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ORIGINAL ARTICLE

The Val66Met polymorphism at the BDNF gene does not influence Wisconsin Card Sorting Test results in children and adolescents with bipolar disorder

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Abstract

Objectives: To assess the role of the Val66Met polymorphism at the brain-derived neurotrophic factor (BDNF) gene on the performance of children and adolescents with bipolar disorder [juvenile bipolar disorder (JBD)] on the Wisconsin Card Sorting Test (WCST). *Methods:* Children and adolescents were assessed by the K-SADS-PL and a clinical evaluation for BD and comorbid conditions. Manic and depressive symptoms were assessed with the Young Mania Rating Scale and the Children Depression Rating Scale – Reviewed. The Val66Met polymorphism at the BDNF was genotyped from a blood sample. Patients' IQ and executive functions were assessed by a standard cognitive flexibility test (WCST). *Results:* Fifty-three subjects were included in the study. No significant difference was observed between the Val/Val and Val/Met+Met/Met groups on any WCST scores in the MANCOVA (F48,5 = .76; p = .59; Perseverative Errors, p = .66; Nonperseverative Errors, p = .58; Categories Completed, p = .34; Attempts to Reach First Category, p=.64; and Percentage of Conceptual Level Responses, p = .99). *Conclusions:* Our findings from this sample of children and adolescents with BD do not replicate results from studies of adults and suggest the existence of differences in the neurobiology of this disorder across the life cycle. Investigations of larger samples are necessary to confirm these data.

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Introduction

Children and adolescents with bipolar disorder [juvenile bipolar disorder (JBD)] present disabling affective and behavioral dysregulation, aggressiveness, and irritability.[1,2] These severe mood swings cause significant developmental impairment. Current studies estimate an occurrence of BD in .6-1% of children and adolescents.[3] The JBD population has a higher propensity of suicide, psychosis, exposure to sexual risks and drug use.[4-6] JBD is also associated with a low symptom remission rate and elevated mood crises recurrence.[7,8]

Neuropsychological studies of adult and pediatric BD have helped elucidate the neurobiological underpinnings of the disorder by revealing deficits in cognitive flexibility (set shifting), planning, working memory, resistance to interference, sustained attention, verbal learning and memory.[9,10] One of the most consistent findings in early-onset BD (although it is not clear whether it is a trait marker in JBD) is a deficit in cognitive flexibility, that is, the ability to modify thinking and behavior in response to changing environmental conditions.[9-13] This ability may be impaired during mood states, when children and adolescents present increased energy and excessive involvement in pleasurable activities and feel irritable due to the inability to interrupt or stop these actions (mania), or during depressive states, when positive events do not impact the child's negative affect or thoughts.

The most widely used test in the assessment of cognitive flexibility in BD is the Wisconsin Card Sorting Test (WCST).[14] A recent investigation of 15 euthymic JBD Type I or II subjects (mean age: 16.4 years) reported they had significantly higher scores in nonperseverative errors in the WCST when compared to healthy controls (n = 15) (p = .038).[13] Another group of investigators compared differences in the WCST performance between JBD children and adolescents (n = 57) and controls (n = 46) and reported trends for differences in perseverative errors, nonperseverative errors and categories completed subtests in WCST, after controlling for Attention-Deficit/Hyperactivity Disorder.[9]

The Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin that plays an important role in neuronal growth, survival, and differentiation during development and is also involved in learning and memory processes.[15,16] The association between JBD and BDNF has been documented by Geller et al.,[16] and the only other gene to have significant positive associations is the SLC6A3 (dopamine transporter) gene (for a review of genetic association studies in JBD, see Mick et al.[17]). Due to the association between BDNF and BD and its participation in the cognitive processes mentioned above, a pioneer study addressed the interaction between BDNF gene polymorphisms and cognitive flexibility in adult patients with BD (n = 54).[18] Patients presenting the Met allele (n = 10) performed more poorly on all of the WCST subtests than patients who were homozygous for the Val allele (n = 44). A subsequent study with a larger sample corroborated the previous conclusions and suggested that the Met allele specifically affects the WCST results of BD patients versus patients with schizophrenia (n = 129) and a control group (n = 160). In another study, the Met allele was also associated with a poor WCST performance only in patients with BD (n = 111).[19,20] Further studies of BDNF revealed that this SNP , a valine-to-methionine substitution at position 66 in the coding region (rs6265), reduces activity-dependent BDNF secretion through disruption in the BDNF-sortilin interaction, which may result in abnormal hippocampal structure and function, and consequently alters cognition processes.[21]

