BRAIN
REVIEW ARTICLE

The amyloid cascade hypothesis: an updated critical review

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Results from recent clinical trials of antibodies that target amyloid-β (Aβ) for Alzheimer's disease have created excitement and have been heralded as corroboration of the amyloid cascade hypothesis. However, while Aβ may contribute to disease, genetic, clinical, imaging and biochemical data suggest a more complex aetiology.

Here we review the history and weaknesses of the amyloid cascade hypothesis in view of the new evidence obtained from clinical trials of anti-amyloid antibodies. These trials indicate that the treatments have either no or uncertain clinical effect on cognition. Despite the importance of amyloid in the definition of Alzheimer's disease, we argue that the data point to Aβ playing a minor aetiological role.

We also discuss data suggesting that the concerted activity of many pathogenic factors contribute to Alzheimer's disease and propose that evolving multi-factor disease models will better underpin the search for more effective strategies to treat the disease.

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Introduction

Fifty-five to sixty million people are estimated to suffer from dementia, and perhaps 30–40 million of them are clinically estimated to have Alzheimer's disease (AD). Dementia kills in the order of a million people every year globally and is one of the most significant tolls on healthcare budgets in the developed world.[1–3] Despite decades of intense research on AD-type dementia, available treatments have short-lived effects.[1,4–6]

The idea that amyloid-β (Aβ) causes AD emerged in the 1980s[7–9] and has dominated research into treatments since then (the amyloid cascade hypothesis, ACH).[10,11] The controversial approval of Biogen's antibody aducanumab (Aduhelm®, ADU) by the Food and Drug Administration (FDA)[12–15] renewed concerns about the ACH, whereas

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on the other hand, a clinical trial using the antibody lecanemab in November 2022 showed possible beneficial effects.[16] For patients and families, this is an important sign of hope, and for some scientists the results are perceived as support for the ACH.[17,18]

AD is a complex disorder with age as the leading risk factor and a broad clinical spectrum of symptoms.[19–22] Many risk modifiers and biochemical pathways contribute to disease.[23–26] AD exhibits diverse histopathological phenotypes that include not just the 'hallmarks' brain amyloid consisting of Aβ peptides[27] and tau depositions,[28–30] but also neurodegeneration, the deposition of TAR DNA-binding protein-43 (TDP-43), α-synuclein and other misfolded proteins.[31–33] In addition, oxidative tissue damage,[34–37] inflammation,[38,39] metabolic abnormalities,[40–45] atherosclerosis,[46–49] cardiovascular effects,[50,51] cerebral hypoperfusion[52,53] and imbalances of intraneuronal calcium[54–56] and other metal ions[25,57–60] participate in the pathology of AD.

AD mainly occurs in the population as sporadic AD (SAD; >95% of cases) with many genetic and lifestyle risk modifiers[61,62] developing slowly first as mild cognitive impairment (MCI),[20,63,64] and subsequently with loss of episodic memory, cognitive skill and identity.[1,65] A few per cent of cases are inherited as autosomal dominant early-onset familial AD (FAD), typically beginning at 30–65 years, whereas SAD usually develops after 65 years.[66,67] The FAD cases are associated with mutations in the genes encoding presenilin-1 (PS1),[68–71] presenilin-2 (PS2),[72] and the amyloid-β precursor protein (APP).[73–77] Despite causing a small subset of AD, FAD mutations have profoundly influenced AD research due to their appealing genotype-phenotype relationships.[11,78–81]

In contrast to these complex aetiologies, the original ACH suggested that the disease is the consequence of a 'machine failure' in the form of overload of brain Aβ depositions. This view substantially impacted AD research, funding and drug development efforts[82] despite consistent concerns expressed by many in the scientific community,[19,83–88] especially after repeated drug failures from 2014 and forward.[89–95]

With the aim of presenting the history, data and discussions of the ACH in a critical context of the recent antibody trials, we systematically searched the literature for papers on the ACH and its history and criticism. Our review led to a list of 15 data-related distinct discussion points necessary for the debate on the hypothesis and how to move forward from the current state of available data.

The amyloid cascade hypothesis

Early foundation and molecular and genetic basis

The ACH can be traced back to the first half of the 1980s. Prusiner[96] and Masters *et al.*[97] noted analogies between brain amyloid deposition in AD patients and proteinopathies found in Creutzfeldt-Jakob diseases, implying a causal gain-of-toxic function. Glenner and Wong[8] purified and sequenced amyloid peptides from cerebral blood vessels (cerebrovascular amyloid, CVA) of AD and Down syndrome patients. They also found that both amyloid forms contained the same primary sequence of β-protein (now known as Aβ peptides) and predicted a genetic risk locus at chromosome 21.[98] In 1985, Masters and colleagues[9] purified plaque $Aβ_{40}$, characterized the N-terminal sequence and related it to the plaques in Down syndrome. The first amyloid-based aetiology of AD was proposed by Glenner and associates in the mid-1980s and posited that CVA compromises the blood–brain barrier and harms neurons.[7,99] With reports indicating that Aβ could be cytotoxic,[100,101] Hardy and Higgins[10] formulated the ACH proposing that Aβ plaque deposition was the main pathogenic factor of AD. Aβ derives from the transmembrane β-APP cloned in 1987 by several groups[102–105] and mutations in the genes encoding APP[106] and PS1[68] may modify the production of Aβ, which is thought to cause early-onset FAD. This was interpreted as supporting the ACH rather than generating interest in the cellular functions of the two proteins and their role in the neurodegeneration of AD.[68,107,108]

In the non-amyloidogenic pathway, APP is cleaved by α-secretase in the middle of the Aβ sequence within APP, precluding the formation of Aβ.[109] However, APP is also cleaved by β-secretase to yield the 99-residue C-terminal fragment of APP (APP-C99) that includes the Aβ sequence.[110–112] Aβ peptides of variable length are formed during consecutive cleavages of APP-C99 by γ-secretase, the four-subunit intramembrane di-aspartyl protease complex having PS1 or PS2 as its multipass transmembrane catalytic subunit.[10,113–116] The cleavage occurs along two pathways, yielding the peptides $Aβ_{49} \rightarrow Aβ_{46} \rightarrow Aβ_{43} \rightarrow Aβ_{40} \rightarrow Aβ_{37}$ and $Aβ_{48} \rightarrow Aβ_{45} \rightarrow Aβ_{42} \rightarrow Aβ_{38}$. The physiological significance of these cleavage products remains poorly understood,[116–119] but evidence indicates that APP and its processing may have important physiological functions.[120–124] This view is also supported by recent findings indicating that $Aβ_{42}$ is associated with normal cognition and preservation of hippocampal volume.[125,126] However, longer peptides (e.g. $Aβ_{42}$) are more hydrophobic, toxic and aggregate more easily,[127] and a surplus of longer peptides (e.g. the $Aβ_{42}/Aβ_{40}$ ratio) has thus been proposed as a key driver of the pathogenic process.[118,128–131]

The tendency of many FAD mutations to cause autosomal dominant AD[76,79,132,133] is often interpreted as a gain-of-toxic function.[134] However, loss-of-function cannot be ruled out as a cause of FAD. For example, in protein misfolding diseases, a peptide's misfolding can cause loss of that protein's normal function while at the same time, generate a toxic interaction with other parts of the cell.[135] Also, one wild-type allele is not necessarily enough to fully compensate for the loss-of-function of the inactivated allele, thereby causing a loss-of-function disorder. This is especially true over time, so this argument is relatively weak when considering a gradually evolving age-dependent disease such as AD.[136] An important example of loss-of-function leading to dementia is progranulin (PRGN) mutations that cause a 50% reduction of the protein (haploinsufficiency), leading to autosomal dominant neurodegeneration and frontotemporal dementia.[137]

