



**“Autumn is really the best of the seasons; and I’m not sure that old age isn’t the best part of life. But of course, like autumn, it doesn’t last.”**

**C.S. Lewis**

## **Chapter 4**

## **Mid-Life**

The photograph below is a very funny and extreme way of looking at mid-life. Now that really is a crisis. The song as good as I once was really sets the tone for this stage of life. It talks about how his body says he caint do things that he used to but his pride will not let him accept it. The movies for this chapter are pretty funny and also depict some extreme mid life crises.

## Chapter Intro

This chapter is about the strange stage known as mid-life. There are many dangers that can overtake you if you are not prepared. Therefore I have taken it upon myself to help you through this stage by warning you in advance of the dangers, and showing you how to cleanly navigate through the obstacles. If you listen closely and do as I say, your transition will be as smooth as butter; which reminds me, you're going to have to start being more modest with the butter intake, but we will get into that later. So here we go.

I just want to start off by saying I understand that this is all happening very fast but if we do not act now it will be too late. You have to understand that mid-life is strange in many different ways. One of the biggest is that you find that people, without your consent, begin to consider you as OLD. It is like you just wake up one day and you are suddenly put in the category of "old person". I know that it is confusing because just yesterday you were no different than the thirty year old in your office but you have are going to have to accept your 'oldness' as a fact. Now I know what thoughts are going through your head right now. You are thinking that you can possibly overcome these thoughts by changing the way you dress something but trust me, you cannot. You must persevere through these thoughts, because if you do not, you are going to get stuck in a crisis situation. You will lose all rational thought and begin acting in very strange ways. You might even spend all your savings on a sports car or motor cycle. It won't change anything though; you will just be an old guy that dresses weird and drive a car worth more than



Well it is getting about lunch time so I figure it is a good time to go over your diet.

Yeah, I know, on top of everything else that is going on you have to watch what you eat. You are going to have to learn the discipline of not getting loose with the salt shaker. Unless you want die you are going to have to take it easy on the sodium. You see when you get old you begin to here this word "cholesterol" in your dreams. You are also going to have to cut down on the fried food and on the butter. I know it sucks but if you want to spend your retirement from a bed then you go right ahead and eat some more hot wings. But that's enough about all this depressing talk, let us move on to the final hurdle.

This final obstacle is actually going to be the most fun. We are going to make a list of the things that you want to do and places you want to go. This may seem unnecessary but trust me, if you don't decide what you want to do you will piss-away the window of time where you are healthy enough to travel. You need to list places that you have always wanted to go and then make plans on how to get there. These can be the best years of your life if you take advantage of the time you have and don't let the fact that you are getting old get in the way. Take this time and enjoy your family and friends and your husband or wife. Live life to the fullest.

As we progress through life, we build a massive mental library of memories. All the things that were important to us - a special present at Christmas, our first kiss, our wedding day, or the birth of a child - we store safely away inside our minds. So it comes as no surprise that diseases such as Alzheimer's, which invariably strip us of our most cherished memories before eventually consuming our lives, scares us so much. The search for a cure is on and has been raging for some time. "Shutting Down Alzheimer's" offers up some examples of the type of work that's being done in hopes of finding a cure for Alzheimer's.

## *Shutting Down Alzheimer's*

The human brain is a remarkably complex organic computer, taking in a wide variety of sensory experiences, processing and storing this information, and recalling and integrating selected bits at the right moments. The destruction caused by Alzheimer's disease has been likened to the erasure of a hard drive, beginning with the most recent files and working backward. An initial sign of the disease is often the failure to recall events of the past few days--a phone conversation with a friend, a repairman's visit to the house--while recollections from long ago remain intact. As the illness progresses, however, the old as well as the new memories gradually disappear until even loved ones are no longer recognized. The fear of Alzheimer's stems not so much from anticipated physical pain and suffering but rather from the inexorable loss of a lifetime of memories that make up a person's very identity.

