Prefrontal and Striatal Volumes in Monozygotic Twins Concordant and Discordant for Schizophrenia

Ulrich Ettinger*,1,2, Anne Schmechtig3, Timothea Touloupolou4, Charmaine Borg3, Claire Orrells3, Sheena Owens4, Kazunori Matsumoto4, Neeltje E. van Haren5, Mei-Hua Hall6, Veena Kumari7, Philip K. McGuire4, Robin M. Murray4, and Marco Picchioni4,8

1Department of Psychiatry, Ludwig-Maximilians-University, Nussbaumstr. 7, 80336, Munich, Germany; 2Department of Psychology, Ludwig-Maximilians-University, Munich, Germany; 3King’s College London, Department of Neuroimaging, Institute of Psychiatry, London, UK; 4King’s College London, Department of Psychosis Studies, Biomedical Research Centre, Institute of Psychiatry, London, UK; 5Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, Utrecht, The Netherlands; 6Psychology Research Laboratory, McLean Hospital, Harvard Medical School, Boston, MA, USA; 7Department of Psychology, Institute of Psychiatry, King’s College London, London, UK; 8King’s College London, St Andrew’s Academic Centre, Institute of Psychiatry Northampton, UK

*To whom correspondence should be addressed; tel: +49 89 5160 3499, fax: +49 89 5160 5789, e-mail: ulrich.ettinger@med.uni-muenchen.de

Frontostriatal networks mediating important cognitive and motor functions have been shown to be abnormal structurally and functionally in schizophrenia. However, the influence of genetic risk for schizophrenia on structural abnormalities in these areas is not well established. This study therefore aimed to investigate prefrontal and striatal volume alterations in schizophrenia and to define the extent to which they are dependent on genetic vulnerability for the condition. We employed structural magnetic resonance imaging (sMRI) in monozygotic (MZ) twins with or without schizophrenia. A sample of 129 twins completed sMRI, consisting of 21 MZ twin pairs concordant for schizophrenia, 17 MZ schizophrenic twins and 18 MZ nonschizophrenic twins drawn from 19 pairs discordant for schizophrenia, and 26 MZ control twin pairs without schizophrenia. Groups did not significantly differ in age, gender, handedness, height, level of education, parental socioeconomic status, and ethnicity. Using a region-of-interest approach, we measured the gray matter volumes (in cm³) of superior, middle, inferior, and orbital frontal cortices (SFC, MFC, IFC, and OFC, respectively); the caudate; and putamen. Covarying for whole-brain volume, age, and gender, we found that concordant but not discordant twins with schizophrenia had significantly lower volumes of MFC and OFC than control twins. In contrast, both patient groups had significantly lower SFC volumes than both groups of nonschizophrenic twins. There were no significant group differences in IFC and the striatum. We conclude that the prefrontal cortex shows a heterogeneous pattern of genetic influences on volumetric reductions in schizophrenia.

Key words: schizophrenia/frontal lobe/putamen/caudate/magnetic resonance imaging/genetics

Introduction

The prefrontal cortex in humans covers about 30% of neocortex. It lies anterior to the precentral sulcus and is thought to play important modulatory and integrative roles in executive function, working memory, attention, response inhibition, and the control of socially appropriate behaviors. Human lesion studies have shown that damage to the prefrontal cortex impairs these cognitive functions and causes significant personality changes. Functional imaging studies have provided corroborative evidence showing increases in brain activation in prefrontal cortex during experimental paradigms of such functions (for recent reviews of frontal cortex structure and function, see 1–6).

Importantly, the prefrontal cortex does not work in isolation but is part of a distributed network of cortical and subcortical areas. For example, prominent white-matter connections exist between prefrontal cortex and the striatum.7 The striatum comprises caudate and putamen and has been linked to a variety of motor and nonmotor behaviors as part of several cortico-striato-thalamo-cortical loops.8

The integrity of frontostriatal circuitry appears compromised in schizophrenia.7,9,10 Schizophrenia can affect virtually every area of human behavior, but particularly impairs complex, coordinated functions that are typically...
controlled by heteromodal cortex and their subcortical projection areas.11,12 Neuroimaging studies using magnetic resonance imaging (MRI) have provided evidence of frontostriatal abnormalities. Some but not all structural MRI (sMRI) studies have detected volumetric reductions in a variety of frontal region-of-interest (ROI) measures13–16 (for review, see 17). Evidence of striatal volume changes in schizophrenia is less consistent, with studies showing larger,14 smaller,18,19 or unchanged20,21 volumes. Striatal volumes are significantly influenced by antipsychotic medication,22–24 with first-generation antipsychotics associated with volume increases (for review, see 25). Both hyper- and hypactivations in frontal cortex are observed in functional neuroimaging studies of schizophrenia. These mixed findings may in part reflect methodological considerations such as task difficulty and differential group performance (for review, see 26,27), although there is also evidence of frontal dysfunction in schizophrenic patients that are independent of group differences in task performance.28 Functional neuroimaging studies have also shown striatal dysfunction in schizophrenia.29,30

