fMRI correlates of state and trait effects in subjects at genetically enhanced risk of schizophrenia

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Summary
Schizophrenia is a highly heritable disorder that typically develops in early adult life. Structural imaging studies have indicated that patients with the illness, and to some extent their unaffected relatives, have subtle deficits in several brain regions, including prefrontal and temporal lobes. It is, however, not known how this inherited vulnerability leads to psychosis. This study used a covert verbal initiation fMRI task previously shown to elicit frontal and temporal activity (the Hayling sentence completion task) to examine this issue. A large (n = 69) number of young participants at high risk of developing schizophrenia for genetic reasons took part, together with a matched group of healthy controls (n = 21). At the time of investigation, none had any psychotic disorder, but on detailed interview some of the high-risk participants (n = 27) reported isolated psychotic symptoms. The study aimed to determine: (i) whether there were activation differences that occurred in all subjects with a genetic risk of schizophrenia (i.e. ‘trait’ effects); and (ii) whether there were activation differences that only occurred in those at high risk who had isolated psychotic symptoms (‘state’ effects). No activation differences were found in regions commonly reported to be abnormal in the established illness, namely the dorsolateral prefrontal cortex or in the temporal lobes, but group differences of apparent genetic cause were evident in medial prefrontal, thalamic and cerebellar regions. In addition, differences in activation in those with symptoms were found in the intraparietal sulcus. No significant differences in performance were found between the groups, and all subjects were antipsychotic naïve. These findings therefore suggest that vulnerability to schizophrenia may be inherited as a disruption in a fronto-thalamic-cerebellar network, and the earliest changes specific to the psychotic state may be related to hyperactivation in the parietal lobe.

Keywords: fMRI; high risk; schizophrenia; sentence completion


Introduction
Schizophrenia is a highly heritable disorder that generally becomes manifest in early adult life. Many studies report that structural and functional abnormalities, principally in the prefrontal and temporal lobes, are associated with the established illness (Frith et al., 1995; Fletcher et al., 1998; Lawrie and Abukmeil, 1998; Shenton et al., 2001). One of the most prominent findings reported in the functional imaging literature is of abnormal prefrontal activation, particularly in response to executive tasks in which patients perform worse than control subjects, such as working memory (see Manoach, 2003), and verbal initiation tasks (Curtis et al., 1998). However, it has been suggested that findings of hypofrontality may be confounded by performance differences between groups (Ebmeier et al., 1995; Weinberger and Berman, 1996; Ramsey et al., 2002). In studies that have controlled for task performance, findings of hypofrontality are less consistently reported (Frith et al., 1995; Fletcher et al., 1998; Ramsey et al., 2002).

Studies on the established illness, however, are not able to clarify the extent to which the abnormal findings relate to the presence of symptoms and/or medication effects, or to the presence of a schizophrenic predisposition or genotype. This has led to the prospective study of relatives of affected individuals, which allows the investigation of whether abnormalities predate development of the illness and reflect genetic vulnerability, or whether
there are changes specifically associated with the manifestation of symptoms. Previous structural and functional imaging studies have indeed suggested that some of the abnormalities associated with the established state are present to some extent in unaffected relatives (Lawrie et al., 1999, 2001; Seidman et al., 1999; Keshavan et al., 2002a, b; Callicott et al., 2003).

The Edinburgh High Risk Study is designed to address such issues and serially examines, in comparison with matched healthy controls, young people (aged 16–25 years at ascertainment) who are at enhanced risk of schizophrenia for genetic reasons (Johnstone et al., 2000). Ten to fifteen percent were predicted to develop schizophrenia by the age of 30 years on the basis of the known frequency of schizophrenia in individuals with this degree of heredity, and the actual occurrence of schizophrenia by the age of 30 years (Johnstone et al., 2002). The first phase of the study (conducted in 1994–1999) employed repeated clinical assessments that have shown increasing psychopathology. Isolated psychotic symptoms are occurring in two to three times as many subjects as are expected to develop schizophrenia, but in those who have done so (as predicted, ~10% at the end of 2002) the florid condition has almost always been preceded by such isolated psychotic symptoms (Johnstone et al., 2002). In the current phase of the study (1999–2004), a covert verbal initiation fMRI activation paradigm previously shown to elicit fronto-temporal activity, the Hayling sentence completion test (Burgess and Shallice, 1997; Lawrie et al., 2002), has been added to the tests used in the first phase. A number of high-risk participants in the current study reported, on direct questioning at standardized interview, isolated and/or transient psychotic symptoms in the presence of unimpaired functioning. Although it is likely that a few of the high-risk participants may still go on to develop schizophrenia or a related disorder, none of the participants in this current study met diagnostic criteria for any psychiatric disorder. All subjects provided written informed consent, and the study was approved by the Psychiatry and Clinical Psychology subcommittee of the Lothian research ethics committee.

