

## Eyes on the prize: Federal Alzheimer's research effort aims to facilitate interventions

Richard J. Hodes, Neil Buckholtz, Vicky Cahan\*, Marcelle Morrison-Bogorad

*National Institute on Aging, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD, USA*

### Abstract

The public Alzheimer's disease (AD) research enterprise began in earnest in the mid-1970s with the creation by Congress of the National Institute on Aging at the National Institutes of Health. Today, AD research is a maturing field of study, with federal effort seeking to encourage the creativity and insights of individual investigators, and targeting special areas for emphasis. It is inspired by the legacy of our friend and colleague Leon Thal, whose innovative and collaborative approach to scientific research serves as a guidepost as we move toward the discovery of new and effective ways to prevent AD or slow its progression. This article describes the progress to date and potentially promising areas of study from the vantage point of the National Institute on Aging.

© 2008 The Alzheimer's Association. All rights reserved.

**Keywords:** Alzheimer's disease; National Institute on Aging; Dementia

### 1. Introduction

It is fitting that this issue of *Alzheimer's & Dementia* honors the life and work of our friend and colleague, Dr. Leon Thal. Leon's research career, in important and perhaps some surprising ways, not only reflects the four-decade journey of one scientist, but also parallels the journey of the research community and of Alzheimer's patients and their families to understand, treat, and prevent the devastation of Alzheimer's disease (AD) and other dementias.

In 1906, Alois Alzheimer first reported on the case of 51-year-old Auguste D with what we now know were the plaques and tangles characteristic of AD. But it was not until neurobiological research entered a new era in the 1960s and 1970s that recognition of Alzheimer's-type dementia as a pathology associated with aging began to take hold, just as a young Leon Thal was in the midst of his medical training [1]. In those years, Leon, a compassionate new physician and young colleague of pioneering researcher Dr. Robert Katzman, was drawn to the human suffering of dementia in late life. The adventurer in him became engaged in the world of scientific discovery that lay ahead, starting as a Fellow in the Interdepartmental Training

Program at Albert Einstein College of Medicine in 1975. Thus began a career involved in virtually every aspect of research on AD, including a critical role in the development of the federal research agenda.

The public AD research enterprise began in earnest when Congress created the National Institute on Aging (NIA) in 1974 as a component of the National Institutes of Health (NIH). The NIA was tasked to broadly address the "aging process and the diseases and other special problems and needs of the aged" through scientific study, training of scientists, and dissemination of information. Early on, the NIA specifically recognized AD and dementia as a research priority. In 1978, the NIA set up a research program, developed and led by Dr. Zaven Khachaturian, to encourage scientific exploration in this area [2]. The program's focus was on the elucidation of basic disease processes, and on defining and measuring common clinical characteristics of the disease for research and diagnostic purposes. Highlighted by the creation of the Alzheimer's Disease Centers (<http://www.nia.nih.gov/Alzheimers/ResearchInformation/ResearchCenters/>) network in 1984, a novel infrastructure to support research was also established.

Today, some 30 years later, the NIA's leadership in a maturing field of AD research seeks to encourage the creativity and insights of individual investigators while target-

\*Corresponding author. Tel.: 301-496-1752; Fax: 301-496-1072.

E-mail address: [cahanv@nia.nih.gov](mailto:cahanv@nia.nih.gov)

ing special areas for emphasis. This approach was a driving force behind the trans-NIH AD Prevention Initiative, launched in February 2006, as well as the conference, “AD: Setting the Research Agenda a Century after Auguste D,” which NIA scientists and colleagues at the NIH hosted in October 2006. Nearly 200 leading investigators and policymakers heard overviews of the status and of future directions in specific areas of research, and engaged in lively discussions concerning what we know about AD, what we have yet to learn, and how to set an agenda to get where we need to be. Presentations covered ongoing research on the role of  $\beta$ -amyloid and tau; the development and use of animal models; genetic and environmental risk and protective factors; the relationship between AD pathology and clinical manifestations; neuroimaging and other biomarkers; drug discovery and development; and clinical trials. Participants also assessed progress and opportunities in emerging areas of interest such as vascular mechanisms; the relationship of normal age-related changes to changes in AD; the use of biomarkers to track disease progression; common mechanisms among neurodegenerative diseases; and the role of neural networks in modulating the effects of AD pathology.

The meeting recognized the enormous progress made and articulated promising avenues ahead. But if one theme emerged, it consisted of a growing appreciation of how pathologically and clinically complex a disorder AD is. Understanding the genetic and environmental influences, including lifestyle and health factors, that put an individual at risk for developing AD will require increasing input from a variety of scientific disciplines. The NIA's broad program of basic, clinical, and social and behavioral studies of AD and other age-related dementias aims at tapping into those disciplines, with growing emphasis on bringing what we learn from the bench to the bedside as rapidly as possible.

## 2. Basic understanding

Research at the cellular and molecular levels has focused from the beginning on understanding the processes which interfere with, or can enhance, the function and survival of neurons and their connections. The aim is to identify targets for developing therapies, so that cell and circuit dysfunction and death can be avoided or reduced, and memory can remain intact. Among the first biochemical changes found to occur in AD were those involving levels of choline acetyltransferase (ChAT) [3], which affect the synthesis of acetylcholine, a neurotransmitter important in the formation of memories. In the 1970s, scientists discovered that levels of acetylcholine fell sharply in people with AD. Leon was actively involved in the design and conduct of the first multisite clinical trial of a drug to inhibit the acetylcholinesterase enzyme that breaks down acetylcholine. The drugs which were developed based on that research are those still primarily used today for the treatment of Alzheimer's symp-

oms. Equally important, the administrative framework devised for that clinical trial, which utilized the Alzheimer's Disease Centers, is still in place; it formed the basis for the Alzheimer's Disease Cooperative Study (ADCS), which was led by Leon from its inception.

Interest in mechanisms at the basic level is ongoing and productive, and the potential roles of  $\beta$ -amyloid and tau in neuronal toxicity continue to be a source of intense investigation. Studies of  $\beta$ -amyloid have moved forward to the point of initial testing in humans of potential therapies aimed at halting  $\beta$ -amyloid formation or degrading early forms before harmful complexes have taken shape. We know a great deal, for example, about the role of secretases in the production of  $\beta$ -amyloid and the potentially toxic effects that plaques exert on neurons and in cellular communication. A new area of focus is investigating how cellular communication may be disrupted well before plaques form. Smaller, soluble forms of amyloid, i.e., oligomers, may be the main culprits in harming neurons, and one recent study described how  $\beta$ -amyloid oligomers target specific synaptic connections between neurons, causing them to deteriorate [4].

