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A potential interaction between COMT and MTHFR genetic variants in Han Chinese patients with bipolar II disorder

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Bipolar II disorder (BP-II), characterized by recurrent dysregulation of mood, is a serious and chronic psychiatric illness. However, BP-II is commonly under-recognized, even in psychiatric settings. Because dopaminergic disturbance is thought to be involved in the development of bipolar disorder (BPD), it seems essential to investigate dopamine-related genes like the catechol-O-methyltransferase (*COMT*) gene, which are involved in dopamine metabolism, and the methylenetetrahydrofolate reductase (*MTHFR*) gene, which may affect COMT methylation and COMT function. The current study examined the association and interaction of the *COMT* Val158Met and *MTHFR* C677T variants with BP-II. Nine hundred seventy-eight participants were recruited: 531 with BP-II and 447 healthy controls. The genotypes of the *COMT* and *MTHFR* polymorphisms were determined using a polymerase chain reaction-restriction fragment length polymorphism analysis. Logistic regression analysis showed a significant interaction effect of the *COMT* Val158Met Val/Val genotype and the *MTHFR* C677T C/T + T/T genotype (P = 0.039) for the protective effect on the odds of developing BP-II. Our findings support preliminary evidence that the *COMT* and *MTHFR* genes interact in BP-II, and they imply the connection of both dopaminergic pathways and methylation pathways in the pathogenesis of BP-II.

Bipolar disorder (BPD), also known as manic-depressive illness, is characterized by periods of elevated mood and periods of depression. The two most commonly known clinical subtypes are bipolar-I disorder (BP-I) and bipolar-II disorder (BP-II). BP-II, characterized by recurrent depressive and hypomanic episodes, is frequently regarded as a "milder form of BP-I"[1]. It is believed that BP-II is greatly under-diagnosed and frequently misdiagnosed in clinical settings[2,3], because patients usually seek treatment during depressive episodes but perceive hypomanic episodes as positive experiences[1,4]. In long-term follow-up studies, patients with BP-II had a more chronic course, more mood episodes, a higher suicidal risk, and shorter inter-episodes than did patients with BP-I[5,6]. Moreover, misdiagnosing BP-II as BP-I or unipolar major depressive disorder leads to inefficacious treatment and a substantial delay in the diagnosis of BP-II, which may increase the suicide rate in BP-II patients[7,8].

Genes that affect the dopaminergic pathway—*COMT*, for example—gene, have been suggested as susceptible genes for BPD[9-11]. Primarily in the frontal cortex[12], the COMT enzyme affects the degradation of dopamine and norepinephrine[13]. The *COMT* gene is located on chromosome 22q11. There is, in codon 158, one functional single nucleotide polymorphism (SNP) (rs4680), which causes an amino acid shift from valine to methionine (Val158Met)[14]. The Val allele encodes for enzymatic activity 3–4 times higher than that of the Met allele; therefore, the SNP distributes trimodal enzymatic activity: low (Met/Met genotype), intermediate (Val/Met genotype), and high (Val/Val genotype)[15]. The low-activity Met allele has been reported to increase susceptibility

Table 1 | Comparison of mean age and gender

Group (n)	BP-II (531)	Controls (447)	Statistics	P
Age (Mean ± SD)	31.9 ± 11.5	35.4 ± 9.7	$t = 5.03$	<0.001***
Gender (Male/Female)	255/276	328/119	$\chi^2 = 64.8$	<0.001***

BP-II: bipolar II disorder; SD, standard deviation.
***P < 0.001.

to BPD[9], which suggests that the disorder is characterized by a high-dopamine state. In addition, the *COMT* Val158Met polymorphism is associated with rapid cycling in BPD[14,16]. However, whether the interaction of the *COMT* Val158Met polymorphism with other dopaminergic genes is associated with the risk of BP-II warrants additional studies.

Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme involved in one-carbon metabolism[17]. The reduction of MTHFR activity may compromise DNA methylation[18]. Therefore, genetic variations of the *MTHFR* gene might affect COMT methylation and COMT function. Decreased *MTHFR* activity has been associated with other psychiatric disorders, for example, schizophrenia[19] and affective disorders[20]. The C677T (rs1801133) variant is a common functional SNP in *MTHFR;* it leads to a substitution of alanine with valine, which reduces enzymatic activity: The TT homozygous variants have only 30% activity and the TC heterozygous variants have only 65% enzyme activity compared with the CC genotype[21]. The low-functioning 677T allele of the common SNP of *MTHFR*, 677C > T, has been significantly associated with the risk of BPD[22–24]. However, others have reported borderline or inconsistent results[25,26].

The well-known theory of the inverted U-shaped relationship between dopamine transmission and prefrontal cortex activation assumes that dopamine signaling either below or above an optimal range can be disadvantageous and be involved in neuropsychiatric pathophysiology[27,28]. Specifically, both the *COMT* Val and *MTHFR* T alleles tend to decrease dopamine below optimal levels (left side of the inverted-U curve), and the *COMT* Met and *MTHFR* C alleles tend to increase dopamine above optimal levels (right side of the curve). This theory provides a context for the possibility that not only the polymorphisms of the *COMT* and *MTHFR* genes, but also their interaction, might be involved in dopamine dysregulation which contributes to the pathogenesis of psychiatric disorder[29]. Association studies have supported the notion that, high dopamine levels contribute to the risk of BPD[9]; thus, based on the inverted U theory, we hypothesize that the high dopamine level, a result of the combination of the low-functioning COMT enzyme (*COMT* Met allele) in the background of the *MTHFR* C allele, contributes to risk of developing BP-II. We also hypothesize that, when dopamine levels are less than optimal, the combination of the *COMT* Val/Val and *MTHFR* T allele protects against BP-II.

We used a gene-to-gene interaction approach to detect weak gene effects in our investigation of the relationship and interaction between the *COMT* and *MTHFR* gene polymorphisms and BP-II, because individual genes may have only a small effect on the pathogenesis of BPD[30,31]. In addition, we tried to control for the clinical heterogeneity of BPD by recruiting only patients with a homogenous subtype of BPD: BP-II.

Methods

Participants. The research protocol was approved by the Institutional Review Board for the Protection of Human Subjects at Tri-Service General Hospital and at National Cheng Kung University Hospital, and the study was carried out in accordance with the nationally approved guidelines. The study conformed to the ethical standards of the 1964 Declaration of Helsinki. After the study had been thoroughly explained to the participants, they all signed written informed consent forms. To minimize the confounding effect of ethnic differences in genetic distribution, we recruited only Han Chinese in Taiwan confirmed to be unrelated.

Between 2007 and 2014, BP-II outpatients and inpatients were recruited from Tri-Service General Hospital and National Cheng Kung University Hospital. Patients were initially evaluated in an interview by an attending psychiatrist. The study protocol was explained to patients with an initial diagnosis of BP-II. Those who agreed to participate in the study and signed informed consents subsequently underwent a more detailed structured interview by a clinical psychologist using the Chinese Version of the Modified Schedule for Affective Disorders and Schizophrenia-Lifetime (SADS-L) version[32], which has good inter-rater reliability[33], to reconfirm that their diagnoses complied with Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria. Inclusion criteria were a diagnosis of BP-II, either first-onset or with previous episodes. Exclusion criteria were (i) any DSM-IV-TR Axis I diagnosis, including organic mental disorders, substance use disorder, and other major and minor mental illnesses other than BP-II, (ii) any significant medical illness, (iii) any neurological disorders, and (iv) any poorly controlled physical illness that might influence the interview and study results.

