Eur Arch Psychiatry Clin Neurosci (2008) 258:350–356 DOI 10.1007/s00406-008-0803-1

ORIGINAL PAPER

San-Yuan Huang · Ming-Teng Lin · Mee-Jen Shy · Wei-Wen Lin · Fang-Yi Lin · Ru-Band Lu

Neither single-marker nor haplotype analyses support an association between monoamine oxidase A gene and bipolar disorder

Received: 23 October 2007 / Accepted: 21 January 2008 / Published online: 24 April 2008

Abstract Monoamine oxidase A (MAOA) abnormality has been suggested as a crucial factor in the pathogenesis of mood disorder, because MAOA is associated with the metabolism of monoamines such as serotonin and norepinephrine. Various *MAOA* gene polymorphisms have been investigated for possible associations with bipolar disorder (BD), but the results are controversial. Our goal was to investigate whether *MAOA* gene polymorphisms, especially the promoter *uVNTR* polymorphism and the *EcoRV* polymorphism, are associated either with BD or with different clinical subtypes of BD. A total of 714 Han Chinese subjects in Taiwan (305 controls and 409 BD patients) were recruited for study. All subjects were interviewed with the Chinese Version of the Modified Schedule of Affective Disorders and Schizophrenia-Lifetime; BD was diagnosed according to DSM-IV criteria. Genotyping for *MAOA* polymorphisms was performed using PCR and restriction fragment length polymorphism. The *MAOA* promoter polymorphisms *uVNTR* and *EcoRV* were not associated with BD or any of its subtypes, in either the frequencies of alleles or genotypes. In multiple logistic regression and haplotype frequency analysis, we confirmed these negative results in both females and males. Our results suggest that *MAOA* polymorphisms do not play a major role in pathogenesis of BD or its clinical subtypes in Han Chinese.

Key words MAOA gene · promoter uVNTR · EcoRV polymorphism · clinical subgroup · bipolar disorder

S.-Y. Huang (✉) · M.-T. Lin · M.-J. Shy · W.-W. Lin · F.-Y. Lin
Department of Psychiatry, National Defense Medical Center
Tri-Service General Hospital
No. 325, Cheng-Kung Road, Sec. 2, Nei-Hu District
Taipei, 114 Taiwan, ROC
Tel.: +886-2/8792-7220
Fax: +886-2/8792-6715
E-Mail: hsy@ndmctsgh.edu.tw

M.-T. Lin
Department of Psychiatry
Taichung Armed Forces General Hospital
Taichung, Taiwan, ROC

M.-J. Shy
Institute of Biology and Anatomy
National Defense Medical Center
Taipei, Taiwan, ROC

R.-B. Lu
Department of Psychiatry, College of Medicine
National Cheng Kung University
Tainan, Taiwan, ROC

EAPCN 803

Introduction

Bipolar disorder (BD) is a severe psychiatric disorder and often seen in primary care [11]. It causes problems in work performance, social/family interactions [7, 33], as well as financial hardship, unemployment, suicide, and family burden [55]. Family, twin and adoption studies suggest that predisposition to BD is hereditary [1, 26, 28], so genetic factors may be crucial in development of BD.

Monoamine oxidase A (MAOA) is an important enzyme associated with the metabolism of biogenic amines, including serotonin, norepinephrine, and dopamine [51]. The catecholamine hypothesis of mood disorder has been extensively investigated [3, 49]. Although there have been inconsistent results, many studies suggest that cerebrospinal fluid or plasma levels of norepinephrine metabolites are increased in manic patients, decreased in depressed patients [29], and decreased in manic patients after lithium treatment [35, 48]. Furthermore, dopamine and serotonin metabolite levels are low in the cerebrospinal fluid of depressed [5, 6, 17] and suicidal patients [47], and increased in manic patients [53]. In animal studies, serotonin and norepinephrine concentrations are increased in brain tissue of *MAOA* knock-out mice [8]. Therefore, the *MAOA* gene is a good candidate gene for a study of the pathogenesis of BD.

Despite evidence for the involvement of *MAOA* polymorphisms in the etiology of mood symptoms,

the role of the *MAOA* gene remains equivocal [43]. Lim et al. [31] first reported a positive association between bipolar affective disorder and the *MAOA* gene, and some subsequent studies have supported this association [18, 25, 38, 42, 45], whereas other studies failed to confirm an association [23, 30, 32, 54]. Most positive associations between BD and the *MAOA* gene are restricted to Caucasian samples or to females [18, 42, 45]. The only study of non-Caucasians which found a positive association involved a Japanese population [25], and this study found other alleles to be over-represented in Japanese BD patients, compared to the studies of Caucasians. There are several possible explanations for these conflicting results, including: differing clinical phenotypes of BD; differing frequencies of the *MAOA* gene in different ethnic groups; differing definitions of what should constitute the healthy control group; differing *MAOA* markers used by different research group; and the limited power that comes from using only a single polymorphism to define the role of the *MAOA* gene in BD.

