Exploring the moderating effects of dopaminergic polymorphisms and childhood adversity on brain morphology in schizophrenia-spectrum disorders

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ABSTRACT

Genetic and environmental etiologies may contribute to schizophrenia and its associated neurobiological profile. We examined the interaction between dopaminergic polymorphisms, childhood adversity and diagnosis (schizophrenia/schizoaffective disorder) on dopamine-related brain structures. Childhood adversity histories and structural MRI data were obtained from 249 (153 schizophrenia/schizoaffective, 96 controls) participants registered in the Australian Schizophrenia Research Bank. Polymorphisms in *DRD2* and *COMT* were genotyped and a dopaminergic risk allelic load (RAL) was calculated. Regression analysis was used to test the main and interaction effects of RAL, childhood adversity and diagnosis on volumes of dopamine-related brain structures (caudate, putamen, nucleus accumbens, dorsolateral prefrontal cortex and hippocampus). A schizophrenia/schizoaffective diagnosis showed significant main effects on bilateral hippocampus, left dorsolateral prefrontal cortex and bilateral putamen volumes. RAL showed a significant main effect on left putamen volumes. Furthermore, across the whole sample, a significant two-way interaction between dopaminergic RAL and childhood adversity was found for left putamen volumes. No brain structure volumes were predicted by a three-way interaction that included diagnosis. Our finding suggests the left putamen may be particularly sensitive to dopaminergic gene-environment interactions regardless of diagnosis. However, larger studies are needed to assess whether these interactions are more or less pronounced in those with schizophrenia/schizoaffective disorders.

Keywords: gene-environment interaction; childhood trauma; neurobiology; DRD2; COMT; structural MRI

1. INTRODUCTION

Schizophrenia is a psychiatric illness that typically emerges during late adolescence, and often results in a lifetime of disability (Lewis and Lieberman, 2000). Although the disorder appears to lack a discretely causal aetiology, dopaminergic abnormalities remain a central hypothesis of the pathophysiology of schizophrenia and are the primary target of current pharmacological treatments (Howes and Kapur, 2009). The dopamine hypothesis of schizophrenia suggests that characteristic symptoms such as hallucinations, delusions and abnormal cognitive functioning are caused by a synergistic imbalance of dopamine neurotransmission in cortical and subcortical brain regions (Howes and Kapur, 2009). Thus, genetic variants and brain regions implicated in the function of the dopaminergic system may contribute to the disorder's aetiology.

A number of variants within genes in the dopaminergic system have been studied, and among them, the dopamine receptor D2 (*DRD2*) and catechol-o-methytransferase (*COMT*) genes have arguably been interrogated the most. *DRD2* was recently identified as one of 108 loci associated with schizophrenia in the largest schizophrenia genome-wide association study to date (Psychiatric Genomics Consortium, 2014). One highly researched *DRD2* variant that occurs at rs1076560 (G > T) determines whether mRNA splices into long or short isoforms (Zheng et al., 2012). Past literature has identified this substitution as a risk-conferring variant for schizophrenia, most likely due to the resultant decrease of dopamine transmission in the frontal cortex (Tallerico et al., 2001; Zheng et al., 2012). Another relevant *DRD2* mutation is located at rs12364283 (T > C). This polymorphism results in enhanced total D2 mRNA expression, which may exacerbate already elevated striatal dopamine transmission in patients with schizophrenia (Bertolino et al., 2009a). A third *DRD2* variant relevant to schizophrenia occurs at rs1801028 (C > G), where cysteine production replaces serine production. A meta-analysis has suggested that there is a link between this

polymorphism and an increased susceptibility to schizophrenia through alteration of D2 receptor physiology and functioning (Glatt et al., 2003). Some of these *DRD2* variants have also been shown to effect schizophrenia-associated intermediate phenotypes such as disrupted prefrontal-striatal activity (Bertolino et al., 2008) and morphological changes including smaller caudate volumes (Bertolino et al., 2009b). Lastly, a mutation in the *COMT* gene at rs4680 (A > G) enhances coding of valine instead of methionine, resulting in a higher enzymatic catabolism of dopamine in the prefrontal cortex (Egan et al., 2001). Whilst some studies have identified *COMT* as a possible candidate gene for schizophrenia (Kunugi et al., 1997; Li et al., 2000; Wonodi et al., 2003), others have revealed no association between the val/met polymorphism and the disorder (de Chaldée et al., 2001; Okochi et al., 2009). As opposed to the manifested clinical outcome, the *COMT* polymorphism is much more strongly associated with schizophrenia-related intermediate phenotypes such as brain morphology; for example, volumetric changes in the hippocampus and dorsolateral prefrontal cortex (DLPFC) (Cerasa et al., 2008; Honea et al., 2009; Kates et al., 2006). It has been widely accepted that these observed gene-brain associations are likely due to the interactive and cumulative effect of molecular mechanisms downstream from genotype (Harrison and Weinberger, 2005), which are heavily affected by environmental influences (van Os et al., 2008a).

One potent and established environmental risk factor for schizophrenia and other psychiatric disorders is childhood adversity (Matheson et al., 2013). Childhood adversity has been defined as any form of emotional or physical ill-treatment, sexual abuse, exploitation or neglect during childhood or teen years (Rosenman and Rodgers, 2004). Perhaps the most frequently reported types of early trauma associated with psychosis are sexual and physical abuse, which are often examined together (Davies-Netzley et al., 1996; Read et al., 2003; Read and Argyle, 1999). Since attributing salience to threatening or adverse environmental stimuli (Kapur, 2003) and stress-mediated responses (Laruelle, 2000) both implicate the

dopamine system, childhood adversity may contribute to pathological dopamine neurotransmission and, in turn, alter neurobiology (Read et al., 2005; Van Winkel et al., 2008; Walker et al., 2008). In those who have experienced early maltreatment, atrophy has been especially noted in the hippocampus (Bremner et al., 1997; Hoy et al., 2012; Rao et al., 2010; Woon and Hedges, 2008) and may occur in other dopamine-related regions such as the prefrontal cortex and striatal structures (Cohen et al., 2006; Frodl et al., 2010; Tomoda et al., 2009). It has been proposed that early physical and emotional adversities each have specific neurobiological targets, as different neural mechanisms and pathways are employed to cope with different types of traumatic experience (Edmiston et al., 2011; Teicher and Samson, 2016). As such, exposure to physical abuse, emotional abuse or emotional neglect may have regionally-specific effects on the brain.