Unfortunately, the computer analogy breaks down: one cannot simply reboot the human brain and reload the files and programs. The problem is that Alzheimer's does not only erase information; it destroys the very hardware of the brain, which is composed of more than 100 billion nerve cells (neurons), with 100 trillion connections among them. Most current medications for Alzheimer's take advantage of the fact that many of the neurons lost to the disease release a type of chemical communicator (or neurotransmitter) called acetylcholine. Because these medicines block an enzyme responsible for the normal decomposition of acetylcholine, they increase the levels of this otherwise depleted neurotransmitter. The result is stimulation of neurons and clearer thinking, but these drugs typically become ineffective within six months to a year because they cannot stop the relentless devastation of neurons. Another medication, called memantine, appears to slow the cognitive decline in patients with moderate to severe Alzheimer's by blocking excessive activity of a different neurotransmitter (glutamate), but investigators have not yet determined whether the drug's effects last more than a year.

More than a decade ago few people were optimistic about the prospects for defeating Alzheimer's. Scientists knew so little about the biology of the disease, and its origins and course were thought to be hopelessly complex. Recently, however, researchers have made tremendous progress toward understanding the molecular events that appear to trigger the illness, and they are now exploring a variety of strategies for slowing or halting these destructive processes. Perhaps one of these treatments, or a combination of them, could impede the degeneration of neurons enough to stop Alzheimer's disease in its tracks. Several candidate therapies are undergoing clinical trials and have yielded some

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promising preliminary results. More and more researchers are feeling hope--a word not usually associated with Alzheimer's.

### **The Amyloid Hypothesis**

THE TWO KEY FEATURES of the disease, first noted by German neurologist Alois Alzheimer 100 years ago, are plaques and tangles of proteins in the cerebral cortex and limbic system, which are responsible for higher brain functions. The plaques are deposits found outside the neurons and are composed primarily of a small protein called amyloid-beta, or A-beta. The tangles are located inside neurons and their branching projections (axons and dendrites) and are made of filaments of a protein called tau. The observation of these anomalies started a debate that lasted throughout most of the 20th century: Are the plaques and tangles responsible for the degeneration of brain neurons, or are they merely markers of where neuronal death has already occurred? In the past decade, the weight of evidence has shifted toward the amyloid-cascade hypothesis, which posits that both A-beta and tau are intimately involved in causing Alzheimer's disease, with A-beta providing the initial insult.

A-beta is a short peptide, or protein fragment, first isolated and characterized in 1984 by George G. Glenner and Cai'ne W. Wong, then at the University of California, San Diego. This peptide is derived from a larger protein called the amyloid-beta precursor protein, or APP. Molecules of APP stick through the cellular membrane, with one part of the protein inside the cell and another part outside. Two protein-cutting enzymes, or proteases--beta-secretase and gamma-secretase--carve out A-beta from APP, a process that

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occurs normally in virtually all cells in the body. The reason why cells make A-beta is unclear, but current evidence suggests that the process is part of a signaling pathway.

A portion of the A-beta region of APP is inside the membrane itself, between its outer and inner layers. Because membranes are composed of water-repelling lipids, the regions of proteins that pass through membranes typically contain water-repelling amino acids. When A beta is cut out of APP by beta- and gamma-secretase and released into the aqueous environment outside the membrane, the water-repelling regions of different A-beta molecules cling to one another, forming small soluble assemblies. In the early 1990s Peter T. Lansbury, Jr., now at Harvard Medical School, showed that at high enough concentrations, A-beta molecules in a test tube can assemble into fiber-like structures similar to those found in the plaques of Alzheimer's disease. The soluble assemblies as well as the fibers of A-beta are toxic to neurons cultured in petri dishes, and the former can interfere with processes critical to learning and memory in mice.