In the work reported here, we used two polymorphisms to clarify the role of the *MAOA* gene in the etiology of BD. Specifically, we used the *uVNTR* promoter polymorphism and the *EcoRV* polymorphism, since this should be more powerful than using only one *MAOA* variant. Our study was designed to test whether the genotype and haplotype of the *MAOA* gene are associated with BD in a Taiwan Han Chinese population. To reduce the risk that genetic heterogeneity of BD could cloud the picture, we also examined clinical subtypes of BD.

Materials and methods

Subjects and clinical assessments

This study was reviewed and approved by the hospital Ethics Committee and was performed in accordance with ethical standards laid down in the 1964 Declaration of Helsinki. To minimize the effects of ethnic differences on gene frequencies, all participants were recruited from the Han Chinese population in Taiwan, and they were matched for ethnicity and geographic origin.

The patient group consisted of 409 patients with BD (217 male and 192 female; mean age 36.56 ± 11.73 years) who were recruited between 2001 and 2007 in the clinic of our hospital. Each patient was initially evaluated by one of the attending psychiatrists (S. Y. H., M. T. L., W. W. L.) and then was interviewed by trained psychologists, using the Chinese Version of Modified Schedule of Affective disorder and Schizophrenia-Lifetime (SADS-L) [15, 36] and DSM-IV diagnosis [2]. The inter-rater reliability κ values of the Chinese Version of Modified SADS-L in our research team were good for major depression (0.79), BD (0.71), anxiety disorder (0.86), schizophrenia (0.95), alcohol abuse and alcohol dependence (1.0), and substance abuse and dependence (0.82) [22]. All patients in this study met the DSM-IV criteria for bipolar I or bipolar II disorder on the basis of interview and a best-estimate procedure that used all available information, including clinical observations, medical records and family information. Further, patients were classified into eight clinical subgroups included 278 individuals with Bipolar I (143 male and 135 female), 131 individuals with bipolar II (74 male and 57 female), 105 individuals with early onset (55 male and 50 female), 304 individuals with late onset (162 male and 142 female), 177 individuals with family history (89 male and 88 female), 232 individuals without family history (128 male and 104 female), 139 individuals with history of suicide attempt (69 male and 70 female), 270 individuals without history of suicide attempt (148 male and 122 female). Family history here indicates one or more first-degree relatives affected for BD or major depression. The age at onset of the initial depressive or manic episode before 18 years was defined as early-onset; otherwise it was defined as late-onset. Suicidal attempt includes any suicidal attempt before recruitment to the study.

The healthy normal control group included 305 healthy volunteers (194 male and 111 female; mean age 37.61 ± 11.91 years) recruited from the community, and each person was interviewed (by S. Y. H.) in the same way as were the patients. The Chinese Version of the Modified SADS-L was used to screen out all psychiatric conditions in the control group. They were free of past or present major or minor mental illness including affective disorder, schizophrenia, anxiety disorder, personality disorder, substance use disorders, and so on. Also, there was no family history of psychiatric disorder in the first-degree relatives of the control subjects.

Blood samples and genotyping DNA extraction

The methods of blood samples and DNA extraction described in our previous study [22]. The 30-bp repeat polymorphism of *MAOA–uVNTR* gene (variable number of tandem repeats located upstream of the promoter region) was investigated using a modification of the polymerase chain reaction (PCR) method described by Zhu et al. [57]. The *EcoRV* (*rs1137070*) polymorphisms in exon 14 of *MAOA* gene were detected using the modified PCR-RFLP (restriction fragment length polymorphism) method described by Hotamisligil and Breakefield [20]. The *MAOA EcoRV* (−) polymorphism remained intact and was 703 bp long, whereas the *MAOA EcoRV* (+) polymorphism was cut into two DNA fragments of 340 and 363 bp by the *EcoRV* restriction enzymes.

Statistical analyses

An independent-samples *t* test was employed to determine the difference in mean age between patients with BD and normal controls. A Pearson χ^2 analysis was used to test for a gender difference between patients and controls. Hardy–Weinberg equilibrium was assessed for each group, and the frequencies of genotype and allele were also compared between patients versus controls using Pearson χ^2 analysis. The Fisher exact test was substituted for the Pearson χ^2 test when cells were smaller than expected values (<5). Multiple logistic regression analysis was used to correct for the effects of possible covariates such as age and different polymorphism. SPSS (version 11.5, SPSS, Taipei, Taiwan) statistical software was used for all analyses and a nominal probability (*P* value) less than 0.05 was considered statistically significant.

