Original Article

Associations between Brain-derived Neurotrophic Factor Val66Met Polymorphism, Melancholic Feature, and Treatment Refractoriness in Patients with Treatment-Resistant Depression

Yu-Shiou Lin, M.D.[1,2], Shih-Jen Tsai, M.D.[1,2], Mu-Hong Chen, M.D., Ph.D[1,2]*

[1]Department of Psychiatry, Taipei Veterans General Hospital, [2]Department of Psychiatry, College of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan

Abstract

Background: Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism is related to the pathophysiology of treatment-resistant depression (TRD). But whether the Val66Met polymorphism is associated with the clinical manifestations of TRD (such as treatment refractoriness and melancholic and anxious distress features) remains unclear. **Methods:** Totally, 106 patients with TRD were genotyped for the *BDNF* Val66Met polymorphism. We used the 17-item Hamilton Depression Rating Scale evaluate depressive symptoms (melancholic and anxious distress features) and Maudsley Staging Method to measure treatment refractoriness. Logistic regression models were constructed to study the relationships among the Val66Met polymorphism, melancholic or anxious distress features, and treatment refractoriness. **Results:** The risk of Val/Met heterozygosity was associated with significantly greater melancholic features than that of Val/Val homozygosity (odds ratio [95% confidence interval (CI)] = (4.67 [1.16–14.24], $p < 0.05$). The melancholic feature in Val/Met heterozygosity was significantly higher to have the risk in treatment refractoriness than that of Val/Val homozygosity odd ratio (95% CI) = (6.42 [1.70–24.25], $p < 0.05$). **Conclusion:** Patients with TRD carrying the *BDNF* Val/Met genotype are more likely to present with melancholic feature, which is in turn related to high treatment refractoriness.

Key words: genetics, heterogenesity effect, major depressive disorder, the brain-derived neurotrophic factor homozygous Met/Met polymorphism

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Introduction

Major depressive disorder (MDD) is a prevalent illness-causing great socioeconomic burden and many medical comorbidities due to its chronic and recurrent nature [1]. Despite the rapid evolution of psychopharmacology over the past 50 years, the concordant care recommended by current guidelines cannot be achieve complete remission with the index antidepression treatment [2]. The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) project revealed that more than 30% of patients failed to achieve remission despite trying all possible options, including switching medications, augmenting treatment, and combination therapy [2]. Treatment-resistant depression (TRD) has thus emerged, and its pathomechanism remains unclear.

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, which plays a crucial rôle in the regulation of neuronal maintenance, differentiation, and survival. BDNF is widely expressed in the human brain and particularly abundant in the hippocampus, cortex, and basal forebrain [3]. Despite not necessarily serving as the initial trigger for depression, BDNF deficiency or dysregulation may result in accelerated cell damage and other associated symptoms [3]. Val66Met (rs6265) is a naturally occurring functional variant found in 25%–30% of humans [4, 5]. The

[1]*Corresponding author. No. 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan.
E-mail: Mu-Hong Chen <kremer7119@gmail.com>

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replacement of Val by Met may impair activity-dependent BDNF secretion, resulting in decreased trafficking of *BDNF* transcripts to dendrites [4]. Thus, studying the *BDNF* Val66Met polymorphism in MDD and TRD is promising [4, 5].

Increasing evidence suggests a role of the *BDNF* Val66Met polymorphism in MDD pathophysiology. A meta-analysis of 14 studies involving 2,812 patients with MDD and 10,843 nondepressed controls revealed significant effects in both the allelic and genotypic analyses in men (odds ratio [OR] (Met allele), 95% confidence interval [CI] = 1.27 (1.10–1.47); OR (Met/Met), 95% CI = 1.67 (1.19–2.36) [6]. However, Li et al. collected mood phenotypic and genetic data from more than 90,000 individuals from diverse ethnic groups; the *BDNF* Val66Met polymorphism was not associated with MDD in European or Asian populations [7]. Those conflicting findings may imply MDD heterogeneity. Therefore, in a study of the *BDNF* Val66Met polymorphism, the symptomology and treatment refractoriness of patients with TRD should be considered.

