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Research report

The effects of BDNF Val^{66}Met polymorphism on brain function in controls and patients with multiple sclerosis: An imaging genetic study

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ABSTRACT

Relatively little is known about genetic determinants of cognitive dysfunction in multiple sclerosis (MS). A growing body of evidence demonstrates that a functional variant of the brain-derived neurotrophic factor (BDNF) gene, the Val^{66}Met polymorphism, contributes to poor hippocampal and prefrontal functions, particularly memory processes, in healthy controls. In contrast, findings from previous association studies examining this polymorphism and memory performance in MS patients yielded conflicting results. However, the way in which this BDNF polymorphism affects brain function in MS patients has not been examined. In line with the "intermediate phenotype" approach, we assessed effects of the BDNF Val^{66}Met polymorphism on brain activity during a spatial working memory task. We used functional magnetic resonance imaging (fMRI) to measure brain responses in a total of 61 subjects comprising 29 relapsing–remitting MS patients and 32 healthy controls. The fMRI results demonstrated association of the BDNF polymorphism with brain activity during working memory, with opposite effects in MS patients and controls. Healthy carriers of the Met^{66} allele showed increased activation of the parieto-prefrontal network and altered disengagement of the ventro-medial prefrontal cortex and hippocampus in comparison with their respective Val^{66} counterparts. Analysis within the group demonstrated that this working memory-related activation pattern was absent in MS patients. Our imaging genetic study demonstrates that the Val^{66}Met polymorphism of the BDNF gene contributes to some of the individual variability in the functional response to a working memory challenge in healthy controls but it does not provide evidence for an MS-specific pattern of gene action.

1. Introduction

Multiple sclerosis (MS) is a demyelinating disease affecting the central nervous system (CNS), being frequently associated with cognitive deficits, which are particularly measurable in tests that are sensitive to frontal lobe function in both early [2] and late stages of the disease [1]. Performances on tasks of attention, working memory and information processing speed are commonly impaired [3,46]. The possibility that the onset of these symptoms may herald future development of a progressive dementing process, means that investigation of factors potentially influencing the cognitive profiles of patients with MS is warranted [13]. One such factor is the brain-derived neurotrophic factor (BDNF), a neurotrophin implicated in almost all aspects of CNS development, including neuronal survival and proliferation, synapse formation and synaptic plasticity [6,30,40,45,57]. There is accumulating evidence for a functional role of BDNF also in the periphery. In fact, not only neurones can produce BDNF but also immune T cells, B cells and monocytes, especially after activation [21,35]. The mRNA of BDNF is readily detectable in peripheral blood mononuclear cells of MS patients

Abbreviations: RRMS, relapsing-remitting multiple sclerosis; BDNF, brain-derived neurotrophic factor; DLPFC, dorsolateral prefrontal cortex; SPL, superior parietal lobule; vmPFC, ventro-medial; ACC, anterior cingulate cortex; EDSS, Expanded Disability Status Scale; FSS, Fatigue Severity Scale; TLL, total lesion load; BPf, brain parenchymal fraction; GMf, gray matter fraction; WMf, white matter fraction; RT, reaction time; BOLD, blood oxygenation level-dependent; Met^{66}+, homozygous Val/Val; Met^{66}−, homozygous Met/Met or heterozygous Val/Met.

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and its level is increased in MS patients compared with patients with other neurological diseases or healthy controls [15]. Among currently used disease modifying therapies in MS, immunomodulatory drugs such as glucocorticoids, interferon-beta, glatiramer acetate (GA) and immunoglobulins play a crucial role. In particular, it has been demonstrated that GA can increase BDNF production through the activation of Th1 and Th2 lymphocytes classes [59]. Current theories propose that GA-activated Th2-cells penetrate the CNS, release anti-inflammatory cytokines to inhibit neighbouring inflammatory cells by a mechanism termed "bystander suppression". This fact, together with the release of BDNF and the expression of the receptor of BDNF, TrkB, in neurons and astrocytes in brain lesions [52] may confer to GA and BDNF a probable protective role in MS therapy.

