Alzheimer Mechanisms and Therapeutic Strategies

Cell

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Review

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DOI 10.1016/j.cell.2012.02.040

There are still no effective treatments to prevent, halt, or reverse Alzheimer's disease, but research advances over the past three decades could change this gloomy picture. Genetic studies demonstrate that the disease has multiple causes. Interdisciplinary approaches combining biochemistry, molecular and cell biology, and transgenic modeling have revealed some of its molecular mechanisms. Progress in chemistry, radiology, and systems biology is beginning to provide useful biomarkers, and the emergence of personalized medicine is poised to transform pharmaceutical development and clinical trials. However, investigative and drug development efforts should be diversified to fully address the multifactoriality of the disease.

Introduction

Alzheimer's disease (AD) is characterized by progressive loss of memory and other cognitive functions. Typically, a decade or so passes before the illness has taken its course and patients die in a completely helpless state. The long duration of AD and its attack on the fragile structures that harbor the very essence of who we are place an enormous emotional and financial burden on patients, their families, and society. AD is estimated to have cost the world $604 billion in 2010 alone (Wimo and Prince, 2010). These costs are staggering, particularly in light of predictions that the worldwide number of AD cases, currently estimated at 36 million, will triple by 2050 (Wimo and Prince, 2010). Few health care systems will be able to cope with this development. This Review highlights some of the most informative developments in AD research and raises major unresolved issues.

Substrates of Cognitive Decline

AD causes a large loss in brain weight and volume and affects some brain regions and neuronal populations more than others (Gómez-Isla et al., 1996). Although AD clearly causes loss of neurons in specific brain regions (e.g., of pyramidal cells in lamina II of the entorhinal cortex and in the CA1 region of the hippocampus), much of the overall loss of brain volume appears to be due to the shrinkage and loss of neuronal processes.

Progress in radiological imaging techniques has advanced morphometric measurements from postmortem tissues to live patients (Hampel et al., 2010). For example, progressive decreases in cortical thickness can be detected in multiple brain regions by magnetic resonance imaging (MRI) in AD patients, correlate with cognitive decline, and predict conversion from mild cognitive impairment (MCI) to AD (Frisoni et al., 2010; Putcha et al., 2011). Consequently, this measure is increasingly used in the early diagnosis of AD and as a biomarker in clinical trials.

Beyond such anatomical alterations, functional MRI (fMRI) has revealed alterations in neural network activities in patients with AD and people at risk for developing the disease. These include abnormal activity and connectivity in the so-called default mode network, which in healthy people is most active when they do not think about anything in particular, and hyperactivation of the hippocampus during the execution of memory tasks (Sperling et al., 2010), which correlates with decreased hippocampal volume and abnormal cortical thinning in AD-vulnerable brain regions (Putcha et al., 2011). Consistent with electrophysiological and biochemical data obtained in related transgenic mouse models (Palop and Mucke, 2010; Verret et al., 2012), these observations suggest that AD does not simply silence neurons and neural networks, but rather causes aberrant network activity that might actively interfere with the intricate processes underlying learning, memory and other cognitive functions. In addition, overstimulation of specific neuronal populations could result in excitotoxicity, which likely contributes to neurodegeneration in AD and related conditions. It is interesting in this regard that AD is associated with an increased incidence of epileptic seizures, which is most evident in patients with early-onset forms of the disease (Palop and Mucke, 2009). Findings in transgenic mouse models suggest that these complications may be the tip of an iceberg, representing an escalation of more subtle alterations of neural network activity (Palop and Mucke, 2010; Verret et al., 2012).

Much evidence suggests that synapses and dendrites, the specializations through which neurons send and receive signals, respectively, are particularly vulnerable to AD. Loss of synapses and dendritic spines correlates better with cognitive decline in AD than loss of neurons (Palop et al., 2006). Synaptodendritic rarefaction is also observed in neuronal cultures and in brains of transgenic mice exposed to factors suspected of causing AD. In these models, the degeneration is preceded by alterations in synaptic function and aberrant network activity (Marchetti and

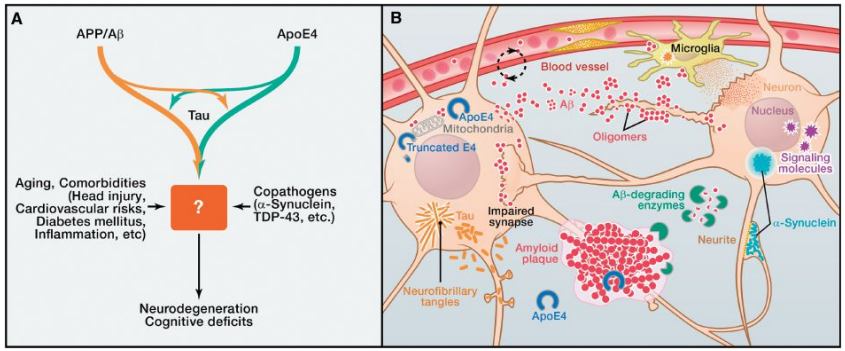

Figure 1. Multifactorial Basis of Alzheimer's Disease Pathogenesis

(A) Alzheimer's disease (AD) is likely to be caused by copathogenic interactions among multiple factors, including APP/Aβ, apoE4, tau, α-synuclein, TDP-43, aging, and various comorbidities. How exactly they conspire to impair neuronal functions and survival remains to be determined.

(B) Aggregation and accumulation of Aβ in the brain may result from increased production of Aβ, decreased degradation by Aβ-degrading enzymes, or reduced clearance across the blood-brain barrier. Aβ oligomers impair synaptic functions and related signaling pathways, changing neuronal activities, and trigger the release of neurotoxic mediators from glial cells. Fibrillar amyloid plaques displace and distort neuronal processes. The lipid transport protein apoE4 impairs Aβ clearance and promotes Aβ deposition. When expressed within stressed neurons, apoE4 is cleaved, to a much greater extent than apoE3, into neurotoxic fragments that disrupt the cytoskeleton and impair mitochondrial functions. Tau, which is normally most abundant in axons, becomes mislocalized to the neuronal soma and dendrites and forms inclusions called neurofibrillary tangles (NFTs). α-synuclein can also self-assemble into pathogenic oligomers and form larger aggregates (Lewy bodies). Both tau and α-synuclein can also be released into the extracellular space, where they may spread to other cells. Vascular abnormalities impair the supply of nutrients and removal of metabolic byproducts, cause microinfarcts, and promote the activation of glial cells.