The etiology of schizophrenia involves a significant genetic component. Twin studies estimate its heritability to be approximately 80%,31–33 Behavioral genetic studies have shown that the frontal lobe is under substantial genetic control in healthy humans, with heritability estimates of frontal volumes of approximately 90%.34,35

What is less clear is the extent to which frontostriatal volume changes in schizophrenia are influenced by the genetic risk for the disorder itself. The subtle yet statistically reliable volumetric changes seen in patients with schizophrenia could reflect the impact of genetic, epigenetic, or environmental factors. Studies of patients’ unaffected family members allow us to partially address this issue, given that they carry genetic risk but do not express the clinical phenotype. A number of MRI studies suggest that patients’ unaffected relatives show qualitatively similar, yet less severe, structural changes in the frontal lobe36–38 and the striatum.39,40 A recent study using magnetic resonance spectroscopy also showed reduced mesial prefrontal glutamate in schizophrenia patients as well as their unaffected co-twins when compared with healthy controls.41 These findings suggest that at least some of the structural brain changes in schizophrenia are likely to be genetically determined, while unique environmental effects, including treatment, also play a role. However, contradicting this view is a recent large-scale study of over 200 siblings of schizophrenia patients that found no significant differences in neuroanatomical measures compared with a similarly large sample of controls,42 suggesting that structural brain changes seen in schizophrenia are not likely to be due to genetic factors.

Studies of monozygotic (MZ) twins, concordant and discordant for schizophrenia, are a complementary and particularly powerful experimental means of addressing questions of genetic influence on brain structural abnormalities in schizophrenia. MZ twins are assumed to be genetically identical. In discordant pairs, one twin has schizophrenia while their co-twin does not; in concordant pairs, both twins have the disorder. Contrasting the unaffected members from discordant pairs and control pairs allows us (complementary to the comparisons of relatives and controls described above) to test to what extent the genetic risk for schizophrenia drives brain morphological changes.

Including MZ concordant twin pairs allows us to address the effects of genetic liability from a different perspective. It has been argued that MZ twins discordant for schizophrenia show a greater genetic liability than discordant pairs who in turn may have a more environmental form of the disorder.43–45 Evidence supporting this notion comes from observations that nongenetic risk factors such as obstetric complications are more common in discordant than concordant pairs.43 Additionally, one study found greater hippocampal abnormalities in discordant than concordant pairs,44 a finding compatible with hippocampal sensitivity to specific environmental insults in schizophrenia.46,47 Thus, brain abnormalities due to schizophrenia risk genes would be expected to be more pronounced in patients from discordant than discordant twin pairs. Adopting this design, we have previously shown that thalamic volume is significantly reduced in concordant but not discordant twins compared with healthy controls, suggesting a genetic influence.48

In this study, we examined 4 prefrontal cortex regional volumes as well as the striatum in MZ twin pairs concordant and discordant for schizophrenia as well as in healthy control MZ twin pairs. The prefrontal cortical segmentation method was previously described,11,13,49 and yields volumes for superior, middle, inferior, and orbital frontal cortices (SFC, MFC, IFC, and OFC, respectively). These areas represent important and functionally as well as structurally dissociable subdivisions of human prefrontal cortex with evidence of dysfunction in schizophrenia.11,50,51 This ROI approach thus allows an anatomically focused test of specific brain regions. Additionally, the ROI approach is advantageous in twin designs as the extraction of regional volumetric data scans enables appropriate treatment of the data by statistical methods that can take into account the relatedness in the sample.

We compared frontal and striatal volumes across groups and expected that structures that are affected by the genetic predisposition for schizophrenia will be (1) reduced in nonschizophrenic co-twins of schizophrenia patients or (2) reduced more strongly in concordant than discordant-affected twins when compared with controls. On the other hand, structures that are more sensitive to environmental factors or illness expression should be similarly reduced in concordant and discordant-affected
Method

Participants

Probands were referred to the study from psychiatric services across the United Kingdom. Control twins were recruited from the Institute of Psychiatry Volunteer Twin Register and by using advertisements in the national media. Exclusion criteria for all participants were a history of neurological illness or of systemic illness with known neurological complication, history of head injury with loss of consciousness of more than 1 minute, and current substance misuse or dependence. Participants provided written informed consent. Ethical permission for this study was granted by the local hospital and by the South East Multi-Centre Research Ethics Committee.

Clinical diagnoses were confirmed by structured clinical interviews performed by two trained psychiatrists using the Schedule for Affective Disorders and Schizophrenia–Lifetime Version augmented with further clinical information to make Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) diagnoses. Current psychotic symptoms in the probands were assessed using the Scale for the Assessment of Positive Symptoms (SAPS) and Scale for the Assessment of Negative Symptoms (SANS). Handedness was determined using the Annett scale, while we determined parental socioeconomic status (SES) using a national standardized scale. The number of years spent in full-time education was established. Monozygosity was first established using twin likeness questionnaires and confirmed using 12 highly polymorphic microsatellite markers. The probands’ medication status was recorded at assessment and chlorpromazine (CPZ) equivalents were calculated to allow an investigation of treatment correlates of regional brain volumes. Probands’ age at first contact with psychiatric services was recorded as a proxy measure of age at illness onset.

