CHAPTER II

REVIEW OF RELATED LITERATURE AND RESEARCH

Alzheimer's diseases (AD)

Alzheimer's disease (AD) is the most common and frequent type of dementia that results in the irreversible loss of neurons, mainly in the cortex and hippocampus (Roberd and Christopher, 2003; Alexis, 2004). AD is the most common dementia, currently affect an estimated 35 million patients world wild (Ferri, et al., 2009). The prevalence of AD in the United States of America is estimated to be approximately 4.5 million and is predicted to increase up to 13.2 million in the next 50 years (Imbimbo, 2005). In 2001, Thailand had around 135,000 AD patients (Sukying, 2007). The pathophysiology of AD is associated with a variety of factors, including the extracellular deposition of β-amyloid (Aβ) plaques, accumulation of intracellular neurofibrillary tangles, oxidative neuronal damage, and inflammatory cascades (Imbimbo, 2005). However, an increase in the production of the Aβ peptide, the main component of amyloid plaques, is central to the pathogenesis of the disease. AD is characterized clinically by progressive decline of cognition, behavior, and functionality that impairs activities of daily living significantly. Morphologically, the disease is characterized by brain atrophy and by enlarged cerebral ventricles. The other typical histopathologic hallmarks of AD are the neurofibrillary tangles within neurons, formed mainly by a filamentous, hyperphosphorylated form of the microtubule-associated protein tau. Whereas most cases of AD occur sporadically, approximately 5% of patients develop the disease early as a result of fully penetrant autosomal dominant gene mutations. Also several studies found mutation or single nucleotide polymorphisms (SNPs) in amyloid precursor protein (*APP*), Presenilin1 (*PSEN1*), Presenilin2 (*PSEN2*) and *APOE* as causative and susceptibility genes in AD patients in many countries.

Diagnosis of Alzheimer's disease

The diagnosis of AD in Thailand is most based on the Thai Mini Mental State Examination (TMSE) (Suparus, et al., 2008). Thai Beck Depression Inventory (BDI)

criteria (Suparus, et al., 2008), National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences (NINDS-AIREN) criteria and the Diagnosis and Statistical manual of Mental Disorders, fourth edition (DMS-IV) using diagnostic criteria for dementia of the Alzheimer's type such as AD patients have the development of multiple cognitive deficits manifested by both memory impairment and one or more of cognitive disturbances: aphasia (language disturbance), apraxia (impaired ability to carry out motors activities despite intact motor function), agnosia (failure to recognize or identify objects despite intact sensory function) and disturbance in executive function (i.e., planning, organizing, sequencing, abstracting), etc. (American Psychiatric Association, 1994; Suparus, et al., 2008). Patients progress from the loss of higher-level activities of daily living, such as the use of public transportation, abnormalities of basic activities of daily living, such as eating, grooming, and using the toilet (Galasko, et al., 1997).

Symptoms and stages of Alzheimer's disease

Early symptoms of AD include confusion, disturbances in short-term memory, problems with attention and spatial orientation, changes in personality, language difficulties and unexplained mood swings. Normally, these symptoms are very mild, and presence of the disease may not be apparent to the person experiencing the symptoms. The three stages given below represent the general progression of the disease. AD generally leads to impairment of cognitive and memory function, communication problems, personality changes, erratic behavior, dependence and loss of control over bodily functions. AD doesn't affect every person in the same way, but symptoms is general by progress in these stages (Alzheimer's research program of the American health Assistance Foundation, 2011).

Stage 1 (Mild)

This stage can last from 2 to 4 years (Figure1). Early in the illness, those with AD tend to be less energetic and spontaneous. The AD patients demonstrate minor memory loss and mood swings, and are slow to learn and react. Plaques and tangles begin to form in brain areas involved in learning and memory and thinking and planning (Alzheimer's Association, 2011). The patients possibly will become

withdrawn, avoid people and new places and prefer the familiar. Individuals become confused, have difficulty organizing and planning, get lost easily and exercise poor judgment. The AD patients may have difficulty performing routine tasks, and have trouble communicating and understanding written material. If the person is employed, memory loss may begin to affect job performance. Some specific examples of behaviors that people demonstrate in this mild stage include;

1. Getting lost
2. Difficulty managing money and paying bills
3. Repetitive questions and conversations
4. Taking longer than usual to finish routine daily tasks
5. Poor judgment
6. Losing things or misplacing them in odd places
7. Noticeable changes in personality or mood (Alzheimer's research a program of the American health Assistance Foundation, 2011).

