Research article
Ερευνητική εργασία

Association of serum BDNF and val66met polymorphism of the brain-derived neurotrophic factor in a sample of first psychotic episode patients

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Psychiatriki 2009, 20:297–304

Polymorphisms in the brain-derived neurotrophic factor (BDNF) gene have been indicated to be associated with schizophrenia. Previous studies have suggested that val66met polymorphism may increase the risk for schizophrenia, although other studies have not confirmed this association. Decreased BDNF levels in the brain and the serum of patients with psychotic disorders have been reported in first episode psychotic (FEP) patients. In our study we investigated the potential genetic association of this polymorphism with schizophrenia in a sample of 38 FEP patients with schizophrenia compared with a sample of 21 normal controls. Furthermore, we assessed serum BDNF levels and investigated whether there was an association between this polymorphism and alterations of serum BDNF levels between the investigated groups. There was a significant difference in genotyped frequencies between cases and controls (p=0.030). The homozygous carriers Met/Met were over-represented in the schizophrenia group (13/31, 41.9%), compared to controls (2/19, 10.5%). The serum BDNF levels in the sample of FEP patients was significantly reduced compared to controls (18.87±8.23 ng/mL *vs* 29.2±7.73ng/mL, U=140, p=0.0). No association was found between alterations of serum BDNF levels and Val66Met polymorphism in the group of patients (p=0.198). Negative correlations were shown between serum BDNF levels of the patients and the PANSS Negative subscale scores (p=0.015). There was found no significant difference between genotypes and memory scores in the sample of patients. Our findings indicate that serum BDNF levels at the onset of schizophrenia and BDNF Val66Met variant may be susceptibility risk factors for schizophrenia.

Key words: BDNF, BDNF val66met polymorphism, first episode, schizophrenia, psychopathology.

Introduction

The brain-derived neurotrophic factor (BDNF) is a member of the neurotrophic family that modulates neurotransmitter synthesis, metabolism and neuronal activity[1] BDNF is also involved in the development of dopaminergic-related systems,[2] and the mesolimbic dopamine systems.[3] Thus, according to both the neurodevelopmental theory[4,5] and the dopamine hypothesis[6,7] in the etiology of schizophrenia, the BDNF genetic locus is a strong candidate gene implicated in the development of this disorder.

The function of BDNF in Central Nervous System (CNS) raises the possibility that this type of neurotrophin is relevant to schizophrenia and a number of studies have reported the potential contribution of BDNF in the pathophysiology of the disorder. Decreased serum BDNF levels have been reported in neuroleptic free patients with schizophrenia when compared to healthy controls,[8,9] and also in serum and in prefrontal cortex in chronic patients with schizophrenia on antipsychotics.[10,11] Increased BDNF levels have been reported in chronicaly medicated patients.[12,13] BDNF levels have also been associated with the severity of positive psychotic symptoms of the patients[8] and with both positive and negative psychotic symptoms.[9]

A number of association studies have been carried out to test correlation between BDNF gene variants and schizophrenia. The two most common studied BDNF polymorphisms were the G196A (val66met) and the C270T.[14–16] Specifically the val66met polymorphism at codon 66, has been reported to influence changes in BDNF expression in the hippocampal area and affect the ability to perform tasks of verbal episodic memory.[16] Furthermore BDNF has been studied as a risk factor for schizophrenia.[17,18] Other genetic studies however have not confirmed this result in various populations of schizophrenic patients.[19–21]

In this study, we investigated whether this polymorphism of the BDNF gene is associated with first psychotic episode of schizophrenia and additionally whether there was a relationship with the alteration of serum BDNF in the group of drug-naive patients. Furthermore, we investigated the correlation of serum BDNF levels with the positive and negative psychotic symptoms of the patients.