In concordant pairs, both members met DSM-IV criteria for schizophrenia or schizoaffective disorder. In discordant pairs, one member (the proband) met DSM-IV criteria for either schizophrenia or schizoaffective disorder, while the other member (the cotwin) was free of any psychotic illness. Controls were required to be free of personal or family history of psychosis or any schizophrenia spectrum disorder. All probands were clinically stable at the time of assessment, with no changes to their medication in the past 6 months. There was only one discordant twin pair that had a confirmed further family history of a schizophrenia spectrum disorder.

Magnetic Resonance Imaging

Participants underwent MRI scanning on a General Electric Signa Advantage scanner at 1.5 Tesla (General Electric Co, Milwaukee, WI). A 3-dimensional T1-weighted, coronal, spoiled gradient of the whole head was obtained (echo time = 5 ms, repetition time = 35 ms, flip angle = 30°, number of excitations = 1, field of view = 200 × 200 mm, voxel dimensions = 1 × 1 × 1.5 mm), yielding 124 contiguous slices 1.5 mm thick. Imaging took place on identical scanners with identical protocols at either of the two sites (St George’s Hospital, London or The Maudsley Hospital, London). In all twin pairs, both members of each pair were scanned at the same site and comparable numbers of twins from each group were scanned at each site ($\chi^2 = 1.59, df = 3, P = .66$).

Images were analyzed in software based on stereological principles (MEASURE). MEASURE superimposes a grid on the image and allows the user to view voxels in three mutually orthogonal planes and manually mark regions of interest. Head tilt was corrected in all brains prior to measurements to align images along the anterior commissure-posterior commissure line and the interhemispheric fissure. For frontal and striatal ROIs, a grid setting of 3 × 3 × 2 was used, with one grid point equaling one voxel. For whole-brain volume, a grid setting of 5 × 5 × 5 was used. Analysis was done blind to group status. See Figures 1 and 2 for an illustration of the ROIs in MEASURE.

Frontal Lobe. The frontal lobe was divided into 4 areas, comprising the SFC, MFC, IFC, and OFC. Surface sulcal landmarks to define boundaries between these regions were based on those described and implemented previously. Neuroanatomical atlases were used to further verify boundaries. Only gray matter was included and all 3 dimensions of image display (coronal, axial, and sagittal) were viewed when rating ROIs. Volumes were obtained for each structure bilaterally, but were also calculated separately for left and right hemisphere by splitting the image midsagittally. If midsagittal grid points occurred that could not be clearly attributed to right or left hemisphere, they were excluded in this calculation. Intrarater (intra-class correlations, ICC > 0.94) and interrater reliabilities (ICC > 0.71) between two raters were obtained from 10 images drawn randomly from the entire database.

The SFC was defined by the following sulcal landmarks: the anterior boundary was the frontomarginal sulcus, the inferior lateral boundary was the superior frontal sulcus, the inferior medial boundary was the cingulate sulcus, and the posterior boundary was the precentral sulcus on the lateral surface and the paracentral sulcus on the medial surface. The MFC was defined superiorly by the superior frontal sulcus, inferiorly by the inferior frontal sulcus, posteriorly by the precentral sulcus, and anteriorly by the superior frontal sulcus merging into the frontomarginal sulcus. The IFC was bounded superiorly by the inferior frontal sulcus, inferiorly by the horizontal ramus of the Sylvian fissure, and posteriorly by the inferior part of
the precentral sulcus. The OFC extended to the ventral cortical surface of the brain and included the rectus, orbital, and frontomarginal gyri. It was defined superiorly by the horizontal ramus of the sylvian fissure and medially by the superior rostral sulcus (suborbital sulcus) as it merges into the frontomarginal sulcus anteriorly. The posterior boundary was the ascending limb of the horizontal ramus (laterally) and a line drawn from the posterior end of the subgenual cingulum to adjacent CSF (medially). As such, our definition of OFC includes different subareas of ventral prefrontal cortex.62

Caudate. The caudate nucleus has clearly visible boundaries on T1-weighted images. Following previous descriptions, our measurements included the head and body but excluded the tail.18,40,63,64 The caudate is medially bounded by the lateral ventricle and laterally by the internal capsule. Measurement of the caudate began on the

Fig. 1. Illustration of Prefrontal ROIs. Figure shows the frontal ROIs painted in red using MEASURE. A, superior frontal cortex; B, middle frontal cortex; C, inferior frontal cortex; D, orbitofrontal cortex. The ROIs are shown in coronal view. ROI, region-of-interest.
most inferior axial slice in which the caudate and putamen were clearly separated by white matter. The rater then continued through each slice superiorly until the caudate body was no longer visible. Coronal and sagittal orientations were additionally viewed to clarify whether individual voxels were part of the caudate. The caudate ratings done in this study preceded the detailed protocol provided recently but are generally compatible with it. Intra- and interrater reliabilities between two raters were obtained from 10 images drawn randomly from the entire database (ICC = 0.99 and ICC = 0.90, respectively).

