WHITE PAPER

Mapping the Road Forward in Alzheimer's Disease

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Alzheimer's disease (AD) is the most common cause of dementia and will lead to a worldwide public health crisis if it continues unchecked. Despite tremendous advances in our scientific understanding of AD, we still do not have effective ways to delay, prevent, or slow this disease. At the 2011 Abelson meeting, a diverse group of scientists discussed current challenges in the AD field and made recommendations in the areas of genetics, clinical trials, protein aggregation, and the cell biology of the nervous system. We hope these recommendations will boost research progress in AD and increase the likelihood of developing effective therapies in the near future.

Alzheimer's disease (AD) is the most common cause of dementia, with current estimates reporting more than 30 million people afflicted with this malady worldwide. This number is expected to quadruple within the next 40 years. In the United States alone, there are more than 5 million AD patients, with 10 million caregivers, and it is the nation's third most expensive disease, already costing the U.S. government close to $200 billion a year. Currently, there is no effective treatment that delays the onset or slows the progression of AD. Without such disease-modifying treatments, the disease threatens not only to cause great personal suffering but also to bankrupt the health budgets of national economies worldwide. Indeed, following the launch of similar plans in Europe (http://www.alzheimer-europe.org/Policy-in-Practice2/National-Dementia-Plans/France), the Obama Administration in the United States is developing the first National Alzheimer's Strategic Plan that will combine research efforts to understand and combat the disease with aid for caregivers of AD patients (http://www.alz.org/documents/national/report_ASG_alzplan.pdf).

Major scientific advances in genetics, biochemistry, cell biology, and neuroscience over the last 30 years have changed the way we think about AD and have provided a better understanding of the pathogenesis of the disease, yet translation of these research advances into new disease-modifying treatments has been disappointingly slow. We know that AD is a disease of protein aggregation involving accumulation in the brain of aggregates of β-amyloid (Aβ) peptide in the form of amyloid plaques and of tau fibrils in the form of neurofibrillary tangles, but other molecules and pathways also have been implicated [for review, see Holtzman *et al.* (*1*)]. To elucidate and tackle the hurdles that are preventing efficient translation of research advances into new treatments for AD, a group of leading researchers working on AD and other neurodegenerative diseases met in April of this year in Washington, D.C. for a 2-day symposium and workshop to discuss our current understanding of AD and to develop a road map to address some of the biggest challenges preventing translation of research advances into clinical benefits (http://www.aaas.org/programs/centers/pe/abelson/). The 1-day symposium, which covered the genetics, cell biology, and clinical aspects of AD and other neurodegenerative diseases such as Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis, was followed by a 1-day workshop where speakers and invited participants broke up into working groups to discuss four different aspects of AD: genetics, clinical trials, protein aggregation, and the cell biology of neurodegenerative diseases and neurodegeneration (Fig. 1). This White Paper is a summary of the discussions of the working groups that we hope will provide a path to boost our understanding of AD pathogenesis, improve our ability to diagnose the disease in its earliest stages, and aid in the development of effective disease-modifying treatments that can be given early enough to prevent emergence of the devastating clinical symptoms of AD.

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USING GENETICS TO UNDERSTAND, PREDICT, AND DIAGNOSE AD

Rigorous genetic studies will help to elucidate the biological pathways that predispose to AD, identify diagnostic combinations of risk factors, and define how genetic factors interact with environmental factors, some of which may act by influencing the epigenome. So far, genetic studies have identified 10 genes that influence risk for AD in people of European origin. Rare hereditary forms of AD are caused by autosomal-dominant mutations in the *APP* and *PSEN* genes encoding amyloid precursor protein and the presenilin proteins, respectively (*2*). Most of these mutations increase total Aβ or Aβ42 production, leading to increased amyloid plaque formation in the brain, a pathological hallmark of AD (*1*). Unlike these rare familial forms of AD, the causes of so-called sporadic or late-onset AD, which accounts for >99% of all AD cases, are not known. The strongest genetic risk factor for most AD cases is an allelic variant of the *APOE* gene encoding the protein apolipoprotein E (apoE), which affects Aβ clearance and Aβ fibril formation. ApoE may have other functions in the brain, such as in lipoprotein trafficking, synaptic remodeling, and inflammation, which may provide additional pathways by which apoE could influence AD pathogenesis (*3*).

