

Funded Research:

advancing the field and the careers of those who help shape it

The Alzheimer's Association is the world's largest private, nonprofit funder of Alzheimer research, having awarded more than \$279 million to 1,900-plus researchers since 1982.

Through its International Research Grant Program, the Association has helped advance broad fields of Alzheimer research—including early detection, prevention, and treatment—as well as the careers of the specific individuals who received funding.

In their own words, scientists Drs. Bruce Lamb, Jaya Padmanabhan, Sanjay Pimplikar, Cheryl Luis and Liqin Zhao describe the impact of being chosen Alzheimer's Association grant recipients.

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Bruce T. Lamb, Ph.D.

Associate Staff, Lerner Research Institute, and Associate Professor, Cleveland Clinic Lerner College of Medicine, Cleveland, Ohio, recipient of a 2008 Investigator-Initiated Research Grant and 2005 Zenith Fellows Awards

Alzheimer's is a complex disease influenced by multiple genetic and environmental factors. A common denominator is the amyloid precursor protein (APP) and its byproduct, beta-amyloid, which seems to be involved in the initiation of the anatomical and functional changes of Alzheimer's. Beta-amyloid is a protein that goes on to accumulate into large clusters called plaques, one of the hallmarks of the disease. My laboratory uses complementary approaches to help unravel the complexities of Alzheimer's disease and identify factors that contribute to the disease and could be targeted by new drugs and therapies.

Through generous support from the Alzheimer's Association over the years, including both Investigator-Initiated Research Grants and a Zenith Award, my laboratory has 1) used novel animal models of Alzheimer's to examine the role of genetic and environmental factors that may trigger specific Alzheimer brain changes, 2) developed more complete animal models of the disease and 3) investigated therapies that might decrease the risk for Alzheimer's. These studies would not have been possible without the support of the Alzheimer's Association.

To identify genetic factors that may trigger brain changes, we have used groups of mice that have

been genetically engineered to carry different genes or combinations of genes associated with Alzheimer's. Mice with a mutant human Alzheimer's gene show a variety of brain changes, including increased APP and beta-amyloid production, development of plaques, activation of the immune system, behavioral abnormalities and abnormal neuronal changes called cell cycle events (CCEs). Depending on the genes they carry, some mice exhibited all, some, minimal or none of these changes. Employing a technique called genetic mapping, we have identified the location of several genes in these mice that influence both the production of beta-amyloid and its accumulation into plaques. Our current focus is identifying the specific genes involved in these events.

In addition to genetic factors, through support from the Alzheimer's Association we have initiated studies to examine the effects of specific environmental factors on the development of Alzheimer brain changes. In particular, we have focused on the effects of high-fat/high-cholesterol diets, as studies in humans have suggested that this may be a risk factor for the development of Alzheimer's.

Our studies have demonstrated that high-fat/high-cholesterol diets similar to those consumed by Western societies increase levels of beta-amyloid in some mouse models of Alzheimer's. The effect of a high-fat/high-cholesterol diet depends entirely on the genetic background of the mice, with some mice

being highly sensitive to the effects of diet, others completely resistant to the effects of diet and others falling somewhere in between. These studies suggest that the effects of an environmental risk factor for Alzheimer's depends on the genetic background of the mice. This has implications for 1) human studies examining the rates of Alzheimer's in different populations, 2) recommendations for specific diets to reduce Alzheimer's risk and 3) the development of therapies to counteract the effects of diet.

Finally, we have been examining whether common medications such as nonsteroidal anti-inflammatory drugs (NSAIDs) have a beneficial effect in Alzheimer mouse models. Notably, we have found that NSAIDs including ibuprofen and naproxen can block inflammation and neuronal CCEs if given before the development of extensive Alzheimer brain changes. However, if NSAID treatments are begun later in disease progression, it does not reverse neuronal CCEs and other brain changes and

