# CURRENT CONCEPTS IN ALZHEIMER'S DISEASE

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**Abstract** Like my presentation, this article focuses exclusively on the role of the amyloid ß protein in Alzheimer's Disease. I first provide an overview of the research findings that have made Aß, more specifically Aß42, a compelling therapeutic target for AD that is being pursued by virtually all pharmaceutical companies. I then discuss specific approaches to Aß-centered therapy. It is widely believed that the best way to manage AD will be through preventive therapy. To implement preventive therapy, it is critically important to be able to identify normal subjects who are at imminent risk for AD. In the last part of this article, I review recent evidence indicating that the measurement of Aß40 and Aß42 in plasma may be useful for identifying those normal subjects who will develop AD.

## Pursuit of the Amyloid ß Protein (Aß) as a Therapeutic Target for Alzheimer's Disease (AD)

Large numbers of plaques (which contain amyloid) and tangles are required for a definite diagnosis of AD. At present, a definite diagnosis of AD can only be made in a demented patient by showing, at autopsy, that there are large numbers of senile plaques and neurofibrillary tangles in the brain. Much of the protein that comprises senile plaques is in the form of amyloid, and amyloid is also deposited in the walls of cerebral and meningeal vessels in many cases of AD.

Amyloid, by definition, is highly insoluble, extracellular protein that is in the form of 5-10 nm wide, relatively straight fibrils. Amyloid is deposited in various organs in many different diseases, and there are many different proteins that can spontaneously assemble into insoluble amyloid fibrils that are deposited in the extracellular space.

In the mid 80s, the amyloid in AD brain and meningeal vessels was isolated, solubilized, and sequenced[1,2]. The amyloid in senile plaques was found to be composed of a then novel peptide now referred to as the amyloid ß protein (Aß). In 1987, several different groups cloned the gene that encodes Aß essentially simultaneously[2-4]. In this gene, which was found to be located on chromosome 21, Aß is encoded as an internal peptide within a much larger protein referred to as the amyloid ß protein precursor (APP). The APP has a large extracellular/intraluminal domain, a single membrane-spanning domain, and a small intracellular tail that extends into the cytoplasm. Subsequent work showed that the APP gene produces several different, alternatively spliced mRNAs. These mRNAs produce APPs with differing extracellular/intraluminal domains, but in each of them, Aß extends from just outside the membrane (amino end) to a position about half way through the single membrane-spanning domain (carboxyl end).

When the APP gene was cloned, it was known that patients with trisomy 21 (Down's syndrome) always develop AD pathology (senile plaques and neurofibrillary tangles) if they live past the age of 40. Subsequent work would show that, in these retarded patients, this AD

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pathology is associated with dementia. This seminal observation meant that there was a gene, or perhaps a combination of genes, on chromosome 21 that could cause AD when an extra copy was present (three chromosomes instead of two). When the APP gene mapped to chromosome 21, it was immediately obvious that the APP gene might be the gene or one of the genes on chromosome 21 that causes AD in patients with trisomy 21. If so it would indicate that the amyloid ß protein, which deposits in senile plaques, plays a major role in AD.

For this reason, a few laboratories including my own turned their full attention to developing a better understanding of the amyloid ß protein precursor. Within a few years, it became clear that the APP was normally cleaved within the Aß protein causing the large extracellular domain to be secreted. Then in the summer of 1992, my laboratory and several others discovered that Aß is also normally secreted[5-8].

Relatively quickly it was found that most secreted Aß has forty amino acids (Aß40) but a small percentage has two extra amino acids at its carboxyl end (Aß42)[9,10]. Cells of all types produce the APP and secrete both Aß40 and Aß42, which are readily detected in human plasma and cerebrospinal fluid. The cells in brain produce particularly large amounts, however, and the concentration of Aß in CSF is 50-times greater than in plasma. This may explain why there is selective amyloid deposition and plaque formation in the brain as opposed to peripheral organs.

