

Grey matter, an endophenotype for schizophrenia? A voxel-based morphometry study in siblings of patients with schizophrenia

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Background: Grey matter, both volume and concentration, has been proposed as an endophenotype for schizophrenia given a number of reports of grey matter abnormalities in relatives of patients with schizophrenia. However, previous studies on grey matter abnormalities in relatives have produced inconsistent results. The aim of the present study was to examine grey matter differences between controls and siblings of patients with schizophrenia and to examine whether the age, genetic loading or subclinical psychotic symptoms of selected individuals could explain the previously reported inconsistencies. **Methods:** We compared the grey matter volume and grey matter concentration of healthy siblings of patients with schizophrenia and healthy controls matched for age, sex and education using voxel-based morphometry (VBM). Furthermore, we selected subsamples based on age (< 30 yr), genetic loading and subclinical psychotic symptoms to examine whether this would lead to different results. **Results:** We included 89 siblings and 69 controls in our study. The results showed that siblings and controls did not differ significantly on grey matter volume or concentration. Furthermore, specifically selecting participants based on age, genetic loading or subclinical psychotic symptoms did not alter these findings. **Limitations:** The main limitation was that subdividing the sample resulted in smaller samples for the subanalyses. Furthermore, we used MRI data from 2 different scanner sites. **Conclusion:** These results indicate that grey matter measured through VBM might not be a suitable endophenotype for schizophrenia.

Introduction

The liability for schizophrenia is heritable,¹ with siblings of patients with schizophrenia being at increased risk (about a 10-fold increase) for the disorder.² Structural brain abnormalities in patients with schizophrenia have been consistently reported. For example, patients show reduced grey matter in the frontal, temporal and thalamic regions (see the meta-analyses by Fornito and colleagues³ and Haijma and colleagues⁴). Previous studies have suggested that part of these grey matter abnormalities might not be related to the illness state, but rather to the genetic risk, and have proposed these abnormalities to be an endophenotype for schizophrenia.⁵⁻⁷ Consistent with this proposal, 4 recent meta-analyses reported grey matter reductions in relatives of patients compared with controls.⁸⁻¹¹ Although the aims of these meta-analyses differed, all of them compared individuals at genetic high risk with controls. How-

ever, the results of these meta-analyses differed substantially, and the thresholds applied were rather low ($p < 0.05$, $p < 0.005$ and $p < 0.001$, uncorrected). Two meta-analyses showed grey matter reductions in relatives compared with controls in the lentiform nucleus^{9,11} and medial prefrontal cortex,⁹ whereas another meta-analysis reported higher levels of grey matter in the medial prefrontal cortex in siblings.¹¹ Furthermore, reductions in the parahippocampal gyrus and anterior cingulate have been reported,⁸ while others reported reductions in the amygdala and hippocampus.¹⁰ Besides these contradictory reports, the 3 largest voxel-based morphometry (VBM) studies in siblings¹²⁻¹⁴ did not report any significant differences in whole brain grey matter between siblings and controls.

In a recent review, several hypotheses were proposed to explain these differences.⁵ The first explanation was that many studies included participants who were already past the critical ages for schizophrenia developing. The onset of

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schizophrenia typically starts before the age of 30 years.¹⁵ Siblings who are past this critical age might therefore no longer be at high risk for schizophrenia, which might reduce the likelihood of finding grey matter abnormalities in this group. Second, the risk for schizophrenia increases as genetic load increases.¹⁶ Relatives from families in which schizophrenia is more common probably share more disease-related genes, which might be associated with larger grey matter differences. Therefore, including relatives with only 1 family member with schizophrenia might lead to negative findings, whereas including participants from families with more than 1 affected member with higher genetic loads could reveal substantial grey matter volume differences. Third, although relatives in previous studies did not have diagnoses of schizophrenia, differences in subclinical psychotic symptoms might have influenced the results.⁵ Previous studies have related subclinical symptoms to higher¹⁷ as well as lower levels of grey matter.¹⁸ Given the idea that the experience of subclinical psychotic symptoms tends to be more frequent in family members of patients with schizophrenia than controls,¹⁹ these symptoms may have confounded previous results. Finally, studies examining grey matter have applied different techniques. Some examined grey matter volume, while others looked at grey matter concentration. Grey matter volume is an estimate of volume, whereas grey matter concentration represents the proportion of grey matter relative to all other tissue types in a brain region.²⁰ Previous research has shown that grey matter volume and concentration results do not necessarily overlap.²⁰ Furthermore, a recent meta-analysis reported that in patients with schizophrenia grey matter concentration reductions are larger and more consistent than grey matter volume reductions.³ Therefore, it would be of interest to examine whether the above-mentioned factors have an impact on grey matter measurements in relatives of patients with schizophrenia.

