The effects of neuregulin1 on brain function in controls and patients with schizophrenia and bipolar disorder

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Abstract

Recent studies have identified neuregulin1 as a probable susceptibility gene for schizophrenia and bipolar disorder. However, little is known about how this gene may affect brain function to increase vulnerability to these disorders. The present investigation examined the impact of neuregulin1 genotype on brain function in patients with schizophrenia, patients with bipolar I disorder and healthy volunteers. We used functional magnetic resonance imaging to measure brain responses during a verbal fluency task in a total of 115 subjects comprising 41 patients with schizophrenia, 29 patients with bipolar disorder and 45 healthy volunteers. We then used statistical parametric mapping to estimate the main effects of diagnostic group, the main effect of genotype and their interaction. We tested the hypothesis that the high-risk variant of neuregulin1 would be associated with altered prefrontal function. In all three diagnostic groups, the high-risk variant of neuregulin1 was associated with greater deactivation in the left precuneus. In addition, there was an interaction between diagnosis and genotype in two regions of the prefrontal cortex. The right inferior frontal gyrus expressed increased activation in individuals with the high-risk variant, but only in patients with schizophrenia. Conversely, the right posterior orbital gyrus expressed increased activation in individuals with the high-risk variant, but only in patients with bipolar disorder. Our results suggest that genetic variation in neuregulin1 has a measurable impact on brain function and provide preliminary evidence for a disease-specific pattern of gene action in different regions of the prefrontal cortex.

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Introduction

Schizophrenia and bipolar disorder are severe psychiatric diseases with a strong genetic component (Owen et al., 2007; Craddock et al., 2006; Berrettini, 2003; Cardno et al., 2002). The identification of susceptibility genes has been problematic because of the complexity of the phenotype and a mode of transmission compatible with a multi-locus model. However, recent association studies have provided converging evidence in favour of a number of positional genes mapped to both schizophrenia and bipolar disorder linkage regions (Park et al., 2004; Lewis et al., 2003; Segurado et al., 2003); amongst the strongest candidates is that encoding neuregulin1 (Harrison and Law 2006).

Neuregulin1 (NRG1), located at 8p21-p22, was originally associated with schizophrenia by Stefansson and colleagues (Stefansson et al., 2003, 2002), who found association of a core-haplotype in the NRG1 gene with a two-fold increased risk for schizophrenia in Icelandic and Scottish populations. Further evidence has since come from a series of studies in Irish, Chinese and South African, mixed British and Dutch samples using the same haplotype region (Tosato et al., 2005), as well as meta-analysis (Li et al., 2006).

In addition, Ophoff et al. have found the strongest linkage in a bipolar I family sample to be in chromosome 8p (Ophoff et al., 2002). More recently, two independent molecular genetic studies (Thomson et al., 2007; Green et al., 2005) have implicated NRG1 in bipolar disorder. First, Green et al. have reported that the “core at-risk haplotype” previously associated with schizophrenia was significantly overrepresented in individuals with either schizophrenia or bipolar disorder compared to controls, with similar odds ratios and confidence
intervals for the two diseases (Green et al., 2005). Thomson et al. have subsequently shown that haplotypes in two regions, one of which overlaps with the “core at-risk haplotype”, are associated with both schizophrenia and bipolar disorder (Thomson et al., 2007). Taken collectively, these studies suggest that NRG1 may confer biological susceptibility across the traditional Kraepelian dichotomy of schizophrenia and bipolar disorder (Cradock et al., 2007).

The functional significance of the NRG1 core at-risk haplotype or any of its markers is currently unknown and it is possible that as yet unidentified mutations in linkage disequilibrium (LD) with it are also responsible for a functional effect. The NRG1 gene spans 1.2 Mb and encodes many structurally and functionally distinct protein isoforms through alternative splicing, grouped in I–IV types, from which type I–III isoforms are known to participate in ErbB signaling (Steinthorsdottrir et al., 2004; Falls 2003). These proteins have been implicated in a diverse range of functions, including neuronal migration and specification, oligodendrocyte differentiation and myelination, hormonal control of puberty, regulation of acetylcholine and expression of glutamate, GABA and other neurotransmitter receptors (Corfas et al., 2004). Disruption of these developmental processes has also been implicated, directly and indirectly, in mental illness (Corfas et al., 2004). The distribution of NRG1 isoforms is widespread in the brain and includes regions implicated in schizophrenia and bipolar disorder by post-mortem and neuroimaging studies, such as the frontal cortex, hippocampus, and cerebellum (Gur et al., 2007; Law et al., 2006). Two recent investigations (Li et al., 2007; Woo et al., 2007) have provided evidence that NRG1 and its receptor erbB4 regulate transmission at brain glutamate and GABA synapses in hippocampal and prefrontal regions, consistent with the notion of synaptic defects in schizophrenia and bipolar disorder.