Material and method

Subjects

Thirty seven unrelated drug-naïve FEP patients (M/F:16/21) with a mean age 26.81±9.22 years old, were recruited from the Psychiatric Departments of the two General Hospitals (General Hospital of Nikea-Pireaus and "ATTIKON" General Hospital, Haidari, Athens) from January 2006 through June 2008. Blood samples were collected at the time of patients' admission. Patients were assessed by SCID-IV,[22] by Positive and Negative Syndrome subscales (PANSS),[23] and by the Wechsler Digit Span forwards and backwards Task.[24] Exclusion criteria included a history of any neurological disease and current substance abuse or dependence in the preceding 6 months as defined by DSM-IV.[25]

Three patients were excluded because they were diagnosed –based on SCID– as suffering from brief psychotic episode and five patients with mania. The patients were followed-up monthly by two experienced psychiatrists. During this period three patients were excluded from the sample because they were diagnosed as suffering with substance abuse. Twenty five patients were suffering from paranoid type of schizophrenic disorder, ten of disorganized schizophrenia and 3 of the catatonic subtype.

The healthy control group consisted of twenty two persons (M/F:13/9) with a mean age 26.81±9.22 years old, which were recruited from the Biochemistry Laboratory Department of Athens Dromokaition Psychiatric Hospital. All controls were candidates for military services and as such were interviewed by one psychiatrist who had excluded the presence of any major psychiatric or neurological disorder. Additionally the exclusion criteria included history of current substance abuse or dependence in the preceding 6 months as defined by DSM-IV (APA, 1994).

Patients were matched to healthy controls regarding gender (Pearson Chi Square=1.386, df=1, p=0.2390), age (Mann Whitney U=354, p=0.405), years of education (Mann Whitney U=360, p=0.412),

marital (Pearson Chi Square=2.091, df=1, p=0.148) and employment status (Pearson Chi Square=0.101, df=1, p=0.750). The study was approved by the ethics committees of the three Hospitals and written informed consent was obtained from all research participants.

BDNF Measurement

Preparation of serum and storage

Human sera were obtained by drawing blood in serum collection Vacutainer tubes (Becton-Dickinson, Rutherford, NJ). The samples were allowed to clot for 30 min before centrifuged at 3500 rpm for 15 min at 15 °C. Serum was carefully separated and stored at –20 °C until analyzed.

Measurement of BDNF levels

Serum BDNF levels were quantitated in the rethawed serum samples by Quantikine Immunoassay Kit (Catalog No. DBD000) of R&D Systems (Minneapolis, MN 55413, USA). This was a double antibody sandwich ELISA method. The manufacturer's instructions were applied to develop the kit to the calibration method and to the measurement of the samples. The absorbance was measured at 450 nm and corrected at 570 nm by Mediators PhL microplate reader (Mediator Diagnostika Gmbh, Vienna, Austria).

Genotyping

DNA for genetic analysis of the BDNF precursor protein gene was extracted from 200μl of whole blood from each patient with the QIAGEN DNA Blood mini kit, according to the manufacturer's instructions. A PCR-RFLP assay was used for the detection of the single nucleotide substitution (A578G) which results in the Val/Met amino acid change in the BDNF precursor protein, as originally described by Maisonpierre et al.[26] A 206bp-long fragment of the BDNF precursor protein gene was amplified using the primers 5′-CTGGAGAGCGTGAATGGGCC-3′ and 5′-TCCAGCAGAAAGAGAAGAGGAGGC-3′, according to the protocol described by Nanko et al.[19] RFLP analysis of the PCR products with the restriction enzyme PmaCI followed and the A578G mutation was detected by the production of two restriction fragments, 70 and 136bp-long respectively. If both restriction patterns were observed (uncut PCR product and the two restriction fragments) the patient was described as a heterozygote for the BDNF precursor protein gene, coding for both normal (Val) and mutated (Met) phenotypes of the protein.

Statistical analyses

Deviation from the Hardy-Weinberg equilibrium was determined using a Pearson's x^2 test. The genotype frequencies of the patients were in accordance with the Hardy Weinberg equilibrium, whereas the respective frequencies in the control population were not. Spearman's test was used to study the correlations between serum BDNF and PANSS-positive and negative subscale scores. Differences in genotype frequencies between FEP patients and healthy control subjects were compared using the chi-square test. The statistical significance was defined by $p<0.05$.