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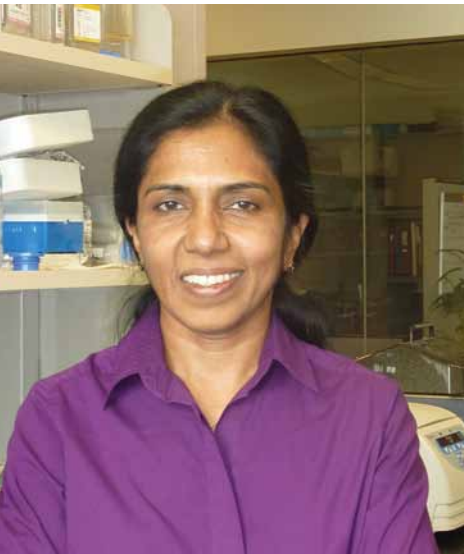
—Bruce T. Lamb, Ph.D.

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One of the first surprises in this project came in a mouse whose own APP gene had been removed and replaced with a mutant human APP gene that causes Alzheimer's. Substituting the human APP gene actually lessened the brain changes of Alzheimer's. This suggests that the mouse APP gene contributes to the development of the brain changes in the transgenic mouse models of Alzheimer's.

has only a minimal effect on other signs of Alzheimer's exhibited by the mice.

These findings are consistent with studies in humans demonstrating that long-term use of NSAIDs for arthritis is associated with a decreased risk for Alzheimer's, while short-term clinical trials with NSAIDs in individuals diagnosed with Alzheimer's have proven largely unsuccessful. These results also suggest that NSAID treatments in humans may need to be initiated as a preventative measure, substantially before the development of extensive brain changes, to be successful.



Jaya Padmanabhan, Ph.D.

Assistant Professor of Molecular Medicine, Johnnie B. Byrd, Sr. Alzheimer's Center & Research Institute, University of South Florida, Tampa, recipient of a 2008 Investigator-Initiated Research Grant

I am grateful to the Alzheimer's Association for providing me with the first independent grant of my scientific career, an Investigator-Initiated Research Grant (IIRG), in 2008. I found out about this while we were traveling north, taking our daughter to a music camp in Vermont. My eyes welled with tears when I read the e-mail from the Association. The award came during a very hard time. The Byrd Alzheimer's Institute had just started, and the money situation was tight. This was when I found out that the Alzheimer's Association was going to fund our research. I am really thankful to this great organization for their timely help.

The major hallmarks of Alzheimer's disease are protein aggregates known as amyloid plaques and neurofibrillary tangles. Our laboratory is interested in understanding the molecular, cellular and biochemical changes in the brain that lead to plaques and tangles. Two proteins associated with plaques and tangles are beta-amyloid, a fragment of the amyloid precursor protein (APP), and tau. The grant from the Alzheimer's Association provides funding to help our laboratory examine the role of inflammatory proteins (proteins associated with inflammation) in Alzheimer's disease. Among these proteins are apolipoprotein E e4 (ApoE-e4),

alpha 1-antichymotrypsin (ACT), cytokines and complement factors. We are currently analyzing the significance of ACT in Alzheimer's disease.

ACT is present in abnormally high numbers in cells called astrocytes that surround plaques in the Alzheimer brain. Higher levels of ACT have been reported in cerebrospinal fluid from people with Alzheimer's, signifying that elevated levels of ACT could serve as a biomarker for early detection and diagnosis of Alzheimer's. Previous studies have shown that ACT helps individual beta-amyloid proteins stick together to form small clumps called oligomers. These clumps are much smaller than plaques and precede the development of plaques.

The combination of ACT and beta-amyloid seems to be more toxic to brain nerve cells tested in cell culture experiments than beta-amyloid itself, suggesting that this combination may have deleterious effects in brains as well as in brain cells. Other studies in mice that were genetically engineered to produce Alzheimer's and that produced excess ACT and APP showed that excess ACT accelerated the development of Alzheimer brain and behavioral changes. How ACT brings about these changes is not clearly understood. Beta-amyloid is generated when APP is clipped first by the enzyme beta-secretase and then by the enzyme gamma-secretase. Whether ACT affects these enzymes is currently being examined.

