Plasma BDNF Level in Major Depression: Biomarker of the Val66Met BDNF Polymorphism and of the Clinical Course in Met Carrier Patients

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Abstract
Aims: Despite the involvement of the brain-derived neurotrophic factor (BDNF) in the physiopathology of major depressive disorder (MDD), the coherence between the components of the BDNF pathway and their link with the clinical features of MDD are insufficiently studied. We aimed to assess in Caucasian depressed patients the impact of the BDNF Val66Met polymorphism on plasma BDNF levels taking into account the clinical characteristics of MDD. Methods: A total of 328 Caucasian adult MDD patients with a current major depressive episode (MDE) were assessed for the BDNF Val66Met polymorphism, plasma BDNF levels and clinical characteristics of the MDD. Results: Plasma BDNF levels were linearly associated with the BDNF Val66Met genotypes (ValVal: 1,525.9 ± 1,183.3 pg/mL vs. ValMet: 1,248.7 ± 1,081.8 vs. MetMet: 1,004.9 ± 952.8; \( p = 0.04 \)), Met carriers having lower BDNF levels than ValVal ones. Significant interactions between the Val66Met polymorphism and 3 clinical characteristics – age at onset (\( p = 0.03 \)), MDD duration (\( p = 0.04 \)), and number of previous MDE (\( p = 0.04 \)) – were evidenced for plasma BDNF levels. Indeed, in Met carriers, but not in ValVal ones, plasma BDNF levels were negatively correlated with age at onset and positively correlated with MDD duration and number of previous MDE. Conclusion: Our results show a measurable, coherent, and functional BDNF pathway based on the BDNF Val66Met polymorphism and plasma BDNF levels in patients with a current MDE. This pathway is
related to the clinical course of major depression, plasma BDNF levels being associated with the long-term history of MDD in Met carriers. Further studies assessing central BDNF are needed to understand the underlying mechanisms of this association.

Introduction

The brain-derived neurotrophic factor (BDNF) is a protein of the neurotrophin family, which increases neuronal plasticity, such as neurogenesis, neurite arborization and synaptogenesis. It has been extensively involved in the pathophysiology of major depressive disorder (MDD) especially in rodents [1]. Indeed, several preclinical data argue for the association of BDNF and major depression: first, decreased BDNF mRNA expression in the hippocampus has been evidenced in different paradigms of major depression (unpredictable mild stress, social isolation, social defeat, forced swim test or maternal deprivation), and second, BDNF injections in the dentate gyrus of the hippocampus induce antidepressant effects [1, 2].

The Val66Met BDNF polymorphism, which exists in humans but not in animals, is a BDNF polymorphism for which a biological impact has been evidenced in vitro [2]. Preclinical studies in knock-in mice show that the Met allele of the Val66Met polymorphism decreases BDNF dendritic trafficking in hippocampal neurons [3] and impairs synaptic transmission and plasticity in the infralimbic medial prefrontal cortex [4], suggesting that the Met allele of the Val66Met polymorphism may be associated with a lower BDNF pathway functionality. However, its functionality has not been assessed yet in depressed patients except in 2 negative studies performed in Asian patients [5, 6], probably because there is no method to assess brain BDNF concentrations in vivo. Nonetheless, some data show that depressed patients have lower plasma BDNF levels than controls [7], suggesting that plasma BDNF may be a proxy of central BDNF in humans [8] and could be associated with the clinical characteristics of MDD [9]. Since the Met frequency of the Val66Met polymorphism is lower in Caucasian than in Asian individuals [10], and ethnicity may interfere with the impact of the Val66Met polymorphism in depressed patients [11], a different effect of the Val66Met polymorphism may exist in Caucasians as compared to Asian patients with MDD. Hence, in this study, we hypothesize that a coherent and functional BDNF pathway, based on the BDNF Val66Met polymorphism and the corresponding peripheral BDNF levels, can be measured in Caucasian depressed patients and that it is related to the clinical characteristics of MDD. Further, we expect that, in Met carriers, lower plasma BDNF levels correlate with severer clinical characteristics of MDD.