### Subjects and methods

#### Subject details

Participants were selected on the basis of being aged between 16 and 25 years when first recruited (1994–1999), and having two or more first or second degree relatives with schizophrenia. The control group had no family history of the illness. At the time of recruitment, all participants regarded themselves as being in good health and functioning well. As part of an ongoing study, this report presents the results from the first 100 subjects reviewed in the second phase of the study, between 1999 to the present. Basic demographic variables are presented in Table 1. Out of the first 100 subjects, six did not participate in scanning. A further four scans were subsequently excluded (two due to minor vascular malformations, and two due to movement artefact; see below), leaving 90 fMRI scans from 21 normal controls and 69 high-risk subjects. On the day of scanning all subjects underwent a structured psychiatric interview (the Present State Examination; Wing et al., 1974). Twenty-seven high-risk subjects reported psychotic symptoms (usually isolated delusions or hallucinations); the remainder of the high-risk group and all of the controls had no such symptoms (Table 1). None of the subjects was on anti-psychotic medication or seeking treatment, or indeed saw themselves as unwell. They therefore could not be said to fulfil diagnostic criteria for any psychiatric disorder. All subjects provided written informed consent, and the study was approved by the Psychiatry and Clinical Psychology subcommittee of the Lothian research ethics committee.

### Scanning procedure

Imaging was carried out at the Brain Imaging Research Centre for Scotland (Edinburgh, UK) on a GE 1.5 T Signa scanner (GE Medical, Milwaukee, WI, USA) equipped with 23 mT/m ‘Echospeed’ gradients having a rise time of 200 μs. After a localizer scan, subjects were imaged with a multislice T2-weighted fast spin-echo sequence [repetition time/echo time (TR/TE) = 6300/102 ms]. Twenty slices (5-mm thickness, 1.5-mm gap), aligned parallel to the anterior commisure–posterior commisure (AC–PC) line, covered the brain. A structural scan with 1-mm pixel size was next acquired using a 3D inversion recovery-prepared T1-weighted sequence [inversion time (TI) = 600 ms]. One hundred and twenty-four slices (thickness 1.7 mm) were aligned perpendicular to the AC–PC line. Finally, axial gradient-echo planar images (EPI) [TR/TE = 4000/40 ms; matrix 64 × 128; field of view (FOV) 220 × 440 mm] were acquired continually during the experimental paradigm. Thirty-eight contiguous 5-mm slices were acquired within each TR period. Each EPI acquisition was run for 204 volumes, of which the first four volumes were discarded. Visual stimuli were presented using a

### Table 1 Demographic details

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 21)</th>
<th>High risk without symptoms (n = 42)</th>
<th>High risk with symptoms (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) [mean (SD)]</td>
<td>26.8 (2.7)</td>
<td>26.8 (3.4)</td>
<td>25.1 (3.1)</td>
</tr>
<tr>
<td>Gender (male:female)</td>
<td>13:8</td>
<td>17:25</td>
<td>13:14</td>
</tr>
<tr>
<td>Mean NART IQ (SD)</td>
<td>97.95 (24.02)</td>
<td>99.56 (18.12)</td>
<td>97.86 (10.60)</td>
</tr>
<tr>
<td>Handedness (R:L:A)</td>
<td>19:2:0</td>
<td>39:2:1</td>
<td>21:4:2</td>
</tr>
<tr>
<td>Genetic liability (1st degree:2nd degree)*</td>
<td>N/A</td>
<td>32:10</td>
<td>16:11</td>
</tr>
</tbody>
</table>

*First or second degree relatives with schizophrenia. N/A = not applicable; R = right; L = left; A = ambidextrous.
Experiment
The participants in the study performed the verbal initiation section of the Hayling sentence completion test (Burgess and Shallice, 1997) in the scanner. Subjects were shown sentences with the last word missing and were asked to silently think of an appropriate word to complete the sentence (i.e. without speaking the word), and press a button when they had done so. Sentences were selected from Bloom and Fischler’s set of sentence completion norms (Bloom and Fischler, 1980). The task was adapted for fMRI to have four levels of difficulty, according to the range of suitable completion words suggested by the sentence context. Examples of each difficulty level are presented in Table 2. Sentences were presented in blocks of fixed difficulty; each block lasted 40 s and included eight sentences. Sentences were presented for a period of 3 s followed by a fixation cross for 2 s. Subjects were instructed to respond at any time (by button press) until the next sentence appeared. The rest condition consisted of viewing a screen of white circles on a black background for 40 s. The order of the blocks was pseudo-random, and each block was repeated four times (different sentences were used for each sentence block). This design allowed both a standard subtraction (sentence completion versus rest) and parametric analysis (examining areas of increasing activation with increasing task difficulty). Verbal instructions were given prior to scanning and were standardized across subjects. During the same scanning session a further functional imaging study was performed, the results of which are not discussed here.

Immediately after scanning, subjects were given the same sequence of sentences on paper and requested to complete each sentence with the word they first thought of in the scanner. Word appropriateness scores were determined from the word frequency list of sentence completion norms (Bloom and Fischler, 1980), which provides respective probabilities of possible responses. A score of 1 was given to the most frequently produced word in the word frequency list, a score of 2 for the next most frequently produced word, etc. Mean scores for both word appropriateness and reaction time were determined for each constraint level. For a number of subjects (six controls, eight high risk without and five high risk with symptoms), no reaction time measures were recorded in the scanner. Since these subjects indicated at debriefing that they had indeed performed the task in the scanner, they were included in the analysis.

