE*) gene represents the major genetic risk factor for AD[26,27]. Humans possess three common *APOE* alleles: *APOE2*, *APOE3* and *APOE4* (REF. 28). The possession of an *APOE4* allele can bring forward considerably the age of disease onset; *APOE3* can be considered to be neutral; and *APOE2* is thought to be protective[29]. How the different APOE proteins mediate their effects in AD is not definitively known, but a compelling series of experiments in PDAPP transgenic mice that harboured the human *APOE* genes indicate that these cognate proteins mediate the clearance of amyloid-β[30], with APOE2, APOE3 and APOE4 being increasingly less effective at clearing amyloid-β[31].

Other AD risk factor genes are being discovered via genome-wide association (GWA) studies[32–34]. Of interest, the genes that cause the autosomal dominant form of the disease have not been detected as risk factors in the GWA studies that have been conducted so far. Although it is beyond the scope of this Review to discuss these results in detail, the most likely explanation for this is that the common genetic variation around these three genes is simply not sufficient to alter their expression in a manner that increases the risk of AD.

Thus, the amyloid cascade hypothesis appears to explain and incorporate several key data that are germane to the disease process, including the pathology, the phenotypes mediated by the genes that cause autosomal dominant disease and the genetic risk conferred by the *APOE* gene status. However, this hypothesis does not consider the interaction of amyloid-β with tau. This was clarified, to some degree, by the discovery that mutations in the tau gene could cause autosomal dominant frontotemporal lobe dementia, chromosome 17-type[35]. The tau pathology in this disease is similar to the tau pathology seen in AD, but without the appearance of amyloid-β plaques. Thus, tau pathology itself can cause neuronal loss. This observation places tau pathology downstream of amyloid-β pathology.

It has also been reported, through studies in *APP*-transgenic mice, that a reduction in endogenous levels of tau can ameliorate some of the behavioural and other deficits that are mediated by amyloid-β[36,37], again placing

Amyloid plaques
Amyloid plaques are deposits of insoluble amyloid-β in the parenchyma of the brain that can be diffuse or compact. If they are associated with dystrophic and degenerating neurons, they are often termed 'neuritic plaques'.

Neurofibrillary tangles
Large deposits of hyperphosphorylated tau (5–9 moles of phosphate per mole of tau) that fill the cell body of the neuron and take its shape. They are composed of both paired helical and straight filaments of hyperphosphorylated tau.

Box 1 | **AD diagnosis and therapy**

Alzheimer's disease (AD) is the major cause of dementia, which is characterized by a gradual onset and progression of deficits in more than one area of cognition, including episodic memory, language, praxis and attention, reflecting those areas of the brain that are initially affected[125]. A definite diagnosis for AD requires the presence of plaques and neurofibrillary tangles in the brain parenchyma following post-mortem analysis, but a probable diagnosis can be achieved on a clinical basis. Various clinical instruments can be used to screen patients for AD, including the mini-mental state exam (and variants thereof), the memory impairment screen, the Blessed test of information, and others[126]. These are rapid tests of memory and cognition that take up to 15 minutes to perform.

To determine the efficacy of a therapy for treating AD, randomized clinical trials must show considerable benefit versus placebo on two co-primary outcomes: first, a measure of cognition, and second, a measure of activities of daily living. For measuring cognition, the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog) clinical instrument is most frequently used[127–129]. For measuring activities of daily living, various tests are used, such as: the Clinical Dementia Rating scale[130]; the US National Institutes of Health's Alzheimer's Disease Cooperative Study Activities of Daily Living (ADCS-ADL) scale[131]; the Disability Assessment for Dementia scale[132]; and the ADCS-Clinical Global Impression of Change (ADCS-CGIS) scale[133]. Other clinical instruments are used as measures of secondary outcome.

In addition to having positive effects on cognition and activities of daily living, the data required to demonstrate that a therapeutic agent genuinely modifies the course of the disease, rather than acting purely on the symptoms, are still a matter of debate. However, data that demonstrate a statistically significant benefit of the therapeutic agent on a relevant biomarker that reflects the underlying disease process — such as a reduction in brain atrophy as measured by magnetic resonance imaging, or a reduction in the levels of total tau or phosphorylated tau in the cerebrospinal fluid of treated patients as evidence of reduced neuronal loss — will almost certainly be required[134]. In addition, various clinical trial paradigms and statistical analyses that might hypothetically demonstrate that the course of the disease has been beneficially deflected — for example, whether the slope of cognitive decline over time is reduced — are being investigated[135,136].

Current symptomatic therapies for AD include the acetylcholinesterase inhibitors (donepezil (Aricept; Eisai/Pfizer), galantamine (Razadyne; Johnson & Johnson) and rivastigmine (Exelon; Novartis)), and a low-affinity NMDA (*N*-methyl-D-aspartate) receptor antagonist (memantine) for moderate to severe AD. The acetylcholinesterase inhibitors mediate their effects by remediating, in part, the cholinergic deficit in AD, whereas the precise mechanism of action of memantine remains to be elucidated. Overall, the effects of these drugs are limited as they modestly improve some of the symptoms but do not treat the underlying causes of the disease.

tau downstream of amyloid-β; however, one must be cautious in extrapolating preclinical data of this nature to human disease. Moreover, the precise temporal and mechanistic relationships between amyloid-β deposition and tau pathology remain to be resolved. A key parameter in this relationship remains the formation and fate of the amyloid-β peptide.

The brain 'amyloid-β economy'

There are currently three main therapeutic intervention strategies aimed at amyloid-β: reducing amyloid-β production, facilitating amyloid-β clearance and preventing amyloid-β aggregation. Key to all of these approaches is a consideration of the amount, rate and reversibility of amyloid-β deposition. There is considerable uncertainty with respect to the amount of amyloid-β that is deposited in the human AD brain, and this stems from the natural heterogeneity of human AD brains and the fact that different regions of the brain can contain different amounts of amyloid-β. For the purposes of this analysis, only Aβx-42 (the 42-amino acid form of amyloid-β (Aβ42) including amino-terminally truncated species) is considered, because this species represents the majority of deposited parenchymal amyloid-β[38].

An estimation of the amount of deposited amyloid-β in the brain requires data that have a sufficient sample size and are derived from quantitative assay systems that are combined with aggressive, formic acid extraction protocols. Assuming that the average weight of an AD brain is 1,150 g[39] and the grey matter of the cortex, which contains the majority of deposited amyloid-β, comprises 42% of the weight of the brain[40], Gravina *et al.*[41] calculated that ~10 mg of amyloid-β per brain is deposited, whereas Naslund *et al.*[42] calculated that ~4 mg of amyloid-β per brain is deposited. Although the result obtained by Naslund *et al.* is lower than other literature estimates[43], for the purposes of this analysis the total amount of Aβx-42 in a human AD brain at end-stage disease is assumed to be derived from 10 mg of Aβ1–42.

It is important to compare the amount of amyloid-β that is deposited in the AD brain with the overall rate of amyloid-β production, to provide a conceptual framework for this aspect of the disease process and to place into context the potential for different amyloid-β-centric therapeutics to mediate a therapeutic effect.

Production rate of amyloid-β. It is difficult to quantify the amount of amyloid-β that is made in the human brain, given the complexity of its catabolism and clearance. The total production of amyloid-β in the human brain can be assessed by extrapolating from rodent cell culture experiments and potentially from SILK technology[44] (BOX 2). It is likely that a proportion of amyloid-β is degraded within the brain parenchyma by enzymes such as neprilysin[45], insulin-degrading enzyme[46] and others[47,48]. Amyloid-β is trafficked via the interstitial fluid (ISF) into the cerebrospinal fluid (CSF) via bulk flow; however, this has been shown to be a minor route for amyloid-β clearance in mice, as it only accounts for approximately 10% of amyloid-β clearance[49]. The other route for amyloid-β clearance is direct trafficking out of the brain via the blood–brain barrier into peripheral circulation.

An important consideration is how the concentration of amyloid-β in the CSF compares with its concentration in the ISF in the brain parenchyma, which is a major site for amyloid plaque formation. This is very difficult to estimate accurately. Cirrito *et al.*[50] used microdialysis to estimate the levels of amyloid-β in PDAPP transgenic mice, but they correctly refer to the analyte as 'exchangeable' amyloid-β, as there will probably be amyloid-β that is extracellularly adsorbed to membranes or matrix proteins and therefore not freely diffusible.