Recently, several large genome-wide association studies (GWAS) have identified variants associated with nine additional genes that modestly influence risk for AD in people of European origin (http://www.alzgene.org/). These genes implicate several biological pathways—including lipid metabolism, innate immunity and endocytic trafficking—that could provide new therapeutic targets for AD (*4*–*6*). The marriage of high-resolution genetic and genomic studies enabled by next-generation sequencing technologies, combined with new stem cell advances and imaging methods, could accelerate progress in understanding mechanisms of AD pathogenesis and may provide new therapeutic leads. We suggest four key initiatives:

(**1**) We need to develop better statistical and phenotyping methods for understanding interactions among multiple genetic variants identified in GWAS and in whole-

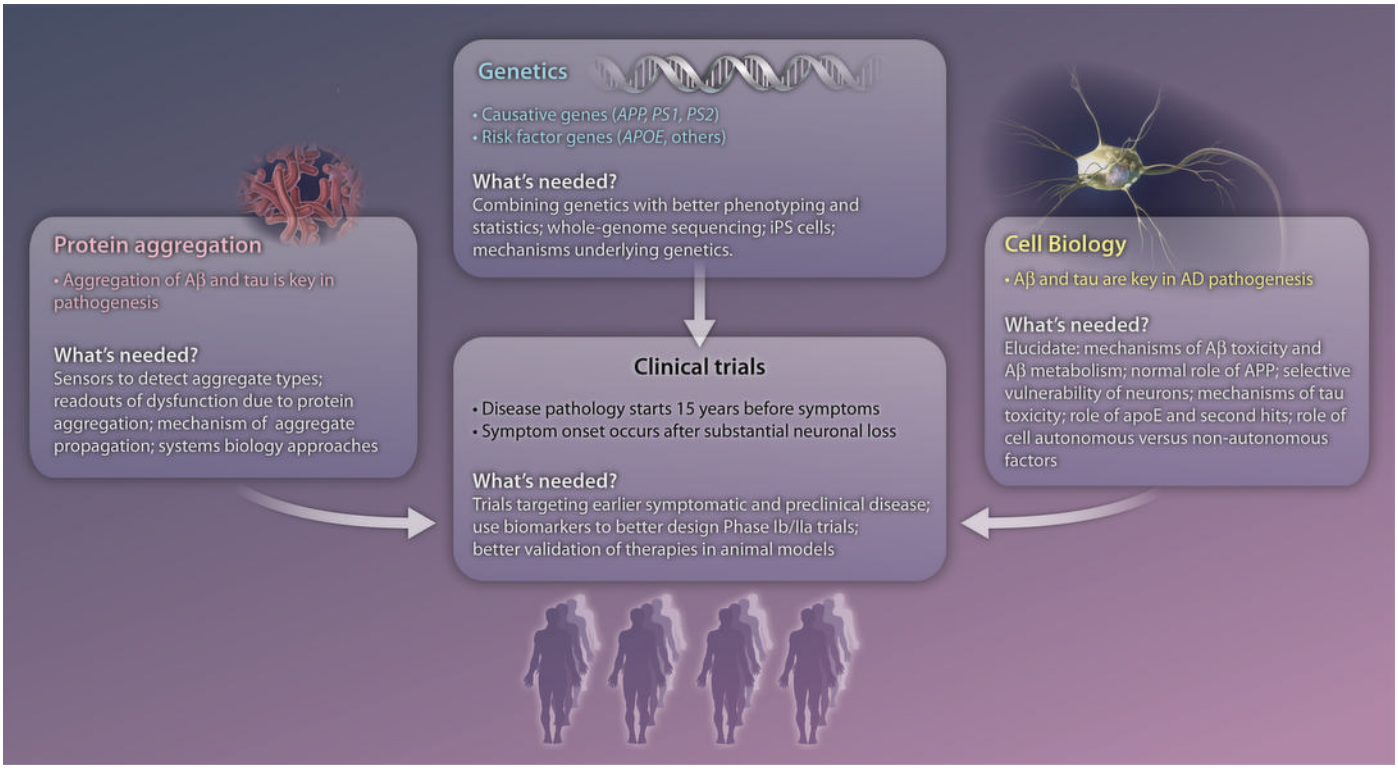

Fig. 1. What do we know and where should we go? AD is strongly influenced by genetic changes that, together with aging and other factors, result in aggregation of specific proteins, such as β-amyloid (Aβ) and tau in the brain. Together, these changes influence neuronal biology, resulting in loss of synapses and death of neurons and leading to brain dysfunction. Better insights into the mechanisms by which genetics, protein aggregation, and the biology of neurons and other cells influence AD pathogenesis will lead to improvements in clinical trial design by enabling the development of informative biomarkers. Such biomarkers will help in selecting specific AD patient cohorts for clinical trials and for monitoring disease progression and the success of disease-modifying treatments. Ultimately, biomarkers will enable early intervention in presymptomatic individuals with the goal of delaying the onset or preventing the cognitive decline seen in AD before the brain is irreversibly injured.

genome sequencing studies. A short-term goal should be to perform meta-analyses and pathway analyses of existing data sets to elucidate the role of recently identified genes and to identify new genes. These studies should also be extended to other correlated phenotypes (behavioral, imaging and biomarker measures, age at onset, rate of decline) using existing well-phenotyped patient data sets to determine the relative importance of genes other than *APOE*. Such studies will identify and