Monoamine oxidase-A polymorphisms might modify the association between the *dopamine D_2 receptor* gene and alcohol dependence

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Objective: Low monoamine oxidase (MAO) activity and the neurotransmitter dopamine are 2 important factors in the development of alcohol dependence. MAO is an important enzyme associated with the metabolism of biogenic amines. Therefore, the present study investigates whether the association between the dopamine *D2 receptor* (*DRD2*) gene and alcoholism is affected by different polymorphisms of the MAO type A (*MAOA*) gene. **Methods:** A total of 427 Han Chinese men in Taiwan (201 control subjects and 226 with alcoholism) were recruited for the study. Of the subjects with alcoholism, 108 had pure alcohol dependence (ALC) and 118 had both alcohol dependence and anxiety, depression or both (ANX/DEP ALC). All subjects were assessed with the Chinese Version of the Modified Schedule of Affective Disorders and Schizophrenia-Lifetime. Alcohol dependence, anxiety and major depressive disorders were diagnosed according to *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition criteria. **Conclusion:** The genetic variant of the *DRD2* gene was only associated with the ANX/DEP ALC phenotype, and the genetic variant of the *MAOA* gene was associated with pure ALC. Subjects carrying the *MAOA* 3-repeat allele and genotype *A1/A1* of the *DRD2* were 3.48 times (95% confidence interval = 1.47–8.25) more likely to be ANX/DEP ALC than the subjects carrying the *MAOA* 3-repeat allele and *DRD2 A2/A2* genotype. The *MAOA* gene may modify the association between the *DRD2* gene and ANX/DEP ALC phenotype.

Objectif : La faible activité de la monoamine oxydase (MAO) et la dopamine neurotransmettrice sont deux facteurs importants de l'apparition de l'accoutumance à l'alcool. La MAO est une enzyme importante associée au métabolisme des amines biogéniques. La présente étude vise donc à déterminer si des polymorphismes différents du gène de la MAO type A (MAOA) ont un effet sur le lien entre le gène du *récepteur D2 de la dopamine* (*RDD2*) et l'alcoolisme. **Méthodes :** On a recruté au total, pour l'étude, 427 hommes chinois Han à Taiwan (201 sujets témoins et 226 atteints d'alcoolisme). Parmi les sujets alcooliques, 108 avaient une dépendance pure à l'alcool (ALC) et 118 avaient à la fois une dépendance à l'alcool et de l'anxiété, de la dépression, ou les deux (ANX/DEP ALC). On a évalué tous les sujets au moyen de la version chinoise du Guide modifié pour le diagnostic des troubles affectifs et de la schizophrénie. On a diagnostiqué la dépendance à l'alcool, l'anxiété et les troubles dépressifs majeurs selon les critères du *Manuel diagnostique et statistique des troubles mentaux*, quatrième édition. **Conclusion :** On a établi un lien entre la variante génétique du gène du *RDD2* et le phénotype ANX/DEP ALC seulement et entre la variante génétique du gène *MAOA* et la dépendance pure à l'alcool. Les sujets porteurs de l'allèle triple *MAOA* 3 et du génotype *A1/A1* du gène du *RDD2* étaient 3,48 fois (intervalle de confiance à 95 % = 1,47–8,25) plus susceptibles d'avoir le trouble ANX/DEP ALC que les sujets porteurs de l'allèle triple *MAOA* et du génotype *A2/A2* du *RDD2*. Le gène *MAOA* a peu modifié le lien entre le gène du *RDD2* et le phénotype ANX/DEP ALC.

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Introduction

Family, twin and adoption studies suggest that heredity plays an important role in alcohol dependence and drinking behaviour and, therefore, that there are genetic risk factors for alcoholism.[1–3] Alcohol dependence is a complex disorder that is probably regulated by several genes.[4] Although several candidate genes have been studied, the results of these studies are controversial[5–12]; the gene-to-gene interaction approach might be more revealing than the single-gene approach in the study of alcoholism.