To date, brain abnormalities in schizophrenia and bipolar disorder have been most evident at “macroscopic” or “system” level, as revealed by neuroimaging (Gur et al., 2007; Phillips and Vieta, 2007; Lagopoulos et al., 2007; McGuire and Matsumoto, 2004; Weinberger et al., 2001). While the effects of NRG1 at molecular and cellular levels are beginning to be unveiled, little is known about how this gene affects the brain at the macroscopic or system level. A recent investigation (Hall et al., 2006) found that relatives of people with schizophrenia with the high-risk variant of the NRG1 gene showed reduced medial prefrontal and temporopolar activation during a sentence completion task compared to relatives with the low-risk variant. However, it is currently unclear whether similar effects would be detected in healthy volunteers without a family history of schizophrenia and how the effect of genotype might interact with that of diagnostic category. For instance, Addington et al. have recently shown that a variant of NRG1 is associated with different longitudinal changes in brain volume in patients with childhood onset schizophrenia and controls (Addington et al., 2007). Similarly, variation in the serotonin transporter gene has been associated with reduced hippocampal volume in patients with depression but not in healthy controls (Frodal et al., 2004). These studies however did not directly test for an interaction between genotype and diagnosis and therefore can only provide anecdotal evidence for disease-specific pattern of gene action.

The aim of the present study was to investigate the impact of NRG1 on brain responses in 3 groups: healthy volunteers, patients with schizophrenia and patients with bipolar I disorder. We used functional magnetic resonance imaging (fMRI) to measure brain responses during a verbal fluency task which required subjects to generate and articulate a word in response to a letter cue. This task was chosen for two main reasons: first, it engages a distributed network of fronto-temporal cortical and sub-cortical brain regions that have been implicated in schizophrenia and bipolar disorder (Fu et al., 2005; Fu et al., 2002; Curtis et al., 2001); second, it taps into executive cognitive processes that are compromised in patients with schizophrenia and, to a lesser extent, patients with bipolar disorder (Daban et al., 2006; Kravariti et al., 2005; Krabbendam et al., 2005; Curtis et al., 2001). A “clustered” image acquisition sequence was used in order to record overt vocal responses in the absence of scanner noise (Fu et al., 2005, 2002) and then model correct and incorrect trials separately in the statistical analysis. Our first hypothesis was that the high- relative to the low-risk variant of NRG1 would have a measurable impact on regional brain activation in the prefrontal cortex. Several converging lines of evidence suggest an effect of NRG1 on prefrontal function: first, the prefrontal cortex is structurally and functionally impaired in patients with schizophrenia; second, the prefrontal cortex shows the most abundant distribution of NRG1 type 1 isoform, particularly in white matter interstitial neurons and a sub-population of GABAergic cortical interneurons (Bernstein et al., 2005); third, there is evidence that NRG1 isoforms are abnormally expressed in the prefrontal cortex of patients with schizophrenia (Bertram et al., 2007; Hashimoto et al., 2004) and affective disorder (Bertram et al., 2007). Our second hypothesis was that the impact of NRG1 on the prefrontal function would vary across the three diagnostic groups as revealed by significant genotype × diagnosis interactions.

Materials and methods

Subjects

A total of 115 subjects were investigated, including 45 healthy volunteers, 41 patients with schizophrenia and 29 patients with bipolar I disorder. All participants were native English speakers and gave written informed consent in accordance with protocols approved by the Local and Multi-centre Research Ethics Committee (LREC, MREC). Healthy volunteers were recruited through local advertisement and had no family history of psychiatric illness as assessed using the FIGS (Family Interview for Genetic Studies). Patients with schizophrenia and bipolar disorder were recruited through the South London and Maudsley NHS Trust and met the relevant DSM-IV criteria, as determined by a detailed clinical interview and a systematic review of their medical records. Full-scale IQ was assessed using the WAIS-III (Wechsler Adult Intelligence Scale–III; Wechsler, 1997), the WAIS-R (Wechsler Adult Intelligence Scale–R; Wechsler, 1981), the WASI-FSIQ-4 (Wechsler Abbreviated Scale of Intelligence; Wechsler 1999) or the Quick Test (Ammons and Ammons, 1962). The WAIS-III correlates highly both with the WAIS-R (93.9%; Wechsler, 1997) and with the WASI-FSIQ-4 (92%; Wechsler, 1999). The Quick test has also been shown to yield comparable results to WAIS (Quick Test, 78±7 and WAIS, 83±6 in schizophrenia; Frith et al., 1991). The proportion of subjects assessed with each method was matched between genotype groups.

