

*Full Length Research Paper*

# **An association study between polymorphism of alcohol dehydrogenase (ADH1B), aldehyde dehydrogenase (ALDH2), cytochrome (CYP4502E1), Catechol-O-Methyltransferase (COMT) and 5-hydroxytryptamine transporter (5-HTT) genes in Yunnan Han population with alcohol dependence**

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Alcohol dependence (AD) is a complex disease resulting from the inheritance of several susceptible genes and multiple environmental determinants. The aim of this study was to identify the genetic risk factors which include alcohol metabolizing genes and neurotransmitter related genes for alcoholism in Yunnan Han population. Eight allelic variants of five genes were genotyped from 332 Yunnan Han individuals (including 118 alcohol-dependent patients (DSM-IV criteria) and 214 controls) using PCR-RFLP method. Those polymorphic sites included alcohol dehydrogenase (ADH1B), aldehyde dehydrogenase (ALDH2), cytochrome P-4502E1 (CYP2E1) PstI, Catechol-O-Methyltransferase (COMT) rs2075507 (5' region), rs737865 (intron1), rs4680 (Val158Met), rs165599 (3' region) and serotonin transporter (5-HTTLPR). Both genotype and allele frequencies of ALDH2 and CYP4502E1 as well as the allele frequency of ADH1B gene differed significantly between AD group and control group. The proportion of ALDH2 \*1/\*2 genotype and \*2 allele was significantly smaller in patients than that in controls ( $\chi^2 = 6.554$ ,  $p = 0.038$ ;  $\chi^2 = 4.906$ ,  $p = 0.027$ ), while the proportion of c2 allele of CYP4502E1 was significantly higher ( $\chi^2 = 4.410$ ,  $p = 0.036$ ). Compared with the controls, the frequencies of the 5-HTTLPR L/L genotype and COMT rs737865 C/C genotype were significantly lower in AD group. Twelve COMT haplotypes (rs2075507, rs737865, rs4680 and rs165599) defined as H1 to H12 were obtained in this major minority population. The prevalence of the haplotype H1 "A-C-A-A" was significantly greater in alcoholics than the prevalence in their respective control group. There were no significant differences in the genotype frequencies of the COMT rs2075507, rs4680 and rs165599 polymorphisms between alcoholics and controls. COMT rs2075507 and rs737865 polymorphisms were in complete linkage disequilibrium in Han population of Yunnan Province. This study indicates that polymorphisms of ADH1B, ALDH2, CYP4502E1, COMT and 5-HTT were significantly associated with AD in Han majority. The ADH1B \*2, ALDH2 \*2 alleles, 5-HTTLPR L/L genotype and C/C genotype of the COMT rs737865 polymorphism had an important role in reducing the risk of AD while the c2 allele of CYP4502E1 increased the risk of AD. Therefore, the A-C-A-A haplotype may be a dangerous factor leading to AD.

**Key words:** Yunnan Han population, polymorphism, alcohol dependence, genetic risk factor.

## **INTRODUCTION**

Alcohol dependence is a complicated disease which is affected by multiple factors. Among factors that may lead

to alcohol addiction, genetic factors play an important role since more than 50% is due to genetic factor (Clarke et al., 2008). Previous genetic studies of alcoholism suggest the existence of inherited functional variant genes including alcohol metabolizing genes that are the best understood factors that influence drinking behavior and they are risk factor for alcoholism. The major genes for alcohol metabolism are alcohol dehydrogenase (ADH) on chromosome 4 and aldehyde dehydrogenase (ALDH) on chromosome 12. These genes exhibit functional polymorphisms and are responsible for the oxidation of ethanol into acetaldehyde and acetic acid in liver. There are reports that ADH1B \*1/\*1 (low-activity ADH) is more frequently found in alcoholics and the number of individuals who have ADH1B \*1 genotype is relatively larger in alcohol addicted group compared to control group (Hines, 2004; Itoga et al., 2004). Several reports have indicated that ADH2\*2 and ALDH2\*2 are considered to protect against alcoholism (Thomasson et al., 1991; Maezawa et al., 1995). Nevertheless, an association has not been observed among Caucasian populations (Gilder et al., 1993). CYP4502E1 is also responsible for the metabolism of ethanol (He et al., 2008; Guo et al., 2005). It metabolizes ethanol fiercely in its non-ADH oxidative pathway. It has been shown that CYP2E1 is less active in c2 allele comparing with c1 allele (Tan et al., 2001; Le Marchand et al., 1999) however, there are also conflicting reports.