Monoamine oxidase (MAO) is an important enzyme associated with the metabolism of biogenic amines and neurotransmitters, including dopamine as well as 5-hydroxytryptamine (5-HT) and norepinephrine.[13,14] Cloninger[15] proposed that the catecholamine neurotransmitters including dopamine, 5-HT and norepinephrine are related to some personality traits that might put an individual at increased risk for drinking behaviour and developing alcohol dependence. For example, low MAO activity might also be a risk factor for impulsive behaviour, personality disorder and alcoholism.[16–19] Therefore, MAO activity may play a critical role in the regulation of catecholamines and in the pathogenesis of psychiatric disorders.[13] A functional 30-base pair (bp) repeat polymorphism in the promoter region of the *MAOA* gene may alter transcriptional efficiency; the allele with 3 copies of the repeat sequence was transcribed about 2 times less efficiently than the allele with 4 copies of the repeat motif.[20,21] The *MAOA* gene is considered to be a candidate gene of alcohol dependence susceptibility, because alcohol dependence is sensitive to allelic variation in the *MAOA* gene.[5–7,22,23] Samochowiec and colleagues[7] and Schmidt and colleagues[23] reported that a low-activity 3-repeat allele of the *MAOA* promoter polymorphism is associated with antisocial alcoholism among German men, and Contini and colleagues[5] confirmed that the 3-repeat allele increased susceptibility to alcohol dependence and antisocial behaviours in a Brazilian sample. However, the existence of an association between the *MAOA* gene and alcoholism with or without antisocial behaviour is not consistently reported. Further, several studies have found no association between alcoholism and the *MAOA* gene.[8,24–26]

In animal studies, alcohol can stimulate dopaminergic neurons in the ventral tegmental area,[27,28] and the density of dopamine D_2 receptors in the limbic system is lower in alcohol-preferring rats than in nonpreferring rats.[29,30] Likewise, the number of striatal dopamine D_2 receptors is less in alcohol-preferring humans than in healthy control subjects.[31] Moreover, brain imaging studies of healthy volunteers have shown that individuals with an *A1* allele of the *DRD2* gene have a reduced number of dopamine D_2 receptors.[32,33] The *A1* polymorphism of the *DRD2 Taq*I A loci has been considered as a risk factor for alcohol dependence,[9,10,34,35] but the association between alcoholism and the *DRD2* gene remains equivocal.[11,12,34,36–38] The confounding effects of *MAOA* and *DRD2* genes on alcohol dependence might be partly due to different definitions of control groups, ethnically or racially mixed study populations and phenotypic heterogeneity of alcoholism.[39–41]

To overcome these possible confounding effects and to reduce the probability of type I and type II errors, we recruited 2 different subtypes of patients dependent on alcohol: a group with pure alcohol dependence and no other comorbid diagnosis (pure ALC) and a group with alcohol dependence and comorbid anxiety, depression or both (ANX/DEP ALC). Our intent was to reduce the phenotypic heterogeneity of the overall sample. We also recruited unrelated healthy control subjects to evaluate the association between *MAOA* and *DRD2* and alcohol dependence in the Han Chinese population of Taiwan.

We hypothesized that, if both the *MAOA* and *DRD2* genes are associated with alcohol dependence, this would be revealed by an association study comparing subjects with pure ALC to well-matched control subjects. However, alcoholism is usually comorbid with anxiety or depression or both, and the mood disturbance might increase drinking behaviour. Thus, we hypothesized that the *MAOA* and *DRD2* genes might increase susceptibility to ANX/DEP ALC. Dopamine is oxidatively deaminated by *MAOA* and, in a rat model, 90% of the metabolism is via deamination by *MAOA* in the corpus striatum to form 3,4-dihydroxyphenyl-acetaldehyde (DOPAL).[38,42] These observations led us to hypothesize that the *MAOA* and *DRD2* genes might interact to increase susceptibility to alcohol dependence and/or its subgroup. We therefore tested whether the relation between the *DRD2* gene and alcoholism is affected by different polymorphisms of the *MAOA* gene.