In addition, we aim to assess in Caucasian depressed patients the impact of the Val66Met polymorphism on plasma BDNF levels, and its relationship with the clinical characteristics of MDD.

Materials and Methods

Design

A total of 328 adult patients with a current major depressive episode (MDE) in a context of MDD were assessed for the Val66Met polymorphism, plasma BDNF levels and MDD characteristics. All of them were Caucasians, i.e. had 2 Caucasian parents. This study was registered by the French National Agency for Medicine and Health Products Safety and the Commission Nationale de l’Informatique et des Libertés, was approved by the Ethics Committee of Paris-Boulogne, France, and conformed to international ethical standards (ClinicalTrials.gov identifier: NCT00526383).

Patients

Patients had a current MDE in a context of MDD (DSM-IV-TR) based on the Mini International Neuropsychiatric Interview [12] and a 17-item Hamilton Depression Rating Scale (HDRS) [13] score of 18 or more. Patients with bipolar disorders, psychotic disorders, current substance abuse or dependence (DSM-IV-TR), organic brain syndromes, unstable medical conditions, and contraindications to cerebral MRI were not included. Patients receiving antipsychotics or mood stabilizers before inclusion and/or for 4 months or more during the last year were excluded.

MDD Characteristics

Clinical characteristics of the MDD such as age at onset (age at the first MDE), previous MDD duration (delay between the beginning of the first episode and clinical assessment), number of previous MDE, presence of previous antidepressant treatments, history of suicide attempts, and the current 17-item HDRS [13] score were assessed.

Genotype

Genomic DNA was extracted from circulating blood leukocytes by using Gentra Puregene Blood Kits according to the manufacturer’s protocol (Qiagen) and was stored at 20.1 °C. The BDNF Val66Met polymorphism (rs6265) was genotyped using TaqMan allelic discrimination [14], with the ABI Prisms 7900HT sequence detection system (Life Technologies). The sequence of interest was amplified using the following primers: 5′-CGCTTTCTCCCT-3′ and reverse 5′-ACCCTCATGGACATGTT-3′. The wild-type allele (Val) was detected using a 5′-FAM-fluorescent probe. For both genes, RT-PCR was performed on a TaqMan ABI PRISM 7900HT detection system (Applied Biosystems) for allelic discrimination.
Plasma BDNF Levels

Fasting blood samples were collected at 8:00 a.m. on ethylene diamine tetraacetic acid tubes. Blood was centrifuged immediately (10 min, 2,000 g at 4 °C), and plasma was stored at −80 °C. Plasma BDNF levels were assessed with an ELISA protocol (QuantiKine®, R & D Systems, Minneapolis, MN, USA). The intra-assay coefficients of variation were 5.0 and 3.8% at concentrations of 476 and 1,258 pg/mL, respectively. The interassay coefficients of variation were 11.3 and 7.6% at concentrations of 528 and 1,338 pg/mL, respectively. There was no impact of storage time on plasma BDNF levels (r = −0.02, p = 0.67). Plasma BDNF levels were preferred to serum BDNF because they are not related to platelet levels [15] and thus have higher correlations with brain BDNF [16].

Statistical Analyses

The statistical analyses were performed using R 3.2.2. The plasma BDNF level was the dependent variable, the Val66Met BDNF polymorphism and MDD clinical characteristics were the independent variables.

After bivariate analyses (χ² tests, ANOVAs and Spearman correlation tests), multivariate linear analyses were performed to analyze the association of the Val66Met polymorphism and plasma BDNF levels and the effects of Val66Met polymorphism and MDD characteristics to explain plasma BDNF levels. On account of previous results showing an association of plasma BDNF levels with age [17], gender [17, 18], and tobacco use [19], all multivariate analyses were adjusted for age, gender, and tobacco use. Results were also adjusted for the presence of a current antidepressant treatment. A p value of 0.05 was considered significant, and no corrections for multiple testing were used according to Bender and Lange [20].