Haplotype frequencies, linkage disequilibrium coefficients, and standardized linkage disequilibrium coefficients between promoter *uVNTR* and *EcoRV* locus in the MAOA gene were estimated using two computer programs: (1) The "Estimating Haplotypes" program and (2) the "Permutation and Model Free Analysis" program [56]. We used Fisher's exact test to examine haplotype frequencies when small cell sizes were encountered. A power analysis was performed using G-Power computer software and the effect size conventions were determined according to the method of Erdfelder et al. [16]. The study power was approximately 0.58 ~ 0.92 to detect a small effect (Effect size = 0.1), and 0.99 to detect either a medium effect (Effect size = 0.3 for medium effect) or a large effect (Effect size = 0.5 for large effect). This is true for both the haplotype and genotype frequencies of the total sample, and for males and females separately.

Result

The mean age of the bipolar II patients and of the bipolar patients with early onset was significant

different from the controls ($P < 0.01$), whereas other group ages were not significantly different from controls. There were significant differences in gender frequency between controls versus total BD or the BD subgroups ($P < 0.05$), except for those patients with bipolar II, BD with early onset, or BD without a family history ($P > 0.05$).

The five repeat polymorphism of the *MAOA* uVNTR gene was not found. The two repeat polymorphism was rare in our study (only four normal controls and one bipolar patient). Therefore, we excluded all subjects with the two repeat polymorphism of *MAOA–uVNTR* fro from further analysis. For data analysis, the study included 408 BD patients (217males, 191 females) and 301 normal controls (190 males, 111 females). Genotype distributions of *EcoRV* polymorphisms of the *MAOA* gene were in Hardy–Weinberg equilibrium, both in the controls and in the patients ($P > 0.05$).

The allele frequency of the *MAOA–uVNTR* and *EcoRV* polymorphisms in total BD patients and the control group was not significantly different ($P > 0.1$, Table 1). Because the MAOA gene is located on the X chromosome, we analyzed males and females separately. Among male subjects, neither the *MAOA–uVNTR* nor the *EcoRV* polymorphisms showed a significant difference in allele frequencies between BD patients and controls, even if they were divided into different clinical subtypes of BD ($P > 0.05$, Table 1). Considering both the *MAOA uVNTR* and *EcoRV* genotype and allele frequencies, there were also no significant associations in female BD patients or controls ($P > 0.05$). Finally, there was no relationship between genotype or allele frequencies considering the clinical subtypes of BD ($P > 0.05$, Table 1; although the genotype frequencies are not shown).

To decrease the risk of negative associations by chance, we used haplotype analysis because it is more powerful and can provide more information in an association study [27]. We calculated linkage disequilibrium between the two investigated polymorphisms, and we found that the two polymorphism site was in strong linkage disequilibrium in each group ($P < 0.001$). However, there are no significant differences in the haplotype frequencies of the *MAOA* gene between controls and total BD patients, or the clinical subtypes of BD in either male or female subjects ($P > 0.1$, Table 2). Using multiple logistic regression

Table 1 Allele frequencies of *MAOA* polymorphisms in bipolar patients and controls

