The α, β, γ's of Alzheimer's Disease

Student group names kept anonymous
Department of Biology, Lake Forest College, Lake Forest, IL 60045, USA

Alzheimer's Disease (AD) is a progressive neurodegenerative disease characterized by memory loss, reasoning impairment, and muscle rigidity and weakness. It is the eighth highest cause of death in the United States, affecting primarily the elderly population. All AD patients have in their brains intracellular protein aggregates called neurofibrillary tangles (NFTs) and extracellular protein deposits named β-amyloid plaques in common. There are two main categories of AD, familial and sporadic. Sporadic, while not fully understood is hypothesized to arise during an individual's life from environmental factors or spontaneous gene mutations. This review focuses on the familial, or inherited, type of AD. Currently evidence of three gene mutations are associated with the familial type: presenilin1, presenilin2, and the amyloid precursor protein (APP). Tau protein hyperphosphorylation leading to NFT formation, and APOE allele type are also integral parts of AD. Each mutation plays a very different role in the progression of AD. The β-amyloid plaques are a result of proteolytic cleavage of APP. The processing of APP is carried out by three secretases regulated by the presenilin genes. Current research focuses on the effects of various combinations of APOE alleles on AD susceptibility, cleavage of APP by α, β, and γ secretases, and the use of immunizations and drug therapy to the combat the fatal disease.

Introduction

Alzheimer's disease (AD) is a dementia disease characterized by insoluble protein deposits in the brain. Alois Alzheimer, a professor of psychology in Germany, first described its clinical symptoms in 1906 after seeing a patient expressing acute memory loss (1). The disease, now named after him, presently affects more than 4 million elderly individuals in the United States, and is responsible for 44,536 deaths annually. Fifteen percent of people who live to the age of 65 will develop some type of dementia disease, with the risk increasing to 35% by age 85 (2).

The principle clinical symptoms of AD are progressive memory loss, deterioration of logic and reasoning capabilities, language impairment and muscle rigidity. AD patients pass through six stages of neurodegeneration, based mainly on the location of neuron damage. Stages one and two include abnormalities in the hippocampus, the region of the brain involved in memory. Stages three and four involve the presence of lesions in the amygdala, altering emotion and aggression. Stages five and six consist of damage to the somatosensory cortex, ultimately impairing language and reasoning (3).

Pathology

AD is classified by two types of insoluble protein aggregations. The first is a result of hyperphosphorylated tau protein. Normal tau protein encoded by the tau gene binds to and promotes the assembly of microtubules responsible for transporting organelles in the cell. The more phosphorylated the protein, the less available it becomes for binding to microtubules. Instead, the protein forms paired α-helical structures called neurofibrillary tangles (NFTs) in the cell bodies of neurons. Loss of tau binding to microtubules inhibits the transport of important organelles in the cell such as mitochondria. The inability of the tau protein to do its job also leads to alterations in cell size, shape, and polarity (4).

The second type of deleterious protein deposits are found between neurons in the extracellular matrix and are composed of toxic Aβ peptides. These Aβ peptides are derived from the cleavage of the amyloid precursor protein (APP) encoded by chromosome 21. Three different classes of secretases are responsible for the processing of the large polypeptide chain. The three classes are called α, β, and γ. Each makes a cut at specific amino

acid sequences on APP, producing specific smaller fragments. Secretase activity is currently a hot topic for research, due to its promising immunization possibilities.

Types of AD

The two known classes of AD are broken down into sporadic and familial. Sporadic AD is not well understood and has not been studied as extensively as its familial counterpart. Like other uninherited diseases, it is caused by spontaneous mutations in the genetic code due to such things as deamination or exposure to ultra-violet light.

The familial form of AD is an autosomal dominant disease. Familial AD (FAD) has been linked to mutations in three genes: APP, PS1, and PS2, with APOE allele type playing a limited role as a risk factor to these mutations. APP was the first gene identified in relation to familial AD (5). Mutations to the amyloid precursor protein (APP) result in early onset of AD as early as anywhere from 43 to 55 years of age. The phenotypes resulting from this mutation are indistinguishable from that of sporadic AD. The presenilins, PS1 and PS2, are encoded from chromosomes 14 and 1, respectively. They have been connected to activation of an enzyme that processes APP. The proteins located on these genes may even be those enzymes. Mutations on these genes are also connected to early onset AD (3). A specific APOE allele type also accounts for earlier AD onset.

