Gray Matter Volume Decrease Distinguishes Schizophrenia From Bipolar Offspring During Childhood and Adolescence

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Objective: There is increasing support toward the notion that schizophrenia and bipolar disorder share neurodevelopmental underpinnings, although areas of divergence remain. We set out to examine gray matter volume characteristics of child and adolescent offspring of patients with schizophrenia or bipolar disorder comparatively.

Method: In this 2-center study, magnetic resonance structural neuroimaging data were acquired in 198 children and adolescents (aged 6-17 years): 38 offspring of patients with schizophrenia, 77 offspring of patients with bipolar disorder, and 83 offspring of community controls. Analyses of global brain volumes and voxel-based morphometry (using familywise error correction) were conducted.

Results: There was an effect of group on total cerebral gray matter volume ($F = 3.26, p = .041$), driven by a decrease in offspring of patients with schizophrenia relative to offspring of controls ($p = .035$). At a voxel-based level, we observed an effect of group in the left inferior frontal cortex/anterior insula ($F = 14.7, p < .001$), which was driven by gray matter volume reduction in offspring of patients with schizophrenia relative to both offspring of controls ($p = .044$) and of patients with bipolar disorder ($p < .001$). No differences were observed between offspring of patients with bipolar disorder and offspring of controls in either global or voxel-based gray matter volumes.

Conclusion: This first comparative study between offspring of patients with schizophrenia and bipolar disorder suggests that gray matter volume reduction in childhood and adolescence may be specific to offspring of patients with schizophrenia; this may index a greater neurodevelopmental impact of risk for schizophrenia relative to bipolar disorder during youth.

Key Words: schizophrenia, bipolar disorder, magnetic resonance imaging

gray matter volume in the left hippocampal–parahippocampal gyrus.\textsuperscript{19} The neuroimaging data so far have been limited by small sample sizes, especially in BdO\textsuperscript{17–19}, ages centered in late, rather than early, adolescence in SzO\textsuperscript{13,15}, and an approach driven by predefined regions of interest (ROI) in the majority of the above studies. In addition, a number of samples have also included other first- and second-degree relatives,\textsuperscript{13–15} limiting extrapolation of findings. An additional shortcoming is that despite reports of increased rates of nonpsychotic mental health disorders in these populations,\textsuperscript{20} this has not been accounted for in many structural neuroimaging studies\textsuperscript{10–13,15,18} so far. Furthermore, no study to date has directly compared child and adolescent offspring of patients with schizophrenia or BD using neuroimaging data. In the present study, we set out to investigate gray matter volume abnormalities in child and adolescent SzO and BdO comparatively, using a whole-brain, voxel-based approach, and assessing the effects of lifetime mental health conditions on the imaging findings. Our aim was therefore to study gray matter volume characteristics of child and adolescent SzO and BdO comparatively, in relation to a community control offspring group (CcO). We also set out to investigate the relationship between gray matter volume and clinical and prodromal symptoms. Based on previous studies, we hypothesized that schizophrenia would display widespread cortical and subcortical volumetric reduction, whereas in BdO, gray matter volume changes would be limited to the hippocampal/parahippocampal cortex.

METHOD

Participants

The study was conducted in the Child and Adolescent Psychiatry Departments of 2 tertiary hospitals in Spain. The protocol was approved by each local ethics review board; all participants provided written informed consent/assent. The recruitment period spanned from January 2008 to December 2013. Patients with schizophrenia or BD from adult psychiatry units with children and adolescents aged 6 to 17 years were identified and offered the opportunity to participate in the study. The exclusion criteria for proband parents were intellectual disability and drug- or medically induced psychosis or mania. All offspring aged 6 to 17 years were invited to participate in the study; exclusion criteria for offspring included intellectual disability, head injury with loss of consciousness, or severe neurological conditions.

Community control parents were recruited through advertisements posted in primary health care centers and other community locations within the same geographical area as the patients. The exclusion criteria were intellectual disability, severe neurological illness, and personal or first-degree family history of schizophrenia or bipolar spectrum disorders. All offspring of community control parents (CcO) aged 6 to 17 years were invited to participate in the study; exclusion criteria were the same as for high-risk offspring.