One fascinating line of research began with the observation that injecting amyloid into AD transgenic mice caused them to form antibodies to  $\beta$ -amyloid, and reduced the number of amyloid plaques in the brain [5]. This seminal observation led to further studies. Ultimately, the work led to immunization treatment trials in humans, although the initial phase 3 trial was halted because of meningoencephalitis in 6% of the immunized participants. Presently, more refined antibody approaches are at the stage of being tested in clinical trials. Research on new ways of harnessing the antibody response continues in the laboratory, encouraged in part by a request issued by the NIA for research applications.

Once thought to have a secondary role in AD, tau is emerging as a leading player in Alzheimer's pathology, and is generating new excitement as an area of study. The recent focus on tau was spurred by the finding that a mutant form of tau is responsible for one form of the second most common cause of dementia, frontotemporal dementia, known as “frontotemporal dementia and parkinsonism linked to chromosome 17” (FTDP-17) [6], indicating that abnormalities in tau can cause dementia. The mutant tau gene was used to form a triple transgenic mouse that also incorporated the mutant amyloid precursor protein and mutant presenilin [7], which, over time, forms plaques and tangles in regions akin to those in AD. In 2005, a new tau knockout transgenic mouse model was developed that made only nonmutant human tau protein, which formed clumps of intracellular tau filaments in a region-specific fashion similar to that of AD in humans [8]. The same study suggested a mechanism for tau pathology different from that previously suspected: that the influence of tau on cell death may have more to do with its interference in the cell-cycle

process than its role in the formation of neurofibrillary tangles. Additional studies of mutant tau accumulation in mice also suggest that the accumulation of tau in insoluble clumps of paired helical filaments may not be the culprit in memory loss, but that some earlier abnormal form of the protein, as seen in  $\beta$ -amyloid, may trigger cell death [9]. Surprisingly, an AD transgenic mouse that normally exhibits plaque neuropathology with age exhibited no such neuropathology when the tau gene was eliminated from its genome [10].

Insights that may be critically important for AD also come from studies of brain abnormalities resulting from common mechanisms in a number of neurodegenerative diseases. For Huntington's disease, amyotrophic lateral sclerosis, AD, Parkinson's disease, and Creutzfeldt-Jacob disease, a hallmark in the brain is the appearance of misfolded and mutant proteins (Huntingtin, TDP-43,  $\beta$ -amyloid,  $\alpha$ -synuclein, and prions) and associated toxicity. The identification last year of TDP-43 as a constituent part of the aggregates that form in amyotrophic lateral sclerosis and some forms of frontotemporal dementia provided the first molecular link between a dementia and a motor neuron disease [11]. Further research will examine the role of the protein in the disease process. Recent efforts to understand similarities between the structures and interactions of protein aggregates in these diseases might point the way to therapeutic interventions that could affect these processes and treat age-related neurodegenerative diseases [12].

Another set of insights derives from the most basic commonality among different neurodegenerative diseases: aging itself. Experiments in worms showed that the introduction of a gene that increases the lifespan also suppresses the toxicity of  $\beta$ -amyloid [12,13]. Age-related changes, such as inflammation and the generation of free radicals, which may also exacerbate AD pathology and age-related reductions in the efficacy of certain synapses, may predispose to failed synaptic communication and cell death in similar regions of AD brains. Likewise, age-related reductions in levels of particular growth factors such as nerve growth factor (NGF) may also predispose to the age-related atrophy of selected cell populations. In nonhuman primates, the specific loss of neuronal activity can be reversed by NGF gene therapy [14]. Because cholinergic-cell populations that are atrophied with aging are the same as those that decline in AD, Tuszynski et al. adapted the techniques originally developed in rat and primate studies for NGF gene-transfer trials in patients with AD [15].

### 3. Genetic causes and risk factors

Until recently, only four of the approximately 30,000 genes in the human genome were conclusively shown to affect the development of AD. Mutations in three genes (*APP* on chromosome 21, *Presenilin 1* on chromosome 14, and *Presenilin 2* on chromosome 1) cause the rare, early-

onset form of familial AD. *Apolipoprotein e4 (APOE e4)* on chromosome 19 is the only known risk-factor gene influencing development of late-onset disease. Since their discovery, the roles of these genes have been the subject of increased scrutiny, and major progress has been made, for example, in clarifying the biology of presenilin proteins even beyond their role in snipping  $\beta$ -amyloid out of its precursor protein. Further study has suggested how presenilins may play a role in short-term memory formation. In an investigation of presenilin 1, knockout mice without presenilin 1 in their forebrain were able to learn normally but retained old memories better than new ones, suggesting that the loss of the presenilin gene may result in an inability to clear old memories from the hippocampus [16]. In theory, this might result in an inability to store new memories, as happens with people who have AD.

Most experts believe that at least half a dozen more genes in addition to *APOE e4* may influence the development of late-onset AD in some way. Recently, a worldwide collaboration of researchers found that variations in *SORL1* may be a risk factor in the development of late-onset AD [17], and research is ongoing to determine the role of *SORL1* in disease pathogenesis. To further such gene-finding efforts, an NIA priority involves support for the collection of biological samples for genetic analyses, matched with important clinical and other phenotypic data, and the maintenance and sharing of databases in this area. A centerpiece of this effort is the NIA Alzheimer's Disease Genetics Initiative, which has recruited nearly 1,000 families with members who have late-onset AD, as well as unaffected family members, with participants providing clinical and neuropsychological data and blood samples.

The National Cell Repository for AD (NCRAD), located at Indiana University (Indianapolis, IN) ([www.NCRAD.org](http://www.NCRAD.org)), serves as the central repository for the initiative, providing DNA and cell lines to qualified investigators for genetics studies. In 2006, the NIA opened the Genetics of Alzheimer's Disease Data Storage Site ([www.niageneticsdata.org](http://www.niageneticsdata.org)) at Washington University (St. Louis, MO), where scientists who use NCRAD samples and data, and AD geneticists funded by the NIA, are asked to submit published data to the storage site for additional analysis by qualified investigators. An initial subset of cases and controls recently underwent a whole-genome scan at a low single nucleotide polymorphism density, and results are now available on the website. The National Institute of Mental Health (NIMH) also has a large sample repository and genetics database, and in 2006, data from the combined NIMH and NIA sample sets became available through a unique data repository shared by the two institutes.

