Familial and environmental influences on brain volumes in twins with schizophrenia

Marco M. Picchioni, MRCP, FRCPsych, PhD; Fruhling Rijsdijk, PhD;
Timothea Toulopoulou, PhD; Christopher Chaddock, PhD; James H. Cole, PhD; Ulrich Ettinger, PhD;
Ana Oses, MD; Hugo Metcalfe, MSc; Robin M. Murray, MD; Philip McGuire, MD, PhD

Background: Reductions in whole brain and grey matter volumes are robust features of schizophrenia, yet their etiological influences are unclear. **Methods:** We investigated the association between the genetic and environmental risk for schizophrenia and brain volumes. Whole brain, grey matter and white matter volumes were established from structural MRIs from twins varying in their zygosity and concordance for schizophrenia. Hippocampal volumes were measured manually. We conducted between-group testing and full genetic modelling. **Results:** We included 168 twins in our study. Whole brain, grey matter, white matter and right hippocampal volumes were smaller in twins with schizophrenia. Twin correlations were larger for whole brain, grey matter and white matter volumes in monozygotic than dizygotic twins and were significantly heritable, whereas hippocampal volume was the most environmentally sensitive. There was a significant phenotypic correlation between schizophrenia and reductions in all the brain volumes except for that of the left hippocampus. For whole brain, grey matter and the right hippocampus the etiological links with schizophrenia were principally associated with the shared familial environment. Lower birth weight and perinatal hypoxia were both associated with lower whole brain volume and with lower white matter and grey matter volumes, respectively. **Limitations:** Scan data were collected across 2 sites, and some groups were modest in size. **Conclusion:** Whole brain, grey matter and right hippocampal volume reductions are linked to schizophrenia through correlated familial risk (i.e., the shared familial environment). The degree of influence of etiological factors varies between brain structures, leading to the possibility of a neuroanatomically specific etiological imprint.

Introduction

Schizophrenia has been linked with reductions in cerebral and grey matter volumes,[1] and there has been variable evidence of white matter changes.[2,3] Given the etiological complexity of the disorder,[4] the extent to which these structural abnormalities are driven by genetic and environmental liability is poorly understood. Similar deficits in unaffected relatives suggests familial, and by extension, genetic effects.[5] However, 2 large studies found no significant grey matter reductions in unaffected relatives, concluding that structural brain changes in individuals with schizophrenia were unlikely to be due to genetic factors.[6,7]

Family cohorts cannot discriminate between genetic and shared environmental effects. Twin studies are a method to circumnavigate this challenge, though the findings of twin studies in schizophrenia have been inconsistent. The majority adopted region of interest methods and reported that monozygotic (MZ) discordant twins with schizophrenia have larger lateral ventricles;[8] smaller grey matter, hippocampal and hypothalamic volumes;[9–12] and smaller thalamic and frontal volumes[13,14] than their nonpsychotic co-twins, implicating unique environmental effects.

The evidence for familial or genetically determined deficits in patients' unaffected co-twins is mixed. Three studies mapped regional grey matter volume differences between the members of MZ discordant pairs[15–17] and reported a variety of familial, genetic and unique environmental deficits, principally in frontal and temporal lobes. A combined European multicentre twin sample suggested that the strongest genetic effect linked to schizophrenia was white matter volume loss.[18]

Regionally, the hippocampus has perhaps the most robust pathophysiological evidence in patients with schizophrenia. Familial risk may determine hippocampal volume loss,[19] which may be both heritable and linked to the specific genetic risk for schizophrenia.[7,20] However, Stefanis and colleagues[21] found no evidence of familial effects once patients with a history of obstetric complications were removed from their analysis,[21] whereas Van Erp and colleagues[22] suggested a gene × environment interaction involving perinatal hypoxic insult to the hippocampus.

In the present study, we first compared whole brain, grey matter and white matter volumes and then focused a complementary region of interest analysis on the hippocampus to explore the regional specificity of volume deficits in that structure. We tested for between-group differences between all twins with schizophrenia, MZ unaffected and dizygotic (DZ) unaffected co-twins, and all healthy control twins, then conducted full genetic modelling. On the basis of previous studies, we hypothesized that twins with schizophrenia would show smaller brain volumes than control twins; that the healthy co-twins from MZ and DZ discordant pairs would occupy an intermediate position, reflecting their shared familial risk, but spared unique environmental effects; that genetic modelling would show volumetric differences linked to the familial and specifically the genetic and common environmental risk for schizophrenia; and that unique environmental risk would further influence brain volume in patients with schizophrenia.

Methods

Participants

Probands were referred from across the United Kingdom by their treating psychiatrists. Control twins were recruited from a volunteer twin register and through national media. Exclusion criteria were a history of neurologic illness or clinically important head injury and any current substance misuse or dependence. We used the MRIs of MZ and DZ twins in this study. In some pairs, the co-twin was not able to complete the MRI examination, or the MRI data were excluded owing to artifact; to optimise the data set, we still included the data from the "surviving" participant in our analysis. We divided the remaining data for subsequent analyses into the following subgroups: patients with schizophrenia, MZ unaffected and DZ unaffected co-twins from discordant pairs, and healthy control twins from MZ and DZ control pairs. Data from subsets of this cohort have been published previously.[18,23] We obtained local and South East (UK) multicentre ethics approval for our study, and all participants gave their written informed consent.

Clinical assessment

Clinical diagnoses and psychotic symptoms were established by 1 of 2 board level–trained psychiatrists using the Schedule for Affective Disorders and Schizophrenia—Lifetime Version augmented with further clinical information to make DSM-IV diagnoses, the Scales for the Assessment of Positive (SAPS) and Negative (SANS) Symptoms, and the Annett Scale to assess handedness. Premorbid schizotypal traits and social development were assessed using the Premorbid Social Scale and Pre-morbid Assessment of Schizoid and Schizotypal Traits. We determined socioeconomic status at birth using a national standardized scale, and we collected information on obstetric complications from participants' parents when available using the Lewis Murray Scale. Patients' current antipsychotic medication use was converted to chlorpromazine (CPZ) equivalents, and we calculated antipsychotic exposure in dose-years.[24] Zygosity was confirmed using 12 highly polymorphic microsatellite markers or a twin likeness questionnaire.

