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Association study of the Brain-Derived Neurotrophic Factor (BDNF) gene C-270T polymorphism with bipolar affective disorder

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Summary

Brain-derived neurotrophic factor (BDNF) has been implicated in the pathogenesis of bipolar disorder. In this paper, the C-270T polymorphism of the BDNF gene has been studied in patients with bipolar disorder (n=380) and in the control group (n=380). Consensus diagnosis was made for each patient, according to DSM-IV, using SCID. Genotypes were determined by PCR-RFLP analysis. No association was found between the polymorphism studied and bipolar disorder. Results were also not significant when analyzed by gender and age at onset. Analysis in subgroups according to disease subtype (I and II) also did not reveal any significant differences between bipolar I and II patients and the control group.

Key words: association, bipolar affective disorder, Brain-Derived Neurotrophic Factor (BDNF) gene, polymorphism

Introduction

Brain-derived neurotrophic factor (*BDNF*) belongs to the neurotrophin family of growth factors [1, 2]. *BDNF* has trophic effects on dopaminergic [3, 4, 5], cholinergic [6, 7] and serotonergic neurons [8, 9, 10]. Moreover, it is essential in the development of sensory ganglia, cerebral cortex, hippocampus and striatum [11]. The protein affects neuronal proliferation, promotes synaptic plasticity and influences hippocampal long term potentation (LTP), learning and memory [12, 13, 14, 15, 16, 17]. Changes in *BDNF* expression were reported in response to antidepressant treatment [18, 19, 20]. Nibuya et al. [21] observed rapid increase in *BDNF* expression in response to chronic electroconvulsive seizures and antidepressant drug treatment, particularly in the hippocampus [22]. In the animal model, Saarelainen et al. [23] found that heterozygous *BDNF* null mice ($BDNF^{+/-}$) were resistant to the effects of antidepressants in the forced swim test, and Smith et al. [24] reported that reduction in *BDNF* expression induced

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by stress in (*BDNF*$^{+/-}$) mice is neutralized by antidepressants. Moreover, Shirayama et al. [25] observed that infusion of exogenous *BDNF* into the hippocampus resulted in antidepressant-like behavioural effects. Chen et al. [26] investigated association of antidepressant medication with increased hippocampal *BDNF* levels in post-mortem brain, concluding that *BDNF* may be regulated by antidepressant medications and might be involved in depressive symptoms in bipolar affective disorder. Clinical studies on depression have demonstrated that serum levels of *BDNF* in drug-naive patients with major depressive disorder are significantly decreased as compared to the control subjects, and that *BDNF* might be an important agent for augmentation of therapy in this disease [27]. Findings mentioned above give evidence that antidepressant medications induce an increase in *BDNF* expression and, subsequently, result in neurotrophic response.

In a recent study, Tsai et al. [28] investigated involvement of *BDNF* in mood regulation. They found that agents inducing manic states also cause an increase of *BDNF* suggesting that its over activity may be implicated in the manic state.

The *BDNF* gene is localized in the short arm of chromosome [11, p13] [29]. The gene consists of four short 5'exons with separate promoters and one 3'exon encoding the mature *BDNF* protein [30, 31]. In the *BDNF*, several polymorphisms have been reported and studied in psychiatric disorders. The most frequently described include dinucleotide repeat polymorphism $(GT)_n$ in the promoter region, 1.4 kb from the transcription start site [32], Val66Met (196G/A) polymorphism [33], -374A/T and -256G/A polymorphisms [34] and -270C/T substitution [35].

The Val/Met polymorphism was studied previously by several research groups with inconsistent results. An association of the Val variant and bipolar affective disorder type I was described by Neves-Pereira et al. [36] and Sklar et al. [37], but the analysis performed by Nakata et al. [38] and Hong et al. [39] did not confirm such an association. In the study from our centre [40], association of Met/Met genotype with the early onset of bipolar disorder was described, in comparison to the patients with heterozygous genotype. Nakata et al. [38] also calculated linkage disequilibrium between the Val/Met and the C-270T polymorphisms but they did not find linkage disequilibrium between these to SNPs, therefore the haplotype analysis was not performed.

The -270C/T polymorphism was investigated with negative results in bipolar disorder [38] and in anorexia and bulimia [41]. In schizophrenia, however, results were inconsistent. Studies by Szekeres et al. [42] and Nanko et al. [43] reported a relationship between the T allele and schizophrenia, but results performed by Galderisi et al. [44] and Szczepankiewicz et al. [45] did not demonstrate any association with this polymorphism.

