

Brain-derived neurotrophic factor gene val66met polymorphism and executive functioning in patients with bipolar disorder

Polimorfismo do gene do fator neurotrófico derivado do cérebro val66met e função executiva em pacientes com transtorno bipolar

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Abstract

Objective: In the present study, we investigate the association between the val66met polymorphism of the brain-derived neurotrophic factor (BDNF) and the performance on the Wisconsin Card Sorting Test in a sample of Caucasian Brazilian patients with bipolar disorder. **Method:** Sixty-four patients with bipolar disorder were assessed and their performance on the Wisconsin Card Sorting Test was compared with the allele frequency and genotype of the val66met polymorphism of the brain-derived neurotrophic factor. **Results:** The percentage of non-perseverative errors was significantly higher among patients with the val/val genotype. There was no association between (BDNF) genotype frequency and other Wisconsin Card Sorting Test domains. **Conclusion:** Our results did not replicate previous descriptions of an association between a worse cognitive performance and the presence of the met allele of the val66met brain-derived neurotrophic factor gene polymorphism.

Descriptors: Bipolar disorder; Cognitive dissonance; Brain-derived neurotrophic factor; Patients; Genetic polymorphism

Resumo

Objetivo: O presente estudo tem por objetivo investigar a associação entre o polimorfismo val66met do gene do fator neurotrófico derivado do cérebro (BDNF) e o desempenho cognitivo no Teste Wisconsin de Classificação de Cartas em uma amostra de pacientes bipolares brasileiros caucasianos. **Método:** Sessenta e quatro pacientes com transtorno bipolar foram avaliados em relação a sua cognição por meio do Teste Wisconsin de Classificação de Cartas que foi comparada com a frequência alélica e genotípica do polimorfismo val66met do gene do fator neurotrófico derivado do cérebro. **Resultados:** O percentual de erros não-perseverativos foi significativamente maior nos indivíduos com genótipo val/val. Não foi encontrada diferença significativa entre a frequência genotípica do polimorfismo do BDNF e os outros domínios do Teste Wisconsin de Classificação de Cartas. **Conclusão:** O estudo do polimorfismo val66met em relação ao desempenho executivo em pacientes bipolares caucasianos de uma amostra brasileira não reproduziu achados anteriores que sugeriam um pior desempenho em indivíduos portadores do alelo met.

Descritores: Transtorno bipolar; Dissonância cognitiva; Fator neurotrófico derivado do encéfalo; Pacientes; Polimorfismo genético

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Introduction

Recent studies showed that serum brain-derived neurotrophic factor (BDNF) levels are decreased during acute mood episodes in bipolar disorder (BD).¹ BDNF levels are negatively correlated to the severity of manic and depressive symptoms,^{1,2} suggesting that peripheral BDNF measures could play a role as a biomarker in BD. In addition, a common polymorphism of the *BDNF* gene, which replaces a valine by a methionine at the codon 66 (val66met) has been associated with BD pathophysiology.³ For instance, in a case-control study with over 3000 individuals there was an association of BDNF val66met polymorphism with susceptibility to rapid cycling.⁴ Another study found an association between *BDNF* val66met polymorphism and altered cognitive performance in 111 patients with BD.⁵ Given that val66met polymorphism affects intracellular packaging of BDNF as well as its activity dependent secretion,⁶ one could hypothesize that met carriers (met/met and val/met genotypes) would present lower levels of serum BDNF than the val/val group does. However, in a recent study we showed that met carriers did not present lower levels of BDNF in their sera.⁷ Thus, BD patients who are met-carriers seem to be more likely to present cognitive impairment and rapid cycling; however, such findings were not correlated with lower BDNF serum levels.

The *BDNF* val66met polymorphism has been associated with altered hippocampal function,⁸ and met carriers showed impaired performance in the WCST.⁵ This finding has attracted a lot of interest in the field, appearing as a possible explanation to why certain patients with BD present impaired cognitive performance. Indeed, impaired cognitive performance has been shown in BD patients even when acute mood symptoms remit.⁹ Moreover, cognitive functions appear to worsen with illness progression,¹⁰ and have a significant impact on general functioning and on peripheral markers.^{11,12} Sustained attention, verbal learning and memory are particularly impaired in bipolar patients.^{13,14} Martínez-Arán et al. examined cognitive functions across all phases of bipolar disorder (manic/hypomanic, depressed, euthymic) and found impaired performance in verbal memory and prefrontal executive tasks in all phases of the disorder as compared to healthy subjects.⁹ The impairment in cognitive function has been pointed out as a possible endophenotypic marker in genetic studies of bipolar disorder. Cognitive impairment can be seen not only in bipolar patients but also in unaffected first-degree relatives.¹⁵

The association of the val66met polymorphism with cognition in bipolar disorder has already been investigated,^{5,16,17} and patients with the val/val genotype obtained superior results in the Wisconsin Card Sorting Test, which assesses the integrity of the executive function. In the present study, we studied the association between

the val66met polymorphism of the BDNF gene and the performance on a test related to executive functioning (the Wisconsin Card Sorting Test) in a well-characterized clinical sample of Brazilian patients with bipolar disorder.