Putamen. The putamen also has clear boundaries on T1-weighted MRI scans. The landmarks were based on previous descriptions. The most inferior axial slice of the putamen was that in which the caudate and putamen were clearly separated by white matter. In the axial view, the superior boundary was white matter above the slice in which the putamen can first be seen between internal and external capsule. The lateral boundary was the external capsule. The medial anterior boundary was the internal capsule, particularly in the more superior slices; otherwise the medial boundary was the lateral medullary lamina of the globus pallidus. Posteriorly, the marking of the putamen was continued until the gray matter structure was no longer discernible against the white matter boundary. Intra- and interrater reliabilities between two raters were obtained from 10 images drawn randomly from the entire database (ICC = 0.97 and ICC = 0.80, respectively).

Cerebrum. The definition of cerebral volume was as implemented previously. Intra- and interrater reliabilities obtained from 10 images drawn randomly from the entire database were high (ICC > 0.97 and ICC > 0.98, respectively).

Statistical Analysis

The genetic relatedness of twin pairs and the resulting within-family correlations violate the assumption of independent observations made in analysis of variance. Therefore, differences between groups (concordant,
discordant schizophrenic, discordant nonschizophrenic, and control) were analyzed with regression models that allowed for correlations within twin clusters and departures from normality by using the robust sandwich estimator to estimate standard errors implemented in Stata 10 (Stata Corporation, College Station, Texas). The significance level was set at 5%.

The robust sandwich estimator provides robust standard errors (and therefore robust confidence intervals and P values), which give accurate assessments of the sample-to-sample variability of the parameter estimates even when the model is misspecified, such as if observations are nonindependent, ie, cluster correlated as is the case in this study of twins. Using robust regression, the parameter estimate may not be unbiased but the 95% confidence interval will be accurate, giving 95% confidence that the true parameter estimate lies within its range.67,68

Group differences in age, years of education, height, and parental SES were examined using linear regression models with robust standard errors. Group differences in gender, ethnicity, and handedness were examined by logistic regressions with robust standard errors of the respective binary dependent variables (gender: male/female; ethnicity: Caucasian/Afro-Caribbean; handedness: right handed/left handed).

To examine differences in clinical data, the two proband groups (concordant and discordant) were compared on SAPS, SANS, age at first contact, and CPZ equivalents using regression models with robust standard errors, and on type of antipsychotic medication (typical and atypical) using logistic regression with robust standard errors.

For group comparisons of ROI volumes (covarying for age, gender, and whole-brain volume), effects of side (right, left) and side-by-group interactions were first evaluated for each ROI. In the absence of any statistical evidence for a dependency of the group difference on side (ie, a significant side-by-group interaction), hemispheres were combined and group comparisons of each ROI volume carried out. The level of significance for the analysis of the 6 ROIs was adjusted using the Bonferroni method (P = .05/6 = .0083). If a group comparison for a given ROI was statistically significant at this level, it was followed by six pairwise post hoc comparisons among the 4 groups again with Bonferroni adjustment (P = .05/6 = .0083) and using age, gender, and whole-brain volume as covariates. Unadjusted effect sizes (Cohen’s d69) were calculated for significant and trend-level pairwise comparisons.

In order to provide an index of the MZ twin similarity of each ROI, ICC were calculated separately for each twin category (concordant, discordant, and control). While these ICCs do not provide an estimate of heritability, they measure the degree of positive correlation between the scores of the members of each twin pair allowing an assessment of the similarity in ROI volumes for each twin category.

Clinical correlates of ROI volumes were investigated separately in the concordant and discordant proband groups using a regression model with robust standard errors predicting ROI volume in separate analyses from SAPS, SANS, age at first contact with psychiatric services; and CPZ equivalents, with age, gender, and whole-brain volume as covariates. For each ROI, Bonferroni correction was carried out for these 4 regression analyses, resulting in a significance threshold of P = .05/4 = .0125. Effects of treatment status on ROI volume were investigated in each proband group by comparing first- and second-generation antipsychotic-treated probands, again using age, gender, and whole-brain volume as covariates. The significance threshold was also determined by the number of ROIs for each group, ie, P = .05/6 = .0083.

Results

Demographic and Clinical Variables

A total of 132 MZ twins completed MRI. Data had to be excluded for two probands and one nonschizophrenic co-twin from 3 discordant pairs due to movement artifact, leaving a final sample size of 129 MZ twins: 21 MZ twin pairs concordant for schizophrenia, 17 MZ schizophrenic twins and 18 MZ nonschizophrenic twins drawn from 19 pairs discordant for schizophrenia, and 26 healthy MZ control twin pairs. While missing data from the discordant group means that not all subjects represent complete pairs of affected-unaffected twins, we decided to retain such individual twins in order to maximize the sample size of this very rare population.

Demographic and clinical variables are summarized in tables 1 and 2, respectively. There were no significant effects of group on age, gender, ethnicity, height, handedness, parental SES, or years of education. The groups were thus matched on a number of possible confounder variables (table 1). Note that there were subtle yet nonsignificant differences in gender distribution across groups; however, the impact of these on group comparisons in ROIs were reduced by using this variable as covariate as described above.