Group	Allele						
	Allele number	Promoter-*VNTR*		*P* value[a]	*Eco-RV* (*rs1137070*)		*P* value[a]
		3R (%)	4R (%)		+	−	
Bipolar disorder (M + F)	599	367 (61.3)	232 (38.7)	0.973	358 (59.8)	241 (40.2)	0.525
Male only (*n*)	217	137 (63.1)	80 (36.9)	0.822	135 (62.2)	82 (37.8)	0.727
Female only (2*n*)	382	230 (60.2)	152 (39.8)	0.690	223 (58.4)	159 (41.6)	0.477
Bipolar I (M + F)	411	250 (60.8)	161 (39.2)	0.921	244 (59.4)	167 (40.6)	0.641
Male only (*n*)	143	91 (63.6)	52 (36.4)	0.914	89 (62.2)	54 (37.8)	0.751
Female only (2*n*)	268	159 (59.3)	109 (40.7)	0.863	155 (57.8)	113 (42.2)	0.589
Bipolar II (M + F)	188	117 (62.2)	71 (37.8)	0.803	114 (60.6)	74 (39.4)	0.508
Male only (*n*)	74	46 (62.2)	28 (37.8)	0.756	46 (62.2)	28 (37.8)	0.807
Female only (2*n*)	114	71 (62.3)	43 (37.7)	0.510	68 (59.6)	46 (40.4)	0.457
Bipolar early onset (M + F)	155	88 (56.8)	67 (43.2)	0.342	84 (54.2)	71 (45.8)	0.444
Male only (*n*)	55	30 (54.5)	25 (45.5)	0.193	31 (56.4)	24 (43.6)	0.580
Female only (2*n*)	100	58 (58.0)	42 (42.0)	0.925	53 (53.0)	47 (47.0)	0.688
Bipolar late onset (M + F)	444	279 (62.8)	165 (37.2)	0.614	274 (61.7)	170 (38.3)	0.240
Male only (*n*)	162	107 (66.0)	55 (34.0)	0.718	104 (64.2)	58 (35.8)	0.479
Female only (2*n*)	282	172 (61.0)	110 (39.0)	0.580	170 (60.3)	112 (39.7)	0.270
Bipolar with FH (M + F)	263	150 (57.0)	113 (43.0)	0.286	147 (55.9)	116 (44.1)	0.632
Male only (*n*)	89	50 (56.2)	39 (43.8)	0.199	50 (56.2)	39 (43.8)	0.491
Female only (2*n*)	174	100 (57.5)	74 (42.5)	0.828	97 (55.7)	77 (44.3)	0.946
Bipolar without FH (M + F)	336	216 (64.3)	120 (35.7)	0.380	210 (62.5)	126 (37.5)	0.189
Male only (*n*)	128	86 (67.2)	42 (32.8)	0.584	84 (65.6)	44 (34.4)	0.357
Female only (2*n*)	208	130 (62.5)	78 (37.5)	0.404	126 (60.6)	82 (39.4)	0.278
Bipolar with suicide (M + F)	209	118 (56.5)	91 (43.5)	0.259	114 (54.5)	95 (45.5)	0.444
Male only (*n*)	69	41 (59.4)	28 (40.6)	0.480	41 (59.4)	28 (40.6)	0.872
Female only (2*n*)	140	77 (55.0)	63 (45.0)	0.505	73 (52.1)	67 (47.9)	0.544
Bipolar no suicide (M + F)	390	249 (63.8)	141 (36.2)	0.433	244 (62.6)	146 (37.4)	0.166
Male only (*n*)	148	96 (64.9)	52 (35.1)	0.901	94 (63.5)	54 (36.5)	0.575
Female only (2*n*)	242	153 (63.2)	89 (36.8)	0.303	150 (62.0)	92 (38.0)	0.150
Control (M + F)	412	252 (61.2)	160 (38.8)		238 (57.8)	174 (42.2)	
Male only (*n*)	190	122 (64.2)	68 (35.8)		115 (60.5)	75 (39.5)	
Female only (2*n*)	222	130 (58.6)	92 (41.4)		123 (55.4)	99 (44.6)	

FH family history
[a]Compared with total controls, male controls and female controls, respectively

Table 2 Haplotype frequency and linkage disequilibrium of the *MAOA uVNTR* and *EcoRV* polymorphism in Chinese Han Bipolar disorder females, and controls

Groups	Haplotype						
	Sample size (2*n*)	Haplotype frequency (%)				χ^2	*P* value[a]
		3R/+	3R/−	4R/+	4R/−		
Bipolar disorder	382	0.565	0.037	0.019	0.379	2.400	0.494
Bipolar I	268	0.559	0.034	0.019	0.387	2.304	0.512
Bipolar II	114	0.578	0.045	0.018	0.359	1.335	0.758
Bipolar early onset	100	0.519	0.061	0.011	0.409	0.828	0.874
Bipolar late onset	282	0.581	0.029	0.022	0.368	3.666	0.300
Bipolar with FH[b]	174	0.540	0.035	0.018	0.407	1.650	0.674
Bipolar without FH	208	0.586	0.039	0.020	0.355	2.136	0.545
Bipolar with suicide	140	0.514	0.036	0.007	0.443	3.035	0.384
Bipolar without suicide	242	0.594	0.038	0.026	0.342	2.717	0.437
Control	222	0.525	0.060	0.029	0.385		

[a]Compared with the control group
[b]*FH* family history

analyses, we further confirmed that *MAOA* polymorphisms do not increase the risk of BD or its clinical subtypes after correcting for age and gene-to-gene interactions ($P > 0.1$, Table 3).

Discussion

We report that the *MAOA* promoter polymorphisms *uVNTR* and *EcoRV* were not associated with BD or any of its clinical subtypes, in either the frequency of alleles or the frequency of genotypes. In multiple logistic regression and in haplotype frequency analysis, we confirmed these negative results in both females and males.

Several different polymorphisms of the *MAOA* gene have been identified [4, 19], but only one functional polymorphism has been found in the promoter region of the *MAOA* gene [46]. A functional polymorphism of three repeats was found to have lower transcriptional activity than a polymorphism of four repeats in cell lines and in postmortem males [12, 46]. In addition, a silent mutation (*EcoRV* polymorphism) in exon 14 has been reported to associate with levels of MAOA enzymatic activity [20]. Therefore, the promoter *VNTR* and *EcoRV* polymorphisms were good candidate genes for genetic study of BD.

