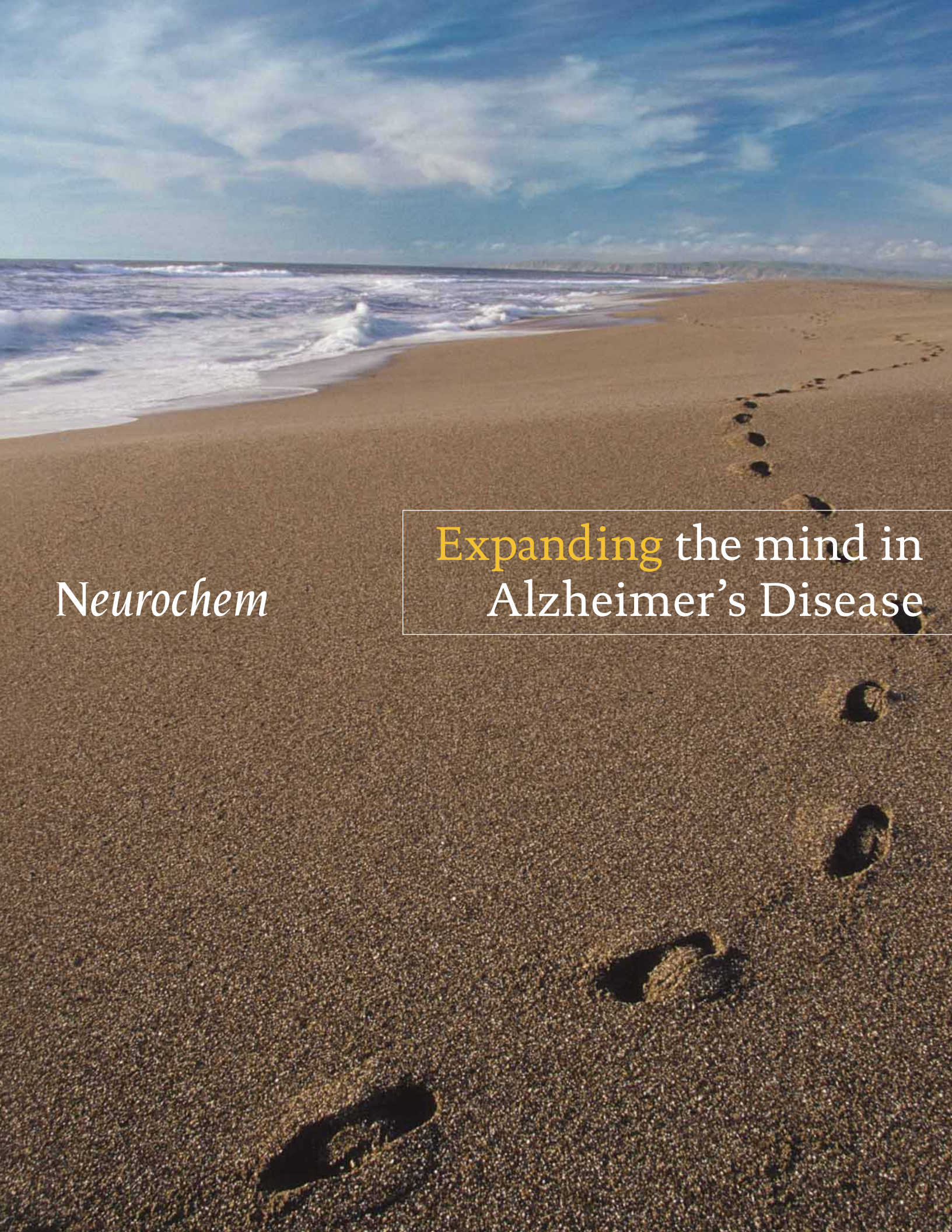
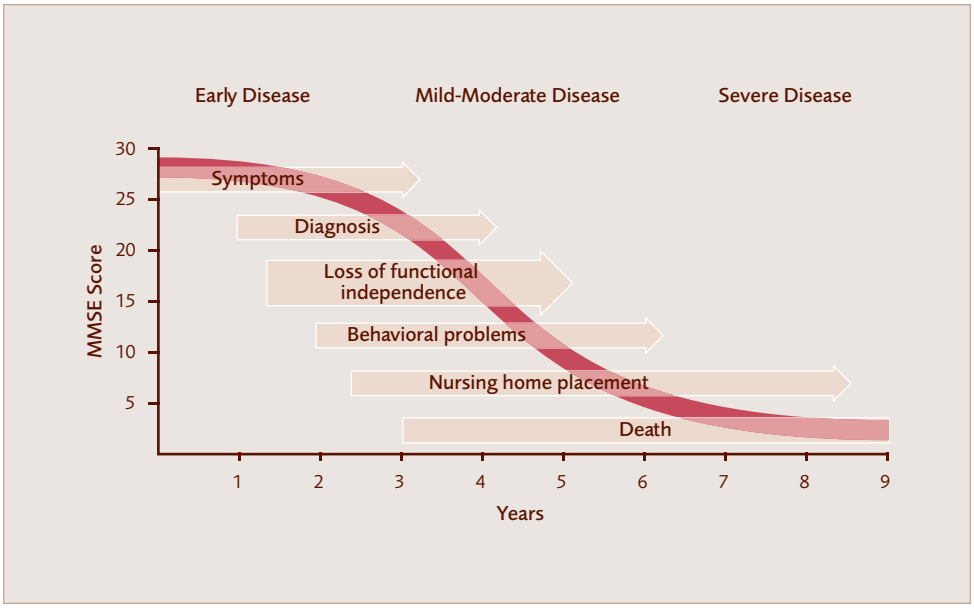