In concordant pairs, both members met DSM-IV criteria for schizophrenia or schizoaffective disorder. In discordant pairs, the proband met schizophrenia or schizoaffective disorder criteria, while the unaffected co-twin was free of any psychotic disorder. The minimum time since schizophrenia onset in the probands from the discordant pairs was set at 4 years, so that it was unlikely that any of these pairs would later become concordant. Some of the discordant unaffected co-twins had a personal history of psychiatric disorders other than schizophrenia-spectrum disorders. Controls were free of any psychotic illness or any schizophrenia-spectrum disorder, but were similarly still included if they had a personal history of other Axis I nonpsychotic pathology. We chose to apply the same exclusion criteria to the controls as the unaffected co-twins with regard to other Axis I illnesses so as not to artificially inflate the differences between these 2 groups. All participants were stable at the time of assessment, with no recent changes in their medications. No unaffected co-twin or healthy control was taking any psychotropic medication.

MRI data acquisition and analysis

Participants were scanned on a 1.5 T General Electric Signa Advantage scanner. We obtained a 3-dimensional (3-D) T_1-weighted, coronal, spoiled gradient (SPGR) of the whole head (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 at 1 of 2 sites: St. George's and Maudsley hospitals, London, UK. Both members of every pair were scanned at the same site. Nine twin pairs were scanned at both sites for data comparison between sites. Images were inspected to exclude significant pathology and movement artifacts.

Image processing and tissue segmentation

Processing was performed using SPM8 software (Wellcome Trust Centre for Neuroimaging, www.fil.ion.ucl.ac.uk/spm/software/spm8/). Segmentation in SPM8 incorporates a unified approach that combines image registration, tissue classification and bias correction. We calculated total grey matter and white matter volumes using the algorithms in SPM8. The volumes were corrected for size changes induced by spatial normalization and summed to produce an estimate of the whole brain volume.

Image preprocessing and analysis

Images were linearly aligned to standard space and resliced into the anterior–posterior orientation. A trained rater blind to diagnosis or twin status outlined each hippocampus manually in MultiTracer using standard landmarks. Outlines were traced using all 3 orientations, from anterior to posterior along contiguous image slices. Volumetric measures were calculated from the centre of the first slice to the centre of the last slice. The anterior hippocampus was delineated from the discrimination of the pes hippocampi to where the crus cerebri separates from the pons. We used the temporal horn cerebrospinal fluid (CSF) and the white matter of the alveus, as viewed in 3D, to establish the separation of the hippocampus from the amygdala anteriorly. The posterior hippocampus was continued until the crus of the fornix. The alveus and fimbria were excluded from hippocampal tracings whenever possible. Inter-rater reliability was established and rechecked based on accepted methods, with intraclass correlation coefficients of 0.869 before and 0.873 after all the ratings were completed. Outlines were traced in the coronal orientation, from anterior to posterior along contiguous image slices and included all hippocampal grey matter, including the dentate gyrus and subiculum. We calculated volumetric measures by summing the segmented areas on each slice and assuming the surface structure between each slice varied linearly based of the square root of the area on that slice.

Statistical analysis

Two-site MRI data

To determine and address the presence of systematic differences in the MRI volumetric data between the 2 scanner sites, we estimated the intersite bias for each brain volume. The method identifies 1 site, in this case the Maudsley Hospital, as the reference site and estimates intersite bias, and from that an intersite correction factor, as the mean difference between the 2 sites for each volume for each of the 18 participants who were scanned at both sites. The intersite correction factor was then applied to data from the second site.

Between-group analyses

For the between-group analyses the participants were allocated into 4 groups. The first group comprised all participants with schizophrenia. The second group comprised the unaffected co-twins from MZ discordant pairs, genetically identical to their co-twins with schizophrenia. The third group comprised the unaffected co-twins from DZ discordant pairs, who share approximately 50% of their genes with their co-twins with schizophrenia. Finally, the fourth group comprised all healthy control twins at low risk for schizophrenia.

The genetic relatedness of twin pairs violates the assumption of independence in analysis of variance. Therefore, we analyzed differences between groups using regression models that allowed for correlations within twin clusters and departures from normality using the robust sandwich estimator in Stata software version 12 (Stata Corporation). This method

provides robust standard errors (SE), confidence intervals (CIs) and *p* values that give accurate assessments of the sample-to-sample variability of the parameter estimates even when the model is mis-specified, including when observations are nonindependent. Using robust regression the parameter estimate may be biased, but the 95% CIs remain accurate, giving 95% confidence that the true parameter estimate lies within its range.[25]

We examined group differences in demographic and clinical variables using linear or logistic regression with robust SEs according to the nature of the data.

If an overall comparison for a given volume was statistically significant, it was followed by 3 planned pairwise post hoc comparisons strictly determined by the experimental hypotheses; these were all patients versus controls, MZ unaffected twins versus controls and DZ unaffected twins versus controls. All contrasts were adjusted for age and sex, and for our analysis of the hippocampus, contrasts were also adjusted for whole brain volume. We investigated etiological correlates of the brain volumes using regression models and the same covariates.

Genetic model fitting

Structural equation modelling specified a model by which the variance of the brain volume phenotypes and the covariance between those traits and the genetic liability for schizophrenia was partitioned between genetic and environmental causes. To study the contribution of genes and environment to covariance between the brain volumes and the liability for schizophrenia, we looked at the cross-trait correlation between these phenotypes. For example, if the cross-trait correlation is greater for MZ than for DZ twins, this implies that additive genetic factors contributed to the phenotypic correlation between the 2 traits, or put another way, that the same genetic factors that increase the susceptibility to schizophrenia cause the brain volume reduction.