Scan processing
The EPI images were reconstructed offline in ANALYZE format (Mayo Foundation, Rochester, MN, USA). Scan analysis was performed using SPM99 (Wellcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/) running in Matlab (MathWorks, Natick, MA, USA), and was performed blind to the group status of the individual. For each subject, EPI volumes were realigned to the first volume in the series using rigid body transformations. Details of within-scanner movement are presented in Table 3; two subjects presented significant motion artefacts and were excluded from further analysis. One subject was excluded on the basis of having moved >3 mm, peak-to-peak, over less than 20 images, and one subject presenting large correlations (>0.5) between movement parameters and task regressors was also excluded. The images were then normalized to the SPM99 EPI template using a linear affine transformation followed by non-linear deformations, and resampled using sinc interpolation to cubic voxels of size 8 mm³. Normalized images were spatially smoothed with a 6 × 6 × 6 mm³ FWHM (full width half maximum) Gaussian filter to minimize residual inter-subject differences, and in order to meet assumptions for statistical analysis regarding the distribution of residuals.

Statistical analysis
Statistical analysis was performed using the general linear model approach as implemented in SPM99. At the individual subject level the data were modelled with five conditions (the four difficulty levels and the rest condition), each modelled by a boxcar convolved with a synthetic haemodynamic response function. The estimates of the subject’s movement during the scan were also entered as ‘covariate of no interest’. Before fitting the model, the subject’s data were filtered in the time domain using both a low-pass [Gaussian kernel, 4 s (FWHM)] and a high-pass (400 s cut-off) filter. Contrasts were constructed to examine all four sentence completion conditions versus rest, and areas of increasing activation with increasing task difficulty (the parametric contrast).

For each contrast of interest (sentence completion versus rest, and parametric effects) one contrast image per subject was entered into a second level random effects analysis to examine areas of activation within each of the three groups (one sample t-test), and differences in activation between the groups (ANOVA). Differences in activation due to symptomatic ‘state’ effects were initially examined by comparing the non-symptomatic groups (controls plus high risk without symptoms) versus high-risk subjects with symptoms (and individual psychotic symptoms), no reaction time measures were recorded in the scanner. Since these subjects indicated at debriefing that they had indeed performed the task in the scanner, they were included in the analysis.

Table 2 Example sentences

<table>
<thead>
<tr>
<th>Difficulty Level</th>
<th>Sentence Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>High constraint</td>
<td>&quot;He mailed the letter without a...&quot;</td>
</tr>
<tr>
<td>Medium high constraint</td>
<td>&quot;The train was still on...&quot;</td>
</tr>
<tr>
<td>Medium low constraint</td>
<td>&quot;Not even the cast liked the...&quot;</td>
</tr>
<tr>
<td>Low constraint</td>
<td>&quot;Rushing out he forgot to take his...&quot;</td>
</tr>
</tbody>
</table>

Numbers 1–4 represent increasing difficulty.

Table 3 Within-scanner movement

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 21)</th>
<th>High risk without symptoms (n = 42)</th>
<th>High risk with symptoms (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max movement in x (mm)</td>
<td>0.80 (0.51)</td>
<td>1.10 (0.83)</td>
<td>1.45 (0.93)</td>
</tr>
<tr>
<td>Max movement in y (mm)</td>
<td>0.80 (0.47)</td>
<td>0.82 (0.38)</td>
<td>0.99 (0.46)</td>
</tr>
<tr>
<td>Max movement in z (mm)</td>
<td>1.11 (0.74)</td>
<td>1.06 (0.65)</td>
<td>1.25 (0.83)</td>
</tr>
</tbody>
</table>

Estimate of movement parameters determined from realignment stage of preprocessing.
Differences due to ‘trait’ effects were initially examined by comparing controls versus all high-risk subjects (and vice versa). This analysis structure was chosen to simplify the reporting of results and to minimize the number of group comparisons. As the groups were matched on demographic variables, and there were no significant differences in movement parameters, we did not include these factors as potential confounds in the model. However, to be confident that these factors were not having a significant effect, further examination of the influence of these variables was performed. Potential confounders [detailed in Tables 1 and 3: age, gender, handedness and within-scanner movement in x (mm)] were examined by entering them as covariates in the second-level random effects analysis. Even at lenient thresholds (regions considered significant at voxel-level \( P < 0.001 \) uncorrected voxel level, extent threshold 50 voxels), none was found to have a significant effect.

In order to further clarify any state/trait related findings, post hoc group comparisons were conducted. In this model groups were also split in order that we could also examine differences according to degree of genetic risk, i.e. those with any first degree, or only second degree, relatives with the disorder. The criteria we followed for differences to be identified as potential state-specific effects were that similar differences should be found between ‘high risk with symptoms versus controls’, and between ‘high risk with versus high risk without symptoms’, but not between ‘high risk without symptoms (\( n = 42 \)); (C) high risk with symptoms (\( n = 27 \)). Activations displayed on 3D ‘glass brain’, maps thresholded at \( P < 0.0001 \) uncorrected voxel level, extent threshold 50 voxels.