*Neurochem*

# Expanding the mind in Alzheimer's Disease

For the first time since Alzheimer's Disease (AD) was originally described almost a century ago, scientific advances in our understanding of the disease offer opportunities to develop disease modifying therapies that may prevent, slow or even halt its progression.

Figure 1: Alzheimer's Disease Natural History (Adapted from Feldman[1])

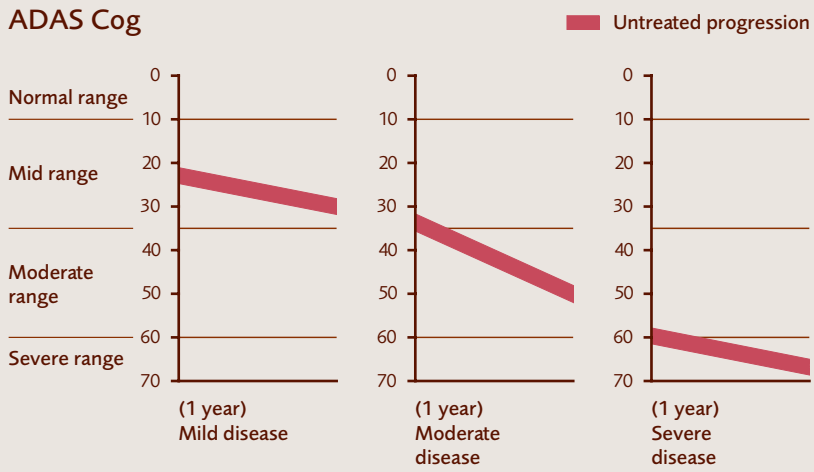



# Alzheimer's disease progression

AD is a progressive form of dementia associated with specific brain pathologies. Mild AD is characterized by mild memory loss, impaired judgment and other cognitive dysfunctions. As patients progress to the moderate phase of the disease, they begin to lose their ability to perform basic everyday tasks, such as cooking and personal care. Behavioral problems such as agitation, aggression, wandering and sleep disorders are often present. In the severe stages, locomotion, speech, continence and the ability to recognize people and objects may all be lost (Figure 1).

Early AD can be very difficult to differentiate from the mild memory and cognitive deficits that characterize either normal aging or mild cognitive impairment (MCI). MCI is a newly defined syndrome characterized primarily by memory loss in the absence of other cognitive deficits. About 40% of persons with MCI will develop AD within 3 years[2]. In clinical practice, there is a failure to diagnose early AD in up to 75% of cases, with an average delay in diagnosis of about 2 years from onset of first symptoms. The clinical progression of AD functional deficits is often initially slow. About 25% of patients per year proceed to the moderate phase of AD[3]. Functional abilities then deteriorate more rapidly as the patient progresses to the severe stages of the disease (Figure 2). Overall mean deterioration as measured on the Alzheimer's disease Assessment Scale cognitive section (ADAS-cog, a 70-point scale) is 9 – 11 points per year[4]. Death generally occurs 8 – 10 years following diagnosis[2].

Figure 2: Change in cognitive subscale scores in AD patients (Adapted from Gauthier[5])




AD is known to be associated with plaques consisting of masses of β-amyloid (Aβ) protein fibrils (Figure 3) and many other proteins that are products of neuronal and glial response to injury.