Nevertheless, we failed to find a difference between patients and controls in the *MAOA* gene or its polymorphisms. We found no differences in *MAOA* gene polymorphisms in males or in females, even when we used multiple logistic regression to correct for the influence of age and gene–gene interaction (Tables 1, 2, 3). This result confirms previous studies of Asian populations [30, 32], but is distinctly different from previous studies of several European populations [18, 31, 38, 42, 45]. A possible explanation is that the *EcoRV* and promoter *VNTR* polymorphisms of the *MAOA* gene do not play a major role in the pathogenesis of BD in Han Chinese. Alternatively, there may be that other genes that contribute to the etiology of BD in Asia populations.

Previous meta-analyses of the association between the *MAOA* gene and BD found that the alleles of the *MAOA* intron 2 and exon 8 polymorphisms are associated with overall BD, or are associated with BD in females, but not in males [42, 45]. However, these studies used two to three markers of *MAOA*, and none of them used haplotype analysis. The statistical power of a single polymorphism is weaker than a haplotype analysis. Our study detected a *uVNTR* polymorphism in the promoter and an *EchoRV* polymorphism in exon 14, and our haplotype analysis region included both the intron 2 and exon 8 polymorphisms. Thus, the haplotype analysis may be more powerful than previous single polymorphism analyses.

Haplotype analysis can increase the statistical power of a study, yet our results contrast with previous reports that showed the haplotype of *MAOA 941T* and four repeats of *MAOA uVNTR* in significant association with BD [38]. One of the reasons for this controversy could be ethnic stratification. The frequency of the three repeat polymorphism in our control subjects was 0.631 for males and 0.572 for females, and in Japanese subjects the frequency was 0.60 [30], essentially the same as in our subjects. In Caucasian populations, however, the frequency of the three repeat allele is around 0.31–0.40 [18, 38, 46, 54], which is much less than in Asian populations. This difference in allele frequency may be partially responsible for the divergent association results. However, it is unlikely that ethnic stratification produced a false-negative result in our study because our subjects were unrelated Han Chinese who were drawn from a population pool in Taiwan that is known to be genetically homogeneous [21, 34], and all of the biological grandparents of our recruited subjects were of Han Chinese ancestry. Our study is also unlikely to be corrupted by use of an inappropriate control group. The healthy controls in our study were interviewed using the Chinese Version of the Modified SADS-L [15, 36] to rule out psychiatric disorders. Thus a false-negative result due to inclusion of affective disorders

Table 3 Logistic regression analysis of the *MAO-A* gene (*promoter* and *Eco RV*) for risk of bipolar disorder, and for risk of its clinical subtypes in female patients

Variable	Groups					
	Bipolar disorder (n = 191)			Bipolar I (n = 134)		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
3/3 Repeat	0.722	0.168–3.114	0.663	0.802	0.175–3.675	0.777
3/4 Repeat	1.412	0.475–4.191	0.535	1.438	0.459–4.505	0.533
+/+	1.882	0.452–7.838	0.385	1.772	0.401–7.839	0.451
+/−	0.918	0.330–2.554	0.870	1.099	0.378–3.190	0.863
Variable	Groups					
	Bipolar II (n = 57)			Bipolar, late onset FH (n = 141)		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
3/3 Repeat	0.273	0.034–2.189	0.222	0.510	0.115–2.256	0.374
3/4 Repeat	0.830	0.209–3.296	0.791	0.889	0.290–2.727	0.837
+/+	3.899	0.534–28.495	0.180	2.666	0.603–11.781	0.196
+/−	0.772	0.212–2.811	0.694	1.266	0.426–3.765	0.672
Variable	Groups					
	Bipolar, with FH (n = 87)			Bipolar, without FH (n = 104)		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
3/3 Repeat	0.569	0.107–3.023	0.509	0.553	0.095–3.218	0.509
3/4 Repeat	0.868	0.234–3.221	0.832	1.645	0.534–5.066	0.386
+/+	1.554	0.303–7.964	0.597	3.320	0.599–18.414	0.170
+/−	1.064	0.306–3.703	0.923	0.968	0.345–2.718	0.951
Variable	Groups					
	Bipolar, with suicide (n = 70)			Bipolar, without suicide (n = 121)		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
3/3 Repeat	0.700	0.112–4.382	0.703	0.597	0.124–2.870	0.519
3/4 Repeat	1.105	0.291–4.199	0.883	1.385	0.455–4.215	0.567
+/+	0.893	0.147–5.409	0.902	3.410	0.737–15.768	0.116
+/−	0.689	0.197–2.412	0.560	1.233	0.436–3.486	0.693

FH family history
Reference group is 4/4 *repeat*, −/− and female, respectively
The P values and Odds ratios of early onset bipolar disorder (n = 50) have not shown in this table, but the risk of MAOA gene for this group is not significant difference, $P > 0.1$

in our control group is unlikely. However, replication studies are still needed to confirm our result.