Despite the differences identified in the neuropsychological profile of the JBD subjects according to BDNF genotype and the potential of BDNF in neurodevelopment, few studies have addressed these issues, and, to our knowledge, no previous research has investigated the association of cognitive performance in JBD and the BDNF gene. Neuropsychological studies may indicate the neural circuitry involved in BD deficits, thus confirming findings from neuroimaging investigations suggesting that brain areas such as the frontolimbic cortex (ventral prefrontal cortex, amygdala, and ventral striatum) are implicated in these alterations.[12,22]

The aim of this investigation was to assess the performance of children and adolescents with BD on the WCST according to their BDNF Val66Met polymorphism status. Our hypothesis is that subjects presenting the Met allele at the BDNF will perform less well on the WCST than those who present the homozygosity of the Val allele.

Methods

Subjects were recruited from the community through press releases, other ongoing JBD studies and the Outpatient Program for Children and Adolescents with Bipolar Disorder (ProCAB). Inclusion criteria included age (5 to 17 years) and diagnoses of BD according to DSM-IV criteria.[23] Exclusion criteria were diagnosis of Pervasive Developmental Disorder, Schizophrenia, Substance Abuse or Substance Dependence, or a severe suicide or homicide risk that excluded the possibility of outpatient treatment. Written informed consent from parents and children's verbal assent were obtained. This study was approved by the ethical committee of the Hospital de Clínicas de Porto Alegre (approved as an IRB by the Office for Human Research Protections, United States of America - IRB 00000921), and is in accordance with the Helsinki Declaration of 1975. The ProCAB is registered at www.clinicaltrials.gov under the identifier NCT00116259.

Diagnostic assessment

Diagnostic assessment was conducted in three stages. First, a child and adolescent psychiatrist with extensive training (GP) interviewed primary caregivers about the presence of DSM-IV mood symptoms and the family history of psychiatric disorders. Subjects who met the DSM-IV criteria for Bipolar I, Bipolar II, or Bipolar NOS then underwent neuropsychological and learning disorder assessments. Also at this stage, the K-SADS-PL (Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version) was administered by trained interviewers.[24] The K-SADS-PL training process was conducted in our center and consisted of several seminars about both child psychopathology in general and the structure of the instrument, live observation of five interviews performed by trained research assistants, and live administration of

the K-SADS-PL interview in five patients with the presence of trained observers. Finally, research assistants performed reliability analyses based on final KSDAS interviews recorded on videotape. Kappa coefficient was calculated as .93 for mood disorders in a previous study.[25] Patients and parents answered the mood and substance use modules separately and the other modules together. A different child and adolescent psychiatrist performed a clinical evaluation of BD and comorbid conditions using DSM-IV criteria with parents and subjects and also checked the positive diagnoses derived from K-SADS-PL. Finally, after the entire clinical assessment, best estimate diagnoses were achieved through a discussion with the entire research group. Priority was always given for the final clinical diagnoses formulated if a diagnostic disagreement occurred in other stages of this process.

Measures

Mania symptoms were assessed with the Young Mania Rating Scale (YMRS).[26] This instrument is an 11-item scale for the assessment of severity of mania symptoms that has similar efficiency across different age groups, including youth aged 4-17 years. The YMRS was translated into Portuguese and validated (Kappa .32-.91, intraclass correlation coefficient .8).[27] The presence and severity of depressive symptoms were rated using the Brazilian version of the Children's Depression Rating Scale – Reviewed (CDRS-R).[28,29] Mood states were defined as follows: mania (YMRS ≥ 7 and CDRS-R < 28), depression (YMRS < 7 and CDRS-R ≥ 28), and euthymia (YMRS < 7 and CDRS-R < 28).[29,30]