Two main therapeutic avenues have been explored based on the ACH: modifying the production of Aβ peptides or neutralizing them after formation. Many pharmaceutical companies have developed β- and γ-secretase inhibitors or, more recently, due to clinical adverse effects of many of these,[138,139] modulators of γ-secretase, changing the relative production of the various forms of Aβ,[140–148] or antibodies intended to neutralize the formed toxic peptides.[149–152]

Current versions of the amyloid cascade hypothesis

The current version of the ACH[11,153,154] differs in several ways from the original one proposed in the 1990s[10] and is summarized in Fig. 1. It describes the oligomers as the most likely pathogenic species, in contrast to plaques, and considers the role of risk factors that modulate penetrance, for example, apolipoprotein E (APOE) and tau-Aβ interactions.[154]

As studies found that senile plaque loads do not correlate with cognitive decline[155–157] and higher Aβ production was not a phenotype of many FAD mutations,[158,159] attention moved to other forms of Aβ as probably more important to disease. Notably, soluble

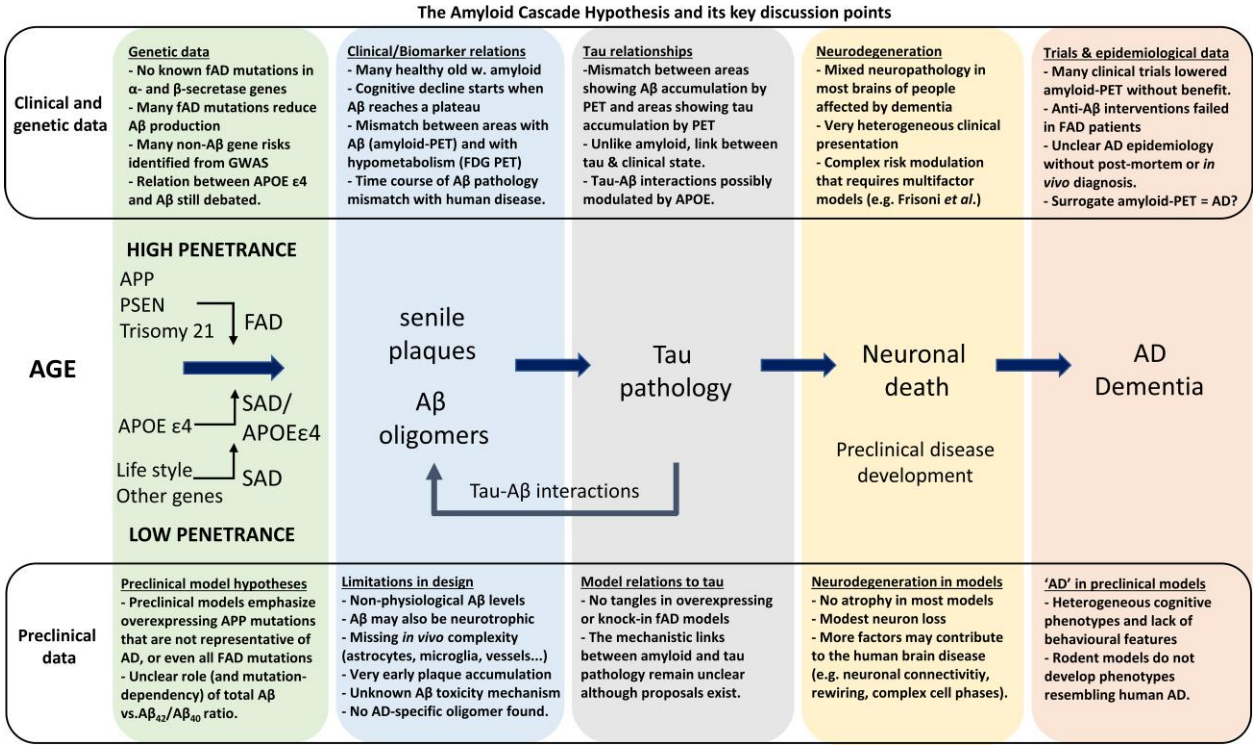

Figure 1 Overview of the amyloid cascade hypothesis and its related controversies. The amyloid cascade hypothesis[11,80,153] has been updated into a model in which APOE ϵ4 and tau pathology affect total penetrance (Frisoni *et al.*[154]).Aβ = amyloid-β; AD = Alzheimer's disease; FAD = familial Alzheimer's disease; GWAS = genome-wide association studies; SAD = sporadic Alzheimer's disease.

oligomers of Aβ[100] are more cytotoxic than fibrillar Aβ plaques.[160–162] The selective toxicity of oligomers in cellular assays was taken as further support for this hypothesis.[163–165] The deposits of senile plaques consist of post-translationally modified, oxidized and truncated forms of Aβ in fibrillar β-sheet structures representing several years of production of Aβ[166] and represent end points of aggregation processes that initiate before clinical onset[167] and are probably less directly involved in pathogenesis compared to their precursor oligomers.[168]

Exposure of the hydrophobic parts of Aβ is likely to cause aggregation and interactions with membranes and other molecules.[169–171] Nevertheless, the molecular mechanisms, location and context of Aβ aggregates causing AD remain elusive despite suggestions[172] that these aggregates cause permeabilization of cell membranes,[169,173–176] dysfunction of neuronal potentiation,[165] oxidative stress,[177] vascular effects,[46,50] disruption of prion-protein interactions and dysfunction of the NMDA receptors,[178] abnormalities of the respiratory chains of mitochondria[179–181] and effects on neuronal calcium and zinc dyshomeostasis.[60,182,183]

An important modification of the ACH is to account for the more complex pathology and many small cumulative genetic, vascular and lifestyle-related factors,[184,185] i.e. abandoning a single-cause determinism of toxic proteinopathy.[44] The 'probabilistic' version of the ACH[154] incorporates additional factors into a model that still retains Aβ as a main causal agent but distinguishes three types of AD (Fig. 1): (i) FAD with very high penetrance due to mutations in PS1, PS2 and APP; (ii) SAD with APOE ϵ4 genotype as an additional risk increasing penetrance; and (iii) SAD without an APOE ϵ4 genotype, and with the latest typical age of onset. The concept of using risk-modifying penetrance resembles other multi-risk models,[44] but the identification of three distinct types of AD makes it a novel and straightforward extension of the ACH.

Another trend is the appreciation of a need for understanding the normal functions of APP, Aβ and presenilin, and the possibility that some loss-of-function pathways can be viewed in connection with the ACH.[11] In combination, these studies reflect a more recent tendency towards a hypothesis that accommodates multiple modifiable genetic, vascular and lifestyle risk factors[154] and loss of the physiological functions of the proteins involved in the proteinopathy, rather than considering the mutant FAD genes as simply pathogenic.[11]

Criticism of the amyloid cascade hypothesis

The main criticisms of the ACH have often been stated to be the lack of correlation between cognition and amyloid deposition[19] and the modest clinical effects of anti-amyloid drugs.[90,186] However, as discussed below, there are many other anomalies, for example, in relation to the genetic data and increasing evidence of other disease risk factors that should weigh heavily.[19,86,89,90,92,136,162,187] Criticism of the ACH has been continuous[19,84,86,89,136,188] but markedly intensified in 2014–2017 after the failure of several high-profile anti-amyloid drug candidates.[89,90,92–94,187,189,190] Some of the main discussion points of the ACH that have emerged are summarized in Table 1 and discussed below. A list of associated representative statements regarding the hypothesis is presented in Box 1.