Demographic data (including age, full-scale IQ, years of education, handedness, gender and ethnicity) and clinical data (including positive and negative symptoms, duration of illness and anti-psychotic medication) are summarised in
Table 1. There were no significant differences across the 3 diagnostic groups with respect to age, handedness, and ethnicity. However the 3 diagnostic groups differed in terms of years of education ($F=3.168, df=2, p=0.046$), IQ ($F=6.733, df=2, p=0.002$) and male:female ratio ($\chi^2=18.736, df=2, p<0.001$). Post hoc t-tests revealed that the group of patients with schizophrenia had fewer years of education, a lower IQ and a higher male:female ratio than both healthy volunteers and patients with bipolar disorder, consistent with previous studies (Krabbendam et al., 2005; Daban et al., 2006). The mean duration of illness (defined as time since the first episode) for patients with schizophrenia was 12.20 years (SD=9.4). These patients were taking regular doses of antipsychotic medication; the mean dose in chlorpromazine equivalents was 579.27 (SD=466.76) and the mean duration of treatment was 12.2 years (SD=9.4). The mean duration of illness for patients with bipolar disorder (defined as time since diagnosis) was 14.4 years (SD=10.5). Only a minority of these patients ($n=7$) were taking regular doses of antipsychotic medication; within this subgroup, the mean dose in chlorpromazine equivalents was 342.86 (SD=305.06). In addition, fourteen of the bipolar patients were taking mood stabilizers, four were taking anti-depressants and seven were medication-free. Patients with bipolar disorder had experienced at least one psychotic episode in the past, with the exception of five participants (three with the high-risk genotype and two with the low-risk genotype).

All participants were genotyped for SNP8NRG221533 (rs35753505) (see Methods below) with C and T being the high-risk and low-risk alleles respectively. The control sample comprised 20 individuals with the CT variant and 25 individuals with the TT variant; the schizophrenic sample comprised 20 individuals with the CT variant and 21 individuals with the TT variant; the bipolar sample comprised 13 individuals with the CT variant and 16 individuals with the TT variant. No individuals with the CC variant were included in the present investigation due to the difficulty of recruiting a sufficiently large sample. Participants in the present investigation were selected from a larger sample of patients and controls ($n=428$) screened for NRG1 genotype, in order to ensure a comparable number of individuals with the low and high risk genotypes within each diagnostic group; thus the present investigation should not be seen as an attempt to replicate the association between rs35753505 in NRG1 and schizophrenia/bipolar disorder.

Age, handedness, ethnicity and years of education did not differ significantly as a function of this genotypic subdivision within each diagnostic group ($p>0.05$). IQ was higher for the high-risk than the low-risk genotypes within the bipolar sample ($F=5.974, df=1, p=0.021$) but not within the control and schizophrenic samples. Within the schizophrenic sample, subjects with the low-risk genotype had a higher dose of antipsychotic medication than those with the high-risk genotype ($F=4.488, p=0.041, df=1$) but the two sub-groups did not differ in terms of duration of illness or treatment. Within the bipolar sample, subjects with the low- and high-risk variants did not differ in terms of anti-psychotic medication or duration of illness ($p>0.05$). Within the schizophrenic sample, SAPS and SANS scores did not differ significantly between high- and low-risk subjects. Within the bipolar sample, the scores on the Altman Self-Rated Mania Scale did not differ as a function of genotype; however, patients with the low-risk variant scored higher than those with the low-risk variant on the Beck Depression Inventory ($F=5.205; df=1; p=0.031$).

Genotyping

DNA isolation and analysis was conducted from blood samples or cheek swabs using standard procedures. Genotyping of the single nucleotide polymorphism SNP8NRG221533 (rs35753505) was performed by KBioscience (http://www.
kbioscience.co.uk) using a competitive allele specific PCR system (CASP). This single nucleotide polymorphism (SNP) was chosen because it was the single marker most significantly associated in Stefansson's original investigation (Stefansson et al., 2002) and furthermore has shown significant association in several follow-up studies (Li et al., 2006; Yang et al., 2003; Stefansson et al., 2003). The genotyping results of a sample of 428 subjects, which included our sample of 115, were under Hardy Weinberg equilibrium and matched the genotype frequencies usually verified in the literature (either for this polymorphism or for others in well characterised schizophrenia risk genes).

Verbal fluency task

During fMRI scanning, subjects performed an overt verbal fluency task involving two main conditions: generation and baseline (Fu et al., 2002). In the generation condition, subjects were presented with a series of letters on a computer screen; the task required them to respond to each letter by generating a word that started with that letter. Letter cues were presented in blocks of seven with a stimulus onset asynchrony of 4000 ms; all cues in a given block were of the same letter but each block involved a different letter. The paradigm thus resembles the classical version of the task used in neuropsychological studies except that each response is cued at regular intervals, rather than the subject responding freely as many times as they can following a single cue. A paced paradigm is more compatible with an fMRI study, as it reduces variation in the timing of overt verbal responses within and between subjects, and reduces the risk that only a small proportion of the block is associated with performance of the task, as would occur if a subject rapidly articulated all their responses in the initial part of the block and then disengaged from the paradigm. A further benefit of using a paced task with a relatively long inter-stimulus interval was that there was less risk of large between-subject and between-group variation in task performance, which could confound interpretation of differences in activation, particularly between the control and patient groups. In the baseline condition, subjects were presented with the visual word “rest” and were required to say “rest” out loud; “rest” cues were also presented in blocks of seven with a stimulus onset asynchrony of 4000 ms. Functional MRI data were acquired during two separate acquisition runs, each including 5 blocks of letters alternating with five blocks of “rest” trials. This resulted in a total of 70 letter stimuli and 70 “rest” trials for each subject. Verbal responses were recorded by means of a microphone that was compatible with the MRI apparatus; this allowed us to identify “incorrect” trials in which the subject did not generate any response or generated repetitions, derivatives or grammatical variations of the previous word.