An a posteriori power analysis was performed with G*Power 3.1.9.2.

Results

Sociodemographic and Clinical Characteristics

In all, 328 adult patients with an MDD and a current MDE were analyzed. Their mean age ± SD was 46.6 ± 12.8 years; 215 (65.6%) were women. Their mean age at onset ± SD was 36.4 ± 14.8 years, and the mean MDD duration ± SD was 10.0 ± 11.6 years; 238 (72.8%) had had previous MDE episodes (recurrent MDD), and the mean number of previous MDE ± SD was 1.8 ± 2.0. Overall, 141 (43.1%) patients had a history of suicide attempts, and 266 (81.4%) had previously received antidepressant drugs. Their mean HDRS score ± SD was 23.8 ± 4.7. The age at onset, the MDD duration, and the number of previous MDE were significantly correlated (age at onset and MDD duration: r = −0.52, p < 0.0001; age at onset and number of previous MDE: r = −0.40, p < 0.0001; MDD duration and number of previous MDE: r = 0.77, p < 0.0001).

Val66Met Polymorphism

In total, 216 (65.9%) patients were homozygote Val-Val, 101 (30.8%) were heterozygote Val/Met, and 11 (3.3%) were homozygote Met/Met carriers, i.e. 34.1% of patients were Met carriers (Met/Met or ValMet). There was no deviation of the Hardy-Weinberg equilibrium (χ² = 0.0, p = 0.99) for the Val66Met genotypic repartition.

As compared to ValVal, Met carriers were older, were less often tobacco users, and had a longer previous MDD duration. They did not differ for gender, age at onset, number of previous MDE, previous antidepressant treatments, history of suicide attempts, and current HDRS score (Table 1).

Plasma BDNF Levels

Plasma BDNF levels were normally distributed (mean ± SD = 1,418.5 ± 1,149.9 pg/mL, IQR = 1,489.9). They were associated neither with age, gender nor with clinical characteristics of MDD in the whole sample.

A significant and linear relationship was shown between the Val66Met genotypes and plasma BDNF levels (Fig. 1), on the basis of a codominant genetic model, the Met allele being associated with lower plasma BDNF levels. Accordingly, in the multivariate linear regression controlled for age, gender, and tobacco use, the Met carriers had lower plasma BDNF levels than the ValVal ones.
Adjustment for the presence of a current antidepressant treatment did not change the results.

Moreover, since ValVal and Met patients differed significantly for the MDD duration and plasma BDNF levels, both the Val66Met polymorphism and clinical characteristics were introduced in the same model to explain plasma BDNF levels. Significant interactions were shown between Val66Met polymorphism and age at onset ($p = 0.03$), MDD duration ($p = 0.04$), and number of previous MDE ($p = 0.04$). Accordingly, in Met carrier patients, plasma BDNF levels were negatively correlated with age at onset ($r = -0.15$, $p = 0.04$), MDD duration ($r = -0.20$, $p = 0.04$), and number of previous MDE ($r = 0.17$, $p = 0.07$), and positively correlated with age at onset ($r = 0.15$, $p = 0.04$), MDD duration ($r = 0.20$, $p = 0.04$), and number of previous MDE ($r = 0.03$, $p = 0.04$)
age, sex, and tobacco use) = 0.03, \( p \) [adjusted for current antidepressant treatment] = 0.03) (Fig. 2). On the contrary, in ValVal patients, no significant association was observed between plasma BDNF levels and clinical characteristics.

Regarding the association between the Val66Met BDNF polymorphism (codominant model) and plasma BDNF, the a posteriori power was 61.7%.

**Discussion**

First, we show a linear relationship between the BDNF Val66Met genotypes and plasma BDNF levels in Caucasian depressed patients. ValMet patients having intermediate plasma BDNF levels. Moreover, we show that the Met allele is associated with lower plasma BDNF levels, independently from other factors such as sex, age, or tobacco use. The effect size is moderate since Met patients have 20% lower plasma BDNF levels than ValVal patients.