characterize new genes and interactions between genes (epistasis) that influence disease risk, age at onset, and rate of cognitive decline as well as additional protective factors, such as *APOE2*, that contribute to healthy cognitive aging. It will be important to determine whether these genes have additive effects on the risk of developing AD or whether some combinations interact in a multiplicative fashion—and whether multiple genes within specific biological pathways contribute to risk. A longer-term goal is to pursue studies in non-European populations given that, to date, *APOE* is the only gene known to influence risk for disease or age at onset in non-European populations. It is essential to determine the gene combinations that affect risk for AD in all major racial and ethnic groups because although *APOE4* is a common risk factor for disease in all populations studied, the allele frequency and effect size do differ. A number of epigenetic studies of aging and dementia are under way (http://www.roadmapepigenomics.org/overview). Including such data in AD genetic studies may improve the accuracy of predicting genetic risk and age at onset. Identification of environmental factors could enable those at high genetic risk to reduce their risk of developing AD through life-style changes such as exercise, cognitive activities, and optimizing both sleep and mental health.

(**2**) We need to perform whole-genome sequencing of individuals and combine this with extensive phenotyping, including clinical, psychometric, pathological, neuroimaging and fluid biomarker measures. Short-term goals include analysis by next-generation sequencing of large families lacking defined *PSEN* or *APP* mutations to identify rare variants causing highly penetrant AD in such families. Even in individuals who are *PSEN* or *APP* mutation carriers or *APOE4* homozygotes, there can be a 25-year range in age at onset for AD. For this type of quantitative trait (age at onset), DNA sequencing of individuals from the extremes of the distribution will help to identify combinations of functional variants. In the longer term, this approach should be applied to large populations, including AD cases and control subjects without dementia who have already been tracked in longitudinal studies; such studies could be enhanced by expression profiling of at-risk individuals over time to identify biomarkers of disease.