Studies have indicated conflicting results regarding the role of the *BDNF* Val66Met polymorphism in the clinical manifestations, including melancholic and anxious distress features, of MDD. Rimay et al. detected the allele frequency of the variant in childhood-onset depression and show no differences in the allele frequencies of patients with and without melancholic features [8]. Quinn et al. discovered no association between the *BDNF* Val66Met polymorphism and the presence of melancholic feature [9]. However, an imaging study has shown significantly lower gray matter volume in the left hippocampi of patients with MDD and melancholic feature with any Met allele than in those with both Val alleles [10]. Furthermore, Ryu et al. investigated the effects of the *BDNF* Val66Met polymorphism and resilience on anxiety and depression symptoms measured with the Hospital Anxiety and Depression Scale in patients with a new diagnosis of type 2 diabetes mellitus and discovered that anxiety and depression scores are remarkably higher in Met carriers than in homozygous Val/Val carriers [11]. Gatt et al. indicated that *BDNF* Met carriers exposed to high early-life stress had lower than normal hippocampal and lateral prefrontal cortical volumes and working memory decline, which are biomarkers of TRD [12]. Moreover, they found that the interaction of the *BDNF* Met allele and early-life stress was related to greater depression and anxiety [12]. However, the complex relations between the *BDNF* Val66Met variant and clinical manifestations, particularly melancholic and anxious distress features, among patients with TRD remain unknown.

Several meta-analyses have indicated a more favorable treatment response to antidepressants in *BDNF* Met carriers than in Val/Val carriers [13-15]. Wissam et al. revealed considerably improved treatment response to escitalopram in Met carriers and found that homozygous Val/Val carriers have high antidepressant resistance [16]. Furthermore, the *BDNF* Val66Met variant may affect the response of patients with TRD to low-dose ketamine infusion. Two studies suggested that patients carrying a Met allele exhibit a lower treatment response to low-dose ketamine infusion than patients with the Val/Val genotype [17, 18]. Therefore, whether the *BDNF* Val66Met variant affects the treatment refractoriness of patients with TRD merits further investigation.

In the current study, we investigated the role of the *BDNF* Val66Met (rs6265) polymorphism in the clinical manifestation of melancholic and anxious distress features and treatment refractoriness among patients with TRD. Given that the replacement of Val by Met in the coding exon of *BDNF* at position 66 (Val66Met) disrupts neuronal processing, trafficking, and activity-dependent secretion of BDNF [19], we hypothesized that patients with TRD carrying any Met allele would have been more likely to have melancholic and anxious distress features and high treatment refractoriness levels compared with those with the Val/Val genotype.

Methods

Participants

In this study, we enrolled adult patients aged between 20 and 64 years who met *the DSM-5* criteria of major depressive disorder and failed to respond to two or more antidepressants with adequate dose (i.e., fluoxetine ≥ 20 mg/day) and treatment duration (≥8 weeks) during the current episode as patients with TRD in our study. Excluded were patients with TRD who had major medical or neurological diseases or a history of alcohol or substance use disorders in the current study. The 17-item Hamilton Depression Rating Scale (HDRS) was used for the evaluation of depressive symptoms. In addition, age at disease onset and duration of illness were also assessed. Given that type 1 error was set as 0.1, power was set as 0.8 and the ratio between Val/Met and Val/Val was about 2.5: 1 in Taiwanese population, the estimated sample sizes of Val/Met and Val/Val were at least 58 and 23, respectively. This study was approved by the institutional review board at Taipei Veterans General Hospital (protocol number = 2012-04-037B and 2016-08-001C as well as date of approval = April 20, 2012, and September 8, 2016, respectively,) with the stipulation of obtaining informed consent from all the participants.