The recent availability of Genome Wide Association Studies (GWAS) and the realization that tens of thousands of samples will be needed to identify the remaining risk factor genes for AD have led geneticists in the field to form a collaborative initiative to pool samples and analyses for

such studies and apply for funding to the NIH. Data from appropriate GWAS studies will be deposited in a database, dbGaP ([www.view.ncbi.nlm.nih.gov/dbgap](http://www.view.ncbi.nlm.nih.gov/dbgap)), set up by the NIH to make GWAS findings on any disease available to other researchers in as timely a fashion as possible. With such efforts, the search for the genetic underpinnings of late-onset disease will intensify, to identify who is at high risk of developing Alzheimer's, to understand the mechanisms at work, and to focus on new pathways amenable to prevention or treatment.

#### 4. Nongenetic risk and protective factors

Epidemiologic and longitudinal research in different populations has contributed to our understanding of the frequency of AD in older persons. Community-based studies that include the most mild forms of AD extrapolate that there may be as many as 4.5 million persons age 65 and older with AD in the U.S. [18]; recent estimates from a nationally representative sample in the Aging, Demographics, and Memory Study (ADAMS), part of the ongoing Health and Retirement Study, suggest that one in seven Americans age 72 and older has dementia—some 2.4 million with AD [19]. The risk of AD increases exponentially with age, and projections suggest that the numbers of people with AD will increase with aging of the population unless effective interventions are found [18]. Estimating the number of persons with AD now and in the future is important in helping to characterize both current and future societal costs of the disease. Equally important, the ability to reliably track trends in AD incidence and prevalence over time may provide insight into potential risk and protective factors and clues to the design of interventions. NIA will continue its interest in research in this area. Recently, around one third of individuals at a mean age of 82 to 85 years without clinical dementia or mild cognitive impairment (MCI) who were studied in two community-based studies, the Religious Orders Study and the Memory and Aging Project, were shown to meet the neuropathologic criteria for intermediate or high likelihood AD [20]. Many persons of similar age with MCI who had not yet advanced to a clinical diagnosis of AD were shown to already have advanced AD pathology [21,22]. Longitudinal positron emission tomography (PET) analysis of cognitively normal persons with Pittsburgh compound-B (PiB), which labels brain amyloid plaques in vivo [23], is ongoing, and preliminary studies show that around 20% of persons with normal cognition, aged 66 to 86 years, manifest positive PiB binding [24,25]. Longitudinal studies will clarify the relationship between AD brain pathology and the eventual diagnosis of MCI and AD, as well as when the diagnosis can be made with certainty.

Epidemiologic studies are also important in identifying potential factors that may contribute to or protect from the risk of AD, separately or interactively with genetics, and these studies are among the most active areas of research.

Two key areas of focus have emerged: lifestyle and the management of chronic illness.

An array of epidemiologic research, complemented by basic studies in animals and limited clinical trials, suggests that exercise and diet may influence the risk of developing AD, although definitive evidence from clinical trials does not yet exist to support this possibility. Americans have heard the evidence that exercise, for example, is beneficial, building muscles, improving heart and lung function, helping to prevent osteoporosis, and improving mood and overall well-being. But does exercise or physical activity prevent AD? Certainly, the epidemiologic evidence is intriguing. Among the most recent studies to suggest an association is a report from the Adult Changes in Thought Cohort (Seattle, WA), which found, after a follow-up of 6 years, that the risk of AD in people who exercised  $\geq 3$  times per week, at least 15 minutes per day, was 35% to 40% lower than in those who exercised  $< 3$  times per week [26]. Investigations of the Honolulu-Asia Aging Study population [27] and of older adults who participated in the Cardiovascular Health Study (CHS) have yielded similar results. In the CHS, for example, the association of exercise with a reduced risk of dementia was stronger in people who did not have the *APOE* e4 allele [28].

Association studies are complicated by difficulties in isolating one component of a lifestyle, such as exercise, and to deem it responsible for a change in risk of a disease. Animal studies to pin down these associations and help explain why exercise might reduce the risk of cognitive decline or dementia have yielded further positive results. One study demonstrated that rats who “exercise” daily or on alternate days showed increased levels of brain-derived neurotrophic factor, even after a period of inactivity [29]. Exercise was also shown in mice to affect the progress of AD pathology. Mice with access to an exercise wheel, one study found, had significantly fewer  $\beta$ -amyloid deposits in the hippocampus and cortex than did mice without the wheel [30].

A few clinical trials in people demonstrated that aspects of both brain function and volume improve with physical exercise in the short term. For example, functional magnetic resonance imaging measures of changes in brain activity in older adults before and after a 6-month program of brisk walking found that the activity of neurons in key parts of the brain increased along with the participants' cardiovascular fitness [31], and a similar aerobic intervention was found to increase brain volume in older adults [32]. Small-scale trials currently underway are looking at the effects of 1-year aerobic fitness training on cognition and brain activity and structure in older adults; on the role of aerobic exercise on electrocortical and behavioral measures in older adults; and on the effects of a short aerobic conditioning program on cognitive function in older adults with MCI. A 3-year study of a group-exercise and health-education intervention in patients with MCI will examine a variety of endpoints, e.g., whether the intervention will slow the progression from MCI to dementia. Additional clinical trials are critical in this

area to determine whether exercise can in fact prevent long-term cognitive decline or AD and, if so, to determine the type and amount of exercise necessary. The NIA is actively supporting research in these areas, and seeks to do more.

The results of these trials, however, will not be available for several years. What can we say to the public about exercise in the meantime? It is well-established that regular physical exercise is a low-risk, low-cost intervention that may help ward off important age-related diseases and conditions such as cardiovascular disease and diabetes. As we seek to demonstrate whether there is a causal relationship between physical activity (or the lack thereof) and the risk of AD, there is every reason to encourage older persons to be active and maintain or improve their fitness.

Further, would combining several components of a healthy lifestyle (e.g., behavioral enrichment, exercise, proper diet, and social interaction) be more protective than exercise alone? Both behavioral enrichment and antioxidant dietary fortification slowed cognitive decline in aged dogs, and the combination treatment was more effective than either alone [33]. The Advanced Cognitive Training for Independent and Vital Elderly Clinical Trial showed that persons who were trained separately in four cognitive domains still showed significant improvements in their particular domain, compared with controls, even after 5 years [34]. There was no transfer of training effect between domains. There was modest improvement in Instrumental Activities of Daily Living, but only the effect of reasoning training on the self-reported performance of daily tasks was statistically significant. Whether any of these interventions would actually delay the onset of MCI or AD is not presently known.