Marie, 2011; Palop and Mucke, 2010), suggesting that these types of dysfunction are early manifestations of the disease and, possibly, also contribute to its progression.

Recent advances in radiological imaging have also enabled the detection of pathological hallmarks of AD in live patients, improving diagnostic accuracy and patient selection for clinical trials. After injection into the blood stream, Pittsburg compound B (PIB) traverses the blood-brain barrier (BBB) and binds to deposits of fibrillar amyloid-β (Aβ) peptides (amyloid plaques), whose abnormal accumulation in brain is a requirement for the pathological diagnosis of AD. PIB binding to amyloid plaques can be detected by positron emission tomography (PET). A multitude of other radiopharmaceutical probes are being developed for the detection of plaques or neurofibrillary tangles (NFTs) (Kim et al., 2010), another pathological hallmark of AD.

While the detection of these pathological hallmarks in live patients clearly has diagnostic value, it is still uncertain whether these hallmarks actually contribute to cognitive dysfunction in AD and whether they represent useful outcome measures for clinical trials. Plaque loads determined histopathologically or radiologically do not correlate well with functional impairments, as illustrated most strikingly by people with substantial plaque burdens and normal cognition (Giannakopoulos et al., 2003). Recent studies have demonstrated an association between increased PIB binding and development of AD 5 years later (Morris et al., 2009), which has been interpreted as a likely cause-effect relationship. However, some cases with autosomal dominant AD show high levels of PIB binding in the basal ganglia but no greater propensity to develop extrapyramidal motor impairments, indicating dysfunction of these brain regions, than cases with sporadic AD, who have different distributions of PIB binding (Villemagne et al., 2009).

Furthermore, inheritance of the E693Δ mutation in Aβ, which inhibits formation of insoluble amyloid fibrils and promotes the formation of soluble Aβ oligomers, causes a syndrome that closely resembles AD clinically but does not appear to be associated with significant increases in cerebral PIB binding (Shimada et al., 2011). Additionally, the pattern of PIB accumulation differs between patients with sporadic late-onset AD and patients with APP locus duplication (Remes et al., 2008). These observations and a large body of data obtained in transgenic mouse models (Palop et al., 2006; Palop and Mucke, 2010) caution against the interpretation that plaques are the main cause and plaque-associated neuritic dystrophy the main substrate of cognitive decline in AD. Although NFTs correlate more closely with cognitive decline in AD than plaques (Giannakopoulos et al., 2003), results obtained in transgenic mouse models indicate that NFTs do not necessarily impair neuronal function and that the microtubule-associated protein tau, the main constituent of NFTs, can cause neuronal dysfunction independently of these structures, as reviewed in (Morris et al., 2011).

Taken together, these studies suggest that aberrant neural network activity, dysfunction and loss of synapses, and degeneration of specific neuronal populations are the main substrates of cognitive decline in AD. As outlined below, it is likely that these abnormalities are caused by copathogenic interactions among diverse factors and pathways (Figure 1).

Multifactional Etiology of AD

Genetics, Epigenetics, and Epidemiology

AD is probably caused by complex interactions among multiple genetic, epigenetic, and environmental factors. Mutations in three genes—*amyloid precursor protein* (*APP*), *presenilin* (*PS*)*-1* and *PS-2*—cause early-onset (<60 years) autosomal dominant AD (Bertram et al., 2010), which probably accounts for less than 1% of AD cases (Campion et al., 1999). The mutations affect APP processing, leading to altered production of different Aβ peptides and, thus, their relative ratios (Bertram et al., 2010). Down's syndrome patients carrying an extra copy of chromosome 21, on which the *APP* gene resides, develop early-onset dementia with pathological hallmarks of AD in their brains (Millan Sanchez et al., 2011), consistent with the idea that overexpression of APP causes early-onset AD. In strong support of this notion, duplication of the *APP* gene alone leads to early-onset AD (Rovelet-Lecrux et al., 2006). Moreover, increased APP gene expression caused by genetic variations in the promoter sequence may be a risk factor for late-onset AD, with levels of APP expression correlating inversely with age of disease onset (Brouwers et al., 2006).

Apolipoprotein (apo) E4 (used here to refer to either the *APOE* ε4 allele or the protein it encodes) has been genetically linked to late-onset (>60 years) familial and sporadic AD, which accounts for most AD cases, and has a gene-dose effect on increasing the risk and lowering the age of onset of the disease (Corder et al., 1993; Farrer et al., 1997). All well-conducted genome-wide association studies (GWASs) on late-onset AD from different populations around the world identified apoE4 as the top late-onset AD gene with extremely high confidence (Bertram et al., 2010). Remarkably, the lifetime risk estimate of developing AD for individuals with two copies of the apoE4 allele (~2% of the population) is ~60% by the age of 85, and for those with one copy of the apoE4 allele (~25% of the population) ~30%. In comparison, the lifetime risk of AD for those with two copies of the apoE3 allele is ~10% by the age of 85. Thus, apoE4 should be considered a major gene, with semidominant inheritance, for late-onset AD (Genin et al., 2011). A recent GWAS also identified apoE4 as the only significant gene associated with age-related cognitive decline in humans (De Jager et al., 2011), which is in line with a longitudinal study showing that age-related memory decline in nondemented apoE4 carriers diverges from that of nondemented noncarriers before the age of 60 years (Caselli et al., 2009). Thus, apoE4's detrimental effect on cognition occurs before the typical signs of AD arise. In contrast, apoE2 may protect against AD in some populations (Farrer et al., 1997). GWAS also identified other genes that modulate the risk of late-onset AD, including *CLU*, *CR1*, *PICALM*, *BIN1*, *SORL1*, *GAB2*, *ABCA7*, *MS4A4*/*MS4A6E*, *CD2AP*, *CD33*, *EPHA1*, and *HLA-DRB1*/*5* (Bertram et al., 2010; Logue et al., 2011). However, the relative contribution of these genes to AD is modest as compared to apoE4.