Also present within the neurons are neurofibrillary tangles, consisting of tau protein. Both plaques and tangles develop preferentially in specific areas of the brain associated with cognition, primarily the hippocampus, the entorhinal cortex and association areas of the neocortex. These same areas of the brain also show atrophy, due to rapid neuron death. This atrophy is associated with the decline of cognitive function seen in AD[6], and can be clearly observed in sequential neuroimaging such as MRI or PET scanning.

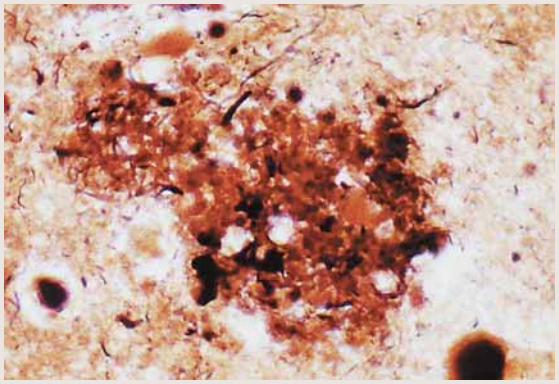


Figure 3: Silver-stained Alzheimer neuritic plaque

"It is widely believed that the cascade of events leading to [β-]amyloid accumulation is at the root of AD pathogenesis and the ensuing dementia."

Roher A., 2003[7]

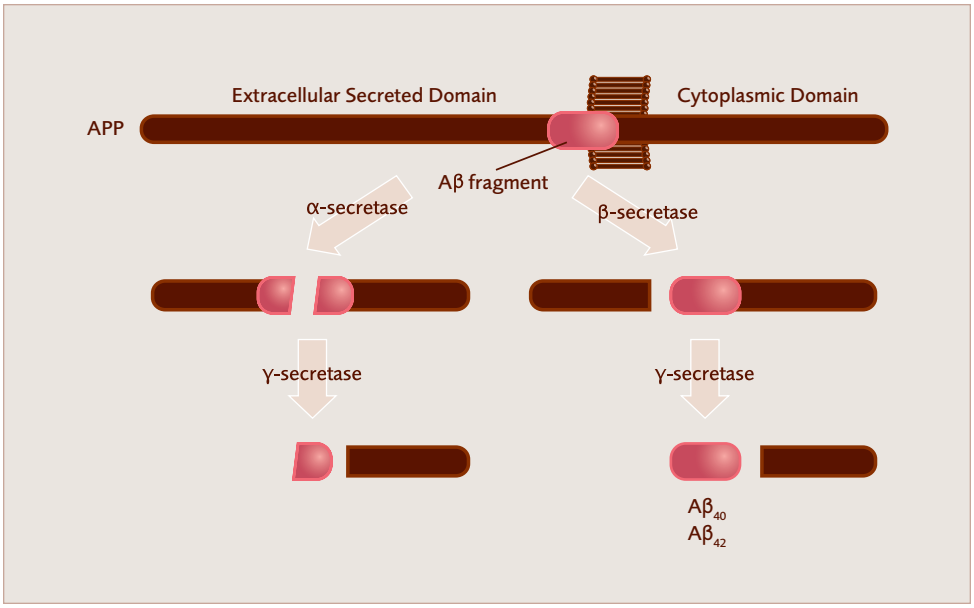
# Understanding the β-amyloid pathway

There has been a long-standing difference of opinion about whether the Aβ plaques are a cause of AD, or merely the result of the degeneration of neuronal tissue.

Elucidation of the biochemistry of Aβ synthesis and clearance has, however, provided evidence for a causative role for Aβ in AD. A great deal of evidence for such a role for Aβ has also come from the field of genetics. Transgenic mice with a mutant form of the human APP gene show both Aβ deposits and behavioral abnormalities[8]. Down's syndrome patients, who carry an extra copy of the APP gene (which is located on chromosome 21), show Aβ deposition as early as age 12, and often develop dementia by their mid-thirties[8]. In a small number of AD cases (1-2%), the disease is known to be directly associated with specific genetic mutations[3]. Mutations resulting in AD have been identified on three separate genes—the APP gene on chromosome 21 (10 mutations), the presenilin 1 (PS1) gene on chromosome 14 (90+ mutations) and the PS2 gene on chromosome 1 (6+ mutations)—each of which directly affects different points along the pathway that leads to the production of Aβ[9]. The APP gene is responsible for the production of amyloid precursor protein (APP), while it is now believed that γ-secretase, mediates the final step in Aβ synthesis[2]. This genetic "smoking gun" reinforces the primary role of Aβ in the pathogenesis of AD, a role that has become increasingly recognized by the research community.