Results

Serum BDNF levels of FEP patients were significantly reduced compared to healthy controls (Mann Whitney U=140, p=0.0). Serum BDNF levels were not correlated in patients to age (onset of disease) (Spearman's rho=0.274, p=0.101) and to the subtype of the schizophrenic disorder (Kruskal Wallis Chi-Square=3.883, p=0.144). Significantly negative correlation was found between serum BDNF levels and PANSS-negative subscale scores (Spearmans' rho= –0.398, p=0.015). There was no correlations observed between serum BDNF levels and PANSS-positive subscale scores (Spearmans' rho=–0.001, p=0.994).

Significant differences in genotype frequencies of BDNF Val66Met polymorphism were observed between FEP patients and healthy control subjects (Pearson Chi-Square=7.013, df=2, p=0.030). Specifically the homozygous mutant Met/Met genotype frequency was higher in the group of patients compared to healthy control subjects. The prevalence of genotypes Val/Val, Val/Met and Met/Met in patients with first psychotic episode was 19.4% (6 of 31), 38.7% (12 of 31) and 41.9% (13 of 31) respectively, with p=0.39 and q=1–p=0.61, whereas the prevalence of these genotypes in the control population were 10.5% (2 of 19), 79% (15 of 19) and 10.5% (2 of 19) respectively, with p=0.5 and q=1-p=0.5 (see table 1). The genotype frequencies of the patients were in

Table 1. Association of val66met genotypes in the group of normal controls and in the group of first psychotic episode patients (p = 0.030).

	BDNF Val66Met variant			*Allele Frequency*	
	Homozygous Val-val	*Heterozygous val-met*	*Homozygous met-met*	*Val allele*	*Met allele*
Normal n = 19	2 (10.5%)	15 (79%)	2 (10.5%)	19 (50.0%)	19 (50.0%)
Patients n = 31	6 (19.4%)	12 (38.7%)	13 (41.9%)	24 (38.7%)	38 (61.3%)

accordance with the Hardy Weinberg equilibrium, whereas the respective frequencies in the control population were not.

Genotype was not associated by age at onset of illness (Kruskal Wallis Chi-Square=0.506, p=0.776), serum BDNF levels (Kruskal Wallis Chi-Square=3.235, p=0.198), PANSS-positive (Kruskal Wallis Chi-Square =3.198, p=0.202) and PANSS-negative (Kruskal Wallis Chi-Square=2.471, p=0.291) subscale scores in the group of patients (see table 2).

Neither the digit span forwards (a measure of sustained attention) nor the digit span backwards scores (a measure of verbal working memory were associated significantly with genotype (p=0.338, p=0.678 respectively) or serum BDNF levels (Spearman's rho=-0093, p=0.732) in the sample of FEP patients (table 2).

Discussion

In the present study we investigated the serum BDNF levels and the presence of BDNF Val66Met polymorphism and their association with psychopathological and memory variables in a sample of drug-naive FEP patients with schizophrenia. This study was a follow up of our project with drug-naïve FEP patients and alterations in serum BDNF levels.

We confirmed the significantly reduced serum BDNF levels in FEP drug-naive patients compared to healthy controls. These results are consistently with our previously published results[9] and with other clinical studies observed in patients not only in the context of schizophrenia[8] but also in the context of mania and major depressive episode.[27,28] This may indicate that BDNF though non specific to schizophrenia, could be a biomarker of clinical importance. Our results offer further support to the prominent role of neurotrophins in the neurodegenerative pathogenetic theory of schizophrenia through their capacity to regulate central neurotransmission as well as to promote neuroplasticity.[3,29,30]

We also found a significant difference in the frequency of BDNF Val66Met variant in the sample of FEP patients (p=0.030), compared to healthy controls. Specifically the homozygous Met/Met carri-

Table 2. Sample characteristics and main effect of genotype in studied variances (means)