Definition of melancholic feature

The melancholic feature was based on ≥ 12 of the total scores at six items of original 17-item HDRS ($HDRS_6$), including depressed mood, feelings of guilt, work and interest, psychic anxiety, general somatic symptoms, as well as psychomotor retardation [20]. Melancholia subscale has been commonly used in previous studies [21, 22].

Definition of anxious distress feature

Anxious distress was defined as major depressive disorder with a total score of 7 or more on the HDRS-anxious distress. Six items from the original 17-item HDRS make up HDRS-anxious distress: psychic anxiety, somatic anxiety, somatic symptoms-gastrointestinal, somatic symptoms-general, hypochondriasis, and insight [23]. HDRS-anxious distress subscale has been used in STAR*D and was proven reliable in assessing anxious distress of depression [24].

Definition of level of treatment refractoriness

As treatment refractoriness in depression involving many dimensions, degrees of treatment refractoriness were measured by a point-based staging model, the Maudsley staging method (MSM) [25]. The MSM contains three factors: treatment history (i.e., numbers of antidepressant treatment failures and if augmentation or electroconvulsive therapy had been used), severity of symptoms based on total scores of HDRS, and duration of presenting episode [25]. Levels of treatment refractoriness were divided based on the total scores of MSM to low (≤ 7), moderate [8–10], and high (≥ 11).

Brain-derived neurotrophic factor Val66Met polymorphism/genotyping

Genomic DNA was extracted from EDTA-containing venous blood samples for BDNF Val66Met polymorphism genotyping. The DNA fragments of interest were amplified using polymerase chain reaction (PCR) with the primers 5′-ACTCTGGAGAGCGTGAAT-3′ and 5′-ATACTGTCACACACGCTC-3′. The PCR was performed in a total volume of 10 μl containing 50 ng of template DNA, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 μM of dNTP, 10 pmol of each oligonucleotide, and 0.25 U of *Taq* DNA polymerase. Amplification conditions consisted of an initial 4-min denaturation step at 94°C, 32 cycles of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C, followed by a final extension of 10 min at 74°C. The Val66Met polymorphism was differentiated with the *Nla*III restriction enzyme. Partial digestion was minimized by an internal restriction site and a control sample of digestible homozygous Val/Val.

Statistical analysis

Demographic analysis was used for the demographic and clinical characteristics, prevalence of melancholic feature and anxious distress, level of treatment refractoriness, and distribution of BDNF Val66Met polymorphism. Logistical regression models with adjustment of age, sex, age at onset, and duration of illness were done for the relationship of BDNF Val66Met polymorphism (Val/Val, Val/Met, Met/Met) with melancholic feature, anxious distress, and level of treatment refractoriness (mild-moderate vs. severe). Additional logistical regression models with adjustment of age, sex, age at onset, duration of illness, and BDNF Val66Met polymorphism were used to investigate the association between melancholic feature, anxious distress, and level of treatment refractoriness. Patients carrying Val/Val were regarded as reference group in the logistical regression models since evidence indicated that the Met allele is the risk allele for major depressive disorder [26]. Sample size estimation and power analysis were done using the G*Power Software.

All data processing and statistical analyses were done using Statistical Package for Social Science software for Windows (SPSS Inc., Chicago, Illinois, USA). The differences between the groups were considered significant if $p < 0.05$.

Results

A total of 106 patients with TRD were included in the study, with a mean age of 47.24 ± 10.71 years, a mean disease onset age of 35.43 ± 11.44 years, an average duration of illness of 12.11±8.18 years, and female predominance (72.6%) (Table 1). The total HDRS scores were 22.67 ± 4.16. Among them, 34 (32.1%) patients.

Exhibited the melancholic feature, whereas 59 (55.7%) had anxious distress feature (Table 1). Levels of treatment refractoriness were measured by Maudsley staging method (MSM), in which 91 (85.8%) patients were defined as mild-moderate treatment refractoriness and fifteen (14.2%) patients were defined as severe treatment refractoriness (Table 1). As for genotyping, patients carrying heterozygous Val/Met accounted for the largest number (55.7%), while patients with Val/Val and Met/Met alleles accounted for 21.7% and 22.6%, respectively (Table 1).