Association research proves that c2 allele is a risk factor for Japanese and Mexican Americans getting alcohol dependent (Tan et al., 2001). However, no association was found in other studies (Carr et al., 1995; Meng et al., 2003). Thus, the role of alcohol metabolism genes in the development of alcoholism in different populations needs further investigations. The strong heritability of alcoholism also suggests the existence of variants of neurotransmitter genes that alter the neurobiology of reward, executive cognitive function, anxiety/dysphoria, and neuronal plasticity such as dopamine, serotonin, glutamate and opioid systems. These neurotransmitter systems which are involved in the different components of alcohol dependence are potential targets for the development of therapeutic drugs for the treatment of alcoholism. Catechol-O-Methyltransferase (COMT) which has a functional genetic polymorphism plays an important role in dopamine metabolism (Chen et al., 2004; Männistö et al., 1999). Due to a rs4680 Valine158Methionine (Val158Met) single nucleotide

polymorphism (SNP), methionine is substituted with valine at 158 site of COMT, resulting in a low-activity COMT (Met/Met homozygotes) and a high-activity COMT (Val/Val homozygotes) and the enzymatic activity of these two types differs by three to four-folds (Sylvänen et al., 1997; Palmatier et al., 1999). Several studies reported an association between the low-activity COMT allele and alcoholism (Tiihonen et al., 1999; Wang et al., 2001). However, these findings were not confirmed in other studies (Ishiguro et al., 1999; Hallikainen et al., 2000; Kweon et al., 2005; Foroud et al., 2007). Other SNPs were found to alter transcription efficiency for the COMT gene resulting in different COMT expression regulation (Brockmoller et al., 1998; Kan et al., 1999). The serotonin system has been thought to play an important role in the impulsivity and craving often seen in alcoholics and is at least partly responsible for alcohol dependence. A number of studies have reported that alcohol dependence is associated with dysfunction of 5-hydroxytryptamine transporter (5-HTT) (Storvik et al., 2007, 2008).

5-HTT gene-linked promoter region, 5-HTTLPR is a polymorphism in the promoter region of the 5-HTT gene which is a functional insertion/deletion variant with two alleles designated as 'long' (L) and 'short' (S). Both Sander et al. (1997) and Lichtermann et al. (2000) reported an association between the S allele and alcoholism as well as two meta-analysis (Gorwood et al., 2004; Feinn et al., 2005) however, Kweon et al. (2005) found an association between the L allele and alcoholism.

Many others had reported negative results (Gelernter et al., 1997; Saiz et al., 2009; Wojnar et al., 2009). This study was focused on polymorphisms of ethanol metabolizing genes and neurotransmitter related genes in 118 alcoholic patients within Yunnan region matched non-alcoholics. We wanted to determine the association between alcohol addiction and ethanol metabolizing genes and neurotransmitter related genes.

## MATERIALS AND METHODS

### Study population

A total of 332 DNA samples from unrelated Chinese Han population in Yunnan region were obtained (214 controls and 118 alcoholics). Participants were recruited from alcohol addiction department in Yunnan Mental Health Centre during 2006~2008. The inclusion criteria were: 1) average age =  $38.9 \pm 10.6$ , male% = 96.25%; 2) the "diagnostic and statistical manual of mental disorder", fourth edition (DSM-IV) criteria. The exclusion criteria were: 1) no current or past diagnosis of mental illnesses such as schizophrenia, schizophreniform disorder, schizoaffective disorder, schizotypal disorder, major depression or bipolar disorder; 2) no current or past history of abuse of other substances (except tobacco). The non-alcoholics were selected from physical examination centre of the first affiliated Hospital of Kunming Medical College (average age =  $32.7 \pm 9.6$ , male% = 95.0%). The inclusion criteria for control participants were exactly the same as alcoholics except for alcohol intake.