These findings supported the amyloid-cascade hypothesis, but the strongest evidence came from studies of families at especially high risk of getting Alzheimer's. Members of these families carry rare genetic mutations that predestine them for the disease at a relatively young age, typically before 60. In 1991 John A. Hardy, now at the National Institute on Aging, and his colleagues discovered the first such mutations in the gene that encodes APP, specifically affecting the areas of the protein in and around the A-beta region. Soon afterward, Dennis J. Selkoe of Harvard and Steven Younkin of the Mayo Clinic in Jacksonville, Fla., independently found that these mutations increase the formation of either A-beta in general or a particular type of A-beta that is highly prone to forming

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deposits. Moreover, people with Down syndrome, who carry three copies of chromosome 21 instead of the normal two copies, have a much higher incidence of Alzheimer's in middle age. Because chromosome 21 contains the APP gene, people with Down syndrome produce higher levels of A-beta from birth, and amyloid deposits can be found in their brains as early as age 12.

Researchers soon discovered other connections between Alzheimer's disease and the genes that regulate the production of A-beta. In 1995 Peter St. George-Hyslop and his colleagues at the University of Toronto identified mutations in two related genes dubbed presenilin 1 and 2 that cause very early and aggressive forms of Alzheimer's, typically appearing when the carrier is in his or her 30s or 40s. Further studies showed that these mutations increase the proportion of A-beta that is prone to clumping. We now know that the proteins encoded by the presenilin genes are part of the gamma-secretase enzyme.

Thus, of the three genes known to cause Alzheimer's early in life, one encodes the precursor to A-beta and the other two specify components of a protease enzyme that helps to manufacture the harmful peptide. Furthermore, scientists have found that people carrying a certain variation in the gene encoding apolipoprotein E--a protein that helps to bring together the A-beta peptides in assemblies and filaments--have a substantially elevated risk of developing Alzheimer's later in life. A variety of genetic factors most likely play a role in the onset of the disease, with each contributing in a small way, and mouse studies indicate that environmental factors may also affect the disease risk (exercise, for example, may lower it).

Scientists still do not understand exactly how the soluble assemblies and insoluble filaments of A-beta disrupt and kill neurons. The evidence suggests, though, that aggregates of A-beta outside a neuron can initiate a cascade of events that include the alteration of the tau proteins inside the cell. In particular, the A-beta aggregates can ultimately change the cellular activity of enzymes called kinases that install phosphates onto proteins. The affected kinases add too many phosphates to tau, changing the proteins' chemical properties and causing them to form twisted filaments. The altered tau proteins somehow kill the neuron, perhaps because they disrupt the microtubules that transport proteins and other large molecules along axons and dendrites. Mutations in the tau gene itself can also generate tau filaments and cause other types of neurodegenerative diseases besides Alzheimer's. Thus, the formation of tau filaments is apparently a more general event leading to neuronal death, whereas A-beta is the specific initiator in Alzheimer's disease.

### **Clamping the Molecular Scissors**

GIVEN THE CRITICAL role of A-beta in the disease process, the proteases that produce this peptide are obvious targets for potential drugs that could inhibit their activity. Protease inhibitors have proved very effective for treating other disorders such as AIDS and hypertension. The first step in the formation of A-beta is initiated by beta-secretase, a protease that clips off the bulk of APP just outside the cellular membrane. In 1999 five different research groups independently discovered this enzyme, which is particularly abundant in brain neurons. Although beta-secretase is tethered to the membrane, it closely resembles a subset of proteases found in the aqueous environments inside and outside cells. Members of this subset--which includes the protease involved in replicating HIV, the virus that causes AIDS—use aspartic acid, a type of amino acid, to catalyze the protein-

cutting reaction. All proteases use water to cut their respective proteins, and enzymes in the aspartyl protease family employ a pair of aspartic acids to activate a water molecule for this purpose.