Methods

Subjects and clinical assessments

The protocol of this study was approved by the Institutional Review Board for the Protection of Human Subjects at Tri-Service General Hospital (TSGH), a medical teaching hospital affiliated with the National Defence Medical Center in Taipei, Taiwan. Written informed consent was obtained from all participants after a full explanation of the study procedures.

To minimize the effects of ethnic differences on gene frequencies, all 427 subjects were recruited from the Han Chinese population in Taiwan; all participants were unrelated and were matched for ethnicity and geographic origin. Alcohol dependence was diagnosed and classified into 2 groups: pure ALC (108 participants) and ANX/DEP ALC (118 participants). A total of 201 participants were healthy control subjects. Subjects with pure ALC had a past or current history of alcohol dependence but no history of other mental disorders, including personality, anxiety, depressive, or affective disorders or illegal drug use disorders. Those with ANX/DEP ALC had a past or current history of major depression or anxiety disorder or both, as well as a diagnosis of alcohol dependence, but no history of other mental disorders or illegal drug use disorders. The healthy control subjects had no past or present major or minor mental illnesses (including affective disorder, schizophrenia, anxiety disorder, personality disorder or substance use disorders) and no family history of alcohol dependence or heavy alcohol consumption in first-degree relatives.

Subjects with alcohol dependence were recruited from the psychiatric clinical population, and control volunteers were recruited from the community. Each subject was interviewed by an attending psychiatrist, who made an initial evaluation, and then by a well-trained research psychologist, who identified the clinical subtype of alcohol dependence and selected the control subjects. All diagnoses of alcohol dependence, anxiety, major depressive disorders and other mental disorders were made according to *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) criteria.[43] A well-trained research psychologist interviewed participants, using the Chinese Version of the Modified Schedule of Affective Disorders and Schizophrenia-LifeTime (SADS-L),[44,45] in order to meet a DSM-IV diagnosis and to exclude other mental disorders (antisocial personality disorder and drug use disorder) in control subjects and in individuals with pure ALC. The interrater reliability kappa values of the Chinese Version of SADS-L were good-to-excellent for major depression (0.79), bipolar disorder (0.71), anxiety disorder (0.86), schizophrenia (0.95), alcohol abuse and dependence (1.00), and substance abuse and dependence (0.82).[38]

Blood samples and DNA extraction

With the informed consent of each participant, 20 mL of whole blood was drawn from the peripheral vein with vacutainer tubes containing 15% (K_3) Ethylendiaminetetraacetic acid (EDTA) solution (Becton Dickinson vacutainer systems). Genomic DNA was extracted from the leukocytes using standard methods.

Genotyping of MAOA and DRD2 genes

The 30-bp repeat polymorphism of the *MAOA-uVNTR* gene (variable number of tandem repeats located upstream of the promoter region) was investigated with a modification of the polymerase chain reaction (PCR) method described by Zhu and colleagues.[46] The *Eco*RV polymorphisms in exon 14 of the *MAOA* gene were detected with the modified PCR-RFLP (restriction fragment length polymorphism) method described by Hotamisligil and Breakefield.[47] The *MAOA Eco*RV *(–)* polymorphism remained intact and was 703 bp long, whereas the *MAOA Eco*RV (+) polymorphism was cut into 2 DNA fragments of 340 bp and 363 bp by the *Eco*RV restriction enzymes.

*Taq*I "*A*" and *Taq*I "*B*" polymorphisms of the *DRD2* gene were genotyped with the PCR-RFLP method. Cycling protocols, which were modified from those described by Castiglione and colleagues[40] and Grandy and colleagues,[48] were carried out on a Perkin Elmer 9700 thermal cycler (Boston, MA). The 310-bp *A1* allele remained uncut, whereas the *A2* allele was cut into 2 DNA fragments of 130 bp and 180 bp. The *459-bp Taq*I *B1* allele remained intact, and the *Taq*I *B2* allele was cut into 2 DNA fragments of 267 bp and 192 bp.