Notably, not all individuals who carry 'risk' polymorphisms or experience childhood adversity develop schizophrenia, suggesting genetic and environmental factors likely interact rather than act alone. As such, the current study used a gene × environment (G × E) framework to investigate the interaction between genetic variation in dopaminergic genes (*DRD2* and *COMT*) and childhood adversity in determining schizophrenia-associated brain morphology. Although there is a wealth of G × E literature examining schizophrenia (Tienari et al., 2004; van Os et al., 2008a; Wahlberg et al., 1997), few studies have utilized this framework to investigate intermediate phenotypes associated with the disorder. The present study focused on brain structures which have shown to be both atrophied in schizophrenia patients and influenced by genetic and environmental factors, thus fulfilling the two defining targets of an intermediate phenotype (Meyer-Lindenberg and Weinberger, 2006). These structures were the hippocampus, DLPFC, nucleus accumbens, caudate nucleus and putamen. It was hypothesised that individuals with a high proportion of dopaminergic 'risk'

polymorphisms, elevated levels of childhood adversity and a diagnosis of schizophrenia-spectrum disorders would have the lowest volumes in these brain regions.

2. METHODS

2.1 Participants

Participant data was obtained from the Australian Schizophrenia Research Bank (ASRB); an ongoing register that commenced data collection in 2010 and is funded by the National Health and Medical Research Council (Loughland et al., 2010). Participants were screened by clinical assessment officers over the telephone. Exclusion criteria for ASRB participation included severe brain injury, organic brain syndrome, movement disorders, mental retardation categorized by an intellectual quotient below 70, current substance dependence or electroconvulsive therapy in the last 6 months. The ASRB also excluded healthy controls if they had a history of any psychosis or bipolar I disorders using the Diagnostic Interview for Psychosis (Castle et al., 2006), or any other mental disorder using a sociodemographic and clinical history schedule. All participants were aged between 18 to 65. Detailed accounts of sampling procedures and consent acquisition can be found elsewhere (Loughland et al., 2010).

In the present study, individuals who met DSM-IV criteria for schizophrenia (n = 116) and schizoaffective disorder (n = 37) were included in the schizophrenia-spectrum cohort, but those with psychotic disorder NOS (n = 34) or other psychiatric illness (n = 6) were excluded. The mean age of the schizophrenia-spectrum cohort was 38.2 years, and comprised 109 males (67%) and 44 females (33%). The healthy cohort contained 96 individuals with a mean age of 41.8 years, and comprised 41 males (43%) and 55 females (57%). Clinical and demographic characteristics of participants are summarized in Table 1.

2.2 Measure of Childhood Adversity

Participants completed the Childhood Adversity Questionnaire (CAQ) (Rosenman and Rodgers, 2004); a retrospective questionnaire that assesses maltreatment before the age of 16. The CAQ comprises 21 yes/no questions that cover the domains of sexual abuse, physical abuse, emotional abuse, emotional neglect, loss, financial disadvantage and family dysfunction. A total unweighted score was calculated for each individual based on the number of times they answered 'yes', ranging from 0 to 21.

2.3 Genotyping and Dopaminergic Risk Allelic Load

DNA was extracted from whole blood using a QIAamp DNA Blood Midi or Maxi Kit (QIAGEN, Chadstone, Australia) according to the manufacturer's instructions. 200ng of gDNA was then amplified and fragmented. The resulting lysate was loaded onto a QIAamp spin column and washed to rid impurities from bound DNA and diluted. Illumina's Goldengate assay (San Diego, CA) was used to genotype three single nucleotide polymorphisms (SNPs) in *DRD2* (rs1076560, rs12364283, rs1801028) and one SNP in *COMT* (rs4680).

For each *DRD2* and *COMT* SNP, a risk allele was nominated based on previous literature (*DRD2* rs1076560: T allele, rs12364283: C allele, rs1801028: G allele and *COMT* rs4680: G allele). An unweighted dopaminergic risk allelic load (RAL) was calculated for each individual based on the number of risk alleles they possessed, with a total score ranging from 0 to 8.

2.4 MRI Acquisition and Processing

Structural MRI was used to obtain whole brain T1-weighted images for each participant using a Siemens Avanto 1.5-Tesla system (Siemens, Erlangen, Germany).

Cortical reconstruction and volumetric segmentation of images were performed using FreeSurfer image analysis suite. The automated volume-based and surface-based streams (Fischl et al., 2002; Fischl et al., 2004) were performed to extract volume estimates for select subcortical and cortical regions This comprised an optimized magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence with the following parameters: 176 sagittal slices of 1mm thickness without gap; field of view = 250 x 250 mm2; repetition time/echo time = 1980/4.3 ms; data matrix size = 256 x 256; voxel dimensions = 0.98 x 0.98 x 1.0 mm3. Exactly the same acquisition sequence was used across all sites. Cortical reconstruction and volumetric segmentation of images were performed using FreeSurfer image analysis suite (version 5.1.0; Martinos Center for Biomedical Imaging, Harvard-MIT, Boston, MA; http://surfer.nmr.mgh.harvard.edu/). The automated volume-based and surface-based streams were performed to extract volume estimates for select subcortical and cortical regions using an automatic labelling system. This procedure assigns a neuroanatomical label to each voxel or vertex on the cortical surface using a probabilistic atlas and a Bayesian classification rule (Fischl et al., 2002; Fischl et al., 2004). Prior to volume extraction, all images were visually inspected for image artifacts and reconstruction errors and, if necessary, were manually corrected using Freesurfer's editing tools according to a standardized protocol.

Volumes (mm^3) from the putamen, caudate, nucleus accumbens, hippocampus and the rostral middle frontal gyrus were selected from each hemisphere as regions-of-interest. The rostral middle frontal gyrus (from the Desikan-Killiany gyral-based atlas) (Desikan et al., 2006) was selected as it most closely corresponds to the DLPFC (Kikinis et al., 2010) and herein will be referred to as the DLPFC.

2.5 Statistical Analysis

2.5.1 Comparison of schizophrenia-spectrum patients and controls

Differences in RAL, CAQ total score, CAQ subscales (physical abuse, sexual abuse, emotional neglect, emotional abuse, loss, financial disadvantage and family dysfunction) and brain structure volumes between schizophrenia-spectrum patients and controls were examined using linear regression.