In this study, the authors have investigated allelic distribution of the C-270T single nucleotide polymorphism (SNP) in the 5' non coding region of the *BDNF* gene in the group of patients with bipolar affective disorder and in the control group. It was analyzed because it is non synonymous and, as it is localized in the promoter region of *BDNF* gene, such substitution may affect gene expression observed in bipolar patients. The authors also examined whether two SNPs, Val66Met analyzed in the previous study

in our centre and C-270T studied in this paper, are in linkage disequilibrium and, if so, to analyze haplotypes.

Subject and methods

Patient population

The study was performed on a Caucasian population of 380 patients with bipolar affective disorder (165 males with a mean age of 43 years, SD=14; 215 females with a mean age of 46 years, SD=13). The group of patients with the type I of the illness was n=311 (170 males with a mean age of 46 years, SD=13; 141 females with a mean of age of 43, SD=15). The group of patients with the type II of the illness was n=69 (43 males with a mean age of 45 years, SD=12; 26 females with the mean age of 44 years, SD=14). The subgroup of n=47 bipolar affective patients with the early age of onset (18 years or earlier) was distinguished. Patients were recruited from inpatients from the Wielkopolska region, treated at the Department of Psychiatry, University of Medical Sciences in Poznań, Department of Psychiatry, University School of Medicine in Bydgoszcz and the Psychiatric Hospital in Kościan. At least two psychiatrists formulated a consensus diagnosis for each patient, according to DSM-IV and ICD-10 criteria using the structured clinical interview for DSM-IV Axis I disorders (SCID) [46].

The control group consisted of 380 subjects (145 males with a mean age of 41.7 years, SD=12; 235 females with a mean age of 40.7 years, SD=11). Control subjects were also recruited from the Wielkopolska region, from the group of blood donors, hospital staff and students of the University of Medical Sciences in Poznań. They were not psychiatrically screened.

Informed consent was obtained from all participants of the study. The local ethics committee accepted the project.

Genotyping

The DNA was extracted from 10 ml of EDTA anticoagulated whole blood using the salting out method [47]. A 223-basepair fragment of the *BDNF* gene was amplified by PCR reaction with a set of primers described by [35] applying the PTC-200 (MJ Research) thermal cycler. A 15 µl amplification mixture contained 150-300 ng of genomic DNA, 0.3 µM of each primer, 0.17 mM of each dNTP, 1.5 mM $MgCl_2$, 75 mM Tris-HCl, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20 and 0.4 U of Taq DNA polymerase (MBI Fermentas). The cycling conditions were: initial denaturation at 95˚C for 3 min. followed by 35 cycles, with a profile of 94˚C for 30 s, 64˚C for 30 s, 72˚C for 30 s, and a final elongation at 72˚C for 10 min. PCR product (4.0 µl) was then digested overnight in a total volume of 6.2 µl at 37˚C with 0.7 U of HinfI restriction endonuclease (MBI Fermentas). Afterwards, digestion products were separated on 3.5% basica LE agarose gel (Prona, Spain) with 90V and visualised by ethidium bromide staining. Band sizes were compared with pUC19DNA/MspI and 50 bp DNA ladder (MBI Fermentas). The uncut PCR product size was 223 bp. After RFLP analysis, the following alleles

were observed: for C allele the bands of 127 and 78 and 18 bp, for T allele the bands of 127, 63, 18 and 15 bp.

Statistical analysis

The Pearson's chi-square (χ^2) test and Fisher's exact test were applied to test differences in the genotypic and allelic (respectively) distribution between groups of schizophrenic patients and controls. Calculations were performed using the computer programme SPSS version 10. Bonferroni correction was made using the formula:

$\alpha' = \alpha/k$,

where α' is the corrected significance level for multiple comparison, α is the significance level (0.05) and k is the number of independent significance tests.

A two-tailed type I error rate of 5% was chosen for analysis. Power analysis was performed using an on-line internet service provided by the UCLA Department of Statistics (http://ebook.stat.ucla.edu/calculators/powercalc/binominal/case-control/b-case-control-power.php). Analysis of linkage disequilibrium between Val66Met and C-270T polymorphisms was evaluated using the Haploview programme available online (http://www.broad.mit.edu/mpg/haploview/index.php).

Results

The genotype distribution was in the Hardy-Weinberg equilibrium for all studied groups.

The genotype and allele distribution for the whole group of patients with bipolar mood disorder did not differ significantly from the control group (p=0.992 and p=0.904, respectively) (Table 1). Comparing genotype and allele frequencies between the groups of bipolar patients according to disease subtype (bipolar I and II), we have found a significant difference in allele distribution between the bipolar II patients and the control group (p=0.102 for genotypes and p=0.031 for alleles). However, after Bonferroni correction, the results were not significant because the corrected p value is 0.016 so p=0.031 for alleles in the group of bipolar II patients lacks statistical significance. The data is shown in table 1.