Logistic regression models with adjustment of age, sex, age at onset, and duration of illness reported that only heterozygous Val/Met alleles of BDNF rs6265 polymorphism were significantly related to melancholic feature (4.67, [1.16–14.24], $p < 0.05$), but not associated with anxious distress feature (1.31, [0.45–3.78]) and levels of treatment refractoriness (1.68, [0.33–8.71]) in patients with TRD (Table 2). Based on that the event rate of Val/Met was 0.424 and type 1 error was set as 0.05 in the current study, the statistical power was 0.845. The homozygous Val/Val and Met/Met alleles were not associated with melancholic and anxious distress feature and levels of treatment refractoriness (Table 2).

Logistic regression models of anxious distress and melancholic features with levels of treatment refractoriness demonstrated that melancholic feature was related to the higher

Table 1. Demographic data, clinical characteristic, and brain-derived neurotrophic factor Val66Met polymorphism in patients with treatment-resistant depression (n=106)

	Patients with TRD, *n* (%)
Age (years), mean ± SD	47.24 ± 10.71
Sex	
Male	29 (27.4)
Female	77 (72.6)
Age at disease onset (years), mean ± SD	35.43 ± 11.44
Duration of illness (years), mean ± SD	12.11 ± 8.18
HDRS (total), mean ± SD	22.67 ± 4.16
Melancholic feature	34 (32.1)
Anxious distress feature	59 (55.7)
MSM	
Mild-moderate	91 (85.8)
Severe	15 (14.2)
BDNF Val66Met polymorphism	
Val/Val	23 (21.7)
Val/Met	59 (55.7)
Met/Met	24 (22.6)

SD, standard deviation; HDRS, 17-item Hamilton Rating Scale for Depression; MSM, Maudsley staging method; BDNF, brain-derived neurotrophic factor; TRD, treatment-resistant depression

Table 2. Logistic regression model of brain-derived neurotrophic factor Val66Met polymorphism with melancholic feature, anxious distress feature, and level of treatment refractoriness[§]

BDNF Val66Met polymorphism	Melancholic feature		Anxious distress feature		Severe treatment refractoriness	
	n (%)	OR (95% CI)	*n* (%)	OR (95% CI)	*n* (%)	OR (95% CI)
Val/Val	4 (17.4)	1 (reference)	14 (60.9)	1 (reference)	2 (8.7)	1 (reference)
Val/Met	25 (42.4)	4.67 (1.16-14.24)*	38 (64.4)	1.31 (0.45-3.78)	8 (13.6)	1.68 (0.33-8.71)
Met/Met	5 (20.8)	1.54 (0.33-7.23)	7 (29.2)	0.33 (0.09-1.24)	5 (20.8)	3.30 (0.53-20.48)

*$p < 0.05$ significantly different
[§]Adjusted for age, sex, age at onset, and duration of illness
OR, odds ratio; CI, confidence interval; BDNF, brain-derived neurotrophic factor

level of treatment refractoriness (6.42, [1.70–24.25], $p < 0.05$) in patients with TRD (Table 3). Anxious distress feature was not associated with the level of treatment refractoriness (Table 3).

Discussion

Our study findings based on a clinical sample of 106 patients with TRD, including 34 with melancholic feature and 59 with anxious distress feature (Table 1), partially supported the study hypothesis that the heterozygous Val/Met genotype, but not the homozygous Met/Met genotype, are significantly related to an increased likelihood of melancholic feature (OR [95% CI] = 4.67 [1.16–14.24], $p < 0.05$) in patients with TRD (Table 2). Furthermore, patients with melancholic feature were significantly more have high treatment refractoriness (OR [95% CI] = 6.42 [1.70–24.25], $p < 0.05$) than those without melancholic feature (Table 3).