Eleven of the nonschizophrenic members of the discordant pairs met criteria for a previous DSM-IV Axis I diagnosis: depression (n = 3); depression and alcohol abuse (n = 1); depression and simple phobia (n = 1); obsessive-compulsive disorder, depression, and drug and alcohol abuse (n = 1); panic disorder, mania, and depression (n = 1); simple phobia (n = 1); simple phobia, panic disorder, and depression (n = 1); generalized anxiety disorder and panic disorder (n = 1); and generalized anxiety disorder, panic disorder, and depression (n = 1). Eight of the control twins met criteria for a previous DSM-IV Axis I diagnosis: depression (n = 3); mania (n = 1); depression and drug and alcohol abuse (n = 2); drug abuse...
Table 1. Demographic Variables by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Concordant (n = 42)</th>
<th>Discordant Schizophrenic (n = 17)</th>
<th>Discordant Nonschizophrenic (n = 18)</th>
<th>Control (n = 52)</th>
<th>Group Comparison Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>35.22 (8.84)</td>
<td>33.28 (12.75)</td>
<td>31.44 (11.23)</td>
<td>35.27 (10.13)</td>
<td>F&lt;sub&gt;3,65&lt;/sub&gt; = 1.15, P = .33</td>
</tr>
<tr>
<td>Male/female twins (n)</td>
<td>34/8</td>
<td>10/7</td>
<td>9/9</td>
<td>34/18</td>
<td>χ&lt;sup&gt;2&lt;/sup&gt; = 6.35, df = 3, P = .10</td>
</tr>
<tr>
<td>Handedness (% right-handed twins)</td>
<td>81.0</td>
<td>81.3</td>
<td>88.9</td>
<td>89.8</td>
<td>χ&lt;sup&gt;2&lt;/sup&gt; = 1.35, df = 3, P = .72</td>
</tr>
<tr>
<td>Years of education</td>
<td>13.07 (2.79)</td>
<td>12.71 (3.57)</td>
<td>13.28 (3.10)</td>
<td>13.87 (2.61)</td>
<td>F&lt;sub&gt;3,65&lt;/sub&gt; = 0.68, P = .57</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.66 (8.20)</td>
<td>174.32 (9.15)</td>
<td>172.10 (9.13)</td>
<td>173.54 (8.90)</td>
<td>F&lt;sub&gt;3,61&lt;/sub&gt; = 0.87, P = .46</td>
</tr>
<tr>
<td>Parental socioeconomic status</td>
<td>2.55 (0.88)</td>
<td>2.41 (1.00)</td>
<td>2.39 (0.98)</td>
<td>2.70 (0.93)</td>
<td>F&lt;sub&gt;3,63&lt;/sub&gt; = 0.37, P = .77</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian twins)</td>
<td>85.0</td>
<td>76.5</td>
<td>83.3</td>
<td>100</td>
<td>χ&lt;sup&gt;2&lt;/sup&gt; = 2.28, df = 2, P = .32</td>
</tr>
</tbody>
</table>

Note: Data reflect mean (standard deviation) unless otherwise stated. There are no significant differences between groups in any variables reported in this table.

*All twins were of either Caucasian or Afro-Caribbean ethnicity.*

There were significant overall effects of group for SFC (F<sub>3,65</sub> = 10.81, P < .0001), MFC (F<sub>3,65</sub> = 5.87, P = .001), OFC (F<sub>3,65</sub> = 5.66, P = .002), but not for IFC, putamen (both P > .15) or caudate (P = .02) after applying the Bonferroni correction (see above). Post hoc comparisons revealed the following pattern.

For SFC, we found that both patient groups had smaller volumes than both nonschizophrenic groups. Specifically, concordant twins had significantly smaller volumes than controls (t = 4.72, P < 0.001; d = −1.29) and discordant nonschizophrenic twins (t = 3.04, P = .003; d = −0.94). Discordant schizophrenic twins had smaller volumes than both discordant nonschizophrenic (t = 3.18, P = .002; d = −0.72) and control twins (t = 3.08, P = .003; d = −1.07). No other comparisons were significant (all P > .34).

For MFC, concordant twins had significantly smaller volumes than discordant nonschizophrenic (t = 3.70, P < .001; d = −1.17) and control twins (t = 3.23, P = .002; d = −0.99), but did not significantly differ from discordant schizophrenic twins (P = .07; d = −0.46). All other comparisons were nonsignificant (all Ps > .11).

On OFC concordant twins also had significantly smaller volumes than controls (t = 4.10, P < .001; d = −1.32), but the reduction compared with the discordant nonschizophrenic twins (P = 0.03; d = −0.80) was not significant after Bonferroni correction. The comparison of the concordant and discordant-affected groups achieved a trend (P = .08; d = −0.44) but no other comparisons were significant (all Ps > 0.19).

**ICCs of ROI Volumes**

The ICCs for the concordant, discordant, and control twin categories are shown in table 4. The observed ICCs ranged from 0.30 to 0.87 and did not consistently

\[(n = 1)\); and specific phobia, agoraphobia, and drug abuse (n = 1). The frequency of psychiatric diagnosis was significantly higher in the discordant unaffected group than in the controls (χ<sup>2</sup> = 9.36, df = 1, P = .003). Importanty, none of the nonschizophrenic twins were unwell at the time of assessment or taking any psychotropic medication.