The minor Aß42 peptide is particularly important in AD. In *vitro*, synthetic Aß42 spontaneously assembles into amyloid fibrils far more rapidly than Aß40[11-13], so Aß42 is far more amyloidogenic than Aß40. In one third of AD brains[14], Aß ending at the 42 position is virtually the only form of Aß deposited and in another third it is by far the major form of Aß deposited. In the remaining third of AD brains, a large amount of Aß ending at position 40 is

deposited in addition to Aß42. The amount of Aß42 deposited varies considerably from brain to brain, but it does not lessen when Aß40 is deposited. Thus, a large amount of Aß42 is deposited in all AD brains, but a substantial amount of Aß40 is deposited in only one-third of AD cases[14].

As the initial research on APP processing took place, rapid progress was also made on the genetics of AD[15-23]. Many families were identified in which AD occurred at an early age (35-60 years) with fully penetrant, autosomal dominant inheritance. Termed early onset familial AD (EOFAD), the AD produced through this simple Mendelian pattern of inheritance constituted a small fraction (<1%) of all AD, but analysis of the EOFAD families proved to be highly informative. Using new methods that had been developed to identify mutations that cause human disease in simple Mendelian fashion, several families were identified in which EOFAD was produced by mutations in the APP gene. The first mutations identified all occurred at the same location several amino acids past the carboxyl end of Aß. Next discovered was a double mutation in the two amino acids just before the amino end of Aß, as well as several additional mutations in the APP gene. Then a large number of causative mutations (over 100) were discovered in the presenilin 1 gene (*PS1*) located on chromosome 14 along with several in the closely related presenilin 2 gene (*PS2*) located on chromosome 1.

Careful study of the genetic changes that *cause* AD by my laboratory and others showed that virtually all of these changes increase Aß42. Trisomy 21 increases both Aß42 and Aß40. The double mutation in the *APP* gene described above, which occurs in a large Swedish family, also increases both Aß42 and Aß40. But most of the many EOFAD mutations in the *APP*, *PS1*, and *PS2* genes selectively increase Aß42. These elevations in Aß40 and Aß42 have been

demonstrated in cells transfected with mutant *PS1*, *PS2* and *APP* genes, the brains of transgenic mice expressing EOFAD mutations, and plasma from both symptomatic and presymptomatic subjects who have EOFAD mutations[24-29].

It is well established that the ApoE4 allele increases risk for AD, and several studies have shown that more Aß is deposited in AD patients with an ApoE4 allele than in those who lack this variant. In mice, ApoE profoundly influences the amount of fibrillar Aß deposited. In mice lacking the ApoE gene, Aß accumulation is reduced and the reduced amount of Aß that is deposited is in the form of diffuse rather than fibrillar deposits. In addition, analysis of human ApoE variants expressed in mouse brain on an ApoE knockout background has shown that human ApoE4 enhances fibrillar Aß deposition relative to human ApoE3. Thus, the only well-established genetic risk factor for AD also enhances Aß aggregation and deposition.

One might argue that trisomy 21, EOFAD mutations, and the ApoE4 allele do all foster Aß aggregation and plaque formation, but that these changes do not cause the neurofibrillary tangles, synapse loss, neuron loss or dementia seen in AD. This line of thinking suggests that it is additional changes caused by these genetic variants but not by elevated Aß42 that actually cause dementia. This seems highly unlikely, however, because an enormous body of evidence has accumulated indicating that aggregated Aß, most likely some form of soluble Aß oligomer, is highly toxic to neurons both *in vitro* and *in vivo*. In addition there is good evidence that Aß accelerates the process of tangle formation in mice expressing a tau mutation (P301L) that causes fronto-temporal parkinsonism with dementia, and this enhancement is particularly prominent in hippocampal pyramidal neurons which are widely believed to be most susceptible to tangle formation in AD.