The sample consisted of 30 families with a parent with schizophrenia, 47 with a parent with BD, and 60 community control parents. The offspring sample consisted of 46 SzO, 90 BdO, and 92 CcO, among whom valid scanning data were available for 38 SzO (83% of the sample), 77 BdO (85%), and 98 CcO (90.2%). Exclusion was due to declination to participate in the scanning or claustrophobia, dental prosthesis, scanner artifacts, 2 cases of asymptomatic hydrocephalus, and 1 congenital malformation of the temporal lobe. There were no significant clinical or sociodemographic differences between offspring who underwent scanning versus those who did not. Within the final sample, 16 SzO (42.1%), 47 BdO (61.0%), and 41 CcO (49.4%) had a sibling in the study; there were no significant differences in the rates of relatedness between groups ($p = .12$).

Clinical Assessment

Members of the research team were all clinically experienced psychiatrists or psychologists. To ensure blindness, the same team member who had initially contacted the families assessed psychopathology of all parents, both the proband and the biological coparent, using the Spanish version of the Structured Interview for DSM-IV (SCID-I).\textsuperscript{21} Socioeconomic status (SES) was estimated using the Hollingshead Scale.\textsuperscript{22} Psychopathology in the offspring was ascertained by child and adolescent psychiatrists blinded to parental status using the Spanish version of the Schedule for Affective Disorders and Schizophrenia for School-Age Children–Present and Lifetime Version (K-SADS-PL),\textsuperscript{23} which was administered separately to parents and children, and in which details on past or current psychopharmacological treatments were also registered. Drug and alcohol use were assessed with the K-SADS-PL and the Teen Addiction Severity Index for Adolescents (Teen-ASI).\textsuperscript{24} Overall clinical severity was assessed with the Clinical Global Impression index (CGI),\textsuperscript{25} and functionality was assessed with the General Assessment of Functioning scale (GAF).\textsuperscript{26} Prodromal symptoms were evaluated with the Structured Interview for Prodromal Symptoms (SIPS).\textsuperscript{27} The Spanish version of the Wechsler Intelligence Scale for Children–Fourth Edition (WISC-IV)\textsuperscript{28} was administered by psychologists blinded to parental status; the General Ability Index, derived from the verbal capacity index and perceptual reasoning index, was used as a measure of general cognitive capacity.

Statistical Analysis of Clinical and Socio-Demographic Variables

Statistical analyses were performed using SPSS v.22.0 software. Socio-demographic and clinical variables were examined across the 3 offspring groups. $\chi^2$ Statistics with the Yates correction and Fisher’s exact test were used to compare percentages of discrete variables. Univariate and multivariate analyses of variance were used to compare continuous variables. Continuous variables that violated assumptions of normality (Kolmogorov–Smirnov $p < .05$) were log transformed and subjected to parametric testing. Post hoc parametric ($\chi^2$ or 1-way analysis of variance) and nonparametric ($\chi^2$ with Fisher’s exact test) analyses were carried out when the 3-group comparison was statistically significant. Significance was set at $p < .05$ using Bonferroni correction; all $p$ values were 2-tailed.

Neuroimaging Analysis

Details of image acquisition are provided in Supplement 1, available online. Neuroimaging analyses were performed using Statistical Parametric Mapping v.8, MatlabR2013. All images were inspected for quality by a physicist. Images were reoriented according to the anterior–posterior commissure line. Customized tissue probability maps were created from the control sample and were then used for tissue segmentation for the whole sample. Normalization to Montreal Neurological Institute space followed diffeomorphic anatomical registration through exponentiated lie algebra procedure, which included modulation of images.\textsuperscript{29} Smoothing was performed with a 4-mm full-width-at-half-maximum kernel.\textsuperscript{30} Between-group comparisons proceeded as described below.