In addition to its effects on beta-amyloid, in cell culture studies of neurons from the cortex region of the brain, we found that ACT induces the protein tau to take on additional phosphate molecules (called phosphorylation). Purified ACT from human plasma was used for these studies. Neurons treated with the purified ACT showed a significant increase in phosphorylation at three particular sites on the tau protein that have been shown to be affected in Alzheimer's disease.

“ We believe that a thorough knowledge of the molecular mechanisms involved in the development of Alzheimer's will help us generate novel therapies targeted toward neurodegenerative diseases, and funding from the Alzheimer's Association will help immensely in reaching this goal. ”

—Jaya Padmanabhan, Ph.D.

Analysis of enzymes called kinases that are involved in tau phosphorylation showed that ACT increases the activity of the kinases ERK (extra-cellular signal-regulated protein kinase) and GSK-3 (glycogen synthase kinase 3). This suggests that one or both of these kinases may be involved in the tau phosphorylation that occurs with excess levels of ACT. This also implies that inhibiting the activity of these kinases may help prevent the changes in beta-amyloid and tau that are hallmarks of Alzheimer's. We hope to pinpoint the molecules involved in this process and use the information to develop drugs targeting these molecules, which may prove beneficial in treating the disease.

that Alzheimer's is like cancer, except that in cancer, cell division leads to an increase in cancer cells, while in Alzheimer's it leads to the degeneration and death of non-dividing neurons. By studying the differences and similarities between dividing cells and non-dividing neurons, we hope to gain information that will help us identify potential drug targets for Alzheimer's and cancer.

The IIRG from the Alzheimer's Association has helped advance both my research and my career. The grant helped me obtain a tenure-track faculty position in the Department of Molecular Medicine at the College of Medicine, University of South Florida, Tampa. I hope to do my best to help the scientific community find a cure or a way to prevent Alzheimer's disease.



Sanjay W. Pimplikar, Ph.D.

Associate Staff, Cleveland Clinic Foundation,
recipient of a 2008 Investigator-Initiated
Research Grant (IIRG), 2002 IIRG and 1995 Pilot
Research Grant

I entered the Alzheimer's disease research field more or less by accident.

Trained as a cell biologist, I was interested in studying how proteins on the outer membrane of a cell reached their correct destination inside a cell. I decided to use amyloid precursor protein (APP) as a marker protein to study this transport process. During these investigations we realized that when APP is clipped by the enzyme gamma-secretase, a fragment called AICD (for "APP intracellular domain") is able to enter the nucleus of the cell and change gene expression (some genes are "switched on" while others are "turned off"). Once I realized APP, a cell surface protein, could communicate with the nucleus of a cell in this fashion and that virtually nothing was known about AICD, I was hooked.

In the early years of AICD research, the attitude in the field oscillated between "AICD is inconsequential" to "AICD is not important." There is still some skepticism about its biological function and controversy about how it affects gene expression. However, the thinking in the field is now changing with the realization that AICD is conserved through evolution (meaning that it is identical in a range of species, from humans to mice) and interacts with several proteins that control communication inside the cell. A number of groups have started to make important contributions to our understanding of AICD biology and how it governs signaling inside cells.

Using genetic engineering techniques, we created mice that had elevated levels of AICD to study the effects of AICD overexpression. We were surprised to see the mice exhibit Alzheimer-like pathological features without increased levels of beta-amyloid or beta-amyloid plaques. Moreover, these features appear in a time-dependent fashion. Our transgenic mice start producing AICD at about 2 weeks. Abnormal activation of glycogen synthase kinase, a protein linked to the tau tangles of Alzheimer's, is seen at age 2 months. At age 3 to 4 months, the mice undergo an abnormal process in which phosphate is added to the tau protein. At this age, we also see abnormal, seizure-like electrical activity in the brain and changes in neuronal circuits, the 'wiring' of the brain that enables it to carry out various functions. Excess accumulation of insoluble tau and memory problems begin at 8 months, and neurons in the hippocampus of the mice start to die by 18 months. Thus, except for the development of beta-amyloid plaques (which are also found in non-demented individuals), the AICD transgenic mice seem to capture the essential pathological features of Alzheimer's.