Bivariate analysis explored any genetic and environmental association between the brain volumes and the genetic liability for schizophrenia. First, a correlation model estimated the familial correlations across phenotypes and schizophrenia. The correlations for schizophrenia ($r = 0.92$ MZ, $r = 0.515$ DZ/sibling) were fixed according to heritability point estimates in population samples to correct for ascertainment ($h^2 = 0.81$, $\chi^2 = 0.19$, $e^2 = 0.08$).[26]

Genetic analysis

Bivariate models between each of the brain volume phenotypes and schizophrenia separated the variance of each trait into additive genetic, common environmental and unique environmental factors (ACE), represented by h^2, χ^2 and e^2. The correlation between each phenotype and schizophrenia liability was partitioned into the different sources of covariation: genetic (rg), common environmental (rc) and unique environmental (re) correlations. As the rg, rc and re correlations do not take into account the traits' heritabilities, it is possible for a large genetic correlation to explain very little of the observed covariation between the traits. Therefore, the model

also combines information from the rg, rc and re with the heritability of each trait to calculate the part of the phenotypic correlation (rph), due to genetic (rph-a), common environmental (rph-c) and unique environmental (rph-e) effects.

Model estimation and evaluation

Before model fitting, we partialled out the effects of age and sex. To fit genetic models we performed structural equation modelling with maximum likelihood estimation of parameters using Open Mx. We compared the fit of the genetic models with that of the correlational models by subtracting the difference in –2 log-likelihood, obtaining a –2 statistic distributed with degrees of freedom equal to the difference in degrees of freedom of the 2 models.

Results

Participants

We obtained MRI data from 44 MZ concordant twins (19 complete and 6 half pairs), 18 MZ discordant twins with schizophrenia and 16 MZ discordant unaffected twins (18 MZ discordant pairs), 8 DZ discordant twins with schizophrenia and 6 DZ discordant unaffected twins (8 DZ discordant pairs), 53 MZ control twins (26 complete and 1 half MZ control pairs), and 23 DZ healthy control twins (11 complete and 1 half DZ control pairs). This yielded a final sample size of 168 participants, which was then divided for subsequent analyses into the following groups: 70 patients with schizophrenia, 16 MZ unaffected and 6 DZ unaffected co-twins from discordant pairs, and 76 healthy control twins from MZ and DZ control pairs.

Eighty-two participants were successfully scanned at St. George's Hospital and 86 at the Maudsley Hospital. There was no significant difference in numbers of patients with

schizophrenia scanned between the 2 sites (χ^2_1 = 3.34, p = 0.07), though more DZ twins were scanned at the Maudsley Hospital (χ^2_1 = 8.85, p = 0.003).

The demographic and clinical variables are summarized in Table 1. Ten participants from the discordant unaffected groups and 6 controls met lifetime criteria for other Axis I psychiatric diagnoses, such as depression. None of these non-schizophrenia participants was unwell at the time of the scanning, and none was taking any psychotropic medication.

The groups did not differ significantly in birth weight, the presence of any obstetric complication or perinatal hypoxia. The patients had been unwell for an average of 12 ± 9.2 years, and as anticipated they had more psychotic symptoms. Twenty-one patients were prescribed a first- and 39 a second-generation antipsychotic, with a mean daily prescribed dose in CPZ equivalents of just over 370 ± 260 mg and a mean of 25.7 dose-years.

Brain volumes

The adjusted mean brain volumes of participants are shown in Table 2.

Group differences in brain volumes

Patients had smaller whole brain (t = –3.86, p < 0.001), grey matter (t = –4.17, p < 0.001) and white matter (t = –2.45, p = 0.016) volumes than healthy controls. Unaffected twins from both MZ and DZ discordant pairs did not differ from healthy controls on any of the 3 global volumes, though there was a trend for healthy co-twins from MZ discordant pairs to have lower white matter volume (t = –1.78, p = 0.08). Patients had lower right hippocampal volumes than healthy controls (t = –2.06, p = 0.040), as did the unaffected twins from DZ discordant pairs bilaterally (t = –3.80, p < 0.001 and t = –3.51, p = 0.001, respectively).

Table 1: Demographic and clinical characteristics of study participants

Characteristic	All schizophrenia (n = 70)	MZ Dc unaffected (n = 16)	DZ Dc unaffected (n = 6)	All controls (n = 76)	Statistic	p value
Age, yr	36.1 ± 9.8	31.9 ± 11.5	36.5 ± 14.9	40.6 ± 12.4	$F_{3,90}$ = 1.92	0.13
Sex, M:F	21:47	8:8	4:4	34:42	χ^2_3 = 5.02	0.17
Race, white:other	62:6	15:1	8:0	76:0	χ^2_1 = 0.14	0.71
Education, yr	13.2 ± 2.9	13.5 ± 3.1	14.9 ± 2.5	14.2 ± 2.5	$F_{3,90}$ = 1.72	0.17
Social class at birth	2.4 ± 0.90	2.5 ± 0.97	1.8 ± 0.71	2.6 ± 1.01	$F_{3,88}$ = 3.45	0.019
Handedness, right:left/ ambidextrous	53:12	14:2	6:2	64:8	χ^2_3 = 1.20	0.75
Birth weight, g	2343 ± 811	2680 ± 773	2449 ± 968	2613 ± 619	$F_{3,60}$ = 0.86	0.47
Signs of hypoxia at birth, yes:no	10:41	3:6	0:5	4:41	χ^2_2 = 3.83	0.15
CSA	2.5 ± 0.7	2.1 ± 0.5	1.7 ± 0.6	1.6 ± 0.4	$F_{3,37}$ = 8.86	< 0.001
ASA	2.8 ± 0.7	2.4 ± 0.5	1.9 ± 0.8	1.6 ± 0.1	$F_{3,37}$ = 14.4	< 0.001
PSST	1.6 ± 0.5	1.3 ± 0.3	1.2 ± 0.2	1.1 ± 0.1	$F_{3,43}$ = 10.1	< 0.001
SAPS	6.4 ± 4.5	1.3 ± 1.8	2.5 ± 4.2	0.01 ± 0.1	$F_{3,82}$ = 43.0	< 0.001
SANS	9.4 ± 5.3	2.5 ± 3.9	1.5 ± 2.8	0 ± 0	$F_{3,82}$ = 57.4	< 0.001

ASA = adolescent social adjustment; CSA = childhood social adjustment; Dc = discordant; DZ = dizygotic; F = female; M = male; MZ = monozygotic; PSST = premorbid schizoid schizotypal traits; SANS = Scale for the Assessment of Negative Symptoms; SAPS = Scale for the Assessment of Positive Symptoms; SD = standard deviation.