This study in Caucasian patients is the first showing an association between BDNF plasma levels and the BDNF Val66Met polymorphism in MDD. In Asian patients, studies assessing the association between BDNF plasma levels and the BDNF Val66Met polymorphism were negative both in MDD [5, 6] and in patients with other disorders [21–25]. Regarding Caucasians, a study in schizophrenia shows results coherent with ours [26] although 2 studies in community samples [16, 27] and 1 in patients with eating disorders [28] were negative.

Regarding the lower plasma BDNF levels in patients with the Met allele, our results are coherent with the preclinical literature showing that the Met allele is associated with a lower brain BDNF functionality [3].

Second, we show that, in Met carriers for the Val66Met polymorphism, who have a dysfunctional BDNF pathway, higher plasma BDNF levels are associated with the MDD history, i.e. earlier age at onset, longer MDD duration and higher number of MDE, but not with severity of the current MDE. Indeed, we show increasing BDNF plasma levels with an increasing duration of MDD and number of MDE in Met patients. This positive finding adds to the existing literature on MDD patients, which shows that neither Val66Met nor peripheral BDNF were individually associated with MDD duration [29–33], age at onset [29–32], or number of MDE [31–33]. However, this finding is unexpected regarding our initial hypothesis, which was that Met carriers would have lower plasma BDNF levels and severer clinical characteristics such as longer duration of the disorder and a higher number of MDE. This unexpected result may be explained by the inflammation process, the association of which with the worst clinical course and higher BDNF levels has been suggested previously [34, 35]. Moreover, Met allele patients had higher depression scores after pro-inflammatory interferon-\( \alpha \) than ValVal [36], suggesting that inflammation could interact with the BDNF pathway. This mechanism needs to be explored in further studies by assessing inflammation biomarkers in addition to the Val66Met BDNF polymorphism, clinical characteristics and plasma BDNF levels in both preclinical studies and clinical studies in depressed patients. Furthermore, MDD is probably a multigenetic disease, and the investigation of a single variation in a single gene may not be sufficient to address the biology of the disorder. Taking into account other genes and epigenetic controls, in addition to Val66Met BDNF polymorphism and plasma BDNF, could improve the biological model of MDD.

Some points argue for the validity of the results observed in this study. In line with previous meta-analyses reporting that the Met allele is associated with depression in men but not in women and in geriatric but not in non-geriatric samples [10, 37], the Met allele is associated with gender and age in our sample. Moreover, the Met allele frequency, 18.8% in this sample, is in line with the Met allele frequency reported in MDD Caucasian patients (19.0%) [10].

However, this study has several limitations. First, we did not assess central BDNF but plasma BDNF levels. Indeed, preclinical studies highlight the role of central BDNF in MDD [1], but, to the best of our knowledge, the brain biopsy is the only method that could assess central BDNF levels. In this study, we only assessed plasma BDNF, because it is noninvasive and because it is associated with the central BDNF level in animals [8, 15]. Second, all patients of this sample were currently depressed. Thus, the effects of the current MDE on plasma BDNF levels were not taken into account and may have hidden other relevant effects. And this point prevents generalization of our results to remitted MDD patients. Third, the power of this study was rather low even if the sample size was reasonable. Fourth, results were not adjusted for all sociodemographic variables (work, family, income, social status, education...), which could be confounders. Fifth, the size effect was rather small due to the lack of penetrance. Sixth, there was a lack of extensive BDNF pathway assessment. Epigenetic controls, and the total gene and molecular cascade could be assessed in further studies.
Conclusion

Our results show a measurable, coherent, and functional BDNF pathway based on the BDNF Val66Met polymorphism and plasma BDNF levels in patients with a current MDE, Met carriers having lower plasma BDNF levels than ValVal ones. This pathway is related to the clinical course of major depression, plasma BDNF levels being associated with the long-term history of MDD in Met carriers. Further studies assessing central BDNF are needed to understand the underlying mechanisms of this association.

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