<table>
<thead>
<tr>
<th>Constraint levels</th>
<th>Mean word appropriateness score (SD)</th>
<th>Mean reaction time (ms) (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Med low</td>
</tr>
<tr>
<td>Controls</td>
<td>6.40 (1.25)</td>
<td>3.25 (0.53)</td>
</tr>
<tr>
<td>High risk without symptoms</td>
<td>6.30 (1.00)</td>
<td>3.17 (0.59)</td>
</tr>
<tr>
<td>High risk with symptoms</td>
<td>6.38 (0.79)</td>
<td>3.35 (0.59)</td>
</tr>
</tbody>
</table>

Constraint levels high to low represent increasing difficulty.

Table 4 Behavioural measures

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**Fig. 1** Sentence completion versus rest: within-group analysis. (A) Controls (\( n = 21 \)); (B) high risk without symptoms (\( n = 42 \)); (C) high risk with symptoms (\( n = 27 \)). Activations displayed on 3D ‘glass brain’, maps thresholded at \( P < 0.0001 \) uncorrected voxel level, extent threshold 50 voxels.
symptoms versus controls’. Criteria for any potential trait-specific effects were that similar differences should be found between ‘controls versus high risk with symptoms’ and between ‘controls versus high risk without symptoms’, but not between ‘high risk with versus high risk without symptoms’. In addition to these more detailed group comparisons, we also performed a masking procedure in SPM to examine regions fulfilling the above criteria for state and trait effects. In order to derive regions signifying potential state effects, an exclusive mask was generated between ‘high risk with versus high risk without symptoms’ and ‘high risk without symptoms versus controls’, at an uncorrected mask threshold of 0.05. This mask was then converted into binary format to define an image to be used to examine areas of overlap between this exclusive mask and the contrast ‘high risk with symptoms versus controls’. Along similar lines, in order to derive regions signifying potential trait effects, an exclusive mask was generated between ‘controls versus high risk without symptoms’ and ‘high risk with versus high risk without symptoms’, which was then used to examine areas of overlap in the contrast ‘controls versus high risk with symptoms’. Finally, interactions between state and trait effects were examined by looking at areas where there were linear effects across the groups, for example controls greater than high risk without symptoms, greater than high risk with symptoms, and vice versa.

Statistical maps were thresholded at a level of $P = 0.001$ uncorrected, and regions were considered significant at $P < 0.05$ cluster level corrected for multiple comparisons, unless otherwise stated. All $P$-values quoted in the text are at the corrected cluster level. Co-ordinates were converted from MNI (Montreal Neurological Institute) to Talairach co-ordinates using a non-linear transformation (http://www.mrc-cbu.cam.ac.uk/Imaging).

Results

Demographic details

There were no statistically significant differences in age, gender, handedness or IQ between the subject groups. There were also no significant differences between the high-risk groups (with and without psychotic symptoms) in terms of genetic liability (Table 1).

Behaviour

All three groups showed the expected pattern of quicker reaction time and higher word appropriateness scores with greater contextual constraint (Table 4). This pattern of reaction time confirms that the subjects were performing the task appropriately during scanning. There were no significant differences between the groups in terms of their performances on either word appropriateness scores or reaction time measures.

Sentence completion versus rest

Figure 1A–C displays the results for the sentence completion versus rest contrast (all levels of difficulty versus baseline) within each of the groups. This demonstrated activation in regions commonly activated with this task, including the left precentral gyrus, inferior frontal gyrus, medial/superior frontal gyrus, middle/superior temporal gyrus, right posterior lobe of the cerebellum and the occipital lobes bilaterally. Visual inspection of these maps indicated an additional region of activation in the high-risk subjects with psychotic symptoms in the left parietal lobe, which was not seen in either of the other two groups.

No significant between-group differences were found between the controls and all the high-risk subjects for the sentence completion versus rest contrast, but, as suggested by the within-group maps described above, there was significantly greater activation in the high-risk subjects with psychotic symptoms versus the non-symptomatic groups in the left parietal lobe ($x = -42, y = -48, z = 48; P = 0.001$; Fig. 2A, B; Table 5). This activation was located in the
intraparietal sulcus, contained mainly within the inferior parietal lobule [Brodman area (BA) 40], and to a lesser extent involved the superior parietal lobule (BA 7). No regions were shown to be relatively less active in the symptomatic group versus the non-symptomatic groups for this contrast.

A more detailed analysis between groups of this parietal lobe difference (using the model with the high-risk groups also split into those with first and second degree relatives) is presented in Table 6. These group comparisons indicated that there was significantly greater activation in the left inferior parietal lobule in the high risk with symptoms versus controls ($x = -42$, $y = -50$, $z = 48; P = 0.001$), and versus the high risk without symptoms ($x = -42$, $y = -48$, $z = 48; P = 0.007$), but not between the controls and high-risk subjects without symptoms (nearest cluster at $x = -34$, $y = -44$, $z = 46$; T score $= 1.74$). The masking procedure confirmed these findings, where regions of overlap were seen in the left inferior parietal lobule.