Evidence of a complex aetiology

In contrast to the complex clinical and neuropathological phenotypes of AD, the original version of the ACH proposed a simple cause-effect relationship between amyloid deposition and

Table 1 Criticism of the amyloid cascade hypothesis, and its counter-arguments

Criticism number	Observation	Context	Notes/statements	Counter-argument
1	Evidence of a complex aetiology[19,191]	Many risk factors, heterogeneous results and clinical spectrum should be addressed[92,192]	Selective interpretation of genetic, biochemical and clinical data	New models begin to account for more risks modulating penetrance[154]
2	The ageing human brain differs from mice	Much evidence based on rodent models[193,194] with distinct neurology and ageing	Antibodies tend to reverse cognitive decline in mice but not in humans.[90] Mouse Aβ confounds results[195]	Primates are expensive and some mechanisms can still be shown in mice
3	Neglect of normal roles of APP, Aβ and PS1[85,92,123,136,196]	Aβ is neurotrophic at normal concentrations, but toxic at high concentrations[197,198]	Soluble $Aβ_{42}$ correlates with cognitive function[125,126] elaborate splicing of APP is clearly there for a reason[123]	γ-Secretase modulators or antibodies may be made selective to harmful forms of Aβ
4	No established causal molecular species; the mechanism of $Aβ_{42}/Aβ_{40}$ ratio is obscure[92]	Despite decades of research, the molecular mode of pathogenesis of Aβ is unclear[11,92,199,200]	The structure, mechanism and cellular location of pathogenic species not established; some oligomers may be artefacts[201]	Some oligomers have been proposed but very hard to identify *in vivo* mechanism[136,162]
5	Poor correlation of Aβ to clinical outcome[157,202]	Aβ in many asymptomatic people[155–157,203] could suggest Aβ is not causal[19,57,90,92,188,204]	At the minimum additional modulators of disease required.[204,205] One proposal is tau-Aβ interactions[154]	Preclinical[206] or cognitive reserves,[186] oligomers rather than plaques[162,189] or tau-Aβ effects[154]
6	Cytotoxicity is not the same as human pathogenicity[86,187]	Studies often used 1000-fold physiological Aβ concentration[19,86,92,187]	Aβ is sub-nanomolar concentrations, toxicity studies often micromolar[19,92]	Some studies[207] showed toxicity at physiological concentrations
7	Absence of α/β secretase FAD mutations[92]	If processing of Aβ was central such mutations are expected	Only seen in APP close to cleavage site, suggesting PS1/PS2 more central	Not considered to the author's knowledge
8	Absence of mutations in key Aβ degrading metalloproteases[92]	Hypomorphic mutations in main Aβ degrading proteases should confer risk	Not seen	Not considered to the author's knowledge
9	Many FAD mutations lead to less Aβ[123,158]	Suggests that 'cascade' of Aβ is not causal[204]	Data e.g. in Sun *et al.* for PS1 mutations[159]	Both Aβ and $Aβ_{42}/Aβ_{40}$ ratio gives disease (i.e. nearly all mutations)
10	Many more FAD mutations in PS1 than elsewhere	Indicates PS1/γ-secretase is more central to disease[208]	Impaired APP cleavage leading to plaques could be correlation, not cause	Most PS1 mutations affect APP cleavage[159]
11	APP mutation have diverse effects[131]	Some APP FAD mutations lower, others raise Aβ	Swedish mutation increases Aβ but is not representative of FAD mutations	Emphasis on longer peptides such as $Aβ_{42}$ and $Aβ_{43}$[209–212]
12	Accounting for the physiological regulators of Aβ balance	Aβ/APP is bound and modified by metal ions[120,213–218]	Metal ions bind, regulate production, aggregation and clearance of Aβ[57,59,122,219–225]	Even within amyloid hypothesis it seems relevant to account for such regulators[122]
13	Interpretation of Down syndrome dementia as due to APP[226]	APP overexpression associates with dementia in Down syndrome patients.	>200 genes on chromosome 21, including BACE2 and APP but also genes of SOD1, proteostasis, calcium binding and ion channel proteins	Regardless of other genes, the excess APP and plaque pathology is consistent with the hypothesis
14	Apparent Aβ removal has not improved cognition[90,92,227,228]	'Bayesian meta-analysis of these trial data provides strong *evidence of absence* of a therapeutic effect'.[227]	Autopsy studies of patients who received Aβ immunization indicate no slowed progression[228]	The hypothesis is ok, but trials failed to target the right species at the right time[189]
15	Blocking Aβ producing enzymes impairs cognition[139]	Not unexpected, because of Aβ's proposed functions[85,123,197,229]	β- and γ-secretase inhibitors cause adverse effects[89]	Not because of neurotrophic Aβ but other substrates[139]

neurodegeneration. However, AD is a complex disorder as indicated by the involvement of many genetic risk factors.[92,192] In the homozygote state, the ApoE ϵ4 allele increases the AD risk by up to 15-fold.[235–237] Many other genetic risk factors have been identified from genome-wide association studies (GWAS),[238,239] including for example, *ABCA7*, *BIN1*, *CLU*, *GAB2*, *GALP*, *PICALM* and *TREM2* that seem implicated in broader functions, such as, lipid metabolism and immune functions.[75,239–241] Lifestyle-related risk factors such as body mass index,[242,243] diabetes,[244–246] hypertension[247] and depression[248,249] also seem to affect and modify the disorder.[250,251] It has been estimated that 40% of dementia cases are caused by 12 modifiable risk factors.[252] These complex pathways then need to be seen in the context of gradual ageing and oxidative stress-induced neuronal insults.[34–37,230] One way to do this is a

Box 1 Statements about the amyloid cascade hypothesis, in chronological order

'This is a controversial theory, however, primarily because there is a poor correlation between the concentrations and distribution of amyloid depositions in the brain and several parameters of AD pathology, including degree of dementia, loss of synapses, loss of neurons and abnormalities of the cytoskeleton'.[19]

'Clearly, it is time to rethink this position and to propose instead that future approaches should focus upon altering the age-related sensitivity of the neuronal environment to insults involving such factors as inflammation and oxidative stress'.[230]

'A detailed review of the relevant data led us to conclude that some data, particularly those from transgenic mice, are inconsistent with the predictions of the amyloid hypothesis. Instead, most data are consistent with the notion that amyloid-β (Aβ) peptide is neuroprotective'.[84]

'By analogy, individuals suffering from altitude sickness nearly always have elevated levels of hemoglobin. However, while hemoglobin is toxic to cells in culture and increased erythropoiesis at sea level can be deadly, it is clear that the increases in hemoglobin occurring at altitude are beneficial. Amyloid, like hemoglobin, may also be beneficial, in this case, following neuronal stress or disease'.[86]

'Implicit in the amyloid hypothesis is that the Aβ peptide harbors neurotoxic properties. Yet, the precise mechanism by which Aβ exerts these putative toxic effects on neurons remains unclear'.[27]

'While the debate over the validity of the amyloid cascade hypothesis will no doubt continue, it remains likely that there are other critical factors playing a role in AD pathogenesis. Metal ions are one such possibility'.[231]

'the amyloid cascade hypothesis has been so modified over time that it is now impossible to confirm or deny. The hypothesis now states, in effect, that invisible molecules target invisible structures. Still relevant, however, are multiple factors that surely cast some doubt but have either been rationalized or overlooked'.[87]

'This elusive soluble Aβ species is in danger of becoming a way to explain inconsistencies in existing models without applying the scientific rigor needed to make real progress'.[162]

'The findings that SDS promotes Aβ dimerization have significant implications for the putative role of low-order oligomers in AD pathogenesis and draw into question the utility of oligomeric Aβ as a therapeutic target'.[201]

