DOI 10.1007/s00702-004-0233-9
J Neural Transm (2005) 112: 885–890

Journal of Neural Transmission
Printed in Austria

Lack of association between two polymorphisms of brain-derived neurotrophic factor and response to typical neuroleptics

S. Anttila[1,4], **A. Illi**[1,2], **O. Kampman**[1,3], **K. M. Mattila**[1,4], **T. Lehtimäki**[1,4], and **E. Leinonen**[1,5]

[1] University of Tampere, Medical School, Tampere,
[2] Department of Psychiatry, Kanta-Häme Central Hospital, Hämeenlinna,
[3] Department of Psychiatry, Seinäjoki Hospital District, Seinäjoki,
[4] Department of Clinical Chemistry, Laboratory of Atherosclerosis Genetics, Centre for Laboratory Medicine, Tampere University Hospital, Tampere, and
[5] Department of Psychiatry, Tampere University Hospital, Pitkäniemi, Finland

Received May 26, 2004; accepted September 14, 2004
Published online November 3, 2004;

Summary. Several studies have connected brain-derived neurotrophic factor (BDNF) with treatment response to neuroleptics. In recent studies, the *BDNF* expression was reduced by typical neuroleptics. We conducted a retrospective study on 94 patients with schizophrenia and 98 controls. The *BDNF* G196A and C270T polymorphisms are not associated with treatment response to typical neuroleptics or with age at first hospitalization. Moreover, these polymorphisms of the *BDNF* gene are not associated with the risk of schizophrenia.

Keywords: BDNF, polymorphism, schizophrenia, pharmacogenetic study, age at onset.

Introduction

Schizophrenia is a complex brain disorder, which presumably results from the interplay of genetic, behavioral, developmental and some other factors. Some studies have also suggested that distinguishing the genetic variation associated with responders and non-responders may be particularly beneficial in finding the genes associated with schizophrenia (Joober et al., 2002). In addition, differences in the response to typical and atypical neuroleptics may have a genetic base (Strange, 2001).

Several studies strongly suggest that the brain-derived neurotrophic factor (BDNF) may have major role in schizophrenia. In patients with schizophrenia BDNF levels may be reduced in prefrontal cortex (Weickert et al., 2003). *BDNF*

polymorphism may also be associated with changes in brain morphology in schizophrenia (Wassink et al., 1999).

Two recent studies show an association between the C270T polymorphism of *BDNF* and schizophrenia (Kunugi et al., 2003; Szekeres et al., 2003). In the study by Egan et al. (2003) the *BDNF* G196A (val66met) polymorphism was not associated with schizophrenia. However, Hong et al. (2003) reported that the val/val genotype was slightly more common in schizophrenia patients.

Several studies have suggested that typical antipsychotics may downregulate BDNF and atypical antipsychotics may have the opposite effect (Dawson et al., 2001; Angelucci et al., 2000; Fumagalli et al., 2003).

The study by Krebs et al. (2000) suggested an association between *BDNF* dinucleotide alleles (172–176 bp) and good response to neuroleptics. Hong et al. (2003) reported that val/val genotype was more frequent in clozapine responding patients.

The aim of the present study was to compare *BDNF* genotypes between two different populations of schizophrenic patients (responding vs. not responding to typical neuroleptics). Moreover, the frequencies of alleles in schizophrenic patients were compared to those of healthy controls and to the age at first hospitalization due to schizophrenia or schizophreniform disorder.

Material and methods

Patients and controls

The patients were 94 unrelated Finnish patients with schizophrenia. All patients met the criteria for schizophrenia of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV. The age at onset was defined as the beginning of the first hospitalization due to schizophrenia or schizophreniform disorder.

Group 1 (responders) consisted of patients with schizophrenia who had experienced sufficient and long-lasting response to treatment with typical antipsychotics.

The patients in group 2 (non-responders) had failed to respond to treatment with at least two different typical antipsychotics. Because of a poor response to the conventional neuroleptics, clozapine was initiated in all the patients in the non-responders' group.

A control sample consisted of 98 blood donors.

The study was performed in compliance with the code of ethics of the World Medical Association (Declaration of Helsinki) and the standards established by the local ethics committee. The participants gave written informed consent. More detailed demographic data on patients and study design can be seen in our earlier publication (Illi et al., 2003).

Genotyping of the BDNF gene

Purification of genomic DNA from whole blood and buffy-coats was performed using standard methods. The genotyping of the G196A (val66met) polymorphism of the *BDNF* was carried out as described by Ventriglia et al. (2002). A 171 bp sequence of the gene containing the polymorphism was amplified using the primers 5′-ACT CTG GAG AGC GTG AAT GG-3′ (forward) and 5′-ACT ACT GAG CAT CAC CCT GGA-3′ (reverse). This was followed by digestion of the PCR products with *PmaC*I restriction enzyme, separation of the fragments generated by agarose gel electrophoresis and identification of the genotypes (G/G (99 bp, 72 bp); G/A (171 bp, 99 bp, 72 bp); A/A (171 bp)).