In studies in rats, using sensitive rodent-specific amyloid-β-targeted antibodies, the concentration of Aβ42 in the CSF was measured at 232±64 pg per ml (mean ± standard deviation), a concentration not dissimilar from the concentration observed in humans (M.M., unpublished observations). However, adsorbed amyloid-β and amyloid-β that is present in the ISF can be released via diethylamine extraction in normal rodent brains. Assuming that the ISF is equal to 18% of brain volume[51] and that all of the amyloid-β released by

Tau
A protein that binds to and stabilizes microtubules within cells and is abundant in neurons. Humans express six isoforms of tau that result from alternative splicing of exons 2, 3 and 10 of the tau gene. Tau can be multiphosphorylated and this regulates its microtubule-binding properties.

Apolipoprotein E
(APOE). A 34-kDa secreted protein that is synthesized predominantly in the liver but is also produced by glial cells in the brain. It acts as a lipoprotein-binding protein and mediates lipid metabolism by binding to the low-density lipoprotein superfamily of receptors.

Box 2 | **Quantifying amyloid-β production**

The rate of amyloid-β production from primary neurons is estimated to be 0.30×10^{-3} pg of amyloid-β 42 (Aβ42; the 42-amino acid form of amyloid-β) per day per cell[137] (M.M., unpublished observations). The human brain contains 86×10^9 neurons, with 13.5×10^9 neurons being in the grey matter of the cortex[40]. Thus, the estimated production of Aβ42 by the human cortex is equal to 0.004 mg of Aβ42 per day. Therefore, 10 mg of Aβ42 deposited in an Alzheimer's disease (AD) brain is equivalent to 6.8 years of total Aβ42 production in the brain. Importantly, notwithstanding the assumptions and extrapolations that are necessary to make this calculation, the strong implication is that the amyloid-β economy explains why it takes many years for the amount of Aβ42 that ultimately becomes deposited in the human AD brain to accumulate and, given the temporal course of the disease, this further demonstrates that a substantial proportion of Aβ42 that is produced becomes accreted into insoluble deposits.

Another approach is to estimate the deposition rate based on the work of Bateman *et al.*[44] who used stable isotope-linked kinetic technology and calculated the fractional clearance rate of newly synthesized amyloid-β to be equal to 8% per hour. Thus, the total production of Aβ42 per day is equal to: 0.08 × 206 (pg per ml of Aβ42 in human cerebrospinal fluid (CSF)[67]) x 150 (ml of CSF[138]) x 24 (hours) = 59 ng of Aβ42 per day. If one further assumes that the 30% reduction in the levels of Aβ42 in the CSF seen in patients with AD[67] represents the amount of Aβ42 that is deposited in the brain, then the amount that is deposited is equal to 59 × 0.3 = 17.7 ng of Aβ42 per day. This small amount is unable to account for the amount of amyloid-β that is deposited in the brains of patients with AD (10 mg) and this therefore suggests that measuring the pool of amyloid-β in the CSF reflects amyloid-β metabolism within the brain parenchyma in a qualitative and correlative manner.

diethylamine was made available in that compartment, the concentration of Aβ42 in the ISF is equal to 25.5 ng per ml, which is approximately a 100-fold higher concentration than in the CSF.

If these data are extrapolated to humans, it is clear that the extracellular environment within the parenchyma could harbour much higher concentrations of amyloid-β than the CSF, which has implications for the potential effect of inhibitors of amyloid-β production and their ability to reduce amyloid-β deposition. Moreover, these findings have to be taken into account when considering to what extent amyloid-β measurements in the CSF reflect the actual situation in the ISF.

Deposition of amyloid-β into plaques and the effects of inhibiting amyloid-β production. It has been very difficult to derive predictive data on the dynamics of plaque deposition. This is because truly quantitative imaging of plaques in humans has not been possible, and *in vivo* models that reflect the human situation accurately are not available. However, Maggio and colleagues[48,52,53] have performed some *ex vivo* experiments that have provided highly relevant data.

Maggio *et al.*[53] developed an *ex vivo* assay in which the binding and dissociation of radiolabelled amyloid-β species was studied using unfixed tissue sections from AD and normal brains. The strength of these studies lies in the appropriate use of relevant human tissue and physiologically relevant concentrations of amyloid-β. There was substantial non-saturated binding of the radiolabelled ^{125}I-Aβ40 species to parenchymal and vascular amyloid-β plaques, which suggests that amyloid-β was not binding to conventional receptors. An investigation of the kinetics of this binding showed that the binding of the amyloid-β monomers followed a 'dock and lock' mechanism. The data indicate that further accretion of amyloid-β will occur once plaques are established, even if the concentration of amyloid-β in the ISF has been substantially reduced, owing to the high avidity of Aβ42 for amyloid-β plaques[52].

There have been *in vivo* studies on the dynamics of amyloid plaque formation using both pharmacological and non-pharmacological intervention. Yan *et al.*[54] used intravital multiphoton microscopy to study the growth of individual amyloid plaques and the effects of a GSI, Compound E, in transgenic mice harbouring familial Alzheimer's disease (FAD)-linked *APP* and *PSEN1* mutated genes (*APP*/*PSEN1*-transgenic mice). This GSI was able to reduce plaque load at the start of the dosing period, when the mice were 6 months old, but was ineffective when it was dosed at 10 months. The inhibitor acted to prevent the development of new plaques but it did not mediate the disaggregation of existing plaques. In a similar study[55], LY-411575 — a potent GSI — was tested in 10–11-month-old *APP*/*PSEN1*-transgenic mice with established plaques. The administration of LY-411575 resulted in statistically significant reductions in the levels of soluble and insoluble Aβ40 and Aβ42. However, despite these reductions in the levels of amyloid-β, there was no reduction or change in amyloid-β plaques.

Hefendehl *et al.*[56] also used multiphoton imaging *in vivo* and followed the deposition of amyloid-β in *APP*/*PSEN1*-transgenic mice from 3–4 months of age for 6 months. They demonstrated that irrespective of size and age, the radii of plaques grew at the same rate, which was consistent with a model in which amyloid-β monomers are constantly added to the surface of the plaques. An interesting aspect of this meticulous study was that the number of new plaques declined with time. The authors hypothesize that as the numbers of plaques increase, the overall surface area of the plaques increases and at some time there will be a competition between the availability of amyloid-β to seed new plaques versus its deposition onto existing plaques. This phenomenon might also explain the plateau demonstrated with the binding of the ^{11}C-Pittsburgh compound B (PIB) in patients with AD[57].

Using LY-411575, a detailed analysis was also performed on the effects of γ-secretase inhibition in transgenic mice that express the Swedish *APP* mutation (known as APP23 mice)[58]. At 6 months of age, APP23 mice are just starting to form plaques, and these plaques are well established by 15 months. In a subchronic dosing paradigm, the administration of LY-411575 to mice for 3 months — from the age of 6 to 9 months — suppressed the levels of Aβ42 in the brain by 77% and reduced the size of the cortical plaque area by 80%. In the same study, APP24 mice, which are transgenic mice that harbour the Swedish and London *APP* mutations and start to form plaques at 8 months, were treated with LY-411575 for 2 months, from 15 months to 17 months of age. In this paradigm, the amount of Aβ42 that was extractable by SDS was limited to the baseline (15-month) level in the cortex of drug-treated mice, with the vehicle-treated group showing statistically significant

Genome-wide association (GWA) studies
This technique enables common, single-nucleotide polymorphic genetic variations to be compared in patients and in controls, to discover whether there is evidence for a genetic predisposition to the disease.

Familial Alzheimer's disease
(FAD). A form of Alzheimer's disease (AD) caused by rare, autosomal dominant mutations that are inherited in a Mendelian fashion within families. Currently identified FAD mutations result in early-onset AD.

increases at 17 months compared to the 15-month baseline. Plaque burden was approximately doubled in the LY-411575 group compared to the baseline, although it was reduced by 30% compared to vehicle-treated mice. These data demonstrate that early treatment with a GSI can prevent or ameliorate amyloid-β plaque deposition, but established plaques are particularly resistant and are not subject to disaggregation even if amyloid-β production is considerably inhibited.