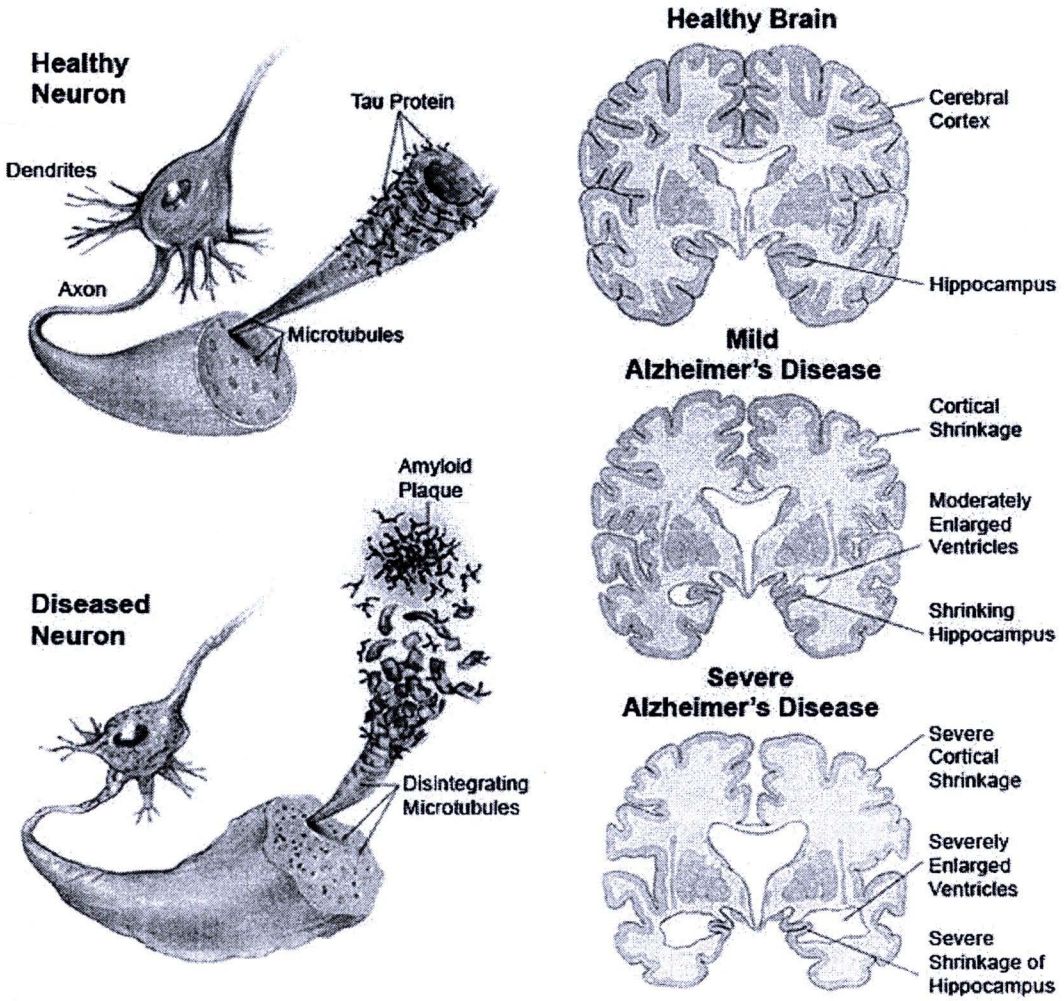

Figure 1 Health and Alzheimer's brain

Source: http://www.ahaf.org/alzheimers/about/understanding/brain-nerve-cells.html

Stage 2 (Moderate): This is normally the longest stage and can last 2 to 10 years. In this stage, the person with AD is clearly becoming disabled. Individuals can still perform simple tasks independently, but may need assistance with more complicated activities. They forget recent events and their personal history, and become more confused and disconnected from reality (Alzheimer's research a program of the American health Assistance Foundation, 2011). Plaques and tangles also spread to areas involved in: Speaking and understanding speech (Alzheimer's