Because beta-secretase clearly falls into this family, researchers were able to exploit the vast knowledge about these proteases, leading to a very detailed understanding of this enzyme and how it might be shut down. Indeed, investigators already know the three-dimensional structure of beta-secretase and have used it as a guide for computer-based drug design of potential inhibitors. Genetic studies suggest that blocking the enzyme's activity will not lead to harmful side effects; deletion of the gene encoding beta-secretase in mice eliminated A-beta formation in the rodents' brains without causing any apparent negative consequences. For the moment, however, beta-secretase inhibitors are not yet ready for clinical trials. The main challenge is to develop potent compounds that are small enough to effectively penetrate the brain. Unlike blood vessels in other parts of the human body, capillaries in the brain are lined with endothelial cells that are very tightly packed. Because there are few gaps between the cells, the protease inhibitors must be able to pass through the cell membranes to reach the brain tissues beyond, and most large molecules cannot breach this so-called blood-brain barrier.

The enzyme called gamma-secretase performs the second step in the formation of A-beta, cutting the stump of APP remaining after the cleavage by beta-secretase. Gamma-secretase accomplishes the unusual feat of using water to cut the protein inside the otherwise water-hating environment of the cellular membrane. Two important clues proved essential to our understanding of this protease. First, Bart De Strooper of the

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Catholic University of Louvain in Belgium found in 1998 that genetically deleting the presenilin 1 gene in mice greatly reduced the cutting of APP by gamma-secretase, demonstrating that the protein encoded by the gene is essential to the enzyme's function. Second, my laboratory, then at the University of Tennessee at Memphis, discovered that compounds in the same chemical category as the classical inhibitors of aspartyl proteases could block gamma-secretase cleavage of APP in cells. This result suggested that gamma-secretase, like beta-secretase, contains a pair of aspartic acids essential for catalyzing the protein-cutting reaction.

Based on these observations, we hypothesized that the presenilin protein might be an unusual aspartyl protease stitched into the fabric of cell membranes. While I was on sabbatical at Harvard in Selkoe's lab and in collaboration with Weiming Xia, we identified two aspartic acids in presenilin predicted to lie within the membrane and demonstrated that they are both critical to the gamma-secretase cleavage that produces A-beta. Subsequently, we and others showed that the inhibitors of gamma-secretase bind directly to presenilin and that three other membrane-embedded proteins must assemble with presenilin to allow it to catalyze. Today gamma-secretase is recognized as a founding member of a new class of proteases that apparently wield water within cellular membranes to accomplish their biochemical tasks. Better yet, the inhibitors of gamma-secretase are relatively small molecules that can pass through membranes, enabling them to penetrate the blood-brain barrier.

Two years ago I spoke to my youngest son's fifth-grade class about the work in my lab, explaining about amyloid and how we hoped to block the responsible enzymes to

discover new medicines for Alzheimer's. One boy interrupted: "But what if that enzyme is doing something important? You could hurt somebody!" This concern, recognized by a 10-year-old, is very real: the potential of gamma-secretase as a therapeutic target is tempered by the fact that this enzyme plays a critical role in the maturation of undifferentiated precursor cells in various parts of the body, such as the stem cells in bone marrow that develop into red blood cells and lymphocytes. Specifically, gamma-secretase cuts a cell-surface protein called the Notch receptor; the piece of Notch released from the membrane inside the cell then sends a signal to the nucleus that controls the cell's fate.

High doses of gamma-secretase inhibitors cause severe toxic effects in mice as a consequence of disrupting the Notch signal, raising serious concerns about this potential therapy. Nevertheless, a drug candidate developed by pharmaceutical maker Eli Lilly has passed safety tests in volunteers. (This kind of test is called a phase I clinical trial.) The compound is now poised to enter the next level of testing (phase II) in patients with early Alzheimer's. Moreover, researchers have identified molecules that modulate gamma-secretase so that A-beta production is blocked without affecting the cleavage of Notch. These molecules do not interact with gamma-secretase's aspartic acids; instead they bind elsewhere on the enzyme and alter its shape.

Some inhibitors can even specifically curtail the creation of the more aggregation-prone version of A-beta in favor of a shorter peptide that does not clump as easily. One such drug, Flurizan, identified by a research team headed by Edward Koo of the University of California, San Diego, and Todd Golde of the Mayo Clinic in Jacksonville, has shown considerable promise in early-stage Alzheimer's patients and is already entering more

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advanced (phase III) clinical trials that will include more than 1,000 such subjects across the country.