Global Brain Volumes. Total cerebral gray matter, white matter, cerebrospinal fluid, and total intracranial volume (TIV) were
calculated from the segmented tissues using a customized script in Matlab. The main effect of group membership (SzO, BdO, CcO) on global brain volumes was tested using linear mixed models, controlling for age, sex, scanner site, and total intracranial volume, including “sibship” as random effects. Significant effects of group were followed up with pairwise comparisons. The effects of lifetime history of psychiatric disorders (present and/or past Axis I diagnoses) and interactions between the effect of group and age and between the effect of group and lifetime history of Axis I diagnosis were also tested.

Voxel-Based Morphometry: Whole Brain Analysis. The effect of group membership (SzO, BdO, CcO) on gray matter voxel-based morphometry (VBM) differences was tested in SPM using analysis of covariance (ANCOVA), where age, gender, TIV, and scanner site were modeled as covariates of no interest, using customized explicit binary gray matter masks (threshold at 0.2). Significant main effect of group membership was followed up with pairwise comparisons (SzO versus CcO, BdO versus CcO, SzO versus BdO). Statistical significance was assessed at the cluster level: spatially contiguous voxels were identified at an uncorrected threshold of $p < .001$, and statistical inferences were then made at $p < .05$, familywise error corrected (FWE). Confirmatory analyses were then performed to account for the effect of sibship and to assess the effect of lifetime psychiatric disorders on voxelwise gray matter volume differences. To this end, mean gray matter volume in clusters surviving FWE correction in the between-group comparison in SPM were extracted for each participant using a customized script. Generalized linear mixed models were then performed in SPSS to test the effect of group on gray matter volume of clusters showing voxelwise differences, with “sibship” as random effects, and controlling for age, sex, TIV, and scanner site. This was also followed up with pairwise analyses. The effect of a lifetime history of psychiatric disorders, and interactions between the effect of group and age and between the effect of group and lifetime history of Axis I diagnosis, were also tested.

Voxel-Based Morphometry: Region-of-Interest Analyses. In keeping with the previous literature, a hypothesis-driven analysis was also performed. The effect of group membership on gray matter voxelwise differences was conducted in SPM using ANCOVA controlling for age, sex, TIV, and scanner site. WFU_PickAtlas-defined ROIs located in the amygdala, hippocampus, and parahippocampal gyrus were used.

Correlation With Symptom Scores. Partial correlations were performed between global and mean gray matter volume of clusters showing voxelwise differences, and prodromal symptom scores, using age, gender, TIV, and scanner site as covariates.

Examination of Potential Confounders. All significant analyses were repeated excluding participants who had any exposure to psychotropic drugs, and adding general cognitive capacity as covariate. Analyses were also repeated in subgroups based on age. To explore effects due to the different scanners and protocol, a compatibility study was carried out between the 2 scanners using data from 7 healthy controls who underwent scanning at each of the 2 sites (see Supplement 2, available online).

RESULTS

Socio-Demographic and Clinical Variables

Table 1 summarizes the clinical and sociodemographic characteristics of the participants. There were trend-level differences in age, and a nonsignificantly different gender distribution between groups. SzO had lower socioeconomic status and lower general cognitive capacity than both CcO and BdO. Both patient offspring groups had a greater prevalence of lifetime Axis I disorders than CcO. No participant met criteria for a bipolar or psychosis spectrum disorder diagnosis or for a prodromal syndrome, although SzO had higher ratings on the positive, negative, and total