BDNF is also one of the most interesting genes currently associated with cognitive function. Within the BDNF gene, a single nucleotide polymorphism (SNP), located at nucleotide 196 (dbSNP rs6265), results in a valine to methionine ($Val^{66}Met$) substitution in the pro-peptide of the BDNF molecule. As a result, the BDNF methionine-containing variant (Met) fails to localize to secretory granules or synapses, resulting in inefficient activity-dependent secretion [12]. The BDNF-Met allele ($Met^{66}-$) has been associated with a wide range of neurodegenerative disorders, which involve a loss of neuronal integrity, including Alzheimer's disease [24], Parkinson's disease [36] and MS [33]. In addition to associations with various neurological disorders, carriers of the $Met^{66}-$ allele have been associated with cognitive impairments in otherwise healthy individuals. In particular, $Met^{66}-$ carriers showed poor cognitive performance during episodic memory tasks [12] and working memory tasks [11,47,48], although conflicting findings have been reported [17,50]. Several functional magnetic resonance imaging (fMRI) studies demonstrated that the $Met^{66}-$ allele is associated with altered neurophysiological activity in specific target areas such as the hippocampus and dorsolateral prefrontal cortex (DLPFC) [12,17,43]. In addition to these observations of altered brain function, reduced grey matter volumes for the DLPFC and superior parietal lobule (both regions known to subserve working memory), have been reported for $Met^{66}-$ carriers compared to Val homozygotes ($Met^{66}+$) [37,43].

The genetic basis of MS-related cognitive impairment is still under investigation and no study has examined the neurocognitive phenotypic correlates of the BDNF $Val^{66}Met$ polymorphism within this disorder. For this reason, the aim of this imaging genetic study is to investigate the effect of the BDNF polymorphism on cognitive-related brain function in a select MS population compared with healthy controls. The available functional, structural and behavioral data from healthy subjects suggest that the Met^{66} allele of the BDNF gene might have a detrimental effect on prefrontal-related cognitive function. Thus, our hypothesis is that the BDNF polymorphism would have a measurable impact on regional brain activation of the neural network subserving working memory. Specifically, we expect that the detrimental effects of this genetic polymorphism on cognitive function should be magnified in MS patients.

2. Materials and methods

2.1. Participants

We studied forty-nine MS outpatients who met the criteria for clinically definite relapsing-remitting (RR) course [31] and compared them with fifty-four healthy subjects recruited from the local population. Both groups underwent fMRI analysis during a spatial working memory task. All subjects gave their written informed consent to participate in this protocol, after approval by the Ethics Committee of the University of Catanzaro, according to the Helsinki Declaration.

Out of a consecutive series of 49 RRMS patients, we retrospectively selected patients who fulfilled the following criteria: (1) disease duration no longer than 10 years; (2) no clinical relapses for at least 1 month prior to study entry; (3) no concomitant therapy with antidepressant, psychoactive, steroid or other disease modifying therapies; (4) no evidence of cognitive impairment as evaluated by a detailed neuropsychological assessment (see next section); (5) no evidence of major depressive episodes or other psychiatric disorders according to the Structured Clinical Interview of the DSM-IV [53]; (6) minimum score of 65% correct response on the 2-back task; (7) minimal clinical disability, Expanded Disability Status Scale (EDSS) [25] score from 0 to 3; (8) right-handedness according to the Edinburgh handedness inventory [41]; (9) completely normal functioning of the right upper limb and optimal visual acuity; (10) no head movement artifacts during fMRI session (see Section 2.7). Twenty-nine RRMS patients fulfilled the inclusion criteria (15 female and 14 men; mean age ± SD = 28.9 ± 7.6 years; mean educational level ± SD = 12.4 ± 3.3 years) and participated in the study. In all patients, fatigue was assessed by a neurologist specialized in MS, according to the Fatigue Severity scale (FSS) [23]. The MS subjects did not receive GA during this study. Four patients were being treated with beta-interferon at the time of the study.

From the initial cohort, thirty-two right-handed healthy volunteers with no previous history of neurological or psychiatric diseases and with normal MRI of the brain (as assessed by structural MRI scanning) were matched for age and education with MS patients (18 female and 14 men; mean age ± SD, 30.5 ± 5.9 years; mean educational level ± SD = 13.75 ± 2.6 years). The demographic and clinical characteristics of all participants are summarized in Table 1.