Further evidence has come from studies of the polymorphic apoE gene. The most common apoE genotype (apoE3) plays a role in cholesterol metabolism and is believed to be involved in clearing Aβ from the brain[10]. The presence of apoE4 alleles is associated with higher levels of Aβ within the brain, suggesting impaired clearance of Aβ in the presence of apoE4 protein. People with one or both apoE4 alleles have been shown to be at higher risk of AD. This again indicates a direct link between Aβ levels and AD[11].

Figure 4: APP and its cleavage



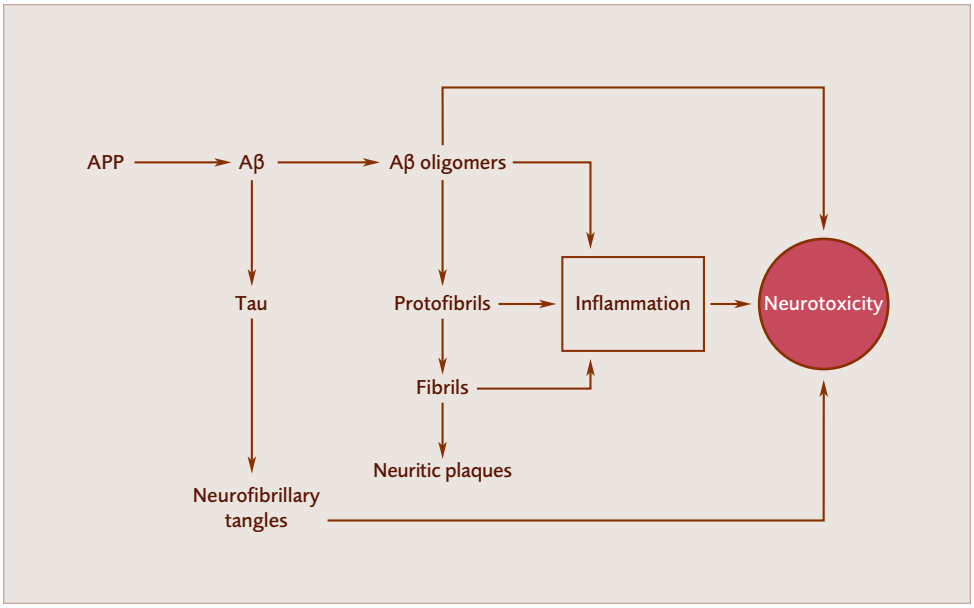

# Understanding the β-amyloid pathway

It is not known whether the accumulation of Aβ precedes the pathological intercellular formation of hyperphosphorylated tau. However, there is increasing supporting evidence, that in AD, the tau alteration follows amyloid plaque formation and deposition rather than the other way around[11]. This is supported by animal studies where transgenic mice (APP + tau) developed neurofibrillary tangles (NFT) as a consequence of Aβ deposition. Additionally, in families with tau mutations, widespread NFT were observed in the absence of amyloid deposits—meaning that high levels of intercellular tau will not necessarily lead to Aβ plaque formation.

## Aβ production

Aβ production begins with amyloid precursor protein (APP), a transmembrane protein found in healthy neurons, believed to be neuroprotective. APP is initially cleaved by either α-secretase or β-secretase, and then by γ-secretase. The more common peptide end products of the α-secretase pathway are soluble and do not result in the formation of Aβ. Among the final products of the β-secretase pathway, however, are several β-amyloid variants, $Aβ_{40}$ and $Aβ_{42}$, consisting of 40 and 42 amino acids respectively (Figure 4). The less common $Aβ_{42}$ is more toxic than $Aβ_{40}$, and has a tendency to aggregate more rapidly into small clusters, or oligomers and ultimately into fibrils and plaques (Figures 3–6). The oligomers are believed to be very toxic to neurons. Several mechanisms of cell damage have been proposed, e.g. activation of apoptosis, depletion of presynaptic APP leading to loss of synaptic transmission, hyperphosphorylation of tau protein causing microtubule collapse, and stimulation of microglia, eliciting a strong inflammatory response[6].