(**3**) In addition to genetic studies aimed at identifying functional variants, experiments are needed to determine the mechanism by which sequence variants influence AD risk,

age at onset, and disease progression. Such functional studies have in the past been performed in model organisms and in cell culture systems. Genetically tractable model systems are particularly attractive because of the possibility of identifying genetic modifiers of these new risk genes that could feed back into human studies, but these model organisms cannot adequately model sporadic AD. We need to take advantage of the ability to reprogram skin fibroblasts from AD patients and healthy individuals to generate induced pluripotent stem (iPS) cells that then can be differentiated into neurons, glia, and other cell populations. Obtaining skin biopsies from well-phenotyped and genotyped/sequenced clinical populations of AD patients will enable functional studies in appropriate cell types, such as neurons and glia, of known genetic composition as well as in mixtures of these cells; eventually, functional studies could be conducted on pieces of human brain tissue generated in vitro. A national repository of these fibroblasts/iPS cells with linked clinical and other data should be created and the cell lines should be made available at a low cost to all researchers (http://www.alzforum.org/new/detail.asp?id=2558).

(**4**) The *APOE* genotype is now used routinely to stratify subjects for clinical trials. However, moving forward, we need to use linked genetic and phenotypic profiles to identify presymptomatic individuals who are at high risk of developing AD for prevention trials and to identify mildly demented individuals predicted to show rapid progression to AD for clinical trials testing disease-modifying therapies. Personalized treatment based on genetic profiles will include advising individuals regarding life-style changes that might moderate genetic risk, determining the age at which clinical monitoring should begin in at-risk individuals, and determining the specific drugs and the dose of those drugs that will provide the optimal therapeutic regimen. For example, individuals who carry *APOE4* alleles are at particularly high risk for dementia after head injury with loss of consciousness; such individuals need to make informed decisions about involvement in activities that have a high risk of repeated head injury (e.g., contact sports or combat service in the military).

IMPROVING OUTCOMES FROM CLINICAL TRIALS

A number of disappointing results from large-scale clinical trials involving symptomatic AD patients with dementia suggest that we may need to change course in the clinical testing of disease-modifying therapies for AD. Genetic studies will be helpful in identifying individuals at high risk for developing AD so that clinical trials can be designed for the earliest stages of AD when disease-modifying agents are likely to be most efficacious (*7*). In addition to genetics, biomarkers are proving to be very useful for both the diagnosis and prognosis of AD. The types of biomarkers that currently appear most useful in large-scale studies are measurements of Aβ, tau, and visinin-like protein-1 (VILIP-1) (*8*) in cerebrospinal fluid (CSF), structural neuroimaging with magnetic resonance imaging (MRI), and positron emission tomography (PET) neuroimaging to detect fibrillar forms of Aβ in the brain (Table 1). Despite recent advances in both basic research and identifying biomarkers for AD, critical barriers remain for the successful execution of clinical trials for testing new treatments to delay, modify, or prevent AD. We need strategies to more effectively select promising therapeutic agents from laboratory testing, to design more efficient "proof of concept" studies in patients with early AD, and to pursue new avenues to prevent or slow neurodegeneration during the earliest stages of the disease. We propose four areas on which to focus:

(**1**) The pathophysiological process of AD begins many years before the onset of clinical symptoms. The phase of disease in which AD pathology is accumulating yet an individual is still asymptomatic is termed "preclinical" AD (*1*, *9*). For example, Aβ deposition and tau aggregation are estimated to begin about 15 and 5 years, respectively, before symptom onset. By the time symptoms emerge, there is already significant neuronal loss in key regions of the brain (*10*, *11*). Despite this, virtually all treatment trials completed to date have studied patients with mild to moderate AD dementia in which AD pathology and resulting neurodegeneration are already well entrenched. Therapeutic agents aimed at presumed early events in AD pathophysiology (e.g., Aβ production, clearance, and aggregation or tau aggregation/dysfunction) are most likely to succeed in preclinical AD populations before neurodegeneration has progressed too far. But there are major challenges to designing trials to detect an effect on clinical progression in these asymptomatic populations; true prevention trials could take longer than a decade to complete. Solutions lie in the selection of preclinical subject populations at high risk for developing AD dementia: (a) asymptomatic autosomal-dominant mutation carriers with evidence of amyloid accumulation (although these subjects represent a very small percentage of AD patients), (b) *APOE*ε4 carriers, particularly homozygotes, who are at high risk for developing amyloid pathology, and (c) older individuals whose biomarkers (for both amyloid and "downstream" neurodegeneration) suggest that preclinical AD is progressing, including individuals with very subtle symptoms who do not yet meet