Association, 2011). Memories of the distant past may be confused with the present, and affect the person's ability to comprehend the current situation, date and time. They may have trouble recognizing familiar people. Speech problems arise and understanding, reading and writing are more difficult. They may no longer be safe alone and can wander. As Alzheimer's patients become aware of this loss of control, they may become depressed, irritable and restless or apathetic and withdrawn. They may experience sleep disturbances and have more trouble in eating, grooming and dressing (Alzheimer's research a program of the American health Assistance Foundation, 2011

Stage 3 (Severe): This stage may last 1 to 3 years. During this final stage, people may lose the ability to feed themselves, speak, recognize people and control bodily functions, such as swallowing or bowel and bladder control. Their memory worsens and may become almost non-existent. They will sleep often and grunting or moaning can be common. Constant care is typically necessary. In a weakened physical state, patients may become vulnerable to other illnesses, skin infections, and respiratory problems, particularly when they are unable to move around (Alzheimer's research a program of the American health Assistance Foundation, 2011). Most of the cortex is seriously damaged. The brain shrinks dramatically due to widespread cell death. Individuals lose their ability to communicate, to recognize family and loved ones and to care for themselves (Figure 1) (Alzheimer's Association, 2011).

Risk factors of Alzheimer's disease

1. **Non-genetic risk factors**

AD is caused by a combination of genetic and environmental factors, and the role of environment cannot be ignored (Imbimbo, et al., 2005). Non-genetics factors are associated with vascular disease, including hypertension, coronary heart disease, smoking, obesity, diabetes and alcohol. Whether these are true causal risk factors for AD, driving the pathogenic processes resulting in plaque and tangle formation, or whether they induce cerebrovascular pathology, which adds to clinically silent disease pathology thus exceeding the threshold for dementia, needs to be established. Some evidence suggests that dietary intake of homocysteine-related vitamins (vitamin B12 and folate), antioxidants, such as vitamin C and E, unsaturated

fatty acids, and also moderate alcohol intake, especially wine, could reduce the risk of AD (Letenneur, 2004), but data so far are not conclusive to enable any general dietary recommendations to be made. Although environmental factors might increase the risk of sporadic AD, this form of the disease has been shown to have a significant genetic background.

2. Genetic risk factors

The genetic discovery was that point mutations in any of three genes could cause autosomal dominant inherited forms of AD that were clinically and pathologically identical to nongenetic forms of the disease except that age of onset was younger (Nussbaum and Ellis, 2003). The first mutations found in the *APP* are on chromosome 21. These mutations incline to cluster near sites where the Aβ peptide itself is cleaved from APP (β-and γ-secretase sites) or where the Aβ peptide itself is cleaved (the α-secretase site). The next group of mutations was found in the genes encoding two proteins called PSEN1 and PSEN2. Subsequently, it was discovered that these two proteins play role in the γ-secretase cleavage of Aβ from APP. Thus, it became clear that the primary consequence of these mutations was an increase in the deposition of the pathogenic form of Aβ, Aβ (1-42), in the brain (Table 1). The cleavage by γ-secretase is release Aβ peptides of difference sizes, with Aβ-40 and Aβ-42 being the most common forms. Aβ-40 and Aβ-42 are both toxic peptides, however the Aβ-42 isoform is insoluble and more capable of aggregating into amyloid plaques (Newman, et al., 2007). The Aβ-42 can aggregate into two different conformation states. There is non-β sheet, non-fibrillar state and the β sheet fibrillar which is cytotoxic and eventually deposits into plaques (Cuajungco and Faget, 2003). These findings combined with the understanding that AD in patients with trisomy 21 provided the most important evidence supporting the amyloid-cascade hypothesis for the pathogenesis of the disease (Bird, 2005). On the other hand, both mutation within the *APP* and presenilin genes result in elevated production and accumulation of the Aβ42 (Scheuner, et al., 1996; Hardy, 1997; Hardy, et al., 1998). The Aβ42 is more sensitive to fibrillization and also sensitive to neuritic plaque formation (Hardy, et al., 1998).

Table 1 Genetic factors predisposing disease: relationships to the β-Amyloid phenotype.

