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Association of brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism with early-onset bipolar disorder

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Abstract

Objectives—Brain-derived neurotrophic factor (*BDNF*) Val66Met (rs6265) functional polymorphism has been implicated in early-onset bipolar disorder. However, results of studies are inconsistent. We aimed to further explore this association.

Methods—DNA samples from the Treatment of Early Age Mania (TEAM) and Mayo Clinic Bipolar Disorder Biobank were investigated for association of rs6265 with early-onset bipolar disorder. Bipolar cases were classified as *early onset* with the definition of first manic or depressive episode at age ≤ 19 years (versus adult-onset cases at age > 19 years). After quality

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control, 69 TEAM early-onset bipolar disorder cases, 725 Mayo Clinic bipolar disorder cases (including 189 early onset cases), and 764 controls were included in the analysis of association, assessed with logistic regression assuming log-additive allele effects.

Results—Comparison of TEAM cases with controls suggested association of early-onset bipolar disorder with the rs6265 minor allele [odds ratio (OR) = 1.55, $p = 0.04$]. Although comparison of early-onset adult bipolar disorder cases from Mayo Clinic versus controls was not statistically significant, the OR estimate indicated the same direction of effect (OR = 1.21, $p = 0.19$). When the early-onset TEAM and Mayo Clinic early-onset adult groups were combined and compared with the control group, the association of the minor allele rs6265 was statistically significant (OR = 1.30, $p = 0.04$).

Conclusions—These preliminary analyses of a relatively small sample with early-onset bipolar disorder are suggestive that functional variation in BDNF is implicated in bipolar disorder risk and may have a more significant role in early-onset expression of the disorder.

Keywords

association; BDNF; bipolar disorder; brain-derived neurotrophic factor; early onset; Val66Met

Early-onset bipolar disorder (EO-BD) has been associated with substantial morbidity characterized by rapid cycling, severe impairment, prolonged time to remission, poor treatment response, and increased psychiatric comorbidity (1–4). It has been estimated that the prevalence of bipolar disorder (BD) among children exceeds 1%, which calls for more research to enhance early identification, understanding, and management of this not-uncommon disease (5). Familial studies provide compelling evidence for a strong genetic contribution to BD (6), with heritability estimates, based on twin studies, of between 64% and 85% (7, 8). EO-BD has been shown to increase the familial risk rate substantially more than late-onset BD (LO-BD) (3). Despite the increased severity of EO-BD, as well as the strong evidence of heritability, the genetic basis of this disorder is undetermined.

BD is a heterogeneous disease that presents considerable challenges in the study of genetic risk factors (9). Investigators have proposed that classifying BD into subgroups with specific subphenotypes may facilitate the determination of associated genotypes (10). EO-BD may represent a clinically distinct BD subgroup with specific genetic risk factors (1). Consistent with this proposition, polymorphisms in at least 17 genes have been reported to be associated with EO-BD, including the brain-derived neurotrophic factor (*BDNF*) gene (2, 3, 11–21).

Brain-derived neurotrophic factor (BDNF) is an important neurotrophic factor in the central nervous system that has a role in growth, maintenance, and differentiation of neural cells (22). The *BDNF* gene is located in chromosome 11 at 11p13. BDNF rs6265 polymorphism is a common functional, nonsynonymous single nucleotide polymorphism (SNP) in which the amino acid valine (Val) is substituted with methionine (Met) (23). Because of its important neurologic functions, *BDNF* has been studied extensively as a candidate gene for BD, as well as other psychiatric illnesses (24).

The BDNF rs6265 (Val66Met) polymorphism has been evaluated in subphenotypes of BD. Two case-control candidate gene studies found an association between *BDNF* and EO-BD that was not identified in LO-BD (25, 26). Several studies have also reported an association of Val66Met with different age at onset among BD patients (27, 28). Non-DNA studies have further implicated reduced lymphocytic *BDNF* gene expression in EO-BD (29). Collectively, these studies are suggestive of a potential association between BDNF Val66Met polymorphism and EO-BD. Further studies in this area could elucidate the role of *BDNF* in EO-BD.

The aim of the present study was to further explore the association of BDNF Val66Met SNP with EO-BD through a case-control candidate gene study.

Methods

Ethics statement

Protocols and procedures were approved by the Mayo Clinic Institutional Review Board and the institutional review boards associated with the Treatment of Early Age Mania (TEAM) study. All participants provided informed consent.