Table 1. Biomarkers for AD.

Markers of Aβ accumulation
Aβ in CSF
Assays for $A\beta_{42}$ or $A\beta_{42}$: $A\beta_{40}$
Amyloid imaging by PET
^{11}C-PiB or ^{18}F radiotracers bind to fibrillar Aβ
Markers of Aβ production or clearance
Aβ in CSF and possibly plasma
Stable isotope labeling kinetics (SILK) for detecting CSF Aβ
Markers of synaptic dysfunction
^{18}F-fluorodeoxyglucose (FDG) PET
Measures resting synaptic function via glucose metabolism
Functional MRI
Measures task-based activation and resting neural connectivity
Indirect measures of neuronal activity and network integrity via blood-oxygen level dependent (BOLD) T2 techniques
Markers of neurodegeneration
Tau, phospho-tau, visinin-like protein-1 (VILIP-1) in CSF
Markers of neuronal loss
MRI to measure:
Cortical thinning in parietal and temporal cortices
Ventricular volume (less specific for AD)
Hippocampal volume (correlates well with memory decline)

the criteria for mild cognitive impairment or very mild dementia (*12*).

Consideration should also be given to more innovative trial designs across the spectrum of AD treatments. Lengthy prevention trials may benefit from using adaptive trial designs, beginning with biomarker assessments of target engagement and adapting dosing, or using subgroups of "responders" for long-term clinical follow-up. Testing of multiple agents in "2x2 Factorial" trial designs may also be advantageous, e.g., combining an antiamyloid agent with an antitau agent. This will likely require cooperation across companies and academia to allow drug-drug interaction testing in early phase studies and working with regulatory authorities to develop efficient safety monitoring protocols for combination therapies.

(**2**) The design and efficiency of "proof-of-concept" Phase Ib/IIa trials in AD needs to be improved. The majority of recent Phase III trial failures did not have convincing Phase II data supporting their continued development. We need to develop better biomarkers, especially biomarkers that can track individual trajectories of disease progression and predict therapeutic responses, so-called theranostic markers. One example of such a marker is stable isotope labeling kinetics in which one can assess the synthesis and clearance rates of proteins such as Aβ in CSF. These biomarkers must also be standardized for use in multiple centers with highly reliable measurements. We must require evidence of target engagement in human studies, with rigorous evaluation using predefined end points. Ideally, we would develop short-term pharmacodynamic markers that predict long-term responses to disease-modifying treatments, such as functional imaging or more sensitive markers of cognition. The majority of current Phase II AD trials are 18 months long and are often insufficiently powered to see a clinical effect. Should we conduct multiple shorter Phase II trials before moving to larger, longer pivotal Phase III trials? Algorithms are needed to define "leveraged" populations for these Phase II studies, such as patients who are likely to manifest rapid clinical decline, perhaps identified by the presence of a particular AD biomarker signature. A critical barrier to successful proof-of-concept trials is the lack of sensitive cognitive measures to assess therapeutic benefit in a shorter time frame that can be reliably administered across multiple sites. It will be especially important to develop and utilize sensitive measures that change in individuals who are in the earliest clinical stages of the disease.