The mortality rate from attempted suicide in BD patients is higher than in the general population [40]. Platelet MAOA activity is lower in suicidal patients than in controls [52], and significant elevation of MAOA activity is present in the hypothalamic region of depressed patients [37] and in postmortem suicide victims [50]. Family, twin and adoption studies suggest that genetic factors are involved in suicide [44]. Courtet et al. [10] found that there were more high-activity *MAOA uVNTR* alleles in violent suicidal men than in non-violent suicidal men, although the haplotype of the *MAOA* promoter and intron 2 did not show a significant difference. Moreover, Du et al. [13] found significantly different genotype distributions of the *MAOA EcoRV* polymorphism in depressed suicide victims. However, our study and other studies [30, 39] did not find this association. Although the association between suicidal behavior and the *MAOA* gene remains equivocal, our study sample used homogeneous Han Chinese and multiple logistic regression to exclude possible bias. Therefore, our study suggests that the *MAOA* gene is not associated with BD with suicide in Han Chinese.

There are several limitations in our study. First, the study design uses a cross-sectional approach, which may not be as sensitive as a longitudinal approach. For example, we cannot predict whether MAOA levels fluctuate over time or whether BD patients without a suicide history might eventually commit suicide. Furthermore, previous studies have suggested that environmental factors and life stresses play an important role in the etiology and pathogenesis of BD [24, 41], and our subjects may not have encountered these factors and stresses when we collected our

sample. Finally, some studies found that depressive symptoms are influenced by gene–environment interactions in patients with major depression [9, 14]. These factors may explain why previous studies have found it hard to identify genes associated with increased risk of mood disorder.

In conclusion, we found that *MAOA* promoter and *EcoRV* polymorphisms do not play a significant role in increasing susceptibility to BD in general or to clinical subtypes of BD in Taiwan Han Chinese. Our results suggest that BD is a complex and heterogeneous disorder, and that genetic factors may have only a modest effect in the pathogenesis of BD and its clinical subtypes. Prospective studies with larger samples are needed, with the effects of ethnic stratification controlled. Furthermore, it will be important to incorporate gene–environment and gene–gene interactions and to use rigorous methodology to confirm our results.

Acknowledgments This study was supported in part by National Science Council Grants NSC94-2314-B-016-016, NSC95-2314-B-016-019 (S. Y. H); by the Department of Health Grants DOH94-TD-D-113-040, DOH96-7D-D-113-031 (S. Y. H); and by Tri-Service General Hospital Grant TSGH-C94-76, TSGH-C97-88 (S. Y. H). The authors thank Mr. Cheng-Chang Huang and Dr. R. Grant Steen for their assistance in the preparation of this manuscript.