**TABLE 1** Socio-Demographic and Clinical Characteristics of the Sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SzO (n=38)</th>
<th>BdO (n=77)</th>
<th>CcO (n=83)</th>
<th>$\chi^2/F$</th>
<th>$p$</th>
<th>Significant Pairwise Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (% female)</td>
<td>13 (34.2)</td>
<td>35 (45.5)</td>
<td>44 (53.0)</td>
<td>3.7</td>
<td>.15</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>11.0 (3.3)</td>
<td>12.4 (3.1)</td>
<td>11.8 (3.2)</td>
<td>2.7</td>
<td>.07</td>
<td></td>
</tr>
<tr>
<td>General cognitive capacity</td>
<td>94.5 (16.1)</td>
<td>104.9 (12.8)</td>
<td>107.3 (12.5)</td>
<td>12.0</td>
<td>&lt;.001</td>
<td>SzO&lt;CcO; SzO&lt;BdO</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>31.8 (14.7)</td>
<td>50.7 (14.3)</td>
<td>51.1 (11.6)</td>
<td>23.8</td>
<td>&lt;.001</td>
<td>SzO&lt;BdO; SzO&lt;CcO</td>
</tr>
<tr>
<td>CGI</td>
<td>2.4 (1.3)</td>
<td>1.5 (0.9)</td>
<td>1.3 (0.7)</td>
<td>17.2</td>
<td>&lt;.001</td>
<td>SzO&lt;BdO; SzO&lt;CcO</td>
</tr>
<tr>
<td>GAF</td>
<td>75.2 (13.0)</td>
<td>83.0 (10.8)</td>
<td>86.0 (7.6)</td>
<td>14.3</td>
<td>&lt;.001</td>
<td>SzO&lt;CcO; SzO&lt;BdO</td>
</tr>
<tr>
<td>Lifetime Axis I diagnoses</td>
<td>21 (55.3)</td>
<td>28 (36.4)</td>
<td>15 (18.1)</td>
<td>17.4</td>
<td>&lt;.001</td>
<td>SzO&lt;CcO; BdO&lt;CcO; SzO&lt;BdO</td>
</tr>
<tr>
<td>Lifetime mood disorders</td>
<td>2 (5.6)</td>
<td>12 (15.6)</td>
<td>4 (4.8)</td>
<td>6.3</td>
<td>.04</td>
<td>BdO&lt;CcO</td>
</tr>
<tr>
<td>Lifetime anxiety disorders</td>
<td>7 (18.9)</td>
<td>8 (10.4)</td>
<td>6 (7.2)</td>
<td>3.7</td>
<td>.16</td>
<td></td>
</tr>
<tr>
<td>Lifetime DBDs</td>
<td>7 (18.9)</td>
<td>3 (3.9)</td>
<td>1 (1.2)</td>
<td>15.9</td>
<td>&lt;.001</td>
<td>SzO&lt;CcO; SzO&lt;BdO</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>17 (45.9)</td>
<td>12 (15.6)</td>
<td>5 (6.0)</td>
<td>28.8</td>
<td>&lt;.001</td>
<td>SzO&lt;CcO; BdO&lt;CcO; SzO&lt;BdO</td>
</tr>
<tr>
<td>Psychopharmacological treatment</td>
<td>9 (23.7)</td>
<td>6 (7.8)</td>
<td>0</td>
<td>20.9</td>
<td>&lt;.001</td>
<td>SzO&lt;CcO; BdO&lt;CcO</td>
</tr>
<tr>
<td>SOPS positive</td>
<td>1.8 (2.5)</td>
<td>0.7 (1.9)</td>
<td>0.3 (0.9)</td>
<td>8.4</td>
<td>&lt;.001</td>
<td>SzO&lt;CcO; SzO&lt;BdO</td>
</tr>
<tr>
<td>SOPS negative</td>
<td>1.7 (3.4)</td>
<td>1.3 (2.7)</td>
<td>0.4 (0.8)</td>
<td>4.8</td>
<td>.009</td>
<td>SzO&lt;CcO; BdO&lt;CcO</td>
</tr>
<tr>
<td>SOPS total</td>
<td>6.7 (8.6)</td>
<td>4.2 (7.6)</td>
<td>1.8 (2.8)</td>
<td>7.4</td>
<td>.001</td>
<td>SzO&lt;CcO; BdO&lt;CcO</td>
</tr>
</tbody>
</table>

Note: Data shown as mean (SD) except where noted. ADHD = attention-deficit/hyperactivity disorder; BdO = adolescent offspring of patients with bipolar disorder; CcO = community control offspring group; CGI = Clinical Global Impression; DBD = disruptive behavior disorder; GAF = General Assessment of Functioning; SOPS = Scale of Prodromal Symptoms; SzO = offspring of patients with schizophrenia.