Although DSM-IV-TR[4] and SADS-L criteria require a 4-day minimum duration of hypomania for the diagnosis of BP-II, we used a 2-day minimum, which is supported by recent epidemiologic data, and it might be more prevalent in community samples[34]. Angst et al.[34] say that the 2-day duration shows comparable clinical significance in 4-day and in 1- to 3-day hypomanic episodes. Therefore, this study used the 2-day limit for hypomania, which has been used in many clinical studies[35,36].

The healthy control group (hereafter, Controls) were volunteers recruited from the community. The SADS-L[32] was used to screen the volunteers for psychiatric conditions. All volunteers were free of major and minor mental illness (schizophrenia, affective disorder, anxiety disorder, substance use disorder, personality disorder, etc.). None had a family history of psychiatric disorder among their first-degree relatives.

Blood Samples and Genotyping. Twenty milliliters of venous blood was collected using venipuncture of the antecubital vein. DNA was isolated from lymphocytes. The *COMT* Val158Met polymorphism was genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis[37]. The genotyping of the *MTHFR* C677T polymorphism was done using a modified protocol described elsewhere[38]. The laboratory technician who did the genotyping, and then retyped and double-checked each sample and recorded the genotype data was blinded to the patients' diagnoses and to whether the samples were from patients or controls. The error rate of genotyping was less than 5%.

Statistical Analysis. Independent *t* tests were used to determine the mean age differences between BP-II patients and Controls. Gender difference was analyzed using χ^2 tests.

Pearson χ^2 analysis (two-tailed) was used to analyze the differences in the genotype and allele frequencies of the *COMT* Val158Met and *MTHFR* C677T polymorphisms between the BP-II and Control groups. Hardy-Weinberg equilibrium was tested for each study group.

To examine the main effects and the interactive effects of gene-to-gene interaction of the *COMT* and *MTHFR* genes for the risk of BP-II compared with Controls, we used logistic regression and controlled for the covariates of age and gender. In the logistic regression model, the diagnosis group (BP-II vs. Controls) was set as a dependent variable (binary outcome). Age, gender, genotypes of *COMT* and *MTHFR*, and the interaction of the *COMT* and *MTHFR* genes were set as independent variables (covariates). The effects of independent variables are expressed as odds ratios (OR) with 95% confidence intervals (CI). Significance was set at $P < 0.05$ (two-tailed). The data were analyzed using SPSS 18.0 (SPSS, Inc., Chicago, IL), the power analysis was done using G-Power 3[39,40], and the effect-size conventions were set as described elsewhere[39].

Results

Thirty-one patients previously diagnosed with BP-II were excluded for not meeting the present study's diagnostic criteria for BP-II (n = 26) or comorbidities of other major mental illnesses (n = 5). Of the 978 participants included in the study, 531 were BP-II patients and 447 were Controls. Both age and gender were significantly different between the BP-II patients and Controls (Table 1).

The genotype and allele distributions of the *COMT* Val158Met and *MTHFR* C677T polymorphisms for each group were in Hardy-Weinberg equilibrium ($P > 0.05$). For *COMT* Val158Met (Table 2), neither the genotypes ($P = 0.075$) nor the allelic distribution ($P =$

Table 2 | Genotype Distributions and Allelic Frequencies of *COMT Val158Met Variants*

Group (n)	BP-II (531)	Controls (447)	χ^2	P
***COMT* Val158Met Genotype (%)**				
Val/Val	272 (51.2)	251 (56.2)	5.19	0.075
Val/Met	224 (42.2)	158 (35.3)		
Met/Met	35 (6.6)	38 (8.5)		
***COMT* Val158Met Allele (%)**				
Val	768 (72.1)	660 (74.1)	0.56	0.45
Met	294 (27.9)	234 (25.9)		

0.45) significantly differed between BP-II patients and Controls. For *MTHFR* C677T (Table 3), neither the genotypes ($P = 0.16$) nor the allelic distribution ($P = 0.13$) were associated with the risk of BP-II.