Figure 5: Pathway of Aβ-induced neuronal damage



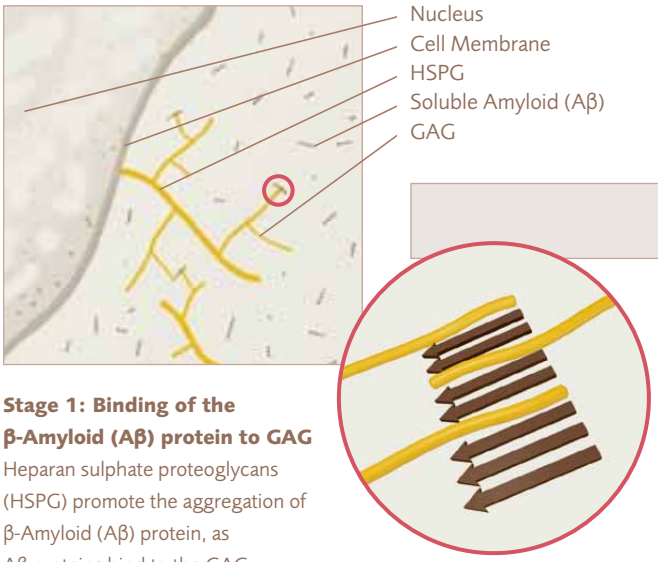

## Neuritic plaque formation

In AD, Aβ fibrils, in association with other elements like ApoE, complement component and heparan sulfate proteoglycans (HSPG), form neuritic plaques, which are found in the extracellular space surrounding the neurons, along with dystrophic neuritis, astroglioses and microglioses[2, 6].

These fibrils have also been shown to be toxic to cell membranes, and elicit inflammatory responses from glial cells. It is believed that their physical presence may also damage neurons.

Figure 6: β-Amyloid fibril

Figure 7: Stages of β-Amyloid (Aβ) fibril formation and deposition




**Stage 1: Binding of the β-Amyloid (Aβ) protein to GAG**
Heparan sulphate proteoglycans (HSPG) promote the aggregation of β-Amyloid (Aβ) protein, as Aβ proteins bind to the GAG

# Understanding the β-amyloid pathway

## The pathophysiology of AD

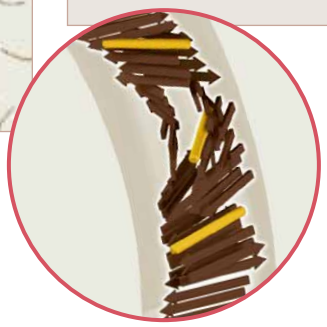
The production of Aβ is not in itself pathological; both $Aβ_{40}$ and $Aβ_{42}$ are found in biological fluids throughout life, so the production of at least some Aβ is part of normal cellular metabolism[9]. It is known that once Aβ begins to assemble in an oligomeric form and to aggregate, it becomes more toxic and more difficult to eliminate due to increasing insolubility, so it is reasonable to assume that the pathological variant of the cascade begins somewhere between the production of Aβ and the appearance of plaques. The abnormality in Aβ metabolism leads to a shift towards increased concentration and aggregation of the most toxic $Aβ_{42}$ form, due either to excessive production or reduced clearance. The result is inflammatory damage to neurons, cell death and the formation of neuritic plaques.

## The role of proteoglycans and glycosaminoglycans

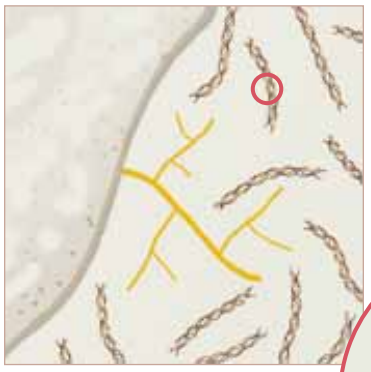
Among recent discoveries that have elucidated the mechanisms of amyloid diseases, one of the most intriguing is the role of glycosaminoglycans (GAGs) in facilitating the aggregation of Aβ.

Proteoglycans (PGs) are widely-distributed molecules found in almost all body tissues. They are part of the extracellular matrix, secreted or membrane-bound. They often serve as cofactors to recruit ligands to their receptors (e.g. Aβ) and to ensure efficient receptor-ligand interactions. PGs consist of a protein core with long side-chains of complex sulfated polysaccharides known as GAGs, which carry a very strong negative charge. In AD, Aβ molecules are attracted to the GAGs on heparan sulfate proteoglycans (HSPG) and bind to them, forming toxic oligomers and insoluble, folded sheets of Aβ protein[12] (Figure 7).