Epigenetic mechanisms may also play a role in AD pathogenesis (Day and Sweatt, 2011). Studies on human postmortem brain samples and peripheral leukocytes, as well as transgenic animal models, have shown that aging and AD are associated with epigenetic dysregulation at various levels, including abnormal DNA methylation and histone modifications (Chouliaras et al., 2010). Although it is unclear whether the epigenetic changes observed in AD represent a cause or a consequence of the disease, twin studies support the notion that epigenetic mechanisms modulate AD risk (Chouliaras et al., 2010). Interestingly, pharmacological inhibition of DNA methylation in the hippocampus after a learning task impaired memory consolidation in mice (Day and Sweatt, 2011), and promotion of histone acetylation improved learning and memory in a mouse model of AD and increased learning-related gene expression in aged wild-type mice (Fischer et al., 2007; Peleg et al., 2010), suggesting epigenetic regulation of learning and memory in health and disease.

Aging is the most important known nongenetic risk factor for late-onset AD. Potential environmental risk factors for late-onset AD include head injury, low educational levels, hyperlipidemia, hypertension, homocysteinemia, diabetes mellitus, and obesity (Barnes and Yaffe, 2011; Rosendorff et al., 2007; Sharp and Gatz, 2011; Van Den Heuvel et al., 2007). However, several of these associations remain controversial (Daviglus et al., 2010). Combinations of apoE4 with one or more of these environmental risk factors may further increase the risks for late-onset AD and age-related cognitive decline (Caselli et al., 2011; Haan et al., 1999).

Aβ and Other APP Products

Aβ peptides, the main constituent of amyloid plaques, and various other metabolites are derived from APP by proteolytic cleavage (Citron, 2010; De Strooper et al., 2010). Diverse lines of evidence support the hypothesis that APP and Aβ contribute causally to the pathogenesis of AD (Figure 2). Overexpression of APP in humans through duplication of its gene or trisomy of chromosome 21 causes early-onset AD (see above), whereas partial trisomy 21 excluding the APP gene does not (Prasher et al., 1998). The catalytic subunit of the γ-secretase protein complex that releases Aβ peptides from its precursor is formed by PS1 or PS2. Autosomal dominant mutations in APP, PS1, or PS2 that alter APP processing and the production or self-aggregation of Aβ, promoting aggregation and accumulation of Aβ in brain cause early-onset AD (Bertram et al., 2010). Neuronal expression of mutant human APP (hAPP) either alone or in combination with mutant PS1 in transgenic rodents causes several AD-like alterations, as reviewed previously (Ashe and Zahs, 2010; Jucker, 2010; Marchetti and Marie, 2011; Palop and Mucke, 2010; Querfurth and LaFerla, 2010) and described further below.

Several lines of evidence suggest that Aβ regulates neuronal and synaptic activities and that accumulation of Aβ in the brain causes an intriguing combination of aberrant network activity and synaptic depression (Figure 2A) (Palop and Mucke, 2010). Impairments of inhibitory interneurons and aberrant stimulation of glutamate receptors, which can result in excitotoxicity, appear to play important upstream roles in this pathogenic cascade (Figures 2B and 2C) (Meilandt et al., 2008; Palop and Mucke, 2010; Sanchez-Mejia et al., 2008; Verret et al., 2012). Aberrant neuronal activity might trigger a vicious cycle by augmenting Aβ production, which is regulated, at least in part, by neuronal activity (Bero et al., 2011). The immediate early gene *Arc*, which directly binds to PS1 to regulate γ-secretase trafficking, is required for neuronal activity-dependent Aβ production (Wu et al., 2011).

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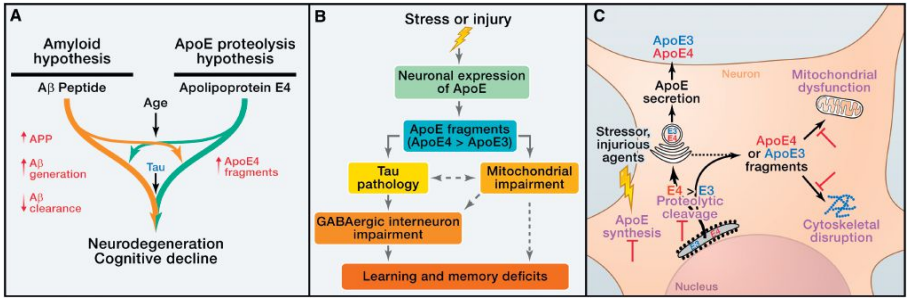


Figure 3. ApoE4 in AD Pathogenesis and Related Therapeutic Strategies

(A) ApoE4 probably has both Aβ-dependent and Aβ-independent roles in AD pathogenesis. ApoE4 impairs Aβ clearance and promotes Aβ deposition (left). In addition, neuronal apoE4 undergoes proteolytic cleavage to generate neurotoxic fragments, contributing to AD pathogenesis independently of Aβ (right).

(B) The apoE proteolysis hypothesis suggests that, in response to stresses or injuries, neuronal apoE expression is triggered to facilitate neuronal repair. However, neuronal apoE undergoes proteolytic cleavage, with apoE4 being more susceptible to the cleavage than apoE3, resulting in the formation of neurotoxic fragments. The apoE4 fragments enter the cytosol and cause tau pathology and mitochondrial impairment. Hilar GABAergic interneurons in the dentate gyrus are particularly vulnerable to apoE4 fragment toxicity and the resulting impairments contribute to learning and memory deficits.

(C) Neuronal expression of apoE and apoE proteolysis-related neurotoxicity represent targets for the development of novel therapeutics. Inhibiting apoE4 expression in neurons should reduce the level of toxic apoE4 fragments and their downstream detrimental effects. Identification of the protease that cleaves apoE in neurons should enable the development of specific protease inhibitors to reduce the production of neurotoxic apoE fragments. It may also be possible to develop drugs to protect mitochondria and the cytoskeleton from attack by the toxic apoE fragments.