The aim of the present study was to examine the possible grey matter volume and concentration abnormalities in siblings of patients with schizophrenia and to investigate the impact of age, genetic loading and subclinical psychotic symptoms on these findings. We therefore performed a voxel-based morphometry (VBM) analysis on a large group of siblings and controls ($n = 170$) — a larger group than in most grey matter studies reporting significant differences between relatives and controls (for example, see the studies by Hu and colleagues,²¹ Tian and colleagues²² and Oertel-Knöchel and colleagues²³). Based on previous large VBM studies^{12–14} we did not expect to find grey matter differences between the general group of siblings and controls. However, we did expect that selecting siblings younger than 30 years with high genetic loading or high schizotypy scores would reveal significant grey matter abnormalities because of their high risk profile.

Methods

Participants

We reviewed structural T_1 -weighted MRI scans of healthy siblings of patients with schizophrenia and healthy control

participants without first- or second-degree family members with a psychotic disorder. We included all siblings and controls from a multicentre (Groningen and Amsterdam) add-on study from the Genetic Risk & Outcome of Psychosis²⁴ (GROUP) project. Notably, our sample is independent from that of Boos and colleagues.¹⁰ We recruited additional control participants via advertisements in shops and at the university. Participants reported no presence or history of any neurologic or psychiatric disorder, which was confirmed with a diagnostic interview. All participants gave written informed consent before participation. Furthermore, the study was approved by the local medical ethics committee.

Diagnostic interviews

During the assessment of the GROUP study (maximum 2 yr before the MRI scan) participants from Groningen were screened using the Schedules for the Clinical Assessment of Psychiatry (SCAN),²⁵ and participants from Amsterdam were screened using the Comprehensive Assessment of Symptoms and History²⁶ to assess the current psychiatric state and psychiatric history of the participants. Participants with a DSM-IV axis-I mood, anxiety or psychotic disorder were excluded from the study. Prior to the MRI session, participants were asked if there were any changes in their psychological well-being since the last GROUP assessment. If participants reported relevant changes in mood, psychotic symptoms or anxiety for which they sought help or received treatment, they were excluded from the study. The additional sample of healthy controls were screened using the SCAN interview before undergoing MRI.

Community Assessment of Psychic Experiences

The Community Assessment of Psychic Experiences (CAPE) is a 42-item self-report questionnaire used to examine subclinical psychotic symptoms.²⁷ The CAPE measures the frequency of positive, negative and depressive symptoms on a 4-point scale (0 = never; 3 = nearly always). Furthermore, when participants report the experience of symptoms (score ≥ 1 on frequency), they are asked how distressed they are by these symptoms (0 = not at all; 3 = very). Total scores of positive, negative and depressive symptoms are calculated by summing the average frequency and average distress scores. The total score was calculated by summation of all the averaged frequency and distress scores. Participants were divided in a high and low subclinical symptom group by means of a median split on the total score. This median split approach was chosen because of the highly skewed CAPE data and is in line with previous studies examining schizotypy/subclinical psychotic symptoms.^{28,29}

Genetic loading

For all participants of the GROUP study, diagnostic information about family members was assessed using the Family Interview for Genetic Studies (FIGS).³⁰ For details on FIGS

assessment in the GROUP study, see the study by Korver and colleagues.²⁴ In the present study, we used the FIGS to determine which siblings had at least 1 first- or second-degree family member other than the affected sibling who had a psychotic disorder. Furthermore, we selected a subsample of healthy controls who did not report any psychiatric problems in first- or second-degree family members.

MRI

Imaging data were acquired using a 3.0 T scanner (Philips Intera) at the University Medical Center Groningen or at the Academic Medical Center in Amsterdam, the Netherlands. Both systems were equipped with an 8-SENSE head coil, and anatomic images were obtained using a sagittal 3-dimensional T_1 -weighted sequence (176 slices, repetition time 9 ms, echo time 3.5 ms, field of view 256 mm, voxel size $1 \times 1 \times 1$ mm, slice thickness 1.0 mm).