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**Abbreviations:** AD, Alcohol dependence; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; COMT, Catechol-O-Methyltransferase; cyt, cytochrome; HTT, hydroxytryptamine transporter.

### Determination of genotype

Informed consent was obtained and the procedures followed were in accordance with the ethical standards. Peripheral blood samples were collected from 214 controls and 118 alcoholics and were kept at -40°C with EDTA anticoagulant until DNA extraction. The frozen blood was thawed and leukocyte DNA was isolated by classical phenol-chloroform method. 30  $\mu$ l TE mucolytic DNA are kept at -20°C. Under its detection  $A_{260}/A_{280}$ , the purity of DNA fits the requirement of PCR amplification.

### Alcohol metabolizing genes

The ADH1B, ALDH2 and CYP4502E1 genotypes were determined by polymerase chain reaction (PCR) followed by analysis of restriction fragment length polymorphisms (RFLP). Primers 5'-AAT CTT TTC TGA ATC TGA ACA G-3' and 5'-GAA GGG GGG TCA CCA GGT TG-3' (Konishi et al., 2004) were used for ADH1B. Primers 5'-CCA GTC GAG TCT ACA TTG TCA-3' and 5'-TTC ATT CTG TCT TCT AAC TGG-3' were used for CYP4502E1. The PCR reactions were performed with 7 to 10 ng genomic DNA, 10 pmol of each primer, 1 unit of TagDNA Pol, 2  $\mu$ L buffer for TagDNA Pol (10  $\times$  with Mg<sup>2+</sup>), 40  $\mu$ mol dNTP mix, 18.7  $\mu$ L pH 8.2 distilled water in total volume of 20  $\mu$ L. The PCR program of ADH1B and CYP4502E1 consisted of 35 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 1 min with a final extension period of 72°C for 5 min. The PCR product was digested with MaeIII and the presence of the ADH1B \*2 allele yielded two fragments of 95 and 60 bp while the \*1 allele produced a single uncut fragment. After Pst I restriction digestion, an uncut fragment of 410 bp of CYP4502E1 was observed when there was a c1 allele while a c2 allele led to two fragments of length 290 and 120 bp. Primers 5'-GTT TGG AGC CCA GTA ACC CTT-3' and 5'-CCC ACA CTC ACA GTT TTT AAT T-3' were used for ALDH2.

The PCR reactions were performed as same as ADH1B. The PCR program consisted of 35 cycles of 94°C for 30 s, 55.5°C for 30 s and 72°C for 30 s with a final extension period of 72°C for 5 min. The PCR products were digested with EcoR I and yield fragments of 90 and 18 bp in the presence of the ALDH \*1 allele or the single uncut 108 bp fragment in the presence of the \*2 allele.

### Neurotransmitter related genes

The COMT rs4680, rs2075507, rs737865 and rs165599 genotypes were performed by PCR-RFLP. Primers 5'- GGA TGA TGG ATT TCG CTC GC -3' and 5'- CTG GTG GGT AGG ACA AAG TGC -3' were used for detecting the rs4680 genotype, primers 5'-TTA TTG CTT ACT GGG AGG ACC-3' and 5'-CAG AAT GAC GGA TGT GAG GG -3' were used for the rs2075507 SNP, primers 5'- CCC TGC TAA CAG ACC TGC TTT-3' and 5'- CCC TTC CCA CTC CCT CAC TC -3' were for rs737865, and primers 5'- GCG ACA GTG CTG CTG AGG T -3' and 5'- AAC TAC AGG GAT GCG GGA G -3' were used for detection of the rs165599 SNP. The PCR program of rs4680 SNP consisted of 35 cycles of 94°C for 30 s, 59.5°C for 30 s and 72°C for 30 s with a final extension period of 72°C for 5 min. The annealing temperatures for the PCR of three SNPs (rs2075507, rs737865 and rs165599) were 60.5°C. The sizes of the PCR products were 117, 245, 254 and 275 bp for rs4680, rs2075507, rs737865 and rs165599 respectively. The genotyping was carried out after RFLP analysis using Bsh1236I (for rs4680), AluI (rs2075507), BseLI (rs737865) and MspI (rs165599) enzymes. The sizes of the RFLP products were as follows, rs4680: G allele (98 and 19 bp), A allele (117 bp); rs2075507: A allele (245 bp), G allele (197 and 48 bp); rs737865: T allele (254 bp), C allele (213 and 41 bp); rs165599: A allele (275 bp), G allele (202 and 73 bp).