Chromosome location	Gene Defect	Phenotypes
21q21.3	*APP* mutations	Increased production of all β-amyloid protein or β-amyloid protein 42
14q24.3	*PSEN 1* mutations	Increased production of β-amyloid protein 42
1q31-42	*PSEN 2* mutations	Increased production of β-amyloid protein 42

Source: Selkoe, 2001

Mutations within the *PSEN1* lead to a particularly aggressive form of the disease having an age of onset between 30 and 50 years. However a polymorphism found within intron 8 of the *PSEN1* was found to be associated with the development of the late-onset form of the disease (Theuns, et al., 2000; Wragg, et al., 1996). To date over 75 mutations have been found within the *PSEN1* in families worldwide that are associated with the early-onset form of the disease (Selkoe, 2001). All mutations within *PSEN1* increase production of the Aβ42 (Hardy, 2001; Scheuner, et al., 1996). Mutations within *PSEN2* have a variable age of onset (40–80 years), result in increase of β amyloid peptide production (Hardy, 2001). Mutations in *PSEN1* have been implicated in 18 – 50 % of autosomal dominant cases with early onset AD (EOAD). The gene has been suggested as a potential risk gene in late-onset AD causes (Theuns, et al., 2000). In addition, mutations in *PSEN1* and *PSEN2* appear to facilitate the cleavage of PSEN. This may be mechanism of which caspases are implicated in the molecular pathology of AD. Finally, it has been reported that APP cleavage by caspases may contribute to amyloid toxicity (Pellegrini, et al., 1999; Weidemann, et al., 1999).

Pathophysiology of Alzheimer's disease

AD is mainly based on genetic and neuropathological findings pointing towards aberrant processing of APP and tau as central molecular events. Mutations in

the genes encoding for APP and PS 1 and 2, which are involved in APP processing, are known to induce early-onset familial AD in rare families (Leonidas, et al., 2010). AD pathology involves increasing the production and accumulation of beta-amyloid (Aβ) peptide, which is central to the pathogenesis of AD (Hardy and Selkoe, 2002). Evidence supporting a pivotal role for Aβ includes the following: mutations in the amyloid precursor protein lead to early-onset AD; all currently known mutations associated with AD increase the production of Aβ; in patients with trisomy 21 (Down's syndrome) and three copies of the gene for amyloid precursor protein, neuropathological characteristics of AD develop by midlife; Aβ is neurotoxic in vitro and leads to cell death; over-expression of human amyloid precursor protein in transgenic mouse models of AD results in neuritic plaques similar to those seen in humans with AD; transgenic mice overexpressing the human amyloid precursor protein have evidence of learning and memory deficits, in concert with the accumulation of amyloid; the apolipoprotein E genotype, a major risk factor for AD, leads to accelerated deposition of amyloid; and the generation of anti-amyloid antibodies in humans with AD seems to ameliorate the disease process (Butterfield, et al., 2002; Hock, et al., 2003). Formation of neurofibrillary tangles, oxidation and lipid peroxidation, glutamatergic excitotoxicity, inflammation, and activation of the cascade of apoptotic cell death are considered secondary consequences of the generation and deposition of Aβ (Hardy and Selkoe, 2002). This hypothesized amyloid cascade underlies attempts to modify the onset and course of AD through identification of anti-amyloid agents, antioxidants, anti-inflammatory drugs or compounds that limit the phosphorylation of tau protein, anti-apoptotic agents, and glutamatergic N-methyl-d-aspartate–receptor antagonists. Cell dysfunction and cell death in nuclear groups of neurons responsible for maintenance of specific transmitter systems lead to deficits in acetylcholine, norepinephrine, and serotonin. Alternate hypotheses regarding the pathophysiology of AD place greater emphasis on the potential role of tau-protein abnormalities, heavy metals, vascular factors, or viral infections.

AD is a degenerative brain syndrome characterized by a progressive decline in memory, thinking, comprehension, calculation, language-learning capacity and judgement and orientation to physical surroundings (Robert and Christopher, 2003). It is important, however, to differentiate the symptoms of AD from normal age-related

decline in cognitive functions. About 5% of men and 6% of women over 60 years of age are affected with AD (Mental and neurological disorders). Longitudinal studies leading to autopsy have shown that the most common neuropathologic findings in elderly patients with these symptoms are neuritic plaques (NPs) and neurofibrillary tangles (NFTs). Plaques are extracellular deposits of Aβ surrounded by dystrophic neurites, reactive astrocytes, and microglia, whereas tangles are intracellular aggregates composed of a hyperphosphorylated form of the microtubule-associated protein tau (Blennow, et al., 2006) (Figure 2).