Statistical analysis

Demographic data were analyzed using SPSS software version 20 (SPSS Inc). We performed 2-sample t tests to examine possible differences between the controls and siblings in age, education, handedness (as measured with the Edinburgh Handedness Inventory [EHI]³¹), subclinical psychotic symptoms and total brain volume. We conducted χ^2 tests to examine possible group differences in sex and scan site. The same tests were used to examine possible group differences between the participants from Groningen and those from Amsterdam in age, education, handedness, total brain volume and sex. We considered results to be significant at $p < 0.05$, 2-sided.

Imaging data were analyzed with unified VBM using Statistical Parametric Mapping (www.fil.ion.ucl.ac.uk) running under Matlab7 (MathWorks Inc). Before processing the data, we checked all images for artifacts, and the image origins were manually set at the anterior commissure. Subsequently, images were segmented into grey matter, white matter and cerebrospinal fluid. We used the DARTEL approach for optimal registration of individual segments to a group mean template. For the grey matter volume analyses, the grey matter segments were modulated by the Jacobian determinants to correct for volume changes in nonlinear normalization. For the grey matter concentration analyses, the grey matter segments were not modulated. The DARTEL-normalized modulated and unmodulated grey matter segments were further normalized to the Montreal Neurological Institute (MNI) space and smoothed using an 8 mm full-width at half-maximum Gaussian kernel. An 8 mm smoothing kernel is optimal for detecting morphometric differences in both large and small neural structures.^{32,33}

Data were analyzed in the context of a general linear model (GLM). Group was included as a dependent variable, and sex, scanner site, age and EHI scores were included as covariates to adjust for their effects on regional brain tissue volumes. Whole brain volume (calculated as the sum of grey and white matter) was entered as a global measure by means of proportional scaling. To examine the effect of age,

we used an additional GLM including only the participants younger than 30 years. In addition, we compared the high genetic loading sibling group with the low genetic loading control group. To examine the effect of subclinical symptoms on grey matter, we created a full factorial model with 2 factors (control/sibling and high/low). Both the grey matter differences between high and low groups as well as the subclinical psychotic symptom \times group interaction (F test, $p < 0.001$, $k > 20$) were examined. All above-mentioned analyses were performed twice, once for grey matter volume and once for grey matter concentration. A grey matter majority optimal threshold mask, created based on the whole sample, was applied to all analyses to eliminate voxels of non-grey matter.³⁴ The abovementioned group comparisons were repeated without including any covariates in the models to examine the possible effect of the covariates on the results. To examine main effects of scanner site, we performed a 2-sample t test between the scanner sites in Groningen and Amsterdam in which whole brain volume was entered as a global measure by means of proportional scaling.

The threshold for all whole brain analyses was set at $p < 0.05$, family-wise error (FWE)-corrected at the cluster level (corrected for nonstationarity of smoothness for VBM data) with an initial voxel threshold of $p < 0.001$.^{35,36} Furthermore, all the above-mentioned analyses also involved region of interest (ROI) analyses. The ROIs were chosen based on previously reported regions in meta-analyses examining grey matter in relatives of patients with schizophrenia:^{8–11} amygdala, anterior cingulate, fusiform gyrus, hippocampus, inferior temporal gyrus, insula, lentiform nucleus (consisting of the putamen, pallidum and thalamus), medial frontal gyrus and parahippocampus, as defined by the Automated Anatomic Labelling system implemented in the Wake Forest University PickAtlas (<http://fmri.wfubmc.edu/software/PickAtlas>). Results from these ROI analyses had to meet a threshold of $p < 0.009$, FWE-corrected for the spatial extent of the ROI to be considered significant. This threshold was chosen to correct for the number of ROIs (i.e., 9) while taking into account their independence from the dependent measure (i.e., total grey matter volume of AAL masks: mean correlation $r = 0.23$, corrected for total brain volume; www.quantativeskills.com/sisa/index.htm).

Results

Demographic and behavioural results

We included 95 healthy siblings of patients with schizophrenia and 75 controls without first- or second-degree family members with a psychotic disorder in our study. All 95 siblings and 51 controls were from the GROUP project, and the remaining 24 controls were recruited via advertisements. Eight participants (4 controls and 4 siblings) were excluded because of poor data quality. Furthermore, 3 participants (2 controls and 1 sibling) were excluded because they were identified as outliers after the homogeneity check (VBM8 toolbox version 435, <http://dbm.neur.uni-jena.de/vbm>). The final sample therefore consisted of 69 controls and 89 siblings. The demographic characteristics of the final sample are shown in Table 1. The controls and siblings

did not differ significantly in sex, age, education and handedness (Table 1). Furthermore, no differences were found between the groups on subclinical psychotic symptoms as measured with the CAPE (Table 1). No significant differences on any of the above-mentioned variables were found between the participants from Groningen and those from Amsterdam (Table 2).