Statistical analyses

The differences in the genotype and allele frequencies of the *MAOA* and *DRD2* genes between the pure ALC and ANX/DEP ALC groups and the control groups were calculated with Pearson's chi-square (2-tailed), and Hardy–Weiberg equilibrium was assessed for each group. Fisher's exact test was substituted for the chi-square test when sample cell sizes were smaller than expected (< 5 subjects). One-way analysis of variance and Bonferroni post hoc test were employed to determine the difference of mean age among these subtypes. The Bonferroni post hoc test, Pearson's chi-square, Fisher's exact test and multiple logistic regression analyses were performed with SPSS (version 11.5, Taipei, Taiwan) for Windows. A p value of less than 0.05 was considered statistically significant. The frequency of the 2-repeat polymorphism of the *MAOA* gene was found in 0 subjects in the ANX/DEP ALC group, 1 subject in the pure ALC group and 4 subjects in the control group. Therefore, we did not include subjects with 2-repeat polymorphism of *MAOA-uVNTR* for data analysis.

Differences in haplotype frequencies, linkage disequilibrium coefficients (D), and standardized linkage disequilibrium coefficients (D') between the *Taq*I *A* and *Taq*I *B* systems of the *DRD2* gene were estimated with the Estimating haplotypes and Permutation and Model Free Analysis computer programs.[49–51] Differences in haplotype frequency between study variables were estimated with Fisher's exact test when the cells were small. Moreover, the power analysis was performed with the use of G*Power computer software, and the effect size conventions were determined according to the method of Erdfelder and colleagues.[52]

Results

There were significant differences in mean age among these 3 study groups ($F = 13.661$; $p < 0.001$) and between the control and pure ALC groups (36.58 [standard deviation {SD} 9.57 yr v. 42.09 [SD 9.82] yr; $p < 0.001$) but not between the control and ANX/DEP ALC groups (36.58 [SD 9.57] yr v. 35.99 [SD10.51] yr; $p = 0.611$).

As shown in Table 1, the haplotype frequencies of *A1B2* and *A2B1* were less than 5%, and strong linkage disequilibrium between the *Taq*I *A* and *Taq*I *B* polymorphisms in the *DRD2* gene ($p < 0.001$) was evident in each of the 3 study groups. Genotype distributions of *Taq*I *A* and *Taq*I *B* polymorphism of the *DRD2* gene were in the Hardy–Weinberg equilibrium, both in the patients and in the control subjects ($p > 0.1$). There are significant differences in the haplotype frequencies of the *DRD2* gene among the 3 study groups ($p = 0.020$). The frequency of the *A1B1* haplotype was significantly higher in the ANX/DEP ALC group than in the control group ($p = 0.006$) but was not significantly different in the pure ALC group versus the normal control group (Table 1). There were no significant differences in the genotype frequency of *MAOA-uVNTR* (in the promoter region of the gene) and in *Eco*RV (in exon 14) polymorphisms among the 3 groups or the ANX/DEP ALC group versus the control group. However, the *MAOA* gene was significantly associated with pure ALC ($p = 0.030$ in *MAOA-uVNTR* and $p = 0.039$ in *MAOA Eco*RV, respectively; see Table 2).

After stratifying the *MAOA-uVNTR* 3-repeat and *MAOA-EchoRV(+)* genotypes, the only significant difference in *DRD2* haplotype was between the ANX/DEP ALC group and the control group. When the *MAOA-uVNTR* 4-repeat and *MAOA-EchoRV(–)* genotypes were stratified, respectively, there were no significant differences in the *DRD2* haplotype between healthy control subjects and each of the other groups, respectively (Table 3).