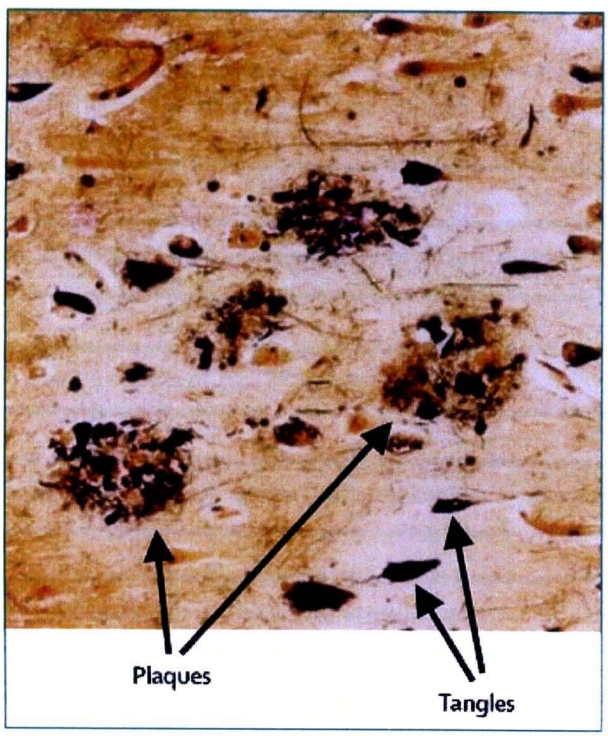

Figure 2 Plaques and tangles in the cerebral cortex in Alzheimer's disease

Source: Blennow, et al ., 2006

All AD patients must have dementia, a progressive loss memory, and at least one other cognitive function that is sufficiently severe to interfere with daily functioning. The genetics of AD is complex. Three genes (*APP*, *PSEN1* and *PSEN2*) have been described in the relatively rare, early-onset, autosomal dominant familial

from of AD (Combarros, et al., 2002). A SNP located in intron 8 of *PSEN1* (rs165932 G/T) has been implicated in sporadic AD. Its location in intron 8 could implicate in alternate splicing of exon 9 or endoproteolysis of *PSEN1* (Belbin, et al., 2008). These are led to hypothesis of the amyloid cascade, which progresses from the generation of the beta-amyloid peptide from the amyloid precursor protein, through multiple secondary steps, to cell death, forms the foundation for current and emerging options for the treatment of AD. APP denotes amyloid precursor protein, and Aβ amyloid (Figure 3) (Jeffrey, 2004).