2.5.2 Multiple linear regression model

A multiple regression model was used to assess the effect of RAL (X_1), CAQ score (X_2) and diagnostic status (X_3) on brain structure volumes (Y). Both main and all two-way interaction effects were estimated for total CAQ score (continuous), RAL (continuous) and diagnosis (dichotomous). In addition, a three-way interaction term (CAQ score*diagnosis*RAL) and covariates (age, gender, scanning site and intracranial volume) were entered into the model.

$$Y = ß_0 + ß_1X_1 + ß_2X_2 + ß_3X_3 + ß_4X_1X_2 + ß_5X_1X_3 + ß_6X_2X_3 + ß_7X_1X_2X_3 + \text{covariates}$$

The results were analysed hierarchically. If the three-way interaction term ($ß_7$) was not significant for any brain structure, two-way interaction terms were examined ($ß_4$, $ß_5$, $ß_6$). If two-way interactions were not significant, main effects were examined ($ß_1$, $ß_2$, $ß_3$).

Preliminary analysis revealed that parametric analysis was not suitable to analyse data because the sample exhibited unequal variances and had a non-normal distribution. Therefore, nonparametric bootstrapping was used in the regression model. 10,000 bootstrap samples were drawn with replacement from the original sample (n = 249) to calculate the 95% bias corrected and accelerated (BCa) confidence intervals (Cl) for the unstandardized regression (B) coefficients. Using this approach, main and interaction effects were considered statistically significant if the 95% BCa CI did not overlap zero.

Multiple comparisons were corrected following the Benjamini and Yekutieli method (Narum, 2006). Pearson correlation analysis revealed that caudate (r = 0.93, $p < 0.001$), nucleus accumbens (r = 0.56, $p < 0.001$), putamen (r = 0.94, $p < 0.001$), hippocampus (r = 0.86, $p = 0.001$) and DLPFC (r = 0.84, $p < 0.001$) volumes were correlated on left and right sides. Since all the investigated brain regions were correlated bilaterally, five independent tests were included in the Benjamini and Yekutieli correction. The critical p threshold of 0.0219 was calculated for five independent tests using the equation

$$\alpha / \sum_{i=1}^{k} (1/i)$$

where k is the number of independent hypothesis tests and α is the significance level (0.05).

2.5.3 Post-hoc analyses

Out of the seven types of adversity that were assessed by the CAQ, physical abuse, emotional abuse, emotional neglect and family dysfunction differed between controls and patients with schizophrenia-spectrum disorders. An exploratory post-hoc linear regression analysis was conducted to investigate the effect of these adversities on the selected brain structures. Covariates of age, gender, scanning site and intracranial volume were included in the model.

3. RESULTS

As indicated in Supplementary Table S1, CAQ scores of the schizophrenia-spectrum cohort ranged from 0 to 19 and had a mean (SD) of 5.53 (4.52) out of 21. These scores were significantly higher ($p < 0.001$) than those of the healthy controls, who had scores ranging from 0 to 14 and a mean (SD) of 2.83 (3.49). CAQ scores did not differ between males and

females (t = - 0.3, p = 0.602). The schizophrenia-spectrum cohort were more likely to report physical abuse (p = 0.009), emotional abuse (p < 0.001), emotional neglect (p < 0.001) and family dysfunction (p < 0.001) than controls. RAL did not differ between the two cohorts (p = 0.674). The schizophrenia-spectrum cohort had a RAL which ranged from 0 to 4 and had a mean (SD) of 1.5 (0.9) out of 8, whilst the control cohort had a RAL which ranged from 0 to 5 and had a mean (SD) of 1.4 (0.9). Those with schizophrenia-spectrum disorder had lower left and right hippocampal (p < 0.001 and p = 0.004, respectively), left DLPFC (p = 0.006) and higher left and right putamen volumes (p = 0.001 and p = 0.003, respectively). A t test revealed that striatal volume was larger in males than in females (t = 7.8, p = 0.038).

As indicated in Supplementary Table S2, a two-way interaction effect between CAQ score and RAL predicted left putamen volumes (B = -18, 95% Cl = -33– -2, p = 0.014), which remained significant after adjusting for multiple comparisons (Figure 1). Examination of main effects across the five brain regions of interest revealed that left putamen size was predicted by a main effect of RAL in which a higher RAL was associated with lower left putamen volume (B = -104, 95% Cl = -176 – -36, p = 0.002). A main effect of schizophrenia-spectrum diagnosis was found for right putamen (B = 199, 95% Cl = 64 – 333, p = 0.004), left putamen (B = 220, 95% Cl = 85 – 362, p = 0.002), left DLPFC (B = - 665, 95% Cl = -1096 – -272, p < 0.001), right hippocampus (B = -146, 95% Cl = -242 – -57, p = 0.006) and left hippocampus (B = -183, 95% Cl = -274 – -98, p < 0.001) volumes.

As seen in Supplementary Table S3, post-hoc analysis revealed that right caudate volumes were predicted by physical abuse (B = 157, 95% CI = 44 – 263, p = 0.008), but no other brain regions were predicted by this adversity type. Emotional abuse, emotional neglect and family dysfunction did not predict the volumes of any brain structures.

4. DISCUSSION

The current study used the dopamine hypothesis of schizophrenia to identify genetic and environmental factors which may play a role in disorder-associated brain morphology. With the exception of the left putamen, there were no brain regions whose size was predicted by a main effect of dopaminergic RAL. This is in contrast with past literature which has found an association between the *COMT* polymorphism and volumetric alterations in the hippocampus and DLPFC (Cerasa et al., 2008; Honea et al., 2009; Kates et al., 2006), and between *DRD2* polymorphisms and alterations in striatal structures (Bertolino et al., 2009b), however, it is important to note that these studies investigated the polymorphisms in isolation and did not calculate a risk score. In addition, there were no brain regions whose sizes were predicted by total childhood adversity. This result was particularly unexpected in terms of the hippocampus, which, in past literature, has repeatedly exhibited volumetric reductions in individuals exposed to childhood trauma (Bremner et al., 1997; Stein et al., 1997; Teicher et al., 2012; Vythilingam et al., 2002). The prefrontal cortex has also previously displayed atrophy in response to these experiences (Frodl et al., 2010; Tomoda et al., 2009). Furthermore, exploratory analysis revealed that out of the subtypes of childhood adversity that differed between controls and schizophrenia-spectrum patients, physical abuse, which predicted right caudate volumes, was the only subtype that exerted an effect on any brain structure. These preliminary results contrast with previous findings that physical abuse, emotional abuse and emotional neglect each impact the size of different brain structures (Edmiston et al., 2011; Teicher and Samson, 2016). Lenze et al. (2008), who also did not find an association between early trauma and neurobiology, suggested that recruiting participants based on psychiatric illness may yield a cohort with a lower severity of trauma than if participants were recruited based on adversity experience. It is possible that this may have contributed to the lack of observed neurobiological effects in the current study. Additionally, a diagnosis of schizophrenia-spectrum disorder predicted smaller left and right hippocampus