Within the MZ concordant group, two individuals, and in the MZ discordant patient group, one individual met lifetime diagnostic criteria for schizoaffective disorder, rather than for schizophrenia. This is a limitation introducing a possible source of heterogeneity into the clinical sample. However, these small numbers prevented us from conducting further meaningful contrasts between these two schizophrenia spectrum disorders.

Table 2 summarizes the clinical variables of the two proband groups. There were no significant differences in type (first, second generation) or dose (CPZ equivalents) of antipsychotic treatment, age at first contact with psychiatric services, or positive (SAPS) and negative (SANS) schizophrenic symptom scores at the time of assessment.

**Group Differences in ROI Volumes**

Table 3 contains the unadjusted means and standard deviations of frontal and striatal ROIs. There were no side-by-group interactions for any ROIs (all P > .07), but there were effects of side for putamen (P = .0005), IFC (P = .003), SFC (P = .0006), MFC (P = .0005), indicating greater left than right hemispheric volumes, but not for OFC (P = .37) or caudate (P = .60). The absence of significant side-by-group interactions indicates that any group effects are independent of hemisphere; therefore, the following analyses were carried out using ROI volumes collapsed across hemispheres.

\[(n = 1)\); and specific phobia, agoraphobia, and drug abuse (n = 1). The frequency of psychiatric diagnosis was significantly higher in the discordant unaffected group than in the controls (χ<sup>2</sup> = 9.36, df = 1, P = .003). Importanty, none of the nonschizophrenic twins were unwell at the time of assessment or taking any psychotropic medication.

Within the MZ concordant group, two individuals, and in the MZ discordant patient group, one individual met lifetime diagnostic criteria for schizoaffective disorder, rather than for schizophrenia. This is a limitation introducing a possible source of heterogeneity into the clinical sample. However, these small numbers prevented us from conducting further meaningful contrasts between these two schizophrenia spectrum disorders.

Table 2 summarizes the clinical variables of the two proband groups. There were no significant differences in type (first, second generation) or dose (CPZ equivalents) of antipsychotic treatment, age at first contact with psychiatric services, or positive (SAPS) and negative (SANS) schizophrenic symptom scores at the time of assessment.

**Group Differences in ROI Volumes**

Table 3 contains the unadjusted means and standard deviations of frontal and striatal ROIs. There were no side-by-group interactions for any ROIs (all P > .07), but there were effects of side for putamen (P = .0005), IFC (P = .003), SFC (P = .0006), MFC (P = .0005), indicating greater left than right hemispheric volumes, but not for OFC (P = .37) or caudate (P = .60). The absence of significant side-by-group interactions indicates that any group effects are independent of hemisphere; therefore, the following analyses were carried out using ROI volumes collapsed across hemispheres.

\[(n = 1)\); and specific phobia, agoraphobia, and drug abuse (n = 1). The frequency of psychiatric diagnosis was significantly higher in the discordant unaffected group than in the controls (χ<sup>2</sup> = 9.36, df = 1, P = .003). Importanty, none of the nonschizophrenic twins were unwell at the time of assessment or taking any psychotropic medication.

Within the MZ concordant group, two individuals, and in the MZ discordant patient group, one individual met lifetime diagnostic criteria for schizoaffective disorder, rather than for schizophrenia. This is a limitation introducing a possible source of heterogeneity into the clinical sample. However, these small numbers prevented us from conducting further meaningful contrasts between these two schizophrenia spectrum disorders.

Table 2 summarizes the clinical variables of the two proband groups. There were no significant differences in type (first, second generation) or dose (CPZ equivalents) of antipsychotic treatment, age at first contact with psychiatric services, or positive (SAPS) and negative (SANS) schizophrenic symptom scores at the time of assessment.

**Group Differences in ROI Volumes**

Table 3 contains the unadjusted means and standard deviations of frontal and striatal ROIs. There were no side-by-group interactions for any ROIs (all P > .07), but there were effects of side for putamen (P = .0005), IFC (P = .003), SFC (P = .0006), MFC (P = .0005), indicating greater left than right hemispheric volumes, but not for OFC (P = .37) or caudate (P = .60). The absence of significant side-by-group interactions indicates that any group effects are independent of hemisphere; therefore, the following analyses were carried out using ROI volumes collapsed across hemispheres.
differ between twin categories (concordant, discordant, and control). Inspection of confidence intervals reveals that these overlapped widely, suggesting that twin categories did not differ in magnitude of ICC.

Clinical Correlates of ROI Volumes

In the concordant group, there were no significant correlations of ROIs with SAPS, SANS, age at first contact, or CPZ equivalents following Bonferroni correction (all \( P > .02 \)). There were no significant differences between treatment groups in ROI volumes, although putamen volumes were larger in typically compared with atypically treated patients (\( P = .04 \); nonsignificant after Bonferroni correction).

In the discordant schizophrenic group, there was an association of SFC volume with SANS (\( P = .007 \)), indicating that greater severity of negative symptoms was associated with smaller SFC volumes. No other correlations or treatment effects for any ROI reached significance in this group (all \( P > .06 \)).