Genotyping

The genotyping of Val66Met polymorphism was performed with PCR as described by Neves-Pereira et al.[31] Blood (5 mL) was drawn from each subject, and DNA was extracted using the high-salt method.[32] Subjects were genotyped for the BDNF Val66Met SNP that determines a valine-to-methionine substitution at position 66 in the coding region. The SNP for the G→A (valine→methionine) variation at position 758 of the BDNF coding sequence was selected from the National Center for Biotechnology Information SNP database (reference number rs6265). A 113-bp segment was amplified by PCR, using the following primers: 5-GAGGCTTGACATCATTGGCT-3 and 5-GTGTACAAGTCTGCGTCCT-3. Target sequences were amplified in a 25 mL reaction solution containing 125 ng genomic DNA; 1 U Taq polymerase (Sigma-Aldrich); 20 mM Tris-HCl (pH 8.4); 50 mM KCl; 1.5 mM $MgCl_2$; 200 mM each of dATP, dCTP, dGTP, and dTTP; and 10 pmol of each primer. After an initial denaturation of the DNA templates for 5 min at 95ºC, 30 cycles were performed, each consisting of 94ºC for 30 s, 60ºC for 30 s, and 72ºC for 30 s. After the last cycle, samples were incubated at 72ºC for 5 min. Samples were then digested overnight with 3 U of Eco721 (MBI Fermentas). The fragments were separated on a 3.5% agarose gel at 100 V, and fragments were visualized with ethidium bromide. The uncut product size was 113 bp (allele A), and allele G comprised the cut bands of 78 and 35 bp.

Assessment

Intelligence Estimation (IQ). The Vocabulary and Block Design subsets of the Wechsler Intelligence Scale for Children – Third Version (WISC-III) were used to estimate the full scale IQ.[33]

The Wisconsin Card Sorting Test (WCST) is a neuropsychological test that addresses the functions that are primarily connected with prefrontal lobe activity, especially cognitive flexibility.[14] The ability to change a reaction due to unawareness of relevant stimuli is measured by the percentage of perseverative errors (WCST-P), the attentional inability to avoid distraction is measured by the percentage of non-perseverative errors (WCST-NP), the ability to utilize new information and previous experiences is measured by the number of correctly completed categories (WCST-CC), the set to the first category measures the ability to formulate a logical conception (WCST-1stCAT), and conceptual thinking ability is measured by the percentage of conceptual level responses (WCST-%CONC). This set of executive functions evaluates abstract thinking and the ability to develop problem-solving strategies in response to changing stimuli or conditions. The test has been adapted for the Brazilian population.[34]

Statistical analysis

Statistical analyses were performed using the software SPSS for Windows, Version 18. Clinical and demographic characteristics were considered to be confounding variables and were entered as covariables in the models when associated with both the independent factor (presence or absence of the Met allele at the BDNF gene) and the outcome measure (WCST subtest scores) with a flexible p-value of .2. To evaluate normal distribution of the variables, the Shapiro-Wilk test was applied. Differences between the two groups of patients were assessed by the Student's t-test in normal distributions and by the nonparametric Mann-Whitney test in abnormal distributions. Correlations were tested with Spearman's rho or Pearson's r according to the sample distributions. Differences between the Val/Val and the Val/Met-Met/Met carriers at the BDNF Val66Met polymorphism were analyzed using Multiple Analysis of Covariance (MANCOVA) models, which included terms for covariables. All tests of hypotheses used a two-sided alpha = .05.

Results

A total of 53 subjects were included in the study. Demographics and clinical characteristics, as well as measures of symptom severity, are presented in Table 1. No significant differences were observed between genotype groups. Due to the design of our outpatient program (a naturalistic setting), patients usually perform neuropsychological testing while presenting symptoms. However, symptom rates or mood states did not differ between groups. One-third of the patients (16 out of 53) were using either mood stabilizers or atypical antipsychotics by the time of evaluation (data available by request).