Other uncertainties about the value of Aβ-lowering drugs relate to the potential physiological functions of APP and different APP metabolites, including Aβ (Duce et al., 2010; Giuffrida et al., 2009; Lee et al., 2010b; Puzzo et al., 2011; Weyer et al., 2011; Yang et al., 2009) and to the question of whether APP itself or APP metabolites other than Aβ (e.g., the C-terminal fragments C99, intracellular domain, or C31) also contribute to the pathogenesis of AD (Ghosal et al., 2009; Harris et al., 2010; Simón et al., 2009).

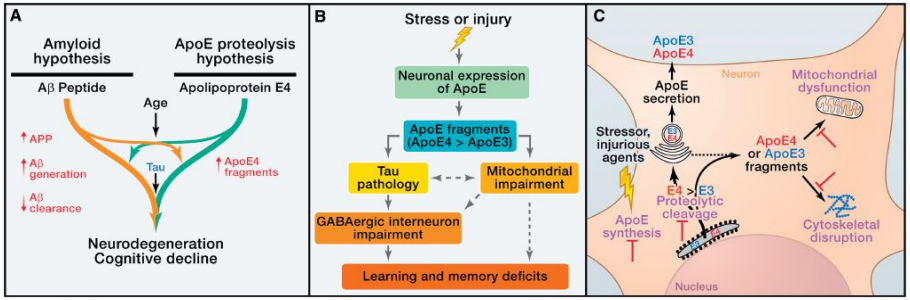
ApoE4

The major impact of apoE4 on AD risk is clearly at odds with the relatively small amount of attention it has received in the field, as compared to, for instance, APP, Aβ, tau, and inflammation. To encourage improvement of this situation, we will review the pathobiology of apoE4 in greater depth here. Since apoE4 was identified as a genetic risk factor for AD, in vitro and in vivo studies have explored its structural properties and functions in neurobiology, its cellular source-dependent physiological and pathophysiological activities in brain, and its Aβ-dependent and independent roles in AD pathogenesis (Figure 3).

ApoE Polymorphisms and Functions in Neurobiology. ApoE is a polymorphic protein with three common isoforms, apoE2, apoE3, and apoE4, in humans. The three isoforms differ from one another by single-amino acid substitutions (Mahley et al., 2006). ApoE has important and diverse roles in neurobiology (Bu, 2009; Huang, 2006; Kim et al., 2009; Mahley et al., 2006). It has isoform-specific roles in neurite remodeling: apoE3 stimulates neurite outgrowth, and apoE4 inhibits it (Huang, 2006, 2010; Mahley et al., 2006). ApoE also takes up lipids generated after neuronal degeneration and redistributes them to cells requiring lipids for proliferation, membrane repair, or remyelination of new axons (Huang, 2006; Mahley et al., 2006). Synaptic and dendritic alterations are observed in apoE-deficient mice (Masliah et al., 1995) and transgenic mice expressing apoE4 in neurons (Buttini et al., 1999). In addition, apoE modulates glutamate receptor function and synaptic plasticity by regulating apoE receptor recycling in neurons, with apoE3 stimulating and apoE4 inhibiting this process (Chen et al., 2010).

Interaction between the N- and C-terminal domains is a unique biophysical property of apoE4 (Zhong and Weisgraber, 2009). Crystallographic and other biophysical studies revealed that this domain interaction is mediated by a salt bridge formation between Arg-61 and Glu-255 (Zhong and Weisgraber, 2009). Mutation of Arg-61 to threonine or of Glu-255 to alanine in apoE4 prevents domain interaction and makes apoE4 more apoE3-like (Zhong and Weisgraber, 2009). The domain interaction is responsible for impaired intraneuronal trafficking of apoE4 (Brodbeck et al., 2011), apoE4's susceptibility to proteolysis (Huang, 2010; Mahley et al., 2006), apoE4-induced impairments in neurite outgrowth, and mitochondrial functions (Brodbeck et al., 2011; Chen et al., 2012), and apoE4-associated astrocytic dysfunction (Zhong and Weisgraber, 2009).

Cellular Source-Dependent Roles of apoE4 in AD Pathogenesis. ApoE derived from different cellular sources has distinct roles in both physiological and pathophysiological pathways (Huang, 2006, 2010; Mahley et al., 2006). Astrocytes have long been recognized as the primary source of apoE in the brain, and expression of apoE in astrocytes is increased during aging and in response to estrogen and activation of liver X receptor or NF-κB (Huang, 2006, 2010; Mahley et al., 2006). In vitro and in vivo studies suggest that astrocyte-derived apoE has isoform-specific effects on Aβ clearance or deposition (Kim et al., 2009), neurite outgrowth (Holtzman et al., 1995), and behavioral performance (Hartman et al., 2001).



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Interaction between the N- and C-terminal domains is a unique biophysical property of apoE4 (Zhong and Weisgraber, 2009). Crystallographic and other biophysical studies revealed that this domain interaction is mediated by a salt bridge formation between Arg-61 and Glu-255 (Zhong and Weisgraber, 2009). Mutation of Arg-61 to threonine or of Glu-255 to alanine in apoE4 prevents domain interaction and makes apoE4 more apoE3-like (Zhong and Weisgraber, 2009). The domain interaction is responsible for impaired intraneuronal trafficking of apoE4 (Brodbeck et al., 2011), apoE4's susceptibility to proteolysis (Huang, 2010; Mahley et al., 2006), apoE4-induced impairments in neurite outgrowth, and mitochondrial functions (Brodbeck et al., 2011; Chen et al., 2012), and apoE4-associated astrocytic dysfunction (Zhong and Weisgraber, 2009).

Cellular Source-Dependent Roles of apoE4 in AD Pathogenesis. ApoE derived from different cellular sources has distinct roles in both physiological and pathophysiological pathways (Huang, 2006, 2010; Mahley et al., 2006). Astrocytes have long been recognized as the primary source of apoE in the brain, and expression of apoE in astrocytes is increased during aging and in response to estrogen and activation of liver X receptor or NF-κB (Huang, 2006, 2010; Mahley et al., 2006). In vitro and in vivo studies suggest that astrocyte-derived apoE has isoform-specific effects on Aβ clearance or deposition (Kim et al., 2009), neurite outgrowth (Holtzman et al., 1995), and behavioral performance (Hartman et al., 2001).