1a Trend-level significance (.05 $< p < .09$)
2 Fisher’s exact test.

Variables were log transformed before applying parametric statistics due to violation of normality assumptions.
prodromal symptom scales compared with CcO, and on the positive ratings compared with BdO. No participant met criteria for substance abuse or dependence.

Nine SzO and 2 BdO were receiving treatment with stimulant medications, whereas 4 BdO were receiving treatment with selective serotonin reuptake inhibitors. One BdO had also received treatment with low-dose risperidone (0.25 mg per day) in the past for behavioral symptoms. Participants receiving psychotropic treatment had more severe CGI and lower GAF scores (all \(p < .05\)) than their nonmedicated counterparts.

**Neuroimaging Data**

**Global Brain Volumes.** There was a main effect of group in total cerebral gray matter volume (Table 2), which was driven by a reduction in SzO relative to CcO. There was no effect of lifetime Axis I diagnosis on these findings, and there were no significant group-by-diagnosis or group-by-age interactions.

**Voxel-Based Morphometry: Whole Brain Analyses.** Results are summarized in Table 3 and Figure 1. We observed a main effect of group in the left inferior frontal cortex/anterior insula, which was driven by gray matter volume reduction in SzO relative to both CcO and to BdO. No differences were observed between BdO and CcO. Linear mixed-model analyses confirmed the main effect of group and subsequent pairwise differences when including the effect of sibship as a random-effects, lifetime Axis I diagnoses as fixed effects and age, gender, center, and total intracranial volume as covariates. There was an effect of lifetime history of Axis I diagnoses on gray matter volume differences in this cluster, but no significant group-by-diagnosis or group-by-age interactions (see Figure S1, available online).

**Voxel-Based Morphometry: Region of Interest Analyses.** No effect of group was observed within any of the preselected volumes.

**Correlation With Symptom Scores.** Neither the CGI nor the GAF scores correlated with the volumetric measures. SOPS negative scores were inversely correlated with total cerebral gray matter volume (\(r = -0.46, p = .017\)), and with mean gray matter volume in the cluster showing voxelwise differences (\(r = -0.41, p = .031\)) in the SzO group, but not in either BdO or CcO; however, these correlations did not survive correction for multiple comparisons.

**Examination of Potential Confounders.** Repeated analyses including general cognitive capacity as covariate and in the medication-free sample revealed an effect of group in global gray matter volume at near trend level, whereas analyses of mean gray matter volume in the cluster derived from VBM analyses revealed between-group differences similar to those in the whole sample and without covarying for general cognitive capacity. Subdivision of the sample according to age demonstrated that for global gray matter volume, there was an effect of group in youth aged 12 years or more, whereas in children aged less than 12 years, differences were nonsignificant. For mean gray matter volume in the cluster derived from VBM analyses, we continued to observe an effect of group membership (SzO, BdO, CcO) in each of the age groups. Further details are provided in Supplement 3, available online.

There were no differences in the proportion of participants per center in each offspring group (number and percentage of participants scanned on the 3-Tesla magnetic resonance imaging [MRI] scanner at the Hospital Clinic of Barcelona: SzO: n = 30, 78.9%; BdO: n = 48, 62.3%; CcO: n = 53, 63.9%; \(p = .18\)). The intersite compatibility study yielded intraclass correlation coefficients qualified as excellent (\(r = 0.97\); global gray matter volume) and near excellent (\(r = 0.86\), cluster in the inferior frontal cortex/anterior insula); further details are provided in Supplement 2, available online.

**DISCUSSION**

To our knowledge, this is the first study to assess gray matter volume characteristics of SzO and BdO comparatively. We demonstrate that child and adolescent SzO showed significant decrease in global gray matter volume in relation to CcO and in voxelwise gray matter volume in the left inferior frontal gyrus/anterior insula in relation to CcO and BdO. Our results suggest that gray matter volume reduction during childhood and adolescence may be specific to SzO.