Participants

The present study contained three participant groups: pediatric and adolescent BD patients from the TEAM study (n = 82), adult BD patients from the Mayo Clinic Bipolar Disorder Biobank (n = 855), and an adult healthy control group from the Mayo Clinic Biobank (n = 857) (30). The TEAM study was a randomized clinical trial comparing antimanic treatments (31). TEAM participants were outpatients [mean (standard deviation) age = 10.1 (2.8) years] with a diagnosis of *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition) (DSM-IV) BD type I, manic, or mixed episodes. The inclusion and exclusion criteria are described elsewhere (31). Blood draw for future DNA studies was done before drug randomization.

Adult cases from the Mayo Clinic Bipolar Disorder Biobank included 855 participants with a diagnosis of BD according to the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition, Text Revision) (DSM-IV-TR), aged 18 to 80 years at enrollment. Cases included participants with diagnoses of BD type I and BD type II. Exclusion criteria were the inability to speak English, inability or unwillingness to provide consent, and actively psychotic or suicidal state. From the Mayo Clinic Biobank, 857 participants were selected to be in the control group (30). Depending on self-report and electronic health records, the exclusion criteria were a current or prior psychiatric condition, including diagnosis of BD, clinical depression, schizophrenia, attention-deficit hyperactivity disorder (ADHD), autism, and Down syndrome. Participants who reported having a first-degree relative with BD were also excluded. Controls were matched to adult cases from the Mayo Clinic BD Biobank for ethnicity/race, age, and gender.

Study assessment

The diagnosis of TEAM participants was confirmed by the Washington University in St. Louis (Missouri) Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) for DSM-IV. In the definition of a manic episode, severity level had to be 4 and a substantial impairment must be present. A score of 60 on the Children's Global Assessment Scale was used to indicate serious impairment. To define a mixed episode, DSM-IV criteria had to be met for both mania and depressive disorders in coinciding time frames. Among the participants, 100% had elated mood or grandiosity; 82.6%, psychotic symptoms; and 95.7%, mixed episodes. Independent raters evaluated children and their primary caretakers. The results were combined through the highest scores from either the children or their primary caretaker ratings. Inter-rater reliability was 90% for psychiatric diagnoses, including mania, on the WASH-U-KSADS.

Adults with BD from the Mayo Clinic BD Biobank were assessed with the Structured Clinical Interview (SCID) for DSM-IV-TR. A patient questionnaire and clinical questionnaire were also used in the assessment, to obtain more information about the past medical and medication history of study participants. A subset of the adult BD cases were classified as *early-onset BD*, defined as first manic or depressive episode at age 19 years ($n = 189$), based on a review of the SCID and electronic medical record, when available. To produce a more homogeneous sample, we set the age threshold lower (19 years) than in earlier studies (1, 13, 14, 32). Demographics and main clinical characteristics of TEAM and adult BD cases are summarized in Table 1.

Genotyping and quality control

DNA specimens were isolated from blood samples. TEAM samples ($n = 82$) were genotyped with the TaqMan assay (Life Technologies) at Washington University; adult BD cases ($n = 855$) and controls ($n = 857$) from the Mayo Biobanks were genotyped using the same TaqMan assay on an ABI 7900 HT instrument (Applied Biosystems) at the Karolinska Institutet. Ten adult BD cases from the Mayo biobank were genotyped at Washington University with the TEAM EO-BD samples for quality control, obtaining 100% concordant genotype calls at the Karolinska Institutet and Washington University for subjects genotyped in duplicate. To reduce the chances of confounding through population stratification, analyses were conducted only on Caucasian participants of European ancestry. After excluding nonwhite participants, as well as the participants whose genotyping had failed, 69 TEAM EO-BD cases, 725 adult Mayo Clinic Bipolar Disorder Biobank cases (189 of early onset), and 764 controls were included in the analyses. The genotype frequencies demonstrated no evidence of departures from Hardy–Weinberg Equilibrium within TEAM cases, adult cases, controls and overall (all p -value > 0.10).

Statistical analysis

To test the hypothesis of association between BDNF Val66Met SNP and EO-BD, we performed four genetic association analyses using logistic regression with log-additive allele effects (i.e., coding genotypes as 0, 1, and 2 representing the number of minor alleles). The first analysis assessed the association between BD in adult cases and the studied SNP, comparing the Mayo Clinic controls with the Mayo Clinic adult cases. The second analysis

compared TEAM samples with controls to test the association of Val66Met with pediatric-onset BD specifically. In the third analysis, we tested for association of EO-BD with the BDNF SNP by comparing the subgroup of early-onset adult Mayo Clinic cases (< 19 years of age at disease onset) with the controls. In the fourth analysis, we compared the controls with the combined group of early-onset cases consisting of the TEAM samples and the early-onset subgroup of adult Mayo cases.