Brain volumes and environmental factors

There was no significant association between antipsychotic exposure (in dose-years) and any of the brain volumes (all $p > 0.49$) in patients with schizophrenia. Across the whole sample, lower birth weight was associated with lower whole brain ($p = 0.047$) and white matter ($p = 0.009$) volumes. Perinatal hypoxia was associated with both smaller whole brain ($p = 0.038$) and grey matter ($p = 0.019$) volumes, but the association with right and left hippocampal volume was not significant ($p = 0.42$ and $p = 0.59$, respectively).

Model fitting analysis

All brain volumes except for the left hippocampus were negatively correlated with schizophrenia (Table 3). For the 3 global volumes the correlations were greater in MZ than DZ pairs, suggesting a genetic ACE model. When tested, genetic factors accounted for a significant part of the total variance in whole brain, white matter and, to a lesser extent, grey matter volumes (Table 4). The heritability of the hippocampal volumes was low and nonsignificant, where com-

mon and unique environmental effects accounted for significant proportions of interindividual differences. The lower limit estimates of the unique environmental effects (e^2) for the hippocampus were larger than the upper limit for each of the 3 global volumes, suggesting a significantly greater impact of unique environmental effects on the hippocampus.

Significant phenotypic correlations existed between schizophrenia liability and reduced whole brain, grey matter, white matter and right hippocampal volumes (Table 5). However, the genetically determined component to these phenotypic correlations (rph-a) were all nonsignificant. Whole brain, grey matter and the hippocampus bilaterally all had high, but nonsignificant, estimates of the phenotypic correlation attributable to correlated shared environment effects (this was up to 95% for the right hippocampus).

Finally, to test for familial effects, the genetic and common environment covariance were dropped simultaneously from the model (whole brain volume: $\Delta\chi^2_2 = 8.70$, $p = 0.013$; grey matter: $\Delta\chi^2_2 = 8.06$, $p = 0.018$; white matter: $\Delta\chi^2_2 = 4.26$, $p = 0.12$; right hippocampus: $\Delta\chi^2_2 = 9.99$, $p = 0.007$; left hippocampus: $\Delta\chi^2_2 = 5.21$, $p = 0.07$). This suggests that there is a significant

Table 2: Summary of adjusted brain volumes and planned between-group comparisons

	Group; brain volume, mean ± SD, mm³				Comparison; pairwise test effect size (95% CI)		
Region	All schizophrenia (n = 70)	MZ Dc unaffected (n = 16)	DZ Dc unaffected (n = 6)	All controls (n = 76)	All schizophrenia v. all controls	MZ Dc unaffected v. all controls	DZ Dc unaffected v. all controls
Whole brain	1134.2 ± 129.6	1144.4 ± 188.6	1145.5 ± 152.8	1181.1 ± 109.6	−56.9 (−104.0 to −9.7) $t = -3.86$ $p < 0.001$	−54.3 (−130.3 to 21.6) $t = -1.42$ $p = 0.16$	35.7 (−48.9 to 120.4) $t = 0.84$ $p = 0.40$
Grey matter	675.3 ± 76.8	688.7 ± 106.2	690.6 ± 74.3	706.2 ± 69.2	−45.9 (−67.7 to −24.0) $t = -4.17$ $p < 0.001$	−19.5 (−56.5 to 17.5) $t = -1.05$ $p = 0.30$	−0.05 (−36.3 to 36.2) $t = 0.00$ $p = 0.99$
White matter	465.0 ± 65.9	448.5 ± 80.0	514.2 ± 91.3	478.7 ± 55.5	−27.2 (−49.2 to −5.2) $t = -2.45$ $p = 0.016$	−31.6 (−66.9 to 3.7) $t = -1.78$ $p = 0.08$	36.6 (−18.8 to 92.1) $t = 1.31$ $p = 0.19$
Right hippocampus	1.85 ± 0.39	1.92 ± 0.26	1.76 ± 0.28	2.02 ± 0.27	−138.7 (−272.3 to −5.0) $t = -2.06$ $p = 0.040$	−92.3 (−231.8 to 47.2) $t = -1.32$ $p = 0.19$	−301.7 (−459.6 to −143.9) $t = -3.80$ $p < 0.001$
Left hippocampus	1.94 ± 0.42	1.94 ± 0.32	1.74 ± 0.31	2.02 ± 0.27	−28.8 (−168.3 to 110.7) $t = 0.41$ $p = 0.68$	−47.0 (−197.1 to 103.1) $t = -0.62$ $p = 0.54$	−348.3 (−545.4 to −151.2) $t = -3.51$ $p = 0.001$

CI = confidence interval; Dc = discordant; DZ = dizygotic; MZ = monozygotic; SD = standard deviation.

Table 3: Cross-twin/sibling within trait and cross-twin/sibling cross-trait brain volume correlations; r (95% CI)

	Within-twin correlations with schizophrenia	Within-twin pair correlations		Cross-twin pair correlation (twin 1) with schizophrenia (twin 2)*	
Region		MZ	DZ	MZ	DZ
Whole brain	−0.26 (−0.39 to −0.11)	0.89 (0.84 to 0.93)	0.65 (0.08 to 0.83)	−0.22 (−0.35 to −0.07)	−0.23 (−0.43 to 0.03)
Grey matter	−0.25 (−0.39 to −0.11)	0.87 (0.80 to 0.91)	0.68 (0.26 to 0.84)	−0.21 (−0.35 to −0.07)	−0.22 (−0.43 to 0.03)
White matter	−0.18 (−0.32 to −0.04)	0.85 (0.77 to 0.91)	0.45 (−0.38 to 0.74)	−0.15 (−0.29 to −0.01)	−0.13 (−0.38 to 0.22)
Right hippocampus	−0.23 (−0.36 to −0.09)	0.64 (0.48 to 0.76)	0.54 (0.02 to 0.77)	−0.22 (−0.36 to −0.09)	−0.34 (−0.62 to −0.07)
Left hippocampus	−0.13 (−0.26 to 0.01)	0.56 (0.37 to 0.70)	0.64 (0.21 to 0.81)	−0.13 (−0.27 to 0.01)	−0.27 (−0.51 to −0.04)

CI = confidence interval; DZ = dizygotic; MZ = monozygotic.
*For schizophrenia the cross-twin correlation (SZtw1–SZtw2) is constrained to be 0.92 in MZ twins and 0.515 in DZ twins based on the genetic point estimates ($h^2 = 0.81$, $\chi^2 = 0.11$, $e^2 = 0.08$) of meta-analysis results, and the thresholds on the liabilities are fixed to reflect a prevalence of 1%.

familial (combined genetic and shared environmental) overlap between increasing schizophrenia liability and reduced whole brain, grey matter and right hippocampal volumes.