References

1. Althoff RR, Faraone SV, Rettew DC, Morley CP, Hudziak JJ (2005) Family, twin, adoption, and molecular genetic studies of juvenile bipolar disorder. Bipolar Disord 7:598–609
2. American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders. American Psychiatric Association, Washington
3. Anand A, Darnell A, Miller HL, Berman RM, Cappiello A, Oren DA, Woods SW, Charney DS (1999) Effect of catecholamine depletion on lithium-induced long-term remission of bipolar disorder. Biol Psychiatry 45:972–978
4. Black GC, Chen ZY, Craig IW, Powell JF (1991) Dinucleotide repeat polymorphism at the MAOA locus. Nucleic Acids Res 19:689
5. Bowden C, Cheetham SC, Lowther S, Katona CL, Crompton MR, Horton RW (1997) Reduced dopamine turnover in the basal ganglia of depressed suicides. Brain Res 769:135–140
6. Brown GL, Linnoila MI (1990) CSF serotonin metabolite (5-HIAA) studies in depression, impulsivity, and violence. J Clin Psychiatry 51:31–41; discussion 42–33
7. Calabrese JR, Hirschfeld RM, Reed M, Davies MA, Frye MA, Keck PE, Lewis L, McElroy SL, McNulty JP, Wagner KD (2003) Impact of bipolar disorder on a U.S. community sample. J Clin Psychiatry 64:425–432
8. Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Pournin S, Muller U, Aguet M, Babinet C, Shih JC et al (1995) Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science 268:1763–1766
9. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301:386–389
10. Courtet P, Jollant F, Buresi C, Castelnau D, Mouthon D, Malafosse A (2005) The monoamine oxidase A gene may influence the means used in suicide attempts. Psychiatr Genet 15:189–193
11. Das AK, Olfson M, Gameroff MJ, Pilowsky DJ, Blanco C, Feder A, Gross R, Neria Y, Lantigua R, Shea S, Weissman MM (2005) Screening for bipolar disorder in a primary care practice. Jama 293:956–963
12. Denney RM, Koch H, Craig IW (1999) Association between monoamine oxidase A activity in human male skin fibroblasts and genotype of the MAOA promoter-associated variable number tandem repeat. Hum Genet 105:542–551
13. Du L, Faludi G, Palkovits M, Sotonyi P, Bakish D, Hrdina PD (2002) High activity-related allele of MAO-A gene associated with depressed suicide in males. Neuroreport 13:1195–1198
14. Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P, Plomin R, Craig IW (2004) Gene-environment interaction analysis of serotonin system markers with adolescent depression. Mol Psychiatry 9:908–915
15. Endicott J, Spitzer RL (1978) A diagnostic interview: the schedule for affective disorders and schizophrenia. Arch Gen Psychiatry 35:837–844
16. Erdfelder E, Faul F, Buchner A (1996) GPOWER: a general power analysis program. Behav Res Methods Instrum Comput 28:1–11
17. Faustman WO, Faull KF, Whiteford HA, Borchert C, Csernansky JG (1990) CSF 5-HIAA, serum cortisol, and age differentially predict vegetative and cognitive symptoms in depression. Biol Psychiatry 27:311–318
18. Gutierrez B, Arias B, Gasto C, Catalan R, Papiol S, Pintor L, Fananas L (2004) Association analysis between a functional polymorphism in the monoamine oxidase A gene promoter and severe mood disorders. Psychiatr Genet 14:203–208
19. Hinds HL, Hendriks RW, Craig IW, Chen ZY (1992) Characterization of a highly polymorphic region near the first exon of the human MAOA gene containing a GT dinucleotide and a novel VNTR motif. Genomics 13:896–897
20. Hotamisligil GS, Breakefield XO (1991) Human monoamine oxidase A gene determines levels of enzyme activity. Am J Hum Genet 49:383–392
21. Hsu YP, Loh EW, Chen WJ, Chen CC, Yu JM, Cheng AT (1996) Association of monoamine oxidase A alleles with alcoholism among male Chinese in Taiwan. Am J Psychiatry 153:1209–1211
22. Huang SY, Lin WW, Ko HC, Lee JF, Wang TJ, Chou YH, Yin SJ, Lu RB (2004) Possible interaction of alcohol dehydrogenase and aldehyde dehydrogenase genes with the dopamine D2 receptor gene in anxiety-depressive alcohol dependence. Alcohol Clin Exp Res 28:374–384
23. Huang YY, Cate SP, Battistuzzi C, Oquendo MA, Brent D, Mann JJ (2004) An association between a functional polymorphism in the monoamine oxidase a gene promoter, impulsive traits and early abuse experiences. Neuropsychopharmacology 29:1498–1505
24. Johnson SL (2005) Life events in bipolar disorder: towards more specific models. Clin Psychol Rev 25:1008–1027
25. Kawada Y, Hattori M, Dai XY, Nanko S (1995) Possible association between monoamine oxidase A gene and bipolar affective disorder. Am J Hum Genet 56:335–336
26. Kendler KS, Pedersen N, Johnson L, Neale MC, Mathe AA (1993) A pilot Swedish twin study of affective illness, including hospital- and population-ascertained subsamples. Arch Gen Psychiatry 50:699–700
27. Kidd KK (1993) Associations of disease with genetic markers: deja vu all over again. Am J Med Genet 48:71–73
28. Kieseppa T, Partonen T, Haukka J, Kaprio J, Lonnqvist J (2004) High concordance of bipolar I disorder in a nationwide sample of twins. Am J Psychiatry 161:1814–1821
29. Koenigsberg HW, Teicher MH, Mitropoulou V, Navalta C, New AS, Trestman R, Siever LJ (2004) 24-h Monitoring of plasma norepinephrine, MHPG, cortisol, growth hormone and prolactin in depression. J Psychiatr Res 38:503–511
30. Kunugi H, Ishida S, Kato T, Tatsumi M, Sakai T, Hattori M, Hirose T, Nanko S (1999) A functional polymorphism in the promoter region of monoamine oxidase-A gene and mood disorders. Mol Psychiatry 4:393–395
31. Lim LC, Powell JF, Murray R, Gill M (1994) Monoamine oxidase A gene and bipolar affective disorder. Am J Hum Genet 54:1122–1124