**Comparison of SzO Versus CcO**

We observed a decrease in total cerebral gray matter volume in SzO relative to CcO; when analyzed by age groups, this was significant only in the older participant group. Our findings resonate with cross-sectional reports from adult patients with schizophrenia and unaffected relatives, and suggest that gray matter reduction related to familial risk for schizophrenia.

| TABLE 2 | Global Brain Volumes [Expressed in Cubic Centimeters] for Offspring of Patients With Schizophrenia (SzO), Bipolar Disorder (BdO), and Community Control (CcO) Offspring |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **SzO** (\(n = 38\)) | **BdO** (\(n = 77\)) | **CcO** (\(n = 83\)) | **Effect of Group*** | **Pairwise Analyses** |
| Gray matter | 756.6 (2.27) | 761.5 (1.6) | 763.3 (1.5) | \(F = 3.26; p = .041\) | SzO<CcO: \(p = .035\) |
| White matter | 487.8 (2.8) | 482.7 (2.0) | 485.0 (1.9) | NS | BdO<CcO: \(p = .224\) |
| Cerebrospinal fluid | 312.3 (3.3) | 312.6 (2.3) | 308.4 (2.2) | NS | |
| Total intracranial volume | 1,517.8 (19.9) | 1,546.9 (14.4) | 1,546.1 (13.2) | NS | |

*Note: Data are mean [standard error] unless otherwise specified. NS = not significant.

*Mixed-model analyses controlling for age, gender, scanner site, and total intracranial volumes (the latter in gray matter, white matter, and cerebrospinal fluid only), and sibship as random effect.
schizophrenia may increase during adolescence as a result of interaction with normative gray matter loss occurring during this period; however, to extract firm conclusions, this will need to be demonstrated with a longitudinal design. Although TIV reduction has been documented in older SzO, and although a sample with a similar age to ours has reported negative findings, no study in child and adolescent SzO so far has provided evidence of global gray matter volume changes.

Our SzO sample also exhibited significant gray matter volume reduction in a cluster located in the left inferior frontal gyrus; abnormalities in this region have been extensively identified in schizophrenia and in genetic risk youth. This cluster extended to the anterior insula: insular volume reduction, and especially the anterior subregion, has been extensively implicated in the neuropathology of schizophrenia and in prodromal patients who later transition to psychosis, in whom it has been associated with baseline negative symptoms. In our study, we also observed an inverse correlation between gray matter volume in this region and negative prodromal symptom scores, although these failed to achieve statistical significance.

Longitudinal data from siblings of patients with schizophrenia also revealed predominantly left-sided reduction in cortical thickness in the prefrontal cortex but not in insula volumes, present in early adolescence, which gradually lessenened as the patients who did not transition to psychosis grew into adulthood. Although both samples are first-degree relatives of patients with schizophrenia, our sample of SzO differs from the young schizophrenia siblings in that they present with clinical markers of heightened risk for the disorder (lower functionality, greater clinical severity, and higher prodromal symptom scores than CcO), thus making it likely that at least a proportion of these individuals will go on to develop the disease. This opens the possibility that the regional volumetric abnormalities that we have observed in SzO may, in some cases, form part of the preclinical phenotype of disease. We may therefore expect to observe different trajectories of gray matter volume development in SzO according to disease outcome, with increasing inferior frontal and insular involvement as these cortices mature and interact with disease-related processes in those individuals who will go on to develop the illness. In individuals who do not develop the disease, the current gray matter volume reduction may constitute age-dependent correlates of genetic risk.

**Comparison of BdO Versus CcO**

To the best of our knowledge, this is the largest study to date to assess child and adolescent BdO using VBM. We found no differences between BdO and CcO, even after applying a lenient threshold for predefined subcortical limbic regions. Ladoucer et al. reported increased gray matter volume in the left parahippocampal cortex in a small sample of healthy BdO, whereas an older sample of BdO revealed increased gray matter volume in the right inferior frontal gyrus. We failed to replicate either of these findings in this larger BdO sample. Our negative findings concur with a recent meta-analysis of bipolar genetic risk studies, including both adult and pediatric samples, which concluded that gray matter volume abnormalities were unlikely to signal genetic liability to BD and may instead be related to environmental exposure or disease-related processes.