and left DLPFC volumes. These results are in line with previous findings (Altshuler et al., 1998; Becker et al., 1996; Copolov et al., 2000; Kikinis et al., 2010; Nelson et al., 1998; Schlaepfer et al., 1994), which highlight them as potentially important morphological intermediate phenotypes for the disorder. Schizophrenia-spectrum patients also exhibited increased left and right putamen volumes relative to controls. Past studies that have recorded similar increases in striatal volume (Chakos et al., 1994; Ho et al., 2011; Hokama et al., 1995) have attributed these increases to prolonged antipsychotic treatment, which, through D2 receptor blockade, may upregulate endogenous dopamine production and induce hypertrophy (Chakos et al., 1998). Thus antipsychotic treatment is likely responsible for the differences between cases and controls in the current study, as 91.5% of the schizophrenia cohort were being treated with antipsychotics.

The interactive effect of dopaminergic RAL, childhood adversity and diagnosis on the volumes of five dopamine-related brain regions was also investigated. Across the entire cohort, an association was found between greater exposure to childhood adversity and lower left putamen volumes that was dependent on RAL in a dose-dependent manner. As an individual's RAL increased, the strength of the association between childhood adversity and left putamen volume also increased, which is illustrated in Figure 1.

The results obtained for the left putamen support the diathesis-stress framework (Walker and Diforio, 1997) in that more pronounced morphological variations occurred in those who were both genetically susceptible and had experienced high levels of stress during a sensitive developmental period. Previous studies which have investigated the isolated effects of dopaminergic polymorphisms (Bertolino et al., 2009b) and childhood adversity (Cohen et al., 2006; Edmiston et al., 2011) on striatal volume have found similar results. Left putamen volumes, however, were not predicted by the interaction term when diagnosis was added, implying that the observed G × E interaction is applicable to healthy individuals as

well as those affected by schizophrenia-spectrum disorder. The theory of intermediate phenotypes suggests that the neurobiological effects of disorder-related aetiologies do not necessarily manifest into clinical phenomenology, which is in line with our findings (Meyer-Lindenberg and Weinberger, 2006).

The putamen is one brain structure which exhibits particularly high neuroplasticity because of its elevated cortico-thalamic input and D2 receptor density (Graybiel, 2004; Hall et al., 1994). Both of these mediating factors may have contributed to the observed volumetric alterations, as childhood adversity is associated with dysfunctional cortico-thalamic pathways related to stress, reward and emotion processing (Dillon et al., 2009; Masten et al., 1999), and the presence of dopaminergic polymorphisms have been shown to affect D2 receptor expression (Duan et al., 2003).

As the putamen seems receptive to genetic and environmental factors, it may be a good candidate for further research into neurobiological intermediate phenotypes in the general population. This cannot be said, however, for the other brain regions that were investigated. Hippocampus, DLPFC, caudate and nucleus accumbens size were not predicted by the genetic and environmental factors of dopaminergic RAL and childhood adversity. Additionally, a three-way interaction between childhood adversity, RAL and diagnosis did not predict the size of any brain structures. These results indicate that early traumatic experiences, when paired with the combined effect of *COMT* and *DRD2* polymorphisms, do not preferentially impact neurobiological intermediate phenotypes in schizophrenia-spectrum patients. Although no previous investigations have examined these particular intermediate phenotypes, other G × E research, including epidemiological and adoption studies, have suggested that dopaminergic genotype moderates sensitivity to environmental stressors in the development of schizophrenia and psychosis (Collip et al., 2011; Stefanis et al., 2007; Tienari et al., 2004; Wahlberg et al., 1997). As there is a relatively established link between these G

× E factors and manifested clinical pathology, it seems that brain morphology did not act successfully as a midway 'marker' between these two phenomena in the current study. The failure of the isolated measures of dopaminergic RAL and childhood adversity to affect most brain structures may provide a clue as to why. Although diagnosis did predict the volumes of several brain regions, as would be expected for intermediate phenotypes, the aetiological factors did not. Instead of neurobiology, perhaps other physiological traits that are more closely linked to genetic substrate and shaped by environmental factors will be better suited as intermediate phenotypes in this kind of research. For instance, a review on psychotic disorders (Van Os et al., 2008b) has asserted that genetic and environmental aetiologies, through the mechanisms of mRNA translation and HPA axis activation, co-participate in sensitizing subcortical dopamine pathways and disrupting global dopaminergic neurotransmission. Future G × E investigations may wish to use functional MRI to study such neural activity-based intermediate phenotypes.

There are several limitations of the present study that should be acknowledged. First, we aimed to investigate four variants in dopaminergic genes that have been heavily researched due to their potential as candidate genes for schizophrenia. A number of other genes associated with the dopaminergic system were not examined, such as *DAT* and *MAOA,* which may possibly also play a role in the neurobiology of this disorder (Meyer-Lindenberg and Weinberger, 2006). Furthermore, the largest genome-wide association study to date has identified 108 schizophrenia-associated genomic loci (Ripke et al., 2014). Many of these loci have no apparent link to the dopaminergic system, suggesting dopamine-related genes will likely explain a small portion of the variance in intermediate phenotypes (Allen et al., 2008). As such, follow-up studies that derive RAL from all of the 108 loci are warranted. Second, due to our modest sample size, an unweighted RAL score was calculated and assumed that each polymorphism contributed equally to phenotypic 'risk'. A weighted RAL is preferred