After the MTHFR C677T genotypes in BP-II patients and Controls had been stratified, the COMT Val/Val variant was significantly more frequently detected in Controls than in BP-II patients (Table 4). Multivariate logistic regression analysis showed a significant interaction between the COMT Val158Met Val/Val genotype and the MTHFR C677T C/T + T/T genotype and their interaction for the risk of BP-II (OR = 0.58, P = 0.034) (Table 5). After controlling for age and gender (Model 2), the protective effect against BP-II was still significantly associated with the interaction of the COMT Val158Met Val/Val genotype and the MTHFR C677T C/T + T/T genotype (OR = 0.57, P = 0.039).

The power analysis showed that the power was 0.81 to detect a small effect, and 1.00 to detect medium and large effects in genotype frequencies ($n = 978$) between BP-II patients and Controls. The power was 0.88 for a small effect, and 1.00 for medium and large effects. For multiple regression analysis, the study had a power of 0.98 to detect a small effect, and 1.00 to detect medium and large effects. The effect-size conventions for this power analysis were determined as follows[39]: small effect size = 0.10, medium effect size = 0.30, large effect size = 0.50 for the χ^2 test; and small effect size = 0.02, medium effect size = 0.15, large effect size = 0.35 for the multivariate regression model ($\alpha = 0.05$).

Discussion

We found initial evidence of interaction between the *COMT* and *MTHFR* genes in the BP-II group. Although both the *COMT* and *MTHFR* genes have been associated with BP, the association of the interaction of these two genes with BP-II has not been previously reported. Our finding supports the notion that genetic factors, especially the interaction of genetic factors, do contribute to the susceptibility for BP-II.

The significant gene-to-gene interaction found in our study suggests that protective effect against developing BP-II was greater for those with both the Val/Val genotype of the *COMT* Val158Met gene and the C/T + T/T genotype of the *MTHFR* C677T polymorphism (OR = 0.57). When stratified by the *MTHFR* C677T C/T + T/T genotype, compared with people with the *COMT* Val158Met Val/Met + Met/Met genotype, those with the *COMT* Val158Met Val/Val genotype had a chance 0.785 times of having BP-II. This finding supports the association of a relatively low dopamine state being protective against the odds of developing BP-II, or at least argues that a relatively high dopamine state is a risk for developing BP-II. This finding is consistent with our hypothesis and the inverted U theory.

MTHFR, as the main enzyme in folate metabolism, provides single carbon moieties for methylation reactions inside the cells. One copy of the 677T allele is associated with a 35% reduction in *MTHFR* activity compared with the 677C allele[21] and is involved in methylating DNA and metabolizing homocysteine in downstream biochemical processes[18]. Therefore, the *MTHFR* 677C/T + T/T genotype, which is less enzymatically active, may have few methyl moieties for the methylating COMT promoter, diminishing *COMT* expression, and augmenting dopamine degradation. In contrast, the Val/Val genotype is associated with high COMT enzyme activity and related to low dopamine levels, compared with the Met/Met and Val/Met genotypes[15]. The interactive effect of these two genotypes (*COMT* Val158Met Val/Val and *MTHFR* C677T C/T + T/T) is a low dopamine level, which is associated with a protective effect against BP-II. This finding is consistent with the inverted U-shaped dopamine theory[27–29]. Most other studies found the interactive effect of the *COMT* Val allele and *MTHFR* T allele associated with prefrontal and cognitive dysfunction in schizophrenia[29,41]. Our finding is contrary to those gene-to-gene interaction studies on schizophrenia, which perhaps provides evidence from genetic and dopaminergic levels that BP-II and schizophrenia are different mental disorders with different degrees of progression and different pathologies of dopamine degradation-relatedness. However, whether the genetic interaction reported in the current study is associated with prefrontal dysfunction in BP-II warrants additional studies.