Other groups have used non-pharmacological methods to reduce amyloid-β production. Jankowsky *et al.*[59] used a tetracyline-responsive gene expression system (that is, a 'tet-off' system) that suppressed the expression of *APP* containing both the Swedish and Indiana FAD mutations when the transgenic mice had doxycycline incorporated into their food pellets. Six-month-old mice were treated with doxycyline for either 3 or 6 months. No difference in plaque load or the levels of amyloid-β extracted by formic acid was observed in the 6-month-old mice, 9-month-old mice (that were treated with doxycycline for 3 months) or 12-month-old mice (that were treated with doxycycline for 6 months). Mice that were allowed to continue to express the mutated form of APP showed a statistically significant increase in plaque burden during the 6–9 month period. These data again demonstrate that reductions in amyloid-β production have little effect on established amyloid-β plaques and stably deposited amyloid-β.

McConlogue *et al.*[60] crossed *Bace*$^{+/-}$ mice with PDAPP transgenic mice; the 50% reduction in BACE activity in these mice led to a 12% reduction in amyloid-β production. Quite remarkably, this led to a 90% reduction at 13 months and a 50% reduction at 18 months in the levels of deposited amyloid-β in the brain. This study has been used to support the concept that modest reductions in amyloid-β production will profoundly reduce plaque deposition in AD. However, although there were insufficient data points for a detailed temporal analysis, the major effect was to delay the onset rather than affect the rate of plaque deposition.

Amyloid-β-specific antibodies are another important potential therapeutic modality for reducing levels of amyloid-β, and there are two basic hypotheses regarding their mechanism of action. First, the 'peripheral sink' hypothesis posits that antibody capture of peripheral amyloid-β prevents the re-entry of amyloid-β from the periphery to the brain and consequently shifts the amyloid-β equilibrium in the brain so as to mediate the dissolution of deposited amyloid-β[61,62]. Second, the binding of amyloid-β-specific antibodies to deposited plaques activates microglia that phagocytose and clear these plaques from the brain[63].

In fact, it is likely that amyloid-β-specific antibodies have mixed mechanisms of action. For example, antibodies such as 3D6 (a mouse monoclonal antibody that was humanized to create bapineuzumab) that recognize amino terminal amyloid-β epitopes will also recognize soluble peripheral amyloid-β in the circulation, and antibodies such as m266 (a mouse monoclonal antibody that was humanized to create solanezumab) that recognize soluble, monomeric amyloid-β are not solely restricted to the periphery but will also enter the central nervous system at a low level that is dependent on the concentration of the antibody in peripheral circulation.

The effects of both passive and active immunization against amyloid-β epitopes have been comprehensively reviewed[64]. In general, most studies that have been carried out have resulted in the prevention of amyloid-β deposition rather than the clearance of existing plaques, but there is good evidence that antibodies that recognize and bind to epitopes on deposited amyloid-β can clear existing plaques[64]. These data therefore point to an active clearance process of plaques in these conditions, which differentiates the immunization-based approaches from the inhibitor-based approaches.

A significant lowering of Aβ42 levels in the CSF, presumably as amyloid-β is deposited into the brain parenchyma, is part of the AD biomarker signature[65]. Furthermore, a subset of normal patients with an *APOE4* genotype showed a statistically significant increase in an AD-like profile. The inference is that these individuals have an increased risk of developing AD and that the deposition of Aβ42 occurs considerably earlier than any overt symptomatology, a finding that has been confirmed neuropathologically[66]. Current investigations in humans of the levels of Aβ42 in the CSF suggest that the rate of deposition is not uniform over time. Rather, there seems to be a prompt conversion from a non-amyloid-β-depositing to an amyloid-β-depositing phenotype (or a substantial acceleration of amyloid-β deposition).

Results from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study[67] and a different diagnosis-independent analysis of the same ADNI data[68] show clear evidence for a bimodal distribution of low levels of Aβ42 in the CSF in normal individuals and in individuals with mild cognitive impairment (MCI), but not in those diagnosed with AD. It might be inferred from the discrete nature of this bimodal distribution that once the first seeding of plaques occurs, amyloid-β is rapidly accumulated into plaques, as modelled in transgenic mice that are predisposed to form insoluble amyloid-β deposits. It has been postulated that the majority of amyloid-β deposition occurs before the onset of decline in cognitive function and hence the extent of amyloid-β deposition in patients with MCI and AD may well be similar[57]. In individuals with normal cognitive function, Fagan *et al.*[69] measured the levels of amyloid-β metabolites in the CSF and correlated these with PIB binding as measured using positron emission tomography (PET) imaging. As expected, reductions in Aβ42 levels in the CSF were correlated with increases in PIB binding.

In summary, the amount of Aβ42 that is deposited in the AD brain is in the low mg range and is equivalent, based on estimates of neuronal Aβ42 production, to approximately 6–7 years of total Aβ42 production. The amount of Aβ42 in the CSF represents a very small fraction of the total amount of amyloid-β that is likely to be produced in the whole brain, and most of the amyloid-β that is produced in the brain is transported via the blood–brain barrier into peripheral circulation from which it is

rapidly cleared. Monomeric amyloid-β is deposited onto plaques in a stable and almost irreversible manner. In transgenic mouse models that lead to amyloid-β deposition, GSIs are able to reduce the formation of new plaques but they do not mediate the removal of existing plaques. Modest reductions in amyloid-β production are able to substantially delay the onset of plaque deposition.

Given the avidity of amyloid-β deposition onto plaques, it is unlikely that even very substantial reductions in the levels of free amyloid-β would mediate a reduction in the formation of amyloid plaques in patients, especially as post-translational modifications of deposited amyloid-β over many years render human amyloid plaques more stable to clearance mechanisms than those plaques that are formed in transgenic mice[70,71]. These data also suggest that antibodies that target amyloid plaques drive an active mechanism that removes plaques from the brain[72].

Amyloid-β peptide ratios and FAD

The drive towards the development of therapeutics that aim to reduce the levels of amyloid-β in the brain has been supported by the evidence underpinning the amyloid cascade hypothesis, which points to the crucial role of amyloid-β in the disease process. Mutations in three genes are currently known to cause FAD: *APP* (mutations include duplications and triplications), *PSEN1* and *PSEN2*. Mutations in the *APP* gene fall into three categories: those at the BACE cleavage site, those at the γ-secretase cleavage site and those in the mid-domain amyloid-β region (FIG. 2).

PSEN1 and PSEN2 are homologous proteins that provide the active site aspartate residues that are needed for the proteolysis of substrates that enter the γ-secretase complex[17,18]. The γ-secretase complex is composed of four proteins that are present at equal stoichiometry: PSEN1 or PSEN2, nicastrin, anterior pharynx defective 1 (APH1) and presenilin enhancer 2 (REF. 73). There are two homologues of APH1 in humans — APH1A and APH1B — so in total there are four different γ-secretase complexes possible[74]. PSEN2-containing γ-secretase complexes do not have a major role in mediating amyloid-β production and therefore it is perhaps not surprising that there are fewer *PSEN2* mutations that lead to FAD[75].

There are currently >180 FAD-linked *PSEN1* mutations (see the AD & FTD Mutation Database) that are scattered throughout the gene and, given that these mutations produce different effects on amyloid-β