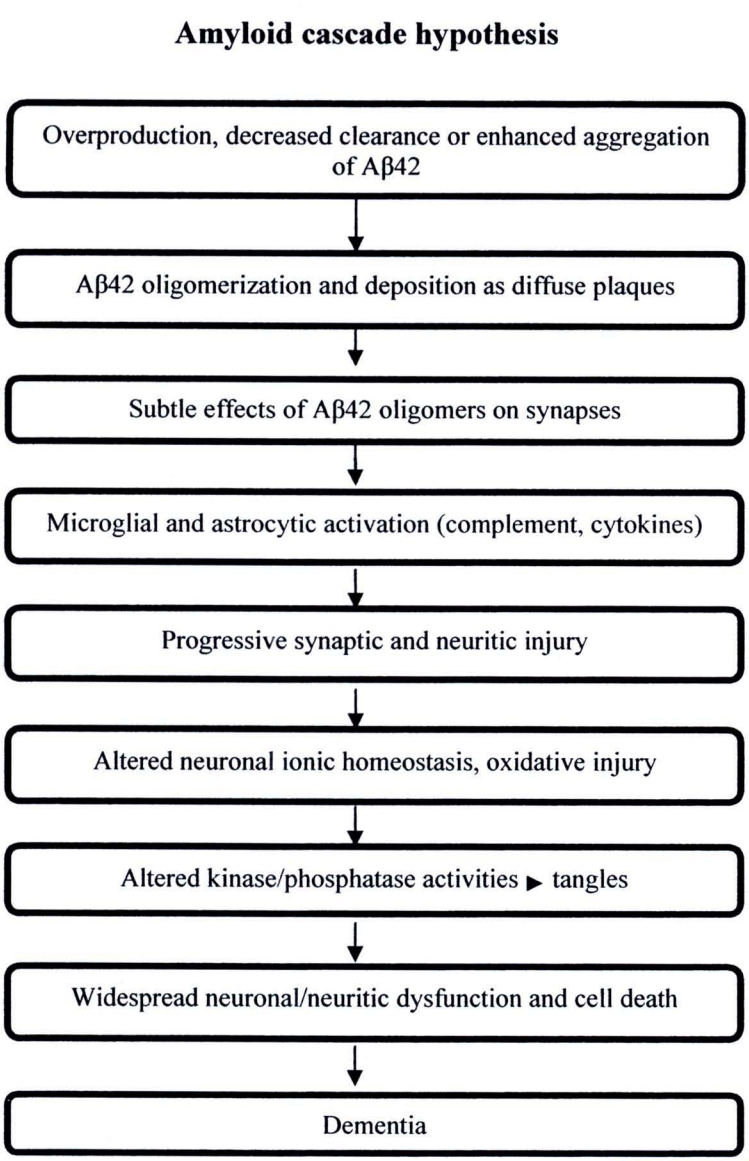

Figure 3 Putative amyloid cascade

The structure and functions of the *PSEN 1*

The *PSEN1* is on chromosome 14 (14q24.3) (Figure 4) (Dewji, 2005). It is located from base pair 72,672,931 to base pair 72,756,861 on the chromosome. The gene provides instructions for producing a protein called PSEN1. The protein is involved in the development of the brain and spinal cord (central nervous system) and the survival of nerve cells (neurons). The PSEN1 is an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (beta-amyloid precursor protein). The other members of the γ-secretase complex having a protease activity may play a role in intracellular signaling and gene expression or in linking chromatin to the nuclear membrane. Under conditions of apoptosis or calcium influx, cleaved E-cadherin promotes the disassembly of the E-cadherin/catenin complex and increases the pool of cytoplasmic beta-catenin, thus negatively regulating Wnt signaling may also play a role in hematopoiesis (Steiner, al 1999; Wolfe, et al., 1999; Berezovska, et al., 2000; Kulic, et al., 2000; Baki, et al., 2001; Wrigley, et al., 2004; Wang, et al., 2006). PSEN1 helps process proteins that transmit chemical signals from the cell membrane into the nucleus. Once in the nucleus, these signals activate genes that are important for cell growth and maturation. PSEN 1 is known for its role in processing APP, which is made in the brain and other tissues. Research suggests that PSEN1 works together with other enzymes to cleave APP into peptides. One of these peptides is called soluble APP (sAPP), and another is called amyloid beta peptide. Recent evidence suggests that sAPP has growth-promoting properties and may play a role in the formation of neurons in the brain both before and after birth. The gene was initially discovered by genetic linkage studies in families with AD. PSEN1 is organized into ten exons that display tissue-specific alternate splicing. The intact forms were shown to be associated with the γ-secretase activity that generates the Aβ fragments of β–APP. *PSEN1* is responsible for up to 80% of familial early-onset AD. Mutations associated with AD (Larner and Doran, 2006) accounts for 30% -70% of EOFAD (Cruts and Broeckhoven, 1998; Campion, et al., 1999; Rogaeva, et al., 2001; Lleo, et al., 2002, Janssen, et al., 2003).

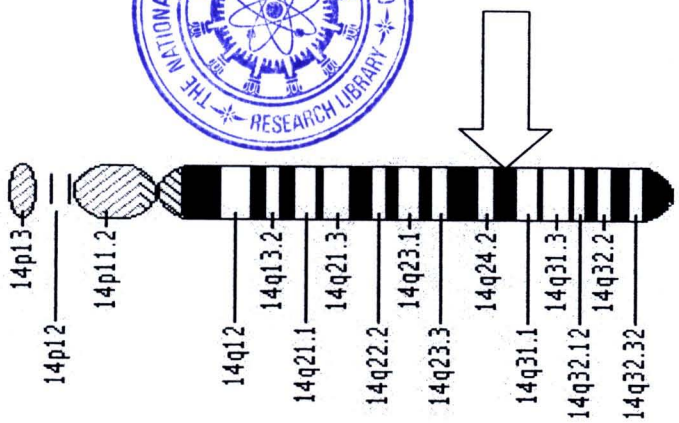

Figure 4 The *PSEN 1* is located on the long (q) arm of chromosome 14 at position 24.3.