We are now studying the underlying mechanisms that cause these pathological features, all of which occur in human Alzheimer's. Our latest findings suggest that inflammation in the brain is likely to be one of the pivotal events induced by AICD. We think that our observations in animals are applicable to human disease because we also find increased

amounts of AICD in the brains of people with Alzheimer's. If further studies confirm that AICD is a significant contributor to human Alzheimer's, we must change our thinking about the therapeutic strategies developed to fight Alzheimer's. Current clinical trials focused on reducing levels of beta-amyloid have met, at best, with limited success.

easy to convince others of ideas that run contrary to the prevailing dogma. In my view, the fact that the Association funded my previous and current studies on AICD demonstrates the Association's willingness to support innovative but risky ideas that more traditional agencies are reluctant to fund. Without the Association's support of my research

“ Without the Association's support of my research program, I doubt that I would have pursued investigations of AICD. In fact, there is a good chance that without the Association's support I would not be doing Alzheimer research at all. ”

—Sanjay W. Pimplikar, Ph.D.

Although a number of factors could account for this, the continued failure of amyloid-focused strategies should prompt us to seriously consider other, non-amyloid causes of the disease. I am organizing a symposium at the 2010 Annual Meeting of the Society for Neurosciences to discuss these alternative mechanisms.

I have been fortunate to receive significant funding from the Alzheimer's Association. It is not always

program, I doubt that I would have pursued investigations of AICD. In fact, there is a good chance that without the Association's support I would not be doing Alzheimer research at all.

I love interacting with the national as well as the local chapter of the Association. In the dark days of research when nothing seems to be going right, it is their commitment to the cause of fighting Alzheimer's that keeps me going.



Cheryl A. Luis, Ph.D.

Associate Clinic Director, the Roskamp Institute,
Sarasota, Florida, recipient of a 2009 New
Investigator Research Grant

The support of the Alzheimer's Association has allowed me to pursue an area of research that I refer to as the other major piece of the puzzle: cost-effective methods of earlier detection of Alzheimer's disease.

Science is moving closer to finding therapies that slow or stop the progression of Alzheimer's, but for these therapies to be truly beneficial, we must start treatment very early in the course of the disease. It makes little sense to halt the progression of the disease when an individual has already suffered considerable cognitive decline and functional impairment. My research at the Roskamp Institute has focused on developing practical methods for earlier detection of dementia.

The New Investigator Research Grant (NIRG) will allow further refinement of a screening approach that I and my colleagues have been studying for several years. This novel approach combines a cognitive test with studies of biomarkers in blood. Biomarkers are physical changes that indicate the presence of a disease or an elevated risk of developing a disease. In addition, this approach may prove useful in measuring the impact of potential new Alzheimer drugs.

Traditional cognitive tests of dementia severity such as the Mini-Mental State Exam (MMSE) are inadequate in detecting the early cognitive signs of the illness and mild cognitive impairment (MCI). MCI is a possible transitional state from normal cognition to Alzheimer's. One of the few cognitive tests that has been consistently effective in identifying those with MCI is the Montreal Cognitive Assessment (MoCA). It is more comprehensive than the MMSE in that it includes items assessing immediate and delayed memory, language fluency, visuospatial skill (the ability to understand and use symbols and maps, for example, and the brain's ability to translate visual signals into a correct impression of where objects are in space), abstract reasoning and divided attention (the ability to pay attention to more than one task at once, such as carrying on a conversation while preparing a meal).

The physical and functional changes of Alzheimer's are intimately linked to the abnormal accumulation of fragments of the amyloid precursor protein called beta-amyloid 1-40 and 1-42. Increased production and decreased removal of beta-amyloid is thought to precede the early symptoms of Alzheimer's, which typically include a decline in episodic memory, the ability to remember the people, places and events of episodes from one's past. As beta-amyloid accumulates, a cascade of inflammation, cognitive and behavioral decline and, ultimately, cell loss ensues.