The comparison of all adult Mayo Clinic cases with controls had 80% power to detect effects with odds ratio (OR) > 1.29. The comparison of TEAM sample and adult EO-BD Mayo sample with the controls had 80% power to detect effects with ORs of 1.78 and 1.47, respectively, while the combined EO-BD sample (TEAM + early onset adult Mayo) provided 80% power to detect ORs of 1.41 when compared with the controls.

Statistical analyses were completed with R statistical computing software. A p-value of 0.05 was predefined as threshold for statistical significance.

Results

Association of Val66Met with EO-BD

Comparison of adult BD cases with controls provided nonsignificant evidence of association of the minor Met allele of Val66Met with BD (OR = 1.19, $p = 0.06$). However, comparison of TEAM early-onset cases with controls showed a stronger association of the minor allele with EO-BD (OR = 1.55, $p = 0.04$). Although comparison of adult EO-BD cases from the Mayo Clinic BD Biobank with controls was not statistically significant, the OR estimate indicated the same direction of effect (OR = 1.21, $p = 0.19$). When early-onset adult BD cases from the Mayo BD Biobank and TEAM early-onset cases were together compared with the controls, the association of the minor allele of rs6265 with EO-BD was statistically significant (OR = 1.30, $p = 0.04$) (Table 2).

Discussion

We performed a case-control candidate gene analysis studying the association of Val66Met in *BDNF* with EO-BD in the largest sample to date. Further, the TEAM cases were patients who were younger than in prior studies.

The present data show statistically significant evidence of association between EO-BD and Val66Met, yet show no significant evidence of association of the SNP with BD when comparing adult cases with controls. Evidence of association of the SNP with EO-BD was statistically significant in the comparison of TEAM cases (age: 6–15 years) vs controls and TEAM plus Mayo adult early-onset cases vs controls, but not in the comparison of controls with only the Mayo adult early-onset adult cases (< 19 years of age at disease onset). These findings could be explained by differences between participants in the TEAM study and adult patients from the Mayo Clinic Bipolar Disorder Biobank who were identified retrospectively as having had EO-BD. Differences between the groups may include age at onset and inclusion criteria, differential diagnosis, and diagnosis confirmation method. Specifically, TEAM cases had earlier onset of BD and thus might include cases with a more

severe type of disease, which may be more genetically influenced than the Mayo Clinic BD Biobank early-onset adult cases. Thus, the early-onset adult cases may be an intermediate group that combines patients with pediatric onset, as well as those with early adult onset. On the other hand, knowing the earlier onset of TEAM cases, the evolution of this younger cohort diagnoses were not followed.

Other differences between the TEAM sample and the Mayo Clinic BD Biobank sample may have contributed to the observed difference in *BDNF* association with EO-BD. For example, ADHD was a common comorbidity in the TEAM sample. Knowing the similarity of the clinical presentation of ADHD and EO-BD, the TEAM sample BD diagnosis was differentiated from ADHD on the basis that 100% had elated mood or grandiosity and 82.6% had psychosis, accounting for symptoms that do not present in patients with ADHD solely. However, because one-third of the TEAM participants had ADHD, the possibility of Val66Met being associated with EO-BD comorbidity in ADHD cannot be ruled out.

Furthermore, TEAM study participants had the diagnosis of BD type I or mixed-episode BD, whereas the adult BD sample held a larger number of diagnoses, including BD type I and BD type II. Of note, the TEAM and early onset BD samples only contain subjects with BD type I, making the early onset group a more homogenous group. In addition, different structured clinical interviews were used to confirm the diagnosis in the two samples, which were WASH-U-KSADS and SCID for TEAM participants and early-onset adults from the Mayo Clinic BD Biobank, respectively. The heterogeneity in the set of diagnoses could further explain the difference in the genetic association results.

Our case-control study results agree with some previous reports in the literature. Skibinska and colleagues (26) found no association between adult LO-BD and Val66Met but reported a significant association when a subgroup of EO-BD cases was compared with the control group. Similar findings were reported by Tang and colleagues (25), where the association was present in the early-onset subgroup and absent in the LO-BD group. However, the studies were inconsistent with respect to the genotype associated with the EO-BD. In the study by Tang et al, the Val/Val genotype was associated with higher risk of EO-BD; however, the data of Skibinska et al indicated lower risk in heterozygotes, with higher risk in both types of homozygotes. When we reanalyzed these data, assuming log-additive allele effects, the association was not statistically significant. This inconsistency could be explained by limitations of the previous studies, including small sample size. The inconsistent results also may reflect different genetic effects in groups with different race/ethnicity.