Can it be fixed?

There are no current effective methods of treating Alzheimer's disease patients or immunizing people to prevent its onset. There is hope, however, with new studies showing successful immunizations and drug therapy in transgenic animals. Today's focus on immunizations is concentrated on the APP processing enzymes' activation and/or inhibition. These immunizations can then be utilized in individuals most susceptible to AD i.e. those with the high-risk APOE alleles.

Apolipoprotein E

Studies conducted in the past several years strongly support a correlation between Apolipoprotein E (APOE) and AD. APOE, found on chromosome 19, plays a role in cholesterol metabolism, storage and transport. It has three alleles; 2, 3, and 4. The affects of APOE and the specific allele combinations have been the focus of many recent AD studies.

It has been found that APOE is present in Aβ plaques and may even serve as a chaperone protein aiding in their formation (5). A 1999 study supporting this hypothesis found that the presence of APOE, either in a +/+ or +/- combination, in transgenic mice expressing a mutant of the human APP led to amyloid deposits. No amyloid deposits were found in the APOE -/- t.g. mice. The absence of APOE greatly reduced the amount of both $A\beta_{40}$ and $A\beta_{42}$ deposits. This study proposes that APOE causes the problems of Aβ, since Aβ, is still present without APOE (6).

Knowing that APOE plays a role in the onset of AD pathologies (e.g., Aβ plaques and NFTs), researchers focused on the specific alleles of APOE. The results of another 1999 study reinforced that APOE must be present for Aβ deposits and neuritic degeneration. Aβ deposits were present in the control mice, but no degeneration was observed. Mice with the e3 or e4 allele of APOE showed Aβ deposits and degeneration within 15 months. More importantly, it was shown that those mice expressing solely the e4 allele had greater than 10 fold increase in deposit density when compared to those mice expressing only the e3 allele (7).

A difference in the complexity and effectiveness of APOE function in normal versus diseased brains was established in a 1998 study. In these diseased brains, impairment of APOE led to accumulation of Aβ and subsequent plaque formations (8).

Scientists have also discovered a relationship between APOE and the protein tau found in the brain. In AD patients, tau becomes tangled and forms NFTs. A study in 2001 found that APOE with a shortened carboxy-terminal stimulates NFT-like formations in neurons. By comparing the e3 and e4 alleles, it was found that individuals with the e4 allele expressed this shortened APOE that induces NFT formation (9).

Scientists have found that the APOE e4 allele is associated with lower metabolism in the brain. A longitudinal study published in 2000 established that a combination of assessing a patient's cerebral metabolic rate and genetic risk factors could present a pre-clinical method for detecting AD (10). Though this detection