but requires large samples from which weights can be derived in a training dataset and then applied to a validation dataset. The modest sample size also means that the power to detect interaction effects is suboptimal. Thus, future studies with larger samples are needed to validate our findings. Third, assessment of childhood adversity relied on retrospective, self-reported accounts, and only accounted for early trauma inflicted inside the family home, excluding potential adversity perpetuated by non-related individuals. Age of exposure to and duration of adversity was not assessed, which are both factors that may also modulate neurobiological processes (Casey et al., 2008; Hodas, 2006; Perry et al., 1995). Development and use of a more comprehensive assessment of childhood adversity assessment tools are needed. Furthermore, patients with schizoaffective disorder were included in the schizophrenia-spectrum cohort. The comorbidity index of schizoaffective disorder and bipolar disorder is similar to that of schizoaffective disorder and schizophrenia (Laursen et al., 2009). Whilst there is preliminary evidence that schizoaffective disorder is more analogous to schizophrenia in terms of psychological and behavioural functioning than bipolar disorder (Reichenberg et al., 2002), it remains unclear whether schizoaffective disorder is more neurobiologically similar to schizophrenia or bipolar disorder. Future studies investigating intermediate phenotypes may wish to focus exclusively on schizophrenia patients. Lastly, the majority (91.5%) of schizophrenia-spectrum patients were taking antipsychotic medication, which previous research has shown could alter striatal structures (Chakos et al., 1994; Ho et al., 2011; Hokama et al., 1995). It is unlikely that the use of antipsychotic medication was responsible for the observed interaction between CAQ score and RAL on left putamen volumes, as this interaction was relevant to the whole sample which included neuroleptic-naïve controls. It would, however, be beneficial to confirm these findings using analyses that account for antipsychotic exposure with measures such as current dosage and cumulative lifetime dosage.

The current study aimed to determine whether the intermediate phenotype of brain morphology, rather than the manifested disorder of schizophrenia, is an outcome measure suited to G × E interaction studies. A three-way interaction between early trauma, dopaminergic risk allelic load and diagnosis did not predict the size of the putamen, caudate, nucleus accumbens, hippocampus or DLPFC. It was found, however, that dopaminergic risk allelic load moderated the association between childhood adversity and left putamen volume in a dose-dependent fashion across the whole sample, which highlights this brain structure as a potential candidate for further exploration.

Given most genetic and environmental factors, on their own, lack the penetrance and predictive power required to be clinically useful, future studies that employ the G × E framework are warranted. These investigations will expand our understanding of intermediate phenotypes and assist in explaining why some but not all of those who experience adversity or have genetic risk factors develop brain morphology abnormalities.

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Conflict of Interest

None

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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Table 1. Demographic characteristics of the cohort

	Schizophrenia patients (n = 153)	Healthy controls (n = 96)	Comparison
Age (years)	38.2 ± 10.1	41.8 ± 13.8	t = 2.4, p = 0.019*
Gender (M/F)[#]	109/44	41/55	χ^2 = 4.6, p < 0.001*
Education (years completed)	14.5 ± 3.1	14.7 ± 3.0	t = 0.4, p = 0.669
Pre-morbid IQ (WTAR)	102.3 ± 13.9	102.2 ± 12.1	t = 0.0, p = 0.984
Current IQ (WASI)	103.6 ± 14.9	116.6 ± 11.9	t = 7.2, p < 0.001*
SANS total score	26.8 ± 19.3	-----	
DIP positive symptoms score (lifetime)	7.5 ± 3.7	-----	
DIP positive symptoms score (present)	2.0 ± 2.9	-----	
Duration of illness (years)	13.1 ± 10.0	-----	
Age of illness onset (years)	22.1 ± 4.2	-----	
Lifetime antipsychotic use (% reported)	91.5	-----	
Typical antipsychotic use[+]	8.5	-----	
Flupentixol[+]	2.3	-----	
Haloperldol[+]	3.1	-----	
Fluphenazine[+]	1.5	-----	
Atypical antipsychotic use[+]	91.0	-----	
Risperidone[+]	10.9	-----	
Olanzapine[+]	32.0	-----	
Aripriprazole[+]	17.1	-----	
Amisulpride[+]	19.5	-----	
Antidepressant use (% reported)	38.5	-----	
Lifetime mood stabilizer/anticonvulsant use (% reported)	15.0	-----	
Lifetime anticholinergic use (% reported)	14.3	-----	
Lifetime anxiolytic/sedative use (% reported)	1.9	-----	
No medication (% reported)	8.5	-----	

WTAR; Wechsler Test of Adult Reading, WASI; Wechsler Abbreviated Scale of Intelligence, SANS; Scale for the Assessment of Negative Symptoms, DIP; Diagnostic Interview for Psychosis