'We show here however that the central conclusion of the amyloid hypothesis, that Aβ is the cause of AD is, at very least, premature'.[89]

'it is increasingly likely that Aβ is merely a marker, a later consequence of upstream changes that lead to neuronal and synaptic losses'.[204]

'while there is fear in the field over the consequences of rejecting it outright, clinging to an inaccurate disease model is the option we should fear most'.[90]

'the large and solid body of evidence accumulated to date points to the waning of the amyloid cascade hypothesis and indicates that the scientific community should still devote its utmost efforts to identify the real culprit of Alzheimer's disease'.[93]

'We're all disappointed, but there are concrete reasons why each failure occurred'.[232]

'Are we going to tie up the whole field for another 15 to 20 years?'[232]

'The general view is that these are the right drugs, but they're too late'[232]

'A holistic view of the available data does not support an unequivocal conclusion that Aβ has a central or unique role in AD'.[94]

'Diagnostic and therapeutic approaches rooted in the 'amyloid hypothesis' have permeated every fiber of AD research for decades by creating a domain of deceiving intellectual monopoly. These unjustified efforts have affected the decisions of funding agencies and editorial offices, and as such, have prevented the exploration of new ideas and avenues of research'.[233]

'As a result, dementia research is estimated to be 15–30 years behind where it could be with conflicting data ignored in favor of the amyloid dogma'.[234]

'annual NIH support for studies labeled amyloid, oligomer, and Alzheimer's' has risen from near zero to $287 million in 2021'.[88]

model that assumes that some of these disease modulators contribute to aggravating Aβ pathology.[184] Recent formulations of the hypothesis have therefore moved towards incorporating these modulators in a penetrance model, with three forms of AD emphasizing the APOE ε4 genotype in particular, as summarized in Fig. 1.[154]

Relying on preclinical familial Alzheimer's disease models

Much support for the ACH came from transgenic rodent models expressing FAD mutants. However, given the marked differences in the ageing processes and neurological development of rodents, results obtained in transgenic mice are easily over-interpreted.[193,194] For example, antibodies tend to reverse cognitive decline in mice but not humans.[90] Even at the level of amyloid aggregation and associated pathology, mouse Aβ itself differs by three substitutions from human Aβ, and endogenous mouse Aβ aggregates to form amyloid at a much lower rate than human Aβ.[195] To overcome the problem, murine AD models have used over-expressed mutant human APP, but this is a non-physiological setting that mainly explores the human peptide's interaction with the host murine brain, as discussed previously.[89,136,253] One of the issues is that AD does not generally feature APP overexpression, which may cause toxicity by itself,[136,254] rather than by a mechanism that relates to AD. Thus, while most models are wrong and only some useful, the distinct ageing and neurological features of humans limit the value of pathogenic processes observed in mice.

Physiological roles of APP, Aβ and PS1/γ-secretase have been neglected

The ACH is not concerned with the normal function of APP, Aβ and PS1 and why humans feature APP and its complex cleavage by

secretases in the first place.[85,123,255–257] APP is expressed as a chondroitin sulphate proteoglycan, which has important brain functions.[258] Plausible functions of APP[124,213] were established at the same time as the ACH, and Aβ concentrations below nanomolar (as encountered within cells) are neurotrophic while higher concentrations (as achieved in many models) are toxic.[197,198,259] Nevertheless, the original ACH focused exclusively on Aβ toxicity. While monomeric Aβ seems important for normal brain function,[85,119,123,196,205,260–262] the exact functions remain elusive but may include effects on neuronal signalling in the synaptic cleft,[229,263] anti-microbial function,[264–266] potassium channel modulation[267] and inhibition of blood–brain barrier leaking.[265] Whereas the N-terminal half of Aβ is hydrophilic, the cleaved C-terminal half is hydrophobic, making $Aβ_{42}$ more hydrophobic than $Aβ_{40}$.[268] Thus, senile plaques tend to be enriched in longer Aβ peptides.[269] Although the potential beneficial roles of Aβ were ignored, toxicity data,[197,259] plaque deposits and FAD mutations pointed to possible loss-of-function[123] and foresaw the adverse effects of indiscriminate amyloid reduction by antibodies and γ-secretase inhibitors.[256,270] Also, Aβ functions in innate immunity[271,272] may be explored in the context of the role of infections in AD.[273–278] A recent study concluded that soluble $Aβ_{42}$ is associated with normal cognitive function, and low $Aβ_{42}$ with cognitive impairment,[125,126] consistent with a loss-of-function of APP/Aβ.[123] There is also good evidence that many PS1 FAD mutations reduce the γ-secretase-mediated epsilon cleavage of many substrates beyond APP-C99, including NOTCH1 and cadherins,[279] and the resulting cytosolic peptides regulate gene expression necessary for neuron function.[280–285]

No established causal molecular amyloid-β mechanism

The ACH has not established the precise pathogenic molecular form of Aβ (some forms of oligomers[164,286,287]), the detailed location of the process in the brain (arguably synapses[288,289]), and the exact molecular mode of pathogenesis.[11,92,199,200] Current strategies assume that we can identify the specific pathogenic form of Aβ and thereby, rationally develop specific antibodies targeting the relevant structural epitopes. Low molecular mass oligomers, such as dimers, may be artefacts of sodium dodecyl sulphate (SDS)-induced dimerization.[201] At the very least, it is clear that Aβ cannot cause disease by itself but needs a modifier. Even if some forms of oligomers are pathogenic, it has been hard to identify relevant *in vivo* mechanisms,[162] and the specific mechanism that makes the $Aβ_{42}/Aβ_{40}$ ratio, rather than the total amount of $Aβ_{42}$ or $Aβ_{40}$, biologically significant remains obscure.[92]

Amyloid biomarkers and their relation to Alzheimer's disease

The ACH proposes that Aβ deposition should be associated with disease,[11,80] possibly with a time delay that is heterogeneous in the type of risk profile of the individual patient.[154] Asymptomatic people could reflect preclinical disease states,[206] but it seems clear that additional modulators are involved.[57,136,186,204,205]

Aβ deposition is measured by PET using tracers such as ^{11}C-labelled Pittsburgh Compound-B[290] or ^{18}F-florbetapir, ^{18}F-florbetaben and ^{18}F-flutemetamol; with distinct chemistry, affinity and specificity.[233,291–295] Whereas amyloid-PET has high sensitivity for β-sheet structured fibrillar depositions (typically 80–100%),[296,297] complex visual and quantitative assessment confounded by spillover from tracer uptake in white matter and partial volume effects complicate use.[295,298] Thus, very heterogeneous specificities (46–88%) with a median estimate of 58% were found in the Cochrane review on ^{11}C-labelled Pittsburgh Compound-B for MCI who eventually convert to AD.[299] Meta-analysis supports a consensus that cross-sectional Aβ-cognition relationships are modest among healthy elderly.[300] Although Aβ is found in many parts of the brain, AD generally begins in the entorhinal/parahippocampal cortices and the hippocampus.[301,302] Aβ deposits correlate only modestly with clinical outcomes.[157,202,303] As regularly mentioned,[57,89,90,204] 30% or more of asymptomatic older people have high loads of Aβ plaques,[155–157,203] and many of these are enough to satisfy AD diagnosis criteria.[304–306] Also, a significant fraction of amyloid-PET positive subjects remain cognitively healthy at 5 years follow-up,[307] and radiotracer uptake is also confounded by covariates such as sleep patterns.[308,309]