Discussion

We aimed to identify the influence of genetic and environmental factors on deficits in brain volume and relate that to the risk for schizophrenia.

There was evidence of significant deficits in whole brain, grey matter, white matter and hippocampal volume in patients with schizophrenia. While these were largely not detected in the discordant unaffected co-twins using standard between-group tests, genetic modelling detected a phenotypic correlation between schizophrenia liability and reductions in 4 of 5 brain volumes tested. These reductions were principally associated with familial risk factors shared with schizophrenia. Reduced hippocampal volume was linked to unique environmental effects, but these were not clearly shared with schizophrenia.

Brain volume in patients with schizophrenia

Individual structural imaging studies, including a meta-analysis[27] and a systematic review,[28] present robust evidence of volumetric deficits in patients with schizophrenia, principally in frontal and temporal lobe grey matter.[3] White matter findings from both volumetric and diffusion tensor imaging standpoints are more mixed.[29]

Familial/genetic risk and the effects of shared familial environment

The association between the volumetric deficits found in patients with schizophrenia and the familial risk, more specifically the genetic risk, for the disorder has remained contentious. We did not find evidence of significant volume deficits in nonpsychotic co-twins compared with healthy controls on the basis of traditional between-group tests for whole brain or grey matter volumes, and we found only a trend for white matter, appearing to implicate unique environmental effects in the patients' volume deficits.

Many studies have attempted to explore the genetic and environmental origins of grey matter volume loss in patients with schizophrenia; the results are complicated. An independent discordant twin study[11] reported reduced whole brain and grey matter volume in the twins with schizophrenia, but not in their nonpsychotic co-twins, compared with healthy controls. A subsequent voxel-based analysis[17] detected grey matter density reductions in both the patients and the unaffected co-twins, with evidence of genetically determined progressive grey matter volume.[30] In another independent and nationally representative twin cohort study, Cannon and colleagues[16] found unique environmental grey matter defits in the dorsolateral and lateral temporal cortex, but also genetically mediated loss in the polar and dorsolateral prefronal cortex, suggesting anatomic specificity to these etiological effects. Using voxel-based morphology we were

Table 4: Additive genetic, common and specific environmental estimates (with 95% CI) of full ACE genetic model*

Region	Estimate (95% CI)†		
	h^2	χ^2	e^2
Whole brain	0.50 (0.14–0.92)	0.40 (0.00–0.75)	0.10 (0.08–0.15)
Grey matter	0.39 (0.06–0.90)	0.48 (0.00–0.80)	0.13 (0.09–0.20)
White matter	0.73 (0.23–0.90)	0.12 (0.00–0.62)	0.15 (0.10–0.23)
Right hippocampus	0.28 (0.00–0.12)	0.38 (0.00–0.99)	0.34 (0.26–0.51)
Left hippocampus	0.02 (0.00–0.08)	0.56 (0.41–0.69)	0.42 (0.29–0.58)

CI = confidence interval.
*Parameters for schizophrenia are fixed based on the genetic point estimates of meta-analysis results (h^2 = 0.81, χ^2 = 0.11, e^2 = 0.08) and the thresholds on the liabilities are fixed to reflect a prevalence of 1%.
†h^2, χ^2 and e^2 are the heritability, shared and nonshared environmental influences, the standardized variance component due to A, C and E factors.

Table 5: Phenotypic correlations between schizophrenia and regional brain activity (rph), and the decomposed sources of the correlations (r_{ph-a}, r_{ph-c}, r_{ph-e}) predicted by the ACE model

Region	Correlation (95% CI)			
	r_{ph-a}	r_{ph-c}	r_{ph-e}	r_{ph}
Whole brain	−0.01 (−0.40 to 0.15)	−0.20 (−0.29 to 0.18)	−0.04 (−0.07 to 0.00)	−0.25 (−0.38 to −0.13)
Grey matter	0.01 (−0.40 to 0.18)	−0.22 (−0.30 to 0.20)	−0.04 (−0.07 to 0.00)	−0.25 (−0.38 to −0.12)
White matter	−0.05 (−0.29 to 0.18)	−0.10 (−0.26 to 0.19)	−0.03 (−0.06 to 0.02)	−0.18 (−0.30 to −0.04)
Right hippocampus	−0.01 (−0.27 to 0.18)	−0.20 (−0.28 to 0.02)	0.00 (−0.05 to 0.05)	−0.21 (−0.34 to −0.08)
Left hippocampus	0.12 (−0.19 to 0.25)	−0.25 (−0.28 to 0.06)	0.01 (−0.00 to 0.18)	−0.12 (−0.23 to 0.02)

CI = confidence interval; r_{ph} = total phenotypic correlation; r_{ph-a}, r_{ph-c}, r_{ph-e} = phenotypic correlation due to additive genetic, shared environmental and specific environmental influence, respectively.

unable, in a smaller subcohort, to detect any regional grey matter deficits in unaffected co-twins, with evidence only of unique environmental effects.[15] A pooled sample of 4 European twin cohorts rejected a significant phenotypic correlation between total grey matter volume and schizophrenia.[18]

Notwithstanding these findings, subtle grey matter volume reductions have been frequently described in individuals unaffected by schizophrenia but at familial risk for the disorder;[31–33] this finding has also been supported by a meta-analysis.[20]

More recently, unaffected sibling data sets have confirmed significant deficits in the patients' grey matter volume, but have either failed to detect significant deficits in their unaffected siblings, or detected only marginal effects.[6,7,34,35] These unaffected sibling studies effectively agree that grey matter volume and subvolumes were heritable but that they are at best weak intermediate phenotypes, a conclusion concordant with our comparative analysis.[36]

The data in the present study confirm a negative phenotypic correlation between the liability for schizophrenia and the volume of 4 of the 5 structures examined. All 3 global volumes were heritable, suggesting that genetic factors are responsible for determining a statistically significant proportion of the variance in their volumes. Further, while all the structures were influenced by unique environmental effects, the effects in the hippocampus were greatest. When we tried to decompose those influences into factors that were shared with the risk for schizophrenia, our sample was probably underpowered. So even for the right hippocampus, where up of to 95% of the correlation with schizophrenia was associated with shared environmental risk with the illness, the power was too low for it to be detected. When we collapsed the genetic and common environmental effects into familial phenotypic correlations, they were significant for whole brain, grey matter and right hippocampal volume. This suggests that there are common familial effects responsible for causing both schizophrenia and the volume reductions in these structures. However, we were unable to decompose these further into genetic and shared environmental effects.