* Significant values

[#] Pearson's χ^2 test performed

Data are expressed as mean ± SD

[+] Data are expressed as % of those using antipsychotics

Antipsychotic data unavailable for 13 patients

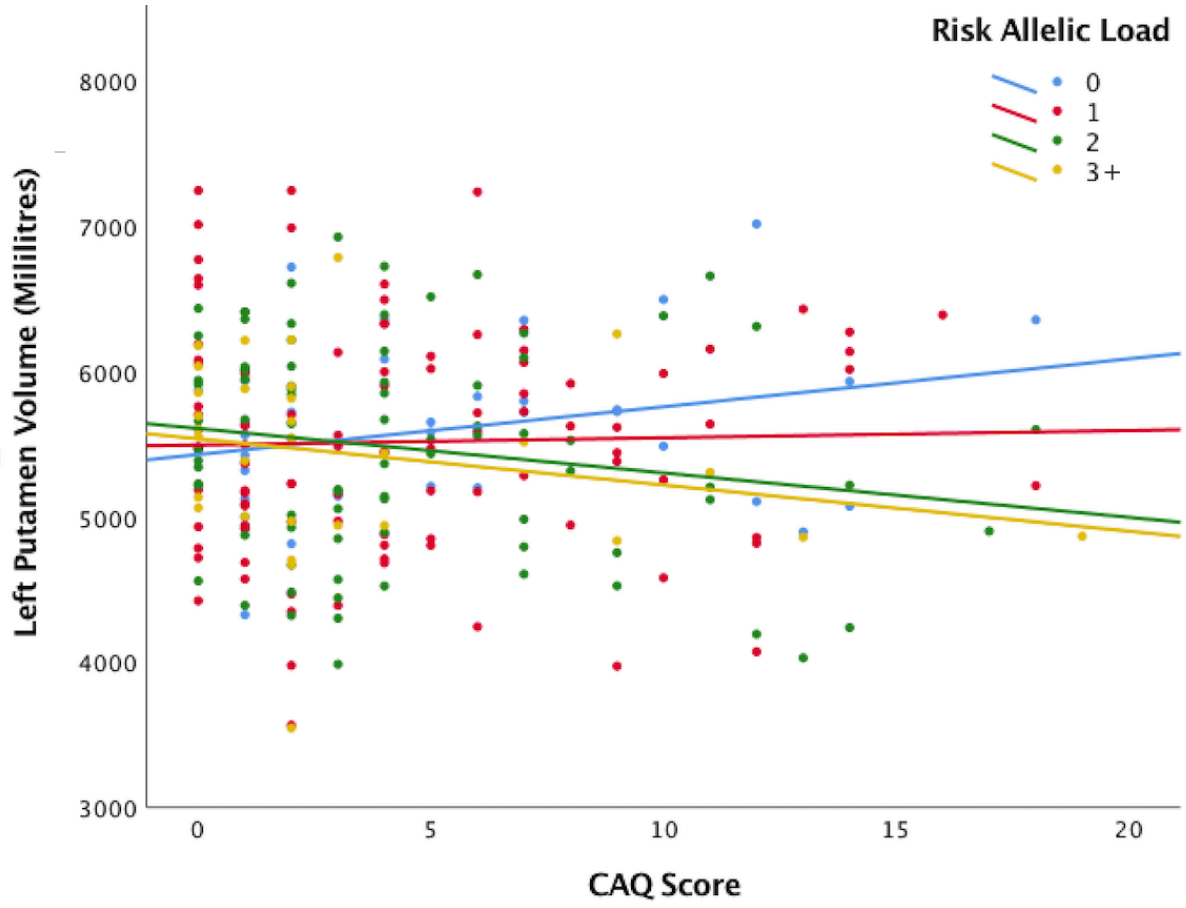

Figure 1. Two-way interaction between dopaminergic risk allelic load (RAL) and Childhood Adversity Questionnaire (CAQ) score for left putamen volumes. A RAL of zero *(n* = 36, R^2 = 0.07), one (*n* = 101, R^2 = 0.0008), two (*n* = 81, R^2 = 0.03), and three or more (*n* = 31, R^2 = 0.05) are shown.

SUPPLEMENTARY MATERIALS

Supplementary Table S1. Comparison of childhood adversity, brain structure volumes and dopaminergic risk allelic load in patients with schizophrenia and healthy controls

	Schizophrenia patients	Healthy controls	Comparison			
			B	SE	BCa 95% Cls	*p*-value
Childhood adversity[#]	5.53 ± 4.52	2.83 ± 3.49	0.1	1.1	-2.0, 2.2	< 0.001*
Sexual abuse[#]	0.04 ± 0.19	0.02 ± 0.14	0.0	0.0	0.0, 0.1	0.182
Physical abuse[#]	0.25 ± 0.49	0.13 ± 0.37	0.1	0.1	0.0, 0.1	0.009*
Emotional abuse[#]	0.84 ± 1.09	0.37 ± 0.90	0.5	0.1	0.3, 0.8	< 0.001*
Emotional neglect [#]	1.16 ± 1.57	0.46 ± 0.99	0.7	0.2	0.4, 1.1	< 0.001*
Loss[#]	0.31± 0.50	0.20 ± 0.45	0.1	0.1	-0.1, 0.3	0.477
Financial disadvantage[#]	0.22 ± 0.42	0.17 ± 0.37	0.1	0.0	0.0, 0.2	0.063
Family dysfunction[#]	2.37 ± 1.48	1.37 ± 1.31	1.2	0.2	0.8, 1.5	< 0.001*
Left caudate	3797.1 ± 527.8	3701.5 ± 516.0	-36.7	52.8	-144.1, 68.1	0.487
Right caudate	3737.2 ± 524.8	3622.5 ± 500.3	-9.8	54.2	-118.7, 98.9	0.855
Left putamen	5668.7 ± 690.0	5234.7 ± 663.9	223.9	66.1	91.1, 355.9	0.001*
Right putamen	5376.7 ± 669.1	4972.1 ± 613.3	191.6	61.5	70.9, 310.9	0.003*
Left nucleus accumbens	536.5 ± 89.6	511.4 ± 89.6	4.9	10.2	-14.5, 25.6	0.644
Right nucleus accumbens	527.1 ± 92.9	511.0 ± 84.1	-5.3	10.3	-24.7, 14.9	0.603
Left hippocampus	4144.6 ± 473.7	4210.1 ± 422.9	-167.3	45.3	-254.8, -74.7	0.001*
Right hippocampus	4288.6 ± 478.3	4330.6 ± 445.5	-145.3	49.1	-239.5, -49.1	0.004*
Left DLPFC	16544.7 ± 2329.4	16617.9 ± 2314.1	-575.7	206.7	-981.8, -172.8	0.006*
Right DLPFC	17089.5 ± 2424.2	17399.2 ± 2396.0	-375.4	225.4	-805.0, 60.2	0.095
Intracranial volume	1628334.7 ± 15635.1	1580817.2 ± 153402.1	-224.8	15635.1	1551117.2, 132667.5	0.014*
Risk allelic load	1.5 ± 0.9	1.4 ± 0.9	0.0	0.1	-0.2, 0.3	0.674

B; unstandardized regression coefficients, SE; standard error, BCa 95% Cls; 95% bias corrected and accelerated confidence intervals

***** Significant values

[#] Adversity data are expressed as mean ± SD CAQ score out of 21

Brain volume data and risk allelic load are expressed as mean ± SD

In multiple linear regression analysis, patients with schizophrenia coded as 1 and healthy controls coded as 0

Supplementary Table S2. Bootstrapped multiple regression analysis of the effect of diagnosis of schizophrenia, childhood adversity and dopaminergic risk allelic load in predicting the volumes of selected brain structures (including covariates of age, gender, scanning location and intracranial volume)