Source: http://ghr.nlm.nih.gov/gene=psen1

Single nucleotide polymorphisms (SNPs) and association studies

SNP (pronounced snip) is a single nucleotide variation in a genetic sequence that occurs when a single nucleotide A, T, C or G in the genome differs between of a biological species or paired or paired chromosomes in an individual (typically greater than 5%) (Joel, et al., 2005) (Figure5). Within a population, SNPs can be assigned a minor allele frequency, the lowest allele frequency at a locus that is observed in a particular population. The types of SNPs can be divided within non-coding region, coding region or in the intergenic regions.

Most of common complex disease like AD is known to be under the influence of multiplex or multifactorial disease. In an effort to identify this association, initial studies involved genotyping of few hundreds or a few thousands of SNPs from regions of interest. These SNP association studies are called candidate-gene approaches. The goal of SNP association studies is to identify the SNPs as bio-markers to the genes that are predisposing individuals to disease.

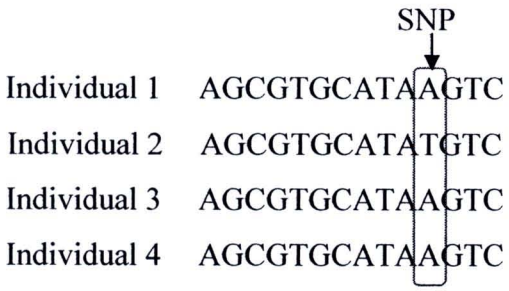

Figure 5 An example of single nucleotide polymorphism (SNP)

***PSEN1* polymorphisms and mutation in Alzheimer's disease**

PSEN1 is the risk for sporadic AD (Combarros, 1999). The pathogenic mutations in *PSEN1* lead to typical AD in a fully penetrant fashion with an onset age of 30-35 (John, 2001). In addition, mutation in *PSEN1* and *PSEN2* cause AD by adopting the production and deposition of amyloid β particularly the most harmful form which contains 42 amino acids (Figure 6), likely by increasing γ-secretase (Figure 6 and Figure 7) cleavage of APP (Holcomb, et al., 1999; Mehta, et al., 1998; Aya, 2006).

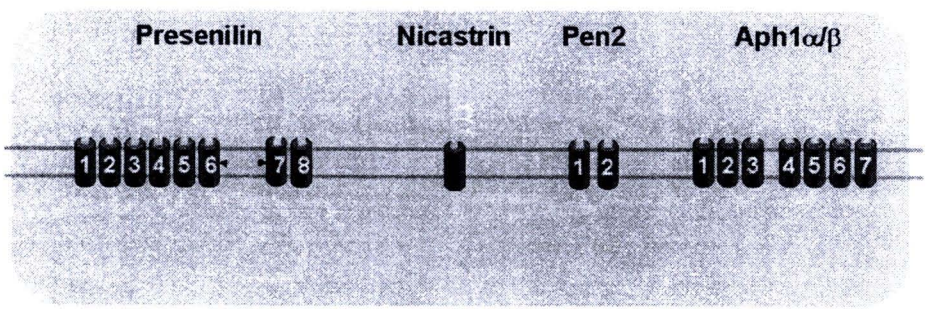

Figure 6 The γ-secretase complex. A schematic model of the γ-secretase complex composed of PSEN, NTC, APH-1 and PSEN2.

Source: http://www.sfb604.uni-jena.de/Aims/Area+B/Project+B11+(Kaether).html

Carriers of two copies of the 1 allele in a non-coding (intron 8) region of *PSEN1* were found to be represented excessively among sporadic AD patients when

compared with control subjects (Wragg, et al., 1996). New polymorphisms recently detected in the promoter and the 5'-non-coding region of *PSEN1* and in intronic and exonic sequences of *PSEN2* are useful in genetic association studies (Cruts, et al., 1998).