**Stage 2: Aggregation of Aβ proteins**
The structural shift of Aβ proteins is responsible for the fibrillogenic aspect of the Aβ protein



Aβ β-sheet formation



**Stage 3: Formation and deposition of Aβ fibrils**
The Aβ fibrils are formed and deposited around cells in affected organs, leading to organ dysfunction

β-Amyloid (Aβ) fibrils

Figure 8: Bielschowsky-stained neurofibrillary tangles in patient with Alzheimer's disease

## The role of tau

*In the healthy neuron, tau protein functions to support the intraneuronal microtubule system responsible for intracellular transport. In the normal course of cell metabolism, phosphate radicals are continually added to and removed from the tau protein. In AD, it is believed that neurotoxic oligomeric Aβ may cause excessive phosphorylation of the tau protein, perhaps as a neuronal response to injury, with the result that the tau detaches from the microtubules and forms neurofibrillary tangles of paired helical filaments (Figure 8). The microtubules subsequently collapse and the affected neurons degenerate and die[6].*

Current FDA-approved treatments for AD provide limited benefits to the patient. Goal of therapy is alleviation of symptoms. The course of the disease is not directly affected.

# Current therapies for AD

**Acetylcholinesterase inhibitors (AChEIs)**

Acetylcholinesterase inhibitors retard the breakdown of acetylcholine (ACh) by inhibiting the enzyme acetylcholinesterase in the synapse, increasing ACh levels at the synapse, resulting in modest improvements in cognition[13, 15]. Patients' cognitive and functional capacities improve in about one-third of patients, and some studies indicate the drugs remain effective as symptomatic treatment for at least 2 years in observational open-label studies[16, 18].

**N-methyl D-aspartate (NMDA) antagonists**

The main effect of memantine is to block excess and toxic calcium entry in neurons at the NMDA glutamate receptor, preventing "excitoxicity," improving neurotransmission and presumably delaying cell death in AD. Clinically, the drug shows a small improvement in cognition and function in moderate to severe AD[19].

**Other therapies**

Other therapies under investigation include estrogen, anti-inflammatory agents, statins, MAO inhibitors and anti-oxidants.

Physicians also commonly prescribe antidepressants, antipsychotics and sedatives to help control the behavioral symptoms commonly seen in AD patients.

Armed with greatly improved knowledge concerning the biochemistry of Aβ and the pathway by which it is produced, research has moved upstream. The goal is the production of disease-modifying modalities with the capacity to slow or arrest the catastrophic progression of AD.

# Treating the causes of AD

The primary focus is now on several potential mechanisms to interrupt either the Aβ synthesis pathway or its fibrillogenic process, to combat its neurotoxicity, or to remove plaques. The present lack of effective treatments for AD is providing a strong impetus for this research; a large number of molecules are currently under investigation.

**AD research approaches – Anti-Aβ agents**

### Immunotherapy

Vaccination against Aβ was postulated to have the effect of promoting the breakdown of existing plaques. However, the clinical program was prematurely terminated due to toxicity. Modified approaches of passive and active immunization are still under investigation[20].

Another possible avenue is to reduce systemic Aβ concentrations by employing an immunotherapy that does not cross the blood-brain barrier. By lowering peripheral Aβ levels, it is possible that the resulting increased concentration gradient may accelerate clearance from the brain, a concept known as a "peripheral sink".

Another non-immune approach which has been suggested to remove amyloid from the brain is the insulin degrading enzyme (IDE)[20].

### Secretase inhibitors

Blocking the breakdown of APP into Aβ protein is intended to act early in the β-amyloid cascade. If the production of Aβ can be prevented by the inhibition of β- or γ-secretase, then further damage due to the toxicity of Aβ could be avoided. Secretase inhibitors have to be highly specific to avoid any undesirable interactions with closely-related secretases and impairment of normal physiological processes.

### Aggregation inhibitors

Preventing the formation of toxic aggregates of Aβ and their deposition in one more step downstream within the amyloid cascade.

A novel approach to inhibit aggregation is via the GAG mimetics.