fMRI data acquisition

The T2*-weighted gradient-echo single-shot echo-planar images were acquired on a 1.5-T, neuro-optimized IGE LX System (General Electric, Milwaukee) at the Maudsley Hospital, London, U.K. Twelve noncontiguous axial planes (7-mm thickness, slice skip: 1 mm) parallel to the anterior commissure–posterior commissure line were collected in a “clustered” acquisition (TE=40 ms, flip angle=70°). A clustered acquisition sequence capitalizes on the delay of the haemodynamic response, which reaches its peak about 3–5 s after stimulus onset (Glover 1999). A letter cue was presented for 750 ms and an overt verbal response could be made over a silent period of 2900 ms; an image was then acquired over 1100 ms resulting in a total repetition time (TR) of 4000 ms.

fMRI data analysis

The analysis of the fMRI data was performed using SPM5 software (Friston, 2003), running under Matlab 6.5. In brief, all volumes from each subject were realigned, normalized to a standard MNI-305 template and spatially smoothed with a 6 mm full width at half maximum isotropic Gaussian kernel. First, the statistical analysis of regional responses was performed in a subject-specific fashion, by convolving each onset time with a synthetic haemodynamic response function (HRF). In order to minimize performance confounds, we modelled correct and incorrect trials separately using an event-related model. This resulted in a total of three experimental conditions: (i) generation, (ii) baseline and (iii) incorrect responses. In order to remove low-frequency drifts, the data were high-pass filtered using a set of discrete cosine basis functions with a cutoff period of 128 s. After the parameter estimates were calculated for all brain voxels using the general linear model, a contrast image for the comparison “generation > baseline” was computed for each subject independently. Second, the subject-specific contrast images were entered into a full-factorial ANOVA to permit inferences at the second level (Penny and Holmes, 2003). Since the superior region of the prefrontal cortex was not scanned in a sub-group of subjects, this region was excluded from the full-factorial ANOVA; the area of the brain included ranged from z=−30 to z=+46. The full-factorial ANOVA allowed us to characterise the impact of the experimental task on brain activation, the main effect of diagnostic group, the main effect of genotype and their interaction. Age, gender, handedness, ethnicity and years of education were included in the statistical analysis as covariates of no interest. The interaction between diagnostic group and genotype was examined by contrasting the effect of genotype in one group against the same effect in another group, using a total of 6 contrasts in SPM5. These included (i) high > low risk in controls > schizophrenic patients; (ii) high > low risk in schizophrenic patients > controls; (iii) high > low risk in controls > bipolar patients; (iv) high > low risk in bipolar patients > controls; (v) high > low risk in schizophrenic > bipolar patients; (vi) high > low risk in bipolar > schizophrenic patients. In regions where a significant interaction was detected, additional t tests were used in order to examine the effect of genotype within each of the three diagnostic groups. All statistical inferences were made in SPM5 using a statistical threshold of p<0.05 with family wise error (FWE) correction for multiple comparisons and an extent threshold of 10 voxels. In order to minimize the chance of false positives, we constrained our search for the main effect of diagnostic group, the main effect of genotype and their interaction to brain areas which were modulated by the experimental task. These areas were identified using an F contrast contrasting verbal fluency against baseline at p<0.05 with FWE correction for multiple comparisons.

Results

Performance

The effects of diagnostic group, genotype and their interaction on the accuracy of verbal responses during fMRI scanning (Table 1) were tested using one-way and multivariate ANOVAs in SPSS (Statistical Package for Social Sciences — version 15.0).
The number of errors significantly differed as a function of diagnostic group ($F=4.101$ $df=2$ $p=0.019$). Post hoc $t$-tests revealed that patients with schizophrenia made significantly more errors than healthy volunteers ($F=9.886$ $df=1$ $p=0.002$). Patients with bipolar disorder made an intermediate number of errors and did not significantly differ from either healthy volunteers or patients with schizophrenia ($p<0.05$). The number of errors did not differ for high- and low-risk genotypes, irrespective of whether the 3 diagnostic groups were considered separately or in combination ($p<0.05$). The interaction between diagnostic group and genotype was not significant ($p<0.05$).