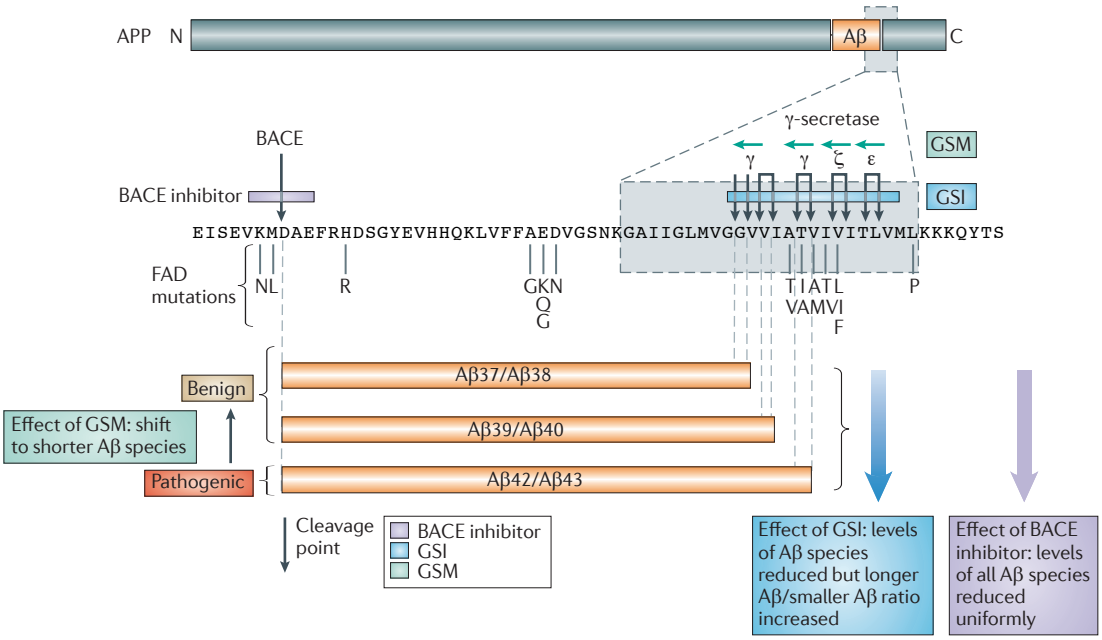

Figure 2 | **Metabolism of APP, FAD mutations and the effects of proteinase inhibition.** The amyloid precursor protein (APP) mutations in Swedish familial Alzheimer's disease (FAD) are distal to the amino terminus of the amyloid-β (Aβ) peptide and result in two amino acid substitutions: K670N and M671L. These mutations increase the rate of proteolysis of APP by β-APP cleaving enzyme (BACE) and hence there is an increased supply of C100 APP for γ-secretase to produce all Aβ species. The mutations around the γ-secretase cleavage site alter the cleavage position of Aβ, such that the ratio of amyloid-β 42 (Aβ42; the 42-amino acid form of amyloid-β)/Aβ40 is tipped in favour of Aβ42 (REF. 148), although quantitative enzymological analysis needs to be carried out to establish the effects of these mutations on absolute levels of Aβ peptides. γ-secretase initially cleaves in tripeptide steps from the ε-cleavage site to produce Aβ peptides[80–82]. The mutations in the mid-domain of Aβ can have various effects that are currently not well understood: indeed, different mutations on the same codon can result in different phenotypes, such as AD, vascular dementia or mixed phenotypes[149,150]. A BACE inhibitor will prevent the production of all Aβ species equally. A γ-secretase modulator (GSM) will shift the production of Aβ peptides in favour of shorter forms of Aβ, but total Aβ peptide production will be unchanged. A γ-secretase inhibitor (GSI) has a complex mode of action, and in this figure it refers to non-competitive compounds that do not bind at the active site but are able to inhibit the production of all Aβ species in a concentration-dependent manner. These compounds often favour the inhibition of shorter Aβ peptides thus increasing the longer Aβ/shorter Aβ ratio.

production, they are particularly informative. It was shown in *Caenorhabditis elegans* gene complementation studies[76] and in cell culture experiments[77] that mutations in *PSEN* cause a partial loss of protein function. The effects of these mutations on amyloid-β production have been characterized by reconstituting their expression in PSEN-deficient fibroblasts[78]. This study revealed that most of the mutations actually reduced the overall amount of amyloid-β produced, and one mutation also reduced the amount of Aβ42 produced. However, in all cases the ratio of Aβ42/Aβ40 was increased. This is consistent with FAD mutations mediating an overall partial loss of protein function[79,80].

In wild-type *PSEN1*, the Aβ40 residue represents the major peptide product from the carboxyl terminus of C100 APP following γ-secretase cleavage. γ-secretase first cleaves Aβ50/Aβ49 residues or Aβ49/Aβ48 residues (ε-cleavage) and then progresses in a stepwise fashion to produce the shorter forms of amyloid-β[81,82] (FIG. 2). After each cleavage event, the remaining peptide is in equilibrium with the aqueous phase and a proportion of the cleaved peptide will diffuse away from the active site as determined by the thermodynamic constraints. If the efficiency of the enzyme is reduced as a consequence of an FAD mutation, the time taken for longer forms of the peptide to diffuse away from the active site between cleavage events increases, and this most probably explains both the reduction in the total amount of amyloid-β and the increase in the proportion of longer forms of amyloid-β.

For the FAD-linked *APP* and *PSEN* mutations, it seems as though there are two potential effects that predispose individuals to early onset AD. If the amount of Aβ42 is increased sufficiently, as mediated by the Swedish *APP* mutation (FIG. 2), then early onset AD can result. For the amyloid-β C-terminal *APP* mutations, the increase in the Aβ42/Aβ40 ratio is sufficient to cause early onset AD. For the *PSEN* mutations, the increase in the ratio of longer Aβ42 or shorter Aβ43 versus shorter Aβ40 or Aβ38 species, even in the context of an overall reduction in amyloid-β production, is sufficient to cause early onset AD. A recent study confirmed that Aβ43 is at least as pathogenic as Aβ42 (REF. 83), and studies in patients demonstrated that the ratio of amyloid-β peptides in the CSF of carriers of the *PSEN1* mutation was substantially different from control patients and patients with sporadic AD (SAD)[84].

It would seem as though shifting the ratio of longer amyloid-β/shorter amyloid-β exerts a profound effect on triggering the disease process, given the very early age of onset of some FAD-linked *PSEN1* mutations[85] (longer amyloid-β species are defined as longer than or the same length as Aβ42 species, and shorter amyloid-β species are shorter than Aβ42 species).

If the ratio of amyloid-β peptides is important to disease onset, then it might be expected that this would be reflected in the nature of amyloid-β deposits seen in the brain. Hellstrom-Lindahl *et al.*[86] carried out a post-mortem analysis to compare the amounts of soluble and deposited amyloid-β peptides in patients with SAD and in patients with FAD caused by *PSEN1* mutations and *APP* mutations. In accordance with the cell culture data, although the levels of insoluble Aβ40 and Aβ42 were substantially lower in patients with FAD caused by *PSEN1* mutations than in patients with SAD, the ratio of insoluble Aβ42/Aβ40 was substantially increased in these groups. This study lends weight to the idea that the ratio of amyloid-β peptides is more important than their absolute levels in promoting the early age of disease onset.

Kim *et al.*[87] used a novel strategy to investigate the roles of Aβ42 and Aβ40. They produced transgenic mice expressing a chimeric protein in which either Aβ40 or Aβ42 was attached to the C-terminal of the integral membrane protein 2B (ITM2B; also known as BRI2); mutations in *BRI2* cause familial British Dementia. These mice produced elevated levels of amyloid-β peptide via the secretory pathway without overexpressing APP. Mice that overproduced BRI2–Aβ40 failed to develop any plaque pathology, whereas a tenfold-lower production of BRI2–Aβ42 resulted in amyloid-β plaques and cerebral amyloid angiopathy. When the mice were crossed, despite having increased amounts of amyloid-β peptides they displayed a substantially suppressed plaque pathology, presumably because BRI2–Aβ40 was able to suppress the deposition of BRI2–Aβ42.

This work was followed up by creating bigenic mice using a prion promoter; mice that express BRI2–Aβ40 were crossed with Tg2576 mice that overexpress the Swedish mutated form of APP. The expression of the Aβ40 peptide via the BRI2 construct dramatically reduced the deposition of parenchymal amyloid-β in the bigenic mice, despite causing considerable elevation of the steady-state levels of Aβ40. The results of these studies are concordant with genetic data, in the sense that the levels of Aβ42 are important for plaque seeding, and in these experiments Aβ40 was able to prevent such seeding.

Meyer-Luehmann *et al.*[88] studied the seeding phenomenon directly. In a seminal experiment, human AD brain extract (10% weight/volume) was injected into the hippocampus of 5-month-old male APP23 host mice and non-transgenic littermates. Mice were analysed after 4 months. The injection of an AD brain extract and a brain extract from an APP23 mouse containing amyloid plaques induced substantial immunoreactive amyloid-β deposition in APP23 host mice. Few or no amyloid-β deposits were detected after injections of brain extract from an aged (95-year-old) control patient or from a wild-type mouse. No amyloid-β deposits were observed after PBS injections or when the brain extract from the transgenic mouse was injected into wild-type mice. Stereological quantification of the amyloid-β load by immunohistochemistry confirmed that brain extracts from patients with AD and transgenic mice induced considerable amyloid deposition compared to control and wild-type brain extracts.