The 5-HTTLPR genotyping was carried out as described previously (Thompson et al., 2000; Gokturk et al., 2008).

By using primers 5'-GGC GTT GCC GCT CTG AAT GC-3' and 5'-GAG GGA CTG AGC TGG ACA ACC -3', the 484 bp fragment was designated as S allele and the 528 and 572bp fragment as L allele.

### Statistical analysis

Statistical analysis was performed using the program SPSS version 11.5. Using the  $\chi^2$  test, we tested the Hardy-Weinberg equilibrium for 3 alleles in alcoholics and controls with the HWSIM program. Allele and genotype frequencies were also compared with  $\chi^2$  tests. Levels of significance for all statistical analyses were set to  $P < 0.05$ .

## RESULTS

All the twenty-four genotype frequencies (eight loci each in alcohol dependence, control groups and total groups) fit Hardy-Weinberg expectations according to  $\chi^2$  tests using a HWSIM program in alcoholics and controls ( $p > 0.05$ ) except that two Hardy-Weinberg tests had  $p$  values smaller than 0.05 ( $p < 0.05$ ) in alcoholics for 5-HTTLPR and  $p < 0.05$  in alcoholics for COMT rs737865). Therefore, the genotype distributions of all selected polymorphisms were in agreement with the Hardy-Weinberg equilibrium and our population was derived from random mating. When alcoholic and control groups were compared, genotypes distribution which contain ADH1B \*1/\*1, ADH1B \*1/\*2, ADH1B \*2/\*2 line in ADH1B locus did not differ significantly ( $P > 0.05$ ). There were still some differences in ADH1B\*1 and ADH1B\*2 alleles ( $P < 0.05$ ). Allele frequency of ADH1B \*2 was higher in the control group. Neither of genotypes in ALDH2 locus nor allele frequencies showed significant difference ( $P > 0.05$ ). In the control group, ALDH2 \*1/\*2 genotype and ALDH2 \*2 allele frequency was higher than in alcoholic group (Table 1). Both genotype and allele frequencies of CYP4502E1 gene differed significantly between AD group and the control group. The proportion of CYP2E1 c2/c2 genotype was significantly higher in patients than in controls ( $P < 0.05$ ) while the proportion of c2 allele of CYP2E1 gene was significantly higher in patients than in the controls ( $P < 0.05$ ) (Table 2). These results suggest that there was an association between polymorphism and alcohol dependence.

The ADH1B \*2 and ALDH2 \*2 alleles significantly reduced the risk of AD while the c2 allele of CYP4502E1 significantly increased the risk of AD. The genotype frequencies of COMT gene rs737865 polymorphism differed significantly between alcohol dependence group and control group ( $P < 0.05$ ). Compared with the controls, the frequencies of the rs737865 C/C genotype in COMT were significantly lower in alcoholics. Logistic regression analysis showed that C/C genotype was associated with alcohol dependence (OR: 0.822,  $P > 0.05$ ) but there was no statistical significance. There were no significant differences in the genotype frequencies of the COMT rs2075507, rs4680 and rs165599 polymorphisms

**Table 1.** Genotyping of alcohol metabolizing genes (ADH1B, ALDH2 and CYP4502E1) and neurotransmitter related genes (COMT, 5-HTT) in Han Chinese alcoholic (case group) and control subjects.