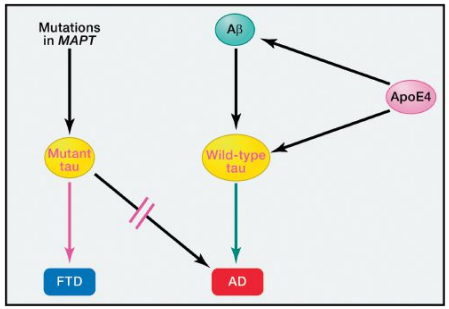

Figure 4. Roles of Tau in Different Tauopathies
Mutations in *MAPT*, the gene that encodes tau, result in the production of mutant tau and cause certain forms of frontotemporal dementia (FTD) but never AD. In AD, Aβ and apoE4 act upstream of wild-type tau and their adverse effects depend, at least partly, on tau whose structure or function may be altered by abnormal posttranslational modification, mislocalization or other variables. Indeed, tau reduction prevents Aβ and apoE4 fragments from causing neuronal and behavioral deficits in mouse models. The exact mechanisms by which tau contributes to neuronal dysfunction and degeneration in AD, FTD and other tauopathies remain to be determined.

leading to mitochondrial dysfunction and neurotoxicity (Figure 3B and 3C) (Chang et al., 2005; Chen et al., 2012). Notably, mitochondrial dysfunction in AD is greater in apoE4 than in apoE3 carriers (Gibson et al., 2000). ApoE4 is also associated with decreased cerebral glucose metabolism in both AD patients and nondemented subjects (Small et al., 2000), which likely reflects apoE4-dependent mitochondrial dysfunctions.

ApoE4-Induced Impairment of GABAergic Interneurons. ApoE4 knockin mice show an age-dependent decrease in hilar GABAergic interneurons, which correlates with the extent of apoE4-induced impairments of adult hippocampal neurogenesis and with learning and memory deficits (Andrews-Zwilling et al., 2010; Li et al., 2009). In transgenic mice expressing neurotoxic apoE4 fragments, the loss of hilar interneurons is more pronounced and also correlates with learning and memory deficits (Andrews-Zwilling et al., 2010). These adverse effects are prevented by tau removal (Andrews-Zwilling et al., 2010). Mice treated with the $GABA_A$ receptor potentiator pentobarbital exhibit normal neurogenesis and learning and memory (Andrews-Zwilling et al., 2010; Li et al., 2009). These findings strongly suggest that apoE4 causes age- and tau-dependent impairment of hilar GABAergic interneurons, leading to decreased neurogenesis in the hippocampus and to learning and memory deficits (Figure 3B).

Dysfunction of the GABAergic system may also contribute to cognitive impairment in humans. AD patients have decreased GABA and somatostatin levels in the brain and CSF, and these alterations are more severe in apoE4 carriers (Grouselle et al., 1998). ApoE4 is associated with increased brain activity at rest and in response to memory tasks (Dennis et al., 2010; Filippini et al., 2009), possibly reflecting impaired GABAergic inhibitory control. A single-nucleotide polymorphism in the somatostatin gene increases the risk for AD in carriers of apoE4 but not of apoE3 (Vepsäläinen et al., 2007). Furthermore, GABA levels in human CSF decrease with aging (Bareggi et al., 1982)—the strongest risk factor for AD. We hypothesize that apoE4 contributes to AD pathogenesis, at least partially, by causing age-dependent impairments of GABAergic interneurons (Figure 3B).

Tau and Other Copathogens

AD is clearly a polyproteinopathy in which multiple proteins assume potentially pathogenic conformations and accumulate separately or together in the brain. Based on the pathological definition of the disease, AD is associated not only with the abnormal accumulation of amyloid plaques, but also with that of NFTs. NFTs form intracellularly and are made up primarily of aggregated tau bearing abnormal posttranslational modifications, including increased phosphorylation and acetylation (Cohen et al., 2011; Iqbal et al., 2010; Min et al., 2010). Several recent findings have challenged the traditional views that tau functions primarily to stabilize microtubules and that its aggregation in AD causes deficits through a loss-of-function mechanism (Morris et al., 2011). Studies in cell culture and genetically modified mouse models suggest that tau may normally facilitate or enhance excitatory neurotransmission by regulating the distribution of synaptic activity-related signaling molecules (Morris et al., 2011). However, when it is abnormally modified and assumes pathogenic conformations, tau becomes enriched in dendritic spines where it can interfere with neurotransmission (Hoover et al., 2010). Aβ oligomers promote this postsynaptic enrichment of tau through a process that involves members of the microtubule affinity-regulating kinase (MARK) family (Yu et al., 2012; Zempel et al., 2010).

Interestingly, tau reduction prevents Aβ from causing neuronal deficits in cell culture and hAPP transgenic mice (Morris et al., 2011). Thus, while Aβ acts upstream of tau, its adverse effects depend in good part on tau (Figure 4). These conclusions are consistent with genetic studies: mutations in *APP* or *presenilins* that cause Aβ accumulation in the brain cause AD with amyloid plaques and NFTs (Bertram et al., 2010), whereas tau mutations cause NFTs but neither amyloid plaques nor AD (Figure 4). The latter mutations cause frontotemporal lobar degeneration instead (Ballatore et al., 2007). ApoE4 and its fragments increase tau phosphorylation and accumulation in the neuronal soma and dendrites (Andrews-Zwilling et al., 2010; Brecht et al., 2004; Harris et al., 2003). Moreover, tau reduction also prevents apoE4-dependent neuronal deficits in vitro and in vivo (Andrews-Zwilling et al., 2010), pinpointing tau as a key mediator or enabler of both Aβ- and apoE4-dependent pathogenesis (Figure 4). As reviewed above, apoE isoforms modulate both Aβ deposition as well as plaque-independent synaptic and cognitive deficits in hAPP mice, with apoE4 enhancing and apoE3 counteracting the abnormalities, further highlighting the copathogenic relationships among Aβ, tau and apoE4.