### Table 5 Random effects analysis: main group differences

<table>
<thead>
<tr>
<th>$P$-value</th>
<th>Extent</th>
<th>$Z$</th>
<th>Peak height ($x$, $y$, $z$)</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sentence completion versus rest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptom ‘state’ contrast: controls, high risk without symptoms ($n = 63$) &lt; high risk with symptoms ($n = 27$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>353</td>
<td>4.50</td>
<td>$-42$, $-48$, 48; $-48$, $-49$, 54; $-30$, $-46$, 36</td>
<td>Left parietal lobe: inferior parietal lobule BA 40; Left parietal lobe: inferior parietal lobule BA 40; Left parietal lobe</td>
</tr>
<tr>
<td><strong>Parametric</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inherited ‘trait’ contrast: controls ($n = 21$) &gt; all high risk ($n = 69$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.033</td>
<td>145</td>
<td>4.01</td>
<td>$14$, $47$, $-1$; $2$, $53$, $5$</td>
<td>Right frontal lobe: medial frontal gyrus BA10/32</td>
</tr>
<tr>
<td>0.004</td>
<td>223</td>
<td>3.97</td>
<td>$-2$, $-78$, $-11$; $-14$, $-78$, $-15$; $-22$, $-73$, $-17$</td>
<td>Left cerebellum; posterior lobe, declive</td>
</tr>
<tr>
<td>0.001</td>
<td>279</td>
<td>3.89</td>
<td>$8$, $-13$, $6$; $-6$, $-15$, $12$; $18$, $-11$, $10$</td>
<td>Right cerebrum: thalamus</td>
</tr>
</tbody>
</table>

### Table 6 Random effects analysis: further examination of potential ‘state’-related effects

<table>
<thead>
<tr>
<th>$P$-value</th>
<th>Extent</th>
<th>$Z$</th>
<th>Peak height ($x$, $y$, $z$)</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sentence completion versus rest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls ($n = 21$) &lt; high risk with symptoms ($n = 27$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>334</td>
<td>4.17</td>
<td>$-42$, $-50$, 48; $-48$, $-48$, 55; $-31$, $-36$, 56</td>
<td>Left parietal lobe: inferior parietal lobule BA 40; Left parietal lobe: inferior parietal lobule BA 40; Left parietal lobe: inferior parietal lobule/postcentral gyrus</td>
</tr>
<tr>
<td>Controls ($n = 21$) &lt; high risk without symptoms ($n = 42$): n/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk without symptoms ($n = 42$) &lt; high risk with symptoms ($n = 27$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.007</td>
<td>235</td>
<td>4.40</td>
<td>$-42$, $-48$, 48; $-40$, $-62$, 54; $-32$, $-46$, 34</td>
<td>Left parietal lobe: inferior parietal lobule BA 40; Left parietal lobe</td>
</tr>
<tr>
<td><strong>Analysis with respect to degree of risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk with first degree relatives ($n = 48$) &gt; high risk with second degree relatives ($n = 21$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.003</td>
<td>274</td>
<td>4.23</td>
<td>$-6$, $-99$, 4; $10$, $-93$, 14; $7$, $-72$, 2</td>
<td>Left occipital lobe: cuneus; Right occipital lobe: cuneus; Right occipital lobe: lingual gyrus BA18</td>
</tr>
<tr>
<td>High risk with second degree relatives ($n = 21$) &gt; high risk with first degree relatives ($n = 48$): n/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n/s = not significant.
lobule in high risk with symptoms versus controls, and versus the high risk without symptoms, but not between controls and high risk without symptoms (x = -42, y = -50, z = 48; P < 0.001).

The analysis with respect to degree of genetic risk indicated no significant differences in the parietal lobe (see Table 6). The analysis examining linear trends across the three groups, however, suggested that parietal lobe activity was lowest in the controls, followed by the high risk without symptoms, and greatest in high risk with symptoms (x = -42, y = -50, z = 48; P = 0.019). There were no significant findings for the reverse contrast.

**Parametric analysis**

For the parametric contrast, each group presented two main regions of increasing activation with increasing task difficulty: the left superior/medial frontal gyrus (BA 6) and the left inferior frontal gyrus (BA 47) (see Fig. 3A–C).

Analysis of group differences for the parametric contrast revealed significantly greater increases in activation with increasing task difficulty in the controls compared with the high-risk group as a whole in several regions: one involving the right medial frontal gyrus and to a lesser extent the anterior cingulate gyrus (x = 14, y = 47, z = -1; P = 0.033), another in the left posterior lobe of the cerebellum (x = -2, y = -78, z = -11; P = 0.004) and finally in the thalamic nuclei (x = 8, y = -13, z = 6; P = 0.001) (Fig. 4A, B; Table 5). No other group differences were observed.