The C270T polymorphism of the *BDNF* was genotyped as reported by Kunugi et al. (2003). The following pair of primers was used to amplify a 223 bp fragment of the *BDNF*: 5′-CAG

AGG AGC CAG CCC GGT GCG-3′ (forward) and 5′-CTC CTG CAC CAA GCC CCA TTC-3′ (reverse). The PCR products were subsequently digested with the *Hinf*I restriction enzyme, the fragments generated separated by agarose gel electrophoresis and the genotypes established (C/C (127 bp, 78 bp); C/T (127 bp, 78 bp, 63 bp); T/T (127 bp, 63 bp)).

Statistical analysis

The association of the distribution of the BDNF polymorphisms was studied using a Pearson Chi-Square test and a Fisher exact test. The association between the age at onset and the BDNF polymorphism was studied using the Kaplan-Meier method and the log rank test for the analysis of survival. The statistical analysis was carried out using SPSS/Win (Version11.0, SPSS Inc., Chicago, IL), and the calculations of the haplotype analysis were made with the Arlequin version 2.000 software.

Results

There was no association between the distribution of the *BDNF* G196A (val66met) and *BDNF* C270T polymorphisms and the response to typical neuroleptics ($p > 0.3$). In addition, there was no difference between the patients and the controls ($p > 0.6$). The results we unchanged when the sample was studied using different subgroups: heterozygous vs. homozygous, A allele carriers vs. those not carrying A allele (G196A polymorphism), and T allele carriers vs. those not carrying that allele (C270T polymorphism). In addition, the results remained insignificant when the smallest subgroups (A/A genotype carriers of G196A polymorphism and T/T genotype carriers of C270T polymorphism), were removed from the analysis. The genotype frequencies of the polymorphisms of the *BDNF* gene are shown in Table 1.

The patients' age at onset was not associated with the distribution of the G196A and C270T polymorphisms (Kaplan-Meier log rank $p = 0.1862$ and $p = 0.5923$, respectively).

There was no difference between responders and non-responders in the haplotype analysis of the *BDNF* polymorphisms [OR = 0.842 (95% CI 0.43–1.64), $p = 0.612$]. However, two patients had such haplotypes that controls did not have (Table 2). One *BDNF* 196A/270T haplotype belonged to a patient considered a responder, the other to a non-responding patient. The haplotype analysis did not show any association between the patients and the controls (data not shown).

Table 1. Distribution of genotypes of the *BDNF*[a]

	G196A			C270T		
	GG	GA	AA	CC	CT	TT
Responders	30 (69.8)	12 (27.9)	1 (2.3)	36 (83.7)	7 (16.3)	0 (0)
Non-responders	35 (68.6)	14 (27.5)	2 (3.9)	43 (84.3)	6 (11.8)	2 (3.9)
Patients	65 (69.1)	26 (27.7)	3 (3.2)	79 (84.0)	13 (13.8)	2 (2.1)
Controls	73 (74.5)	20 (20.4)	5 (5.1)	85 (86.7)	12 (12.2)	1 (1.0)

[a] Numbers in the parentheses indicate percentages

Table 2. Results of the haplotype analysis of *BDNF* G196A (val66met) and C270T polymorphisms[a]

G196A	C270T	Responders	Non-responders	Patients	Controls
G	C	66 (76.4)	75 (73.5)	141 (74.9)	152 (77.6)
A	C	13 (15.4)	17 (16.7)	30 (16.1)	30 (15.3)
G	T	6 (7.3)	9 (8.9)	15 (8.1)	14 (7.1)
A	T	1 (0.9)	1 (0.9)	2 (0.9)	0 (0)

[a] Numbers in parentheses indicate percentages

Discussion

The main aim of our study was to identify such different polymorphisms in the BDNF gene which may predict treatment response to typical neuroleptics. However, the *BDNF* G196A (val66met) and C270T polymorphisms were not associated with treatment response. Neither were there any haplotypes of the *BDNF* gene which could predict response to typical neuroleptics in schizophrenia. The result may be in conflict with the two recent studies by Krebs et al. (2000) and Hong et al. (2003). However, Krebs et al. (2000) studied a different polymorphism and Hong et al. (2003) studied the association between the *BDNF* G196A polymorphism and response to clozapine.

We analyzed the effects of the *BDNF* polymorphisms on the risk of schizophrenia. Consequently, the *BDNF* G196A (val66met) polymorphism is not associated with schizophrenia, which is in line with the earlier results of Egan et al. (2003) and Hong et al. (2003). The frequency of val/val genotype in the controls is 74.5%, which is higher in the present study than reported in three other populations (Japan: 33.8%, Italy: 48.7%, and USA: 68.4%) (Shimizu et al., 2004). Interestingly, the authors suggest that *BDNF* G196A (val66met) polymorphism may explain different ethnic mental traits (Shimizu et al., 2004).