A growing body of evidence suggests that certain health conditions may influence AD in a number of possible ways. Metabolic or structural changes that occur in the brain in response to chronic diseases such as heart disease, stroke, high blood pressure, and diabetes may contribute to the development of AD, affect its severity, or cause vascular dementia, which can contribute to clinical severity [35,36]. One report indicated that AD may be exacerbated by other cerebrovascular problems such as small strokes [37], and another linked untreated high blood pressure in midlife with an increased risk of dementia in later life [38]. Findings from at least four long-term studies link diabetes with a decline in cognitive function [39–42].

It has been difficult, however, to untangle causal relationships between these diseases and dementia from epidemiologic studies alone. Given the epidemiologic evidence and the availability of well-tested interventions for cardiovascular disease and diabetes, the NIA is moving ahead with clinical trials to see if managing these conditions might reduce the risk of cognitive decline and dementia. Ongoing trials cover a range of interventions for cardiovascular disease, such as statin drugs, vitamins, and exercise. Two

studies examining cardiovascular-related treatments from the ADCS were recently completed, and the data are being analyzed. One of the trials tested whether simvastatin (Zocor), a commonly prescribed cholesterol-lowering drug, can safely and effectively slow the rate of disease progression in people with mild to moderate AD. The other ADCS trial examined whether a reduction of homocysteine levels with high-dose supplements of folate, vitamin B6, and vitamin B12 will slow the rate of cognitive decline in older adults with AD. Three clinical trials to examine directly whether diabetes-related interventions might be effective in preventing or delaying the progression of cognitive decline or AD are also in progress with NIH support. These include a substudy of the National Heart, Lung and Blood Institute (NHLBI) funded Action to Control Cardiovascular Risk in Diabetes (ACCORD) Trial, called ACCORD-MIND (Memory IN Diabetes), which will test whether intensive glucose, blood-pressure, and lipid management can reduce rates of cognitive decline and structural brain change in 2,800 diabetics over a 4-year period.

## 5. Diagnosis

The testing of interventions can be expensive, time-consuming, and labor-intensive. Currently, the only way to measure the effectiveness of an intervention is to compare over time the emergence of Alzheimer's clinical symptoms or their progression in treated subjects versus those untreated. We now know, however, that the earliest AD pathology begins to develop in the brain long before clinical symptoms yield a diagnosis. Therefore, it is critical to find a way to detect signs of the disease at the earliest point possible, so that we can test interventions and, ultimately, treat the disease as early as we can.

Toward that end, the NIA has embarked on ambitious efforts to find new ways to measure changes in the brain and cerebrospinal fluid or in other fluids, including blood, that occur preclinically and during the course of MCI and AD. These programs are already yielding results. Improvements in brain imaging, coupled with the development of more sensitive cognitive tests, allow for diagnosis in the research setting with greater precision than ever before. The discovery of compounds such as PiB [23] and, more recently, FDDNP [43] that enable the visualization of the disease's characteristic amyloid plaques (PiB and FDDNP) and neurofibrillary tangles (FDDNP) in the living brain—an impossibility only a few years ago—will not only provide a means to diagnose AD earlier, but may also help researchers and clinicians develop new treatments and monitor their effectiveness, as well as reduce the time and sample size for clinical trials [44].

The Alzheimer's Disease Neuroimaging Initiative (ADNI), supported by a unique public-private partnership led by the NIA, is currently a major venue for facilitating neuroimaging and biomarker research regarding AD ([www.adni-info.org](http://www.adni-info.org)).

The initiative, funded federally by the NIA and the National Institute of Biomedical Imaging and Bioengineering and by the private sector through the Foundation for the National Institutes of Health, brings together experts from academia and industry to share an intellectual vision of this path of Alzheimer's research. Early results from the use of ADNI data, reported at the Alzheimer's Disease Prevention Conference in June 2007 (<http://www.nia.nih.gov/NewsAndEvents/PressReleases/>), show that, in addition to aiding early diagnosis, researchers may be able to reduce the time and expense associated with clinical trials by improving methods and developing uniform standards for imaging and biomarker analysis. For example, one study found that a standard physical model can be used successfully to monitor performance of magnetic resonance imaging (MRI) scanners at many different clinical sites; this will help ensure the accuracy of MRI images produced from ADNI volunteers. Other investigators compared changes over time in PET scans of brain glucose metabolism in people with normal cognition, MCI, and AD. They reported that the scans correlated with symptoms of each condition, and that images from different clinical sites were consistent across sites, suggesting the validity of PET scans for monitoring the effectiveness of therapies in future clinical trials. The clinical core essential to the operational success of ADNI is based on the infrastructure set up for the ADCS by Leon at the University of California at San Diego.

As with the genetics initiative, an important aspect of ADNI is the creation of a publicly accessible database available to qualified researchers worldwide. The database contains thousands of MRI and PET scan brain images and clinical data, and will include biomarker data obtained through blood and cerebrospinal fluid analyses. To date, over 200 researchers have signed up for database access. Researchers can also apply to propose new add-on experiments and to utilize the biological samples stored in the Biomarkers Core at the University of Pennsylvania (Philadelphia, PA) for novel biomarker analyses. The results of these ancillary analyses will also be available through the publicly accessible database.

## 6. Translational research

The only way to determine whether a pharmacologic or nondrug approach is effective, based on what we have learned from basic studies and epidemiologic observations, is to conduct a clinical trial. The ADNI seeks to facilitate and speed up clinical trials in one way, while the NIA's concerted efforts in translational initiatives aim to increase greatly the number of possible alternatives for AD therapies and, eventually, the number of trials to test them in humans. In 2004, the NIA launched its multi-component Translational Research Initiative through program announcements for drug discovery and drug development applications, partnering with the Alzheimer's Drug Discovery Foundation.

The ultimate goal of this initiative is to facilitate the submission of investigational new drug (IND) applications to the Food and Drug Administration (FDA), so that more clinical trials to test promising therapies can be started sooner. The NIA also supports toxicology services for investigators or small companies that have a potentially viable candidate drug for AD treatment, but lack the resources to begin the formal drug toxicology testing process needed for an IND.