The fluid biomarkers offer useful complementary insight, most notably the CSF $Aβ_{42}/Aβ_{40}$ ratio with a clear tendency of decline with age and in AD.[206,304,310] The $Aβ_{42}/Aβ_{40}$ ratio is typically inversely related to the amyloid PET signal, which is interpreted as due to plaques being enriched in the longer more hydrophobic and aggregation-prone $Aβ_{42}$ peptide.[311] However, this relationship has not been robust in antibody trials that offer the highest level of evidence (randomized controlled trials) for these relationships. While most antibodies lower amyloid-PET, bapinezumab and gantenerumab did not clearly affect CSF $Aβ_{42}/Aβ_{40}$.[312–314] The inverse relationship between the two biomarkers is strong for aducanumab and lecanemab.[16,315]

In recognition that tau, at least, is also involved, the clinical criteria for AD diagnosis by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD)[316] include Braak tau pathology and Aβ pathology not directly captured by standard amyloid PET.[293] The National Institute of Aging-Alzheimer's Association (NIA-AA) diagnostic guidelines for AD criteria included in 2011, amyloid positivity as one element of AD diagnosis, at least in research,[317] and more recently, in practice.[65] The International Working Group (IWG) emphasized clinical markers as well, although maintaining that amyloid (and tau) positivity together with specific AD phenotypes is a precondition for diagnosis.[318]

The correlation between amyloid and tau pathology is complex and not fully understood, with tau pathology typically correlating better with clinical course.[184] A recent paper by Frisoni *et al.*[154] includes a detailed discussion of this 'spatial paradox' and the tau-amyloid relationship that may be modulated by APOE ϵ4 genotype.[319] It seems likely as more data accrue that the complexity will not be explained by amyloid, tau and APO ϵ4 alone.

Cell toxicity is not the same as human pathogenicity

Some of the main data supporting the ACH originated from studies evaluating Aβ effects on cell viability or *in vitro* aggregation assays.[86,187] However, many reports of Aβ toxicity[320] and aggregation tendency[321] occurred at micromolar concentrations,[136] although biological concentrations of the peptides are in the (sub) nanomolar range, i.e. the models used amounts of peptide that represent perhaps 1 year of total brain production administered instantaneously at 1000-fold higher than biological concentrations.[19,86,187,322,323] This is unlikely to occur with a slowly developing, age-induced human disease of the brain. Although some toxic modes at physiologically relevant concentrations were reported,[207] the implications of these studies for a complex neurodegenerative disorder remain highly doubtful, and even if the used cells had ageing processes and phenotypes that resemble human neurons,[324,325] the multicellular context and interplay of connected neurons, astrocytes, microglia, etc. is not caught by such models,[38,89,326] including the central role played by synapses in this interplay.[89,289,327]

Absence of familial Alzheimer's disease mutants of α- and β-secretase

One of the most surprising claims is that the FAD genetic evidence for the ACH is almost indisputable.[328] Accordingly, PS1 and APP mutations indicate a role for these genes in FAD, but do not prove that a gain-of-function mechanism or that Aβ is central to the aetiology. Meanwhile, no known mutations in the α- or β-secretases appear to associate with FAD,[92] despite the crucial roles these two enzymes play in determining whether Aβ is formed. Mutations are found only in PS1, the catalytic subunit of γ-secretase making the final cleavage in the production of Aβ but also cleaving more than a hundred other substrates.[118,329] If Aβ (or an associated cleavage product of APP) were central to AD pathogenesis, one would expect FAD mutations in α- and β-secretases that control the non-amyloidogenic versus amyloidogenic pathways to modulate the risk of AD.

Absence of mutations in key amyloid-β degrading metalloproteases

If Aβ overload and aggregation were critical to AD aetiology, one would expect hypomorphic mutations in the main proteases responsible for degrading Aβ intracellularly and in the extracellular matrix[330] to confer risks of AD, yet this is not the case to any large extent.[92] These proteases include, for example insulin-degrading enzyme,[331,332] neprilysin[333–335] and other zinc proteases.[57,333] The absence of such mutations suggests that impaired clearance of Aβ is not a substantial problem, supporting that Aβ has either a spectator, minor or even a loss-of-function role in AD.[125,136]

Most familial Alzheimer's disease mutations reduce amyloid-β due to loss-of-function

For a long time, a cornerstone of the ACH was that FAD mutations lead to more Aβ (e.g. the Swedish mutation[336]). However, most FAD-causing PS1 variants are hypomorphic, displaying impaired enzyme activity[123,158,279] (as expected on evolutionary grounds, as the enzyme is already optimized), yet often increase the $A\beta_{42}/A\beta_{40}$ ratio.[134,159,337–339] These hypomorphic phenotypes of PS1/PS2 FAD mutants[134,159,337–339] substantially challenge a gain-of-function hypothesis.[204] The Fit-Stay-Trim (FIST) model of γ-secretase[338,340–342] explains why the loss-of-function increases the $A\beta_{42}/A\beta_{40}$ ratio: the PS1 transmembrane helix 'fingers' produce two conformation states, a semi-open conformation with good affinity for APP-C99, maximal staying time and trimming to shorter Aβ peptides, and an open conformation with looser binding, less staying time, less and inaccurate cleavage and higher $A\beta_{42}/A\beta_{40}$ ratios.[67,159,338,342,343] Despite high heterogeneity in clinical outcomes, assayed $A\beta_{42}/A\beta_{40}$ ratios of mutants[159] seem to correlate with the age of symptom onset for carriers of the PS1 mutations.[344] This could imply that impaired presenilin/γ-secretase function may be clinically significant, as suggested by adverse effects of γ-secretase inhibitors,[5,345] and the presenilin hypothesis.[208,346–348] An inhibition of function by FAD mutations could affect both γ-secretase-independent presenilin processes[349–354] and the γ-secretase cleavage of many substrates involved in neuronal homeostasis.[114,284,329,355]

Familial Alzheimer's disease mutations suggest that presenilin/γ-secretase could be central

FAD mutations are more common in PS1/PS2 than in APP, and PS1 mutations are often more severe.[76] This suggests that PS1/γ-secretase function could be important to AD pathogenesis.[208,348,356] Although the ACH is supported by FAD mutations,[162] many of them have insignificant or unclear effects on APP-C99 processing.[158,159] If APP-C99 processing were central, one would expect mutations in all secretases and arguably in other parts of APP not directly related to PS1 interaction. In contrast, most FAD mutations scatter across PS1 and tend to impair protein compactness and stability,[338] which explains their effect on APP-C99 cleavage.[342] The (much fewer) APP mutations are all located in areas that involve PS1 engagement. A slow membrane protease, like γ-secretase, occupied with more APP-C99, could spend less time on other substrates, for example, Notch.[357] Thus, we need to explore whether FAD APP mutations work by disrupting PS1 function rather than the other way around. The original paper on the ACH also discussed the possible impact of other APP cleavage products than Aβ.[10] For example, soluble APP fragment (sAPP) has important functions that could be affected by aberrant APP cleavage.[358]