Logistic regression analysis of the *DRD2 Taq*I *A* polymorphism as a risk factor for alcohol dependence and correction for age showed that the *DRD2 A1/A1* and *A1/A2* genotypes were associated with higher risk for ANX/DEP ALC than the *DRD2 A2/A2* genotype (approximately 1.98–2.59-fold), but the *DRD2 A1/A1* genotype was not significantly associated with pure ALC (model 1, Table 4). After stratifying *MAOA* genotypes and correcting for age, the association of the *DRD2 A1/A1* and *A1/A2* genotypes with ANX/DEP ALC persisted only after stratification by *MAOA-uVNTR* 3-repeat (odds ratio [OR] = 3.48, $p = 0.005$ for *A1/A1* genotype; OR = 2.53, $p = 0.008$ for *A1/A2* genotype) and *MAOA-EchoRV(+)* genotypes (OR = 2.80, $p = 0.017$ for *EchoRV(+)* genotype), but the association had not been found under stratification of the *MAOA-uVNTR* 4-repeat and *MAOA-EchoRV(–)* genotypes, respectively (Table 4). These results led us to suggest that the *DRD2* gene plays an important role in the ANX/DEP ALC group and that the *MAOA* gene might modify the association between the *DRD2* gene and alcoholism.

The study power was around 0.41–0.56 to detect a small effect and 0.99 to detect a medium and large effect in the haplotype and/or genotype frequencies. With a power of 0.93, we detected an effect size of 0.15 for detecting a significance difference in haplotype distributions. After stratification and logistic regression analysis, this study had a power of 0.22–0.35 to detect a small effect, 0.98–0.99 to detect a medium effect and 0.99 to detect a large effect. In the present power analysis, effect size conventions were determined according to the method of Erdfelder and colleagues,[52] as follows: small effect size = 0.10, medium effect size = 0.30, large effect size = 0.50 ($\alpha = 0.05$).

Discussion

We found that the *DRD2* gene is associated with ANX/DEP ALC and that the frequency of the *A1/B1* haplotype is higher in subjects with alcohol dependence. These results are consistent with our previous studies in mixed-sex subjects,[38] but results differ from those of the small study of 20 subjects with alcoholism and mood disorder.[53] We hypothesized that, if the *DRD2* gene is associated with alcohol dependence, this would be revealed by an association study of subjects with pure ALC and well-matched control subjects, but these results do not support this association. The foregoing observations led us to suggest that the *DRD2* gene is associated with alcoholism only in patients with anxiety and depression among the Han Chinese population of Taiwan. Thus, it may be easier to detect an association between the *DRD2* gene and alcohol dependence in specific population subgroups.

The association of *MAOA Eco*RV polymorphism with alcoholism has been reported in the Han Chinese population[6] but remains controversial.[8,24] We found that the polymorphism of

Table 1: Haplotype frequency and linkage disequilibrium of the *DRD2 Taq*I *A* and *Taq*I *B* polymorphism in Han Chinese men with alcohol dependence and in control subjects

Groups/haplotypes	Sample size (2*n*)	Haplotype frequency, %				*p* value*	Linkage disequilibrium		*p* value†
		A1B1	*A1B2*	*A2B1*	*A2B2*		D	D´	
Control subjects	402	0.333	0.018	0.048	0.601	0.020‡	0.217	0.999	< 0.001
Pure ALC	216	0.356	0.005	0.033	0.606	0.486¶	0. 221	0.999	< 0.001
ANX/DEP ALC	236	0.470	0.009	0.030	0.491	0.006**	0.239	0.999	< 0.001

DRD2 = dopamine D_2 receptor; *Taq*I *A* = rs1800497; *Taq*I *B* = rs1079596; 2*n* = double strain; D = linkage disequilibrium coefficients; D´ = standard linkage disequilibrium coefficients; pure ALC = pure alcohol dependence; ANX/DEP ALC = alcohol dependence and anxiety or depression or both.
**p* value of Fisher s exact test.
†*p* value of linkage disequilibrium in each of the 3 study groups.
‡Control subjects v. pure ALC v. ANX/DEP ALC; χ^2 value = 14.388, *df* = 6.
¶Control subjects v. pure ALC.
**Control subjects v. ANX/DEP ALC.