Components of the effort include grant solicitations to stimulate the discovery, development, and preclinical testing in cellular, tissue, and animal models of novel compounds for the prevention and treatment of the cognitive impairment and behavioral symptoms associated with AD and MCI, as well as for age-related cognitive impairment. To date, the NIA has funded over 30 grants through these solicitations. The projects explore a variety of approaches toward the identification of novel compounds directed at multiple therapeutic targets such as amyloid, tau, TGF- $\beta$  signaling, nitric oxide (NO)-mediated cAMP response element binding protein (CREB) signaling, and various neurotransmitter and neurotrophin receptor systems. For example, some anti-amyloid strategies include the identification of  $\alpha$ -secretase activators, liver X-receptor activators, and different metal-complexing strategies. Anti-tau approaches include the discovery and development of microtubule-stabilizing drugs. Projects that pursue cognitive-enhancer approaches include the discovery of novel NO-mimetic non-steroidal anti-inflammatory drugs, NO/cyclic guanosine monophosphate/CREB pathway enhancers, and dual  $\gamma$ -aminobutyric acid and nicotinic receptors activators. The initiatives also fund the preclinical development of novel first-in-class, orally bioavailable anti-inflammatory drugs with neuroprotective properties, and the development of novel small-molecule neurotrophin receptor activators for the treatment of AD. The first cooperative-agreement awards under the drug development initiative were made in 2006. In September 2007, the NIA convened the First Annual Investigators Meeting for Translational Research, in an effort to provide guidance and foster interactions among investigators who are funded under the drug discovery and drug development initiatives.

## 7. Clinical trials

Currently, the NIA is supporting approximately 28 AD-related clinical trials (Table 1 lists these clinical trials). These studies examine lifestyle interventions and treatments for cardiovascular disease or diabetes, as previously described, as well as interventions potentially affecting brain  $\beta$ -amyloid or tau or other aspects of AD pathophysiology. It is hoped that one or more of these approaches will reduce the risk or delay the progression of MCI and AD, or manage behavioral symptoms and ease the burden on caregivers.

Table 1  
Ongoing NIA-funded AD/MCI/Cognitive Decline Prevention and Treatment Clinical Trials

Class of intervention	Principal investigator	Trial name	Intervention	Population
Antioxidants	William Markesbery	PREADVISE (Prevention of Alzheimer's Disease by Vitamin E and Selenium)*	Vitamin E, Selenium, Vitamin E+ Selenium	Older men, ages 60–90
	Steven DeKosky	GEM (Ginkgo Evaluation of Memory)†	Ginkgo biloba	Older adults, ages 75+
	Arthur Dalton	Vitamin E in Aging Persons With Down Syndrome	Vitamin E	Down Syndrome, at high risk of developing AD, ages 50+
	Ira Lott	Alzheimer's Disease in Down Syndrome: Antioxidant Trial	Vitamins E and C, alpha-lipoic acid	Down Syndrome and AD, ages 40+
	Douglas Galasko	Antioxidant Trial‡	Vitamins E & C, alpha lipoic acid, coenzyme Q	AD
Cardiovascular	Lenore Launer	ACCORD-MIND (Action to Control Cardiovascular Risk in Diabetes—Memory in Diabetes)*	Intensive glucose, blood pressure, and lipid management	Type 2 diabetes mellitus, Ages 40–79
	Mary Sano	Cholesterol Lowering Agent to Slow Progression (CLASP) of Alzheimer's Disease Study‡	Simvastatin	AD
	Cynthia Carlsson	ESPRIT (Evaluating Simvastatin's Potential Role in Therapy)	Simvastatin	Middle-aged adults at high risk of AD (family history), Ages 35–69
Omega-3 fatty acids	Emily Chew & John Paul SanGiovanni	AREDS2 (Age-Related Eye Disease Study 2)†	Macular xanthophylls (lutein and zeaxanthin) and/or omega -3 fatty acids (DHA and EPA)	Age-related Macular degeneration (AMD) in both eyes, or advanced AMD in one eye, Ages 50–85
	Joseph Quinn	DHA (Docosahexaenoic Acid), an Omega 3 Fatty Acid, in Slowing the Progression of Alzheimer's Disease‡	DHA	AD
Passive immunization	Norman Relkin	Intravenous Immunoglobulin (IVIg) for treatment of Alzheimer's Disease*	IVIg	AD
Hormones	Sanjay Asthana	KEEPS-CA (Kronos Early Estrogen Prevention Study - Cognitive and Affective Study)*	Oral conjugated equine estrogen (CEE or Premarin) and transdermal 17 $\beta$ -estradiol (tE2)	Healthy perimenopausal women, Ages 42–58
	Howard Hodis	ELITE (Early Versus Late Intervention with Estradiol)	17 $\beta$ -estradiol	Healthy early (less than 6 years) or late (10 years +) menopausal women
	Monique Cherrier	Testosterone Supplementation in Men with MCI	Testosterone	Older men with MCI and low testosterone
	Victor Henderson	Raloxifene for Women With Alzheimer's Disease	Raloxifene (selective estrogen receptor modulator or SERM)	Older women with AD
	Michael Vitiello	SMART (Somatotrophics, Memory, and Aging Research Trial)	growth hormone releasing hormone (GHRH)	MCI and healthy older adults, Ages 55–80
	Carey Gleason	Alzheimer's Disease: Potential Benefit of Isoflavones	Novasoy (soy isoflavones – phytoestrogens)	AD
Diabetes	Suzanne Craft	RECALL (Rosiglitazone Effects on Cognition for Adults in Later Life)	Rosiglitazone	MCI
	Suzanne Craft	SNIFF 120 (Study of Insulin to Fight Forgetfulness, 120 Days)	Intranasal insulin	MCI and AD
	Suzanne Craft	Glucose Regulation and Memory in Alzheimer's Disease	Improved insulin resistance, 3 studies: 1. diet, 2. triglyceride emulsion, 3. Rosiglitazone	AD (studies 1–3), age-matched healthy older adults (study 1)
Exercise	Robert Krikorian	Exercise and Cognitive Aging	Aerobic exercise	MCI
	Linda Teri	Exercise & Health Promotion for MCI: A Controlled Trial	Two exercise programs (one developed for individuals with MCI and the other developed for cognitively intact older adults)	MCI

Table 1  
Continued

Class of intervention	Principal investigator	Trial name	Intervention	Population
Exercise and Cognitive Training	Mark Espeland	Seniors Health and Activity Research Program Pilot (SHARP-P)	Physical activity, cognitive training, physical activity + cognitive training	Older adults at risk for developing MCI, Ages 70–85
Other	Paul Newhouse	Transdermal Nicotine Treatment of MCI	Nicotine patch	MCI
	Paul Aisen	Huperzine A in Alzheimer's Disease	Huperzine A	AD
	Pierre Tariot	VALID (Valproate in Dementia)‡	Valproate	AD
	Dev Devanand	Antipsychotic Discontinuation in Alzheimer's Disease	Risperidone	AD
	Jiska Cohen-Mansfield	TREA (Treatment Routes for Exploring Agitation)	TREA – systematic approach to individualizing non-pharmacological interventions for persons with dementia	AD/dementia, nursing home residents

NOTE. For information on new and currently recruiting trials please see the following websites: Alzheimer's Disease Education & Referral Center (ADEAR): <http://www.nia.nih.gov/alzheimers>; ClinicalTrials.gov: <http://clinicaltrials.gov/>

\* NIA funded add-on trials: PREADVISE (add-on to National Cancer Institute's SELECT trial); ACCORD-MIND (add-on to National Heart Lung and Blood Institute's ACCORD trial); KEEPS-CA (add-on to Kronos Longevity Research Institute KEEPS trial).