In summary, an increase in the ratio of longer amyloid-β/shorter amyloid-β is a key phenotype in FAD, even in the context of an overall reduction in total amyloid-β production and brain deposition. In preclinical models, Aβ42 is the species that results in plaque deposition, whereas Aβ40 is comparatively benign and

may even be protective. Seeding of plaque components accelerates plaque deposition in transgenic mice that are predisposed to form plaques. However, data on amyloid-β species are constrained by the availability of specific antibodies for detecting amyloid-β levels in quantitative assays: thus, longer forms of amyloid-β resulting from the initial γ-secretase ε-cleavage may also have an important role, given their increased hydrophobicity. The data therefore suggest that absolute levels of amyloid-β are not a key determinant in the age of onset of AD.

From amyloid-β deposition to dementia

AD is neuropathologically defined as requiring the formation of amyloid-β plaques and neurofibrillary tangles of tau, and the relationship between these pathognomonic signs has been of considerable debate for many years. Braak and Braak[89] showed that tau pathology occurs first in the transentorhinal cortex, often in the absence of plaque pathology. The pattern of tau pathology is highly regular, whereas amyloid-β plaque pathology is much more varied.

In a compelling study involving 97 elderly individuals with no symptoms of dementia and with an average age of 84.3 years[66], the incidence of AD neuropathology was determined to be between 20–40%, depending on which of the following four neuropathological criteria were used: the Khachaturian criteria; the modifications of the Khachaturian criteria used by Washington University; the CERAD (Consortium to Establish a Registry for Alzheimer's Disease) criteria or the National Institute of Aging (NIA)–Reagan criteria. There was an age-related statistically significant increase in the presence of neurofibrillary tangles, but no association between age and amyloid-β plaque burden was observed. There was no relationship between neocortical plaque burden and the density of limbic neurofibrillary tangles: thus, there was no correlation between neurofibrillary tangles and plaque burden.

This mismatch between amyloid-β plaque burden and the presence of neurofibrillary tangles has been noted by many groups to occur in AD as well, and Gomez-Isla *et al.*[90] investigated this observation very thoroughly. A stereological analysis of 34 AD brains compared with 17 age-matched controls was carried out; this revealed that the average total number of neurons in the superior temporal sulcus volume was substantially reduced by 53% in the AD group compared to the control group. There was a statistically significant correlation between the loss of neurons and the increase in the presence of neurofibrillary tangles with disease progression; however, no correlation was observed between amyloid-β load and either disease duration or neuronal loss. Importantly, at a quantitative level the neuronal loss was greatly in excess of the number of neurofibrillary tangles.

The explanation for this could be that neurofibrillary tangles are associative but not causative of neuronal loss. This seems to be unlikely, as tau mutations that result in neurofibrillary tangle pathology are directly responsible for the neuronal death and dementia that is observed in frontotemporal lobe dementia[35]. Neurofibrillary tangles may therefore present an end-stage of tau pathology and neurons could be lost before this stage; alternatively, it is possible that the majority of neurofibrillary tangles are cleared after neuronal death.

Savva *et al.*[91] showed that cerebral atrophy — which is caused by the loss of neurons and synapses — followed by tau pathology correlated best with clinical symptomatology. Amyloid-β plaque burden does not, in most studies, correlate with either disease duration or cognitive status. Furthermore, PIB-positive individuals can present with normal magnetic resonance imaging (MRI) scans —therefore showing no evidence for atrophy — which implies that amyloid-β deposition per se need not result in neuronal loss or dementia. In the transition from normal cognition to MCI and then to definite AD, although the rate of brain atrophy is accelerated, PIB binding and lowered levels of amyloid-β in the CSF remain abnormal but unchanged[92].

The studies carried out by Braak and Braak[89] on the temporal appearance and location of AD pathology concluded that tau pathology, in contrast to amyloid-β pathology, followed a uniform and invariant pattern in the brain. Furthermore, these authors suggested that this might result in a specific 'neuron to neuron' mechanism of transmission of tau pathology. Some preclinical data are now available to support this theory. Tau aggregates that were derived from P301L mice (transgenic mice expressing the P301L human mutant tau) were injected into the brain of transgenic mice expressing wild-type human tau, which does not normally lead to tau pathology[93]. This treatment resulted in the development of tau pathology at the site of the injection and along the injection tract.

In summary, amyloid-β aggregation and deposition is a very early event that can trigger tau pathology and will do so in many individuals, but the deposition of amyloid-β does not correlate with the presence of neurofibrillary tangles, cell loss or dementia. Tau pathology follows a regular neuroanatomical path that correlates well with the observed sequence of cognitive defects. Tau pathology also correlates with neuronal loss and cerebral atrophy, which in turn correlates strongly with dementia. In addition, there is preclinical evidence to suggest that in the absence of plaques, tau pathology is able to spread through the brain.

Given the current knowledge discussed above, and recognizing that there are considerable gaps in our understanding of AD pathology, one hypothesis that accommodates most of the known data is that amyloid-β aggregation and deposition triggers a process that leads to neuronal loss via the formation of paired helical filaments of tau. Nonetheless, it remains to be clarified how deposited amyloid-β triggers or accelerates tau pathology and how tau pathology ultimately leads to neuronal loss. However, a recent study demonstrated that the formation of intracellular amyloid-like fibrils resulted in the sequestration of key intracellular proteins, which subsequently led to the derangement of essential cellular processes[94]. It is possible that intracellular aggregates of tau act in an analogous manner.

Paired helical filaments
Hyperphosphorylated tau filaments that can be visualized in neurons using electron microscopy.

FAD — age of onset and duration of disease

In some cases, FAD mutations bring forward the age of onset of disease to as low as 26 years[85]: to put this into context, approximately 57% of the population aged 85 years and older is likely not to have AD[95]. What has attracted less attention is the effects that FAD mutations have on the duration of the disease process, which is defined as the time from the diagnosis of symptoms to death, and how this compares to SAD. Precise data on disease duration are difficult to ascertain because the clinical presentation of patients with memory or other cognitive defects is variable. Another confounding aspect is whether patients actually die of AD — many patients die of other causes that are linked to the changes in activities of daily living that are brought about by the disease process[96]. Nonetheless, several groups have studied the duration of AD, from the initial diagnosis of dementia to the time of death.

With respect to SAD, the duration of the disease is strongly correlated with the age of onset. This is not surprising, as older cohorts are more likely to have additional risk factors and their life expectancy is lower. However, a sufficient number of patients with SAD develop the disease before they are 75 years old, therefore a valid comparison can be made with FAD[97–99]. Based on these studies, the average duration of disease is approximately 8.5 years for the younger patients with SAD. How does this duration compare with FAD?

Some reviews of the literature suggest that FAD is a more aggressive form of the disease, with a shorter duration — as defined by the time from diagnosis to death[100]. Furthermore, studies with small numbers of patients with FAD have suggested that the duration of disease is shorter in FAD than in SAD[101]. However, several large studies have demonstrated that disease duration in FAD is not significantly different to disease duration in SAD[85,102–105].

The major effect of carrying an *APOE4* allele is to lower the age of onset of AD[106]. Some studies suggest that APOE4 also reduces disease duration[107], but others do not report this effect[108]. A recent meta-analysis that included 17 studies encompassing 1,733 participants — of whom 975 carried an *APOE4* allele — concluded that the *APOE4* genotype had no effect on either the rate of cognitive decline or the time to death[109].

Pastor *et al.*[110] also studied the effect of the *APOE* genotype in 249 members of the Columbian PSEN1 E280A cohort. The mean age of disease onset in this cohort was 45.2 years. In this study, *APOE4* carriers within the E280A kindred developed FAD earlier than non-*APOE4* carriers such that 50% of the *APOE4* carriers had developed FAD 3 years in advance of the non-*APOE4* carriers. In this sample, the mean duration of disease was 11.1 years and the *APOE4* genotype had no effect on disease duration. Thus, even when this major genetic risk factor was added to an FAD-linked *PSEN1* mutation, the average duration of disease was unaffected. If it can be assumed that APOE4 mediates its effects on AD by increasing amyloid-β deposition or reducing amyloid-β clearance[111], it would seem that there is sufficient evidence for this effect bringing forward the age of disease onset, but far less data supporting it having a direct role in disease progression and duration.