Variable	*Val/Val*	*Val/Met*	*Met/Met*	*Chi-Square*	*p*
Patients (31)	6	12	13		
Age of onset	26.50	27.08	26.53	0.506	0.776
Ser BDNF	17.06	22.65	16.96	3.235	0.198
Panss-pos	35.33	36.58	32.23	3.198	0.202
Panss-neg	31.16	30.33	33.46	2.471	0.291
B mem sc	33.66	19.60	19.33	0.776	0.678
F mem sc	23.33	24.40	33.71	2.170	0.338

B mem Sc: Backwards memory scores
F mem Sc: Forwards memory scores

ers showed a 41.9% over-represented with respect to the heterozygous type. Our study confirms the association of BDNF Val66Met polymorphism with schizophrenia. Other studies have confirmed the implication of this polymorphism to schizophrenia[31] and the age of onset of the disease.[32] The first study was a meta-analysis with chronic schizophrenic patients and the second referred to a sample of 42 FEP African-Americans patients. However other studies have failed to associate this polymorphism with schizophrenia in either Caucasians or Asian populations.[21,33–35]

Although there are contradictory results about the association of BDNF Val66Met variant to schizophrenia, other studies have linked this polymorphism to brain morphology, cognitive function and psychiatric symptoms in schizophrenia.[36–38] Met allele carriers had significantly greater reductions in frontal gray matter volume, with reciprocal volume increases in the lateral ventricles than Val homozygous patients. This is a result that seems to be in association with changes in cognition and clinical symptoms in schizophrenia.[39–43] Although we found no significant differences between genotypes status of patients and other variables like age of onset, alteration of serum BDNF levels, PANSS-Positive and PANSS-Negative subscale scores, we cannot rule out that other polymorphisms of BDNF gene could be related to these features of schizophrenia.

Our study also revealed significant negative correlations between serum BDNF levels of the patients and the PANSS-Negative subscale scores. Correlations of BDNF levels with PANSS-Positive subscale scores have been reported in previous studies.[8,27] Additionally our study reports a negative correlation between PANSS negative subscale scores and serum BDNF levels. As mentioned before, reduced serum BDNF levels in FEP patients might reflect an abnormally functioning dopaminergic-related signaling system, which leads to the emergence of psychotic symptoms. The negative correlation with PANSS-Negative but not with PANSS-Positive subscale scores may reflect the abnormally functioning dopaminergic-relating signaling system of mostly negative symptoms which are the core symptoms of schizophrenia. Therefore, it can be suggested that BDNF levels might be linked to the formation of these symptoms. These correlations may also indicate that BDNF is associated with the severity of psychotic symptoms of schizophrenia.

We found no significant difference in both digit span forwards and digit backwards scores. In a study of Egan et al, the same BDNF val66met polymorphism was found to have an effect on memory function and modulation.[16] This effect though not specific to schizophrenia seems to influence cognitive function which is found to be abnormal in certain forms of schizophrenia and also, as mentioned above, to aspects of brain morphology.[41] Therefore BDNF variations may influence memory function by impacting on an underdeveloped abnormal frontal gray brain matter. Despite the fact that in our sample of FEP patients the Met/Met carriers had the lowest digit forward digit backwards scores compared to the two other carriers, this hypothesis cannot be substantiated from our data.

Among the limitations of our study is the rather small though well balanced sample size. However, it must be stated that drug-naïve first-episode patients with schizophrenia are difficult to ascertain. BDNF levels were assessed in serum, thus representing an indirect measurement of brain BDNF levels. However, preclinical studies have confirmed the relationship between BDNF levels in the peripheral blood and the brain.[42,43]

Conclusions

Our results reinforce the finding that decreased serum BDNF levels are strongly associated in drug-naive first psychotic patients with schizophrenia reflecting pathophysiological processes related to the onset of the disease. The significant evidence for association between the BDNF Val66Met polymorphism and schizophrenia in a pure sample of greek nationals, provides evidence that this polymorphism is associated with schizophrenia in this caucasian population as well. Further analysis of other polymorphisms with the BDNF gene are needed to be investigated in order to ascertain the relationship between specific genotype status, alterations of BDNF levels and psychopathology in patients with schizophrenia.