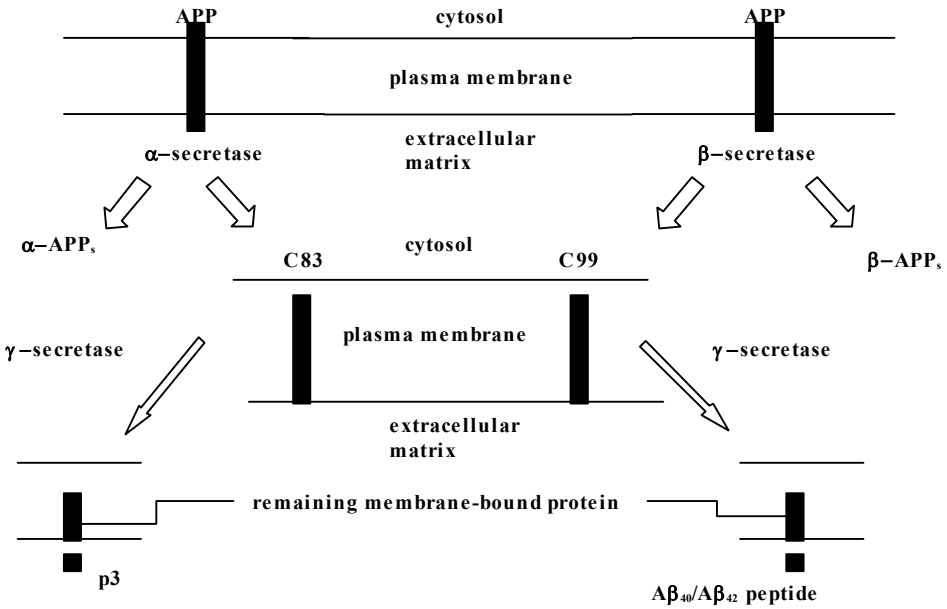

Figure 1: Secretase Activity in Neurons. The different activities of the secretases and a depiction of the way in which Aβ plaques accumulate on the exterior of neurons in AD patients.

method is promising, it isn't foolproof. Thus more direct research into AD pathologies, especially Aβ plaque formation, could lead to a better diagnosis method. One such area would be secretases, which have been implicated in this plaque formation.

The secretase trio, at a glance

Much of the scientific research on AD has recently been directed towards understanding the three enzymes implicated in the cleavage of the transmembrane protein APP, whose function is currently unknown. These enzymes, collectively referred to as secretases, are responsible for the cutting of APP at specific sites on the APP.

Each secretase catalyzes a different reaction (see Fig 1); therefore, they have names to differentiate them. α–secretase is the enzyme that cuts the APP to produce a soluble α-APP fragment and the carboxy (COOH)-terminal fragment C83 that remains membrane-bound. β–secretase performs a similar cut on APP, except the soluble fragment made is referred to as β–APP and the COOH-terminal fragment left behind, bound in the plasma membrane, is C99. γ-secretase's substrates are C83 and the C99. The secretase proteolytically cleaves the C83 to release the innocuous p3 fragment from the membrane. However, its catalytic action on the C99 fragment leads to the formation of two peptides: $A\beta_{40}$ and $A\beta_{42}$. The $A\beta_{40}$ (containing 40 residues) is soluble in the extracellular space; however, the $A\beta_{42}$ (containing 2 additional residues) is hydrophobic and tends to aggregate and form Aβ plaques on the outsides of cells (1).

This is where the direct link to AD exists. Many mutations in the APP at the cleavage sites as well as possible mutations in the enzymes themselves could lead to increased accumulation of Aβ plaques outside neurons. Therefore, studying these secretases will potentially lead to a way to prevent plaque formation and ultimately AD.

A closer look at α secretases

Until around 1997, only the existence of α secretases had been noted. Their amino acid sequence, physical properties, and APP cleavage site were all unkown. Upon investigation, it was discovered that it was the α secretases responsible for the creation of nonamyloidogenic (non Aβ forming) APP fragments. This sparked a race to discover all there was to know about this secretase in hopes that it would lead to possible Alzheimer's treatments.

Researchers knew that the α secretases were metalloproteases, requiring metal to cut proteins. They also knew that APP was located in the plasma membrane of neurons. Thus, they were searching for metalloproteases also located in the plasma membrane. The first candidate for an α secretase study was TACE (ADAM 17), a

member of the ADAM (a disintegrin and metalloprotease) family.

In a study published in 1998, results showed that TACE played an important role in α cleavage of APP (11). The α secretase active site on APP is between Lys^{687} and Leu^{688} residues (5). In the study, TACE was able to cleave a synthetic peptide between Lys and Leu, therefore indicating that it might be able to cut APP in the same way. With manipulation and interference of TACE in cells, α cleavage was abolished further supporting that TACE was an α secretase (11).

A second contender for the α secretase is another metalloprotease in the same family as TACE, called ADAM 10. A study published in 1999 showed that the active form of this protease was found in the plasma membrane on the cell surface, and therefore geographically capable of cutting APP. As also consistent with α secretase activity, ADAM 10 was shown to proteolytically cleave between Lys and Leu (12).

The understanding of specific α secretases could be important in creating immunizations for AD. Since the inhibition of both ADAM 10 and 17 resulted in the elimination of α secretase activity, there is an obvious connection between the proteases and α secretases if they are not α secretases themselves In both studies, the specific enzymes were overexpressed by activation of the protein kinase C using phorbol esters. Overexpression of the proposed α secretases showed an increase in α APP processing, with a larger number of nonamyloidogenic fragments produced. Immunization possibilities stemming from this research include the stimulation of α secretase expression in neurons (11, 12, 13).