Table 2: Genotype frequencies of *MAOA* polymorphism in Han Chinese men with alcoholism and control subjects

Groups/genotypes	Sample size, no.	*Promoter-VNTR*; no (and %)		χ^2	*p* value	Sample size, no.	*EcoRV*; no. (and %)		χ^2	*p* value
		3-*repeat*	4-*repeat*				+	–		
Control subjects	197	123 (62.4)	74 (37.6)	5.160	0.160*	201	125 (62.2)	76 (37.8)	5.174	0.159*
Pure ALC	107	53 (49.5)	54 (50.5)	4.736	0.030†	108	54 (50.0)	54 (50.8)	4.283	0.039†
ANX/DEP ALC	118	72 (61.0)	46 (39.0)	0.063	0.802‡	118	73 (61.9)	45 (38.1)	0.003	0.954‡

MAOA = monoamine oxidase-A; VNTR = variable number of tandem repeats; *Eco*RV = restriction enzyme rs1137070; pure ALC = pure alcohol dependence; ANX/DEP ALC = alcohol dependence and anxiety or depression or both.
*Control subjects v. pure ALC v. ANX/DEP ALC; *df* = 3.
†Control subjects v. pure ALC.
‡Control subjects v. ANX/DEP ALC.

promoter and *Eco*RV in the *MAOA* gene are associated with pure ALC but not with other subgroups of alcohol dependence. The significant association between the *MAOA* gene and pure ALC is consistent with previous studies on alcoholism[6,22] but contradicts certain other reports.[8,24,25] Moreover, our finding of no *MAOA* gene association with ANX/DEP

Table 3: *DRD2* haplotype frequencies of the *Taq*I *A* and *Taq*I *B* polymorphisms in the 4 groups with stratification of the *MAOA* genotypes

Groups/haplotypes	Sample size (2*n*)	Haplotype frequency (%)				χ^2	*df*	*p* value
		A1B1	*A1B2*	*A2B1*	*A2B2*			
***MAOA* 3-repeat:**								
Control subjects	246	0.308	0.017	0.041	0.634	13.184	6	0.028*
Pure ALC	106	0.358	0.010	0.057	0.575	1.689	3	0.669†
ANX/DEP ALC	144	0.486	0.007	0.028	0.479	12.006	3	0.005‡
***MAOA* 4-repeat:**								
Control subjects	148	0.378	0.014	0.062	0.546	6.839	6	0.301*
Pure ALC	108	0.361	0.005	0.009	0.625	5.088	3	0.148†
ANX/DEP ALC	92	0.445	0.011	0.033	0.511	1.739	3	0.661‡
***MAOA Eco*RV(+):**								
Control subjects	250	0.323	0.021	0.041	0.615	9.871	6	0.109*
Pure ALC	108	0.342	0.010	0.047	0.601	0.694	3	0.908†
ANX/DEP ALC	146	0.472	0.007	0.028	0.493	8.901	3	0.024‡
***MAOA Eco*RV(–):**								
Control subjects	152	0.348	0.014	0.060	0.578	7.309	6	0.246*
Pure ALC	108	0.370	0.000	0.019	0.611	3.590	3	0.282†
ANX/DEP ALC	90	0.466	0.012	0.034	0.488	3.636	3	0.285‡

DRD2 = dopamine D_2 receptor; *Taq*I *A* = rs1800497; *Taq*I *B* = rs1079596; *MAOA* = monoamine oxidase; 2*n* = double strain; pure ALC = pure alcohol dependence; ANX/DEP ALC = alcohol dependence and anxiety or depression or both; *Eco*RV = restriction enzyme rs1137070.
*Control subjects v. pure ALC v. ANX/DEP ALC.
†Control subjects v. pure ALC.
‡Control subjects v. ANX/DEP ALC.
§*p* value of Fisher's exact test.