Parameter	n	Genotype (%)			Allele (%)	
		*1/*1	*1/*2	*2/*2	*1	*2
ADH1B						
Case group	80	29 (36.25)	37 (46.25)	14 (17.50)	95 (59.38)	65 (40.62)
Control group	100	22 (22.00)	50 (50.00)	28 (28.00)	94 (47.00)	<b>106 (53.00)<sup>a</sup></b>
ALDH2						
Case group	80	66 (82.50)	13 (16.25)	1 (1.25)	145 (90.63)	15 (9.37)
Control group	100	66 (66.00)	33 (33.00)	<b>1 (1.00)<sup>b</sup></b>	165 (82.50)	<b>35 (17.50)<sup>c</sup></b>
CYP4502E1Rsa I						
Case group	80	56 (70.00)	24 (30.00)	0 (0.00)	136 (68.00)	24 (12.00)
Control group	100	84 (84.00)	16 (16.00)	<b>0 (0.00)<sup>d</sup></b>	184 (92.00)	<b>16 (8.00)<sup>e</sup></b>
COMT rs2075507						
Case group	107	7 (6.54)	46 (42.99)	54 (50.47)	60 (28.04)	154 (71.96)
Control group	214	21 (9.81)	84 (39.25)	109 (50.94)	126 (29.44)	302 (70.56)
COMT rs737865						
Case group	107	45 (42.06)	58 (54.21)	4 (3.73)	148 (69.16)	66 (30.84)
Control group	214	120 (56.08)	73 (34.11)	<b>21 (9.81)<sup>f</sup></b>	313 (73.13)	115 (26.87)
COMT rs4680						
Case group	107	61 (57.01)	40 (37.38)	6 (5.61)	162 (75.71)	52 (24.29)
Control group	214	106 (49.53)	96 (44.86)	12 (5.61)	308 (72.00)	120 (28.00)
COMT rs165599						
Case group	107	23 (21.50)	52 (48.60)	32 (29.90)	98 (45.79)	116 (54.21)
Control group	214	34 (15.89)	112 (52.34)	68 (31.77)	180 (42.06)	248 (57.94)
5-HTTLPR						
Case group	118	83 (70.34)	26 (22.03)	9 (7.63)	192 (81.4)	44 (18.6)
Control group	214	124 (57.95)	77 (35.98)	<b>13 (6.07)<sup>g</sup></b>	325 (75.9)	103 (24.1)

The significant differences of genotypes and alleles frequencies are shown by bold font and the significant  $p$  and  $\chi^2$  values as follow: a  $\chi^2 = 5.459$ ,  $p = 0.019$ ,  $df = 1$ ; b  $\chi^2 = 6.554$ ,  $p = 0.038$ ,  $df = 2$ ; c  $\chi^2 = 4.906$ ,  $p = 0.027$ ,  $df = 1$ ; d  $\chi^2 = 5.040$ ,  $p = 0.025$ ,  $df = 2$ ; e  $\chi^2 = 4.410$ ,  $p = 0.036$ ,  $df = 1$ ; f  $\chi^2 = 13.165$ ,  $p = 0.001$ ,  $df = 2$ ; g  $\chi^2 = 6.920$ ,  $p = 0.031$  and  $df = 2$ .

between alcoholics and controls ( $P > 0.05$ ) (Table 1). Table 2 shows the analysis of haplotypes for rs2075507, rs737865, rs4680 and rs165599 in Yunnan Han population.

Twelve COMT haplotypes defined as H1 to H12 were obtained in this major minority population (frequency of each haplotype was greater than 1% at least in one group). The prevalence of the haplotype H1 "A-C-A-A" was significantly higher in alcoholics than in their respective control groups which might be a risk factor leading to alcohol dependence syndrome (OR: 2.865,  $p = 0.003347$ ). COMT rs2075507 and rs737865 polymorphisms were in complete linkage disequilibrium in our population ( $D > 0.8$ ) (Figure 1). Table 1 indicates that the frequency of 5-HTTLPR L/L genotype in the control group was higher than in alcohol dependent group.