Method

Sixty-four patients with BD type I were consecutively selected from a bipolar disorder outpatient clinic (Clinical Hospital of Porto Alegre, Brazil). The Local Ethics Committee approved all the procedures described in this report (04-457). Written informed consent was obtained from all patients prior to any study procedures. All subjects were self-assigned Caucasians.

We excluded patients who had received electroconvulsive therapy (ECT), with history of alcohol or drug abuse in the 6 months prior to the testing, lifetime history of neurological disease, and those who were illiterate. Psychiatric diagnoses were based on a clinical interview performed by qualified psychiatrists and confirmed with the Structured Clinical Interview for DSM-IV-Axis I. Manic and depressive symptoms were assessed using the Young Mania Rating Scale (YMRS)¹⁸ and the Hamilton Depression Rating Scale (HDRS),¹⁹ respectively.

1. Genotyping

DNA was obtained from peripheral blood and genomic DNA was extracted using standard procedures. The genotyping of *BDNF* val66met SNP polymorphism was performed using 5' nuclease Taqman allelic discrimination assay on the ABI 7000 Sequence Detection System (Applied Biosystems, CA, USA). Investigators who were blind to the subjects' clinical status performed the genotyping.

2. Cognitive test

The Wisconsin Card Sorting Test (WCST) has been widely used as a neuropsychological test that measures prefrontal lobe function.²⁰ An experienced psychologist administered the WCST. WCST domains that were used in the analyses included the number of correctly completed categories, the percentage of perseverative errors, the percentage of non-perseverative errors, and the percentage of conceptual level responses.

3. Statistical analysis

WCST scores were skewed and therefore a Mann-Whitney's U analysis was used. Correlations were tested with Spearman's rho. Groups were compared using ANOVA. Current mood status was controlled for using an ANCOVA model, including depressive and manic symptoms as covariates. Correlations were tested with

Table 1 - Clinical characteristics of patients with bipolar disorder (n = 64)

| Characteristic | Val/val (n = 35) | Val/met and met/met (n = 29) | p value |
|--|------------------|------------------------------|---------|
| Clinical state^a | | | |
| Hamilton Depression Rating Scale score | 9 (3-20) | 9 (4-16) | 1 |
| Young Mania Rating Scale score | 3 (1-7) | 4 (0-6.5) | 0.50 |
| Intelligence quotient^b | 97.3 ± 9.7 | 98.2 ± 12.0 | 0.76 |
| WCST^{b,c} | | | |
| Percentage of perseverative errors | 30.9 ± 18.3 | 30.3 ± 21.1 | 0.59 |
| Percentage of non-perseverative errors | 25.5 ± 17.8* | 16.7 ± 11.8 | 0.048** |
| Number of correctly completed categories | 2.5 ± 2.1 | 3.6 ± 2.4 | 0.08 |
| Percentage of conceptual level responses | 40.4 ± 24.2 | 47.7 ± 24.8 | 0.20 |

^a Results are shown as median (interquartile range)

^b Results are shown as means (± standard deviations)

^c Wisconsin Card Sorting Test

** p < 0.05 for difference between groups

Pearson's r . All tests were two-tailed and were carried out using a significance level of $\alpha = 0.05$, with 80% of power, as in previous studies.¹⁶

Results

A total of 64 subjects were recruited, of which 14 men and 50 women. Patients showed a mean age of 42.3 years (± 11.1) and 9.9 (± 4.4) mean schooling years (see Table 1 for clinical characteristics). The sample genotype distribution did not deviate significantly from the Hardy-Weinberg equilibrium ($X^2 = 0.423$, $p = 0.50$). The prevalence of allele frequencies was consistent with those previously reported in the Caucasian population (val/val = 35 (54.7%); val/met = 26 (40.6%); met/met = 3 (4.7%). Groups of patients having val/met ($n = 26$) and met/met ($n = 3$) were merged for analytical purposes considering the dominant effect of alleles, hypothesized on the basis of evidence coming from functional studies.⁶

In the whole sample, only the percentage of non-perseverative errors was significantly higher for the val/val genotype ($Z = 1.99$, $p = 0.048$). There was no association between genotype and the number of correctly completed categories ($Z = 1.78$, $p = 0.08$), the percentage of perseverative errors ($Z = 0.55$, $p = 0.59$), set to first category ($Z = 1.27$, $p = 0.21$), and the percentage of conceptual level responses ($Z = 1.30$, $p = 0.20$) (see Table 1). When co-varied for depressive and manic symptoms, results remained largely the same. Duration of illness was not associated with either genotype ($Z = 1.72$, $p = 0.09$) or executive function ($p > 0.3$ for all WCST categories).