Table 4: Multiple logistic regression analysis of the *Taq*I *A* polymorphisms of the *DRD2* gene for risk of alcohol dependence with stratification of the *MAOA* genotype

Groups/ variables	ANX/DEP ALC			Pure ALC		
	Odds ratio	95% CI	*p* value	Odds ratio	95% CI	*p* value
Model 1						
DRD2 A1/A1	2.59	1.34–5.02	0.005	1.02	0.50–2.10	0.951
DRD2 A1/A2	1.98	1.17–3.34	0.011	1.14	0.69–1.90	0.602
Model 2						
***MAOA* 3-repeat:**						
DRD2 A1/A1	3.48	1.47–8.25	0.005	1.70	0.64–4.51	0.283
DRD2 A1/A2	2.53	1.28–4.99	0.008	1.22	0.59–2.51	0.598
***MAOA* 4-repeat:**						
DRD2 A1/A1	1.58	0.55–4.48	0.394	0.56	0.18–1.79	0.328
DRD2 A1/A2	1.36	0.58–3.18	0.475	1.49	0.66–3.40	0.341
Model 3						
***MAOA Eco*RV(+):**						
DRD2 A1/A1	2.00	1.20–6.51	0.017	1.27	0.47–3.43	0.631
DRD2 A1/A2	1.94	0.99–3.77	0.051	1.05	0.52–2.14	0.889
***MAOA Eco*RV(-):**						
DRD2 A1/A1	2.29	0.78–6.67	0.129	0.77	0.25–2.39	0.660
DRD2 A1/A2	1.98	0.84–4.69	0.118	1.66	0.73–1.23	0.226

*Taq*I *A* = rs1800497; *DRD2* = dopamine D_2 receptor; *MAOA* = monoamine oxidase-A; ANX/DEP ALC = alcohol dependence and anxiety or depression or both; pure ALC = pure alcohol dependence; CI = confidence interval; *Eco*RV = restriction enzyme rs1137070.
Note: parameter coding of *DRD2 Taq*I *A* genotypes are *A1/A1 = 1, A1/A2 = 2, A2/A2 = 0;* reference group is *DRD2 A2/A2*.
Model 1: no stratification of MAO-A genotype, but controlling for age. Model 2: stratification of *MAO-A-uVNTR* and controlling for age. Model 3: stratification of *MAO-A-Eco*RV and controlling for age.

ALC is consistent with the some reports[8,24] but not others.[6,23]

There are several possible reasons for these contradictory results. First, definition of the "normal control" group varies between studies. Some studies use a "super-control" (that is, the healthy control subjects had no past or present major or minor mental illnesses, including affective disorder, schizophrenia, anxiety disorder, personality disorder or substance use disorders), while others do not.[10] In genetic association studies, use of suitable control subjects is very important.[10,54,55] Several studies have shown that the *MAOA* and *DRD2* genes are associated with several substance abuse or mood disorders.[56–59] Previous studies suggested that the prevalence of the *A1* allele is significantly higher in unscreened control subjects (not excluding people with alcoholism or nicotine addiction) than in assessed control subjects (with exclusion).[10] Using unscreened individuals as control subjects may unwittingly include an excess of patients with *A1* alleles and further attenuate the association between the *DRD2 A1* allele and alcohol dependence.[55,60] Thus, the control group should probably exclude subjects with substance use disorders, other major or minor mental disorders and/or a family history of mental disorders. In this study, all potential control subjects were screened by an attending psychiatrist and interviewed by a well-trained psychologist to reduce the confounding factors. If comorbid disorders were found, these participants were excluded. In the study by Lu and colleagues,[24] patient subtype of alcohol dependence was not determined, even though alcohol dependence is a complex phenotype with a heterogeneous etiology.[6,24] To establish a precise phenotype, Cloninger[15] proposed a neurobiological learning model that subdivided alcoholism into 2 subtypes. People with type I alcoholism (late-onset) often have a high incidence of comorbidity with mood disorders; they show high harm-avoidance and low novelty-seeking behaviours. People with type II alcoholism (early-onset) often have antisocial personality traits and show low harm-avoidance and high novelty-seeking. Cloninger's classification was not confirmed by subsequent studies.[61–64] The use of Cloninger's Tri-dimensional Personality Questionnaire (TPQ) lacks a cut-off point to distinguish the various subtypes of alcoholism, and the definition of personality traits may vary according to sociocultural differences.[65] In recent studies, the DSM-IV criteria have been considered more reliable for clinical use and have also been used in clinical research.[7,23,38] It is important to use a well-defined or quantitative phenotype in the association studies of candidate genes of complex disorders because it can lead to a dramatic increase in statistical power.[66] Thus, we suggest that using the SADS-L for initial assessment and the DSM-IV diagnosis to further subgroup patients might reveal novel associations between candidate genes and specific subtypes of alcohol dependence. Another potential confounding factor in prior studies is that there are racial and ethnic differences in gene frequency. The frequencies of *DRD2* and *MAOA* genes are known to vary among different racial or ethnic groups.[10,12,39–41] The *DRD2 Taq*I *A* polymorphism of the *A1* allele frequency in our control samples (35.1%) is similar to other Asian populations (35%–37%)[53,67] but is much higher than in Caucasian samples (11%–20%).[10] Finally, the *MAOA* promoter polymorphism, high-activity allele (4-repeat) has a prevalence of 40% in Asian populations[24,68] but in Caucasian populations is as high as 60%–70%.[7,23,25] These differences may be partially responsible for the divergent association results.