It is conceivable that the FAD mutations do accelerate the disease process but this is counterbalanced by age. Thus, the onset of disease is triggered by amyloid-β deposition, but because this is occurring in a younger and more resilient brain in FAD, the duration of the disease, as measured by cognitive decline and time to death, is relatively increased and indistinguishable from SAD. However, given that the age of onset of FAD varies extensively, in our view it is unlikely that brain resilience and age of onset would always be so perfectly balanced as to produce no net difference in disease duration.

In summary, the major effect of all FAD mutations is to lower the age of onset of disease and there are no compelling data to suggest that the duration of disease is reduced in FAD to the same extent as the age of onset (FIG. 3). There are convincing data that the *APOE4* genotype lowers the age of disease onset rather than having an effect on disease progression. Thus, it might be

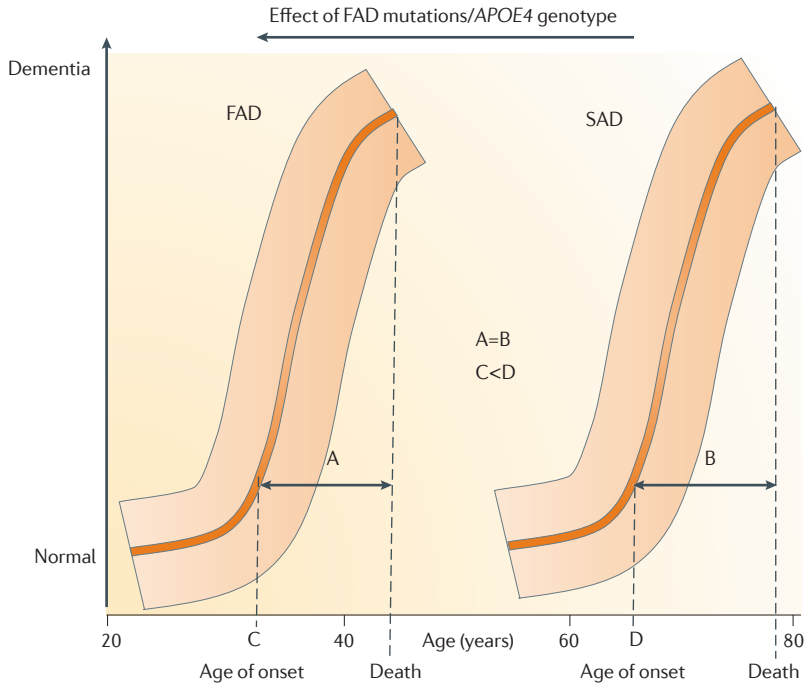

Figure 3 | **Age of onset versus disease duration of FAD and SAD.** The major effect of familial Alzheimer's disease (FAD) mutations and the apolipoprotein E4 (*APOE4*) genotype is to decrease the age of onset of the disease, with little evidence for an effect on disease duration. If deposited amyloid-β had a constant and direct neurotoxic role, it would be expected that with FAD mutations (and perhaps with *APOE4* carriers) both the age of onset and the duration of the disease would be affected. Interpreted in this way, it seems as though the deposition of amyloid-β is a trigger for other events that actually mediate neuronal loss. Nevertheless, the concept of the amyloid cascade is still appropriate, in the sense that one event leads to another, but the term 'amyloid trigger' captures the temporal separation of the events more accurately[112]. A and B represent the durations of FAD and sporadic Alzheimer's disease (SAD), respectively, from onset of cognitive decline to death. Most data suggest that A is not different from B (A=B). C and D represent the ages of onset of FAD and SAD, respectively. The age of onset of FAD can be very much lower than SAD (C<D).

inferred that after the initiation of AD, absolute levels of amyloid-β are not important for disease progression[112] and that the progression of the disease follows a relatively stereotypical path.

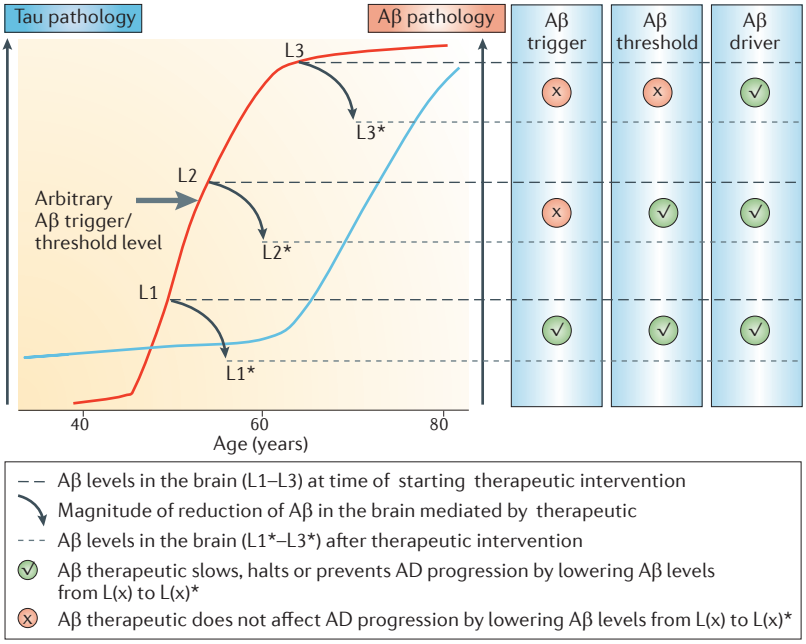

Figure 4 | **Potential amyloid-β scenarios and treatment effects.** This figure shows the administration of an amyloid-β (Aβ) therapeutic that is able to lower the levels of Aβ deposited in the brain. The Aβ therapeutic is illustrated as being administered at various stages during the Alzheimer's disease (AD) process: L1, L2 and L3. The L1 stage is temporally before L2 which in turn is before L3. Over time the therapeutic reduces levels of Aβ down to the level indicated with an asterisk (L1*, L2* or L3*). The effect of an Aβ therapeutic depends on the relationship between deposited Aβ and tau pathology, which is assumed to be the primary pathology that results in neuronal loss. The relationship between the arbitrary Aβ trigger/threshold and tau pathology is purely illustrative and not designed to be temporally accurate. A successful therapeutic intervention would be required to reduce the extent of tau pathology, but for simplicity this is not shown. In the Aβ trigger scenario, at some level of Aβ deposition (indicated by the bold arrow on the graph), there is sufficient 'aggregate stress' to initiate or accelerate tau pathology that then becomes self-sustaining and Aβ-independent. Here, any therapeutic that is administered to reduce Aβ levels will only affect the disease process if it is given before the trigger point. Although difficult to determine, the point at which this threshold is reached could potentially be at a very low level of Aβ deposition, with subsequent increases in Aβ plaque load being irrelevant to the disease process. Only one intervention point — at the L1 stage — prevents levels of Aβ from reaching the 'trigger' point, and this is the only circumstance in which therapeutic intervention results in a clinical benefit. Although the administration of the therapeutic at the L2 stage lowers Aβ levels in the brain to below the trigger level, by this time tau pathology and other AD pathology is Aβ-independent. In the Aβ threshold scenario, Aβ aggregate stress reaches a threshold level (indicated by the bold arrow on the graph) that initiates or accelerates tau pathology. An Aβ-directed therapeutic will only be effective if Aβ aggregate stress can be reduced to below the threshold, but tau pathology and other AD pathology do not become Aβ-independent. Here, interventions at the L1 and L2 stage will be clinically effective as both will result in Aβ levels being lowered to or kept to a level that is below the threshold. In the Aβ driver scenario, some Aβ deposition is continuously required for AD progression in a direct and concentration-dependent manner. Here, an Aβ-directed therapeutic will be effective in proportion to its ability to reduce Aβ levels in the brain at any point in the disease process. Here, interventions at all three stages — L1, L2 and L3 — will be clinically effective. In the Aβ irrelevant scenario, Aβ deposition is an epiphenomenon of another disease-relevant mechanism (or mechanisms) that are affected by familial AD (FAD) mutations and the apolipoprotein E genotype. In this scenario, an Aβ-directed therapeutic cannot be effective under any circumstance (this scenario is not modelled).

Testing the amyloid cascade hypothesis

Whether or not a therapeutic intervention can be said to test the amyloid cascade hypothesis is dependent on the role of amyloid-β in mediating AD. Various scenarios can be considered: the 'amyloid-β trigger' scenario, the 'amyloid-β threshold' scenario, the 'amyloid-β driver' scenario and the 'amyloid-β irrelevant' scenario (FIG. 4).

