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Functional magnetic resonance imaging of BDNF val66met polymorphism in unmedicated subjects at high genetic risk of schizophrenia performing a verbal memory task

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ABSTRACT

Multiple strands of evidence suggest a role for Brain Derived Neurotrophic Factor (BDNF) in the pathophysiology of schizophrenia. It is not yet clear, however, how BDNF may contribute to altered brain function seen in the disorder, or in those at high genetic risk. The current study examines functional imaging correlates of the BDNF val66met polymorphism in a population at high genetic risk of schizophrenia. Subjects at high genetic risk for the disorder ($n = 58$) provided both BDNF genotyping and fMRI data while performing a verbal memory task. During encoding, participants were presented with a word and asked to make a 'living'/'non-living' classification. During retrieval, individuals were requested to make an 'old'/'new' word classification. For encoding, we report decreased activation of the inferior occipital cortex and a trend in the cingulate cortex in Val homozygote individuals relative to Met carriers. For retrieval, we report decreases in activation in the prefrontal, cingulate cortex and bilateral posterior parietal regions in Val homozygote individuals versus Met carriers. These findings add to previous evidence suggesting that genetic variation in the BDNF gene modulates prefrontal and limbic functioning and suggests that it may contribute to differences in brain function seen in those at high risk of the disorder.

1. Introduction

The hypothesis that neurotrophic factors are involved in the pathogenesis of schizophrenia is based on the theory that the neurodevelopmental abnormalities seen in the disorder could arise from abnormal cell migration, disconnection, disturbances in neurotransmitter systems, and changes in neural plasticity caused by alterations in the expression and or functioning of these molecules. The impact of such changes is postulated to span not just early developmental phases but also changes in adult neural plasticity and could result in compromised responses to environmental factors in adulthood such as reactions to stress or anxiety provoking stimuli (Thome et al., 1998; Durany and Thome, 2004; Aguilera et al., 2009; Alleva and Francia, 2009). The neurotrophin hypothesis thereby encompasses interactions between genetic predisposition and environmental influences typically described in complex models of schizophrenia.

BDNF is the most widely distributed neurotrophin in the central nervous system (Yan et al., 1997; Bath and Lee, 2006). Although the precise molecular and signalling mechanisms are not completely understood, it is considered to be involved during neurodevelopment, and in the regulation of structure and function of neural circuits throughout life, including long-term potentiation (LTP), the molecular substrate of learning and memory (Bath and Lee, 2006; Bekinschtein et al., 2008). Numerous lines of evidence have suggested that BDNF may play a role in the pathophysiology of the disorder. Studies of post-mortem tissue from patients have indicated altered expression levels of BDNF mRNA in the prefrontal cortex (Weickert et al., 2003; Hashimoto et al., 2005), the hippocampus (Takahashi et al., 2000; Knable et al., 2004), and the cingulate cortex (Takahashi et al., 2000). As circulating BDNF levels are considered to reflect BDNF levels in the brain, other studies have examined serum and or plasma levels in patients with the disorder. The majority report decreased levels in patients (Toyooka et al., 2002; Pirildar et al., 2004; Palomino et al., 2006; Buckley et al., 2007; Grillo et al., 2007; Ikeda et al., 2008), although some report increases or fail to find a difference versus controls (Shimizu et al., 2003; Huang and Lee, 2006; Gama et al., 2007), possibly relating to heterogeneity of samples, particularly given the potential impact of medication on BDNF levels (Lipska et al., 2001; Angelucci et al., 2005; Grillo et al., 2007). BDNF is also considered important in the development and function of neurotransmitter systems considered to be dysregulated in schizophrenia (Connor and Dragunow, 1998; Lessmann, 1998; Harrison, 1999; Djalali et al., 2005; Guillin et al., 2007).

A naturally occurring single nucleotide polymorphism in the human BDNF gene has recently been identified leading to a valine (Val) to methionine (Met) substitution at nucleotide 196 in the 5′ prodomain (val66met). This polymorphism has been shown to be functionally

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relevant, affecting intracellular trafficking and activity-dependent secretion of BDNF, human episodic memory, and hippocampal functioning (Egan et al., 2003; Hariri et al., 2003; Chen et al., 2004). It has also been linked with susceptibility to various neuropsychiatric diseases, including schizophrenia (Neves-Pereira et al., 2005; Rosa et al., 2006), schizoaffective disorder (Lencz et al., 2009), bipolar disorder (Sklar et al., 2002; Muller et al., 2006) and with anxiety-related personality traits (Lang et al., 2005). One study reported significant excess of the Val allele in a large Scottish sample of patients with schizophrenia (Neves-Pereira et al., 2005). Another reported an over-transmission of the Val allele to affected offspring (Rosa et al., 2006). It should be noted however that other studies have reported no association between this polymorphism and risk to the disorder (Kanazawa et al., 2007; Kawashima et al., 2009). Inconsistencies may be attributable to sample size and population differences, particularly with regard ethnicity (Qian et al., 2007; Petryshen et al., 2009). Further, studies of this polymorphism in healthy individuals suggest that it is the Met allele, rather than the Val allele that is associated with poorer episodic memory performance and hippocampal function (Egan et al., 2003; Hariri et al., 2003). It may appear therefore that the Met allele confers impaired memory function in healthy controls, yet the Val allele may confer risk to schizophrenia.