VBM results

No significant differences in total brain volume were found between the 2 groups (Table 1) or between the 2 scanner sites (Table 2).

The VBM results revealed no significant regional differences in grey matter volume or concentration between the siblings and healthy controls in the whole brain analyses. Furthermore, no significant differences were found in the selected ROIs, except for a higher grey matter volume in the anterior cingulate cortex (ACC) in siblings compared with controls (MNI coordinates: $x, y, z = 8, 33, 15$; $k = 429, Z = 3.72, p = 0.012$). However, this finding did not survive the multiple comparison threshold ($p < 0.009$). No grey matter volume or concentration differences were found between the scans obtained in Groningen and those obtained in Amsterdam.

Age

Selecting participants aged younger than 30 years resulted in a subsample of 33 controls and 40 siblings. These 2 groups did not differ in sex, age, education, handedness, scanner site and CAPE scores (all $p > 0.21$). The VBM results did not reveal any significant group differences on grey matter volume or concentration in the whole brain or the ROI analyses.

Genetic loading

Selecting siblings with at least 1 additional first- or second-degree family member with a psychotic disorder resulted in a subgroup of 20 siblings. These 20 siblings were compared

with 21 healthy controls with no reports of any psychiatric problems in first- or second-degree family members. The groups did not differ in sex, age, education, handedness or scanner site (all $p > 0.11$). However, there was a small, but nonsignificant, difference in the CAPE positive symptom scores, with higher median scores in the sibling group than in the control group (0.61 v. 0.27, $p = 0.08$). The VBM results did not show significant grey matter volume or concentration differences between the high genetic risk siblings and the low genetic risk controls in the whole brain or the ROI analyses.

Subclinical psychotic symptoms

Owing to missing or incomplete CAPE data, 6 controls and 7 siblings had to be excluded from the analysis, leaving a sample of 63 controls and 82 siblings; they did not differ on demographic variables (all $p > 0.25$). Furthermore, siblings and healthy controls did not differ in CAPE scores (Table 1). To subdivide the groups based on high and low subclinical symptoms, we performed a median split based on total median CAPE scores per group (2.4 in the control group v. 2.4 in the sibling group). The resulting high subclinical symptoms group ($n = 72$) did not differ from the low subclinical symptoms group ($n = 73$) in sex, age, education, handedness and scanner site (all $p > 0.43$). The high and the low subclinical symptoms groups did differ in median CAPE positive symptoms (0.59 v. 0.13, $p < 0.001$), negative symptoms (1.7 v. 0.49, $p < 0.001$), depressive symptoms (1.9 v. 0.67, $p < 0.001$) and CAPE total scores (4.2 v. 1.3, $p < 0.001$). The full factorial analysis revealed that high subclinical symptom individuals did not differ significantly from low subclinical symptom individuals on grey matter volume or concentration. Furthermore, the interaction analysis did not reveal a CAPE \times group interaction on grey matter volume or concentration in the whole brain or the ROI analyses (F test, $k < 20, p < 0.001$).

Repeating the analyses without including sex, scanner site, age and handedness as covariates did not significantly alter any of the above-mentioned findings.

Table 1: Group differences between healthy controls and unaffected siblings of patients with schizophrenia on demographic variables, total brain volume and schizotypy

Variable	Group, mean \pm SD*		Test	p value
	Control, $n = 69$	Siblings, $n = 89$		
Male sex, no. (%)	38 (55)	41 (46)	$\chi^2 = 1.26$	0.26
Age, yr	33.5 \pm 10.2	32.1 \pm 8.1	$t = 0.93$	0.36
Education, yr	6.1 \pm 0.8	5.9 \pm 0.8	$t = 1.23$	0.22
Scan site, no. (%) Amsterdam	28 (41)	44 (50)	$\chi^2 = 1.23$	0.27
Handedness, % right	87	82	—	—
EHI	65.3 \pm 48.6	60.6 \pm 52.8	$t = -0.57$	0.57
Total brain volume	986 \pm 65	982 \pm 63	$t = 0.33$	0.74
CAPE†				
Positive symptoms	0.33 \pm 0.54	0.40 \pm 0.61	$t = -0.70$	0.48
Negative symptoms	1.03 \pm 0.85	1.15 \pm 0.86	$t = -0.82$	0.41
Depressive symptoms	1.30 \pm 0.93	1.35 \pm 0.88	$t = -0.31$	0.76
Total score	2.70 \pm 1.97	2.90 \pm 1.99	$t = -0.59$	0.56

CAPE = Community Assessment of Psychic Experiences; EHI = Edinburgh Handedness Inventory; SD = standard deviation.