† Co-funded trial: GEMS (National Center for Complementary and Alternative Medicine lead institute); AREDS2 (National Eye Institute lead institute).

‡ Alzheimer's Disease Cooperative Study (ADCS) trial.

A cornerstone of the NIA's efforts in clinical-trials research is the Alzheimer's Disease Cooperative Study (ADCS) (<https://adcs.ucsd.edu>). In 1991, the NIA launched the ADCS due in large part to the insight of Leon that a national network might facilitate clinical-trials research and be better able to address special issues surrounding the study of cognitively impaired patients. The consortium of about 70 research sites around the U.S., including most of the Alzheimer's Disease Centers, focuses on trials of compounds either off-patent, patented and marketed for another use but perhaps useful in treating AD, or developed by individual investigators or small companies without adequate resources for clinical trials. A critical component of the ADCS is its development of new methods for conducting dementia research.

The ADCS was led by Leon until his death, and investigators and observers will always recall his orchestration of extensive discussions about data, approaches, and issues, wielding the microphone for these discussions as if leading a symphony. For the most recent NIA grant renewal, Leon requested and received suggestions for trials from academic investigators as well as from small biotechnology companies, and several were selected to be included in the grant application. In October 2006, this new round of ADCS studies was funded. It will investigate:

- Docosahexaenoic acid (DHA): An omega-3 fatty acid found in algae and fish, DHA will be examined to see whether it will slow decline in AD. Observational studies associated high fish consumption with a reduced risk of AD, and studies in mouse models show that dietary DHA reduces brain levels of  $\beta$ -amyloid,

oxidative damage associated with  $\beta$ -amyloid, and neurotoxicity.

- Intravenous immunoglobulin (IVIg): There is increased interest in passive immunization strategies against AD. Intravenous immunoglobulin contains naturally occurring antibodies against  $\beta$ -amyloid, and preliminary studies showed that IVIg may improve cognition. In addition, research demonstrated that IVIg increased levels of anti- $\beta$ -amyloid antibodies in plasma, and promoted the clearance of  $\beta$ -amyloid from cerebrospinal fluid. The new ADCS trial will more definitively demonstrate whether IVIg is useful clinically for treating AD.
- Lithium: The biological activity of lithium, which was shown in animal models to block abnormal changes in tau, has created interest in lithium as a novel treatment for AD. The ADCS investigators will undertake a pilot biomarker study to see whether the drug can lower tau and  $\beta$ -amyloid levels in cerebrospinal fluid and be safely tolerated in older AD patients.
- Home-based assessment: Older individuals, particularly the very elderly, may have physical, social, and health limitations that make it difficult for them to take part in research trials in which they have to travel to clinical centers to be assessed. This study, conducted in people aged  $\geq 75$  years, will examine the use of mail-in questionnaires, automated telephone technology, and computerized data collection to assess cognitive, functional, and other factors in the home environment, to see how home-based assessments might be used in primary prevention trials. Such an approach

could significantly reduce the cost and increase the feasibility of participation in these long-term, costly clinical trials.

- Resveratrol: An early-phase pilot study will evaluate the safety and tolerability of resveratrol and its effects on cognitive and other clinical outcomes, as well as on biological markers of AD pathology in patients with mild-to-moderate AD.

## 8. Coping with AD

Leon understood the struggle of caregivers to cope with the devastation and disorder that AD brings to its victims and their families. He was first and foremost a *physician-researcher*, who treated patients as he directed the Alzheimer's Disease Research Center at the University of California at San Diego. In his leadership of the AD Cooperative Study clinical-trials efforts, the management of behavioral symptoms of AD was always an important issue for consideration. One trial, for example, is testing whether valproate, used to treat epilepsy and some psychiatric disorders, might slow decline or help delay the agitation and psychosis that often accompany dementia.

Beyond the management of patient cognitive symptoms and behaviors, research on caregiving aims to reduce stress and burden in other ways. The Resources for Enhancing Alzheimer's Caregiver Health (REACH) II study, jointly sponsored by the NIA and the NIH's National Institute of Nursing Research, successfully demonstrated interventions in ethnically diverse populations [45], and follow-up studies are planned to see how these practices might be integrated into existing networks of health and aging services.

To help move these and other research findings into clinical practice and into the hands of patients, families, and caregivers, the NIA supports a major communications effort on AD. The NIA's Alzheimer's Disease Education and Referral Center is the central federal resource for information about research, participation in clinical trials, clinical practice, and various aspects of caregiving through its website ([www.nia.nih.gov/alzheimers](http://www.nia.nih.gov/alzheimers)), publications, exhibits, and a variety of public health outreach activities.

## 9. Charting a course for the road ahead

It is difficult to predict what the pace of science will be and how soon new discoveries will be made that bring interventions for preventing or delaying the onset and progression of AD. That said, the momentum is palpable, and the mood about the promise of Alzheimer's research is the most optimistic in 30 years of study. We have built an enormous base of knowledge, and can now see how what we learn may lead to treatment and prevention. To sustain our momentum, we can move ahead on a number of fronts.

### 9.1. Genetics

By the end of the genetics initiative, we should know the major risk-factor genes for AD and, in related work, those for cognitive decline as well. The overlap will be informative in terms of how much genetic risk is shared between cognitive decline and AD. The risk factor genes will be useful in several ways:

- Identifying new pathways that contribute to the early development of AD. This is an important first step in developing new therapeutic approaches based on modulating these pathways.
- Identifying persons at the highest genetic risk for developing cognitive decline/AD. Recruiting these persons into prevention trials would result in smaller, shorter, and less expensive trials, and will allow persons at the highest genetic risk to be given prevention therapies at an early stage, and could help determine the utility of particular therapies (pharmacogenomics), accounting for the genetic differences between individuals.