We have been studying beta-amyloid in blood for its potential use as a marker of disease onset and progression because obtaining a blood sample is far more cost-effective and applicable for wide-scale use in routine healthcare settings than lumbar puncture and beta-amyloid imaging of the brain. Using a series of blood samples, we have shown that these markers in blood are less variable in

Furthermore, Alzheimer's is associated with increased levels of inflammation-causing proteins around beta-amyloid deposits in the brain. We have shown that a blood sample tested for the biomarkers beta-amyloid, inflammation-related proteins CD40 and CD40L, and Alzheimer's risk gene apolipoprotein E-e4 (ApoE-e4) has a high degree of accuracy in diagnosing Alzheimer's.

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— Cheryl A. Luis, Ph.D.

people with Alzheimer's than in healthy, nondemented individuals. We and others have also shown that levels of beta-amyloid in the blood are predictive of cognitive decline in healthy at-risk seniors and in individuals with MCI. In addition, in a group of individuals at high-risk for Alzheimer's whom we have been studying for several years, we have shown that beta-amyloid levels in blood are impacted by vascular disease.

Moreover, we have shown that beta-amyloid levels in blood are associated with the cognitive changes seen in Alzheimer's. In individuals with MCI or early Alzheimer's, higher levels of beta-amyloid 1-42 in blood are associated with poorer performance on episodic memory tests while higher levels of beta-amyloid 1-40 are correlated with impaired language fluency and processing speed, as well as impaired divided attention, which are indicators of disease progression.

Without funding from the Alzheimer's Association, I would not be able to move forward with this important line of investigation, which will examine how accurately the combined use of MoCA and blood tests of beta-amyloid, CD-40 and CD-40L, and APOE-e4 detect the presence or absence of Alzheimer's. We hope that further refinement of this screening method will lead to funding from the National Institutes of Health to take this approach into the primary care setting for further study.

Wide-scale, cost-effective methods for very early detection of Alzheimer's will allow us to start disease-modifying treatment early and hopefully prevent the significant loss of cognitive abilities currently experienced by people with Alzheimer's.



Liqin Zhao, Ph.D.

Research Assistant Professor of Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, recipient of a 2005 New Investigator Research Grant

I often think about a very emotional conversation with a close friend regarding his 69-year-old father, who had just been diagnosed with early-stage Alzheimer's disease and had failed his driver's license exam. My friend was deeply hurt by the mean way in which his father communicated with him after failing the exam. "He is perfectly fine in all other areas, and I can't believe that he was so mean to me," my friend repeated, even after I had spent hours explaining to him how a human brain's structure and function can be altered by Alzheimer's and that these changes can affect the individual's judgment and the way they communicate and act.

Many months later, my friend was able to put these feelings behind him, but then was overwhelmed with emotion as he witnessed the disease slowly taking his father away from their family. While living in a nursing home over the next five years, his father continued to worsen until he could no longer recognize the people around him, even family members.

Those conversations with my friend solidified my passion for Alzheimer research and finding a cure so others would not experience the emotional struggles my friend had experienced. The

Alzheimer's Association opened a door to help turn my passion into reality. The New Investigator Research Grant (NIRG) from the Alzheimer's Association enabled me to pursue my own ideas and conduct the basic science research that has led to the discovery of a promising therapy for Alzheimer's. This novel therapy is being studied in a clinical trial funded by the National Institute on Aging (NIA).

Despite the attention given to Ronald Reagan, Charlton Heston, Sargent Shriver and other men who developed Alzheimer's, elderly women are still by far the principle victims of the disease, accounting for 68 percent of cases. Both basic science and clinical research suggest that ovarian estrogens may help prevent neurodegeneration in women. The loss of estrogen during menopause could be a risk factor for cognitive impairment and dementia such as Alzheimer's. Moreover, research suggests that timely initiation of an estrogen-containing hormone therapy (HT) could help prevent Alzheimer's and alleviate other estrogen deficiency-associated symptoms in menopausal and postmenopausal women. However, since current forms of HT may increase women's risk of breast cancer, stroke and blood clots, it's imperative that we develop an alternative HT that is both effective and safe.