The Val/Val genotype composed 75.5% of the sample (40 subjects), the Val/Met genotype 22.6% (12 subjects), and Met/Met only 1.9% (1 subject). In male subjects (n = 33), 26 presented the Val/Val genotype, 6 presented the Val/Met, and 1 presented the Met/Met. In the female group (n = 20),

Val/Val was found in 14 subjects, Val/Met in 6 patients, and there were no Met/Met cases. As the Met/Met genotype at the BDNF was present in only one subject, the Met/Met and Val/Met carrier groups were merged and classified into Met carriers, as has been performed in other investigations.[18] The distributions of genotypes did not deviate from Hardy–Weinberg equilibrium (x^2 = 2.74, p = .26).

The Wisconsin Card Sorting Test scores presented asymmetrical distribution, and their values were normalized using log transformation. Data from WCST-T1C and WCST-%CLR from five patients was not available, and they were thus excluded from the MANCOVA. Current use of medication was not associated to any WCST subtest scores (WCST-P p = .32; WCST-NP p = .83; WCST-CC p = .31; WCST-%CONC p = .28; WCST-1stCAT p = .90) and was not included in the MANCOVA model.

No significant difference was observed between the Val/Val and Val/Met+Met/Met groups and the WCST results in the MANCOVA ($F_{48,5}$ = .76; p = .59, Partial Eta Squared = .08), or WCST specific categories (Perseverative Errors, p = .66, Partial Eta Squared < .01; Nonperseverative Errors, p = .58, Partial Eta Squared < .01; Categories Completed, p = .34,

Partial Eta Squared = .02; Attempts to Reach First Category, p = .64, Partial Eta Squared < .01; and Percentage of Conceptual Level Responses, p = .99, Partial Eta Squared < .01). Results are presented in Table 2. Even though comorbidity with ADHD was not considered to be a confounding variable in our comprehensive approach, its inclusion in the model did not change our findings significantly (p = .49).

Discussion

This was the first investigation to assess the role of a gene on neuropsychological tests in children and adolescents with bipolar disorder. We were not able to detect any differences in a test of cognitive flexibility (WCST) and the Val66Met polymorphism at the BDNF gene.

Attentional ability to avoid distraction, to utilize new information and previous experiences, to formulate a logical conception, to form a conceptual thinking and inability to change reaction due to unawareness of relevant stimuli were not mediated by the presence of the Met allele at the BDNF. Although robust data exist suggesting that children

Table 1 Demographics and clinical characteristics of the sample.

		Val/Val (n = 40)	Val/Met (n = 12) or Met/Met (n = 1)	
		Mean ± SD	Mean ± SD	p-value
Age		11.90 ± 2.61	10.07 ± 3.01	.07
Duration of disorder		5.03 ± 2.87	5.25 ± 2.54	.84
Intelligence quotient		86.27 ± 14	90.25 ± 16.2	.45
CGAS		47.78 ± 11.96	51.00 ± 10.80	.38
Assessment scale				
YMRS		24.41 ± 13.45	27.22 ± 9.08	.46
CDRS-R		41.84 ± 14.52	39.55 ± 14.8	.68
		Frequency: n (%)	Frequency: n (%)	
Socio-economical status		C: 25 (62.5%)	C: 11 (84.6%)	.37
Gender	Male	26 (65%)	7 (53.8%)	.52
Ethnicity	Caucasian	34 (85%)	12 (92.3%)	.6
Bipolar disorder subtype	Type I	26 (65%)	11 (84.6%)	.41
	Type II	6 (15%)	1 (7.7%)	
	NOS	8 (20%)	1 (7.7%)	
Comorbidities	ADHD	35 (87.5%)	10 (77%)	.39
	Disruptive disorders	25 (62.5%)	8 (61.5%)	.5
	Anxiety disorders	21 (52.5%)	5 (38.5%)	1.00
	Enuresis	7 (17.5%)	4 (30.8%)	.43
Current medication use		10 (25%)	6 (46.1%)	.18

CGAS: Children's Global Assessment Scale; YMRS: Young Mania Rating Scale; CDRS-R: Children's Depression Rating Scale, Revised; NOS: Not otherwise specified; ADHD: Attention Deficit/Hyperactivity Disorder.

Table 2 Comparison of the Val66Met polymorphism at the BDNF gene in the results of the Wisconsin Card Sorting Test in children and adolescents with bipolar disorder.