(**3**) The past two decades have seen a number of disappointing failures in AD treatment trials based on promising epidemiological data. This highlights the "disconnect" between results from large epidemiological cohorts (studied over many years) and randomized clinical trials in clinically impaired individuals. For example, whereas epidemiological data suggest that anti-inflammatory medications such as nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the risk for AD, treatment trials involving NSAIDs and patients with AD dementia have not shown efficacy. There may be many reasons for this, including the possibility that NSAIDs may act differently at different stages of disease (*13*). To better translate promising findings from epidemiological studies into successful randomized clinical trials, epidemiological studies need to incorporate biomarkers from CSF sampling, PET imaging of amyloid, or another biomarker modality to better interpret outcomes. Meanwhile, biomarker studies and randomized clinical trials that typically recruit "samples of convenience" need to select populations that are fully representative of individuals at risk for dementia. The goal would be to launch several large studies of middle-aged and early late-life subjects recruited with epidemiological principles and embed a number of sensitive biomarkers collected on at least a representative subset of the cohort. If these cohorts are large enough, one might be able to later recruit from within these studies for randomized trials of promising new therapeutic agents. One possibility would be to consider cohort studies within large managed health care plans or even within the Medicare system to take advantage of existing data collection systems.

(**4**) Another barrier is selection of the most promising therapeutics from preclinical animal studies for testing in large-scale clinical trials. Current animal models are not ideal for all purposes, as they are primarily models in which some, but not all, aspects of AD pathogenesis are present. For example, the majority of mouse models are based on genetic forms of autosomal-dominant AD characterized by overproduction of Aβ. There are drugs that reduce Aβ production, with a decrease in Aβ concentrations in the brain presented as evidence of "target engagement." Decreasing production of Aβ may ultimately prove to be an effective therapy; however, recent evidence suggests that sporadic AD may be related to impaired clearance of Aβ rather than increased production of Aβ (*14*). More importantly, tau aggregation and tau-mediated neurodegeneration are not present in most mouse models that overproduce Aβ. Thus, even if Aβ is reduced, these mouse models cannot indicate whether this change will reduce downstream neurodegeneration. Furthermore, although several double and triple transgenic mouse models have been shown to develop both Aβ and tau pathology (*15*), they typically do not manifest the frank neuronal loss seen in human AD patients. We need new models in genetically tractable animals, such as mice that develop both Aβ and tau pathology as well as neurodegeneration with neuronal loss. We also need additional worm and fly models that are useful for high-throughput drug screening. A public resource providing access to all preclinical animal models should be available.

We also need more rigorous evidence of preclinical efficacy before we move into human studies. Ideally, there would be conclusive evidence of target engagement (e.g., a drug that lowers Aβ production or decreases Aβ plaque burden, decreased tau, or evidence of a reduction in neurodegeneration, etc.) in more than one animal model and rigorous standardized operating procedures for assessment of preclinical behavioral or other functional effects. We must develop better pharmacodynamic markers that can more easily translate from animal into human physiological and behavioral studies and set stringent standardized thresholds for decisions to move into clinical development.

PROTEIN AGGREGATION IN NEURODEGENERATIVE DISEASE

The aberrant aggregation of Aβ and tau proteins is the hallmark of AD, but protein misassembly and aggregation is an early event common to many other neurodegenerative diseases, including Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, the transthyretin amyloidoses, and prion diseases (*16*). There are four key areas that need further study:

(**1**) Protein aggregates form several different structures in these neurodegenerative diseases, including oligomers, protofibrils, and fibrils. However, it remains unclear which forms of these aggregated proteins are present in each disease, which forms are toxic, and what their locations are in the cell and the whole organism. Thus, a major goal

is to develop tools and sensors to distinguish between the different forms of these proteins in vitro and in vivo. Sensors could be small molecules, antibodies, modified aggregation-prone proteins, or polymers such as sulfated glycosaminoglycans. Devices for diagnosis—e.g. a microfluidic gel filtration device to distinguish the spectrum of protein aggregates in plasma or CSF—would also be useful. It will be important to make structure-pathology correlations for each species of aggregate that is sensed and to understand aggregate dynamics. Ex vivo sensors would be appropriate for proof-of-concept studies, but ideally they would need to be designed so that they could be used in vivo as well. It would be desirable to monitor multiple misfolding-prone and aggregation-prone proteins simultaneously for a given pathology. The goal is to connect structural data with nomenclature and with biological (pathological) consequences. Success will require an influx of chemists, physicists, and engineers to collaborate with biologists.