Functional MRI

**Effects of generation vs. baseline**

We identified the effects of generation relative to baseline using a statistical threshold of $p<0.05$ (corrected). Consistent with our previous reports (Fu et al., 2005, 2002), a distributed network of regions including bilateral prefrontal, superior temporal and subcortical areas expressed increased activation during generation relative to baseline (Fig. 1 and Table 2). Conversely, the precuneus, the anterior cingulate gyrus and the temporo-parietal junction were more engaged during baseline than generation (Fig. 1 and Table 2); this set of regions correspond to the so-called “resting state” or “default” network that typically shows a relative decrease in neuronal responses during a wide range of active tasks compared to a low level baseline (McKiernan et al., 2006, 2003; Binder et al., 1999; Shulman et al., 1997; McGuire et al., 1996).

**Main effect of diagnostic group**

Patients with schizophrenia expressed greater activation relative to controls in the left angular gyrus ($x=-48$ $y=-60$ $z=36$ $Z$-score=4.7 $p=0.002$ after FWE correction for multiple comparisons). In this region, there was also a non significant trend for greater activation in patients with bipolar disorder than controls ($x=-48$ $y=-60$ $z=36$ $Z$-score=2.8 $p=0.062$ after FWE correction for multiple comparisons). In contrast, there were no statistical differences between patients with bipolar disorder and controls. A direct comparison between patients with schizophrenia and patients with bipolar disorder did not reveal any significant effect.

**Table 2**

<table>
<thead>
<tr>
<th>Co-ordinates and $Z$-score</th>
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<tr>
<td><strong>Generation &gt; baseline</strong></td>
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<tr>
<td>Left inferior frontal gyrus &amp; $-44.628$ ($8.5$)</td>
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<tr>
<td>Right inferior frontal gyrus &amp; $44.1610$ ($5.3$)</td>
</tr>
<tr>
<td>Left middle frontal gyrus &amp; $34.226$ ($7.8$)</td>
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<tr>
<td>Right middle frontal gyrus &amp; $40.3826$ ($5.4$)</td>
</tr>
<tr>
<td>Left anterior insula &amp; $32.224$ ($7.7$)</td>
</tr>
<tr>
<td>Right anterior insula &amp; $36.222$ ($7.6$)</td>
</tr>
<tr>
<td>Left precentral gyrus &amp; $-44.430$ ($8.5$)</td>
</tr>
<tr>
<td>Left anterior superior temporal gyrus &amp; $-50.160$ ($7.8$)</td>
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<tr>
<td>Right anterior superior temporal gyrus &amp; $48.160$ ($7.3$)</td>
</tr>
<tr>
<td>Left superior posterior temporal gyrus &amp; $-50.426$ ($4.9$)</td>
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<td>Left angular gyrus &amp; $-40.424$ ($7.7$)</td>
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<tr>
<td>Left thalamus &amp; $-10.28$ ($7.5$)</td>
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<tr>
<td>Right thalamus &amp; $12.210$ ($6.2$)</td>
</tr>
<tr>
<td>Left caudate &amp; $-16.016$ ($7.8$)</td>
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<tr>
<td>Right caudate &amp; $20.022$ ($7.6$)</td>
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<tr>
<td>Cingulate gyrus &amp; $2.432$ ($6.6$)</td>
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<tr>
<td><strong>Baseline &gt; generation</strong></td>
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<tr>
<td>Precuneus &amp; $2.5234$ ($8.7$)</td>
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<tr>
<td>&amp; $-14.6022$ ($8.0$)</td>
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<tr>
<td>&amp; $14.6022$ ($7.8$)</td>
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<tr>
<td>Anterior cingulate gyrus &amp; $4.4656$ ($7.4$)</td>
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<tr>
<td>&amp; $-2.384$ ($7.4$)</td>
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<tr>
<td>Left temporo-parietal junction &amp; $-50.6424$ ($7.8$)</td>
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<tr>
<td>Right temporo-parietal junction &amp; $46.5424$ ($6.5$)</td>
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<tr>
<td>Left posterior insula &amp; $38.2038$ ($4.9$)</td>
</tr>
<tr>
<td>Right posterior insula &amp; $40.1614$ ($5.7$)</td>
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</table>

All effects survived a statistical threshold of $p<0.05$ (corrected for multiple comparisons) and an extent threshold of 10 voxels.

Fig. 1. Areas activated for "generation > baseline" and "baseline > generation" consistently across all diagnostic groups and genotypes.
Main effects of genotype

There were a significant effect of genotype in the left precuneus (x = -6 y = -68 z = 42 Z-score = 4.3; p-value = 0.012 after FWE correction for multiple comparisons). This region expressed decreased activation during generation relative to baseline; however this deactivation was greater in subjects with the high-risk genotype relative to the low-risk genotype as shown graphically in Fig. 2. Inspection of the group-specific values revealed that this effect was present in all three diagnostic groups (controls: x = -12 y = -62 z = 32 Z-score = 3.7; schizophrenic patients: x = -4 y = -68 z = 38 Z-score = 3.6; bipolar patients: x = -6 y = -70 z = 44 Z-score = 2.9). A general linear model analysis of parameter estimates in SPSS revealed that NRG1 genotype accounted for 14.2% (p < 0.001), 7.8% (p = 0.009) and 10.3% (p = 0.01) of inter-subject variability in this region within the control, schizophrenic and bipolar samples respectively.