Up close and personal with β secretases

Similar to the α secretases, not much was known about the β secretases until about three years ago. There was only evidence of another cleavage site in addition to the α site. Three years ago, β secretase was identified as the protein responsible for the additional cut. It was deemed an aspartyl protease, cleaving APP between residues 671 and 672 at an aspartate (5).

Near the end of 1999, the protease BACE (β-site APP-cleaving enzyme) was studied as the first β secretase. The study used human embryonic kidney cells to establish BACE as a β secretase candidate due to its location in the cell, active site and pattern of mRNA expression. All were consistent with that of β secretase. The presence of the soluble βAPP fragment was used to determine the amount of β secretase activity. With over expression of BACE in cells, the amount of the fragment increased significantly. Thus, there was an increase in β secretase activity with BACE overexpression (14).

In 2000, BACE2, a homolog of BACE was studied as another β secretase. The researchers established that BACE2 was located in neural tissue in the regions of the brain also expressing BACE and APP. They knew that β secretase activity occurred optimally under acidic conditions. It was determined that BACE2 cleaved the most efficiently under those same acidic conditions. In fact, they discovered that BACE2 could cleave at the β site more efficiently than BACE while producing less of the potentially toxic C99 fragment (15). Thus it would be interesting to investigate the effects of regulating BACE and BACE2 expression on the amount of Aβ produced.

BACE activity has been inhibited in the lab through the use of competitive inhibitors. Researchers know the exact active site on APP that β secretases cleave at. Thus they can mimic the active site on synthetic proteins and inject them into the cell. These types of inhibitors compete with APP to bind to the β secretases. As β secretases bind to the injected synthetic protein, less APP is pretolytically cleaved at the β site. This causes a decrease in the formation of the C99 fragment and ultimately Aβ and therefore is a viable immunization possibility (14).

Close-up on γ–secretase

Relatively little was known of γ-secretase a few years ago. All that was known was that it was somehow responsible for the production of the $A\beta_{40}$ and $A\beta_{42}$ peptides from APP. Its coding sequence (and consequently its amino acid sequence), its mechanism of cleavage, its degree of substrate specificity, and its reaction to genetic mutations in APP or other molecules involved in its proteolytic activity remained largely uninvestigated until the year 1999. After this year, many of these γ-secretase mysteries were elucidated through research. An important hypothesis emerged from this research that is still being worked out in 2002. γ-secretase requires PS2 (presenilin 2) or PS1 (presenilin 1)

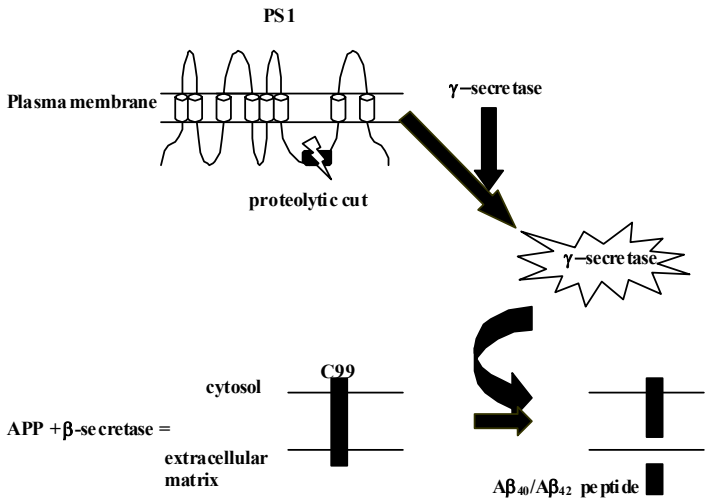

Figure2: Aβ formation. The proposed pathway for Aβ peptide formation via proteolytic cleavage by γ-secretase in AD patients. It is known that cleavage of the transmembrane protein PS1 at the site indicated is necessary for the secretase's activation; however it is not known whether γ-secretase is an enzyme different from PS1 or if γ-secretase is actually PS1.

to make the necessary cut on the C99 fragment to produce the Aβ peptides (see Fig 2).