Wisconsin Card Sorting Test	Val/Val (n = 38)	Val/Met (n = 9) or Met/Met (n = 1)	p-value	Partial Eta Squared
	Mean ± SD	Mean ± SD		
Percentage of Perseverative Errors	20.39 ± 10.95	18.90 ± 10.64	.66	< .01
Percentage of Nonperseverative Errors	22.37 ± 12.24	24.50 ± 12.68	.58	< .01
Number of Correctly Completed Categories	3 ± 1.98	3.60 ± 2.01	.34	.02
Set to the First Category	34.07 ± 35.67	25.5 ± 18.22	.64	< .01
Percentage of Conceptual Level Responses	44.15 ± 20.36	44.61 ± 24.05	.99	< .01

* Multivariate Analysis of Variance ($F_{48,5}$ = .76; p = .59- Partial Eta Squared = .08).

and adolescents with BD have deficits in executive functions, such as abstract thinking, problem-solving skills, and cognitive flexibility, which can result in maladaptive behavior, the previously documented reduction in activity-dependent BDNF secretion did not translate into additional cognitive flexibility deficits in our sample.[9,10,21] This finding was surprising, due to previous results in studies with adult populations. In adults with bipolar disorder, the poorest performance in the Wisconsin Card Sorting Test was observed for BDNF Met allele carriers when compared subjects with BD, schizophrenia and typically developing adults.[18-20] Moreover, other researchers have not found an association between the BDNF Val66Met polymorphism and performance in the WCST in a quantitative transmission disequilibrium test (QTDT) analyses in adults with schizophrenia,[35] or in a comparison of homicidal and non-homicidal male adults with schizophrenia.[36] As this is a pioneer study in children and adolescents, we cannot draw age-specific comparisons.

Although BDNF has been widely associated with the neurobiology of adult BD, it is possible that its measurable effects on neuropsychological functions are not present in children due to the ongoing development of frontolimbic areas (and consequently executive functions) through adolescence, as has been suggested by neuropsychological and neuroimaging studies of JBD.[22,37,38] This speculation is partially supported in our sample by the positive correlation between age and performance in all WCST categories. It is also possible that consecutive mood episodes may be responsible for progressive cognitive deterioration, as decreased BDNF peripheral levels are present in adults with late-stage BD, as compared to patients in the early stages of the disorder.[39] Longitudinal studies that include measurement of BDNF peripheral levels in children and adolescents may provide an answer to this issue.

We cannot rule out that WCST was not able to detect cognitive flexibility deficits because executive function weaknesses in JBD are milder and less consistent than in other childhood disorders such as ADHD.[38] In addition, some patients were using mood stabilizers and antipsychotics, and we cannot quantify the effect of these drugs on the test results. However, current neuropsychological findings regarding juvenile bipolar disorder persist regardless of the state of the illness or medication status, although such findings are limited and inconclusive.[40] Futures studies should address these issues through the use of different neuropsychological tests and on drug-naïve subjects.

Our findings should be considered in light of some limitations. The Met allele frequency in our sample was lower than in the other previous studies, as was the percentage of females in our sample. However, no change was found when the gender variable was included in the model. Although our sample size was comparable with the original study in adults (n = 54), we cannot completely exclude the possibility that lack of power affected our results. Our effect size estimates for all WCST scores (eta partial squares) were extremely small; this finding suggests no relevant effects of this polymorphism on the WCST scores. In addition, to reach 80% power in all WCST subtests, with the means and standard deviations found in our sample, the minimum sample size we would need for statistical significance would be 1,113 subjects in WCST-PE, 31,005 subjects in WCST-NP, 3,339 in WCST-CC, 1,680 in WCST-1stCAT, and 100,800 in WCST-%CLR. These extremely elevated numbers support the nonsignificance of the differences between the genotypes in our sample. Statistical control for potential sociodemographic and clinical confounding variables was broad and strongly suggests that our findings were not influenced by other variables.

Further investigations with larger samples, including a more comprehensive battery of neuropsychological testes, may provide a better understanding of the neurobiological pathways that are involved in this highly disabling disorder .

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Disclosures

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