Hence, although there are many possible genetic loci proposed for schizophrenia, evidence points to the potential importance of the BDNF genotype in the pathophysiology of the disorder and phenotypes associated with the illness. It is unclear, however, to what degree this polymorphism contributes to structural and functional brain abnormalities seen in the disorder. Imaging studies of unaffected relatives of patients with schizophrenia suggest the presence of less severe structural and functional brain abnormalities than those seen in the established disorder. There is also evidence of a positive relationship between the genetic proximity and the severity of some of these abnormalities, suggesting they may be inherited as part of a state of vulnerability or 'intermediate phenotype'. These studies are also not confounded by anti-psychotic medication. In the current study we sought to determine if the val66met BDNF polymorphism would be associated with altered functioning of prefrontal and limbic regions, including the hippocampus, in a cohort of individuals at high genetic risk of schizophrenia from the Edinburgh High Risk Study (EHRS). Specifically, we hypothesised that there would be differences in prefrontal and limbic functioning in high risk individuals homozygous for the Val allele versus Met carriers. The study involved fMRI scanning during performance of a verbal memory task which has previously been shown to recruit prefrontal and limbic regions (Whyte et al., 2006; Whalley et al., 2007).

2. Methods

2.1. Subject details

The Edinburgh High Risk Study (EHRS) examined young adults at enhanced genetic risk of schizophrenia over the period at which they are at greatest risk of becoming ill. Full recruitment details have been presented previously (Hodges et al., 1999; Johnstone et al., 2000). Briefly, high risk participants were selected on the basis of being aged between 16 and 25 years when first recruited (1994–1999), and having one first or second degree relative with schizophrenia and a minimum of one further genetic relative with the illness. None of the subjects were on anti-psychotic medication or seeking treatment, or indeed saw themselves as unwell and therefore did not fulfil diagnostic criteria for any psychiatric disorder. This report presents results obtained during the second phase of the study (1999–2004) when fMRI was introduced into the protocol. All subjects were supplied with detailed written information regarding the study and provided written informed consent. The study was approved by the Psychiatry and Clinical Psychology subcommittee of the Lothian research ethics committee. A total of 86 high risk subjects provided a usable fMRI scan during performance of a verbal memory paradigm (Whyte et al., 2006). Of these, 58 high risk subjects provided genotyping data. At the time of scanning all subjects underwent the Present State Examination (PSE) (Wing et al., 1974; Whalley et al., 2004) and the PANSS (Kay et al., 1987) conducted by two experienced psychiatrists. Four subjects with fMRI data subsequently became ill after the baseline scan (Whalley et al., 2006), however only one of these subjects provided both genotyping and fMRI data hence it was not possible to meaningfully address subsequent illness effects in the current analysis.

2.2. Genotyping

Genomic DNA was extracted from venous blood samples using standard protocols. SNPs were genotyped by the Wellcome Trust CRF Genetics Core Facility using TaqMan assay-by-design assays. Genomic DNA was isolated from whole blood. The Target sequence for the SNP was submitted to the Assay By Design Service for SNP Genotyping Assays. Assays from the service consist of a mix of unlabelled primers and TaqMan MGB probes. Genotyping was performed in 384 well plates, using the TaqMan polymerase chain reaction-based method. The final volume PCR reaction was 5 μl using 20 ng of genomic DNA, 2.5 μl of Taqman Master Mix and 0.125 μl of 40× Assay by design Genotyping Assay Mix. The cycling parameters are as follows: 95° for 10 min, followed by 40 cycles of denaturation at 92° for 15 s and annealing/extension at 60° for 1 min. PCR plates were then read on ABI PRISM 7900HT instrument with SDS v2.1 software. Subjects were typed for the G/A (valine–methionine) at position 758 of the BDNF gene (MIM 113505) ref rs6265.

2.3. Verbal memory task

Details of the verbal memory task have been presented previously (Whyte et al., 2006). This was an event-related paradigm comprising two phases; encoding and retrieval. During encoding single words were presented in the scanner and subjects were asked to classify them as 'living' or 'non-living' by pressing the relevant button. A total of 36 words were presented, 18 referring to living things and 18 to non-living things. During retrieval single words were presented, randomly selected from those shown during the encoding phase along with matched similar new words, and subjects were requested to classify them as either 'new' or 'old' words and signify their response by pressing the relevant button. In total 72 words were presented, 36 words which were presented previously (old) intermixed with another 36 matched new words. Stimuli were presented for 2 s, followed by a variable fixation period of 2–10 s. Both parts of the task were preceded by a practice session with feedback.

2.4. Scanning procedure

Imaging was carried out at the Brain Imaging Research Centre (BIRC) for Scotland on a GE 1.5 T Signa scanner (GE Medical, Milwaukee, USA). The imaging protocol consisted of a localiser scan, followed by a T2-weighted fast spin-echo sequence, and a structural T1 weighted sequence followed by the functional imaging paradigms. Functional images were collected using an EPI sequence (TR/TE = 2000/40 ms; matrix = 64 × 64; field of view (fov) 220 × 220 mm). Twenty-four contiguous 5 mm axial (horizontal) slices were collected at an oblique angle aligned with the anterior and posterior commissure. Data were acquired during 2 sessions, consisting of 104 volumes for the first (encoding) and 204 volumes for the second session (word retrieval), for each session the first four volumes were discarded.