(**2**) Observations about protein misfolding and aggregation derived from these new sensors and tools will need to be correlated with dysfunctional readouts in multiple organisms as a function of age and disease. Such readouts of dysfunction could include electrophysiology, altered brain connectivity, changes in protein homeostasis (proteostasis) in different subcellular compartments, alterations in vesicular trafficking, and compromised proteasomal and lysosomal activity. How do protein aggregates cause dysfunction and ultimately neuronal loss and which aggregates are important? Is neurodegeneration driven by a neuronal-based (cell autonomous) or a non-neuronal based (cell non-autonomous) mechanism? The aggregation sensors developed need to be correlated with disease phenotypes in animal models and in humans to establish biomarkers for diagnostic and prognostic tests. Molecules that prevent or alter the aggregation of intrinsically disordered proteins or of structured proteins that have undergone disordering need to be developed and then interrogated with these tools for their ability to ameliorate disease. Possibilities include small organic molecules that stabilize the nonaggregated form of the protein (*17*), as well as molecules (such as peptides or peptidomimetics) that specifically block the formation of aggregate structures that compromise the integrity and function of tissues.

(**3**) Murine and primate studies suggest that aggregates can spread from one cell to another by an apparent seeding mechanism. Both cell autonomous and non-autonomous mechanisms may be involved in such aggregate propagation, and propagation may be both an early and a late event. Understanding the mechanisms of propagation using both in vitro and in vivo models may provide new targets for interfering with the spread of aggregates and the development of pathology in the brain (*18*).

(**4**) We need to elucidate how information from models of neurodegenerative diseases correlates with human pathological observations with respect to the proteostasis network, genes conferring risk, inflammatory pathways, degenerative mechanisms, and other cellular or immune functions. It will be useful to recruit systems biologists to work with cell and molecular biologists and medicinal chemists to integrate data across cellular and multicellular neurodegenerative disease models to discern which pathways are most critical to neurodegeneration in humans.

THE CELL BIOLOGY OF NEURODEGENERATION

Aberrant protein aggregates play a prominent role in AD pathogenesis, but how they elicit the cascade of events that results in neurodegeneration and cognitive impairment remains unclear. Four general areas of pursuit and the questions that need to be addressed in each area are outlined for near-term studies to address this chasm in our understanding:

(**1**) Aβ aggregation seems to be a key event in AD pathogenesis that can be instigated by baseline differences among individuals in Aβ production, aggregation, and clearance (*1*, *19*). Genetic or other factors may lead to a first "hit" in which Aβ accumulation into different toxic forms is initiated. However, a variety of other factors or events appear to be necessary for the cognitive decline characteristic of AD to ultimately take place. Regarding Aβ itself, there is still much we do not know about its role in AD, for example: Which forms of Aβ are toxic, which pathways are most relevant to toxicity, how can the toxic species be monitored, are both intracellular and extracellular Aβ species toxic, and how is Aβ metabolized? Could some aspect of APP metabolism exclusive of its processing to Aβ contribute to AD? To elucidate the role of Aβ in AD, we need better animal models that do not rely on overexpression and that mimic multiple aspects of AD, including Aβ aggregation, tau abnormalities, neuronal network dysfunction, and the loss of synapses and neurons. For example, it would be useful to understand why most of the available mouse models that develop Aβ aggregates and associated pathology do not develop substantial neuronal death.

