CHAPTER V

DISCUSSION AND CONCLUSIONS

Sample size determination

Cases (AD patients) and controls were calculated with Quanto Version 1.2 (Jim and John, 2007) for sample size determination. This study focused on highest MAF tagSNPs from identified tagSNPs in *PSEN1* in HapMap. The highest MAF is rs165933. It was determined in sample size determination. There are two alleles at the *PSEN1* locus, denoted 'Null' (allele G/A) and non-Null (allele G/G (ancestral allele)). It is thought that only those with the Null/Null (G/A) genotype are at increased risk for disease (Jim and John, 2007). The proportion of subjects in the population with the Null/Null (allele G/A) genotype is estimated to be 38.9% (Table 4 identified tagSNPs in *PSEN1*). Assuming Hardy-Weinberg equilibrium, the prevalence of the Null allele in the population has a frequency (prevalence) of the rs165933 in the population (Han Chinese from Beijing, China) = $38.9^{1/2}$ = 0.6237. The inheritance model is recessive therefore only the heterozygous carrier is assumed to at increased risk. The relative risk for (G/A allele) carriers, compared to normal, is 2.0 (Rg). Desired power is 80%, at a significance level of 0.05 with a 2-sided alternate hypothesis. Therefore, the study was to perform with 135 AD patients and 135 controls in order to performed association study in *PSEN1* in Thai AD patients. However, this study used only 15 AD patients and 15 controls because AD in the Memorial Clinic, Chaingmai Neurological Hospital was limited and most AD patients usually have co-symptom with vascular disease and alcoholism, etc., which then were excluded (Memorial Clinic, 2011). The average ages of controls and AD patients are 58.33 and 71.73 years old, respectively. The AD patients were classified in lately onset Alzheimer's disease (LOAD) (Memorial Clinic, 2011).

Alzheimer's disease patients and control subjects collection

Study samples comprise of AD patients and healthy controls. The AD patient subjects were recruited from Memorial Clinic, Chiangmai Neurological Hospital,

Chiangmai, Thailand under ethic committee of the hospital (certificate of approval EC 017-54) and human ethic committee of Naresuan University, Phitsanulok, Thailand (certificate of approval HE 54-Ep1-0024). The diagnosis of AD patients was performed by medical doctor in expert of neurological disease. The diagnosis of AD in the clinic is 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, Clinical Practice Guideline for Dementia (Prasat Neurological Institute, 2008) 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) et al. (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). The control groups were selected from the hospital and they were confirmed healthy and neurologically normal by medical history, general examinations and Thai mini-mental state examination (TMSE). An informed consent to participate was obtained from each subject. All patients with evidence of an autosomal dominant AD trait, or where a first degree relative had been diagnosed with familial AD, were excluded. However, most AD patients in the clinic were diagnosed in AD with other symptoms such as AD-alcoholism, AD-vascular disease. These co-symptoms were also excluded. Therefore, the study investigated only 15 AD patients and 15 controls as the initial study.

SNP identification on HapMap website

SNPs are highly abundant, stable, and distributed throughout the genome. These variations are associated with diversity in the population, individuality,

susceptibility to disease, and individual response to medicine (Shastry, 2002). The International HapMap Project is a partnership of scientists from Canada, China, Japan, Nigeria, the United Kingdom and the United States to develop a public resource that will help researchers find genes associated with human disease and response to pharmaceuticals. The goal of the International HapMap Project is to develop a haplotype map of the human genome, the HapMap, which will describe the common patterns of human DNA sequence variation, frequencies, and correlations between them, in DNA samples from populations with ancestry from parts of Africa, Asia and Europe. The project offers tools that will allow the indirect association approach to be applied to any functional candidate gene in the genome, to any region suggested by family-based linkage analysis, or ultimately to the whole genome for scanning for disease risk factors (The International HapMap Consortium, 2003). The HapMap is expected to be a key resource for researchers to use to find genes affecting health, disease, and responses to drugs and environmental factors. The HapMap has efficiency of genetic association studies that can be increased by typing informative SNP-haplotype tagging SNPs (htSNPs) that are in linkage disequilibrium with several other SNPs, thus a small fraction or subset of SNPs at the locus or gene of interest are sufficient to capture the vast majority of the genetic variation. Therefore, this research used the HapMap website to identify tagSNPs in *PSEN1* for genotyping. The tag SNP available on the HapMap (HapMap Tutorial, 2007) website used to select tag SNPs present at a minor allele frequency (MAF) > 5 % and with a pairwise LD cut-off value of $r^2 > 0.6$. Coverage of the entire gene (90 kbp) was achieved by rs3025780 (Intron 3), rs214273 (Intron 3), rs362340 (Intron 4), rs165932 (Intron 8), rs165933 (Intron 8) and rs10146743 (Intron 9). The highest MAF of the identified 6 tagSNPs in *PSEN1* was rs165933 (Intron 8) with 38.9 %. The rs165933, therefore, might be used as a marker of SNPs in *PSEN1* in Thai AD patients.