### **Clearing the Cobwebs**

ANOTHER STRATEGY for combating Alzheimer's is to clear the brain of toxic assemblies of A-beta after the peptide is produced. One approach is active immunization, which involves recruiting the patient's own immune system to attack A-beta. In 1999 Dale B. Schenk and his colleagues at Elan Corporation in South San Francisco made a groundbreaking discovery: injecting A-beta into mice genetically engineered to develop amyloid plaques stimulated an immune response that prevented the plaques from forming in the brains of young mice and cleared plaques already present in older mice. The mice produced antibodies that recognized A-beta, and these antibodies apparently prompted the brain's immune cells--the microglia--to attack aggregates of the peptide. The positive results in mice, which included improvements in learning and memory, quickly led to human trials.

Unfortunately, although the injection of A-beta passed initial safety trials, several patients in the phase II tests developed encephalitis--inflammation of the brain--forcing a premature halt to the study in 2002. Follow-up research indicated that the therapy might have caused the inflammation by prompting the T cells of the immune system to make overaggressive attacks on the A-beta deposits. Nevertheless, the investigation confirmed that many patients produced antibodies against A-beta and that those who did showed subtle signs of improved memory and concentration.

The safety concerns about active immunization led some researchers to try passive immunization, which aims to clear the peptide by injecting antibodies into patients. These antibodies, produced in mouse cells and genetically engineered to prevent rejection in humans, would not be likely to cause encephalitis, because they should not trigger a harmful T cell response in the brain. A passive immunization treatment developed by Elan Corporation has already advanced to phase II clinical trials.

How active or passive immunization can remove A-beta from the brain is somewhat mysterious, because it is unclear how effectively the antibodies can cross the blood-brain barrier. Some evidence suggests that entry into the brain may not be required: sopping up A-beta in the rest of the body may lead to an exodus of the peptide from the brain, because molecules tend to move from high concentrations to lower ones. Although passive immunization now seems to hold the most promise, active immunization is not out of the running. Preliminary studies headed by my Harvard colleague Cynthia Lemere show that immunization with selected parts of A-beta, instead of the entire peptide, can stimulate the antibody-producing B cells of the immune system without triggering the T cells responsible for the encephalitis.

Other researchers are pursuing nonimmunological strategies to stop the aggregation of A-beta. Several companies have identified compounds that interact directly with A-beta to keep the peptide dissolved in the fluid outside brain neurons, preventing the formation of harmful clumps. Neurochem in Québec is developing Alzhemed, a small molecule that apparently mimics heparin, the natural anticoagulant. In blood, heparin prevents platelets from gathering into clots, but when this polysaccharide binds to A-beta,

it makes the peptide more likely to form deposits. Because Alzhemed binds to the same sites on A-beta, it blocks the heparin activity and hence reduces peptide aggregation. The compound has shown little or no toxicity even at very high doses, and the treatment has resulted in some cognitive improvement in patients with mild Alzheimer's. Phase III clinical trials for this drug candidate are already well under way.

### **Targeting Tau**

AMYLOID, HOWEVER, is just one half of the Alzheimer's equation. The other half, the tau filaments that cause neuronal tangles, is also considered a promising target for preventing the degeneration of brain neurons. In particular, researchers are focused on designing inhibitors that could block the kinases that place an excessive amount of phosphates onto tau, which is an essential step in filament formation. These efforts have not yet resulted in candidate drugs for clinical trials, but the hope is that such agents might ultimately work synergistically with those targeting A-beta.