# The aggregation inhibitor approach – GAG mimetics

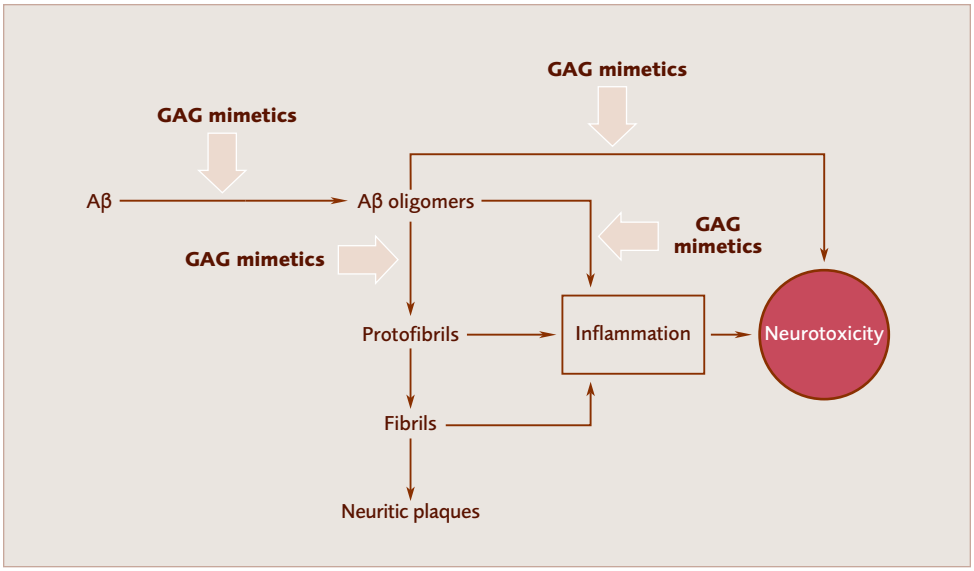
It was hypothesized that inhibition of the binding of Aβ to GAG would inhibit the formation of toxic Aβ aggregates, thus attacking what are believed to be the two most serious disease processes in AD: the induction of neurotoxicity by Aβ oligomers and the formation of neuritic plaques.

# The aggregation inhibitor approach – GAG mimetics

The pathological formation of amyloid deposits is seen, not only in AD, but also in more than 20 diseases, as diverse as type 2 diabetes and Creutzfeldt-Jakob disease, in many body organs and systems. In each case, a different amyloid protein is responsible: Aβ in AD, cerebral amyloid angiopathy (CAA) and Down's syndrome; immunoglobulin light chain in multiple myeloma; and $\beta_2$-microglobulin in hemodialysis-associated amyloid disease[21]. Amyloid deposits, moreover, are believed to be abnormal, misfolded proteins and are not known to serve any normal physiological function. The interruption of the pathological cascade at the point where amyloid aggregation occurs, therefore, seems a logical and promising strategy.

The glycosaminoglycan (GAG) side-chains of HSPG may help in Aβ oligomers formation and their organization as plaques. It was hypothesized that inhibition of the binding of Aβ to GAG would subsequently inhibit the formation of toxic Aβ aggregates, thus attacking what are believed to be the two most serious disease processes in AD: the induction of neurotoxicity by Aβ oligomers and the formation of neuritic plaques. Research has been done to develop small molecules that are intended to mimic the anionic properties of the GAG and bind to Aβ molecules, inhibiting the formation of Aβ aggregates. GAG mimetics could also interfere with the ability of soluble Aβ to bind to cell surfaces, impairing its ability to exert toxic effects. Furthermore, Aβ clearance may be increased and Aβ concentrations in the brain may be reduced[12] (Figure 9).

Figure 9: The potential role of GAG mimetics




The GAG mimetic approach has other interesting characteristics. The small GAG mimetic molecules intended to treat AD cross the blood-brain barrier, are not metabolized, show potential for low toxicity and can be administered by the oral route.

## Neurochem's research focus

Neurochem is investigating the therapeutic use of GAG mimetics in several amyloid-related diseases.

Neurochem's lead product candidate is currently undergoing Phase III investigation. This study assesses safety and clinical efficacy of the compound on the cognitive function and global performance in patients with mild to moderate AD. The effect of the drug on disease progression will also be evaluated using structural MRI.

# Socioeconomic implications of modifying the course of AD

AD shares with stroke the dubious honor of third place in the list of the most common causes of death in the US[6]. About 3% of people between 65 and 74 in the US have AD. The prevalence over the age of 85 is nearly 50% (NIA Alzheimer's Disease Education & Referral Center).

Demographic projections predict an enormous increase in the prevalence of AD during the next few decades. A modeling study by Sloane demonstrated that expected advances in the treatment of AD could have correspondingly dramatic effects in ameliorating this epidemic[3].

Sloane calculated the likely prevalence rates for AD in 2050 if treatments were developed by 2010 that a) would delay the onset of AD and/or b) would delay the progression of AD in a manner comparable to other chronic diseases, scenarios which are considered highly probable in the light of current advances in the understanding of the disease process.

Because there is neither a definitive diagnostic test for AD nor a formal reporting system for diagnosed cases, estimates for the current and future prevalence of the disease vary widely. However, Sloane reviewed the available data for current AD rates and derived a range of values for prevalence and staging. To provide the most accurate predictions for future trends in AD, he examined the impact that treatment advances have had on two major chronic diseases, congestive heart failure and Parkinson's disease.