The manner in which γ-secretase makes its cut is vital to develop the PS1 hypothesis. In one study published in 1999, the effects of mutations in APP between the residues of 43 and 51 in the Aβ domain on γ-secretase activity were investigated. The results showed that, depending on the mutation, the amount of $A\beta_{40}$ and $A\beta_{42}$ peptide produced varied. From this, a detailed model for the γ-secretase cleavage site was established (16). However, it has been shown that the ability of γ-secretase to cut at this site and generate Aβ peptide is dependent on the endoproteolytic cleavage and subsequent formation of the activated heterodimeric PS1 complex. Mutations made to either of the aspartate residues in transmembrane domains 6 and 7 (TM6 and TM7) on PS1 prevented the intramolecular cutting of the loop between these two domains and therefore caused the PS1 to remain inactive. This inactivity led to the accumulation of COOH terminal fragments and the absence of Aβ peptides in the cells that lacked the active PS1, implying that γ-secretase activity was being inhibited. It was concluded that PS1 must undergo endoproteolytic cleavage in order for it to serve as a cofactor for γ-secretase or for it to become γ-secretase itself (17, 18).

γ-secretase activity is not totally dependent on the endoproteolysis of PS1. It has been shown that cells expressing PS1 unable to autoactivate themselves and having mutations linked to AD still show pathological levels of $A\beta_{42}$ peptide (19). Specifically, this implies that endoproteolysis is not absolutely necessary for some degree of γ-secretase activity. Broadly, this implies that the interaction between PS1, γ-secretase, and mutations in APP and PS1 is complex and does not produce a straightforward model for the effects they have on Aβ production.

To further understand γ-secretase and its vital connection to PS1 and PS2 activity, research has been done on the consequences of the total absence of the presenilins from cells. Organisms expressing no PS1 or PS2 were aborted during the early stages of embryonic development (1). This affirms that PS1 and PS2 are indispensable and required for normal cellular processes. PS1and PS2 knockout embryonic stem cells provided an important step in understanding the presenilins' connection to γ-secretase. In three separate studies done on these cells, significant or total inactivation of γ-secretase was observed (20, 21, 22). That is, there was little to no production of Aβ peptides detected in these cells. Again, this establishes that some amount of PS1 and PS2 are required for γ-secretase activity.

As a consequence of all this research, γ-secretase's role in $A\beta_{42}$ peptide production and accumulation has become better understood. Because the exact structure of γ-secretase remains unresolved, nothing can be definitively said about its active site--just that it must have

loose sequence specificity in order to still be able to produce Aβ peptides despite APP mutations (1, 23).

It is apparent that many gaps in knowledge still exist despite the progress made. Future research must be done to determine whether γ-secretase is an enzyme independent from the PS1 structure or if γ-secretase is PS1. A hypothesis regarding the role that PS2 plays in the development of AD would be informative, since so much work has already been done on PS1. Finally, the current research has led to the question of whether Aβ plaque formation could be better attributed to proteins responsible for its degradation or clearance rather than simply its production. Before much more is done on γ-secretase, it may be extremely rewarding to investigate this area.

While this proposed work for the future is being done, research on possible AD drug therapies using the knowledge gained from the studies already done on γ-secretase and the presenilins should be undertaken. Peptidomimetics, compounds that are deliberately made to resemble a protein, have already been shown as potential inhibitors of γ-secretase (23). They inhibited the protease's activity as an Aβ producer by competing with the C99 for γ-secretase's active site.

This result implies that Aβ plaque formation could be prevented or at least reduced in AD patients. This result implies that Aβ plaque formation could be treated with these compounds. PS1-based peptidomimetics were also successfully used to inhibit γ-secretase activity (24). This is just one of the many approaches being undertaken towards AD drug therapy.

Immunization confronted

Research done in 1999 explored the possibilities of an AD vaccine. The goal of a vaccine is to prevent the further development of AD in patients who are diagnosed with AD and to prevent the development of the disease in people who are susceptible to AD.

