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 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.
to BPD\textsuperscript{2}, which suggests that the disorder is characterized by a high-dopamine state. In addition, the \textit{COMT} Val158Met polymorphism is associated with rapid cycling in BPD\textsuperscript{4,14}. However, whether the interaction of the \textit{COMT} Val158Met polymorphism with other dopaminergic genes is associated with the risk of BP-II warrants additional studies.

Methylenetetrahydrofolate reductase (\textit{MTHFR}) is a crucial enzyme involved in one-carbon metabolism\textsuperscript{17}. The reduction of \textit{MTHFR} activity may compromise DNA methylation\textsuperscript{18}. Therefore, genetic variations of the \textit{MTHFR} gene might affect \textit{COMT} methylation and \textit{COMT} function. Decreased \textit{MTHFR} activity has been associated with other psychiatric disorders, for example, schizophrenia\textsuperscript{19} and affective disorders\textsuperscript{20}. The \textit{G677T} (rs1801133) variant is a common functional SNP in \textit{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\textsuperscript{21}. The low-functioning \textit{677T} allele of the common SNP of \textit{MTHFR}, \textit{677C} > \textit{T}, has been significantly associated with the risk of BP-II\textsuperscript{22–24}. However, others have reported borderline or inconsistent results\textsuperscript{25–28}.

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\textsuperscript{29,30}. Specifically, both the \textit{COMT} Val and \textit{MTHFR} T alleles tend to decrease dopamine below optimal levels (left side of the inverted-U curve), and the \textit{COMT} Met and \textit{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 \textit{COMT} and \textit{MTHFR} genes, but also their interaction, might be involved in dopamine dysregulation which contributes to the pathogenesis of psychiatric disorders\textsuperscript{31}. Association studies have supported the notion that, high dopamine levels contribute to the risk of BPD\textsuperscript{3}; thus, based on the inverted U theory, we hypothesize that the high dopamine level, a result of the combination of the low-functioning \textit{COMT} enzyme (\textit{COMT} Met allele) in the background of the \textit{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 \textit{COMT} Val/Val and \textit{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 \textit{COMT} and \textit{MTHFR} gene polymorphisms and BP-II, because individual genes may have only a small effect on the pathogenesis of BPD\textsuperscript{30,32}. In addition, we tried to control for the clinical heterogeneity of BPD by recruiting only patients with a homogenous subtype of BPD: BP-II.

\section*{Methods}

\textbf{Participants.} The research protocol was approved by the Institutional Review Board for the Protection of Human Subjects at Tri-Service General Hospital and 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\textsuperscript{33}, which has good inter-rater reliability\textsuperscript{34,35}, 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\textsuperscript{36} 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\textsuperscript{37,38}. Angst et al.\textsuperscript{39} 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\textsuperscript{40,41}.

The healthy control group (hereafter, Controls) were volunteers recruited from the community. The SADS-L\textsuperscript{42} 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.

\textbf{Blood Samples and Genotyping.} Twenty milliliters of venous blood was collected using venipuncture of the antecubital vein. DNA was isolated from lymphocytes. The \textit{COMT} Val158Met polymorphism was genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis\textsuperscript{43}. The genotyping of the \textit{MTHFR} Val158Met polymorphism was done using a modified protocol described elsewhere\textsuperscript{44}. 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\%.

\textbf{Statistical Analysis.} Independent \textit{t} tests were used to determine the mean age differences between BP-II patients and Controls. Gender difference was analyzed using \textit{χ}\textsuperscript{2} tests.

Pearson \textit{χ}\textsuperscript{2} analysis (two-tailed) was used to analyze the differences in the genotype and allele frequencies of the \textit{COMT} Val158Met and \textit{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 \textit{COMT} and \textit{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 \textit{COMT} and \textit{MTHFR}, and the interaction of the \textit{COMT} and \textit{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 \textit{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 3.1.3, and the effect-size conventions were set as described elsewhere\textsuperscript{45}.

\section*{Results}

Thirty-one patients previously diagnosed with BP-II were excluded for not meeting the present study’s diagnostic criteria for BP-II \textit{n} = 26 or comorbidities of other major mental illnesses \textit{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 \textit{COMT} Val158Met and \textit{MTHFR} C677T polymorphisms for each group were in Hardy-Weinberg equilibrium (\textit{P} > 0.05). For \textit{COMT} Val158Met (Table 2), neither the genotypes (\textit{P} = 0.075) nor the allelic distribution (\textit{P} = 0.093)
the C/T polymorphism of the MTHFR genes have been associated with BP, the association of the MTHFR genes in the BP-II group. Although both 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\(^5\): small effect size = 0.10, medium effect size = 0.30, large effect size = 0.50 for the \(\chi^2\) 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 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\(^7\) and is involved in methylating DNA and metabolizing homocysteine in downstream biochemical processes\(^8\). Therefore, the MTHFR C677T 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\(^9\). 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\(^10\). Most other studies found the interactive effect of the COMT Val allele and MTHFR T allele associated with prefrontal and cognitive dysfunction in schizophrenia\(^11\). 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.\(^12\) 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\(^13\). 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\(^14\). However, these contradictory hypotheses require further investigation.

In the Pearson \(\chi^2\) 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

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

| Group [n] | BP-II (531) | Controls (447) | \(\chi^2\) | 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)     |        |    |

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

| Group [n] | BP-II (531) | Controls (447) | \(\chi^2\) | 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)     |        |    |
focusing on other SNPs or haplotypes of the C677T polymorphism was associated with BP-II. Our finding differed from others' which reported that the low-functioning 677T allele is a risk factor for BPD. In other words, the Val allele is involved in lower prefrontal dopaminergic activity, and those with the COMT Val158Met 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 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 COMT 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 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 catalysis, 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, it is possible that, using DSM diagnostic criteria, some of our patients would be diagnosed with unipolar depression. The Bridge study 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 multivariable 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 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. 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 catalysis 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 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 (%) | χ² | P | OR | 95% CI |
|-----------------------------|-----|-----------|-------------|----|---|----|-------|
| **MTHFR C677T C/C Genotype** |     |           |             |    |   |    |       |
| COMT Val158Met Genotypes    |     |           |             |    |   |    |       |
| 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    |     |           |             |    |   |    |       |
| 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.

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

| Variable | Model 1 |         | P | Model 2 |         | P |
|----------|---------|---------|---|---------|---------|---|
|          | Odds Ratio | 95% CI |   | Odds Ratio | 95% CI |   |
| Age      | 0.96     | 0.95–0.98 | <0.001*** | 0.96     | 0.95–0.98 | <0.001*** |
| Gender (Male vs. Female) | 0.30 | 0.23–0.40 | <0.001*** | 0.30 | 0.23–0.40 | <0.001*** |
| COMT Val/Val genotype | 1.05 | 0.74–1.51 | 0.747 | 1.05 | 0.74–1.51 | 0.747 |
| MTHFR C/T + T/T genotype | 1.06 | 0.72–1.52 | 0.816 | 1.06 | 0.72–1.52 | 0.816 |
| 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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Authors Contributions
L.J.Wood 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.

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