As the evidence described above accumulated,

many pharmaceutical and biotechnology companies began preclinical work to develop novel strategies to treat AD using Aß as the therapeutic target. This effort was further galvanized by studies that clarified the cellular processing that results in Aß secretion and that identified the proteases that cleave the APP to release Aß40 and Aß42. Now, virtually all pharmaceutical companies have major programs focused on Aß as a therapeutic target for AD.

The many studies that have investigated APP processing indicate that processing begins as α secretase or ß secretase cleave full-length APP. ß secretase, which cleaves just before the first amino acid in Aß, generates two fragments: sAPPß (secreted APP generated by ß secretase) and CTFß (carboxyl-terminal fragment generated by ß secretase), which has Aß at its amino end. Similarly, α secretase, which cleaves between the 16th and 17th amino acid in Aß, generates sAPPα and CTFα, which begins at the 17th amino acid in Aß. Subsequently, γ secretase cleaves CTFß to produce secreted Aß and CTFγ. γ secretase also cleaves CTFα to produce secreted P3 (a ~3 kDAß species, which begins at the 17th amino acid of Aß) and CTFγ. The CTFγ generated by γ secretase is released into the cytoplasm from which it enters the nucleus altering nuclear transcription. Both α and ß secretase have been cloned, and extensive study of γ secretase indicates that it is a multi-subunit proteolytic complex that includes presenilin 1, which is the catalytic subunit.

## Approaches to AD therapy using Aß as the therapeutic target.

### ß secretase inhibition.

Inhibition of ß secretase is a highly attractive approach to the therapy of AD that has been pursued aggressively by many pharmaceutical companies. When ß secretase is knocked out, Aß is no longer present in the brain or peripheral organs, indicating that ß secretase is essential for

Aß production. ß secretase knockout mice survive normally and ß secretase has no known substrates except for APP, so mechanism-based toxicity has not been a major concern. The challenge in developing ß secretase inhibitors lies in identifying inhibitors with pharmacokinetic properties that permit oral administration, penetration of the blood barrier, and maintenance of an adequate CNS concentration using acceptable dosage regimens. Large compound libraries screened for inhibition of ß secretase using cultured cells failed to identify lead compounds and X-ray crystallographic analysis showed that ß secretase has a large binding pocket, so the development of effective ß secretase inhibitors has been slow and frustrating.

### γ secretase inhibition.

Many lead compounds that inhibit γ secretase were identified through early screening of compound libraries using cultured cells. The primary difficulty with γ secretase as a therapeutic target is that it cleaves the protein Notch, which plays an important role in the immune system, in addition to APP CTFs. Because of this mechanism-based toxicity, many γ secretase inhibitors show unacceptable toxicity in animal models. The challenge, therefore, has been to develop γ secretase inhibitors that are selective for CTFß and dosage regimens with an acceptable toxicity profile.

### Selective inhibition of Aß42 production.

In 2001 the laboratories of Eddie Koo and Todd Golde collaborated to show that several commonly used non-steroidal anti-inflammatory drugs, including ibuprofen, sulindac, and indomethacin, selectively reduce Aß42 secretion[30]. In addition, it has been shown that chronic administration of ibuprofen can reduce Aß deposition in a transgenic model of AD[31]. The precise mechanism for the Aß42 reducing effect of the NSAIDs is still under investigation, but the effect is independent of cyclooxygenase inhibition, the primary anti-inflammatory effect of these drugs. NSAIDs do not change the total Aß secreted, rather they shift cleavage producing a shorter 38-amino acid Aß species instead of Aß42. There is evidence that inflammation may play a role in the pathogenesis of AD as well as evidence from multiple epidemiologic studies that some NSAIDs may prevent AD when taken chronically. Thus, this class of agents could, in principal, be effective by reducing inflammation, Aß42, or both. Any compound in this class that is given chronically to elderly patients must avoid the GI bleeding associated with these agents as well as the cardiac toxicity that has more recently been associated with drugs of this type. One drug in this class that may avoid these toxic effects, R-Flurbiprofen, showed great promise in phase II clinical trials but there was no evidence of efficacy in the phase III clinical trial that followed. This trial may have failed because the concentration of drug present in the brain under the dosage regimen employed was insufficient to reduce Aß42 to the extent required. Additional selective Aß42 inhibitors with greater efficacy in lowering Aß42 are in development.