In the experiment, predominant amyloid precursor protein (PDAPP) transgenic mice over expressing mutant human APP were immunized with $A\beta_{42}$. Aβ deposits were mostly prevented as a result of this immunization. The immunogens used were either synthetic human $A\beta_{42}$ or serum amyloid-P component (SAP), a protein linked to amyloid plaques in AD. Seven of the nine mice immunized with $A\beta_{42}$ exhibited little to no plaque formation (25). Immunization with SAP had no affect on $A\beta_{42}$. This study provided scientists with a way to combat the pathological symptoms, but the study did not look into clinical symptoms (e.g., deficiencies in memory and spatial learning).

A subsequent study looked at how immunization affects the clinical symptoms of AD. Spatial learning and memory were the two areas investigated. The study revealed that immunization prevented spatial learning deficits, which have been correlated to plaque formation. The TgCRND8 mice used exhibited spatial learning deficits within 3 months. Increasing levels of SDS-soluble Aβ and increasing plaque density were observed in mice showing learning deficits. TgCRND8 mice and non-Tg littermates were vaccinated at various stages. $A\beta_{42}$ and islet-associated polypeptide (IAPP) were the vaccines used.

The IAPP was selected because it has similar biophysical properties to Aβ, but is associated with a non-central nervous system amyloidogenesis (26). As weeks passed, detectable antibody concentration continually increased. The water maze used to examine spatial learning deficits and memory revealed that immunization with Aβ hampers the formation of plaques and prevents the decline of learning and memory (27).

Another Aβ immunization study used a different spatial and memory test to determine whether the decline of learning and memory could be curbed. The researchers used a newly designed working-memory test that combines elements of a radial-arm maze and a water maze. This new test accurately detects learning and memory deficits that develop in AD transgenic mice. The maze consisted of a circular pool with six swim lanes known as arms. At the end of one of the arms, there was a submerged escape platform. Each day, the platform was placed at the end of a different arm. The mice were allowed to learn the location of the platform during the first four runs. Thirty minutes later, the mice were allowed to run through again to test their memory of where the platform was located.

In the studies where improvements in memory and learning were observed (via the tests discussed), it is hypothesized that the injected vaccine significantly altered or restricted Aβ peptide formation and shape in the transgenic mice (28).

" Age" old question answered

Another group of researchers took a totally different approach. Instead of testing whether immunization would prevent the decline of learning and memory, they concentrated on whether age has any relation to spatial learning and Aβ plaque formation in AD mice. Their results support the hypothesis that learning deficits increase with age and that the greater density of Aβ plaques resulting from aging is the cause of these increased learning deficits. To run their experiment, they used a revolutionary water maze training protocol (29).

Immunization defaults

Excited by the progress made using AD transgenic mice, scientists were quick to start human trials. Studies were suspended, however, when four patients exhibited signs of CNS inflammation. The trials were then stopped altogether when eleven more patients showed the same signs (30). The reasons as to why the vaccines failed in the human trial are still at a mystery.

Conclusion

Evidently much work must still be done on the countless angles of AD. The research possibilities are truly overwhelming, but it is apparent that the focus is centered on understanding the formation of Aβ plaques through secretase cleavage of APP and the reduction/elimination of these plaques via immunization. In addition to this, AD researchers have become fascinated with the convoluted role that APOE alleles play in increasing individual susceptibility to AD. Only time will give the answer to whether a viable cure exists for AD.

Acknowledgements

We would like to thank to Dr. Shubhik DebBurman, Jen Mcguire, Angie Eakley, the Lake Forest College librarians, the creators of the Cell and Molecular Biology research home page and the helper at Finch Medical School.

