CHAPTER 1

INTRODUCTION

1.1 General background

Current alcohol consumption of Thai people was increased every year. The yearly alcohol consumption per capita has been increased from 20.2 litres in the year 1989 to 58.0 liters in the year 2003 (Sornpaisarn, 2006). According to WHO report, Thailand was the fifth of the world's alcohol consumption per capita, Additionally of age of first drink in teenager was decreasing, age range in the male was 15-19 years old and 20-24 years old in female (CAS, 2005). Thai adults were estimated to consume 2.0 litres of pure ethanol per capita. The alcohol consumption is also related to traffic accident deaths and illness. It was estimated that the hospital admission of the patients due to alcohol-related diseased costed over 20,000 baht/ person in the year 1992 and this caused tremendous lost in economic and social aspects of the country (WHO 2004).

Continuous alcohol consumption are known as the cause of alcohol dependence. This condition characterized by compulsory repeated alcohol usage leading to physiological dependence. The patients will received adverse physical and psychological consequences and prone to experience the serious accidents, violence even suicide. The high alcohol intake can cause undesirable effects such as gastrointestinal irritation, cirrhosis, central and peripheral nervous system damage.

1.2 Diagnostic criteria for alcohol dependence

Substance dependence is defined as a cluster of cognitive, behavioral and physiological symptoms of an individual who continue to use the substance regardless of substance-related problems. The repeated self-administration that results in the development of tolerance or withdrawal with or without physiological dependence (Andreasen *et al.,* 2006).

According to the diagnostic criteria from checklist requiring that the patients with alcohol dependence must have symptoms in at least 3 of 7 at anytime during 12-month period for DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) by the American Psychiatric Association or 3 of 6 for International Classification of Disease: ICD10 (Kapland *et al.,*1994; Zering *et al,* 2000). Another diagnostic tools that suitable for alcohol dependence according to DSM-IV or ICD 10 such as Mini International Neuropsychiatric Interview (M.I.N.I) (Sheehan *et al.,* 1998).

The Mini International Neuropsychiatric Interview (M.I.N.I) is the structure diagnostic interview according to American Psychiatric Association and WHO. It was developed to assess the diagnoses of psychiatric disorders. It is a short but accurate structured interview with an administration time about 20-30 minutes. Therefore, it is suitable for research purposes. This diagnostic interview was developed by the cooperation of 2 groups of psychiatrists and clinicians. The first group was Professor David V. Sheehan from University of South Florida College of Medicine. The second group was Dr. Yves Lecrubier from Hospital de la Salpetriere. The M.I.N.I was translated into many languages including Thai version. The M.I.N.I contains 16 modules identified by letters for each category. Each diagnostic module (except for psychotic disorders module) started with 1-2 screening questions shown in gray box.

The patients have to answer Yes or No. If they rejected the screening questions, the interviewer must move to the next module until the diagnostic criteria are met. In conclusion, we can make a summary that the patient was indicated in the disorders. Moreover, we can identified the time frame of disorders in current, past or lifetime (Kittirattanapaiboon and Khamwongpin, 2005; Srisurapanont *et al.,* 2001).

M.I.N.I. was developed to reduce time in assessment and recording data but accurate. Kittirattanapaiboon and Khamwongpin, 2005 translated and validated the M.I.N.I Thai version 5.0.0 on both face and contents. To compare the criterion-related validity between psychiatric nurses and psychiatrists by using the normal subjects and patients from psychiatric hospital. They tested for statistic of Cohen's kappa, sensitivity, positive predictive value (PPV), negative predictive value (NPV) and efficiency. They found that Cohen's kappa, sensitivity and PPV on the module of current major depressive episode, current suicide risk, lifetime psychotic disorder and current generalized anxiety disorder were very high (>0.75, >0.81, >0.81 respectively). They also found that specificity, NPV and efficiency were very high (>0.81) in all module conformed to the study of Sheehan *et al.,*1997 that have compared the inter-rater and test-retest reliability of M.I.N.I and SCID-P. In consequence, M.I.N.I Thai version 5.0.0 is suitable for diagnose the psychiatric disorders in Thai population. The limitation in Thai population was few patients on the module of anxiety disorder Thus, the specific groups of patients and general health care setting should be considered in the further study.