	Comparison					Comparison			
	B	SE	BCa 95% CIs	*p*-value		B	SE	BCa 95% CIs	*p*-value
Left Putamen					*Right Putamen*				
Constant	2464.9	481.0	1563.8, 3348.1	< 0.001	Constant	2724.8	447.6	1888.9, 3523.1	< 0.001
Diagnosis	220.3	68.9	85.4, 362.4	0.002*	Diagnosis	199.0	68.6	64.3, 333.2	0.004*
CAQ	2.5	7.6	-12.7, 17.8	0.716	CAQ	-0.3	7.9	-17.1, 17.4	0.964
RAL	-104.1	36.0	-176.3, -35.7	0.002*	RAL	-63.9	39.1	-139.7, 9.0	0.090
Diagnosis × RAL	83.3	73.5	-72.3, 236.9	0.229	Diagnosis × RAL	54.4	79.3	-110.7, 226.6	0.480
CAQ × RAL	-18.2	8.1	-33.4, -1.6	0.014*	CAQ × RAL	-6.4	9.5	-23.9, 10.9	0.499
Diagnosis × CAQ	-7.5	15.6	-36.8, 23.4	0.610	Diagnosis × CAQ	0.9	16.0	-29.2, 28.3	0.952
CAQ × RAL × Diagnosis	34.1	16.3	0.6, 68.9	0.024	CAQ × RAL × Diagnosis	18.1	19.2	-20.2, 59.4	0.339
Age	-18.2	2.9	-24.2, -12.4	< 0.001	Age	-18.5	2.7	-23.9, -13.1	< 0.001
Gender	-148.8	82.1	-306.3, 15.8	0.072	Gender	-201.4	73.3	-349.8, -51.4	0.006
Scanning Location	48.5	31.2	-13.3, 107.4	0.121	Scanning Location	44.5	28.5	-11.2, 96.8	0.120
Intracranial Volume	0.0	0.0	0.0, 0.1	< 0.001	Intracranial Volume	0.0	0.0	0.0, 0.0	< 0.001
Right DLPFC					*Left DLPFC*				
Constant	3649.1	1672.2	451.7, 6988.0	0.027	Constant	2400.6	1552.8	-540.4, 5169.9	0.125
Diagnosis	-305.5	249.5	-776.2, 151.9	0.209	Diagnosis	-665.3	215.9	-1096.2, -272.4	< 0.001*
CAQ	-17.3	28.6	-72.4,38.2	0.519	CAQ	20.8	24.8	-24.8, 65.0	0.384
RAL	-14.5	104.9	-229.4, 202.4	0.895	RAL	22.3	100.5	-178.9, 257.1	0.812
Diagnosis × RAL	28.0	201.9	-377.5, 441.8	0.884	Diagnosis × RAL	5.3	197.1	-363.6, 349.2	0.981
CAQ × RAL	-16.2	24.2	-65.7, 31.1	0.467	CAQ × RAL	12.1	25.4	-38.5, 59.5	0.612
Diagnosis × CAQ	-2.4	55.9	-109.1, 89.0	0.962	Diagnosis × CAQ	-52.4	49.7	-153.0, 31.2	0.226
CAQ × RAL × Diagnosis	63.9	48.7	-32.4, 158.5	0.150	CAQ × RAL × Diagnosis	-36.9	50.0	-130.1, 57.6	0.424
Age	-63.7	9.6	-83.6, -44.6	< 0.001	Age	-62.1	8.3	-79.3, -44.9	< 0.001
Gender	94.2	264.8	-421.8, 620.9	0.718	Gender	234.1	241.8	-236.9, 711.8	0.344
Scanning Location	52.2	93.5	-131.2, 229.1	0.580	Scanning Location	2.8	86.6	-165.3, 154.3	0.976
Intracranial Volume	0.0	0.0	0.0, 0.0	< 0.001	Intracranial Volume	0.0	0.0	0.0, 0.0	< 0.001

	Comparison					Comparison			
	B	SE	BCa 95% CIs	*p*-value		B	SE	BCa 95% CIs	*p*-value
Left Caudate					*Right Caudate*				
Constant	1558.9	400.6	792.7, 2324.0	< 0.001	Constant	1590.2	425.2	801.3, 2410.4	< 0.001
Diagnosis	-28.2	58.5	-141.7, 76.0	0.621	Diagnosis	-12.7	58.0	-126.1, 97.2	0.820
CAQ	-1.4	7.4	-16.3, 15.5	0.840	CAQ	3.3	7.3	-11.5, 19.4	0.637
RAL	10.5	35.5	-59.1, 73.3	0.751	RAL	-12.1	33.2	-73.4, 43.4	0.707
Diagnosis × RAL	54.2	69.5	-93.3, 225.6	0.423	Diagnosis × RAL	51.4	64.1	-84.2, 208.9	0.411
CAQ × RAL	-1.8	8.8	-20.4, 12.4	0.811	CAQ × RAL	-0.4	8.2	-18.0, 14.1	0.957
Diagnosis × CAQ	25.6	14.5	-2.6, 49.4	0.066	Diagnosis × CAQ	27.3	14.3	-0.1, 53.3	0.047
CAQ × RAL × Diagnosis	-3.0	17.8	-35.1, 46.3	0.859	CAQ × RAL × Diagnosis	-1.6	16.5	-31.7, 46.3	0.916
Age	-13.6	2.3	-18.1, -8.9	< 0.001	Age	-11.8	2.4	-16.7, -6.9	< 0.001
Gender	6.5	62.5	-112.2, 124.3	0.916	Gender	-8.7	61.5	-125.3, 104.8	0.890
Scanning Location	-38.6	22.6	-83.6, 6.4	0.085	Scanning Location	-54.0	24.3	-104.1, -0.7	0.026
Intracranial Volume	0.0	0.0	0.0, 0.1	< 0.001	Intracranial Volume	0.0	0.0	0.0, 0.1	< 0.001
Right Hippocampus					*Left Hippocampus*				
Constant	1726.1	347.2	1055.7, 2372.1	< 0.001	Constant	1575.2	327.0	948.9, 2238.0	< 0.001
Diagnosis	-146.1	51.7	-242.4, -57.1	0.006*	Diagnosis	-183.3	49.8	-274.3, -98.6	< 0.001*
CAQ	-2.1	6.1	-14.1, 8.9	0.723	CAQ	2.6	5.6	-8.7, 13.8	0.623
RAL	26.3	23.9	-20.9, 72.9	0.249	RAL	35.3	24.7	-15.5, 81.9	0.132
Diagnosis × RAL	84.5	48.9	-13.1, 176.1	0.073	Diagnosis × RAL	67.1	49.7	-30.1, 157.3	0.163
CAQ × RAL	-9.1	6.5	-22.1, 2.9	0.133	CAQ × RAL	-5.6	6.2	-17.4, 7.2	0.334
Diagnosis × CAQ	-8.7	12.2	-31.8, 10.9	0.458	Diagnosis × CAQ	-0.1	11.2	-21.4, 17.7	0.993
CAQ × RAL × Diagnosis	9.1	12.6	-14.9, 32.5	0.443	CAQ × RAL × Diagnosis	-3.0	12.2	-26.3, 17.9	0.787
Age	-4.9	2.1	-9.3, -0.6	0.025	Age	-5.9	2.0	-9.8, -2.1	0.003
Gender	-11.7	61.7	-133.8, 114.8	0.853	Gender	13.8	58.3	-102.3, 123.6	0.809
Scanning Location	6.2	19.7	-29.9, 39.1	0.743	Scanning Location	-12.6	18.9	-48.2, 21.1	0.500
Intracranial Volume	0.0	0.0	0.0, 0.1	< 0.001	Intracranial Volume	0.0	0.0	0.0, 0.1	< 0.001