References

1. Esler, William P. and Michael S. Wolfe. A Portrait of Alzheimer Secretases--New Features and Familiar Faces. *Science*. **293**, 1449-54 (2001).
2. St. George Hyslop, P.H. Piecing Together Alzheimer's. *Scientific American* 76-83 (2000).
3. Price, D.L. and Sisodia, S.S. Mutant Genes in Familial Alzheimer's disease and Transgenic Models. *Annual Review Neuroscience* **21**, 479-505 (1998).
4. Ebneth, A. *et al.* Overexpression of Tau Protein Inhibits Kinesin-dependent Trafficking of Vesicles, Mitochondria, and Endoplasmic Reticulum: Implications for Alzheimer's Disease. *The Journal of Cell Biology*. **143**, 777-794 (1998).
5. Scriver, et al. The Metabolic and Molecular Bases of Inherited Disease. New York: McGraw Hill Medical Publishing, (2001).
6. Bales et. al., Apolipoprotein E is essential for amyloid deposition in the APPv717f transgenic mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences*. **96**, pp. 15233-15238, (1999).
7. Holtzman et. al., Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proceedings of the National Academy of Science* **97**, pp. 2892-2897, (2000).
8. Russo et. al., Opposite roles of Apolipoprotein E in normal brains and in Alzheimer's disease. *Proceedings of the National Academy of Sciences* **95**, pp. 15598-15602, (1998).
9. Huang et. al., Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons. *Proceedings of the National Academy of Sciences* **98**, pp. 8838-8843, (2001).
10. Small et. al., Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. *Proceedings of the National Academy of Sciences* **97**, pp. 6037-6042, (2000).
11. Buxbaum, J.D. *et al.* Evidence that Tumor Necrosis Factor α Converting Enzyme Is Involved in Regulated α-Secretase Cleavage of Alzheimer Amyloid Protein Precursor. *The Journal of Cell Biology*. **273**, 27765-27767 (1998).
12. Lammich, S., et al. Constitutive and regulated α-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc. Natl. Acad. Sci.* **96**, 3922-27 (1999).
13. Parvathy, S., et al. Alzheimer's amyloid presursor protein α-secretase is inhibited by hydroxamic acid-based zinc metalloprotease inhibitors: similarities to the angitensin converting enzyme secretase. *Biochemistry*. **37**, 1680-5 (1998).
14. Vassar, R. and B.D. Bennett. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science*. **286**, 735-41 (1999).
15. Farzan, M. et al. BACE2, a β-secretase homolog, cleaves at the β-site and within the amyloid-β region of the amyloid-β precursor protein. *Proc. Natl. Acad. Sci.* **97**, 9712-7 (2000).
16. 2. Lichtenthaler, S.F., et al. Mechanism of the cleavage specificity of Alzheimer's disease γ-secretase identified by phenylalanine-scanning mutagenesis of the transmembrane domain of the amyloid precursor protein. *Proc. Natl. Acad. Sci.* **96**, 3053-8 (1999).
17. 3. Wolfe, M.S., et al. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature*. **398**, 513-8 (1999).
18. 4. Yu, Gang, et al. Mutation of conserved aspartates affects maturation of both aspartate mutant and endogenous presenilin 1 and presenilin 2 complexes. *Journal of Biological Chemistry*. **275**, 27348-53 (2000).
19. 5. Steiner, H., et al. Amyloidogenic function of the Alzheimer's disease-associated presenilin 1 in the absence of endoproteolysis. *Biochemistry*. **38**, 14600-05 (1999).
20. 7. Herreman, A., et al. Total inactivation of γ-secretase activity in presenilin-deficient embryonic stem cells. *Nature Cell Biology*. **2**, 461-2 (2000).
21. 8. Zhang, Z, et al. Presenilins are required for γ-secretase cleavage of β-APP and transmembrane cleavage of Notch-1. **2**, 463-4(2000).
22. 9. Strooper, B.D., et al. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature*. **391**, 387-90 (1998).
23. 10. Wolfe, M.S., et al. Peptidomimetic probes and molcular modeling suggest that Alzheimer's γ-secretase is an intramembrane-cleaving aspartyl protease. *Biochemistry*. **38**, 4720-27 (1999).
24. 11. Esler, W.P., et al. Transition-state analogue inhibitors of γ-secretase bind directly to presenilin-1.*Nature Cell Biology*. **2**, 428-434 (2000).
25. Schenk et al. Immunization with amyloid- attenuates alzheimer-disease-like pathology in the PDAPP mouse. *Nature* **400**, 173-177 (1999).
26. Janus et al. Aβ peptide immunization reduces behavioral impairment and plaques in a model of Alzheimer's disease. *Nature* **408**, 979-982 (2000).
27. Chapman. Model behavior. *Nature* **408**, 915-916 (2000).
28. Morgan et al. Aβ peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* **408**, 982-985 (2000).
29. Chen et al. A learning deficit related to age and β-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* **408**, 975-979 (2000).
30. Steinberg. Companies Halt First Alzheimer Vaccine Trial. *The Scientist* **16**, 22-23 (2002).