Further examination of these activation differences (using the model with the high-risk groups also split into those with first and second degree relatives) are presented in Table 7. The largest number of differences were seen between the controls and high-risk subjects without symptoms in several regions, including: the right medial frontal gyrus (x = 6, y = 53, z = 12; P < 0.001) and left posterior lobe of the cerebellum (x = -14, y = -79, z = -22; P = 0.001). While we did not find thalamic differences at the standard threshold of P = 0.001 uncorrected, at a lower threshold of P = 0.005 uncorrected, differences also emerged between the controls and high-risk subjects without symptoms in this region (x = 8, y = -13, z = 10; P < 0.001). Also at this lower threshold,
significant differences between controls and high risk with symptoms were seen in the thalamus ($x = 6, y = -14, z = 4; P < 0.001$) and left posterior lobe of the cerebellum ($x = -4, y = -81, z = -14; P = 0.033$). No differences were seen between the two high-risk groups at either statistical threshold. The masking procedure produced consistent findings, whereby regions of overlap were seen in controls versus high risk with symptoms and high risk without symptoms, but not between high risk with and without symptoms in the region of the cerebellum ($x = -4, y = -80, z = -8; P = 0.049$), and at a trend level, in the thalamus ($x = 8, y = -14, z = 4; P = 0.075$).

Table 8 presents analysis of the potential 'trait'-related effects with respect to degree of genetic risk. These results indicated greater increases in activation with increasing difficulty in those with first degree relatives versus those with second degree in the right medial frontal gyrus ($x = 16, y = 32, z = 40; P = 0.021$). No significant differences were observed for the reverse contrast. The analysis examining linear trends
High risk with first degree relatives (n = 48) > high risk with second degree relatives (n = 21)

- **P-value**: 0.021
- **Extent**: 173
- **Z**: 4.47
- **Peak height (x, y, z)**: 16, 32, 40
- **Region**: Right frontal lobe: medial/superior frontal gyrus BA8

- **P-value**: 0.063
- **Extent**: 132
- **Z**: 4.40
- **Peak height (x, y, z)**: 24, 32, 35; 21, 19, 30
- **Region**: Right frontal lobe: middle frontal gyrus BA8/9; Right frontal lobe

High risk with second degree relatives (n = 21) > high risk with first degree relatives (n = 48): ns

- **P-value**: ns
- **Extent**: ns
- **Z**: ns
- **Peak height (x, y, z)**: ns
- **Region**: ns

ns = not significant.

Discussion

We used fMRI in combination with a sentence completion test to examine trait and state effects of brain activation in subjects at high risk of schizophrenia. This report presents the results as part of a study of these individuals over the time period at which they are at the greatest risk of becoming ill. Importantly, none of these subjects was considered ill at the time of testing; those referred to as high risk with psychotic symptoms had reported isolated or transient psychotic symptoms in the setting of unimpaired function. They did not meet diagnostic criteria for any psychiatric disorder, and all subjects were antipsychotic naive at the time of investigation. We found that the high-risk subjects with isolated psychotic symptoms demonstrated significantly increased activation in the left inferior parietal lobule compared with controls and non-symptomatic high-risk subjects. The controls showed significantly greater task-related increases in activity in medial prefrontal, thalamic and cerebellar regions compared with the high-risk group as a whole.

The differences in brain activation patterns occurred against a background of closely similar task performance. This has important implications in relation to interpretation of the data. Many functional imaging studies examining patient populations (often medicated) reveal activation differences alongside performance differences. Thus it is difficult to determine whether activation differences are due to a core neuronal abnormality, or whether they are a secondary effect of poor performance (and/or medication). Functional imaging studies have often reported hypofrontality (in dorsolateral regions) in schizophrenic subjects, particularly with executive tasks in which the patients perform worse than controls. Studies that have controlled for task performance, however, suggest that hypofrontality may only become evident when performance fails (Frith et al., 1995; Fletcher et al., 1998). This is in agreement with the current findings, where we did not find evidence for decreased dorsolateral prefrontal activity in the presence of matched task performance. Others have suggested a more complex relationship between performance and either decreased or increased prefrontal cortex activation, particularly with reference to increasing working memory load (see Manoach, 2003).

An alternative explanation is that abnormal dorsolateral prefrontal activation may be specifically associated with deficits relating to the established illness. Results from other functional imaging studies of high-risk subjects are at present inconsistent. One study of clinically unaffected relatives reported increased activation of the right dorsolateral prefrontal cortex associated with increased risk in the absence of performance deficits during a working memory paradigm (Callicott et al., 2003). This was reported to involve BA 9/10/46, and so would appear to be more lateral than the prefrontal region reported to be positively associated with increased familial risk in this study (high-risk subjects with first degree relatives versus second degree relatives; Table 8; BA 8). In contrast, other studies of unaffected relatives report no differences in prefrontal cortex activation during a verbal fluency test (Spence et al., 2000).

It is, however, important to consider, since schizophrenia is a disorder with a restricted age of onset, that the subjects in the four studies mentioned above were generally outside the period of maximum risk (mean ages 34, 32, 39 and 55 years, respectively, and including a number of individuals >40 years old), and were therefore unlikely to develop the disorder. Our younger high-risk subjects will therefore presumably be in different risk strata than these older...
relatives, which may account for differences between these four studies and the current findings.