32. Lin S, Jiang S, Wu X, Qian Y, Wang D, Tang G, Gu N (2000) Association analysis between mood disorder and monoamine oxidase gene. Am J Med Genet 96:12–14
33. Lish JD, Dime-Meenan S, Whybrow PC, Price RA, Hirschfeld RM (1994) The National depressive and manic-depressive association (DMDA) survey of bipolar members. J Affect Disord 31:281–294
34. Lu RB, Lin WW, Lee JF, Ko HC, Shih JC (2003) Neither antisocial personality disorder nor antisocial alcoholism is associated with the MAO-A gene in Han Chinese males. Alcohol Clin Exp Res 27:889–893
35. Manji HK, Lenox RH (2000) The nature of bipolar disorder. J Clin Psychiatry 61(Suppl 13):42–57
36. Merikangas KR, Stevens DE, Fenton B, Stolar M, O'Malley S, Woods SW, Risch N (1998) Co-morbidity and familial aggregation of alcoholism and anxiety disorders. Psychol Med 28:773–788
37. Meyer JH, Ginovart N, Boovariwala A, Sagrati S, Hussey D, Garcia A, Young T, Praschak-Rieder N, Wilson AA, Houle S (2006) Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. Arch Gen Psychiatry 63:1209–1216
38. Muller DJ, Serretti A, Sicard T, Tharmalingam S, King N, Artioli P, Mandelli L, Lorenzi C, Kennedy JL (2007) Further evidence of MAO-A gene variants associated with bipolar disorder. Am J Med Genet B Neuropsychiatr Genet 144:37–40
39. Ono H, Shirakawa O, Nishiguchi N, Nishimura A, Nushida H, Ueno Y, Maeda K (2002) No evidence of an association between a functional monoamine oxidase a gene polymorphism and completed suicides. Am J Med Genet 114:340–342
40. Osby U, Brandt L, Correia N, Ekbom A, Sparen P (2001) Excess mortality in bipolar and unipolar disorder in Sweden. Arch Gen Psychiatry 58:844–850
41. Paykel ES (2003) Life events and affective disorders. Acta Psychiatr Scand Suppl 418:61–66
42. Preisig M, Bellivier F, Fenton BT, Baud P, Berney A, Courtet P, Hardy P, Golaz J, Leboyer M, Mallet J, Matthey ML, Mouthon D, Neidhart E, Nosten-Bertrand M, Stadelmann-Dubuis E, Guimon J, Ferrero F, Buresi C, Malafosse A (2000) Association between bipolar disorder and monoamine oxidase A gene polymorphisms: results of a multicenter study. Am J Psychiatry 157:948–955
43. Preisig M, Ferrero F, Malafosse A (2005) Monoamine oxidase A and tryptophan hydroxylase gene polymorphisms: are they associated with bipolar disorder? Am J Pharmacogenomics 5:45–52
44. Roy A, Nielsen D, Rylander G, Sarchiapone M, Segal N (1999) Genetics of suicide in depression. J Clin Psychiatry 60(Suppl 2):12–17, discussion 18–20, 113–116
45. Rubinsztein DC, Leggo J, Goodburn S, Walsh C, Jain S, Paykel ES (1996) Genetic association between monoamine oxidase A microsatellite and RFLP alleles and bipolar affective disorder: analysis and meta-analysis. Hum Mol Genet 5:779–782
46. Sabol SZ, Hu S, Hamer D (1998) A functional polymorphism in the monoamine oxidase A gene promoter. Hum Genet 103:273–279
47. Samuelsson M, Jokinen J, Nordstrom AL, Nordstrom P (2006) CSF 5-HIAA, suicide intent and hopelessness in the prediction of early suicide in male high-risk suicide attempters. Acta Psychiatr Scand 113:44–47
48. Schildkraut JJ (1965) The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry 122:509–522
49. Sher L, Carballo JJ, Grunebaum MF, Burke AK, Zalsman G, Huang YY, Mann JJ, Oquendo MA (2006) A prospective study of the association of cerebrospinal fluid monoamine metabolite levels with lethality of suicide attempts in patients with bipolar disorder. Bipolar Disord 8:543–550
50. Sherif F, Marcusson J, Oreland L (1991) Brain gamma-aminobutyrate transaminase and monoamine oxidase activities in suicide victims. Eur Arch Psychiatry Clin Neurosci 241:139–144
51. Shih JC, Thompson RF (1999) Monoamine oxidase in neuropsychiatry and behavior. Am J Hum Genet 65:593–598
52. Simonsson P, Traskman-Bendz L, Alling C, Oreland L, Regnell G, Ohman R (1991) Peripheral serotonergic markers in patients with suicidal behavior. Eur Neuropsychopharmacol 1:503–510
53. Swann AC, Secunda S, Davis JM, Robins E, Hanin I, Koslow SH, Maas JW (1983) CSF monoamine metabolites in mania. Am J Psychiatry 140:396–400
54. Syagailo YV, Stober G, Grassle M, Reimer E, Knapp M, Jungkunz G, Okladnova O, Meyer J, Lesch KP (2001) Association analysis of the functional monoamine oxidase A gene promoter polymorphism in psychiatric disorders. Am J Med Genet 105:168–171
55. Wyatt RJ, Henter I (1995) An economic evaluation of manic-depressive illness—1991. Soc Psychiatry Psychiatr Epidemiol 30:213–219
56. Zhao JH, Sham PC (2002) Faster haplotype frequency estimation using unrelated subjects. Hum Hered 53:36–41
57. Zhu QS, Grimsby J, Chen K, Shih JC (1992) Promoter organization and activity of human monoamine oxidase (MAO) A and B genes. J Neurosci 12:4437–4446