Several findings from our study suggest that *MAOA* genes modify the association between the *DRD2* gene and alcoholism. The *DRD2* gene was associated with ANX/DEP ALC before stratifying by *MAOA* genotype; after stratification, an association with the *MAOA* 3-repeat and *MAOA Eco*RV(+) genotypes was revealed, even though the *DRD2* gene was not associated with ANX/DEP ALC in those with *MAOA* 4-repeats and *Eco*RV(–) genotypes (Table 3). After stratifying *MAOA* genotypes and correcting for age, multiple logistic regression analysis showed that the risk of alcohol dependence differed in people with different *DRD2 Taq*I A genotypes. The risk for ANX/DEP ACL was much higher in subjects with *A1/A1* and *A1/A2* genotypes (2.53–3.48 times) than in those with the *A2/A2* genotype. That difference in risk was significant only after stratification into the *MAOA-uVNTR* 3-repeat genotype and into those with the *Eco*RV(+) *genotype* (Table 4). Thus, the *MAOA* genes appear to modulate the effect of the *DRD2* gene in the ANX/DEP ALC group but not in the pure ALC group. A possible reason for this result is that dopaminergic tone might be lower in people with alcoholism who have mood disorders than in control subjects and others with different subtypes of alcohol dependence. The lower reinforcement of the dopamine-related reward system might be compensated for by the low-enzyme activity genotype of the *MAOA* gene. Our results seem to favour a hypothesis that *MAOA* genes modulate the relation between the *DRD2* gene and ANX/DEP ALC, but additional studies are needed. Such studies should use large samples in different populations to determine whether *DRD2* and *MAOA* genes are jointly involved in the development of alcohol dependence.

A potential weakness of our study is that only men were recruited. This is because men with alcoholism are about 10 times more frequent than women with alcoholism in the Han Chinese population in Taiwan,[69] and the *MAOA* gene locus is located on the short arm of the X chromosome (Xp11.23).[70] Therefore, the relation is easier to study in men than in women. Nevertheless, alcoholism is more frequent in women with depression and anxiety than in women without mood disorders.[38] Thus a study is needed to investigate the relation between the *MAOA* gene and the *DRD2* gene in women with alcoholism.

The ANX/DEP ALC subtype of alcohol dependence might play an important role in a candidate gene study of alcoholism. The present study suggests that the *MAOA VNTR* allelic variants may modify the effect of the *DRD2* gene in subjects with ANX/DEP ALC. However, dopamine is not only degraded by MAO but is also subjected to *O*-methylation by catechol-*O*-methyltransferase (*COMT*) and aldehyde dehydrogenase (*ALDH*). Our results suggest that the effect of other genes on alcoholism, including gene variants of metabolic enzymes involved in the metabolism of dopamine (e.g., *ALDH* and *COMT* genes), should be further investigated.

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