Interaction between diagnostic group and genotype

The interaction between diagnostic group and genotype was examined by contrasting the effect of genotype in one group against the same effect in another group, using a total of 6 contrasts (see Methods for details). This revealed two regions of the prefrontal cortex which expressed a significant diagnosis x genotype interaction at p < 0.05 (after FWE correction for multiple comparisons).

The first region to show a significant diagnosis x genotype interaction was the right inferior frontal/insular cortex, where there was a greater effect of high- relative to low-risk genotype in schizophrenic patients compared to controls (x = 36 y = 2 z = 24 Z-score = 3.9 p = 0.042 after FWE correction; see Fig. 3 and Table 3). In this region, there was also a non-significant trend for a greater effect of high- relative to low-risk genotype in schizophrenic compared to bipolar patients (Z-score = 2.7 p = 0.850 after FWE correction). Examination of the effects of genotype within each diagnostic category revealed greater activation in right inferior frontal/insular cortex for high- relative to low-risk genotype in schizophrenic patients (Z-score = 3.9 p = 0.043 after FWE correction) but not in either controls or bipolar patients (Table 3). In this region, genotype accounted for 2.6% (p = 0.114), 17.8% (p < 0.001) and 0.4% (p = 0.632) of variance in BOLD activation within the control, schizophrenic and bipolar samples respectively and the interaction between genotype and diagnostic group accounted for 9.3% (p = 0.003) of variance. We explored the possibility that the effect of genotype in the right inferior frontal/insular cortex of schizophrenic patients might be explained by the significant differences in anti-psychotic medication between high- and low-risk subjects. However, there was no correlation between brain activation in this region and the dose of antipsychotic medication, even at a very liberal statistical threshold (p < 0.05 uncorrected).
The second region to show a significant diagnosis × genotype interaction was the right posterior orbital gyrus, where there was a greater effect of high-risk relative to low-risk genotype in bipolar patients compared to controls ($z = 28 \ y = 24 \ z = -20$ $Z$-score = 4.4 $p = 0.008$ after FWE correction; see Fig. 3 and Table 3). In this region, there was also a non significant trend for a greater effect of high-risk relative to low-risk genotype in bipolar compared to schizophrenic patients ($Z$-score: 3.6 $p = 0.119$ after FWE correction). Examination of the effects of genotype within each diagnostic category revealed greater activation in the right posterior orbital gyrus for high-risk relative to low-risk genotype in bipolar patients ($Z$-score = 3.8 $p = 0.077$ after FWE correction) but not in either controls or schizophrenic patients (Table 3). In this region, genotype explained 5.7% ($p = 0.010$), 3% ($p = 0.108$) and 13.5% ($p = 0.003$) of variance within the control, schizophrenic and bipolar samples respectively and the interaction between genotype and diagnostic group accounted for 9.9% ($p = 0.001$) of variance. We explored the possibility that the effect

![Brain regions showing significant interactions between diagnostic group and NRG1 genotype. Top: right inferior frontal area where increased activation was found in individuals with the high-risk variant, but only in patients with schizophrenia. Bottom: right posterior orbital area where increased activation was found in individuals with the high-risk variant, but only in patients with bipolar disorder. For each region, the parameter estimates for the contrast “generation − baseline” are shown for all six experimental groups. High=high-risk NRG1 genotype; Low=low-risk NRG1 genotype.](image)

**Table 3**

Co-ordinates and $Z$-scores (in brackets) of the brain regions that expressed a significant interaction between diagnostic group and genotype

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<th>Genotype × group interactions</th>
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<tr>
<td>Right inferior frontal gyrus/insula</td>
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<td>36 2 24 (3.9)*</td>
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<td>36 2 24 (2.7)</td>
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<tr>
<td>Effects of genotype specific to schizophrenics</td>
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<td>Right posterior orbital gyrus</td>
<td>n.s.</td>
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<td>28 24 −20 (3.8)</td>
<td>28 24 −20 (4.4)*</td>
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<td></td>
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<td>28 26 −20 (3.6)</td>
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</table>

The effects that survived a statistical threshold of $p < 0.05$ (after FWE correction for multiple comparisons) are highlighted with an asterisk (*). The C=control sample; S=schizophrenic sample; BD=bipolar disorder sample. n.s.=not significant.
of genotype in the right posterior orbital gyrus of bipolar patients might be explained by the significant differences in IQ or depressive symptoms between high- and low-risk subjects. However, there was no correlation between brain activation in this region and IQ or scores on the Beck Depression Inventory, even at a very liberal statistical threshold \( p < 0.05 \) uncorrected.

Finally, when the effect of genotype in patients with schizophrenia was contrasted against the effect of genotype in patients with bipolar disorder, no significant diagnosis \( \times \) genotype interaction was detected using our statistical threshold of \( p < 0.05 \) (corrected).