If the amyloid-β trigger scenario is true, then a therapeutic agent would have no efficacy if it is administered after some amyloid-β deposition has occurred and aggregate stress has triggered the disease process, even if the therapeutic was eventually able to lower the levels of deposited amyloid-β to below the original trigger point. For the amyloid-β threshold scenario, the amyloid cascade hypothesis can be tested either by administering therapeutic agents before the amyloid-β threshold has been reached or by administering agents that act to facilitate the resolution of amyloid plaques, if they are administered after the threshold has been reached. If this is not achieved, it could be argued that an insufficient effect of the therapeutic on amyloid-β allowed the disease to proceed. For the amyloid-β driver scenario, any therapeutic agent that reduced levels of amyloid-β would demonstrate a clinical benefit in proportion to the magnitude of its amyloid-β-lowering effect.

Although it is always preferable to treat any disease as early as possible, if the amyloid-β trigger scenario is true, and if the trigger event is the onset of amyloid-β deposition, then testing the amyloid cascade hypothesis would require a clinical trial in cohorts with normal PIB scans who are at risk of developing AD. This represents a huge clinical challenge, including the selection of the clinical trial population, ethical considerations, trial duration, regulatory hurdles, and so on. However, extrapolating from the effect of the FAD mutations, early intervention might also be extraordinarily effective (FIG. 5).

Key to the scenarios above is an understanding of the mechanism and the temporal relationship by which amyloid-β mediates neuronal loss. As described earlier, most clinical data show a disconnection among the levels of amyloid-β in the brain, the location of amyloid-β in the brain, neuronal loss and dementia. Thus, it seems likely that amyloid-β mediates its deleterious effects in AD via another mechanism, with the triggering of tau pathology being a strong candidate. If deposited amyloid-β is required to activate tau pathology, then most preclinical data would suggest that lowering amyloid-β production — for example, with a GSI or BACE inhibitor — will have very little effect once amyloid-β seeding has been initiated because there is such a strong thermodynamic drive for amyloid-β deposition. Most studies in transgenic mice show that if amyloid-β production is reduced — either pharmacologically or genetically — amyloid-β plaques remain very resistant to dissolution. In this case, the amyloid-β plaques themselves will have to be disaggregated via some form of therapeutic intervention.

A modification of the amyloid cascade hypothesis is supported by increasing evidence from the literature that smaller, soluble oligomeric species of amyloid-β mediate either neuronal death or affect synaptic

neurotransmission[113–115]. This is an attractive concept, as it neatly resolves the issues mentioned earlier regarding the lack of correlation among deposited plaques, tau pathology and neuronal loss. Thus, oligomeric or dimeric amyloid-β might diffuse through the brain parenchyma and mediate neuronal stress or perhaps cause direct toxicity. However, this remains a highly complex area of science and there are several issues that remain to be clarified.

Many of the bioassays that are used to test the toxicity of the oligomeric forms of amyloid-β use human amyloid-β that is applied to rodent primary neurons in culture. In some cases, high concentrations of amyloid-β are used *in vitro* and their physiological relevance is questionable. In addition, numerous transgenic mouse models that overexpress human APP bearing FAD mutations, sometimes in combination with FAD-linked *PSEN1* mutations, do not generally display neuronal loss, thus questioning the relevance of the *in vitro* experimental paradigms.

A precise biophysical characterization of the oligomeric and dimeric species is lacking and, although it might be known what is added to cell cultures, as the oligomeric species are not covalently linked it is unclear how robust they remain when they are diluted. In addition, a comprehensive study on the biochemistry of oligomers derived from synthetic amyloid-β peptides has suggested that the characterization of oligomers is substantially confounded by subtle changes in detergent concentration, as is the appearance of amyloid-β oligomers under the electron microscope[116].

Finally, the relationship between soluble and insoluble amyloid-β probably reflects a thermodynamic equilibrium, and it is unlikely that the two pools can be considered to be independent of each other. Given the above issues, we consider it appropriate to sustain the concept that both deposited and soluble forms of amyloid-β contribute to the aetiology and pathogenesis of AD, until compelling science suggests otherwise. However, we acknowledge that the link to tau pathology remains obscure. Recent GWA studies suggest that the immunoinflammatory system as well as lipid, synaptic and cell membrane processes contribute to the risk of developing AD[32–34,117]; it is certainly conceivable that inflammatory processes that are continually activated as a consequence of aggregate stress ultimately result in tau pathology in sensitive neuronal populations.

Out of the various scenarios discussed, there seem to be more preclinical and clinical data supporting the amyloid-β trigger and amyloid-β threshold scenarios than the other scenarios. For the amyloid-β irrelevant scenario, there is a hypothesis that the FAD mutations cause a failure in PSEN function and this mediates the neuronal loss that is observed in AD[118]. If this were the case, one might have expected that FAD mutations would have been found in other substrates of γ-secretase[5] rather than just APP, but this has not been demonstrated to date. The recent finding of total loss-of-function mutations in subunits of the γ-secretase complex that are not associated with dementia[119,120] also favours a scenario in which abnormal amyloid-β peptide generation is necessary for AD pathology.

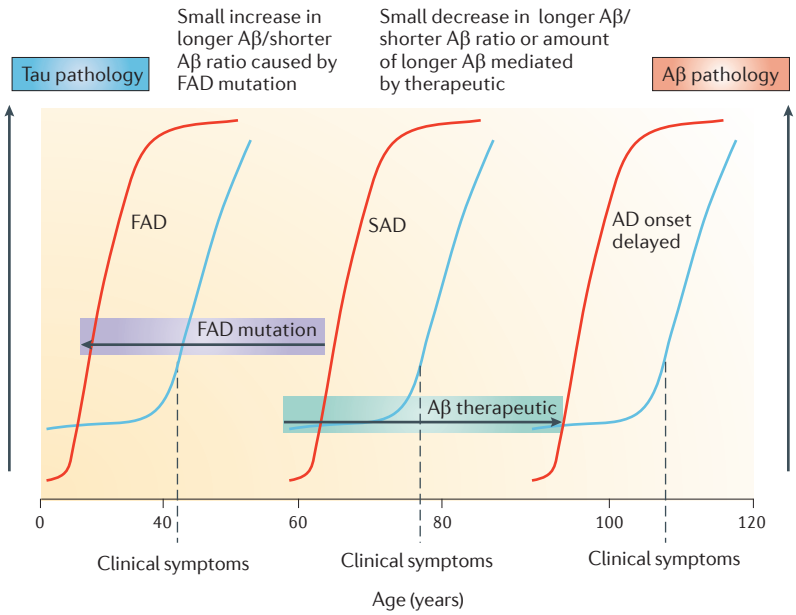

Figure 5 | **Hypothetical scheme showing the effect of an amyloid-β therapeutic that is administered before the amyloid cascade.** Familial Alzheimer's disease (FAD) mutations that result in modest changes in the ratio of amyloid-β (Aβ) peptides in favour of longer forms of Aβ have a very dramatic effect on the age of onset of FAD. Logically, if a therapeutic is administered before the Aβ deposition trigger or threshold point and it modestly affects Aβ ratios in the opposite direction (that is, in favour of shorter forms of Aβ as predicted for a γ-secretase modulator), or reduces longer forms of Aβ (as predicted for a β-APP cleaving enzyme inhibitor), such a therapeutic would be expected to delay the onset of disease equally radically. In this situation, a high therapeutic efficacy (for example, complete suppression of longer forms of Aβ) would not be required for a substantial therapeutic benefit, although it is currently difficult to predict how far in advance of the trigger point any medication would need to be given to be effective. SAD, sporadic Alzheimer's disease.

Conclusions

The human brain produces substantial quantities of amyloid-β and, although such calculations are heavily dependent on making large assumptions and extrapolations, it would seem that the amount of deposited amyloid-β in the AD brain is equivalent to approximately 7 years of total amyloid-β production. Biochemical and preclinical studies support the concept that plaques are stable and provide surfaces for pseudo-irreversible docking and locking of amyloid-β peptides such that even when the concentration of amyloid-β in the ISF is reduced, it is unlikely that plaques will be disaggregated. Preclinical data from *APP*/*PSEN1*-transgenic mice that have been treated with GSIs and mice in which BACE has been genetically ablated support the concept that these interventions only demonstrate efficacy in preventing deposition if they are administered before the establishment of plaques.