The finding of white matter deficits in the patients and a trend toward white matter deficits in the unaffected co-twins from MZ discordant pairs is a replication of an earlier finding by Hulshoff Pol and colleagues.[11] It was also heritable and was phenotypically correlated with schizophrenia liability, though the familial and genetic decomposed sources were nonsignificant. Diffusion tensor imaging results in patients with schizophrenia remain inconsistent, though a meta-analysis supports localized white matter deficits,[37] a situation mirrored in the small number of studies of unaffected relatives that reported lower fractional anisotropy in the inferior frontal, posterior cingulate and internal capsule, but increased fractional anisotropy in the anterior cingulate.[38–41]

Unique environmental effects

Previous region of interest studies of discordant MZ twin pairs have detected unique environmentally determined volume deficits in frontal,[13] hippocampal[9,42] and lateral ventricular volumes[10] and in the insula, superior temporal gyrus, pos-

terior cingulate, left superior and medial frontal gyrus and right anterior cingulate gyri.[15] Cortical surface mapping has revealed environmentally driven differences in grey matter density in the dorsolateral prefrontal cortex, superior temporal gyrus and superior parietal lobule.[16]

In the present study, genetic modelling detected evidence of significant unique environmental effects in each of the brain volumes chosen. These unique environmental estimates were greater for the hippocampus bilaterally than any of the 3 global brain volumes. Medial temporal lobe and hippocampal volume deficits are widely recognized in patients with schizophrenia.[43] Evidence from high-risk cohorts has linked this structure specifically to the onset of psychosis,[44] with evidence also of a link with illness progression.[43,45,46] Sibling and twin studies have detected volume deficits in unaffected relatives,[20] which suggests an interaction between genetic and familial environment.[22,47] It may be that the hippocampus is vulnerable to a variety of environmental factors, including obstetric complications[48] and stress,[49] varying according to the hormonal milieu[50] that is then linked to schizophrenia.[51]

We confirmed our hypothesis of an association between low birth weight and hypoxia and smaller whole brain, white matter and grey matter volumes. It is accepted that perinatal obstetric complications have an effect on brain structure, function[52] and neurodevelopment.[53] The association of such complications with schizophrenia, either at an etiological or pathophysiological level, is more contentious.[54] Links with brain structure in patients with schizophrenia and their unaffected relatives[22] and these results suggest that the impact of environmental factors is influenced by the underlying genetically determined vulnerability.[55]

We did not detect any significant effects of antipsychotic medication in this study. Antipsychotic drugs can induce structural change,[56–58] though there may be some anatomic specificity to these effects. First-generation drugs seem most closely linked to increases in the basal ganglia and cingulate, while second-generation agents are linked to the thalamus and temporal cortex.[56,59] It is possible that in this relatively small sample of patients and with heterogeneous effects the study was underpowered to detect an effect.

Limitations

We collected structural MRI data at 2 centres, using the same acquisition sequence on identical scanners. Numerous approaches have been deployed to address this method in the past. Both members of each twin pair were scanned on the same day at the same site. We scanned a subsample on both scanners and were able to test for a systematic bias between the 2 centres and incorporate a correction factor to address this. We chose not to include a voxel-based analysis of these multisite data owing to that method's sensitivity to interscanner variance; however, we recognize that techniques have been developed to address this challenge.

Although the total sample was relatively large, the individual groups were of modest size. As a result, we cannot exclude the possibility that we were unable to detect some group differences owing to a lack of statistical power.

It is possible that the volume deficits in the unaffected groups, as with other studies, could be related to the presence of other psychiatric disorders as opposed to familial risk for schizophrenia. It may be that those other psychiatric disorders are themselves a clinical expression of that familial risk,[60] which would hypothetically be greater in the MZ than DZ unaffected co-twins. However, the number of these twins in our sample was too small to formally test. All were clinically well and medication-free at the time of scanning, and the groups were otherwise well matched.

Conclusion

We detected typical volumetric abnormalities in patients with schizophrenia compared with healthy controls. Genetic modelling showed that the 3 global volumes — whole brain, grey matter and white matter volume — were heritable and that there was a significant familial overlap between whole brain, grey matter and right hippocampal volume loss in patients with schizophrenia. The hippocampus was the structure most influenced by environmental factors. The degree of influence of etiological factors varied between the brain structures, leading to the possibility of a neuroanatomically specific etiological imprint underlying schizophrenia.

Affiliations: From the St. Andrew's Academic Department, Institute of Psychiatry Psychology and Neuroscience, King's College London, UK (Picchioni); the Department of Psychosis Studies, Institute of Psychiatry Psychology and Neuroscience, King's College London, UK (Picchioni, Toulopoulou, Chaddock, Murray, McGuire); the Department of Forensic and Neurodevelopmental Sciences, Institute of Psychiatry Psychology and Neuroscience, King's College London, UK (Picchioni, Metcalfe); the Social, Genetic & Developmental Psychiatry Centre, Institute of Psychiatry Psychology and Neuroscience, King's College London, UK (Rijsdijk); the Department of Psychology, The University of Hong Kong, Hong Kong (Toulopoulou); the Computational, Cognitive & Clinical Neuroimaging Laboratory, Department of Medicine, Imperial College London, UK (Cole); the Department of Psychology, University of Bonn, Bonn, Germany (Ettinger); and the Centro de Salut Mental del Ripolles, Institut d'Assistencia Sanitaria de Girona, Girona, Spain (Oses).