### Interference with Aß aggregation.

Several reports have appeared[32-35] reporting encouraging results using direct aggregation inhibitors in animal models. Because the agents employed to interfere with Aß aggregation are peptide-like, it may prove difficult to develop good drugs from these compounds.

One of the most promising approaches to AD therapy has been the immunization strategy pioneered by Dale Schenk and his colleagues. Both active and passive immunization have been shown to reduce Aß deposition in multiple animal models[36-40]. The precise target(s) and mechanism(s) involved in anti-Aß therapy are unknown. Possible beneficial effects of antibodies to Aß include (i) interference with the effect of

toxic oligomers, (ii) prevention of fibril formation, (iii) disruption of fibrils, and (iv) increased removal of Aß. Several groups have shown that anti-Aß therapy can improve memory deficits. This improvement occurs with remarkable rapidity and can take place without any obvious change in the amount of Aß deposited. These findings suggest that antibodies to Aß may be able to ameliorate some cognitive deficits by targeting a toxic Aß oligomer rather than Aß deposited as amyloid. Other studies also suggest that Aß oligomers, which are also referred to as Aß derived diffusible ligands (ADDLs) and protofibrils, may be neurotoxic[41-43]. The implication of these observations for human AD are unclear, however, because the relationship between cognitive deficits in humans, where there is extensive tangle formation as well as substantial neuron and synapse loss, and deficits in mouse models, where pathology is primarily restricted to plaque formation, is uncertain.

When there was no indication of toxicity in phase I trials, a phase II study using active immunization was begun several years ago. Unfortunately, meningo-encephalitis developed in ~5% of the phase II study group. This unfortunate toxicity dealt a severe blow to the direct immunization approach. No data have been published clarifying the mechanism of the meningo-encephalitis that developed, but it is widely believed that it occurred because of a cytotoxic T-cell mediated response to Aß. On this basis, passive immunization and immunization with peptides too small to provoke a T-cell response are being pursued as alternative approaches to anti-Aß therapy.

It is worth noting that individuals in the discontinued phase II trial who had high anti-amyloid titers showed some clinical improvement compared to those with low anti-amyloid titers. This observation and the many positive results in animal models suggest that additional efforts to develop safe anti-Aß therapy are warranted.

Two additional drugs that may target Aß aggregation, Clioquinol and Alzhemed, have been investigated clinical trials and should be noted. Clioquinol, which is postulated to inhibit Aß fibril formation by binding zinc and copper, markedly reduced Aß deposition in APP transgenic mice after several months of treatment[35]. Alzhemed is targeted on interfering with the interaction between Aß and glycosaminoglycans.

## Pursuit of the Amyloid ß Protein (Aß) as a Premorbid Biomarker

There is a growing consensus that the best way to manage Alzheimer's Disease (AD) will be through preventive therapy similar to that employed for atherosclerotic heart disease. To facilitate preventive therapy, it is important to develop AD-related premorbid biomarkers that can be used to identify at risk individuals in the same way that LDL and HDL levels are used to identify those who are at risk for atherosclerotic heart disease.

We have shown that plasma Aß is elevated in first-degree AD relatives, where risk for AD increases ~ 4-fold, and several groups have shown that Aß increases with aging over the age of 65 where risk for AD increases progressively. These observations suggest that elevated plasma Aß42 may be an important risk factor in the early presymptomatic period. It is important to emphasize, however, that plasma Aß42 is not useful as a *diagnostic* biomarker. Plasma Aß42 is not significantly different in AD patients compared to normal controls, and many studies have shown that CSF Aß is significantly *decreased* both in typical late onset AD (LOAD) and in mild cognitive impairment (MCI), which often precedes overt LOAD. In a transgenic mouse model of AD that we analyzed, CSF Aß and plasma Aß declined in parallel as Aß was deposited in the brain[44]. Decreases in plasma Aß42 that occur as Aß42 is deposited in the brain may explain why plasma Aß42 is not

elevated in LOAD or MCI despite the AD risk factors that are associated with elevated Aß42.