**Discussion**

In the present investigation, we examined the effect of NRG1 genotype on brain function during a verbal fluency task which engages brain regions and cognitive processes impaired in the two conditions (Daban et al., 2006; Krabbe et al., 2005; Wachtel et al., 2005; Fu et al., 2002; Curtis et al., 2004). Based on previous neuroimaging studies of the neural correlates of psychosis as well as post mortem studies of the distribution of NRG1 isoforms in controls and patients, we predicted an effect of NRG1 in the prefrontal cortex.

Independent of diagnostic group, the left precuneus showed greater deactivation during task performance relative to baseline in individuals with the high-risk than those with the low-risk variant \( (p < 0.05 \) after FWE correction). The left precuneus is part of the “resting state” or “default” network that shows a decrease in activation during task performance relative to baseline in the present investigation as well as in several previous studies (McKiernan et al., 2006, 2003; Binder et al., 1999; Shulman et al., 1997; McGuire et al., 1996). This network of regions is thought to be implicated in ongoing internally generated processes that occur during any low-level baseline but are disrupted when an exogenous task is imposed on the subject (McKiernan et al., 2006, 2003; Binder et al., 1999). Task-related deactivation within this network is thought to be the result of a reallocation of cognitive resources that occurs when a low-level baseline is followed by an effortful active task (McKiernan et al., 2006, 2003; Binder et al., 1999). The implication for our findings is that greater deactivation in the left precuneus of individuals with the high-risk variant might reflect greater reallocation of cognitive resources to accommodate task-related demands. In other words, the active task might have produced greater interference with ongoing internally generated processes in individuals with the high-risk variant. In neurophysiological terms, greater deactivation in the left precuneus of individuals with the high-risk variant might be the result of altered effective connectivity from the prefrontal cortex.

Two distinct regions within the prefrontal cortex showed a significant genotype \( \times \) diagnostic group interaction. The right inferior frontal/insular cortex expressed greater activation in individuals with the high-risk variant compared to those with the low-risk variant, but only in patients with schizophrenia. Exploration of the parameter estimates (Fig. 3) revealed that this interaction was driven by a decrease in activation during task generation relative to baseline which occurred in schizophrenic patients with the low-risk alleles but not in either healthy controls or bipolar patients with the same alleles; this suggests the possible presence of a moderating variable that might influence brain physiology differentially among schizophrenic individuals with the low-risk genotype. The right inferior frontal/insula cortex is normally engaged by executive and working memory processes and is structurally and functionally altered in patients with schizophrenia and their non-psychotic relatives (McGuire and Matsumoto 2004; Fusar-Poli et al., 2007). Furthermore, this region is part of the prefrontal cortex in which NRG1 isoforms are abnormally expressed in patients with schizophrenia and affective disorder relative to healthy controls (Bertram et al., 2007; Hashimoto et al., 2004). Conversely, the right posterior orbital gyrus expressed increased activation in individuals with the high-risk variant, but only in patients diagnosed with bipolar disorder. Exploration of the parameter estimates (Fig. 3) revealed that this region was more active during generation than baseline in bipolar patients with the high-risk genotype but showed the reverse effect in bipolar patients with the low-risk genotype. The right posterior orbital gyrus is part of a neural system that mediates emotional processing (Cardinal et al., 2002) and decision making (Elliott et al., 2000) and has been implicated in bipolar disorder by neuroimaging and post-mortem studies (Cotter et al., 2005; Dunn et al., 2005; Haldane and Frangou 2004; Monks et al., 2004; Blumberg et al., 1999; Drevets 1999). In addition, this area is densely interconnected with the amygdala and the hypothalamus which are implicated in motivational, chronobiologic and endocrinologic processes (Hikosaka and Watanabe, 2000). It has been previously suggested that disrupted modulation of the amygdala and the hypothalamus by orbitofrontal cortex may contribute to dysregulation of these processes in bipolar disorder (Blumberg et al., 1999).

Previous neuroimaging studies (Addington et al., 2007; Frodl et al., 2004) had provided anecdotal evidence of a disease-specific influence of genotype on brain structure, but had not formally tested for an interaction between the effects of genotype and diagnosis. The observation of two interactions between diagnostic group and rs35753505 variant in the present investigation demonstrates that the same gene may have different effects in controls and patients and that these effects may differ between different disorders. This is an important conceptual point because the vast majority of functional genomic studies in the past have been conducted on healthy volunteers, with the implicit assumption that similar effects may operate in patients. Little is known about the possible mechanisms which might lead to a different effect of rs35753505 between controls and patients and between different diagnostic categories. One possibility is that the effects of rs35753505 genotype may depend on the presence or absence of specific environmental risk factors (Caspi and Moffitt, 2006). Alternatively the expression of the NRG1 gene in the brain might be modulated by allelic variability in one or more other genes that also contribute to vulnerability to mental illness. Consistent with this hypothesis, a recent investigation has demonstrated that the functional COMT Val158Met polymorphism influences NRG1-mediated cell migration (Sei et al., 2007). A final possibility is that the effects of NRG1 genotype may interact with the effects of a disorder to affect the development and function of the brain.