Συσχέτιση των επιπέδων νευροτροφικού παράγοντα ορού BDNF και του γενετικού πολυμορφισμού val66met του ίδιου παράγοντα σε μια ομάδα ασθενών με πρώτο ψυχωσικό επεισόδιο σχιζοφρενικής διαταραχής

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Ψυχιατρική 2009, 20:297–304

Η παρουσία αρκετών λειτουργικών πολυμορφισμών στο γενετικό τόπο του νευροαναπτυξιακού παράγοντα BDNF έχει συσχετισθεί με την ανάπτυξη της σχιζοφρενικής διαταραχής. Ειδικότερα σε προηγούμενες μελέτες έχει βρεθεί ότι η παρουσία του πολυμορφισμού Val66Met αυξάνει τον κίνδυνο ανάπτυξης σχιζοφρενικής διαταραχής, αν και άλλες μελέτες δεν το επιβεβαιώνουν. Μειωμένα επίπεδα BDNF στον εγκέφαλο και στον ορό ασθενών με ψυχωσικές διαταραχές έχουν αναφερθεί σε ασθενείς με πρώτο ψυχωσικό επεισόδιο. Στην παρούσα μελέτη μας ερευνήσαμε την πιθανή γενετική σχέση αυτού του πολυμορφισμού σε ένα πληθυσμό 38 ασθενών με πρώτο ψυχωσικό επεισόδιο σχιζοφρενικής διαταραχής σε σύγκριση με έναν άλλο πληθυσμό 21 υγιών εθελοντών. Επιπροσθέτως, μετρήσαμε τα επίπεδα BDNF στον ορό και μελετήσαμε την πιθανή σχέση μεταξύ του συγκεκριμένου πολυμορφισμού και των μεταβολών των επιπέδων του BDNF στον ορό και των δύο υπό μελέτη ομάδων. Βρέθηκε στατιστικά σημαντική σχέση στις συχνότητες των γονοτύπων μεταξύ των ασθενών και των υγιών (p=0,030). Οι ομόζυγοι φορείς Met/Met υπερεκπροσωπούνταν στην ομάδα των ασθενών με σχιζοφρενική διαταραχή (13/31, 41,9%), σε σύγκριση με την ομάδα των υγιών (2/19, 10,5%). Τα επίπεδα ορού του BDNF στην ομάδα των ασθενών ήταν μειωμένα σε σχέση με τα αντίστοιχα επίπεδα στον ορό των υγιών σε στατιστικά σημαντικό βαθμό (18,87±8,23 ng/mL έναντι 29,2±7,73 ng/mL, U=140, p=0,0). Δεν βρέθηκε συσχέτιση μεταξύ των μεταβολών των επιπέδων του BDNF στον ορό και της παρουσία του γενετικού πολυμορφισμού Val66Met στην ομάδα των ασθενών (p=0,198). Αρνητικές συσχετίσεις βρέθηκαν μεταξύ των επιπέδων του BDNF στον ορό και του σκορ στην κλίμακα PANSS αρνητικών συμπτωμάτων της σχιζοφρένειας (p=0,015). Δεν βρέθηκαν σημαντικές διαφορές μεταξύ των γονοτύπων και των μνημονικών σκορ στην ομάδα των ασθενών. Τα αποτελέσματά μας καταδεικνύουν ότι τα επίπεδα του νευροαναπτυξιακού παράγοντα BDNF και ο γενετικός πολυμορφισμός του BDNF Val66Met αποτελούν παράγοντες κινδύνου για την ανάπτυξη της σχιζοφρενικής διαταραχής.

Λέξεις ευρετηρίου: Νευροτροφικός παράγοντας, BDNF, γενετικός πολυμορφισμός, πρώτο επεισόδιο, σχιζοφρένεια, ψυχοπαθολογία.

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