*Unless otherwise indicated.

†Based on a subsample of 63 controls and 82 siblings.

Discussion

The aim of the present study was to examine putative grey matter differences between siblings of patients with schizophrenia and controls and to investigate whether the previously suggested factors age, genetic loading and subclinical psychotic symptoms influence these grey matter differences. The results revealed no significant differences in grey matter volume or concentration between siblings and controls. Furthermore, selecting subsamples for analyses based on age, genetic loading or subclinical psychotic symptoms did not alter our findings.

The finding of nonsignificant grey matter differences between siblings and controls is in accordance with results of 3 previous large studies on grey matter in relatives of patients with schizophrenia.^{12–14} This agreement suggests that enhanced genetic risk for schizophrenia might not be related to substantial differences in grey matter. Although large studies have been unable to find grey matter alterations in relatives of patients with schizophrenia, smaller studies with lower thresholds have often reported grey matter differences between relatives and controls,^{23,37} which may explain the positive (albeit inconsistent) effects found in previous meta-analyses.^{8–11} These discrepancies have previously been proposed to be due to differences in age, genetic loading and subclinical psychotic symptoms.⁵ However, our results indicate that these factors might not explain these discrepancies.

Our results revealed that selecting participants younger than 30 years — a critical age in the development of schizophrenia — did not result in grey matter differences between controls and siblings, indicating that the null finding in the total sample was not caused by including siblings older than 30 years. This finding is consistent with that of a previous study in which including participants younger than 30 years also did not result in significant grey matter differences between controls and relatives of patients with schizophrenia.¹⁴

Previous studies have examined individuals at high genetic risk for schizophrenia. However, the present study is, as far as we know, the first VBM study comparing individuals at high genetic risk (e.g., 1 affected sibling and at least 1 other affected first- or second-degree family member) to low-risk controls with no reported psychiatric problems in first- or second-degree family members. Our results showed that even when comparing a high genetic risk group of siblings and a low genetic risk group of controls, no grey matter vol-

ume or concentration differences were found. This finding is in line with those of studies that were unable to find grey matter differences in unaffected monozygotic twins of patients with schizophrenia,^{38,39} who are at the highest possible genetic risk for schizophrenia.

The present findings revealed no significant CAPE \times group (control or siblings) interaction on grey matter. Previous studies have reported grey matter differences related to subclinical symptoms.^{17,18} However, the present study is, as far as we know, the first to examine the symptoms \times genetic risk interaction on grey matter. The fact that no significant interaction was found indicates that subclinical symptoms might not interact with group on grey matter. In line with this finding, Boos and colleagues¹⁰ proposed that solely subclinical symptoms might be more related to grey matter differences than the genetic risk for psychosis. However, our findings did not reveal any significant grey matter abnormalities related to subclinical psychotic symptoms, whereas previous studies have reported both higher¹⁷ and lower¹⁸ levels of grey matter in high subclinical symptom groups. These conflicting findings indicate that more research on the association between subclinical psychotic symptoms and grey matter is necessary, as the results to date are inconclusive.

We are not certain whether the affected siblings of our genetic risk group had grey matter abnormalities, as this was not investigated. However, previous reports have reliably documented grey matter abnormalities in patients with schizophrenia.³⁴⁰ Furthermore, in a study by Boos and colleagues¹³ grey matter abnormalities in patients were reported, whereas their nonaffected siblings did not show these abnormalities.