A large proportion of AD cases also have abnormal accumulations of the presynaptic protein α-synuclein and of the RNA-binding protein TDP-43 in the brain (Higashi et al., 2007). As with tau, mutant forms of these proteins do not cause AD, but other neurodegenerative disorders. Aβ enhances the misfolding and accumulation of α-synuclein in vitro and in vivo, and studies in doubly transgenic mice suggest that these molecules can

synergize to more severely impair neuronal functions (Masliah et al., 2001). Likewise, apoE4 fragments may enhance the accumulation of TDP-43 in neurons of FTD patients (Vossel et al., 2012).

Potential Common Mechanisms

Like Aβ, tau and α-synuclein can exist in different assembly states, and several lines of evidence suggest that smaller aggregates such as soluble oligomers are more pathogenic than larger, insoluble, highly ordered, fibrillar aggregates (Morris et al., 2011; Winner et al., 2011). An interesting question that remains unresolved is whether the in vivo pathogenicity of these and other proteins associated with neurodegenerative disorders depends on specific conformations (e.g., β-pleated sheet structures) and whether the proteins require self-aggregation or binding to other proteins to assume these conformational states. At least for tau and α-synuclein, both of which are intrinsically disordered proteins, it is conceivable that even monomers could assume pathogenic conformations under circumstances that promote protein misfolding. Experimental evidence for such a scenario has recently been obtained for huntingtin bearing disease-associated polyglutamine repeat expansions (Miller et al., 2011).

Whether the various pathogenic protein conformations or assemblies impair neuronal functions through common, distinct, or partly overlapping mechanisms also remains to be determined. The copathogenic interactions that have been identified among tau, apoE4, Aβ, and α-synuclein (see above) suggest that there are probably downstream mechanisms on which the effects of these proteins converge. Abnormalities in the activity or distribution of neurotransmitter receptors and downstream signaling cascades, excitotoxicity, and dysregulation of intracellular calcium homeostasis, alterations in the intracellular transport of critical cargoes, mitochondrial impairments, epigenetic dysregulation, and engagement of pathogenic glial loops are among the possible convergence points. It is unfortunate that the identification of such diverse possibilities is often misinterpreted as controversy or lack of understanding. After all, even normal proteins often have multiple functions and different activities under different circumstances, cytokines such as TGF-β being a good example. There is no reason why the activity of pathogenic proteins should be less complex. Therefore, the various mechanisms listed above are clearly not mutually exclusive. In fact, some of them may be related, e.g., intracellular transport deficits and mitochondrial dysfunction.

In addition, abnormal folding and accumulation of multiple proteins could nonspecifically stress and ultimately overload the cellular protein handling machinery. Much evidence suggests that autophagy is the main mechanism by which cells clear abnormal protein aggregates (Harris and Rubinsztein, 2011). In the most common form of autosomal dominant AD, mutant PS1 may disrupt autophagy directly by impeding lysosomal proteolysis, whereas in other forms of AD autophagy impairments may involve different genetic or environmental factors (Nixon and Yang, 2011). Attempts to restore normal lysosomal proteolysis and autophagy in mouse models of AD have yielded promising therapeutic effects, lowering Aβ levels and improving neuronal function as well as cognitive performance (Nixon and Yang, 2011; Pickford et al., 2008; Yang et al., 2011a).

Another interesting aspect of Aβ, tau, α-synuclein and other proteins associated with neurodegenerative disorders is their ability to perpetuate pathology through cell-to-cell spread and seeding of abnormal protein aggregation in experimental models. These properties have been compared to those of prions, different forms of which cause Jacob-Creutzfeldt disease, scrapie, and mad-cow disease (Braak and Del Tredici, 2011; Jucker and Walker, 2011). However, prions differ fundamentally from the other proteins in that they cause diseases that are communicable, which AD and most other neurodegenerative disorders are not.

It is well established that neurodegenerative disorders are strongly linked to aging. However, it remains uncertain whether this link is specifically caused by aging-related processes or simply reflects the time required for the relevant pathogenic processes to unfold. Genetic changes that accelerate the accumulation of pathogenic proteins in the brain can clearly override the aging "requirement" and cause AD or other neurodegenerative conditions in middle-aged or even young people. Nonetheless, diverse lines of experimental evidence support the notion that AD and other neurodegenerative conditions may be enabled by specific aging-related factors, such as gradual failure of neuroprotective or protein clearance mechanisms and the emergence of comorbidities (Herrup, 2010; Mawuenyega et al., 2010; Villeda et al., 2011). Inflammation may be a key component of unhealthy aging (Sastre et al., 2011).

Last but not least, both Aβ and apoE4 can contribute to network and cognitive dysfunction by impairing specific populations of inhibitory interneurons that normally regulate the activity of excitatory principal cells (Figures 2B, 2C, and 3B). Fast-spiking parvalbumin-positive GABAergic interneurons in the parietal cortex of hAPP mice have reduced levels of voltage-gated sodium channel (VGSC) subunits (Figures 2B and 2C), and similar alterations are present in patients with AD (Verret et al., 2012). Increasing the level of these sodium channel subunits in the transgenic mice improves gamma oscillations, network activity and cognitive functions. Exploratory activity also increases gamma intensity and reduces network hypersynchrony in some of the mice. ApoE4 and its fragments cause age- and tau-dependent impairments of somatostatin-positive GABAergic interneurons in the hilus of the dentate gyrus, leading to learning and memory deficits (Figure 3B). Stimulation of GABA signaling reverses these deficits (Andrews-Zwilling et al., 2010). Thus, improving the function of interneurons may be an interesting new entry point for therapeutic interventions in AD.