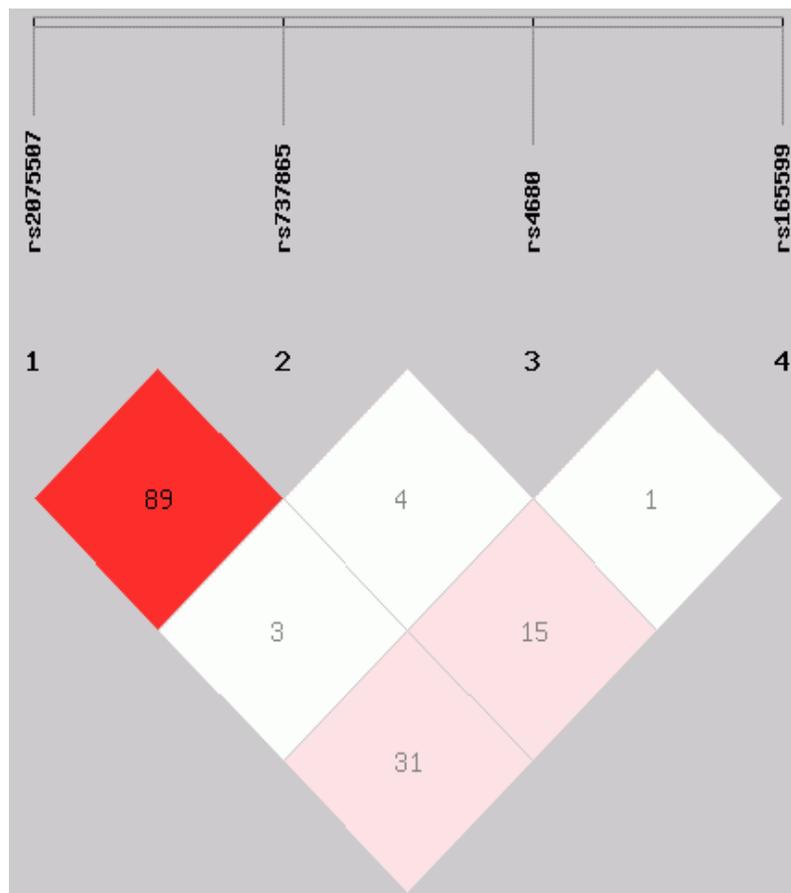
Logistic regression analysis showed that 5-HTTLPR L allele had an association with alcohol dependent patients which suggested that it might be a factor responsible for decreasing susceptibility for alcohol dependence in Chinese population (OR = 0.581,  $p = 0.026$ ).

## DISCUSSION

Our data suggest that in comparison between alcoholic and control groups, there was a diversity in the distribution of ADH1B \*1 and ADH1B \*2 alleles. ADH1B \*2 allele frequency was higher in control group and this result is not quite accordant with other findings. However, it was similar to the high frequency procession in Asians. This finding supports the hypothesis that ADH1B\*2 allele is a considerable influential factor in alcohol addiction. It has been reported to have a protective effect in the etiology of alcohol dependence (Fan et al., 1998; Shen et al., 1997). ALDH2 \*2 allele frequency is 16% in Koreans (Lee et al., 1997) and 27% in Japanese (Maezawa et al., 1995) subjects. Furthermore, 50% are low-activity genotypes ALDH2 \*1/\*2 and ALDH2 \*2/\*2 and it was very low in Caucasians (Gilder et al., 1993). The ALDH2 \*2 allele frequency was 25% in population in Shanghai (Muramatsu et al., 1995). In the Taiwanese subjects, ALDH2\*2 allele frequency was 30% in healthy groups (Chao et al., 1993); in the study of Han majority in Wuhan

**Table 2.** Estimated four-locus haplotype frequencies of COMT gene in Han Chinese.

Order	Haplotype	Case group (%)	Control group (%)	$\chi^2$	P	OR	Confidence interval (95%)
H1	A-C-A-A	18.02 (0.084)	13.16 (0.031)	8.614	0.003347	2.865	1.379~5.948
H2	A-C-A-G	0.01 (0.000)	15.25 (0.036)	6.955	0.008377	0.001	0.000~0.017
H3	A-C-G-A	31.51 (0.147)	52.22 (0.122)	0.708	0.399964	1.227	0.762~1.975
H4	A-C-G-G	16.45 (0.077)	29.54 (0.069)	0.105	0.745552	1.109	0.593~2.076
H5	A-T-A-A	2.50 (0.012)	21.77 (0.051)	6.133	0.013288	0.218	0.058~0.815
H6	A-T-A-G	11.71 (0.055)	34.58 (0.081)	1.536	0.215211	0.651	0.328~1.290
H7	A-T-G-A	25.81 (0.121)	67.51 (0.158)	1.721	0.189512	0.723	0.444~1.176
H8	A-T-G-G	47.98 (0.224)	67.96 (0.159)	3.867	0.049273	1.511	1.000~2.283
H9	G-T-A-A	11.34 (0.053)	27.10 (0.063)	0.304	0.581289	0.818	0.401~1.670
H10	G-T-A-G	8.41 (0.039)	6.44 (0.015)	3.623	0.056987	2.648	0.936~7.492
H11	G-T-G-A	26.80 (0.125)	61.87 (0.145)	0.522	0.469933	0.836	0.514~1.360
H12	G-T-G-G	13.44 (0.063)	25.77 (0.060)	0.009	0.924868	1.033	0.523~2.042

