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

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ABSTRACT

BACKGROUND: Gray matter, both volume and concentration, has been proposed as an endophenotype for schizophrenia given a number of reports of gray matter abnormalities in relatives of patients with schizophrenia. However, previous studies on gray matter abnormalities in relatives have produced inconsistent results. The aim of the current study was to examine gray matter differences between controls and siblings of patients with schizophrenia, and to examine whether the age, genetic loading or schizotypy scores of selected individuals could explain the previous reported inconsistencies.

METHODS: To this extent, 89 healthy siblings of patients with schizophrenia and 69 healthy controls, matched for age, gender and education, were compared on gray matter volume and gray matter concentration using voxel-based morphometry (VBM). Furthermore, subsamples were selected based on age (below 30 years old), genetic loading and schizotypy to examine whether this would lead to different results.

RESULTS: The results showed that siblings and controls did not significantly differ on gray matter volume nor concentration. Furthermore, specifically selecting subjects on age, genetic loading or schizotypy did not alter these findings.

CONCLUSION: These results indicate that gray matter measured through VBM might not be a suitable endophenotype for schizophrenia.
INTRODUCTION

The liability for schizophrenia is heritable (Cardno et al., 1999) with siblings of patients with schizophrenia being at increased risk (around tenfold increase) for developing schizophrenia (Gottesman, 1991). Structural brain abnormalities in patients with schizophrenia have been consistently reported. For example, patients show reduced gray matter (GM) in the frontal, temporal and thalamic regions (for meta-analyses see Fornito et al., 2009; Haijma et al., 2013). Previous studies have suggested that part of these GM abnormalities might not be related to the illness state, but to the genetic risk, and proposed these abnormalities to be an endophenotype for schizophrenia (Moran et al., 2013; Prasad and Keshavan, 2008; Turner et al., 2012). Consistent with this proposal, four recent meta-analyses reported GM reductions in relatives of patients compared to controls (Boos et al., 2007; Cooper et al., 2014; Fusar-Poli et al., 2014b; Palaniyappan et al., 2012). Although the aims of these meta-analyses differed, all meta-analyses compared subjects at genetic high risk with controls. However, the results between these meta-analyses differed substantially and the thresholds applied were rather low (p<.05, p<.005 and p<.001, uncorrected). Two meta-analyses showed GM reductions in relatives compared to controls in the lentiform nucleus (Cooper et al., 2014; Palaniyappan et al., 2012) and medial prefrontal cortex (Palaniyappan et al., 2012), whereas another meta-analysis reported higher levels of GM in the medial prefrontal cortex in siblings (Cooper et al., 2014). Furthermore, reductions in the parahippocampal gyrus and anterior cingulate have been reported (Fusar-Poli et al., 2014b), while others reported reductions in the amygdala and hippocampus (Boos et al., 2007). Besides these contradictory reports, the three largest voxel-based morphometry (VBM) studies in siblings of patients (Boos et al., 2012; Honea et al., 2008; Job et al., 2003) did not report any significant differences in whole brain GM between siblings and controls.

In a recent review, several hypotheses were proposed to explain these differences (Moran et al., 2013). The first explanation was that many studies included subjects which already past the critical ages for developing schizophrenia. The onset of schizophrenia typically starts before the age of 30 (Beratis et al., 1994). Siblings who are past this critical age might therefore not be at high risk for schizophrenia anymore, which might reduce the likelihood of finding GM abnormalities in this group. Second, the risk of developing schizophrenia increases as genetic load increases (Keshavan et al., 2005). Relatives from families in which schizophrenia is more common probably share more disease-related genes, which might be associated with larger GM differences. Therefore, including relatives with only one family member with schizophrenia might lead to negative findings while including subjects from multiple affected families, with higher genetic loads, could lead to substantial GM volume differences. Third, although relatives in previous studies were not diagnosed with schizophrenia, differences in subclinical psychotic symptoms might have influenced the results (Moran et al., 2013). Previous studies have related subclinical symptoms to higher (Modinos et al., 2010a) as well as lower GM (Ettinger et al., 2012). Given the idea that the experience of subclinical psychotic symptoms tends to be higher in family members of patients with schizophrenia (Vollme et al., 2002), these symptoms may have confounded previous results. Finally, studies examining GM have applied different techniques. Some examined GM volume (GMV), while others looked at GM concentration (GMC). GMV represents an estimate of the volume of GM, whereas GMC represents the proportion of GM relative to all other tissue types in a region (Taki et al., 2013). Previous research has shown that GMV and GMC results do not necessarily overlap (Taki et al., 2013). Furthermore, a recent meta-analysis reported that in patients with schizophrenia GMC, reductions are larger and more consistent compared to GMV reductions (Fornito et al., 2009). Therefore, it would be of interest to examine whether abovementioned factors have an impact on gray
matter measurements in relatives of patients with schizophrenia.