Preclinical and Clinical Trials

Acetylcholine Esterase Inhibitors and Memantine

Drugs that are currently approved by the FDA for the treatment of AD inhibit acetylcholine esterase to increase the levels of the neurotransmitter acetylcholine, which is depleted in AD brains, or antagonize NMDA-type glutamate receptors to prevent aberrant neuronal stimulation (Cummings, 2004). The impact of these drugs on disease manifestations is modest and transient, although observational studies suggest that combination treatment may increase the time before patients require nursing

the identification of phenotypes with potential relevance to schizophrenia (Brennand et al., 2011) and AD (Israel et al., 2012). These exciting developments have renewed interest in cell culture models. Even conventional cell culture models have proved very useful in AD research. For example, they have taught us much about APP processing, Aβ production, and differential effects of apoE isoforms, and many of the findings obtained in these models have held up well in animal models and humans (Bertram et al., 2010; Citron, 2010; De Strooper et al., 2010; Golde et al., 2011; Huang, 2006, 2010; Kim et al., 2009; Mahley et al., 2006; Morris et al., 2011; Palop and Mucke, 2010). Nevertheless, some caveats deserve to be mentioned. While dispersed cultures are well suited for studying cell-autonomous processes, they disrupt the intricate relationships that exist among diverse cell types in the brain and can drastically alter the activity of neurons and glia and their responses to pathogens. In addition, it remains to be determined how best to simulate brain aging in these models, which has such an important impact on the development of AD and other neurodegenerative disorders.

These aspects of the human condition are more easily simulated in animal models. Mice or rats genetically engineered to express human APP/Aβ, tau, apoE isoforms, α-synuclein, or other AD-related factors in brain cells, either individually or in combination, have been particularly informative. Some of the insights provided by these models were highlighted in the text above and many others were reviewed previously (Ashe and Zahs, 2010; Huang, 2006, 2010; Ittner and Götz, 2011; Jucker, 2010; Kim et al., 2009; Mahley et al., 2006; Marchetti and Marie, 2011; Morris et al., 2011; Palop and Mucke, 2010; Querfurth and LaFerla, 2010). The following selection highlights but a few examples.

Neuronal expression of hAPP carrying mutations that cause autosomal dominant AD causes a range of AD-like abnormalities in transgenic mice, including pathologically elevated levels of Aβ in brain, impairments of learning and memory, behavioral alterations, synaptic deficits, aberrant network activity, alterations in neuronal activity-dependent proteins, amyloid plaques, neuritic dystrophy, vasculopathy, astrocytosis, and microgliosis. Notably, several unexpected molecular alterations identified in hAPP transgenic mice have been validated in the human condition, including depletion of voltage-gated sodium channel subunits in cortical interneurons (Verret et al., 2012), depletions of calbindin and EphB2 in dentate granule cells (Cissé et al., 2011; Palop et al., 2003), and hippocampal increases in metenkephalin, activated group IVA phospholipase A_2, and collagen VI (Cheng et al., 2009; Meilandt et al., 2008; Sanchez-Mejia et al., 2008).

In spite of these striking similarities, several concerns have been raised about the relevance of these models to the human condition, in part because hAPP and Aβ are overexpressed in the mice but probably not in most cases of AD. However, overexpression of hAPP and Aβ also occurs in humans with APP gene duplications, and this overexpression causes syndromes that closely resemble sporadic AD (Rovelet-Lecrux et al., 2006). Another concern raised about hAPP mice is that most lines show little, if any, neuronal loss (Ashe and Zahs, 2010; Karran et al., 2011), a clear difference from the human condition. This discrepancy might represent a true species difference but might also suggest that Aβ simply does not kill neurons in vivo or that it requires longer to do so than the usual lifetime of a mouse.

It has also been pointed out that none of the treatments that have shown benefits in hAPP mice have so far shown benefits in the human condition (Karran et al., 2011). As outlined above, there are numerous reasons for drug failure in humans with AD that have nothing to do with species barriers. It is also true that the design of many preclinical trials is not rigorous enough and sensible recommendations to rectify this problem have been made (Jucker, 2010; Shineman et al., 2011). It should further be noted in this context that most transgenic models aim to evaluate specific pathogens in isolation, which is one of their strengths but could clearly be a weakness in the assessment of drugs for AD. Targeting a single factor in a multifactorial pathogenic cascade may well result in detectable benefits when this factor is isolated in an experimental model, but not when it is accompanied by other copathogens in the complex human condition.

Several reasons might account for why cognitive deficits can be detected in hAPP mice before plaque formation and in humans presumably only after plaque formation, including differences in the sensitivity of cognitive tests used, in the ability of mice and humans to compensate for hippocampal deficits, and in numerous variables that affect the deposition of Aβ into amyloid plaques or the formation of Aβ oligomers. Formation of plaques and Aβ oligomers can be reliably dissociated only in experimental models, where functional deficits have been shown to be plaque-independent (Palop et al., 2006; Palop and Mucke, 2010; Tomiyama et al., 2010).

Another concern is that the effect of murine apoE on Aβ metabolism clearly differs from human apoE, particularly with respect to Aβ deposition, which is more prominent in hAPP transgenic mice expressing murine apoE than in those expressing human apoE isoforms (Bien-Ly et al., 2011; Holtzman et al., 2000). Compared with mice lacking apoE, both human apoE3 and apoE4 stimulate Aβ clearance in mice, whereas murine apoE stimulates Aβ deposition (Bien-Ly et al., 2011; Holtzman et al., 2000). This difference has significant implications for understanding the effect of apoE on Aβ metabolism and for validating and interpreting clinical trials of anti-Aβ therapy. The great majority of preclinical trials related to Aβ were performed in hAPP mice expressing endogenous murine apoE (Zahs and Ashe, 2010). If murine apoE differs from human apoE in regulating Aβ metabolism, drugs that work well in hAPP mice with murine apoE might not work well in AD patients with human apoE. hAPP mice expressing different forms of human apoE may have greater predictive value.

Conclusions and Outlook

There often are no simple solutions to complex problems and AD is sadly a good case in point. Although progress in diverse disciplines of science, technology, and medicine has helped unravel many important aspects of this mysterious illness, the field has yet to translate these insights into more effective treatments for AD. We humbly submit the following suggestions as to how this process might be accelerated. Premature clinical

tions. To benefit from these steps before the predicted rise in AD cases overwhelms health care systems around the world, they would have to be implemented expeditiously and on a broad scale. Concerted global efforts and substantial resources will be required to make this happen.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants AG011385 and AG022074 and a gift from the Stephen D. Bechtel, Jr. Foundation. We thank the authors' lab members for many stimulating discussions of these topics, John Carroll for preparation of graphics, and Monica Dela Cruz for administrative assistance. L.M. serves on the scientific advisory board of iPierian and has received research funding from Bristol-Myers Squibb for a tau-related project.