There is compelling genetic evidence that amyloid-β deposition has a critical role in AD. Increases in the Aβ42/Aβ40 ratio (or, more appropriately, the longer amyloid-β/shorter amyloid-β ratio) or increases in the levels of Aβ42 or Aβ43 in the brain will predispose individuals to developing AD. However, the duration of the disease appears to be unaltered by these genetic factors. This implies that changes in the amount of amyloid-β or

Box 3 | **Considerations for future AD clinical trials**

The Alzheimer's disease (AD) field is intensively engaged in redefining AD from being a clinicopathological entity to being a disease process that has a long, preclinical, asymptomatic phase during which several pathological processes are underway[139]. To distinguish between these phases, a nomenclature that refers to the pathological processes of AD (AD-P) and the clinically observable functions of AD (AD-C) has been proposed. For the AD-P phase, a conceptual model has been described that features three phases. The first phase is asymptomatic amyloidosis, which is detected by positive positron emission tomography (PET) amyloid tracer retention and low levels of amyloid-β 42 (Aβ42; the 42-amino acid form of amyloid-β) in the cerebrospinal fluid. The second phase is amyloidosis with neurodegeneration (neuronal dysfunction that is measured using ^{18}F-deoxyglucose–PET scanning, high levels of tau/phosphorylated tau in the cerebrospinal fluid, and cortical thinning and/or hippocampal atrophy). The third phase is amyloidosis plus neurodegeneration plus subtle cognitive decline; the evidence for cognitive decline is based on a prior baseline measurement[140]. Currently, there are insufficient data to use these measures in a diagnostic or predictive manner.

Large natural history studies enrolling young, normal participants will be required to determine how biomarkers reflect the changes that occur during the AD-P phase and how or whether they could be used to project the likely clinical trajectory of any given individual. To that end, several such studies are either underway or planned[141]. Such studies might also enable clinical trials to be performed on well-characterized cohorts that are already participants in natural history studies, although there are considerable logistical and ethical hurdles that must be overcome in order to achieve this. Ideally, these long-term studies might enable a battery of biomarkers and sensitive assays of cognitive performance to detect individuals who are at the earliest stage of the AD-P phase, at which time they would be most responsive to therapeutic intervention[142,134].

However, therapeutic interventions that are used in such clinical trials would have to be very safe as the benefit–risk equation would be undefined at this stage; furthermore, even if they are untreated, some individuals may never proceed to develop AD. The safety of a medicine only becomes apparent after it has been used in clinical practice for several years and such data will not be readily available. Clinical trials of this design would have very different measures of outcome than those that are currently required by regulatory authorities for AD therapies. Perhaps, for example, a delay in the development of mild cognitive impairment, or a delay in the progression of an AD-P biomarker change, such as the preservation of normal levels of Aβ42 in the CSF, would be sought as an indication of efficacy.

Finally, a new model of drug development would be required as it will be difficult for pharmaceutical companies to engage in lengthy disease prevention trials. Given the current timelines for the development of a new medicine, it would not be inconceivable that just as a therapeutic has been demonstrated, to the satisfaction of the regulatory authorities, to prevent or delay the onset of AD, there might be insufficient patent life remaining for the pharmaceutical company to be able to recoup the cost of the drug discovery and development programme.

amyloid-β ratios after seeding are not important modifiers of the disease process. The reduction in Aβ42 levels in the CSF is an early event in the disease process but there are very few studies demonstrating that once amyloid-β deposition has occurred, the amount of deposition — or its neuroanatomical location — can be correlated with neuronal loss, paired helical filaments of tau or dementia.

With respect to amyloid-β-modifying therapeutics, the question that remains to be answered is: which of the scenarios described herein most accurately reflects the pathology of human disease? Of course, any amyloid-β-directed therapeutic that shows efficacy in placebo-controlled trials of sufficient size and duration will prove the amyloid cascade hypothesis, but a failure to demonstrate efficacy might not disprove this hypothesis unless certain conditions are met.

If the amyloid-β trigger scenario posited above is true, then it has profound implications for the development of AD therapeutics. Some of the therapeutics that are currently being tested in Phase II/III trials in mild to moderate AD — such as GSIs and solanezumab — would not show efficacy unless they were administered before the trigger event. This trigger event may be the reduction in Aβ42 levels in the CSF, which heralds amyloid-β deposition into the brain. This event occurs many years before dementia can be robustly detected by clinical instruments.

If the amyloid-β threshold hypothesis is correct, then therapeutics that are currently under development would also not show efficacy unless they were able to reduce the levels of deposited amyloid-β to below the critical threshold. Thus, a therapeutic agent — such as bapineuzumab — that directly targets the amyloid-β plaque itself may clear amyloid-β plaques as well as seeding sites to below a critical threshold such that the disease process might be favourably deflected. A combination of drugs that target amyloid-β production and clearance might also be a very effective strategy in this scenario.

In a Phase II multiple-ascending-dose trial of bapineuzumab, exploratory analyses showed some positive effects on the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog) and the disability assessment for dementia. In addition, pooled data from two Phase II studies showed a statistically significant reduction in the levels of phosphorylated tau 181 with a trend towards a substantial reduction in the levels of total tau after 51–52 weeks of treatment with bapineuzumab[121]. Another study measured the effects of bapineuzumab on deposited amyloid-β levels in the brain using the PET ligand PIB[122]. In a comparison of pooled bapineuzumab-treated patients versus pooled placebo-treated patients, there was a statistically significant reduction in PIB–PET binding in the bapineuzumab-treated patients at 78 weeks, which is suggestive of a reduction in the levels of deposited amyloid-β in the brain.

Although these studies are encouraging, it must be emphasized that these are preliminary findings in small numbers of patients. Some post-mortem studies on eight patients who were immunized with Aβ42 to produce amyloid-β-specific antibodies have also been analysed[123]. These studies showed qualitative evidence of plaque removal, but no supportive evidence of a delay in cognitive decline. However, these data must be treated with caution as there was no relevant control group and no estimate of pre-immunization plaque load in the analysed patients, and the sample size was very small.

The most robust test of the amyloid cascade hypothesis will be the prevention of amyloid-β deposition in patient populations that are at risk of developing AD: this is also, by several orders of magnitude, the most challenging test (BOX 3). If individuals are at risk of developing the disease but amyloid-β deposition has not yet occurred, then modest reductions in Aβ42 production or a decrease in the longer amyloid-β/shorter amyloid-β ratio would be expected to prevent the onset of disease within a normal lifetime.

In terms of judging the potential efficacy at the end of Phase II clinical trials of amyloid-β-lowering agents that are administered to patients with mild to moderate AD, it will be important to seek changes in biomarkers

that reflect the underlying disease process: for example, measuring phosphorylated tau and/or total tau in the CSF or measuring the brain volume using MRI scanning, as opposed to measuring reductions in amyloid-β, although the latter biomarker would be important as proof of target engagement. With reference to an agent that targets plaques — such as bapineuzumab and other therapeutic agents that are currently in development[124] — one might anticipate that if the agent is effective the levels of amyloid-β in the CSF of patients with AD would increase as plaque seeding sites are removed, thus allowing amyloid-β clearance in the CSF to be normalized. Assaying such a rise in the concentration of amyloid-β in the CSF might be confounded by the therapeutic modality: for example, amyloid-β-specific antibodies might form complexes with free amyloid-β levels in the CSF and thus compromise enzyme-linked immunosorbent assay (ELISA) quantification. It might also be possible to detect plaque-specific amyloid-β components in the CSF and possibly even in the plasma as plaques are disaggregated. A test of the amyloid-β trigger scenario is beyond the resources of a single pharmaceutical company, but if this truly reflects the AD process, then a radically different model of pharmaceutical development will need to be established to prevent the public health disaster that awaits us.

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Acknowledgements
B.D.S. is the Bax-Vanluffelen Chair for Alzheimer's Disease and is supported by a Methusalem grant (FWO-Flanders). The authors would like to thank S. M. Paul and J. Hardy for their careful reading of the manuscript.

Competing interests statement
The authors declare competing financial interests: see Web version for details.

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AD & FTD Mutation Database: http://www.molgen.ua.ac.be/ADMutations
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