Investigators are also exploring whether the cholesterol-lowering drugs called statins, which are widely used to cut the risk of heart disease, could become a treatment for Alzheimer's as well. Epidemiological studies suggest that people taking statins have a lower risk of acquiring Alzheimer's. The reason for this correlation is not entirely clear; by lowering cholesterol levels, these drugs may reduce the production of APP, or perhaps they directly affect the creation of A-beta by inhibiting the activity of the responsible secretases. Phase III trials are trying to establish whether statins such as Pfizer's Lipitor can truly prevent Alzheimer's.

Another exciting recent development involves cell therapy. Mark Tuszynski and his colleagues at U.C.S.D. took skin biopsies from patients with mild Alzheimer's and inserted the gene encoding nerve growth factor (NGF) into these cells. The genetically modified cells were then surgically placed into the forebrains of these patients. The idea was that the implanted cells would produce and secrete NGF, preventing the loss of acetylcholine-producing neurons and improving memory. The cell-based therapy was a clever strategy for delivering NGF, a large protein that could not otherwise penetrate the brain. Although this study included only a handful of subjects and lacked important controls, follow-up research showed a slowing of cognitive decline in the patients. The results were good enough to warrant further clinical trials.

Although some of these potential therapies may not fulfill their promise, scientists hope to find at least one agent that can effectively slow or stop the gradual loss of neurons in the brain. Such a breakthrough could save millions of people from the inexorable decline of Alzheimer's disease and set the stage for regenerative medicine to restore lost mental functions.

Targeting A-beta may block the onset of Alzheimer's or retard it early in its course, but whether this strategy will treat or cure those with more advanced stages of the disease remains unclear. Still, researchers have good reason for cautious optimism. The recent spate of discoveries has convinced many of us that our quest for ways to prevent and treat Alzheimer's will not be in vain.

Everyone wants to find a way around death, but we, as rational beings generally accept that fact that there's no getting away from it. Aging, on the other hand, is something that just might be preventable. If death is inevitable, why not at least spend the time one has on the earth in a relative amount of good health? This plausible question has been a topic of concern for many years, and the hope of scientists has been to find the proverbial fountain of youth. Today, the approach to the secret of longevity has turned toward understanding our genes. Some of the more recent developments in age-related genes are presented in the following article.

## *Unlocking the Secrets of Longevity Genes*

A handful of genes that control the body's defenses during hard times can also dramatically improve health and prolong life in diverse organisms. Understanding how they work may reveal the keys to extending human life span while banishing diseases of old age

You can assume quite a bit about the state of a used car just from its mileage and model year. The wear and tear of heavy driving and the passage of time will have taken an inevitable toll. The same appears to be true of aging in people, but the analogy is flawed because of a crucial difference between inanimate machines and living creatures: deterioration is not inexorable in biological systems, which can respond to their environments and use their own energy to defend and repair themselves.

At one time, scientists believed aging to be not just deterioration but an active continuation of an organism's genetically programmed development. Once an individual achieved maturity, "aging genes" began to direct its progress toward the grave. This idea has been discredited, and conventional wisdom now holds that aging really is just wearing out over time because the body's normal maintenance and repair mechanisms simply wane. Evolutionary natural selection, the logic goes, has no reason to keep them working once an organism has passed its reproductive age.

Yet we and other researchers have found that a family of genes involved in an organism's ability to withstand a stressful environment, such as excessive heat or scarcity of food or water, have the power to keep its natural defense and repair activities going strong regardless of age. By optimizing the body's functioning for survival, these genes maximize the individual's chances of getting through the crisis. And if they remain activated long enough, they can also dramatically enhance the organism's health and extend its life span. In essence, they represent the opposite of aging genes--longevity genes.

We began investigating this idea nearly 15 years ago by imagining that evolution would have favored a universal regulatory system to coordinate this well-known response to environmental stress. If we could identify the gene or genes that serve as its master controllers and thereby act as master regulators of an organism's life span, these natural defense mechanisms might be turned into weapons against the diseases and decline that are now apparently synonymous with human aging.

Many recently discovered genes, known by such cryptic names as daf-2, pit-1, amp-1, clk-1 and p66Shc, have been found to affect stress resistance and life span in laboratory