Table 1

Genotype distribution and allele frequencies of the *BDNF* C-270T polymorphism for patients (males and females) with bipolar affective disorder and the control group.

	Genotype C/C n (%)	Genotype C/T n (%)	Genotype T/T n (%)	Total genotypes n (%)	Allele C n (%)	Allele T n (%)	Total alleles n (%)
Bipolar	345 (90.8%)	34 (8.9%)	1 (0.3%)	380 (100%)	724 (95.3%)	36 (4.7%)	760 (100%)
Bipolar I	277 (89.1%)	33 (10.6%)	1 (0.3%)	311 (100%)	587 (94.4%)	35 (5.6%)	622 (100%)
Bipolar II	68 (98.6%)	1 (1.4%)	0 (0.0%)	69 (100%)	137 (99.3%)	1 (0.7%)	138 (100%)
Control	346 (91.1%)	33 (8.7%)	1 (0.3%)	380 (100%)	725 (95.4%)	35 (4.6%)	760 (100%)

Difference, bipolar I+II vs. control – χ^2=0.16, df=2, p=0.992 for genotypes, p=0.904 for alleles; bipolar I vs. control – χ^2 =0.760, df=2, p=0.684 for genotypes, p=0.391 for alleles; bipolar II vs. control – χ^2 =4.574, df=2, p=0.102 for genotypes, p=0.031 for alleles.

When groups were analyzed by gender, we did not find any significant difference in genotype and allele distribution: in the male group p=1.000 for genotypes and p=1.000 for alleles, respectively; in the female group p=0.948 for genotypes and p=0.772 for alleles, respectively.

Comparing genotype and allele distribution according to the early (18 years or earlier) age of onset, we did not find any significant differences between bipolar patients and control subjects (p=0.178 for genotypes and p=0.128 for alleles. In analysis of genotype and allele distribution by late age of onset (after 18 years old), we did not observe any association with bipolar mood disorder, either (p=0.911 and p=0.796, respectively).

The power to detect an association was established to be of 54% in the whole group of patients and 8% in the group of patients with bipolar disorder type II and 23.4% in the group of patients with early age of onset for a relative risk of 1.75.

There was no linkage disequilibrium between two studied SNPs, Val66Met and C-270T so haplotype analysis was not applicable.

Discussion

In our study we have not found any significant association between the C-270T polymorphism of *BDNF* gene and bipolar affective disorder in the whole group of patients. When we divided our sample into subgroups by subtype of disease (bipolar I and II), significant difference was found between the bipolar II patients and the control group (p=0.102 for genotypes, p=0.031 for alleles). However, after considering the Bonferroni correction for multiple testing the results were not significant for the studied group. In the subgroups analyzed according to gender and the age of onset we have not found any association. However, due to insufficient power of the tests performed for the group of bipolar II patients (8%) and patients with early age at onset (23.4%) (a small number of subjects), we cannot exclude the results as being falsely negative. Further analysis in a larger group is required to revise our findings.

The functional significance of the C-270T substitution in the prodomain of the *BDNF* gene is not known. Changes in *BDNF* protein level in brain or serum of bipolar affective patients were reported (21, 49-51), but there is no evidence whether the C-270T polymorphism might be responsible for such alterations in protein expression.

The results obtained in this analysis are consistent with the previous study on the subject [38] where no association of bipolar affective disorder with C-270T polymorphism was found. There, the group of n=192 patients with bipolar disorder was genotyped for two polymorphisms in the *BDNF* gene (C-270T and Val66Met), but the analysis revealed no association between any of these polymorphisms and bipolar disorder in a Japanese population. Also, the two polymorphisms analyzed by Nakata et al. [38], Val66Met and C-270T were not in linkage disequilibrium and, therefore,

haplotype analysis was performed. This is consistent with the results obtained in this paper.

The lack of association of C-270T polymorphism with bipolar affective disorder presented here is consistent with the previous findings on this subject [38]. Their analysis, however, was performed in the smaller sample (n=192), therefore results obtained on the larger group in our study (n=380) are a certain confirmation of their findings. To our knowledge, no other studies including C-270T polymorphism in *BDNF* gene in bipolar patients were reported.

The results presented in this paper do not support the possible association of promoter polymorphism of *BDNF* gene with bipolar disorder. Therefore, further studies are necessary to identify other polymorphisms in the *BDNF* gene that may be involved in the aetiology of bipolar affective disorder.

Conclusions

The results presented here do not support the possible association of the C-270T polymorphism of *BDNF* gene with susceptibility to bipolar affective disorder. However, the *BDNF* gene has been postulated as an important factor in neurodevelopment, and further studies are required to identify novel functional polymorphisms that may affect its expression and lead to changes of *BDNF* level observed in bipolar patients.

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