Reasoning that elevated plasma Aß42 was likely to be an important risk factor for LOAD in the early presymptomatic period and that plasma Aß42 was likely to decline selectively as Aß42 deposits in the brain in the presymptomatic period, we analyzed plasma Aß40, Aß42 and the Aß42/Aß40 ratio in a large longitudinal study to determine if any of these measures might be useful biomarkers for identifying elderly subjects who will develop MCI or AD. Aß42, Aß40, and the Aß42/Aß40 were analyzed in the first blood sample taken 563 normal subjects who were followed for up to 12 years. Survival analysis was employed to determine if any of these measures was a useful predictor of conversion to MCI or LOAD.

In the 563 subjects that we followed, the median age was 78 at the start of follow-up. In this cohort, the estimated cumulative incidence was 5% at four years. Cumulative incidence reached 11% (95% CI: 7% to 14%) at six years, 18% (95% CI: 12 to 24%) at 8 years, and was 30% (95% CI: 19% to 39%) at 10 years of follow-up. As expected, both advancing-age and presence of the ApoE4 allele, two well-established risk factors for AD, showed evidence of association with incidence of MCI/AD.

Neither Aß42 nor Aß40 were significantly associated with conversion to MCI/AD, but the plasma Aß42/Aß40 ratio did show association. The risk of MCI/AD for patients with an Aß42/Aß40 ratio in the lowest quartile was estimated to be approximately three times the risk for subjects with a ratio in the highest quartile (p=0.010), and this association persisted (p=0.038) after adjusting for age, and ApoE4.

There was a striking relationship between the Aß42/Aß40 ratio and cumulative incidence of AD. Subjects whose Aß42/Aß40 ratio was in the lowest quartile (Q1: <0.05) reached a 10% incidence by three years, followed by those in

Q2 who took approximately 5 years, and those in Q3 and Q4 who took around 8 years to reach 10% cumulative incidence.

Elderly subjects with an ApoE4 allele are at increased risk for AD, but more than 50% of those with an ApoE4 allele did not develop AD by 10 years. In our series, the Aß42/Aß40 ratio was highly effective in separating the ApoE4 carriers who developed MCI/AD from those who did not. After 5 years of follow-up, over 20% of subjects with an ApoE4 allele and a low (below median) Aß42/40 ratio, had developed AD. In contrast, only 3% of the ApoE 4 carriers with a high (above median) Aß42/Aß40 ratio above developed AD by five years. Remarkably, more subjects without an ApoE 4 allele who had a low ratio had developed AD by five years (10%) than subjects with an ApoE 4 allele who had a high Aß42/Aß40 ratio (3%).

Combining age and the Aß42/Aß40 ratio was also highly effective in separating subjects who developed disease from those who did not. In our series, 20% of older subjects (age ≥ 80 years) with a low (below median) Aß42/40 ratio had developed AD/MCI by 5 years. In contrast, less than 5% of all other subjects developed AD within five years

The results of this study suggest that cognitively normal people having low plasma Aß42/Aß40 ratios are at increased risk of developing MCI or AD. As of this writing, there have been four additional studies of elderly Caucasian subjects in which findings similar to those described above have been reported. Thus it is increasingly likely that measurement of plasma Aß42 and Aß40 may become an important factor for developing and implementing a preventive approach to therapy. Our results suggest, for example, that prevention trials in normal subjects over 80 could be facilitated substantially by performing them in subjects with Aß42/40 below the median in our study, where we found the cumulative incidence of MCI/AD to

be far greater than in those with ratios above this value.

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