To our knowledge, four meta-analyses of the *BDNF* Val66Met association with BD have been completed. Although 1 meta-analysis found a significant association (33), the other three indicated a lack of association (24, 34, 35). Most prior studies did not account for BD subphenotypes, such as EO-BD, which may have contributed to the lack of consistent findings. Moreover, most previous studies found association (although inconsistent) between the Val allele with BD, with stronger evidence in rapid cycling subgroup (36, 37). However, the Met allele appears deleterious and more functionally relevant. For example, Met carriers have been shown to have a smaller hippocampus with poorer functioning, impaired *BDNF*

cellular trafficking and lower BDNF secretion (38–40). These observations are in line with our finding of the Met allele being associated with EO-BD. One possible explanation for this is epigenetic modifications. In one of the first epi-genome wide association studies, BD subjects with Val/Val genotype were found to have increased methylation of BDNF promotor IX in the prefrontal cortex (41). The authors suggested that the possible association of epigenetic changes with DNA variation is relevant to the inconsistency in genetic association studies of BD. Another explanation might be the possible implication of gene–environment interaction (e.g., childhood adversity) (42).

Although our sample provided statistically significant evidence for the association between Val66Met and EO-BD, these results must be interpreted with caution, given the study limitations. The present study contained a large sample for the control group; however, the EO-BD group still had a relatively modest size ($n = 258$), despite being the largest sample of early-onset cases analyzed to date for association with *BDNF*. Furthermore, a potential retrospective assessment bias might be present in classifying our early-onset adult cases. In addition, methods requiring genome-wide SNP data to control for population stratification (e.g., principal components) were not used in these analyses. However, all subjects were of self-reported European ancestry reducing the chance of confounding by population structure. Nevertheless, the study also had important strengths, including a well-characterized (albeit small) sample of EO-BD cases from the TEAM study.

Our finding of a significant association between a subgroup of BD with early onset and a candidate SNP adds to previous successful studies with similar design based on defining more homogeneous disease subgroups (10). The present study provides further evidence that BD is a heterogeneous disease, and identification of the contributing genetic risk factors may require a more careful approach to phenotyping.

In conclusion, these preliminary analyses are suggestive of association between Val66Met and risk of EO-BD. Future studies need to evaluate this association in larger samples to increase statistical power and allow investigation of secondary phenotypes related to EO-BD. Utilizing enriched EO-BD samples, studying BDNF (i.e., Val66Met variant) gene–environment interactions, as well as the correlation with BDNF methylation patterns is warranted.

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Table 1

Demographics by bipolar disorder case, control, and age at onset status

Characteristic	Controls	TEAM BD	Adults EO-BD only	All adults BD
	(n = 764)	(n = 69)	(n = 189)	(n = 725)
Age at enrollment, years, mean (SD)	41.3 (14.6)	10.5 (2.9)	35.8 (12.7)	42.5 (15.1)
Gender, male, n (%)	312 (40.8)	30 (43.5)	64 (33.9)	299 (41.2)
BD type I, n (%)	–	69 (100)	189 (100)	543 (74.9)
Rapid cycling, n (%)	–	69 (100)	134 (70.9)	420 (58.4)
Psychosis, n (%)	–	57 (82.6)	107 (58.2)	319 (44.9)
Mixed episodes, n (%)	–	66 (95.7)	27 (14.3)	75 (10.5)

TEAM BD = Treatment of Early Age Mania; EO-BD = early-onset bipolar disorder; BD = bipolar disorder; SD = standard deviation.

Table 2

Case-control association of Val66Met variant with bipolar disorder

Case definition	Cases n	Controls n	Cases MAF	Controls MAF	Genotype	Cases n (%)	Controls n (%)	OR	p-value
Adult BD cases	725	764	0.207	0.179	Val/Val	459 (63.3)	513 (67.2)	1.19	0.06
					Val/Met	232 (32)	228 (29.8)		
					Met/Met	34 (4.7)	23 (3)		
TEAM cases	69	764	0.254	0.179	Val/Val	41 (59.4)	513 (67.2)	1.55	0.04
					Val/Met	21 (30.4)	228 (29.8)		
					Met/Met	7 (10.1)	23 (3)		
Early-onset adult BD cases	189	764	0.216	0.179	Val/Val	121 (64)	513 (67.2)	1.21	0.19
					Val/Met	57 (30.2)	228 (29.8)		
					Met/Met	11 (5.8)	23 (3)		
TEAM + early-onset adult BD cases	258	764	0.229	0.179	Val/Val	162 (62.8)	513 (67.2)	1.30	0.04
					Val/Met	78 (30.2)	228 (29.8)		
					Met/Met	18 (7)	23 (3)		

BD = bipolar disorder; MAF = minor allele frequency; OR = odds ratio; TEAM_i = Treatment of Early Age Mania.