	Comparison					Comparison			
	B	SE	BCa 95% CIs	*p*-value		B	SE	BCa 95% CIs	*p*-value
Left Nucleus Accumbens					*Right Nucleus Accumbens*				
Constant	162.6	67.3	33.4, 282.9	0.017	Constant	241.3	72.0	102.6, 380.1	0.001
Diagnosis	7.3	11.6	-14.0, 27.2	0.531	Diagnosis	0.5	10.5	-18.8, 18.6	0.962
CAQ	0.3	1.4	-2.6, 3.7	0.782	CAQ	-0.9	1.4	-3.6, 2.1	0.485
RAL	-2.2	6.1	-15.1, 8.3	0.695	RAL	-11.5	6.7	-24.4, 1.2	0.076
Diagnosis × RAL	12.6	12.5	-10.6, 40.6	0.288	Diagnosis × RAL	14.6	13.6	-12.2, 43.6	0.262
CAQ × RAL	1.1	1.7	-2.3, 4.1	0.484	CAQ × RAL	-0.8	1.6	-3.9, 1.9	0.579
Diagnosis × CAQ	1.9	2.9	-3.0, 6.1	0.508	Diagnosis × CAQ	2.3	2.8	-2.8, 6.9	0.413
CAQ × RAL × Diagnosis	5.2	3.4	-0.8, 13.1	0.100	CAQ × RAL × Diagnosis	5.4	3.2	-1.1, 12.7	0.075
Age	-2.2	0.4	-3.1, -1.3	< 0.001	Age	-2.2	0.4	-3.1, -1.3	< 0.001
Gender	1.4	12.2	-21.7, 24.3	0.915	Gender	-7.4	12.2	-32.0, 17.9	0.547
Scanning Location	0.8	4.0	-6.5, 8.3	0.830	Scanning Location	-4.4	4.2	-12.8, 2.6	0.300
Intracranial Volume	0.0	3.8E-5	0.0, 0.0	< 0.001	Intracranial Volume	0.0	3.8E-5	0.0, 0.0	< 0.001

B; unstandardized regression coefficients, SE; standard error, BCa 95% Cls; 95%; bias corrected and accelerated confidence intervals, CAQ; childhood adversity questionnaire, RAL; risk allelic load; DLPFC; dorsolateral prefrontal cortex

* Significant values relevant to investigation according to the Benjamini and Yekutieli correction

In multiple linear regression analysis, patients with schizophrenia coded as 1 and healthy controls coded as 0

Supplementary Table S3. Bootstrapped regression analysis of the effect of diagnosis of physical abuse, emotional abuse, emotional neglect and family dysfunction in predicting the volumes of selected brain structures (including covariates of age, gender, scanning location and intracranial volume)

	Comparison					Comparison			
	B	SE	BCa 95% CIs	*p*-value		B	SE	BCa 95% CIs	*p*-value
Physical Abuse					*Emotional Abuse*				
Right Putamen	29.5	67.0	-106, 150	0.644	Right Putamen	-41.9	32.4	-105, 32	0.187
Left Putamen	-6.1	76.1	-158, 144	0.938	Left Putamen	-18.3	33.0	-83, 49	0.561
Right Caudate	157.4	60.6	44, 263	0.008*	Right Caudate	12.1	28.4	-46, 83	0.676
Left Caudate	88.5	60.7	-28, 205	0.134	Left Caudate	-10.7	27.9	-67, 53	0.689
Right Nucleus Accumbens	8.5	14.4	-17, 37	0.560	Right Nucleus Accumbens	-3.4	5.6	-14, 10	0.542
Left Nucleus Accumbens	10.1	12.3	-13, 33	0.402	Left Nucleus Accumbens	0.3	5.8	-10, 13	0.955
Right Hippocampus	17.3	64.8	-103, 133	0.792	Right Hippocampus	-4.1	22.7	-51, 44	0.865
Left Hippocampus	24.9	47.0	-67, 104	0.587	Left Hippocampus	27.3	25.1	-18, 78	0.222
Right DLPFC	-30.0	255.1	-501, 441	0.900	Right DLPFC	-61.9	102.0	-255, 116	0.535
Left DLPFC	300.5	204.4	-71, 627	0.131	Left DLPFC	-38.5	85.1	-207, 107	0.649
Emotional Neglect					*Family Dysfunction*				
Right Putamen	11.7	23.3	-32, 59	0.597	Right Putamen	6.2	22.3	-39, 50	0.776
Left Putamen	8.9	66.6	-38, 55	0.698	Left Putamen	5.8	22.7	-37, 49	0.790
Right Caudate	26.6	20.8	-14, 67	0.191	Right Caudate	23.7	19.0	-15, 64	0.213
Left Caudate	15.1	19.9	-23, 52	0.493	Left Caudate	9.8	18.0	-26, 50	0.587
Right Nucleus Accumbens	-1.4	3.3	-7, 5	0.655	Right Nucleus Accumbens	-2.0	4.1	-9, 6	0.631
Left Nucleus Accumbens	0.4	4.1	-7, 9	0.927	Left Nucleus Accumbens	1.5	3.7	-5, 7	0.689
Right Hippocampus	-4.2	19.5	-38, 26	0.831	Right Hippocampus	-20.0	18.5	-55, 12	0.277
Left Hippocampus	7.0	18.1	-28, 40	0.676	Left Hippocampus	-3.2	18.1	-36, 29	0.864
Right DLPFC	-41.6	82.9	-83, 803	0.139	Right DLPFC	-62.1	78.6	-215, 91	0.429
Left DLPFC	55.0	62.7	-64, 154	0.673	Left DLPFC	15.6	71.3	-118, 140	0.950

B; unstandardized regression coefficients, SE; standard error, BCa 95% Cls; 95%; bias corrected and accelerated confidence intervals; DLPFC; dorsolateral prefrontal cortex
* Significant values according to the Benjamini and Yekutieli correction
In linear regression analysis, patients with schizophrenia coded as 1 and healthy controls coded as 0