The severity of alcohol dependence questionnaires (SADQ) was developed to measure the degree of dependence in patients. This questionnaires consist of 5 issues (physical withdrawal symptoms, affective symptoms of withdrawal, craving and

withdrawal-relief drinking, typical daily consumption and reinstatement of withdrawal symptoms after a period of abstinence), each were separated into 4 questions (Dawe *et al.,* 2002). SADQ is self-rating questionnaires that have good reliability and accuracy (Davidson, 1986). The score from SADQ was significantly correlated to the severity of alcohol withdrawal and drinking pattern (Stockwell, Murphy and Hodgson 1983).

Moreover, the SADQ is a short questionnaires (approximately 5-10 minutes to complete), easy score counting and suitable to assessment the severity in problem drinkers. It has been suggested that the cut-off point at 30 can separate the severe dependence from mild/moderate dependence (Stockwell *et al.,* 1994). Ee Heok *et al.,* 1990 found that the SADQ can be used with a range of culture groups.

Cloninger *et al.,* 1981 was defined the types of alcohol dependence into 2 groups according to clinical manifestation and heritability pattern. The type I alcohol dependence was characterized by the less severe with adult-onset form. This type was greatly effected by the environment factor with little or no positive family history. Their clinical distinctions of dependence and onset are shown over 25 years old and easy to detoxification. The type II dependence was characterized by more severe and early onset form (age of dependence was less than 25 years old) with a strong positive family history or high heritability. This type II also has characteristics of impulsive and antisocial personality disorder with poor response to treatment (Cloninger, 1995).

1.3 Genetics related to alcohol dependence

As the alcohol dependence is the complex genetic and environment interaction (Edenberg and Kranzler, 2005; Luo *et al.,*2005). There are strong evidences supported a role for genetics. The individual with close relative of alcohol dependence showed high incidence 3-4 times of disorder.The studies in subjects with positive family history found that the offspring of alcoholics being 3-5 times to develop dependence more than the non-alcoholics offspring (Cotton, 1979).

The studies of twin and adoption were used to separated the effects of genetics from environment factors. The results revealed that genetics may affect alcohol dependence including 50-60% in individual (McGue, 1991; Dick and Foroud, 2003). Twin studies were found that monozygotic twins have greater rate to develop alcohol dependence than dizygotic twins. The heritability of alcohol dependence in male twins ranges between 49-64% of the genetic variance (Picken *et al.,* 1991; Kendler *et al.,* 1994; Heath *et al.,* 1997). The adoption studies also found that adoptees with alcohol dependence biological parent can develop 3-4 folds increase risk of dependence although they were raised by non- alcohol dependence adoptive parent (Bohman *et al.,* 1981; Cloninger *et al.,* 1981). These previous studies reflected that genetics may contribute to the alcohol susceptibility in individuals.

The variation of nucleotide sequences in DNA called “DNA polymorphism”. This phenomenon may effect the protein structure and/or function. There are two types of polymorphism, first type called Single Nucleotide Polymorphism (SNPs) which was the single nucleotide variation on DNA strand. SNPs can define as single base substitution, deletion and addition. Secondly, the repetitive DNA sequence gene high copy numbers which can be found in non-coding region. SNPs are the most

abundant type of polymorphisms in the human genome. More than five million SNPs have been collected in databases and four million have been validated. On average there should be one SNP for every 300 bases. Their enormous abundance makes them interesting as markers for genes that underlie complex diseases, pharmacogenetics, and forensic genetics (Gabriel et al. 2002).