Several studies have suggested the rôle of BDNF in the presence of melancholic feature in patients with MDD [27]. Primo de Carvalho Alves et al. assessed 151 in patients with severe depression and discovered a negative correlation between BDNF levels and $HDRS_6$ scores but no correlation between BDNF levels and other depressive symptom scores, such as those for depressed mood, psychic anxiety, or somatic symptoms [27]. Furthermore, Kotan et al. have reported a negative correlation between BDNF levels and the number of depressive episodes in patients with severe melancholic depression [28]. Because the Met allele at position 66 (Val66Met) disrupts BDNF secretion and results in low serum BDNF levels [19], our results may partially explain the association between the *BDNF* Val66Met variant and melancholic feature. Counterintuitively, we found no association between the homozygous Met/Met genotype and melancholic feature. But whether BDNF levels are related to the Val66Met polymorphism remains uncertain [29]. Colle et al. demonstrated that plasma BDNF levels are linearly associated with *BDNF* Val66Met genotype, but Kumar et al. have discovered no association of the variant with serum BDNF levels [29, 30]. In addition, Rimay et al. and Quinn et al. have uncovered no differences in the allele frequencies of the *BDNF* Val66Met variant between patients with depression with and without melancholic feature [8, 9]. Further studies are required to elucidate the roles of the *BDNF* Val66Met polymorphism in the presence of melancholic feature and TRD.

Table 3. Logistic regression of anxious distress and melancholic features with level of treatment refractoriness[§]

	Severe treatment refractoriness	
	n (%)	OR (95% CI)
Melancholic feature		
Absent	5 (6.9)	1 (reference)
Presence	10 (29.4)	6.42 (1.70-24.25)*
Anxious distress feature		
Absent	4 (8.5)	1 (reference)
Presence	11 (18.6)	3.29 (0.79-13.69)

*$p < 0.05$ significantly different
[§]Adjusted for age, sex, age at onset, duration of illness, and BDNF Val66Met polymorphism.
OR, odds ratio; CI, confidence interval; BDNF, brain-derived neurotrophic factor

Our results indicated that melancholic feature is related to treatment refractoriness among patients with TRD, a result compatible with the findings of the STAR*D study [31]. Rush et al. revealed that the prevalence of melancholic feature is increased with the use of more advanced treatment strategies, from 19.7% after Step 1 (citalopram monotherapy) to 30.5% after Step 4 (venlafaxine and mirtazapine combination therapy) [31]. A prospective study of 1,455 outpatients with depression demonstrated that patients with melancholic feature are more severely depressed and had more depressive episodes than those without melancholic feature [32].

Study limitations

The readers are warned against over-interpreting our study results because this study has three major limitations:

- The small sample size of 106 patients with TRD with only 24 Met/Met carriers may limit the power of our study findings. But our study is one of the few studies to explore the rôle of the *BDNF* Val66Met variant in the clinical manifestations of TRD. Further studies with large sample sizes are necessary to corroborate our results.
- Whether our results are generalizable to patients of other ethnicities, particularly to Caucasian patients, requires further investigation because of the considerable ethnic difference in Met allele frequency [6, 33].
- Previous studies suggested that BDNF Met carriers may more expose to early-life psychosocial stress compared with Val carriers, which may be related to the increased

risk of subsequent antidepressant resistance [34]. Early-life psychosocial stress was unavailable in this study; therefore, we could not assess the impact in our study. Further studies are necessary to clarify the complex issues among BDNF Val66Met polymorphism, early-life stress, and antidepressant resistance.

Summary

Patients with TRD carrying the heterozygous Val/Met genotype are more likely to have melancholic feature than are those with the homozygous Val/Val genotype. Furthermore, melancholic feature is further associated with increased treatment refractoriness in patients with TRD. Further prospective studies are required to investigate causal relationships between genes and clinical presentations in patients with MDD and TRD.

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Conflicts of Interest

Shih-Jen Tsai, an editorial board member at *Taiwanese Journal of Psychiatry* (Taipei), had no role in the peer review process of or decision to publish this article. The other authors decalared no conflicts of interest in writing this paper.

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