Discussion

We employed sMRI to study prefrontal and striatal ROI volumes in MZ twins with and without schizophrenia. We detected a heterogeneous pattern of prefrontal volume reduction in twins with schizophrenia. While two regions (MFC and OFC) showed significant volumetric reductions in twins with schizophrenia only if they were concordant but not discordant for the disorder, relative to controls, other areas showed no statistically significant differences (after Bonferroni correction) across groups (IFC) or were reduced in schizophrenia patients regardless of their concordance status (SFC). The striatum did not show any significant differences across groups. In the putamen, there was evidence of treatment effects, suggesting there may be volumetric increases with typical relative to atypical neuroleptic treatment.

As outlined above, the study allowed us to test the influence of genetic effects on brain volume deficits in schizophrenia in two ways; first, by comparing the genetically identical but unaffected co-twins of schizophrenia patients to the proband group of that twin with schizophrenia. The discordant twin group provides an additional two-way analysis, comparing the unaffected co-twin to the schizophrenia twin of the proband. This provides for the greatest level of variance in the genetic makeup of the group, allowing a more thorough understanding of the influence of genetic factors on brain volume deficits in schizophrenia.

Table 2. Clinical Variables by Proband Group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Concordant (( n = 42 ))</th>
<th>Discordant Schizophrenic (( n = 17 ))</th>
<th>Group Comparison Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAPS</td>
<td>6.51 (4.35)</td>
<td>7.14 (4.31)</td>
<td>( F_{1,34} = 0.22, P = .65 )</td>
</tr>
<tr>
<td>SANS</td>
<td>10.33 (4.42)</td>
<td>9.29 (6.07)</td>
<td>( F_{1,34} = 0.34, P = .57 )</td>
</tr>
<tr>
<td>Age at first contact (y)</td>
<td>21.40 (4.80)</td>
<td>21.40 (5.50)</td>
<td>( F_{1,35} = 0.00, P = 1.00 )</td>
</tr>
<tr>
<td>CPZ equivalents (mg)</td>
<td>613.14 (449.99)</td>
<td>478.57 (346.25)</td>
<td>( F_{1,33} = 1.11, P = .30 )</td>
</tr>
<tr>
<td>Type of medication (N atypical/typical)</td>
<td>17/16 (9 n/a)</td>
<td>8/4 (5 n/a)</td>
<td>( \chi^2 = 0.66, df = 1, P = .42 )</td>
</tr>
</tbody>
</table>

Note: Data reflect mean (standard deviation) unless otherwise stated. SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms; CPZ, chlorpromazine; n/a, not available. There are no significant differences between groups in any variables reported in this table.

Table 3. Prefrontal and Striatal ROI Volumes by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Concordant (( n = 42 ))</th>
<th>Discordant Schizophrenic (( n = 17 ))</th>
<th>Discordant Nonschizophrenic (( n = 18 ))</th>
<th>Control (( n = 52 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFC</td>
<td>51.05 (7.50)</td>
<td>52.39 (8.43)</td>
<td>58.12 (7.56)</td>
<td>60.98 (7.87)</td>
</tr>
<tr>
<td>MFC</td>
<td>24.72 (4.55)</td>
<td>27.12 (6.50)</td>
<td>29.98 (4.36)</td>
<td>30.11 (6.07)</td>
</tr>
<tr>
<td>IFC</td>
<td>18.62 (3.92)</td>
<td>18.90 (3.50)</td>
<td>20.58 (4.17)</td>
<td>20.75 (3.70)</td>
</tr>
<tr>
<td>OFC</td>
<td>34.07 (4.56)</td>
<td>36.44 (7.11)</td>
<td>37.84 (5.09)</td>
<td>40.06 (4.50)</td>
</tr>
<tr>
<td>Caudate</td>
<td>7.45 (1.01)</td>
<td>8.08 (1.07)</td>
<td>8.06 (1.05)</td>
<td>8.42 (1.09)</td>
</tr>
<tr>
<td>Putamen</td>
<td>8.58 (1.48)</td>
<td>8.22 (1.60)</td>
<td>7.64 (1.54)</td>
<td>8.43 (1.00)</td>
</tr>
</tbody>
</table>

Note: Data reflect mean (standard deviation) of unadjusted ROI volumes in cm\(^3\) by group. SFC, superior frontal cortex; MFC, middle frontal cortex; IFC, inferior frontal cortex; OFC, orbitofrontal cortex.
Patients with healthy controls, and second, by examining the differential profile of concordant and discordant schizophrenic twins in relation to healthy control twins. Nonschizophrenic co-twins from MZ discordant pairs are expected to share 100% of the DNA sequence variation with their schizophrenic co-twins and thus to show endophenotypic features of their genetic liability for schizophrenia. This design complements the traditional family study design, where relatives who share 50% of DNA sequence variation with patients are compared with healthy controls. Previously we have shown that MZ co-twins show impaired antisaccade performance compared with MZ control twins.\(^{70}\) The observation of a more frequent behavioral or neurobiological feature in relatives than controls constitutes an important criterion in the validation of an endophenotype or intermediate feature of a more frequent behavioral or neurobiological feature of the illness rather than merely genetic vulnerability to the disorder. Our findings support this interpretation, suggesting a regionally specific illness but not a genetic effect. Functional MRI evidence is also compatible with this interpretation. For example, a study of saccadic endophenotypes provided evidence of genetic mechanisms underlying functional deficits in the middle frontal gyrus (within our MFC area), whereas activation deficits in the supplementary eye field (within our SFC area) were associated with disease status.\(^{74}\)