Fifty-nine patients were taking mood stabilizers during assessment. Twenty-two patients were taking atypical antipsychotics, twenty were in use of typical antipsychotics and 15 patients were treated with antidepressant drugs. There was no statistical difference between genotype groups in relation to number and group of medication (Table 2) or between genotype groups and age. There were no patients with manic symptoms (YMRS > 8), however, some patients presented a HAM-D > 8 which could influence the results of cognitive tests. There was no association between executive functioning and depressive symptoms ($p > 0.3$ for the 5 correlations) or between genotype and depressive symptoms ($p = 0.29$). There were no significant statistical differences between the allele frequencies and genotype distribution in male and female patients (see results in Table 3).

Discussion

We were unable to replicate the association of worse executive functioning among met-carriers BD patients as described previously.^{5,16,17} This is not surprising given the inheritance pattern

Table 2 - Number of medication and medication frequencies in different genotype of bipolar patients studied ($n = 64$)

| Medication | Val/val ($n = 35$) | Val/met and met/met ($n = 29$) | p value |
|--|-------------------------|-------------------------------------|------------|
| Number of medications used for bipolar disorder ^a | 2.7 \pm 1.1 | 2.6 \pm 1.1 | 0.72 |
| Mood stabilizer | 91.4% | 93.1% | 0.80 |
| Antidepressants | 25.7% | 20.7% | 0.64 |
| Benzodiazepines | 34.3% | 27.6% | 0.56 |
| Atypical antipsychotics | 31.4% | 37.9% | 0.59 |
| Typical antipsychotics | 28.6% | 34.5% | 0.61 |

^a Results are shown as means (\pm standard deviations)

Table 3 - Allele and genotype BDNF frequencies in a sample of male and female bipolar I patients

| | Allele | | Genotype | | |
|-------------------------|-------------|-------------|---------------|---------------|-------------|
| | Val | Met | Val/Val | Val/Met | Met/Met |
| Male (14) | 22 | 6 | 8 | 6 | 0 |
| Female (50) | 74 | 26 | 27 | 20 | 3 |
| Total frequency (64) | 96 (75%) | 32 (25%) | 35 (54.7%) | 26 (40.6%) | 3 (4.7%) |

of BD features is probably of the Complex type, which involve several genes of small effects that interact in order to express the phenotype. Moreover, the three other reports suggesting impaired executive functioning among met-carriers were conducted by the same group within an Eastern European sample and have not been replicated in differential studies. Apart from not replicating previous findings,^{5,16,17} our study showed an opposite trend towards impaired performance in the non-perseverative errors dimension of the WCST among val/val patients. An explanation for the lack of association between the val66met polymorphism and executive dysfunction in our study may be that the sample size did not suffice to detect small effects. However, replication studies are more likely to give effect sizes smaller and closer to the true effect size than initial published reports of an association. False-positive results caused by chance in initial studies have been considered the chief cause for negative replications.²¹ Moreover, the present finding of impaired performance in the val/val sample (the opposite of what has been described) pertain to the non-perseverative-errors dimension of the WCST, which is not primarily related to executive functioning and reflects random errors that are related to a non-specific attentional impairment.

As BDNF is associated with neuroplasticity, it could be part of an interesting rationale to link BDNF expression and cognition.¹² Even though some studies have suggested an association of the val66met polymorphism and BDNF expression, negative reports on this association have been described.^{7,22} In any case, genetic influences on cognition might be subtler, and involve synergic polymorphism interaction, such as the one recently demonstrated in the general population between the RE1-silencing transcription factor (REST) gene and the BDNF val66met polymorphism.²³

Our study has other limitations that must be acknowledged. As happens in all case-control genetic studies, we must be aware of false-positive and false-negative findings. In addition to the sample size limitation discussed above ethnic stratification is an important limitation of this study. We attempted to control for this variable including only self-assigned Caucasians but as previously demonstrated ethnic ancestry cannot be easily assessed in Brazil only on the basis of physical examination.²⁴ Another limitation is the fact that patients who are using psychotropic drugs may present psychomotor slowness and may achieve a poorer performance, but since the patients participating in our study used different medications, sometimes even more than one, it was impossible to isolate any drug interference. Nevertheless, those medications were equally distributed between genotypes.

Cognitive endophenotypes for psychiatric disorders have been subject of a growing body of research and debate. Attempts to replicate results regarding the association of genetic polymorphisms and cognition in differential settings and populations may help to elucidate this matter.

Disclosures

| Writing group member | Employment | Research grant ¹ | Other research grant or medical continuous education ² | Speaker's honoraria | Ownership interest | Consultant/ Advisory board | Other ³ |
|---------------------------------|------------|---------------------------------------|---|---|--------------------|----------------------------|--------------------|
| Juliana Fernandes Tramontina | - | CAPES FIPE-HCPA | - | - | - | - | - |
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* Modest

** Significant

*** Significant. Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.

Note: UFRGS = Universidade Federal do Rio Grande do Sul; CAPES = Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; FIPE = Fundo de Incentivo à Pesquisa e Ensino; HCPA = Hospital de Clínicas de Porto Alegre; CNPq = Conselho Nacional de Desenvolvimento Científico e Tecnológico; INCT-TM = Instituto Nacional de Ciência e Tecnologia Translacional em Medicina; NARSAD = National Alliance for Research on Schizophrenia and Depression.

For more information, see Instructions for authors.

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