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References

1. Shepherd AM, Laurens KR, Matheson SL, et al. Systematic meta-review and quality assessment of the structural brain alterations in schizophrenia. *Neurosci Biobehav Rev* 2012;36:1342-56.
2. Lawrie SM, Abukmeil SS. Brain abnormality in schizophrenia — a systematic and quantitative review of volumetric magnetic resonance imaging studies. *Br J Psychiatry* 1998;172:110-20.
3. Honea R, Crow TJ, Passingham D, et al. Regional deficits in brain volume in schizophrenia: a meta-analysis of voxel-based morphometry studies. *Am J Psychiatry* 2005;162:2233-45.
4. Picchioni MM, Murray RM. Schizophrenia. *BMJ* 2007;335:91-5.
5. Whalley HC, Whyte MC, Johnstone EC, et al. Neural correlates of enhanced genetic risk for schizophrenia. *Neuroscientist* 2005;11:238-49.
6. Honea RA, Meyer-Lindenberg A, Hobbs KB, et al. Is gray matter volume an intermediate phenotype for schizophrenia? A voxel-based morphometry study of patients with schizophrenia and their healthy siblings. *Biol Psychiatry* 2008;63:465-74.
7. Goldman AL, Pezawas L, Mattay VS, et al. Heritability of brain morphology related to schizophrenia: a large-scale automated magnetic resonance imaging segmentation study. *Biol Psychiatry* 2008;63:475-83.
8. Reveley AM, Reveley MA, Murray RM. Cerebral ventricular enlargement in non-genetic schizophrenia — a controlled twin study. *Br J Psychiatry* 1984;144:89-93.
9. Suddath RL, Christison GW, Torrey EF, et al. Anatomical abnormalities in the brains of monozygotic twins discordant for schizophrenia. *N Engl J Med* 1990;322:789-94.
10. Baare WFC, van Oel CJ, Hulshoff Pol HE, et al. Volumes of brain structures in twins discordant for schizophrenia. *Arch Gen Psychiatry* 2001;58:33-40.
11. Hulshoff Pol HE, Brans RGH, van Haren NEM, et al. Gray and white matter volume abnormalities in monozygotic and same-gender dizygotic twins discordant for schizophrenia. *Biol Psychiatry* 2004;55:126-30.
12. Koolschijn P, van Haren NEM, Hulshoff Pol HE, et al. Hypothalamus volume in twin pairs discordant for schizophrenia. *Eur Neuropsychopharmacol* 2008;18:312-5.
13. Ettinger U1, Schmechtig A, Toulopoulou T, et al. Prefrontal and striatal volumes in monozygotic twins concordant and discordant for schizophrenia. *Schizophr Bull* 2010;38:192-203.
14. Ettinger U, Picchioni M, Landau S, et al. Magnetic resonance imaging of the thalamus and adhesio interthalamica in twins with schizophrenia. *Arch General Psychiatry* 2007;64:401-9.
15. Borgwardt SJ, Picchioni MM, Ettinger U, et al. Regional gray matter volume in monozygotic twins concordant and discordant for schizophrenia. *Biol Psychiatry* 2010;67:956-64.
16. Cannon TD, Thompson PM, van Erp TGM, et al. Cortex mapping reveals regionally specific patterns of genetic and disease-specific gray-matter deficits in twins discordant for schizophrenia. *Proc Natl Acad Sci U S A* 2002;99:3228-33.
17. Hulshoff Pol HE, Schnack HG, Mandl RCW, et al. Gray and white matter density changes in monozygotic and same-sex dizygotic twins discordant for schizophrenia using voxel-based morphometry. *NeuroImage* 2006;31:482-8.
18. Van Haren NEM, Rijsdijk F, Schnack HG, et al. The genetic and environmental determinants of the association between brain abnormalities and schizophrenia: the Schizophrenia Twins and Relatives Consortium. *Biol Psychiatry* 2012;7:915-21.
19. Job DE, Whalley HC, Johnstone EC, et al. Grey matter changes over time in high risk subjects developing schizophrenia. *NeuroImage* 2005;25:1023-30.
20. Boos HB, Aleman A, Cahn W, et al. Brain volumes in relatives of patients with schizophrenia. *Arch Gen Psychiatry* 2007;64:297-304.
21. Stefanis N, Frangou S, Yakeley J, et al. Hippocampal volume reduction in schizophrenia: effects of genetic risk and pregnancy and birth complications. *Biol Psychiatry* 1999;46:697-702.
22. Van Erp TGM, Saleh PA, Rosso IM, et al. Contributions of genetic risk and fetal hypoxia to hippocampal volume in patients with schizophrenia or schizoaffective disorder, their unaffected siblings, and healthy unrelated volunteers. *Am J Psychiatry* 2002;159:1514-20.
23. Van Haren NEM, Picchioni MM, McDonald C, et al. A controlled study of brain structure in monozygotic twins concordant and discordant for schizophrenia. *Biol Psychiatry* 2004;56:454-61.
24. Andreasen NC, Pressler M, Nopoulos P, et al. Antipsychotic dose equivalents and dose-years: a different method for comparing exposure to different drugs. *Biol Psychiatry* 2010;67:255-62.
25. Williams RL. A note on robust variance estimation for cluster-correlated data. *Biometrics* 2000;56:645-6.

26. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait — evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* 2003;60:1187-92.
27. Arnone D, Cavanagh J, Gerber D, et al. Magnetic resonance imaging studies in bipolar disorder and schizophrenia: meta-analysis. *Br J Psychiatry* 2009;195:194-201.
28. Shepherd AM, Laurens KR, Matheson SL, et al. Systematic meta-review and quality assessment of the structural brain alterations in schizophrenia. *Neurosci Biobehav Rev* 2012;36:1342-56.
29. Kubicki M, McCarley R, Westin CF, et al. A review of diffusion tensor imaging studies in schizophrenia. *J Psychiatr Res* 2007;41:15-30.
30. Brans RGH, van Haren NEM, van Baal GCM, et al. Heritability of changes in brain volume over time in twin pairs discordant for schizophrenia. *Arch Gen Psychiatry* 2008;65:1259-68.
31. Lawrie SM, McIntosh AM, Hall J, et al. Brain structure and function changes during the development of schizophrenia: the evidence from studies of subjects at increased genetic risk. *Schizophrenia Bulletin* 2008;34:330-40.
32. Rosso IM, Makris N, Thermenos HW, et al. Regional prefrontal cortex gray matter volumes in youth at familial risk for schizophrenia from the Harvard Adolescent High Risk Study. *Schizophrenia Research* 2010;123:15-21.
33. Francis AN, Seidman L, Jabbar GA, et al. Alternations in brain structures underlying language function in young adults at high familial risk for schizophrenia. *Schizophr Res* 2012;141:65-71.
34. Goldman AL, Pezawas L, Doz P, et al. Widespread reductions of cortical thickness in schizophrenia and spectrum disorders and evidence of heritability. *Arch Gen Psychiatry* 2009;66:467.
35. Turner JA, Calhoun VD, Michael A, et al. Heritability of multivariate gray matter measures in schizophrenia. *Twin Res Hum Genet* 2012;15:324-35.
36. Owens SF, Picchioni MM, Ettinger U, et al. Prefrontal deviations in function but not volume are putative endophenotypes for schizophrenia. *Brain* 2012;135:2231-44.
37. Ellison-Wright I, Bullmore E. Meta-analysis of diffusion tensor imaging studies in schizophrenia. *Schizophrenia Research* 2009;108:3-10.
38. Camchong J, Lim KO, Sponheim SR, et al. Frontal white matter integrity as an endophenotype for schizophrenia: diffusion tensor imaging in monozygotic twins and patients' nonpsychotic relatives. *Front Hum Neurosci* 2009;26:35.
39. Muoz Maniega S, Lymer GKS, Bastin ME, et al. A diffusion tensor MRI study of white matter integrity in subjects at high genetic risk of schizophrenia. *Schizophr Res* 2008;106:132-9.
40. Knochel C, Oertel-Knoechel V, Schoenmeyer R, et al. Interhemispheric hypoconnectivity in schizophrenia: fiber integrity and volume differences of the corpus callosum in patients and unaffected relatives. *NeuroImage* 2012;59:926-34.
41. Phillips OR, Nuechterlein KH, Asarnow RF, et al. Mapping corticocortical structural integrity in schizophrenia and effects of genetic liability. *Biol Psychiatry* 2011;70:680-9.
42. Van Erp TGM, Saleh PA, Huttunen M, et al. Hippocampal volumes in schizophrenic twins. *Arch Gen Psychiatry* 2004;61:346-53.
43. Adriano F, Caltagirone C, Spalletta G. Hippocampal volume reduction in first-episode and chronic schizophrenia: a review and meta-analysis. *Neuroscientist* 2012;18:180-200.
44. Velakoulis D, Wood SJ, Wong MTH, et al. Hippocampal and amygdala volumes according to psychosis stage and diagnosis — a magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra-high-risk individuals. *Arch Gen Psychiatry* 2006;63:139-49.
45. Koolschijn PCMP, van Haren NEM, Cahn W, et al. Hippocampal volume change in schizophrenia. *J Clin Psychiatry* 2010;71:737-44.
46. Chakos MH, Schobel SA, Gu HB, et al. Duration of illness and treatment effects on hippocampal volume in male patients with schizophrenia. *Br J Psychiatry* 2005;186:26-31.
47. Narr KL, van Erp TGM, Cannon TD, et al. A twin study of genetic contributions to hippocampal morphology in schizophrenia. *Neurobiol Dis* 2002;11:83-95.
48. McNeil TF, Cantor-Graae E, Weinberger DR. Relationship of obstetric complications and differences in size of brain structures in monozygotic twin pairs discordant for schizophrenia. *Am J Psychiatry* 2000;157:203-12.
49. Arango C, Kirkpatrick B, Koenig J. At issue: stress, hippocampal neuronal turnover, and neuropsychiatric disorders. *Schizopr Bull* 2001;27:477-80.
50. Panizzon MS, Hauger RL, Eaves LJ, et al. Genetic influences on hippocampal volume differ as a function of testosterone level in middle-aged men. *NeuroImage* 2012;59:1123-31.
51. Chetty S, Friedman AR, Taravosh-Lahn K, et al. Stress and glucocorticoids promote oligodendrogenesis in the adult hippocampus. *Mol Psychiatry* 2014;19:1275-83.
52. Narberhaus A, Lawrence E, Allin MP, et al. Neural substrates of visual paired associates in young adults with a history of very preterm birth: alterations in fronto-parieto-occipital networks and caudate nucleus. *NeuroImage* 2009;47:1884-93.
53. Nosarti C, Mechelli A, Herrera A, et al. Structural covariance in the cortex of very preterm adolescents: a voxel-based morphometry study. *Hum Brain Mapp* 2011;32:1615-25.
54. Clarke MC, Harley M, Cannon M. The role of obstetric events in schizophrenia. *Schizophr Bull* 2006;32:3-8.
55. Haukvik UK, Saetre P, McNeil T, et al. An exploratory model for G x E interaction on hippocampal volume in schizophrenia; obstetric complications and hypoxia-related genes. *Prog Neuropsychopharmacol Biol Psychiatry* 2010;34:1259-65.
56. Lieberman JA, Tollefson GD, Charles C, et al. Antipsychotic drug effects on brain morphology in first-episode psychosis. *Arch Gen Psychiatry* 2005;62:361-70.
57. Smieskova R, Fusar-Poli P, Allen P, et al. The effects of antipsychotics on the brain: What have we learnt from structural imaging of schizophrenia? A systematic review. *Curr Pharm Des* 2009;15:2535-49.
58. Ho BC, Andreasen NC, Ziebell S, et al. Long-term antipsychotic treatment and brain volumes: a longitudinal study of first-episode schizophrenia. *Arch Gen Psychiatry* 2011;68:128-37.
59. Thompson PM, Bartzokis G, Hayashi KM, et al. Time-lapse mapping of cortical changes in schizophrenia with different treatments. *Cerebral Cortex* 2009;19:1107-23.
60. Argyropoulos SV, Landau S, Kalidindi S, et al. Twins discordant for schizophrenia: psychopathology of the non-schizophrenic co-twins. *Acta Psychiatr Scand* 2008;118:214-9.