The location and pattern of DNA polymorphism can affect the protein synthesis process and its quality in different ways. The polymorphism in regulartory region can cause the difference quantity of protein. This incident in non-coding region will effect on the protein quality (Kelada *et al.,* 2003). The variation of mRNA stability or mRNA splicing or alternative splicing was the process that can deliver more than one pattern of mRNA from the same gene. This phenomenon can diversified the structure and function of proteins in synthesis process (Moroy and Heyd, 2007).

Many studies found that DNA polymorphism in genome was related to susceptibility and severity of alcohol dependence. The candidate genes were focused on alcohol metabolism genes and neurotransmitter genes (Dick and Foroud, 2003; Kohnke, 2008).

1.4 Dopamine reward system

The mesocorticolimbic dopamine system in ventral tegmental area (VTA) of the brain plays an important role on reward mechanisms. (Tupala and Tiihonen, 2004; Kohnke, 2008). The addictive drug including alcohol can overstimulate dopamine release in nucleus accumbens (NAC). This phenomena reinforced self-repeated administration of substance. The chronic overstimulation of dopamine causes

tolerance that the user must increase the amounts of substance to achieve desire effects, leading to addiction (Bear *et al.,*2007; Chandler, 2003; Markianos *et al.,* 2000) as shown in Figure 1.

The mutation at 3' or 5' untranslated region of the gene may effect the translation efficiency and mRNA stability (Browning *et al.,* 1988; Kabnick *et al.,* 1988) that may associate to the change in gene products.

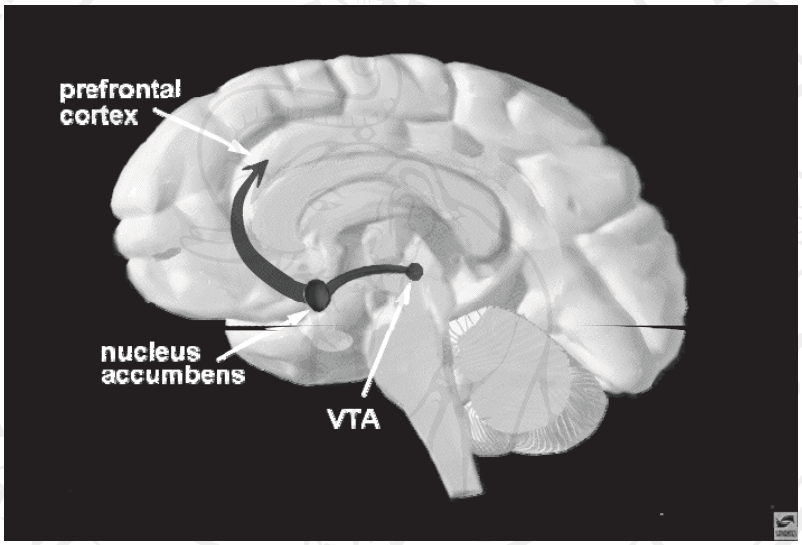

Figure 1 The brain region associated with reward pathway.

VTA : ventral tegmental area

http://www.nida.nih.gov/pubs/teaching/largegifs/slide-9.gif

Korpi *et al.,* 1987 found that the D2 receptor density (*Bmax*) in the nucleus caudate (NC) of ethanol-preferring AA strain rat was lower than the ethanol-nonpreferring rat. Stefanini *et al.,* 1992 found that there was lower density of D2 receptor in nucleus caudate , nucleus accumbens and olfactory tubercle compared to nonpreferring sNP strain. The human brain section studies showed that in the dorsal striatum, nucleus accumbens and amygdala of type 1 alcohol dependence had the

lower D2 receptor density than in control group (Volkow *et al.,* 1996; Kuikka *et al.,*2000). The comparison of D2 receptor density also revealed that the type 2 alcohol dependence had 19.6-21.4% of density lower than controls (Volkow *et al.,* 2002).