### 9.2. Basic studies

Research continues to refine our mechanistic understanding of AD. The general outlines for a number of pathways have emerged, but pieces of the puzzle are still entirely missing. The basic research endeavor will remain critical to the search for effective new therapies, by improving our understanding about the biology of the aging brain. How much do age-related changes in the brain form the substrate for age-related cognitive decline and onset of dementia? Another promising area may involve understanding more about the molecular basis of cognition, and how it changes with age. Behavioral or molecular interventions targeting cognitive decline may also prove helpful in delaying the onset of AD. Another approach is to study how the brain adapts to injury. What are the components important to adaptability, and what is the molecular basis of cognitive reserve, and can building this up prevent the negative consequences of AD pathology? The Cognitive Aging Summit held by the NIA in Washington, D.C., in October 2007, and funded by a generous donation from the McKnight Brain Research Foundation, will help identify areas of research most likely to be fruitful along these lines.

### 9.3. Imaging and other biomarkers

By the end of the ADNI, we should know which clinical/neuropsychological, imaging, and biomarker combinations are best for predicting who will progress from MCI through various stages of AD, and how this progression occurs. These efforts will help by:

- Identifying molecules in pathways, through the discovery of novel biomarkers, previously not known to be involved in AD, giving us new ideas for potential therapies.

- Speeding up and refining the conduct of clinical trials. Biological or imaging markers will eventually cut down the time needed to know whether a drug is having an effect or not. The shorter, smaller clinical trials likely possible with these new approaches should increase our ability to fund more clinical trials of promising approaches.
- Predicting with greater accuracy the time course of an individual's decline into full-blown AD. We should be able to start eventual therapies much earlier in the disease, when changes in the brain associated with AD are still minimal.

#### 9.4. Translational research and clinical trials

Translational initiatives and federally supported clinical trials should provide important new approaches for study and intervening to prevent or treat AD by:

- Making sure that researchers with promising therapeutic approaches have the chance to develop these approaches. The stepped-up push for the initial development and testing of new compounds for safety and efficacy, with the most promising to be referred to the FDA for IND approval for clinical trials, is an important catalyst for innovation. With over a dozen new approaches to therapy and many more in the pipeline, this joint effort by the NIA and partners in the non-profit sector to fund early-stage, high-risk approaches will more rapidly identify those promising enough for broader support and testing in clinical trials.
- Funding the most speculative stages of intervention testing, in the hope that the results from some phase 1 and 2 trials will be encouraging enough to be picked up for phase 3 testing, and thus increase the number of total trials funded by NIH and private industry. Other new NIH trials will economize on money and effort by piggybacking cognitive testing onto selected trials supported by other programs within the NIA or by other NIH institutes.

#### 9.5. Supporting and developing research infrastructure and resources

In addition to the specific genetics, neuroimaging, and biomarker initiatives discussed previously, the NIA will continue to provide broader venues through which investigators can conduct interdisciplinary and collaborative AD research. Of particular note is the expansion of the National Alzheimer's Coordinating Center (NACC) and the development of the Uniform Data Set. More than 7,000 individuals are now being followed through the NACC, which pools and shares data collected by the Alzheimer's Disease Centers. The growing dataset, maintained at the University of Washington, Seattle (Seattle, WA) ([www.alz.washington.edu](http://www.alz.washington.edu)), is available to qualified researchers for any number of studies on AD.

We intend to continuously assess the basic, translational, and clinical research effort, to maximize the public's investment, particularly in the context of a year-by-year drop in constant dollars available for all NIH-sponsored research funding since 2003. These decisions will be informed by the recent conclaves on AD in 2006 and the Cognitive Aging Summit held more recently, as well as by more focused workshops and sessions sponsored by us and others to examine specific areas of study. We will continue to work with the Foundation for the National Institutes of Health to identify opportunities, such as the ADNI, for cofunding; with industry for appropriate collaboration with the ADCS and other NIH-funded clinical trials; with private Foundations such as the Alzheimer's Association and the Alzheimer's Drug Discovery Foundation on specific initiatives; and colleagues at the Centers for Disease Control, the FDA, and other collaborative groups to ensure we move as quickly as possible to find the combination of behavioral and drug interventions that can best fight AD in the majority of our elderly.

Just a few months before he died, at the time the latest round of ADCS funding and studies was announced, Leon was asked what aspect of the new award he would like to comment on in a press release being prepared by the NIA. He chose to note that the selection of compounds for testing was enhanced by seeking ideas from the biotechnology sector as well as from individual investigators and the consortium's members. He said, "We have been able to bring together a larger universe of people studying therapies for Alzheimer's, and I think this group of studies reflects new thinking in how to approach the disease."

"Bring together." "New thinking." These are the words that Leon left us with, and they are certainly guideposts as we move forward into a new era of research on AD.

#### Acknowledgments

The authors thank NIA colleagues Dr. Samuel Speciale, Dr. Laurie Ryan, Robin Walter, and Patricia Lynch for their invaluable assistance with this project.

#### References

- [1] Katzman R. The prevalence and malignancy of Alzheimer's disease: a major killer. *Arch Neurol* 1976;33:217–8.
- [2] Khachaturian ZS. A chapter in the development of Alzheimer's disease research: a case study of public policies on the development and funding of research programs. *Alzheimers Dement* 2007;3:243–58.
- [3] Davies P, Maloney AJF. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* 1976;II:1403.
- [4] Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, et al. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci* 2007;27:796–807.
- [5] Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 1999;400:173–7.