The striatum did not show any group differences that survived Bonferroni correction. Previous striatal MRI studies of the putamen and caudate volumes in schizophrenia have provided mixed evidence.\(^{14,18–21}\) The most consistent pattern of findings emerging from these studies is that striatal volumes increase with antipsychotic medication, particularly first-generation antipsychotics (for review, see\(^{25}\)). The majority of patients in our study were treated with antipsychotics, perhaps explaining the lack of differences with controls, and there was evidence of treatment effects in the putamen.

### Table 4. Intraclass Correlations (ICC) by Twin Category

<table>
<thead>
<tr>
<th>Twin Category</th>
<th>Concordant</th>
<th>Discordant</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFC</td>
<td>0.46 (0.12–0.80)</td>
<td>0.49 (0.12–0.87)</td>
<td>0.47 (0.17–0.77)</td>
</tr>
<tr>
<td>MFC</td>
<td>0.49 (0.16–0.82)</td>
<td>0.43 (0.02–0.83)</td>
<td>0.62 (0.39–0.86)</td>
</tr>
<tr>
<td>IFC</td>
<td>0.30 (0.00–0.69)</td>
<td>0.57 (0.24–0.90)</td>
<td>0.61 (0.36–0.85)</td>
</tr>
<tr>
<td>OFC</td>
<td>0.73 (0.54–0.93)</td>
<td>0.69 (0.43–0.94)</td>
<td>0.65 (0.43–0.88)</td>
</tr>
<tr>
<td>Caudate</td>
<td>0.63 (0.36–0.89)</td>
<td>0.73 (0.50–0.96)</td>
<td>0.87 (0.77–0.96)</td>
</tr>
<tr>
<td>Putamen</td>
<td>0.69 (0.42–0.92)</td>
<td>0.45 (0.06–0.84)</td>
<td>0.65 (0.42–0.87)</td>
</tr>
<tr>
<td>SAPS</td>
<td>0.22 (0.00–0.65)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SANS</td>
<td>0.33 (0.00–0.73)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Note:** The table shows the ICC coefficients (and 95% confidence interval) by twin category. Abbreviations are explained in the first footnote to table 2.
The present study did not include dizygotic (DZ) twins, thus not allowing a precise calculation of brain structural heritability measures. Instead, we calculated ICC in the MZ pairs to provide an estimate of within-pair similarity, which can be assumed to be largely genetic in origin. The frontal and striatal structures’ within-pair ICCs were comparable with previously reported findings for other regions of interest, though lower than whole-brain volumes and global frontal volumes reported in other work. These higher heritabilities in larger structures could reflect methodological considerations such as measurement error that is less prominent in larger structures compared with smaller, anatomically more specific regions.

Several limitations of the current study should be noted. First, the fundamental assumption that differences between patients with schizophrenia from MZ concordant and discordant pairs is attributable to greater genetic risk in the concordant pairs and greater unique environmental risk in the discordant group is contentious. This assumption is supported by work showing that obstetric complications, an environmental risk factor for schizophrenia, are more common in discordant than concordant twin pairs. Also, hippocampal volume, a sensitive index of hypoxic environmental insult, is smaller in discordant than concordant twins, supporting the greater involvement of these environmental factors in discordant pairs.

Second, the majority of patients in the study were pharmacologically treated at the time of assessment and had been ill for a number of years. This is a common feature in studies of patients with schizophrenia, giving rise to possible effects on the dependent variables of antipsychotic treatment and disease chronicity. However, the two patient groups in this study (concordant and discordant affected twins) did not significantly differ from each other in terms of any clinical variable. Third, two MRI scanners were used in this study, introducing possible confounds. However, both scanners were identical and used identical MRI image acquisition protocols with comparable participant numbers from each twin category scanned at each site. Fourth, an important assumption underlying our study is that monozygotic twins are genetically identical. However, this assumption is undermined by evidence that postzygotic epigenetic factors, such as DNA methylation, may underlie complex disease phenotypes instead of or in addition to DNA sequence variation.

Fifth, the study included only MZ twins. While the comparison of concordant and discordant pairs is clearly of interest, the inclusion of DZ twin pairs would have further strengthened the study by allowing formal calculation of heritability in ROI volumes and assessing the magnitude of the genetic correlation between frontal lobe volumes and schizophrenia.

Finally, the anatomic selectivity of the reported group differences should be interpreted with caution. Given the relatively small group sizes and the conservative correction for multiple testing, some group differences were not statistically significant despite medium effect sizes. Such differences should not be discarded as trivial and may reflect important anatomic changes at a more subtle level that simply failed to achieve statistical significance in this study.

References