The effects of rs35753505 in NRG1 reported here do not appear to be attributable to differences in age, gender, handedness, ethnicity or years of education, since these factors were modelled in the statistical analysis as covariates of no interest. Nevertheless, the present investigation presents a number of limitations which require careful consideration.
First, we genotyped our subjects for rs35753505 which was the only marker individually showing a significant association with schizophrenia in Stefansson’s original investigation (Stefansson et al., 2002) and in several follow-up studies (Li et al., 2006; Yang et al., 2004; Stefansson et al., 2003). However, within the NRG1 gene, several other SNPs have been identified by genetic studies and there is no agreement as to which is the most significant marker. The function of the different SNPs is still unknown and it is therefore difficult to make predictions in terms of their functional impact in the brain. Furthermore, linkage disequilibrium within the risk haplotype is significant and makes findings with a single SNP difficult to interpret. Law et al. recently demonstrated changes in mRNA expression associated with a different SNP (rs6994992) (Law et al., 2006). However this functionality cannot be regarded as a specific property because rs6994992 is part of a four marker haplotype block including rs35753505 and two other SNPs which are all in significant linkage disequilibrium. Based on these considerations, it is possible that the results reported in the present investigation are not unique to rs35753505. An alternative approach might be to use the risk haplotype rather than SNPs in order to characterize the effect of NRG1 on brain function. However there are methodological problems with this strategy as well, since haplotypes are not absolute measures in phase-unknown populations but are assigned by probability using a complex likelihood calculation. The use of estimated haplotypes can inflate the false positive rate in genetic studies, especially when genotyping error is present, their use can entail extensive multiple testing, which decreases the efficiency and power of association tests through the sharply increased degrees of freedom, and there are unresolved technical problems in assigning haplotypes which means that different algorithms can give very divergent estimates (Curtis and Xu, 2007; Moskvina and Schmidt, 2006).

Second, the low-risk and the high-risk genotypes were not balanced for dose of anti-psychotic medication within the schizophrenic sample and for scores on the Beck Depression Inventory and IQ within the bipolar sample. We explored the possibility that the genotype x diagnostic group interactions that we report might be explained by one or more of these variables. However we did not detect any significant correlation between medication, scores on the Beck Depression Inventory or IQ and brain activation in genotype-sensitive areas, even when lowering the statistical threshold to \( p < 0.05 \) (uncorrected).

Third, individuals with two risk alleles (CC) constitute only a small fraction of the population and were not included in the present investigation. Nevertheless we were able to examine the effect of rs35753505 in NRG1 by comparing individuals with one risk allele (CT) against those with none (TT). This means that our data cannot reveal whether the action of the risk allele on brain functioning is best described by a dominant or an additive model.

Fourth, reaction times were not measured during scanning and could not be modelled in the statistical analysis. Thus, we cannot exclude the possibility that some of the effects of diagnosis or genotype that we report might be explained by differences in performance speed. In addition, we cannot comment on whether poor performance accuracy in patients relative to controls was associated with shorter or longer reaction times. However we modelled correct and incorrect trials separately thereby minimizing the potential confounding impact of performance accuracy.

Fifth, the gender distribution in the present investigation was different across diagnostic groups, with fewer females in the schizophrenic sample and fewer males in the bipolar sample. While we attempted to minimise the impact of gender by modelling this variable as a covariate of no interest, we were unable to obtain reliable estimates of the gender by genotype interaction because there were not enough male and female subjects within each experimental group. Given that both schizophrenia and bipolar disorder have been reported to show gender differences in their nature and incidence, it is important that future studies should examine the extent to which the impact of candidate genes on brain physiology is modulated by gender.

Sixth, the superior region of the prefrontal cortex was not included in our statistical analysis because this region was not scanned in a sub-group of subjects (see Methods); based on the present investigation it is therefore unclear whether this region also expresses an interaction between diagnostic group and genotype. A final limitation of the present investigation is that different scales were used to acquire clinical data from the schizophrenic and bipolar samples. This meant that we were unable to examine the extent to which effects of genotype on brain activation were related to specific symptoms as opposed to the diagnostic categories. It would be interesting in future studies to examine the relationship between genotype, symptom profile and brain activation.

In conclusion, we have shown for the first time that NRG1 has a measurable impact on brain responses in healthy volunteers without a family history of mental illness and in patients with schizophrenia and bipolar disorder. Our findings support the idea of a disease-specific pattern of genetic action in different regions of the prefrontal cortex. A more comprehensive understanding of how variation in NRG1 affects brain function to increase susceptibility to mental illness will require investigation of interactions with other candidate genes and with environmental risk factors.

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