**Comparison of SzO Versus BdO**

Gray matter volume reduction in the left inferior frontal gyrus/anterior insula was also reduced in SzO relative to BdO. Increases in gray matter volume in the inferior frontal gyrus/anterior insula also observed an inverse correlation between gray matter volume in this region and negative prodromal symptom scores.
gyrus have also been identified in an older sample of BdO and in the early stages of the disorder. However, this was not observed in the comparison of BdO versus CcO, which prevents us from reporting on an effective gray matter volume increase in this region in BdO, possibly related to the younger age of our BdO. Between-group differences in the inferior frontal gyrus concur with reports from clinical samples comparing patients with schizophrenia and BD and adult unaffected relatives. Taken together, our results extend these findings to child and adolescent offspring, suggesting that reduced inferior fronto-insular gray matter volume may distinguish SzO from BdO during adolescence.

**Methodological Considerations**

This sample differs from previous investigations in terms of its size, its young age, and its hypothesis-free approach and stringent statistical correction. It also stands out due to a strict recruitment process through the parents, whereby only offspring were included, and all participants were assessed by experienced clinicians blinded to parental status. Another factor that distinguishes this from previous reports is that controls were from the community and thus presented with a range of mental health conditions at rates comparable to those present in the general population of similar ages. This allowed us to confirm that the current findings were related to group status and not to the effect of pre-existing mental health conditions.

This study also has several limitations. First, it was designed as a multicenter study, given the difficulties forecast in recruitment, as patients with schizophrenia in particular are known to have lower fertility rates. This is particularly relevant when dealing with neuroimaging data, hence the undertaking of an intersite compatibility study to assess the effect of intersite differences in the current dataset. Second, we did not assess prodromal symptoms for BD, given the lack of available tools for measuring these symptoms when the study was designed. Third, as it was a naturalistic study, a proportion of patients received psychotropic medications. Analyses were therefore repeated in a medication-free sample; however, risk of excluding the more severely affected patients and subsequently those at greater risk for the disorder must also be considered.

Taken together, our findings add support to the notion that developmental impact may differentiate the premorbid phenotype of schizophrenia and BD. SzO may carry a larger neurodevelopmental “load” derived from genetic and early environmental influences, evident in this sample on cognitive, clinical, and functional levels, in combination with the current neuroanatomical findings. Conversely, brain structural abnormalities in BdO may emerge at a later age through regionally specific gray matter volume increases, which will require longitudinal examination. Only follow-up studies will be able to elucidate which of the current baseline findings are predictive of disease.

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**FIGURE 1** Brain regions showing a main effect of group in gray matter volume are displayed (minimum K = 200 voxels).

Note: Cross-hairs identify cluster showing a significant effect of group, which is driven by gray matter volume reduction in offspring of patients with schizophrenia relative to offspring of patients with bipolar disorder and offspring of community controls. Data are presented in SPM Montreal Neurological Institute (MNI) space and overlaid on an average brain.
REFERENCES


SUPPLEMENT 1

Image Acquisition
The Hospital Clinic of Barcelona site (HCP) used a 3 Tesla Siemens Magnetom TrioTim syngo MR B13, and the Hospital General Universitario Gregorio Marañon site (HGUGM) used a 1.5T Philips Intera. The details of the magnetic resonance imaging (MRI) acquisition sequence for each site were as follows. HCP: T1-weighted protocol using the Magnetization Prepared Rapid Acquisition Gradient Echo sequence, 175 sagittal slices, TRTE (ms) = 2050.41; voxel dimensions (mm^3): 1 × 0.94 × 0.94. HGUGM: T1-weighted protocol using 3D Gradient Echo Sequence, 175 sagittal slices, acquisition matrix: 256 × 256, TRTE (ms) = 259.2; voxel dimensions (mm^3): 1 × 0.94 × 0.94; acquisition time: 6 minutes. T2 images were also acquired and evaluated by independent neuroradiologists to rule out structural pathology.