Over the past decade, a group of plant-derived, non-steroidal estrogen-like compounds, known as phytoestrogens, have been found to have similar

functional activity to ovarian estrogens and have received enormous attention by both researchers and clinicians. This increased interest in phytoestrogens has been attributed in large part to the health-promoting effects suggested by epidemiological

American and 70 percent of European women. In addition, historically, breast and prostate cancer rates in Asia have been much lower than in Western countries. These positive observational findings, however, lack confirmation from well-controlled,

“ In addition to its tremendous impact on my research, the NIRG award from the Alzheimer’s Association has monumentally advanced my career as an independent Alzheimer researcher. I was appointed to a research faculty position soon after I received this honorable award, and the funded research has resulted in several peer-reviewed publications and two U.S. patent applications. No words can describe how grateful I am to the Alzheimer’s Association for its instrumental role in making this happen. ”

—Liqin Zhao, Ph.D.

studies of soy-based foods, which are rich in phytoestrogens and regularly consumed in Asian countries. For example, a number of epidemiological studies conducted in populations across the continents revealed a 2.5 times lower prevalence of Alzheimer’s in Japan and China than in North America and Europe, where individuals consume less than 1 milligram of phytoestrogens per day compared with 20–80 milligrams per day in Asian individuals.

Moreover, epidemiological studies suggest that this difference in phytoestrogen intake may also contribute to differences in the incidence of other sex hormone-related disorders seen in Asian versus North America and European populations. In particular, recent statistics show that only 25 percent of Japanese and 18 percent of Chinese menopausal and postmenopausal women experience hot flashes compared with 85 percent of North

randomized clinical trials, which have produced inconsistent and inconclusive data.

Growing up in China and then living in the United States has given me a firsthand opportunity to appreciate the differences in the diets between these two distant parts of the world. Capitalizing upon such knowledge, I hypothesized that the discrepancies between observational studies focusing on soy-derived foods and interventional studies examining soy-derived extracts could have originated from the differences between the natural forms of soy foods and pharmacological preparations of soy extracts.

The bottom line is that soy foods and soy extracts are not the same. In recent years, many soy extract products have become available over the counter. Most of these are advertised as dietary supplements for use by women to lessen menopausal

symptoms such as hot flashes. However, these supplements are unregulated by the U.S. Food and Drug Administration, so their safety and efficacy are questionable.

The greatest problem is that the processing of soy extract products is not standardized. An analysis of a number of commercial soy extract supplements revealed an abundance of substances of unknown origin. Some of these substances may have been created through the extraction process. In this process, the organic solvents and high temperatures used may cause chemical reactions leading to the generation of substances not naturally present in soy. These substances could produce an undesirable effect, counteracting the favorable health-giving properties of other substances in the supplement.



Funding from the Alzheimer's Association enabled me to test this hypothesis and attempt to develop a phytoestrogen formulation that is safe and effective. The research included screening a database of 25,000 phytoestrogens and resulted in the discovery of a formulation we've called phyto-beta-SERM that was found to be neuroprotective. A nine-month study of female mice engineered to develop Alzheimer's that were given the formulation provided additional support for the therapeutic potential of the formulation in slowing or reducing the cognitive decline and neurophysiological changes associated with Alzheimer's. In contrast, mice

receiving a commercial soy extract-containing diet did not show these improvements.

To my tremendous excitement, these benchside scientific discoveries have led to an NIA-funded, recently launched, first-in-humans pilot trial of phyto-beta-SERM. The trial evaluates the formulation's safety, its effect on hot flashes and cognition in menopausal and postmenopausal women and how it is absorbed, distributed, and metabolized (its pharmacokinetics) in the body. The trial consists of two studies to be completed in the next three years. The Phase I study is designed to determine the pharmacokinetics of the formulation and which doses are safe and well-tolerated. The Phase IIA study is a proof-of-concept study that will continue to examine safety and best dosages but also examine the formulation's effect on hot flashes and cognition. The success of these early stage human studies will lay important foundations for later stage, long-term efficacy studies in Alzheimer patients.

For many years, one of the largest unmet medical needs in the health of menopausal and postmenopausal women has been an estrogen therapy that is both effective and safe. I am hopeful that my research could lead to a breakthrough to fill the gap. The formulation may also help preserve cognitive function in men.