Our study indicates that grey matter measured through VBM might not be a suitable endophenotype for schizophrenia. One important aspect of an endophenotype is that it should be present in unaffected relatives of patients. The present study and previous reports^{12–14} question the idea that grey matter abnormalities are present in relatives of patients with schizophrenia. However, our ROI analysis did reveal significantly higher grey matter volume in the ACC in siblings of patients with schizophrenia. This finding is inconsistent with previous reports of lower ACC volume in relatives of patients with schizophrenia.^{12,14} However, these studies found lower ACC volume only when lowering the significance thresholds or when performing ROI analyses. Furthermore, others have failed to show any volumetric differences in the ACC.^{21,41} The lack of reproducibility of these

Table 2: Group differences between participants from the 2 scanner sites on demographic variables and total brain volume

Variable	Control, n = 69		Test	p value	Siblings, n = 89		Test	p value
	Groningen, n = 41	Amsterdam, n = 28			Groningen, n = 45	Amsterdam, n = 44		
Male sex, no. (%)	21 (51)	17 (61)	$\chi^2 = 0.61$	0.47	22 (49)	19 (43)	$\chi^2 = 0.29$	0.59
Age, mean \pm SD, yr	33.7 \pm 10.7	33.2 \pm 9.6	$t = 0.20$	0.85	30.9 \pm 7.9	33.2 \pm 8.3	$t = -1.3$	0.19
Education, mean \pm SD, yr	6.0 \pm 0.7	6.1 \pm 0.8	$t = -0.44$	0.66	5.8 \pm 0.8	6.0 \pm 0.9	$t = -0.87$	0.39
Handedness, % right	68.0 \pm 44.9	61.3 \pm 54.2	$t = 0.56$	0.58	63.1 \pm 46.5	58.1 \pm 59.0	$t = 0.45$	0.65
Total brain volume, mean \pm SD	986 \pm 59	984 \pm 75	$t = 0.15$	0.88	981 \pm 58	983 \pm 69	$t = -0.14$	0.89

SD = standard deviation.

and other previously reported findings, as indicated by the different results in 4 separate meta-analyses^{8–11} raises further doubts about the value of grey matter as an endophenotype for schizophrenia. For example, only 1 of 4 meta-analyses reported lower ACC volume in siblings of patients with schizophrenia.⁸ This divergent finding may not be fully explained by differences in age, subclinical psychotic symptoms and genetic loading, because specifically selecting participants based on these factors did not reveal any grey matter differences between siblings and controls in the present study. One possibility could be that only specific schizophrenia related genes are associated with grey matter abnormalities, such as aberrant ACC volume. For example, previous research has shown that *DISC1* risk allele carriers have lower grey matter volume in the ACC,⁴² and *CNNM2* risk allele carriers have higher ACC volume.⁴³ Future research should examine whether these genetic variations can explain the divergent findings on grey matter abnormalities in relatives of patients with schizophrenia.

Limitations

Several limitations of the present study need to be addressed. First, by subdividing our sample, the group sizes for our subanalyses became smaller. In particular, the analysis on genetic loading had lower power than the other analyses. However, the power of this analysis was still sufficient to detect medium effects.⁴⁴ Furthermore, selecting based on high versus low genetic loading increased the sensitivity to detect differences related to genetic risk. Hence, smaller sample sizes would be sufficient to detect grey matter differences between these groups. Second, the group was too small to select participants based on 2 factors combined (e.g., age < 30 yr and high genetic risk). Future research should consider selecting participants specifically based on the combination of these factors to examine whether this has an effect on grey matter. Third, participants were scanned using 2 different scanners. Although the reliability of multiscanner VBM has proven to be good when adding scanner as a covariate,^{45,46} including scan site as a covariate may have lowered the statistical power of detecting between-group differences. Therefore, we repeated the analyses without including any covariates, which did not change the results. Fourth, all participants with an axis-I mood, anxiety or psychotic disorder were excluded from our study. This selection method may have resulted in excluding the most vulnerable siblings. However, this method was chosen to ensure that possible grey matter abnormalities were not due to comorbid psychiatric disorders. Our findings show that the differences between previously reported findings may not be explained by differences in age, genetic loading or subclinical psychotic symptoms among studies or by examining either grey matter volume or concentration. However, it is still possible that methodological differences (e.g., T_1 acquisition, VBM method, correction for total brain volume) may explain these divergent findings in the literature. We therefore encourage future research on the possible influence of these methodological differences.

Conclusion

The present study provides further support for the hypothesis that grey matter volume or concentration, as measured with VBM, might not be an endophenotype for schizophrenia and that it might be more related to the illness itself. Future research should focus more on brain connectivity and functional neuroimaging as possible endophenotypes, as these seem to differ more consistently across unaffected relatives of patients with schizophrenia.^{47,48} Furthermore, research should examine the role of specific genetic variations on grey matter, specifically in the ACC.

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