Likewise, the *BDNF* C270T polymorphism is not associated with schizophrenia. This result is in conflict with an earlier publication by Szekeres et al. (2003) who found an excess of T alleles in Caucasian patients with schizophrenia. Moreover, Kunugi et al. (2003) reported more frequent T alleles in patients with schizophrenia than in controls in a Japanese sample. These conflicting results may be due to the different ethnic populations. In fact, the controls in the samples of Szekeres et al. (2003) and Kunugi et al. (2003) have considerably lower frequencies of C/T genotypes (5.9% and 4.5% respectively) than in our sample (12.2%) (Table 1). Moreover, the patients in our sample were either good or poor responders to typical neuroleptics. Thus, our different study design may have affected the results. Therefore, the results may be different if the study did not focus in the treatment response to typical neuroleptics.

In the haplotype analysis, there is no difference between responders vs. non-responders and patients vs. controls. Interestingly, one haplotype was only seen in two patients. However, the relatively small sample size of the present study does not allow us to evaluate the importance of this result.

In the present study, the *BDNF* G196A and C270T polymorphisms are not associated with the age of onset in schizophrenia. Krebs et al. (2000) reported that the age at first contact with a practitioner for psychiatric reasons was associated with the *BDNF* gene dinucleotide polymorphism. As the definition of age at onset and the polymorphism studied were different from ours, one cannot compare these results.

A relatively small sample size introduces the major limitation of the present study. Moreover, the retrospective study model did not allow the use of standardized psychiatric rating scales in defining the treatment response.

In summary, the present study suggests that the *BDNF* G196A and C270T polymorphisms are not associated with the treatment response to typical neuroleptics or with the age of first hospitalization. As there are no differences in allele frequencies between the patients and controls, these polymorphisms and haplotypes of the *BDNF* gene may not represent a risk for schizophrenia. However, these results should be considered preliminary because of the small sample.

Acknowledgements

The authors wish to thank Dr. V. Lassila, Dr. M. Roivas, and Dr. T. Ristilä for their assistance in the patient screening and recruitment, and Ms. M. Koli and Ms. N. Kilkku for their skillful technical assistance.

References

Angelucci F, Mathe AA, Aloe L (2000) Brain-derived neurotrophic factor and tyrosine kinase receptor TrkB in rat brain are significantly altered after haloperidol and risperidone administration. J Neurosci Res 60: 783–794

Dawson NM, Hamid EH, Egan MF, Meredith GE (2001) Changes in the pattern of brain-derived neurotrophic factor immunoreactivity in the rat brain after acute and subchronic haloperidol treatment. Synapse 39: 70–81

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112: 257–269

Fumagalli F, Molteni R, Roceri M, Bedogni F, Santero R, Fossati C, Gennarelli M, Racagni G, Riva MA (2003) Effect of antipsychotic drugs on brain-derived neurotrophic factor expression under reduced N-methyl-D-aspartate receptor activity. J Neurosci Res 72: 622–628

Hong CJ, Yu YW, Lin CH, Tsai SJ (2003) An association study of a brain-derived neurotrophic factor Val66Met polymorphism and clozapine response of schizophrenic patients. Neurosci Lett 349: 206–208

Illi A, Mattila KM, Kampman O, Anttila S, Roivas M, Lehtimäki T, Leinonen E (2003) Catechol-O-methyltransferase and monoamine oxidase A genotypes and drug response to conventional neuroleptics in schizophrenia. J Clin Psychopharmacol 23: 429–434

Joober R, Boksa P, Benkelfat C, Rouleau G (2002) Genetics of schizophrenia: from animal models to clinical studies. J Psychiatry Neurosci 27: 336–347

Krebs MO, Guillin O, Bourdell MC, Schwartz JC, Olie JP, Poirier MF, Sokoloff P (2000) Brain derived neurotrophic factor (BDNF) gene variants association with age at onset and therapeutic response in schizophrenia. Mol Psychiatry 5: 558–562

Kunugi H, Hirasawa H, Kato N, Nabika T, Kobayashi S, Nanko S (2003) Brain-derived neurotrophic factor gene and schizophrenia: polymorphism screening and association analysis. Schizophr Res 62: 281–283

Shimizu E, Hashimoto K, Iyo M (2004) Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. Am J Med Genet 126B: 122–123

Strange PG (2001) Antipsychotic drugs: importance of dopamine receptors for mechanisms of therapeutic actions and side effects. Pharmacol Rev 53: 119–133

Szekeres G, Juhasz A, Rimanoczy A, Keri S, Janka Z (2003) The C270T polymorphism of the brain-derived neurotrophic factor gene is associated with schizophrenia. Schizophr Res 65: 15–18

Ventriglia M, Bocchio Chiavetto L, Benussi L, Binetti G, Zanetti O, Riva MA, Gennarelli M (2002) Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. Mol Psychiatry 7: 136–137

Wassink TH, Nelson JJ, Crowe RR, Andreasen NC (1999) Heritability of BDNF alleles and their effect on brain morphology in schizophrenia. Am J Med Genet 88: 724–728

Weickert CS, Hyde TM, Lipska BK, Herman MM, Weinberger DR, Kleinman JE (2003) Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia. Mol Psychiatry 8: 592–610

Authors' address: S. Anttila, MD, Department of Psychiatry, Tampere University Hospital, FIN-33380 Pitkäniemi, Finland, e-mail: samia@koti.soon.fi