Kontis et al.[42] explored the hypotheses that the putative interaction between the *COMT* and *MTHFR* genes on cognition occurred because of two pathophysiological mechanisms: expression of the *COMT* gene and catabolization of dopamine. The first hypothesis predicts that the *MTHFR* C677T T allele increases the expression of the hyperfunctional Val variant of the *COMT* Val158Met polymorphism by decreasing COMT promoter methylation and prefrontal dopamine[41]. The second hypothesis predicts that *MTHFR* C677T T allele reduces the methyl moieties. The resultant COMT enzyme does not have the necessary methyl groups to catabolize prefrontal dopamine; thus, dopamine levels increase[43]. However, these contradictory hypotheses require further investigation.

In the Pearson χ^2 analysis, we found a non-significant association between the *COMT* Val158Met polymorphism and BP-II. However, when stratified by the *MTHFR* polymorphism, only in the MTHFR

Table 3 | Genotype distributions and allelic frequencies of *MTHFR* C677T polymorphisms

Group (n)	BP-II (531)	Controls (447)	χ^2	P
***MTHFR* C677T Genotype (%)**				
C/C	287 (54.0)	215 (48.1)	3.61	0.16
C/T	206 (38.8)	199 (44.5)		
T/T	38 (7.2)	33 (7.4)		
***MTHFR* C677T Allele (%)**				
C	780 (48.2)	629 (49.8)	2.30	0.13
T	282 (51.8)	265 (50.2)		

Table 4 | Genotype distribution of genotypes of the *COMT Val158Met* polymorphism after stratification of the *MTHFR* C677T genotypes

Gene polymorphisms and Group	(n)	BP-II (%)	Controls (%)	χ^2	P	OR	95% CI
***MTHFR* C677T C/C Genotype**							
***COMT* Val158Met Genotypes**		(n = 287)	(n = 215)				
Val/Val	282	163 (56.8)	119 (55.3)	0.104	0.785	1.026	0.877–1.200
Val/Met + Met/Met	220	124 (43.2)	96 (44.7)			1	
***MTHFR* C677T C/T + T/T Genotype**							
***COMT* Val158Met Genotypes**		(n = 244)	(n = 232)				
Val/Val	241	109 (44.7)	132 (56.9)	7.11	0.008**	0.785	0.656–0.939
Val/Met + Met/Met	235	135 (55.3)	100 (43.1)			1	

OR = odds ratio; CI = confidence interval.
**P < 0.01.

C/T + T/T genotype was there a protective effect against BP-II (OR = 0.785) in those with the *COMT* Val158Met Val/Val genotype. This agrees with other[44–46] findings that controls without BP-II carried significantly more Val/Met and Met/Met genotypes of the *COMT* Val158Met polymorphisms than of the Val/Val genotype. It implies increased central dopamine availability in those with BP-II[14]. In other words, the Val allele is involved in lower prefrontal dopaminergic activity, and those with the *COMT* Val158Met Val/Val genotype may be less vulnerable to BP-II because of the interaction effect of the *MTHFR* gene. Therefore, we hypothesize that the *COMT* gene is the candidate gene for BP-II only in those who carry the *MTHFR* C677T C/T + T/T genotypes. Additional association studies of BP-II focusing on other SNPs or haplotypes of the *COMT* gene on the background of the *MTHFR* C677T polymorphism are needed.

We found, after controlling for age and gender, that the C/T + T/T genotype of the *MTHFR* C677T polymorphism was not associated with the risk of BP-II. Our finding differed from others'[22–24] which reported that the low-functioning 677T allele is a risk factor for BPD. Our study included substantially more participants and a more homogeneous group of patients with BP-II only. Perhaps the discrepancy can be attributed to their focus on the *COMT* Val158Met polymorphism and its influence on a definition of BPD that does not discriminate between BP-I and BP-II. However, after we stratified *COMT* Val158Met polymorphism, we found that the *MTHFR* C677T polymorphism was also associated with BP-II. We hypothesize that the association of the *MTHFR* gene and BP-II is modulated by the *COMT* gene. The *MTHFR* gene has been reported[47] to be greatly involved in brain function and neurodevelopment. We also hypothesize that neurodegeneration and epigenetic mechanisms such as DNA methylation, through the pathway of dopamine catabolism, are important in the etiology of BP-II.