Which specific cell types contribute most to Aβ production and clearance, and do different cell types (e.g., monocytes and microglia) preferentially clear monomers, oligomers, and aggregates? Is there some way to activate endogenous cells that clear Aβ? Is there a receptor for Aβ and, if so, which cell types produce the receptor, and do specific forms of Aβ bind to the same or a different receptor? Does Aβ-mediated neuronal/synaptic dysfunction contribute to AD independently of neuronal death, and does neuronal death follow loss of synaptic interactions?

(**2**) We need to clarify at the level of macro-brain circuitry and microcellular connectivity the major sites of neuronal dysfunction during the early, mid- and late phases of AD. We need to elucidate precise molecular mechanisms of cellular dysfunction and dismantling of the neural circuitry in neurodegeneration by identifying the signaling pathways, proteases, and cytoskeletal programs involved. On the other hand, we also need to determine whether neurogenesis could help in slowing or preventing AD. Although there is no direct evidence in humans that neurogenesis is impaired in AD, mouse models that develop amyloid deposition do show impaired neurogenesis (*20*). We need to elucidate whether genetic changes could predispose individuals to AD by altering neurogenesis and whether glia contribute to this process.

(**3**) Other key cell biological hallmarks and the time course of neuronal, glial, and neurovascular abnormalities that contribute to brain dysfunction in AD remain unclear. Such hallmarks include tau aggregation and neurofibrillary tangle formation, synaptic and axonal loss, gliosis, amyloid angiopathy, and impaired vascular function. How is tau protein aggregation linked with neuronal and synaptic dysfunction? Does soluble tau as well as aggregated tau play a role in neurodegeneration in AD? We need to nail down how Aβ exacerbates tau aggregation and neurodegeneration. Can tau be targeted as a therapy for AD by lowering its concentration using small molecules and by decreasing its aggregation and spread using biological agents such as antibodies? In

addition, we still do not understand mechanistically how apoE influences the risk for developing AD. ApoE affects Aβ metabolism and aggregation, but how does it do this? Does it also affect Aβ toxicity? Could primary abnormalities of lipid metabolism that are influenced by apoE or other factors, either in the central nervous system (CNS) or systemically, contribute to AD? Could modifying CNS lipid metabolism slow or prevent neurodegeneration? Does apoE affect synaptic function or pruning of synapses independent of any effect on Aβ?

(**4**) Finally, what are the key factors and stressors underlying susceptibility to neurodegeneration in AD, the so-called "second hit"? We know that individuals with seemingly similar Aβ and tau accumulation in the brain can be clinically affected to differing degrees. This suggests that in addition to AD pathology other factors (the second hit) are important. Such factors may include aging, brain trauma/ischemia, bioenergetic stress, noncanonical mitochondrial functions, synaptic activity, selective neuronal vulnerability to Aβ and tau, and elements of the cellular environment that could promote neuronal dysfunction. Animal models that develop AD pathology concomitant with decreased brain function and neuronal loss should be used to determine which of these second hits exacerbates or protects against neurodegeneration. Little work has been done on the role of bioenergetics in AD pathophysiology. We do not know whether abnormalities in glucose utilization contribute to AD, why regions of high aerobic glycolysis in the brain are the regions of highest Aβ plaque accumulation (*21*), and whether defects in mitochondrial function contribute to AD. Other important areas that are understudied include life-style factors such as stress, sleep, and exercise and how they relate to bioenergetics and AD risk.

SUMMARY

There have been tremendous advances in our understanding of the scientific underpinnings of AD over the last 30 years. Major advances in genetics, cell and molecular biology, systems neuroscience, and biomarkers have set the stage for the development of the first truly effective therapies for AD. However, transitioning from scientific understanding to treatments to help AD patients has been painfully slow. Much more work is needed in the following areas: the cell and molecular biology of protein aggregate formation and neurodegeneration; genetics; developing informative biomarkers and reliable methods of early diagnosis; and the design and implementation of new clinical trials. We believe the stage is set to accomplish these goals and that there is still reason to be optimistic that truly effective disease-modifying treatments for AD can be developed in the next decade.

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