The heterogeneous APP mutation effects

A central support for the ACH is the existence of APP mutations that cause FAD, located in the neighbourhood of (e.g. the Swedish and London mutations) or within the Aβ (e.g. the Dutch and Italian mutation) portion of APP.[131] The Swedish double mutation produces very high Aβ levels and has been much used in AD mouse models as it fits the 'overload' concept of the early hypothesis well.[193] Correspondingly, the protective A2T variant (using Aβ sequence numbering)[359] was taken as a strong support of the ACH,[153] as a useful counter-example to the Swedish mutation and the same-site pathogenic substitution A2V.[360] However, these examples are not representative of the FAD mutations in APP, whose effects are extremely heterogeneous.[131] In response to the observation of many FAD phenotypes with lower overall amyloid production, the emphasis turned to the $A\beta_{42}/A\beta_{40}$ ratio.[209–212] While some APP mutations increase this ratio, others seem to lower it. A2V and D7N lead to $A\beta_{42}/A\beta_{40}$ ratios similar to wild-type, but E22G, E22K and E22Q lower the $A\beta_{42}/A\beta_{40}$ ratio.[131] The London mutation causes more severe (earlier onset) disease despite producing much less Aβ than the Swedish mutation.[188] Some variants have high Aβ aggregation tendencies, others low. Some mutations are more toxic in cell assays than the wild-type, others not significantly so.[131] Accordingly, the Swedish mutation used in much AD research is not representative of FAD APP mutation phenotypes broadly.[131]

Accounting for the physiological regulators of amyloid-β balance

As the ACH revolves around Aβ production, degradation, homeostasis and aggregation, it would be reasonable to include biochemical regulators of this balance. As one well-documented group of regulators, metal ions Cu^{2+} and Zn^{2+} bind naturally to APP and Aβ in high-affinity sites and modulate the aggregation and production of Aβ,[120,213–218] as documented in many reviews.[57,59,122,219–225] As mentioned above, no FAD mutations are found in the Aβ-degrading enzymes, but furthermore, these proteases are typically zinc proteases, indicating perhaps a role of zinc in regulating these pathways.[57,361,362] It would seem natural to include these regulators, and possibly others, in the disease models.[57,121,231,363,364]

Interpretation of Down syndrome dementia as due to amyloid overload

One of the early claims of evidence in favour of the ACH[9] was the development of dementia and plaque burden in patients with Down syndrome, which have three copies of chromosome 21 where

the APP gene is situated.[365] However, there are more than 200 genes on chromosome 21, including *BACE2* and *APP* but also the gene coding for Cu, Zn-superoxide dismutase 1 (*SOD1*) whose mutations cause familial amyotrophic lateral sclerosis,[366,367] proteostasis genes, calcium-binding proteins and ion channels important to neurological homeostasis; all these genes may be upregulated in a process that could give dementia in trisomy-21 by many pathways. A proposed alternative explanation is that the additional chromosome (or parts of it) exhausts proteostasis in neurons,[44] accelerating disease processes that lead to neurodegeneration by proteostatic dysfunction.[368,369] In this case, aberrant amyloid deposition would be a marker of such dysfunction, not the primary cause of dementia.

Amyloid-β antibodies: small benefits with adverse effects

The ultimate test of any disease mechanism is the development of treatments based on it, but this approach has repeatedly failed.[5,90–92,189,191,227,228,370] High profile antibodies before lecanemab, such as solanezumab[151,371] and bapineuzumab,[313] did not show cognitive benefit[151] and produced adverse effects[150] despite lowering Aβ levels as assessed by changes in CSF.[372,373] As with aducanumab, subsequent *post hoc* analysis of solanezumab data was interpreted as possible clinical benefit,[374,375] which led to an expensive extra trial (EXPEDITION 3) before giving up.[376,377] This has been explained by suggesting that the trials have been incorrectly designed and targeted irrelevant forms of Aβ too late in the disease process.[189,226,232] Yet, many trials targeting MCI also failed,[139,378] and a 2019 report of autopsy follow-up performed 4 months to 14 years after the first active Aβ immunization showed plaque removal but progression to severe dementia in most AD patients.[228] In March 2023, it was announced that solanezumab targeting pre-symptomatic subjects only showing PET-evidence of brain amyloidosis (the A4 study)[379] did not slow cognitive decline. In trials specifically addressing the most direct genotype-phenotype relation of the hypothesis via studying FAD patients, antibodies gantenerumab or solanezumab, despite targeting Aβ, also failed to produce clinical effect versus placebo; solanezumab in some patients generated even worse outcomes than placebo.[380]

Also, amyloid-related imaging abnormalities (ARIA) in monoclonal antibody trials are a major concern.[150] When administering aducanumab, ARIA affected 41% of participants versus 10% in the placebo group, including microhaemorrhages in 19% (placebo group: 7%) and siderosis in 15% of patients (placebo group: 2%).[381] ARIA was consistently more frequent in the high-dose group, indicating a dose-dependent adverse effect of the highly specific Aβ antibody.[381] ApoE ϵ4 carriers had these adverse effects twice as often as non-carriers.[381] Gantenerumab also showed no effect but led to vasogenic oedema or inflammation in some patients,[382] and ARIA risk was higher for APOE ϵ4 genotypes in the gantenerumab[312] and bapineuzumab trials.[313] It also speaks against a strong disease-modifying role that while ARIA follows a clear dose-response relationship with biomarker changes,[151,381] the dose-response correlation for clinical benefits on cognition in the same trials tend to be weaker.

Blocking amyloid-β producing enzymes often impairs cognition

While β- and γ-secretase inhibitors failed to show clinical effects, they often caused adverse effects,[139] which could be due to the inhibition of action towards other substrates or other functional APP cleavage products such as sAPP,[383,384] with Aβ deposition being a correlating observation. Blocking Aβ producing enzymes commonly impaired cognition,[89,139] raising the possibility that normal protein functions need to be preserved.[85,123,197,229] Recent Bayesian analysis of historic anti-amyloid trials indicates strong evidence for a null hypothesis.[227,385] We note that this analysis included trials without precise target engagement by biomarkers or advanced stage patients for whom treatment may come too late.[386] However, even for the newer monoclonal antibody trials of donanemab, aducanumab and lecanemab with highly selected early-disease cohorts and robust biomarker data, the benefits were below the threshold typically considered clinically meaningful[387,388] and the risk/benefit ratio remained uncertain.[389]

Amyloid-PET as a diagnostic tool

Concerns have been raised that surrogate-based approvals without clear clinical benefit[13] could negatively impact dementia research[94,234] and efforts to combat other diseases such as cancer.[390] Yet, the field increasingly focuses on amyloid removal as a surrogate of treatment benefit. This is potentially a concern because the ACH has not yet been proven true, and because techniques used to measure amyloid have limitations: the heterogeneous and modest specificity,[299] differences between tracers, and large non-specific PET signals raise questions about the appropriate use and limitations of amyloid PET, not only for diagnostic purposes, but also in therapeutic trials.[153,203,233,292,293] Amyloid-PET currently has limited ability to detect amyloid over time in relation to disease course and post-therapy evaluations.[294,391,392] There are concerns about the spatial resolution being too low to identify low concentration plaque in early disease[203,291,293] and tracer uptake in white matter, spillover and partial volume effects may complicate analysis.[291–293,393]

PET mainly targets fibrillar β-sheet structures and is unlikely to measure the soluble pathogenic forms of Aβ[168,207,394] that may vary in morphology and contain less β-sheet structure.[292] Reduced uptake of tracers can also be due to many other causes than removal of plaque deposits, including morphological changes or changing the conformation of the plaques.[293,294,395] Combined with the substantial indication of no clinical effect of many other immunizations,[227] FDA approval of a surrogate PET marker with a median specificity of 58% according to the Cochrane topic review[299] seems erroneous due to suboptimal specificity of the surrogate[293,395] and the underlying causal premise that it reflects disease state.[155–157,203,304,305]

Aducanumab: a case study of unmaterialized hope

The focus on amyloid-PET as a surrogate for determining disease status and assessing the clinical benefit of anti-amyloid therapies has especially raised concern in relation to aducanumab.[13]