The previous studies about the 5 dopamine receptor subtype genes (*DRD1-DRD5*) found that *DRD2* which is in chromosome 11q22-11q23 region was related to alcohol dependence. Blum *et al.,* 1990 compared typing of the polymorphism at *Taq*I A site on 3' untranslated region from the DNA samples between alcohol dependence group and controls by PCR-RFLP technique (Polymerase Chain reaction and Restriction Fragment Length Polymorphism). They found that the polymorphism at *Taq*I A site (rs1800497) in *Ankyrin Repeat and Kinase Domain Containing 1* (*ANKK1*) (as shown in Figure 2) was associated with the alcohol dependence. The percentage of A1 allele found in patient was (69%) higher than in controls (20%). The allelic frequency of A1 allele was significantly difference (p = 0.002) between alcohol dependence (37%) and controls (13%). The A1 allele carriers (TT and TC genotypes) were significantly higher in dependence group than controls (Amadeo *et al.,*1993; Blum *et al.,* 1991; Comings *et al.,* 1991; Lawford *et al.,* 1997; Neiswanger *et al.,* 1995; Noble *et al.,* 1998a; Samochowiec *et al.,* 2000). The studies in multiple ethnic population studies were conformed these association (Cloninger *et al.,* 1991; Uhl *et al.,* 1992; Noble *et al.,* 1991; Parsian *et al.,* 1991; Arinami *et al.,* 1993; Lawford *et al.,* 1997). The studies in Asian population were conducted in Japanese, Korean and Chinese (Arinami *et al.,* 1993; Ishiguro *et al.,* 1998; Kono *et al.,* 1997; Namkoong *et al.,*2008).

Arinami *et al.,* 1993 studied the association of A1 allele incidence and severity of alcohol dependence in patients and unscreened controls found that there was 77%

of A1 allele in more severe dependence. The percentage in the less severe dependence and controls were similar (59% and 60% respectively). The severity tended to increase in A2A2, A1A2 and A1A1 respectively (Blum *et al.,*1991; Noble *et al.,* 1998a; Noble *et al.,* 2000).

Connor *et al.,* 2002 compared the A1 allele frequency between dependence groups and found that the A1 allele was related to the severity of disorder in term of the parameter of alcohol quantity (g per day), alcohol consumed per week (g), alcohol dependence scale score (ADS), age of problem drinking onset, years developing problem drinking and more detoxification attempts as shown in Table1 (Blum *et al.,*1991; Connor *et al.,*2002).

Table 1 Comparison of parameters between A1+ allele patients and A1- allele patients (Connor *et al.,* 2002).

Table I. Drinking and treatment parameters by DRD2 allele* status.

Measure	*A_1^+ Allele*		*A_1- Allele*		*Significance*
	Mean	*S.D.*	*Mean*	*S.D.*	
Consumption					
Alcohol quantity (g per day)	220	104	163	81	$P = .004$
Drinking frequency (days per week)	6.39	1.22	6.07	1.36	$P = .255$
Alcohol consumed per week (g)	1409	738	1008	571	$P = .003$
Dependence					
Alcohol dependence scale score	32.9	9.0	29.2	8.8	$P = .048$
Chronology					
Age of first drink	13.4	4.6	14.8	4.9	$P = .185$
Age of problem drinking onset	20.7	5.9	26.7	8.5	$P = .0001$
Years of alcohol consumption	17.4	8.6	15.2	10.9	$P = .327$
Years developing problem drinking	7.27	5.47	12.0	7.42	$P = .002$
Detoxifications					
Proportion of patients utilizing 5 or more detoxification attempts in each allele group	$^{12}/_{35}$ (34%)		$^{10}/_{71}$ (14%)		$P = .023$

*A_1^+ allele subjects ($n = 35$) include those with the A_1/A_1 and A_1/A_2 genotypes. A_1- allele subjects ($n = 71$) include those with the A_2/A_2 genotype.

Noble *et al.,* 2000 compared allelic distribution in Caucasian alcohol dependence of more severe and less severe patients and found that the more severe

type had the higher percentage of A1 allele than less severe group as shown in Table 2.

Table 2 The A1 allelic distribution studies compared in more severe and less severe dependences (Noble *et al.,* 2000).

