No association of the Val66Met polymorphism of brain-derived neurotrophic factor (BDNF) to multiple sclerosis

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Abstract

Brain-derived neurotrophic factor (BDNF), a neurotrophin produced by neurons and immune cells, promotes neuronal survival and repair during development and after CNS injury. The BDNF-Val66Met polymorphism is functional and induces abnormal intracellular trafficking and decreased BDNF release. Therefore, we investigated the impact of the BDNF-Val66Met polymorphism on the susceptibility and clinical course in a case–control study of 224 multiple sclerosis (MS) Spanish patients and 177 healthy controls. We found no evidence for association to susceptibility or severity of the disease in our population. Moreover, we did not observe, in a subgroup of 12 MS patients, that the methionine substitution at position 66 in the prodomain had negative impact in the capacity to produce BDNF by peripheral blood mononuclear cells (PBMC).

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Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS), with both inflammatory and neurodegenerative components. Although the mechanisms underlying both processes remain unclear, genetic and environmental factors seem to contribute to the etiology and the course of the disease [5]. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, which promotes neuronal survival and repair during development and after CNS injury [1]. Thus, BDNF is immunolocalized in inflammatory cells in active MS lesions and immune cells are the main source of BDNF, supporting the concept that inflammation may have a neuroprotective role in MS [9,10,16,18].

A frequent single nucleotide polymorphism in the exon 5 of the human BDNF gene (GenBank accession no. NM170735) determines a valine to methionine substitution at amino acid position 66 of the prodomain of BDNF. This change, Val66Met, has been associated with abnormal intracellular trafficking and decreased BDNF release in an activity-dependent manner, and with deleterious influence in several neuropsychiatric diseases [6–8,12,17].

In the present study, we analyzed the effect of the BDNF-Val66Met polymorphism on the susceptibility and the clinical course of the disease in a Spanish MS population. Furthermore, we assessed the impact of these genotypes in the production of BDNF by immune cells.

In a cross-sectional manner, we collected clinical and genetic data of 224 unrelated MS patients of Spanish origin, diagnosed by standard criteria [3], recruited from the outpatient Service of Neurology of three centers, and followed according to standardized protocol. One hundred and forty of them were previously described in a study that analysed the influence of NOS2A polymorphism to MS [3]. The demographic and clinical data collected were: age, gender, type of MS, age at onset, disease duration, disease severity according to the expanded disability status scale (EDSS) score, annualized relapse rate, time between first and second relapse, time to reach an EDSS score of 4.0 and

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BDNF gen, Germany). Allelic variants at codon 66 (SNP# rs6265) of controls was isolated using QIAmp DNA Blood Mini Kit (QIAstudy. The Ethical Committee of the participant centers approved the study. Informed consent was obtained from all patients and ethnicity and of Spanish origin were included as controls. One hundred and seventy-seven age- and sex-matched unrelated healthy blood donors from the same geographical area were included in each run.

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Genomic DNA from whole blood samples from patients and controls was isolated using QIAPrep DNA Blood Mini Kit (Qiagen, Germany). Allelic variants at codon 66 (SNP# rs6265) of BDNF gene were genotyped using TaqMan® SNP Genotyping Assay (PE Applied Biosystems, Foster City, CA, USA) following the manufacturer’s instructions. Control samples of each genotype were included in each run.

Patients and controls were in Hardy–Weinberg equilibrium (MS $x^2 = 0.14, 2df, p = 0.92$; and controls $x^2 = 2.23, 2df, p = 0.33$). As shown in Table 2, the alleles and genotypes distribution frequencies of the BDNF-Val66Met polymorphism of the MS patients were not different from those of the control population examined by using chi-square test and the Fisher's exact test. The heterozygosity was 0.37 in MS patients and 0.36 in controls. No differences were found in the allelic and genotypic frequencies among the three participant hospitals.

Neither allele nor genotype was associated with the demographic or clinical variables analysed. Thus, the genotype distribution frequencies were similar in the different clinical form of disease, and between genders. Kruskal–Wallis test did not identify differences in baseline EDSS or PI. By using ANOVA test, a difference among the three genotypes was not found for age, age at onset, disease duration, annualized relapse rate, time between first and second relapse, and MSSS. Moreover, the time to achieve an EDSS of 4.0 and 6.0, and the time to develop a secondary progressive MS was studied by using Kaplan–Meier curves, and after log rank test comparison between genotypes no differences were found. In a model of logistic regression, disease duration was associated with severe disease outcome, analysed by MSSS (OR = 1.1 per year, 95% CI 1.06–1.15, $p < 0.0001$).

After correction for this variable, BDNF-Val66Met polymorphism remained without association with disability outcome measured by PI or MSSS.

We previously reported BDNF levels in serum and culture supernatant of peripheral blood mononuclear cells in 14 rapidly evolving MS patients who underwent autologous hematopoietic stem cell transplantation [2]. Here, in an exploratory study, we analyzed the genotype of this homogeneous group of patients to evaluate its influence in the BDNF production. Nine of the 12 analyzed patients were Val/Val and 3 Val/Met heterozygous. Baseline serum levels were not different between both groups (31.46 ng/ml versus 30.5 ng/ml, respectively, $p = 0.64$). By using Mann–Whitney test, a non-significant increased level was found in the supernatant of unstimulated PBMC of the Val/Met genotype patients (905.13 pg/ml versus 623.45 pg/ml, $p = 0.2$). After PBMC stimulation with anti-CD3 and soluble anti-CD28 antibodies (stimulation that mimics a physiological mode of T-cell activation), the BDNF production of Val/Met patients was 4.43-fold higher than that of Val/Val homozygous patients ($p = 0.017$).

Taken together, our data suggests that the methionine substitution at position 66 in the prodomain has no negative impact on the capacity to produce BDNF by immune cells, considering the...
of the disease.

The genotype distribution in our population is similar to those reported in other Spanish samples and European and American populations [4,7,11,14]. Therefore, the lack of association we found does not seem to be related to a specific genetic background of our population.

Although it is well known the negative effect of BDNF-Met 66 in regulated BDNF secretion from neuronal cells, reducing the amount of BDNF released in an activity-dependent manner, this effect has not been demonstrated in other cell populations such as endothelial cells [6]. Moreover, in vivo analysis on effects of BDNF genotype by using proton magnetic resonance spectroscopic imaging showed lower hippocampal levels of N-acetyl-aspartate in Val/Met heterozygous compared to Val/Val subjects, but no effect of BDNF genotype in other brain regions [13]. In spite of the limitations of our study on the functional effect of BDNF genotype in immune cells, all these data would support that the BDNF-Val66Met polymorphism is related to a selective impairment. In any case, our study suggests that this functional polymorphism is not influencing the clinical course of the disease.

References