**Figure 1.** Linkage disequilibrium analysis of COMT gene in Han Chinese.

city of China, ALDH2 \*2 allele frequency was found to be 12% (Luo et al., 2001). Data collected in this research suggest that possession of ALDH2 \*1/\*2 and ALDH2 \*2/\*2 was 34% in the control group. ALDH2 \*2 allele frequency was 17.5% higher than in alcohol addicted

group. Results of this study showed that c1/c2 frequency and c2 allele frequency rose in alcohol addicted group. These findings indicate that genotype c1/c2 is associated with alcohol dependent syndrome and c2 allele also has effect on alcohol dependence. Our results support Tan et

al. (2001) point of view on the other side. COMT gene has been reported to have biologically functional SNP (Val158Met: rs4680). Yunnan Han population have very low frequency of rs4680 A allele with low activity COMT (Table 1) and this frequency is similar to the findings obtained in oriental populations.

Japanese: 29%; Chinese in other region: 27% (Zhao et al., 2000; Kunugi et al., 1997), whereas the A allele frequency is 51 to 53% in the Caucasian people (Daniels et al., 1996; Hoda et al., 1996). However, our data show that no association exists between rs4680 and alcoholism. These results are consistent with previous findings (Ishiguro et al., 1999; Hallikainen et al., 2000; Kweon et al., 2005; Foroud et al., 2007). The lack of association may be due to biased samples such as small sample sizes especially samples taken for AD. Rare reports about rs2075507 and rs165599 have been found, although they may play a role in COMT expression regulation (Brockmoller et al., 1998; Kan et al., 1999). This study found no difference in the frequency of two SNPs between the alcohol-dependent group and control group in Yunnan Han Chinese.

The rs737865 SNP is located in intron 1 of the COMT gene and a number of studies investigated this SNP related to expression regulation. Table 1 shows that the frequencies of rs737865 C/C genotype were significantly lower in alcoholics compared with the controls. These data suggest that rs737865 was associated with alcohol dependence and there are biologically functional SNPs in the intron1.

Haplotype analysis is a powerful and increasingly popular tool for identifying candidate genes for complex disorders as more information can be obtained from haplotype analysis than from single-locus analysis. With single SNP analysis, only the rs73765 has been found to be associated with alcoholism in Han Chinese. However, the COMT haplotypes showed significant difference between the case and control groups. The prevalence of the haplotype H1 "A-C-A-A" was significantly higher in alcoholics than in their respective control groups. We might speculate that genes are associated with each other, resulting in complex interactions between the genetic risk factors and development of alcoholism. We also studied the association between 5-HTTLPR and alcohol dependence and found significant differences in the 5HTTLPR genotypes suggesting that LL genotype may be a factor responsible for decreasing susceptibility for alcohol dependence in Chinese population. We might speculate that individuals who carry the L allele with high activity 5-HTT regulate and maintain the 5-HT level in the synapses leading to reducing drinking behavior and the risk of alcoholism. Etiology of alcohol dependence is complex and is incompletely associated with chain effects of gene factors. Alcohol metabolic genes (alcohol dehydrogenase, acetaldehyde dehydrogenase) and DRD2 (dopamine D2 receptor, DRD2) gene are associated with each other (Huang et al., 2004). Single factor research is limited and this kind of research will

presumably lead to more contradictory results since it can not disclose precisely the truth.

Systematical research of alcohol metabolic genes and joint pharmacodynamic genes such as dopamine should be done with a careful analysis. Mutual interaction of each gene should also be carefully considered. Moreover, geographical, environmental and ethnical factors should also be taken into account in future research.

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