Table II. *Taq*I A DRD2 allelic distribution in studies of more severe and less severe Caucasian alcoholics

	More severe alcoholics			*Less severe alcoholics*			*Odds ratio*
	$A1^+$	$A1^-$	% $A1^+$	$A1^+$	$A1^-$	% $A1^+$	
Bolos et al. [11] a	9	11	45.0	6	14	30.0	1.91
Parsian et al. [57] b	6	4	60.0	7	15	31.8	3.21
Blum et al. [8] b	33	19	63.5	15	29	34.0	3.36
Gelernter et al. [26] c	12	11	52.2	7	13	35.0	2.03
Cook et al. [19]d	4	11	26.7	1	4	20.0	1.45
Turner et al. [69] d	4	18	18.2	5	20	20.0	0.89
Noble et al. [55] d	19	15	55.9	15	15	50.0	1.27
Geijer et al. [24] e	20	36	35.7	3	15	16.7	2.78
Geijer et al. [24] f	6	4	60.0	3	6	33.3	3.00
Lawford et al. [37] b	23	20	53.9	49	109	31.0	2.56
Hietala et al. [31] d	23	29	44.2	7	11	38.9	1.25
Total subjects (n = 706)	159	178	47.2*	118	251	32.0*	1.90

Note: $A1^+$ allele subjects include AlAl or A1A2 genotypes; $A1^-$ allele subjects include A2A2 genotype only. More severe (n = 337) and less severe (n = 369) alcoholics were differentiated by a variety of means: a: The Michigan Alcoholism Screening Test (MAST); b: The presence or absence of medical complications of alcoholism; c: Alcohol consumption; d: Severity of Alcohol Dependence Questionnaire (SADQ); e: DSM-III-R criteria (P2 group vs. P1 minus P2 group); f: Autopsy determination (P6 group vs. P5 minus P6 group); * The prevalence of the Al allele was significantly higher in the more severe than in the less severe alcoholic group (χ^2 = 16.4, 95% confidence limits: 1.38 and 2.61, $P = 5.00 \times 10^{-5}$).

1.5 *ANKK1*

Ankyrin Repeat and Kinase Domain Containing 1 (*ANKK1*) is the gene that encodes the Ser/Thr protein kinase family involved in signal transduction pathways. The *Taq*I A SNP which actually located 10 kb downstream from *DRD2* was determined to be located in exon 8 of *ANKK1*. This SNP site causes an amino acid substitution in the 11th ankyrin repeat (Glu713Lys) that may affect structural integrity and substrate binding specificity. Neville *et al.,* 2004 suggested that *ANKK1* may involved in dopaminergic reward pathway through signal transduction. The

mechanism may describe the previous associations between *Taq*I A polymorphism and the addictive behavior.

Dick *et al.,* 2007 studied the association of *DRD2* and *ANKK1* gene by multiple SNPs genotyping and conducting the family-based association to test for the phenotypes related to severity of alcohol dependence in the literature. The results showed that *ANKK1* may involve in the risk for severe form of dependence.

Ponce *et al.,* 2008 investigated the contribution of *TaqI* A in *ANKK1* and C957T in *DRD2* polymorphisms in alcohol dependence patients. They also found a significant association when both risk genotypes were present (A1+ and CC) at $p < 0.001$. This data may reflect a relationship between D2 receptor and kinase ANKK1 that this protein could effect on dopaminergic neurotransmission. Since *TaqI* A polymorphism has been associated with increased striatal activity of aromatic L-amino acid decarboxylase which is the final enzyme in dopamine biosynthesis. Thus, *ANKK1* and *DRD2* could be related or somehow interact in dopamine pathways that affecting to addiction and antisocial traits in alcohol-dependent patients.