SUPPLEMENT 2

Intersite Compatibility Study
To explore interscanner effects, 7 healthy control participants underwent scanning in each of the 2 scanners used in this study. Images were processed with Statistical Parametric Mapping (SPM) version 8, Matlab R2013. An affine coregistration was undertaken between the images of each of the 2 scanners for each participant, which were then segmented using the New Segment tool. The gray matter segmented images were then normalized using the Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra toolbox, and smoothed with a 4-mm full-width-at-half-maximum kernel. We calculated type C (consistency) intraclass correlation coefficients (ICC) at a voxel level, using the segmented and normalized gray matter volume images with a customized script in MatlabR2013. The ICC map for gray matter volume overlaid on a standard T1 template is presented in Figure S1.

We then set out to investigate ICC of total and regional gray matter volumes for the 7 control participants. For total gray matter volume (using the segmented gray matter volume tissue), the overall ICC for the 7 participants was r = 0.98. We next calculated the mean ICC for the cluster of significant group differences on the basis of the ICC map created above, revealing a value of 0.86, which approached the excellent range (ICC > 0.90).1

SUPPLEMENT 3

Effects of General Cognitive Capacity, Psychotropic Medication, and Age Group
Given the association between brain volume and intelligence,2,3 linear mixed model analyses were repeated, adding index of general cognitive capacity as a covariate. For global gray matter volume, the effect of group was significant at a near trend level (F = 2.55, p = .082), driven by a reduction in offspring of patients with schizophrenia (SzO) relative to offspring of community controls (CcO; p = .077). For the voxel-based data, there was a significant effect of group in mean gray matter volume of the cluster located in the left inferior gyrus anterior insula (F = 8.39, p < .001). Pairwise analyses confirmed that this was driven by decreased gray matter volume in SzO relative to CcO (p = .003) and relative to offspring of patients with bipolar disorder (BdO; p < .001).

To explore the effects of medication on the current findings, neuroimaging analyses were repeated in the medication-free sample: 29 SzO, 71 BdO, and all CcO (n = 83) had never received any form of psychotropic medication. For global gray matter volume, there was an effect of group significant at a near trend level (F = 2.43, p = .093). Pairwise analysis confirmed that differences were driven by the SzO less than CcO comparison (p = .090).

For the voxel-based data, linear mixed-model analyses within the medication-free sample showed an effect of group in mean gray matter volume of the cluster located in the left inferior gyrus anterior insula (F = 7.79, p = .001). Pairwise analyses confirmed that this was driven by decreased gray matter volume in SzO relative to CcO (p = .003) and relative to BdO (p = .001).

Given the age range of the sample, analyses were performed in subgroups by subdividing the sample between those up to 11 years of age and those 12 years or more (age <12 years: SzO, n = 19 [50%]; BdO, n = 26 [33.5%]; CcO, n = 45 [54.2%]). In both age groups, SzO showed numerically lower values of global gray matter volume relative to CcO. However, the effect of group (SzO, BdO, CcO) was significant only in the older participants (F = 3.1, p = .049; SzO less than CcO: p = .05), whereas the younger participants showed a nonsignificant effect of group (F = 1.40, p = .25).

There was also a main effect of group (SzO, BdO, CcO) in the cluster located in the inferior frontal gyrus anterior insula, both in children aged less than 12 years of age (effect of group: F = 5.8, p = .004, SzO less than CcO: p = .035; SzO less than BdO: p = .003), and in youth 12 years or more (effect of group: F = 8.89, p < .001; SzO less than CcO: p < .001; SzO less than BdO, p = .002).

SUPPLEMENTAL REFERENCES
FIGURE S1  Map of intraclass correlation coefficients for gray matter volume for the 7 participants in the intersite compatibility study. Note: Color scale represents values of voxelwise intraclass correlation coefficients. Map is overlaid on a standard T1 template.