- [6] Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5'-splice-site mutations in *tau* with the inherited dementia FTDP-17. *Nature* 1998;393:702–5.
- [7] Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron* 2003;39:409–21.
- [8] Andorfer C, Acker CM, Kress Y, Hof PR, Duff K, Davies P. Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. *J Neurosci* 2005;25:5446–54.
- [9] Tanzi RE. Tangles and neurodegenerative disease—a surprising twist. *N Engl J Med* 2005;353:1853–5.
- [10] Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science* 2007;316:750–4.
- [11] Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006;314:130–3.
- [12] Morimoto RI. Stress, aging, and neurodegenerative disease. *N Engl J Med* 2006;355:2254–5.
- [13] Cohen E, Bieschke J, Perciavalle RM, Kelly JW, Dillin A. Opposing activities protect against age onset proteotoxicity. *Science* 2006;313:1604–10.
- [14] Smith DE, Roberts J, Gage FH, Tuszyński MH. Age-associated neuronal atrophy occurs in the primate brain and is reversible by growth factor gene therapy. *Proc Natl Acad Sci USA* 1999;96:10893–8 [erratum appears in *Proc Natl Acad Sci USA* 1999;96:14668].
- [15] Tuszyński MH, Thal L, Pay M, Salmon DP, HS U, Bakay R, et al. A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nat Med* 2005;11:551–5.
- [16] Feng R, Rampon C, Tang YP, Shrom D, Jin J, Kyin M, et al. Deficient neurogenesis in forebrain-specific presenilin-1 knockout mice is associated with reduced clearance of hippocampal memory traces. *Neuron* 2001;32:911–26.
- [17] Rogava E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, et al. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nat Genet* 2007;39:168–77.
- [18] Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch Neurol* 2003;60:1119–22.
- [19] Plassman BL, Langa KM, Fischer GG, Heeringa SG, Weir DR, Ofstedal MB, et al. Prevalence of dementia in the United States: the Aging, Demographics, and Memory Study. *Neuroepidemiology* 2007;29:125–32.
- [20] Bennett DA, Schneider JA, Arvanitakis Z, Kelly JF, Aggarwal NT, Shah RC, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology* 2006;66:1837–44.
- [21] Bennett DA, Schneider JA, Bienias JL, Evans DA, Wilson RS. Mild cognitive impairment is related to Alzheimer disease pathology and cerebral infarctions. *Neurology* 2005;64:834–41.
- [22] Galvin JE, Powlishta KK, Wilkins K, McKeel DW Jr, Xiong C, Grant E, et al. Predictors of preclinical Alzheimer disease and dementia: a clinicopathologic study. *Arch Neurol* 2005;62:758–65.
- [23] Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B. *Ann Neurol* 2004;55:306–19.
- [24] Mintun MA, Larossa GN, Sheline YI, Dence CS, Lee SY, Mach RH, et al. [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. *Neurology* 2006;67:446–52.
- [25] Rowe CC, Ng S, Ackermann U, Gong SJ, Pike K, Savage G, et al. Imaging beta-amyloid burden in aging and dementia. *Neurology* 2007;68:1718–25.
- [26] Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, et al. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. *Ann Intern Med* 2006;144:73–81.
- [27] Abbott RD, White LR, Ross GW, Masaki KH, Curb JD, Petrovitch H. Walking and dementia in physically capable elderly men. *JAMA* 2004;292:1447–53.
- [28] Podewils LJ, Guallar E, Kuller LH, Fried LP, Lopez OL, Carlson M, et al. Physical activity, APOE genotype, and dementia risk: findings from the Cardiovascular Health Cognition Study. *Am J Epidemiol* 2005;161:639–51.
- [29] Berchtold NC, Chinn G, Chou M, Kesslak JP, Cotman CW. Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. *Neuroscience* 2005;133:853–61.
- [30] Adlard PA, Perreau VM, Pop V, Cotman CW. Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. *J Neurosci* 2005;25:4217–21.
- [31] Erickson KI, Colcombe SJ, Wadhwa R, Bherer L, Peterson MS, Scalf PE, et al. Training-induced functional activation changes in dual-task processing: an FMRI study. *Cereb Cortex* 2007;17:192–204.
- [32] Colcombe SJ, Erickson KI, Scalf PE, Kim JS, Prakash R, McAuley E, et al. Aerobic exercise training increases brain volume in aging humans. *J Gerontol [A] Biol Sci Med Sci* 2006;61:1166–70.
- [33] Milgram NW, Head E, Zicker SC, Ikeda-Douglas CJ, Murphey H, Muggenburg B, et al. Learning ability in aged beagle dogs is preserved by behavioral enrichment and dietary fortification: a two-year longitudinal study. *Neurobiol Aging* 2005;26:77–90.
- [34] Willis SL, Tennstedt SL, Marsiske M, Ball K, Elias J, Koepke KM, et al. Long-term effects of cognitive training on everyday functional outcomes in older adults. *JAMA* 2006;296:2805–14.
- [35] Luchsinger JA, Reitz C, Honig LS, Tang MX, Shea S, Mayeux R. Aggregation of vascular risk factors and risk of incident Alzheimer disease. *Neurology* 2005;65:545–51.
- [36] Curb JD. The tangled story of plaques and arteries. *J Am Geriatr Soc* 2005;53:1257–8.
- [37] Schneider JA, Boyle PA, Arvanitakis Z, Bienias JL, Bennett DA. Subcortical infarcts, Alzheimer's disease pathology, and memory function in older persons. *Ann Neurol* 2007;62:59–66.
- [38] Freitag MH, Peila R, Masaki K, Petrovitch H, Ross GW, White LR, et al. Midlife pulse pressure and incidence of dementia: the Honolulu-Asia Aging Study. *Stroke* 2006;37:33–7.
- [39] Crooks VC, Buckwalter JG, Petitti DB. Diabetes mellitus and cognitive performance in older women. *Ann Epidemiol* 2003;13:613–9.
- [40] Luchsinger JA, Reitz C, Patel B, Tang MX, Manly JJ, Mayeux R. Relation of diabetes to mild cognitive impairment. *Arch Neurol* 2007;64:570–5.
- [41] Peila R, Rodriguez BL, White LR, Launer LJ. Fasting insulin and incident dementia in an elderly population of Japanese-American men. *Neurology* 2004;63:228–33.
- [42] Schnaider BM, Goldbourt U, Silverman JM, Noy S, Schmeidler J, Ravona-Springer R, et al. Diabetes mellitus in midlife and the risk of dementia three decades later. *Neurology* 2004;63:1902–7.
- [43] Shoghi-Jadid K, Small GW, Agdeppa ED, Kepe V, Ercoli LM, Siddarth P, et al. Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with Alzheimer disease. *Am J Geriatr Psychiatry* 2002;10:24–35.
- [44] Jack CR Jr, Shiung MM, Gunter JL, O'Brien PC, Weigand SD, Knopman DS, et al. Comparison of different MRI brain atrophy rate measures with clinical disease progression in AD. *Neurology* 2004;62:591–600.
- [45] Belle SH, Burgio L, Burns R, Coon D, Czaja SJ, Gallagher-Thompson D, et al. Enhancing the quality of life of dementia caregivers from different ethnic or racial groups: a randomized, controlled trial. *Ann Intern Med* 2006;145:727–38.