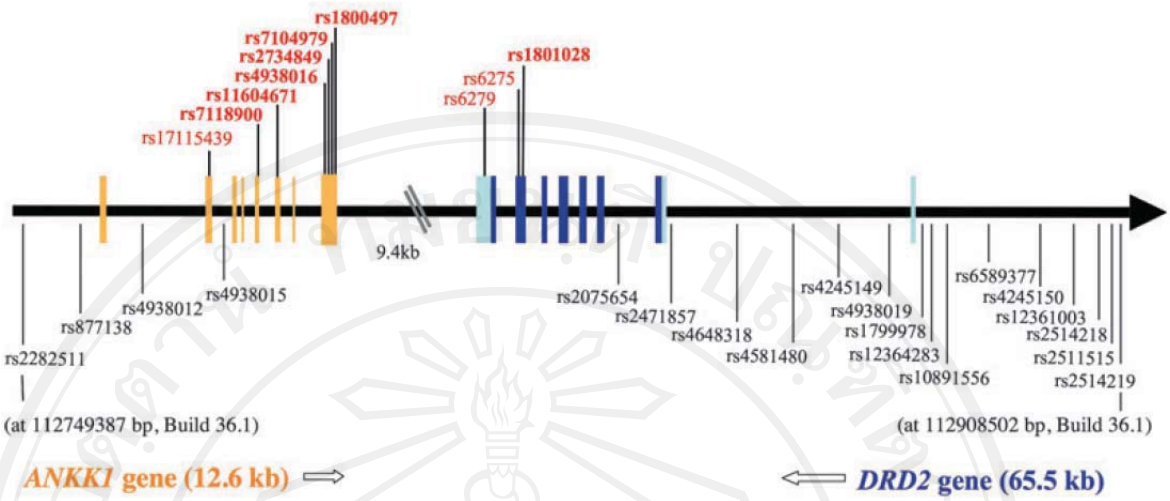

Figure 2 Location of the SNPs across the region of *DRD2* and *ANKK1* gene (Dick *et al.,* 2007)

Although there were many studies found the association of *DRD*2 *Taq*I polymorphism with alcohol dependence, some negative association were revealed. The A1 allele can differ between ethnic populations. The family-base and case control studies were performed to abstain difficulties with population some showing negative results. Lawford *et al.,* 1997 found that there was no significant difference of A1 allele frequency among alcoholic patients from detoxification unit compared to 4 controls groups ($p > 0.088$). Samochowiec *et al.,* 2006 conducted the family-base and case-control study of *DRD*2*, DAT, 5HTT, COMT* genes on 100 Polish families also revealed that the genotype distribution of *DRD*2 between alcoholics and controls was not significantly different.

The study of Lu *et al.,* 1996 in three distinct Taiwanese subjects (Chinese Han, Ayatal and Ami) also found no significantly difference of both allele and genotype of *Taq*I A polymorphism between alcohol dependence group and non alcoholic in all

population. Chen *et al.,* 1997 compared the allelic frequency of *DRD*2 *Taq*I A and *Nco*I N1 among severe dependence with ethnically matched in 4 aboriginal groups (Atayal, Ami, Bunun and Paiwan) and Han (Chinese) in Taiwan. They found that the higher prevalence of *DRD*2 *Taq*I A and *Nco*I N1 alleles was not associated with severity of dependence. No significant difference was found in genotype frequencies, allelic frequencies and prevalence of *Taq*I A allele.

Gelernter *et al.,* 1991 compared the allele frequencies in alcoholics and controls. They did not found an excess of A1 allele in alcoholic samples. Even though they have divided the patients into subgroups (using family history, presence of diagnosis of antisocial personality, age of onset under or over 25 years old, standard drinks consumption, presence of physical withdrawal symptoms), none of the subgroups showed significant difference in allele frequencies.

1.6 Purposes of this study

1. To investigate the allele and genotype at *Taq*I A (rs 1800497; C/T) polymorphism on dopamine D2 receptor gene in alcohol dependence groups (severe and moderate dependence) and control groups.

2. To compare the percentage of genotype and allele, allele frequency and proportion of T/C allele between alcohol dependence and control groups.

3. To find the relationship of *Taq*I A (rs 1800497; C/T) polymorphism with the susceptibility and severity of dependence in alcoholic patients.