According to alleles, each group (control and MS) was categorized into three groups: the homozygous Val/Val group ($Met^{66}+$) (control: $n = 18$, MS: $n = 15$), the Val/Met-BDNF group (control: $n = 12$; MS: $n = 13$), and the remaining homozygous Met-BDNF group (control: $n = 2$; MS: $n = 1$). The allelic distribution of BDNF gene was in Hardy–Weinberg equilibrium in both groups (MS group; $\chi^2 = 0.83$; p-level = 0.363; control group; $\chi^2 = 0.0$; p-level = 1.00). Because of the low number of subjects with homozygous Met-BDNF, and since heterozygosity produces a 70% reduction of secretion efficiency [8], we combined heterozygous Val/Met and homozygous Met/Met subjects into a single group ($Met^{66}-$). To reduce the possibility of artifactual association caused by ethnic stratification, the final sample only

Table 1
Allele distribution, demographic data and behavioral performances during the fMRI task.

	MS-$Met^{66}-$	MS-$Met^{66}+$	HC-$Met^{66}-$	HC-$Met^{66}+$	Allele F (p)	Diagnosis F (p)	Allele by diagnosis F (p)
Number of subjects	14	15	14	18			
Gender (m/f)	7/7	5/10	8/6	7/11			
Age (mean ± SD)	31.1 ± 8.9	26.2 ± 5.4	30 ± 6.4	30.9 ± 5.5	1.37 (0.25)	1.16 (0.28)	2.8 (0.1)
Education (y) (mean ± SD)	11.5 ± 2.9	13.1 ± 3.6	13.3 ± 2.8	14 ± 2.4	2.5 (0.14)	2.03 (0.16)	1.01 (0.32)
Disease duration (m) (mean ± SD)	40.5 ± 31.3	26.7 ± 31.2	–	–	1.53 (0.22)		
EDSS (*Median* (*range*))	1.5 (1–3)	1.5 (1–2.5)	–	–	96 (0.97)		
FSS (*Median* (*range*))	20.5 (11–42)	27.5 (9–49)	–	–	61 (0.59)		
TLL (cm^3) (mean ± SD)	4.3 ± 1.4	4.02 ± 1.2	–	–	78 (0.36)		
BPf (mean ± SD)	0.8 ± 0.02	0.82 ± 0.02	0.82 ± 0.01	0.81 ± 0.02	3.99 (0.051)	6.05 (0.01)*	3.83 (0.055)
GMf (mean ± SD)	0.43 ± 0.02	0.45 ± 0.02	0.46 ± 0.01	0.45 ± 0.01	2.6 (0.11)	7.53 (0.008)*	6.8 (0.01)*
WMf (mean ± SD)	0.37 ± 0.02	0.37 ± 0.01	0.36 ± 0.01	0.37 ± 0.02	2.04 (0.15)	0.11 (0.74)	0.32 (0.57)
2-Back RT (ms) (mean ± SD)	473.7 ± 228.3	414.3 ± 166.3	363.1 ± 140.2	333.5 ± 121.1	1.08 (0.3)	5.01 (0.03)*	0.01 (0.72)
2-Back accuracy (%) (mean ± SD)	73.2 ± 5.1	75.2 ± 8.2	80.2 ± 14.3	83.1 ± 14.5	0.58 (0.44)	3.01 (0.09)	0.01 (0.91)

Table 1 shows results of demographic variables and behavioral data according to diagnostic status and BDNF allele. p-Values are reported for comparisons between groups that reached significance at $p < 0.05$. MS: multiple sclerosis; HC: healthy controls; EDSS: Expanded Disability Status Scale; FSS: Fatigue Severity Scale; TLL: total lesion load; BPf: brain parenchymal fraction; GMf: gray matter fraction; WMf: white matter fraction. RT: reaction time. Data are expressed as mean values (±SD), or median values (range) when appropriate. Demographic, volumetric and behavioral differences between groups were tested with one-way ANOVAs followed by Bonferroni correction for pair-wise comparisons or with the Mann–Whitney U-test when appropriate. The asterisk (*) indicates a significant difference.