Primer design and PCR amplification

Primers were designed in the sense or antisense sequences, and checked hairpins loops, self-annealing, melting temperature (Tm) using Oligo Analyzer 1.0.3 (Teemu Kuulasma, 2001-2002) and PRIMER BLAST investigation for 5 tagSNPs (rs165932, rs3025780, rs10146743, rs214273 and rs165933). The failed rs362340

might be due to its short nucleotide (65 bp) sequence when compared with others. This could be improved by using designed primers which would result a longer nucleotide sequence.

Genotyping of identified 5 tagSNPs in *PSEN1* by DNA sequencing techniques

Normally, the identical alleles of human gene are present on both homologous chromosomes (Lawrence, 2008). This study found that 97.33% are homozygous alleles, and only showed 2.66% homozygous alleles in rs165933 for all controls and Alzheimer's disease patients. This study, the homozygous and heterozygous alleles in rs165933 (GG, GA and AA) were shown as the same allele pattern as African ancestry in Southwest USA (ASW),Utah residents with Northern and Western European ancestry from the CEPH collection (CEU), Han Chinese in Beijing, China (CHB), Chinese in Metropolitan Denver, Colorado (CHD), Gujarati Indians in Houston, Texas (GIH), Japanese in Tokyo, Japan (JPT), Luhya in Webuye, Kenya (LWK), Mexican ancestry in Los Angeles, California (MEX), Maasai in Kinyawa, Kenya (MKK), Tuscan in Italy (TSI) and Yoruban in Ibadan, Nigeria (YRI) as reported on HapMap project (HapMap project).

Association studies of 5 tagSNPs in *PSEN1* in Thai AD patient and controls

Many genetic studies of disease association rely heavily on linkage disequilibrium (LD) patterns between pairs of markers to detect susceptibility markers (Steven et al., 2007). Therefore, in the study, LD cut-off r^2 more than 0.6 and MAF more than 5% in identification of SNPs in *PSEN1* on HapMap was achieved: rs3025780 (Intron 3), rs214273 (Intron 3), rs362340 (Intron 4), rs165932 (Intron 8), rs165933 (Intron 8) and rs10146743 (Intron 9). The highest MAF of the identified 6 tagSNPs in *PSEN1* was rs165933 (Intron 8) with 38.9 %. However, 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 showed that the *PSEN1* intronic polymorphism associated with AD (Taddei, et al., 1998). In United Kingdom, SNP located in intron 8 of *PSEN1* (rs165932G/T) has also been implicated in sporadic AD (Belbin, et al., 2008). 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). In Thailand, as this study reported, only 2.66% was found as GA heterozygous in rs165933 for both AD patients and controls. In AD, the GA heterozygous was about 20 %, when only 6.66% showed in the controls. Association studies in the study using odd ratio (95% CI) of the 5 tagSNPs in *PSEN1* showed genotypes of GG, GA and AA alleles as 3.00 (95% CI = 0.2478 - 36.3266), 0.2381 (95% CI = 0.0188 - 3.0117), and 4.2 (95% CI = 0.332-53.1253), respectively. The odd ration (OR) of less than 1 means the SNPs is protective from AD, when OR is equal to 1 means the genotype in the SNPs is equal in both groups (AD patients and controls). When OR is more than 1, it suggested that this genotype is the risk for AD. Therefore, the rs165933 genotypes (GA alleles) is a potential SNP marker of *PSEN1* in Thai population which is different from other ethnic groups (Bagli, et al., 1999).

Conclusions

This study is the first association studies and frequency reports of 5 tagSNPs (rs3025780 (Intron 3), rs214273 (Intron 3), rs165932 (Intron 8), rs165933 (Intron 8) and rs10146743 (Intron 9)) genotypes in *PSEN1* in Thailand. The main aim of this study was to identify and investigate whether single nucleotide polymorphisms in *PSEN1* related with the risk of AD. Although, only the rs165933 (GA alleles) might be a possible SNP marker of *PSEN1* in Thai population. Further study of large sample size needs to be evaluated.