Aducanumab is a monoclonal antibody that selectively targets Aβ oligomers over monomers *in vitro*, and it is hoped, also *in vivo*.[396] The rationale was to reduce the pathogenicity of oligomers and the seeding by oligomers of fibrillar plaques.[397] Results of early aducanumab trials were promising as the drug significantly reduced the amount of brain amyloid depositions.[398,399] The following two, nearly identical, phase 3 trials, named EMERGE and ENGAGE, yielded contradictory clinical results (little versus no effect) and were halted based on futility analysis from the first half of the enrolled patients as announced on 21 March 2019.[400,401] However, on 22 October 2019, Biogen announced that *post hoc* analysis of trial data[315] indicated that EMERGE met its primary clinical end point for more prolonged exposures of aducanumab, while ENGAGE did not. Based on this, Biogen sought FDA approval.[402,403] FDA then approved aducanumab as Aduhelm via the accelerated

approval pathway, based on reduction in amyloid plaques as a surrogate of clinical effect despite weak evidence of such,[404] a decision that caused widespread controversy.[13,14,405–408]

The move by FDA executives to use amyloid-PET tracer uptake as a surrogate of clinical efficacy assumes that the ACH has been proven. The surrogate claim has never been tested scientifically as it was not submitted to the advisory panel.[13,409,410] Setting aside the limitations of measuring amyloid and the dispute over the ACH itself, the amyloid-clinical relationship is well accepted as being confounded by additional interactions such as APOE ε4 genotype and tau pathology,[154,184] i.e. FDA's claim that reduced amyloid-PET tracer uptake is a surrogate of clinical effect lacks scientific support.

Lecanemab: promise with limitations

Lecanemab,[411] a humanized form of the mAb158 mouse antibody attacking protofibrils in mouse models,[412] although not meeting its primary end points in phase 2, showed promising clinical results in follow-up analysis.[411] Lecanemab was made specific towards the Arctic APP mutation (E22G) that presumably forms protofibrils very quickly and seems to bind protofibril more strongly than aducanumab.[413] The published 18-month study trial data[16] indicated a statistically significant clinical effect on several end points. A significant outcome was observed for the primary end point [change in the 18-point Clinical Dementia Rating–Sum of Boxes (CDR-SB) score] and for secondary end points, such as the Alzheimer's Disease Assessment Scale (ADAS-cog14).[16]

In terms of CDR-SB scores, patients receiving lecanemab declined by 1.21 points, while patients receiving placebo declined by 1.66 points. This is a difference of 0.45 on an 18-point scale (27% relative effect but only 2–3% absolute effect). For the ADAS-cog14 outcomes, the difference was −1.44.[16] Although statistical significance was maintained or even expanded at late time points, this effect might reverse or reduce after the trial period. Whether the observed 0.45-point improvement compared to controls translates into a clinically meaningful difference is uncertain[414]: establishing clinical meaningful changes can be tricky, but for the primary end point CDR-SB score a cut-off with real-world clinical implications has been estimated to be ∼1.[387,388] Moreover, lecanemab is not superior to the acetylcholinesterase inhibitor donepezil, which for example, has shown a 2-point difference versus placebo on the ADAS-cog scale,[415] despite being symptomatic rather than amyloid-modifying.

Lecanemab was also estimated to provide cognitive and functional symptom improvement corresponding to half a year disease progression based on data obtained during the trial.[16] However, this depends on whether the trajectory of any potential benefits continues after 18 months. Similarly, the cumulative adverse effects of ongoing treatment are unknown and could ultimately curtail treatment time.

Also of note are the very heterogeneous subgroup results. For some end points, simultaneous use of symptomatic medicine gave as large an effect as lecanemab. Males had much more benefit across all end points, whereas females exhibited little benefit (12% for the primary end point), a large and unexplained effect. Also, lecanemab showed by far the smallest effect in younger patients (6% for the primary end point in < 65 years), in contrast to the assumption that the drugs must be applied early to work. Data for composite groups (e.g. young females, female APOE ε4 carriers) were missing, but the effects separately suggest that efficacy could approach zero in many groups. The lower benefit to APOE ε4 carriers is unexplained. This heterogeneity is important for labelling of the drug (as some patients may have no benefit) and for illustrating that population covariates affect disease development as much as Lecanemab itself.

Lecanemab was originally welcomed as associated with relatively less adverse effects than some other antibodies,[411] but 12.6% of treated patients still displayed ARIA.[16] Also, although analysis was not described in detail in the paper,[16] previous MRI data indicated consistent brain volume changes.[411] Of concern, during the trial period, at least two deaths have been reported.[416,417] The lecanemab study indicated similar death rates in the treatment and placebo group (one more in the latter) and no deaths associated with ARIA,[16] but independent autopsies[417] indicated otherwise, so careful investigation of these red flags is needed. A third death indicates a risk associated with the use of anticoagulants (heparin) simultaneously with lecanemab administration.[418]

Although the authors did perform sensitivity analysis for this possibility,[16] unblinding of patients due to ARIA protocols could potentially cause bias in the cognitive performance scores that would correspond to a weakening of the clinical disease progression delay.[419] Of further interest are the adverse events that caused participants to drop out of treatment. This number was twice as large in the treatment group compared to the placebo group,[16] and the outcomes of these patients need to be analysed in total together with the long-term outcomes of participants, as essential information for the treatment perspective. The efficacy was evaluated against a consistently reducing cohort during the trial period, not the final cohort at 18 months, which may also produce bias.[420]

Finally, it is very important to understand the mechanisms behind both the beneficial and adverse effects (ARIA[381,421]) on the molecular level. It is, for example, possible that the benefits are not due to removal of oligomers or protofibrils *per se*, but for example, reconstitution of functional monomers[123] or Aβ-independent pathways, depending on the molecular affinity and specificity of the antibodies versus other molecular targets. $A\beta_{42}$ increased substantially in the CSF due to lecanemab treatment,[16] which for a given level of amyloidosis has recently been associated with a net positive clinical effect by itself,[125,126] consistent with loss-of-function aetiologies of the disease.[84,86,136,188,422] Thus, the contributions of beneficial and harmful forms of Aβ need attention when dissecting the information provided by the data. The clinical effects were not correlated with the change in amyloid load and were much more modest,[16] i.e. only an absolute 2–3% clinical effect as elaborated above.

Donanemab: debated efficacy

Donanemab is another monoclonal antibody developed by Eli Lilly that also bears some promise in recent trials.[423,424] Like the trials of lecanemab and aducanumab, the donanemab trial used early-disease cohorts and consistent use of biomarker data. Mintun *et al.*[423] indicated a borderline significant outcome for their primary end point (the iADRS scale[425]), which is a composite of two of the other four scores used as secondary end points [CDR-SB, ADAS-Cog13, ADCS-iADL and Mini-Mental State Examination (MMSE)] in none of which the change after 76 weeks was significant. The change in the primary end point iADRS score after 76 weeks was −6.86 versus −10.06 with placebo, i.e. a difference of 3.2 (equal to 3.2/149 or 0.22%). However, clinically meaningful changes in iADRS are estimated to be >5 for MCI and >9 for mild AD dementia.[426]

At the same time, treatment-related ARIA-E and ARIA-H were frequent in treated versus placebo patients: 26.7% versus 0.8% and 8.4% versus 3.2%, respectively. As with lecanemab, unexplained brain volume changes and possible atrophy associated

with treatment are of concern and need to be understood,[423] noting a new meta-analysis that finds these effects to be systematic and significant.[427]

Also, the small (below clinically meaningful) benefits may be affected by potential biases from unblinding due to ARIA protocols[419] and differential dropout biases.[420] However, these effects are all below the threshold typically considered clinically meaningful[387,388] and the risk/benefit ratio remains uncertain.[389] Recently, the FDA declined accelerated approval for donanemab due to too few patient data, but traditional approval will likely be sought by Lilly based on the confirmatory phase 3 TRAILBLAZER-ALZ 2 trial later in 2023.[428] However, as for aducanumab, the cost-effectiveness of donanemab for treating mild AD is uncertain and plausibly unfavourable,[429] and the current debate on the monoclonal antibody trials[389,414,430–432] is likely to continue. The additional TRAILBLAZER-ALZ trial results coming over the next years will add useful insight to the current debate on monoclonal antibody treatments.