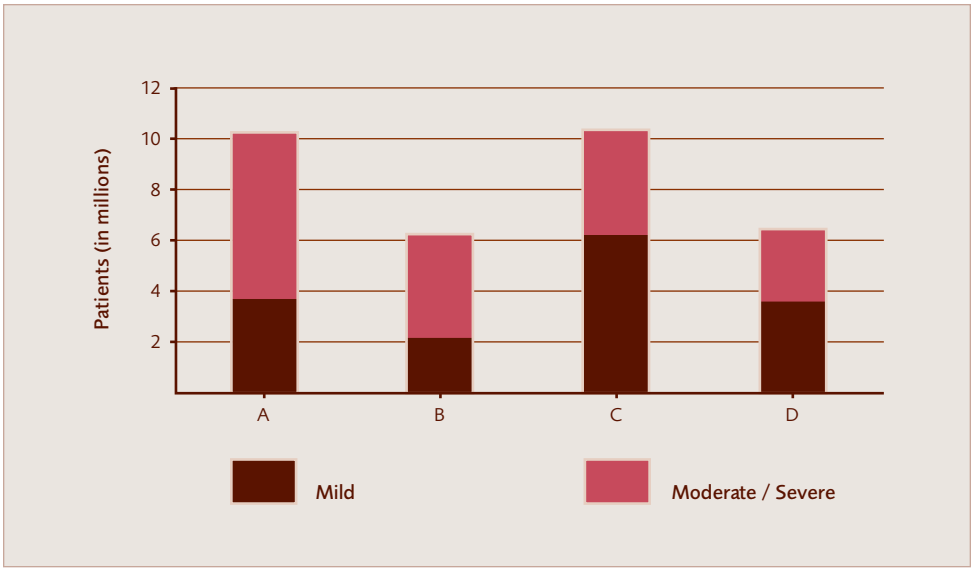
**Scenario A:**
No advancements in treatment

**Scenario B:**
Therapy in 2010 delaying disease onset by 6.7 years

**Scenario C:**
Therapy in 2010 delaying rate of progression from mild to moderate/severe from 28% to 10% per year

**Scenario D:**
Therapy in 2010 delaying both disease onset and rate of disease progression

Figure 10: Prevalence of AD in 2050 (Adapted from Sloane)




The estimated prevalence of AD in the United States in 2000 was between 2.17 and 4.78 million cases, with an incidence of 360,000 cases per year. (The 2004 global prevalence of AD is estimated at 15.3 million). At an estimated annual cost of $38,000 per patient (excluding lost employment income), the total annual direct cost in the U.S. is $65 billion. In the absence of significant advances in treatment, the prevalence of AD in 2050 is predicted to be 8 to 13 million, a four-fold increase. If, however, a treatment is developed in 2010 that delays disease onset by an average of 6.7 years, the number of cases in 2050 is projected to be around 6 million, a 38% reduction. If new treatments delay the rate of progression from mild to moderate/severe from 28% to 10% per year by 2010, the disease rates will remain the same, but the proportion of mild cases will increase to 59%, compared to a projected 33% without new treatments. If both types of treatment are developed, the two effects will be additive (Figure 10).

Even in the best case scenario, the total number of AD patients will triple compared to today, and AD will inevitably remain a major public health concern in the future. Consequently, the reduction in cases resulting from even a modest delay in disease onset, combined with a decrease in severity for many patients with a slowing of disease progression, would represent a large reduction in the future burden on total healthcare costs including hospitalization and nursing home care[3, 22]. At the time of the study, 1994 nursing home care costs for AD patients in the U.S. were estimated at over US$8 billion. It has been estimated that only a six-month delay in the onset of AD would lead to a saving of nearly US$18 billion in direct healthcare costs after 50 years. The indirect costs of AD would also be greatly reduced; it is estimated that one-half to two-thirds of the cost of AD care stems from unpaid caregivers (often family members), who spend 16-35 hours per week looking after a relative with AD.

# The future

Thanks in large part to advances in medical science, more and more people can expect to enter their seventh, eighth or even ninth decade of life. As longevity increases, so do concerns about the quality of life that might be expected in these additional years. Alzheimer's disease is one of the major hazards faced by the aging population. As medicine begins to understand and to conquer the causative factors of the disease, the prospects for treating one of the greatest threats to the enjoyment of our final years will increase considerably. Disease modifying therapies are on the horizon.

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