A matter that requires consideration is where our symptomatic high-risk subjects lie in relation to well, asymptomatic individuals and individuals with schizophrenia. These subjects did not have schizophrenia according to any diagnostic criteria. Their psychotic symptoms were fleeting and non-disabling, and their functioning good, but they do of course have an enhanced liability to the disorder. It has recently been reported that fleeting psychotic symptoms like these occur in ~10% of the normal population at some time (Verdoux and van Os, 2002). In this high-risk sample the occurrence lies nearer 40% (reported over the previous 18 months). Some of those 40% may still go on to develop schizophrenia, as it has been reported that the cases who do have often, but not always, had transient or partial psychotic symptoms prior to becoming ill (Johnstone et al., 2000). We conclude that in this sample the presence of these transient or partial psychotic symptoms is indicative of a state of genetically induced vulnerability to schizophrenia that, in some cases, will translate into psychosis.

Although we did not find lateral prefrontal differences, medial prefrontal-thalamic-cerebellar regions of reduced activation were seen in our high-risk group as a whole compared with control subjects. More detailed group comparisons also indicated differences between controls and both high-risk groups (but not between the two high-risk groups) in thalamic and cerebellar regions (although some only became evident at lower thresholds). Medial prefrontal differences were, however, only observed between controls and high-risk subjects without symptoms. Similar results were also reflected in the masked analysis. It is interesting and unexpected that more differences were observed between the high risk without symptoms versus controls than between the high risk with symptoms versus controls. This may be due to the larger number of subjects in this group, giving greater statistical power, particularly in a contrast examining incremental differences associated with increasing task difficulty rather than in the simpler contrast of sentence completion versus rest. Nevertheless, as a whole, these results are consistent with findings that this group of regions may be dysfunctional in schizophrenia (Andreasen et al., 1996), and in part with another study of unaffected relatives (Callicott et al., 2003) that also reported decreased activation in medial frontal, thalamus and cerebellar regions in unaffected relatives (although some only became evident at lower thresholds). Medial prefrontal differences were, however, only observed between controls and high-risk subjects without symptoms. Similar results were also reflected in the masked analysis. It is interesting and unexpected that more differences were observed between the high risk without symptoms versus controls than between the high risk with symptoms versus controls. This may be due to the larger number of subjects in this group, giving greater statistical power, particularly in a contrast examining incremental differences associated with increasing task difficulty rather than in the simpler contrast of sentence completion versus rest. Nevertheless, as a whole, these results are consistent with findings that this group of regions may be dysfunctional in schizophrenia (Andreasen et al., 1996), and in part with another study of unaffected relatives (Callicott et al., 2003) that also reported decreased activation in medial frontal, thalamus and cerebellar regions in unaffected relatives. Our favoured interpretation of these findings is that as task difficulty increases, healthy controls are able to increase activation in a network of areas involving medial prefrontal-thalamic and cerebellar regions, but those at genetically enhanced risk of schizophrenia were less able to do so. We suggest that the presence of the schizophrenia genotype (i.e. the high-risk trait) is associated with a restricted ability to activate this network, but this may only have behavioural consequences on more demanding tasks where the deficit cannot be compensated. Although we did not find increasing or decreasing degree of risk to be associated with activity in these specific regions within the high-risk group using the categorical measure of inheritance described, greater familial risk was found to be associated with increased activity in a region with the maxima located in the right medial/superior prefrontal region (BA 8). This region was slightly more superior and posterior to the medial prefrontal region referred to above (BA 10/32). It should also be considered that, although the finding of increasing activation with increasing task difficulty in the thalamus met criteria for a genetically mediated effect, the analysis examining linear differences across the groups suggested that this increased activation was greatest in the controls, followed by the high risk without symptoms, followed by high risk with symptoms. This indicates this effect may not be purely trait related, and that there may be some state modulation of trait effects with regards this finding.

Other studies of the Hayling sentence completion test in normal subjects have reported areas of activation similar to those reported here (Nathaniel-James et al., 1997; Lawrie et al., 2002). The study by Lawrie et al. (2002) compared schizophrenics (n = 8) and controls (n = 10), but did not report any functional localization differences between groups. However, Lawrie et al. (2002) examined medicated subjects with established schizophrenia who were not specifically selected to be at enhanced genetic risk of the disorder. Alternatively, the present, much larger, study was perhaps able to distinguish groups, because of increased statistical power.

We used the verbal initiation section of the Hayling test because it had previously been shown to demonstrate differences between schizophrenic and control subjects (in terms of functional connectivity; Lawrie et al., 2002), and is considered to be a refinement of the verbal fluency test, which is commonly found to elicit functional imaging abnormalities in schizophrenia (Frith et al., 1995; Yurgelun-Todd et al., 1996; Curtis et al., 1998; Spence et al., 2000). PET studies of verbal fluency report a relative failure to deactivate the left superior temporal gyrus in schizophrenic subjects compared with controls (Frith et al., 1995), while fMRI studies report decreased left dorsolateral prefrontal activation (Yurgelun-Todd et al., 1996; Curtis et al., 1998). Differences in scanning methodologies, particularly with respect to the requirement for covert responses in fMRI, may account for inconsistencies in temporal lobe activations (Curtis et al., 1998). fMRI paradigms that involve overt speech in response to a stimulus can be problematical, since the associated movement can cause large image distortions. Indeed, one limitation of the current study is that, although we collected within-scanner measures of performance by way of reaction time, on-line task performance in terms of word appropriateness involved covert word generation. At the time the study began, novel paradigms that allow overt responses cued in the gap between image acquisitions (see Henson et al., 2002) were not readily available, or indeed widely used.