The present study has several limitations. First, the taxonomy of BP-II in this study differs from that provided in the DSM-IV and DSM-5, which require a minimum duration of 4 days of hypomania for a BP-II diagnosis. Although the 2-day duration for hypomania used in the current study has been widely used in many clinical studies[34,48], it is possible that, using DSM diagnostic criteria, some of our patients would be diagnosed with unipolar depression. The Bridge study[49] has validated that 1- to 3-day hypomania criterion provides better discrimination between BP-II and major depressive disorder. Unfortunately, our current data cannot tell us whether there is a genetic difference in BP-II subgroups with <4 days and ≥4 days of hypomania. Thus, our findings must be cautiously compared with those of other genetic studies of BP-II. In addition, significant differences in age and gender were found between the patient groups and the controls. To control for the differences in age and gender, we used multivariate logistic regression to analyze both the genetic main effect and the effect of gene-to-gene interaction. Although comparing age- and gender-matched controls is ideal, having an older control group reduces the risk of future BPD onset. Third, a 10-year follow-up study[50] reported that 7.5% of patients with BP-II switched from hypomanic to manic episodes (BP-I). Therefore, a confirmed diagnosis of BP-II may require a long-term follow-up. Finally, only one SNP of the *COMT* gene and only one of the *MTHFR* gene were examined in this study. Other polymorphisms of the *COMT* (rs2075507 and rs165599) and *MTHFR* genes (A1298C) are also reported to be involved in the pathogenesis of BPD[26,51]. The findings in our study cannot be generalized into an association between BP-II and other polymorphisms of the COMT and MTHFR genes. Therefore, additional comprehensive association studies of other dopamine-related genes, potentially involving methylation pathways, are needed to support our hypotheses.

Our study serves as initial evidence of a gene-to-gene interaction between the *COMT* and *MTHFR* genes in BP-II, which implies the involvement of dopaminergic pathways and methylation pathways in the pathogenesis of this disorder. However, whether the interaction of these genes advance dopamine dysfunction and how that influences the etiology of BP-II still require further investigation. Because COMT is responsible for dopamine catabolism and MTHFR is essential in brain function, neurodevelopment, etc., the association of the *COMT* and *MTHFR* genes and other neurodegeneration-related genes with BP-II warrants additional studies.

Table 5 | Multivariate logistic regression analysis of *COMT* and *MTHFR* genes and their interaction for the risk of bipolar-II disorder

Variable	Model 1			Model 2		
	Odds Ratio	95% CI	P	Odds Ratio	95% CI	P
Age				0.96	0.95–0.98	<0.001***
Gender (Male vs. Female)				0.30	0.23–0.40	<0.001***
***COMT* Val/Val genotype**	1.05	0.74–1.51	0.747	1.09	0.75–1.58	0.764
***MTHFR* C/T + T/T genotype**	1.06	0.72–1.52	0.816	1.06	0.72–1.57	0.669
***COMT* Val/Val × *MTHFR* C/T + T/T genotype**	0.58	0.35–0.96	0.034*	0.57	0.33–0.97	0.039*

OR = odds ratio; 95% CI = 95% confidence interval. Covarying for age and gender; reference groups are *COMT* Val/Met + Met/Met, *MTHFR* C/C genotypes, and healthy control groups.
*P < 0.05, ***P < 0.001.

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L.J.W. and S.Y.L. wrote the first draft. L.J.W., S.L.C., Y.H.C. and T.Y.W. managed the lab work and statistics. S.Y.L., T.Y.W., P.S.C., S.Y.H., N.S.T., K.C.C., I.H.L. and Y.K.Y. managed participant recruitment. R.B.L. supervised this work and edited the manuscript. All authors reviewed the manuscript.

Additional information

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