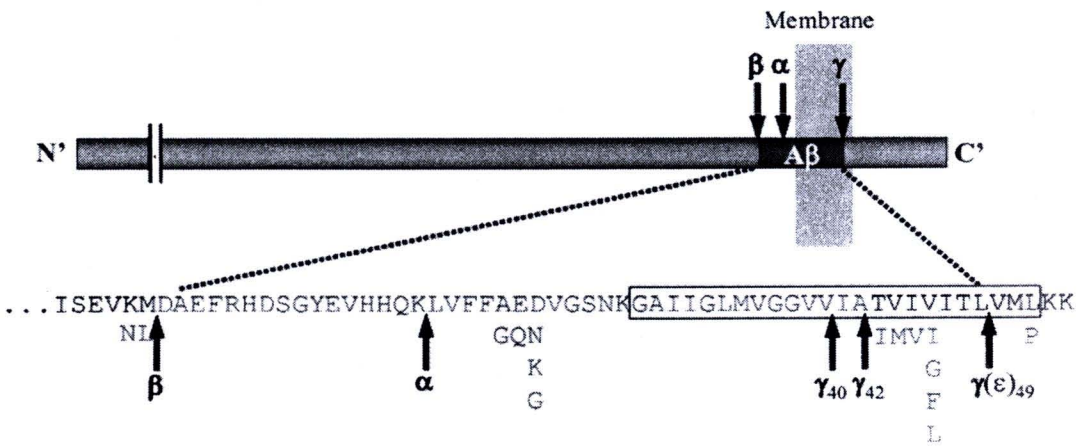

Figure 7 γ-secretase cleavage of APP

Source: Aya, 2006

Nevertheless, the current availability of high-throughput genotyping platforms of single nucleotide polymorphisms (SNPs), large-scale genetic studies will eventually generate additional knowledge about the genetic risk profile for AD (Brouwers, Sleegers, and Broeckhoven, 2008). In Italy, the homozygosity of an allele in the *PSEN1* was associated with a doubling of the risk for LOAD (Sorbi et al., 1997). The study in Australian population shows that the *PSEN1* intronic polymorphism is associated with AD (Taddei, et al., 1998). Mann and colleagues (1997) show that 8 intronic PSEN1 polymorphism does not influence the pathological phenotype of AD. In United Kingdom, SNP located in intron 8 of *PSEN1* (rs165932G/T) has also been implicated in sporadic AD (Belbin, et al., 2008). On the other hand, in Germany, healthy subjects and depressed patients showed no significant differences in the distribution of the *PSEN1* genotypes and allele frequencies between AD patients and controls (Bagli, et al., 1999). And also, the intron 8 polymorphism of

the *PSEN1* does not appear to be important risk factors for sporadic AD in Caucasians originating from a limited geographical area in northern Spain population (Combarros, et al., 1999). The *PSEN1* intronic polymorphism does not influence the amount or molecular form of amyloid β in AD patients (Sodeyama, et al., 1998).

However, differing results exist for the association between 8 intronic polymorphism and AD probably due to the *PSEN1* among different ethnic groups (Bagli, et al., 1999). Therefore, we were to investigate the possible involvement of SNPs of the *PSEN1* in the risk for Thai AD patient and control subjects using identify and genotype proper tag SNPs to find frequency in *PSEN1* which association with the AD subjects.

Alzheimer's disease treatments

Researchers have identified several new treatment strategies that might have the potential to change its course. A number of experimental therapies based on the amyloid hypothesis and other targets have reached various stages of clinical testing in human volunteers. Despite the current lack of disease-modifying therapies, studies have consistently shown that active medical management of AD can significantly improve quality of life through all stages of the disease for diagnosed individuals and their caregivers. Active management includes appropriate use of available treatment options, effective integration of coexisting conditions into the treatment plan, and utilization of supportive services such as counseling, activity and support groups, and adult day programs. Scientists consider the emerging field of prevention one of the most exciting recent developments in the dementia research arena. A growing body of evidence suggests that the health of the brain, one of the body's most highly vascular organs, is closely linked to the overall health of the heart and blood vessels. Management of cardiovascular risk factors, such as high cholesterol, type 2 diabetes, high blood pressure, and overweight, might help avoid or delay cognitive decline. Additional evidence points to a significant role for regular physical exercise in maintaining lifelong cognitive health. More limited data suggest that a low-fat diet rich in fruits and vegetables might support brain health, as might a strong social network and a lifetime of intellectual curiosity and mental stimulation.