In the current study we did not find any activation differences in either the left dorsolateral prefrontal cortex or
left superior temporal cortical region, which is consistent with another study of relatives of schizophrenic subjects (Spence et al., 2000). Rather, we found parietal lobe over-activation in symptomatic high-risk subjects. Further examination of this over-activation suggested that this was a state-related effect. Differences were found between controls and high-risk subjects with psychotic symptoms, and between the two high-risk groups, but not between controls and asymptomatic high-risk subjects, even at more lenient thresholds. These results were also confirmed using the masked analysis. However, although the parietal lobe hyperactivity fulfilled criteria for state-specificity, the analysis regarding linear differences across the three groups suggested that the parietal activation was greatest in the high risk with symptoms, followed by high risk without symptoms, followed by controls. Hence there may also be an interaction between with genetically mediated and symptom-related effects in this region.

The majority of imaging studies in schizophrenia have focused on prefrontal and temporal brain abnormalities, but there are a number that report abnormalities of parietal lobe regions. In an early study investigating the relationship between hippocampal pathology and prefrontal hypofunction in monozygotic twins discordant for schizophrenia, there was an inverse relationship between hippocampal volume and activation in the parietal cortex in the affected twin group (Weinberger et al., 1992). In a more recent study the earliest structural deficit (associated with early onset schizophrenia) was reported to occur in the parietal lobe, which only later progressed to involve frontal and temporal lobes (Thompson et al., 2001). Several other functional imaging studies have reported increased activation in parietal lobe regions in schizophrenic subjects compared with controls during a variety of paradigms, including memory tasks (Crespo-Facorro et al., 1999) and verbal fluency (Curtis et al., 1998), decision making (Paulus et al., 2002), and in a study of voluntary movements in patients with symptoms specifically attributed to self-monitoring failures (Spence et al., 1997).

One interpretation of the relative over-activation of the parietal lobe in our symptomatic high-risk subjects is that this region may be recruited to enable them to perform the task at a similar level to the other groups. Since we did not find that activation in the left intraparietal area was linearly related to task difficulty, we suggest that the relative over-activation in this region represents a general dysfunction in those at high risk with psychotic symptoms. In other words, the presence of the early symptomatic state is associated with a compensatory over-activation of the parietal lobe at all levels of task engagement. The intraparietal region is considered to be involved in attentional maintenance (Corbetta et al., 2000; Hopfinger et al., 2000), response preparation (Snyder et al., 1997) and response monitoring (Garavan et al., 1999; Menon et al., 2001), functions that have been shown to be abnormal in schizophrenia (Frith, 1992; Schatz, 1998). A compensatory additional activation in the parietal lobe in the high-risk subjects with symptoms may therefore be related to attentional aspects of the task and the preparation and monitoring of suitable responses. This interpretation is consistent with reports that subjects in the prodrome to schizophrenia commonly report difficulties focusing attention and a reduced sense of control of behaviour (McGhie and Chapman, 1961; Klosterkötter et al., 1997). Alternatively, regions of the posterior parietal cortex have been implicated in the role of distinguishing between self and others (Meltzoff and Decety, 2003). Hyperactivity in this region in our symptomatic high-risk subjects may therefore suggest deficits of this neural system. Misattribution of internally generated actions as being externally generated are considered to be a potential neurophysiological basis of positive symptomatology. Indeed, findings of hyperactivity in parietal areas in subjects with the established illness experiencing passivity phenomena are consistent with this hypothesis (Spence et al., 1997). Regardless of the origin of this abnormality, a relative over-activation of the parietal lobe could represent one of the earliest pathological changes by which the trait of high genetic risk of schizophrenia switches to the state of incipient psychosis.

It is conceivable that the differences in male:female ratios across the groups could contribute to the reported findings, especially since there is evidence to suggest gender differences in brain activation during language-based tasks (Shaywitz et al., 1995). However, there were no statistically significant differences in gender between the groups; furthermore, we were unable to demonstrate differences in activation due to gender at the second level.

Overall, these results indicate that there are state and trait features of schizophrenia that can be demonstrated with functional imaging. Group differences of apparent genetic cause were found in medial prefrontal-thalamic-cerebellar regions, and differences were found in subjects at high risk with isolated psychotic symptoms in the parietal lobe. These patterns of activation reveal information about the pathophysiology of the state of vulnerability to schizophrenia, and about the mechanisms involved in the development of schizophratic symptoms.

Acknowledgements
The authors would like to thank those involved in subject recruitment (Richard Cosway, Lesley Harrison, Kirsten Russell), Heba Lakany for programming the paradigm, the radiographers, and the participants themselves. We also wish to thank Norma Brearley for the careful preparation of the manuscript. This study was funded by an MRC programme grant. Scanning was carried out at the Brain Imaging Research Centre (BIRC) for Scotland. Both BIRC and CFIS are funded by SHEFC.

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