Important considerations regarding anti-amyloid therapies

It is difficult to ascertain the extent to which amyloid removal can be considered beneficial, given the early data from the clinical trials. While currently approved treatments, such as donepezil, show benefits in clinical studies,[415,433] improvements in clinical practice are limited and even then, last for a limited time. The benefit of Aβ antibodies should be measured against the same real-world benchmarks, considering their significant adverse effects in some patients and the fact that they seem to work in distinct subsets of patients only, and the modest effects of these drugs may not be cost-effective for early AD.[429] We need more work to determine the reproducibility of results, alerted by the different aducanumab results in the parallel, similarly designed EMERGE and ENGAGE trials.[401,406] We should also work to understand the mechanisms behind the adverse effects (ARIA) and the paradoxical brain volume changes and concern about atrophy triggered by these compounds,[427] as well as the specific mechanisms behind any potential clinical benefits.

There will be pressure to apply the drugs well outside their tested parameters, and there is concern that the risk-benefit analysis would not favour such unfettered use.[429,434] The patients in the CLARITY trial[16] were a carefully selected and followed cohort, and excluded the dropouts noted above, arguably increasing the tendency towards a positive benefit in the trial. The field will need to carefully follow real-world clinical experience with these drugs, including the long-term risk profile and magnitudes of meaningful, beneficial impact on specific patient groups with diverse real-life experiences of dementia. Caution on the implementation of these drugs into practice is warranted until these analyses have been generated.

In summary, it would be unwarranted at this stage to double down on the ACH based on the current data. We are concerned that enormous pressure is leading to fast-track approval of anti-amyloid therapies, fuelled by a view that Aβ is synonymous with dementia in AD, as implied by the FDA's scientifically untested adoption of the amyloid-PET surrogate for clinical efficacy.[13,295] We do not yet know the long-term effects of lecanemab use (positive or negative) or whether effects persist or revert after the drug is ceased. We recommend follow-up of patients enrolled in these trials over the next decade and careful assessments of real-world use, to address these questions.

Moving forward: options for integrating theories and therapies

Lack of therapeutic success in AD and other major neurodegenerative disorders such as Parkinson's disease and amyotrophic lateral sclerosis may relate to the absence of convergent molecular models of disease and strict definitions of disease criteria, and the lack of clear distinction between pathology and pathogenicity. No monocausal treatment has ever produced disease reversal, stagnation or even general patient-wide robust clinical effects.[227]

It is crucial to acknowledge the historic importance of the ACH in establishing AD as a single disorder that may be targeted therapeutically.[89,94] The hypothesis followed a series of exceptional observations, discoveries and reasoning. It was supported by the best available evidence at the time.[11] However, the data reviewed above suggest a need to replace the ACH with a more complex hypothesis integrating the many polygenic, epigenetic, environmental, vascular, neuroinflammatory and metabolic factors, ideally in more predictive holistic models.[154,185] The misfolding of proteins may be a sign, not always cause, of molecular abnormalities.[94,136,218] More realistic models that acknowledge the many modulators of the disease[44,154,185] yield room for individual disease trajectories based on the patient's personal aggregate risk.

Understanding the function of APP is essential, as it may increase our understanding of the main pathways and mechanisms involved in FAD. The function of presenilins is important for lysosome proteolysis. Notably, PS1 mutations impair this function[435] and functions of the vascular system.[436] The accumulation of undegraded waste proteins and peptides resembles the pathology of lysosomal storage diseases that would assign senile plaques to a dysfunction of the proteasome and lysosome,[110,437] with a plausible role of presenilins in controlling calcium access to the lysosomes and preserving proteostasis competency.[435,437,438]

We should consider that patients exhibit individual, overlapping but heterogeneous disease trajectories.[49,89,92,94,136] Ignoring these multiple factors and pathways in favour of magic 'silver bullets' is detrimental. We urge great care and caution in considering the whole individual and reiterate the need for developing and applying individualized care and treatment programmes for patients.

The next decade will be an exciting time to be in AD research as we work towards new understandings and effective therapeutics. Efforts are now required to establish the molecular granularity and aetiological heterogeneity but relatedness of condition subtypes as framing the complexity of the disease, including both loss- and gain-of-function pathways,[19,123,422,439] with ageing being a main factor of pathogenicity.[57,89,205]

Hypotheses relating to infections, vascular disease and dysmetabolism must be explored further as possible drivers, among others.[266,275,440] In these scenarios amyloid may, for example, be a marker of increased APP turnover, as a response to insult rather than a disease driver. Importantly, a recent trend in drug pipelines towards more diverse disease pathways[82] lends promise of more complex and novel future applications of treatment regimes.

The ageing human proteome features reduced gene expression related to synaptic function, calcium homeostasis and vesicular transport but increased expression of genes involved in inflammation and stress tolerance,[441] a human ageing phenotype largely absent in research models. Chemical ageing is relatively straightforward to include in preclinical models and is known to modulate Aβ production and clearance and aggregation.[57,219,442]

On the regenerative front, promising areas are emerging. Converging data support a role for insulin signalling in

dementia.[443,444] These pathways offer opportunities to integrate metabolic pathways with other protein-specific pathways. For instance, glucagon-like peptide-1 receptor (GLP-1R) analogues have been tested in preclinical models and clinical trials.[445,446] Additional therapeutic venues, ideally in combination, can be expected from non-neuronal targets like neuroinflammation and the microglia-astrocyte axis.[26,440]

Finally, we envision that data-backed artificial intelligence and systems biology tools can help address the challenge of unravelling personalized disease trajectories. These approaches have helped define degenerative subprocesses inside and outside the CNS. For instance, growing evidence supports a gut-brain connection,[447,448] microbiota's role in neurodegeneration,[266,275] and the role of lipid and energy-related dysmetabolism in AD.[40,44,185,449] Artificial intelligence may combine the various personal-medicine trajectories and account for the biochemical and clinical heterogeneity in perhaps more complete, realistic and predictive AD models.

Concluding remarks

Motivated by the recent trials of monoclonal antibodies against AD, we have reviewed the history of the ACH and genetic, biochemical, biomarker and clinical data underlying it. We suggest that these data show that Aβ, while important in AD pathology, plays a minor role in the aetiology and thus treatment of the disease. Despite decades of research, the normal functions of APP and Aβ and the precise pathogenic mechanism and structure of Aβ have not been established. Whether clinical trials produce positive or adverse results, we are largely in the dark regarding the molecular underpinnings of these outcomes.

Anti-amyloid therapies may at best support a notion that the amyloid cascade participates in some pathologically relevant processes of AD. However, Aβ-pathways as targeted in the current form by antibodies in no way account for the aetiological complexity and real-world clinical needs of patients, and thus AD is better described within a modern network-based anti-reductionist disease view.[450]

To resolve the current therapeutic impasse, efforts are required to explore the molecular granularity of condition subtypes to capture the full complexity of the disease and the many disease trajectories and converging biochemical pathways and complex cellular interactions in the brain. We envision that artificial intelligence-aided approaches along with systems biology and precision medicine can help to refocus our attention toward generating a significant impact on the lives of the millions of patients affected by this terrible disease.

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Competing interests

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