REFERENCES

Andrews-Zwilling, Y., Bien-Ly, N., Xu, Q., Li, G., Bernardo, A., Yoon, S.Y., Zwilling, D., Yan, T.X., Chen, L., and Huang, Y. (2010). Apolipoprotein E4 causes age- and Tau-dependent impairment of GABAergic interneurons, leading to learning and memory deficits in mice. J. Neurosci. *30*, 13707–13717.

Ashe, K.H., and Zahs, K.R. (2010). Probing the biology of Alzheimer's disease in mice. Neuron *66*, 631–645.

Asuni, A.A., Boutajangout, A., Quartermain, D., and Sigurdsson, E.M. (2007). Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements. J. Neurosci. *27*, 9115–9129.

Bales, K.R., Verina, T., Cummins, D.J., Du, Y., Dodel, R.C., Saura, J., Fishman, C.E., DeLong, C.A., Piccardo, P., Petegnief, V., et al. (1999). Apolipoprotein E is essential for amyloid deposition in the APP(^{V717F}) transgenic mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. USA *96*, 15233–15238.

Bales, K.R., Liu, F., Wu, S., Lin, S., Koger, D., DeLong, C., Hansen, J.C., Sullivan, P.M., and Paul, S.M. (2009). Human APOE isoform-dependent effects on brain beta-amyloid levels in PDAPP transgenic mice. J. Neurosci. *29*, 6771–6779.

Ballatore, C., Lee, V.M.-Y., and Trojanowski, J.Q. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat. Rev. Neurosci. *8*, 663–672.

Bareggi, S.R., Franceschi, M., Bonini, L., Zecca, L., and Smirne, S. (1982). Decreased CSF concentrations of homovanillic acid and gamma-aminobutyric acid in Alzheimer's disease. Age- or disease-related modifications? Arch. Neurol. *39*, 709–712.

Barnes, D.E., and Yaffe, K. (2011). The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol. *10*, 819–828.

Bien-Ly, N., Andrews-Zwilling, Y., Xu, Q., Bernardo, A., Wang, C., and Huang, Y. (2011). C-terminal-truncated apolipoprotein (apo) E4 inefficiently clears amyloid-β (Abeta) and acts in concert with Abeta to elicit neuronal and behavioral deficits in mice. Proc. Natl. Acad. Sci. USA *108*, 4236–4241.

Bien-Ly, N., Gillespie, A.K., Walker, D., Yoon, S.Y., and Huang, Y. (2012). Reducing human apolipoprotein E levels attenuates age-dependent Aβ accumulation in mutant human amyloid precursor protein transgenic mice. J. Neurosci. *32* 10.1523/jneurosci.0033-12.2012.

Bousquet, J., Anto, J.M., Sterk, P.J., Adcock, I.M., Chung, K.F., Roca, J., Agusti, A., Brightling, C., Cambon-Thomsen, A., Cesario, A., et al. (2011). Systems medicine and integrated care to combat chronic noncommunicable diseases. Genome Med. *3*, 43.

Braak, H., and Del Tredici, K. (2011). Alzheimer's pathogenesis: is there neuron-to-neuron propagation? Acta Neuropathol. *121*, 589–595.

Braak, H., Thal, D.R., Ghebremedhin, E., and Del Tredici, K. (2011). Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. J. Neuropathol. Exp. Neurol. *70*, 960–969.

Brecht, W.J., Harris, F.M., Chang, S., Tesseur, I., Yu, G.-Q., Xu, Q., Dee Fish, J., Wyss-Coray, T., Buttini, M., Mucke, L., et al. (2004). Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. J. Neurosci. *24*, 2527–2534.

Brennand, K.J., Simone, A., Jou, J., Gelboin-Burkhart, C., Tran, N., Sangar, S., Li, Y., Mu, Y., Chen, G., Yu, D., et al. (2011). Modelling schizophrenia using human induced pluripotent stem cells. Nature *473*, 221–225.

Brodbeck, J., McGuire, J., Liu, Z., Meyer-Franke, A., Balestra, M.E., Jeong, D.E., Pleiss, M., McComas, C., Hess, F., Witter, D., et al. (2011). Structure-dependent impairment of intracellular apolipoprotein E4 trafficking and its detrimental effects are rescued by small-molecule structure correctors. J. Biol. Chem. *286*, 17217–17226.

Brouwers, N., Sleegers, K., Engelborghs, S., Bogaerts, V., Serneels, S., Kamali, K., Corsmit, E., De Leenheir, E., Martin, J.J., De Deyn, P.P., et al. (2006). Genetic risk and transcriptional variability of amyloid precursor protein in Alzheimer's disease. Brain *129*, 2984–2991.

Brunden, K.R., Trojanowski, J.Q., and Lee, V.M. (2009). Advances in tau-focused drug discovery for Alzheimer's disease and related tauopathies. Nat. Rev. Drug Discov. *8*, 783–793.

Brunden, K.R., Zhang, B., Carroll, J., Yao, Y., Potuzak, J.S., Hogan, A.M., Iba, M., James, M.J., Xie, S.X., Ballatore, C., et al. (2010). Epothilone D improves microtubule density, axonal integrity, and cognition in a transgenic mouse model of tauopathy. J. Neurosci. *30*, 13861–13866.

Bu, G. (2009). Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. Nat. Rev. Neurosci. *10*, 333–344.

Buttini, M., Orth, M., Bellosta, S., Akeefe, H., Pitas, R.E., Wyss-Coray, T., Mucke, L., and Mahley, R.W. (1999). Expression of human apolipoprotein E3 or E4 in the brains of *Apoe*$^{-/-}$ mice: isoform-specific effects on neurodegeneration. J. Neurosci. *19*, 4867–4880.