The aim of the current study was to examine the possible GMV and GMC 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 VBM analysis on a large group of siblings and controls (n=170), larger than most GM studies reporting significant differences between relatives and controls (e.g. Hu et al., 2013; Oertel-Knöchel et al., 2012; Tian et al., 2011). Based on previous large VBM studies (Boos et al., 2012; Honea et al., 2008; Job et al., 2003) we did not expect to find GM differences between the general group of siblings and controls. However, we did expect that selecting siblings below the age of 30, with high genetic loading or high schizotypy scores, would result in significant GM abnormalities because of their higher risk profile.

METHODS

Participants

Structural T1-weighted MRI scans of 95 healthy siblings of patients with schizophrenia and 75 healthy control subjects without first or second degree family members with a psychotic disorder were included. All 95 siblings and 51 healthy controls were included from a multi-center (Groningen and Amsterdam) add-on study from the GROUP project [Genetic Risk & Outcome of Psychosis (Korver et al., 2012)]. Notably, our sample is independent from Boos et al. (2012). An additional number of 24 control subjects were recruited via advertisements in shops and at the university. Participants reported no presence or history of any neurological or psychiatric disorder, which was confirmed with a diagnostic interview. All participants gave written informed consent prior to participation. Furthermore, the study was approved by the local medical ethical committee.

Behavioral measurements

Diagnostic interviews

During the assessment of the GROUP study (max. two years prior to the MRI scan) participants from Groningen were screened with the SCAN interview [Schedules for the Clinical Assessment of Psychiatry (Wing et al., 1990)] and participants from Amsterdam with the CASH [Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992)] to assess the current psychiatric state and psychiatric history of the participants. Participants with an 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 with the SCAN interview prior to the MRI scan.

Community Assessment of Psychic Experiences

The Community Assessment of Psychic Experiences (CAPE) is a 42-item self-report questionnaire used to examine schizotypy (Stefanis et al., 2002). 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
of 1 or more 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 score. The schizotypy total score was calculated by summation of all the averaged frequency and distress scores. Subjects were divided in a high and low schizotypy group by means of a median split on the total schizotypy score. This median split approach is in line with previous studies examining schizotypy (e.g. Nitzburg et al., 2014; van Dongen et al., 2011).

Genetic loading
From all participants of the GROUP study, diagnostic information about family members was assessed with the Family Interview for Genetic Studies (FIGS; Maxwell, 1992). For details on FIGS assessment in the GROUP study see Korver et al. (2012). In the current study the FIGS was used to examine which siblings had at least one other first- or second degree family member with a psychotic disorder, besides their affected sibling. Furthermore, a subsample of healthy controls was selected whom did not report any psychiatric problems in first- or second degree family members.

Image acquisition
Imaging data were acquired using 3.0 Tesla magnetic resonance imaging systems (Philips Intera, Best, NL) located at the University Medical Center Groningen and at the Academic Medical Center in Amsterdam. Both systems were equipped with an 8-SENSE head coil and anatomical images were obtained using a sagittal 3-dimensional T1-weighted sequence (176 slices; TR=9 msec; TE=3.5 msec; FOV=256 mm, voxel size=1 x 1 x 1 mm; slice thickness=1.0 mm).

Statistical analyses
Demographic data were analyzed using SPSS 20 (SPSS Inc, Chicago, Illinois). Two-sample t-tests were calculated to examine possible differences between the controls and siblings on age, education, handedness (as measured with the Edinburgh Handedness Inventory (EHI); Oldfield, 1971), subclinical psychotic symptoms (schizotypy) and total brain volume. Chi-square tests were applied to examine possible group differences on gender and scan site. The same tests were used to examine possible group differences between the subjects from Groningen and Amsterdam on age, education, handedness, total brain volume and gender. Significance was set at p<.05, two-sided.

Imaging data were analyzed with unified voxel-based morphometry (VBM) using Statistical Parametric Mapping (SPM8) (http://www.fil.ion.ucl.ac.uk) running under Matlab7 (The MathWorks Inc., Natick, MA, USA). Before processing the data, all images were checked for artifacts and the image origins were manually set at the anterior commissure. Subsequently, images were segmented into gray matter, white matter, and cerebrospinal fluid. The Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) approach was used for optimal registration of individual segments to a group mean template. For the GMV analyses, the GM segments were modulated by the Jacobian determerts to correct for volume changes in nonlinear normalization. For the GMC analyses, the GM segments were not modulated. DARTEL normalized modulated and un-modulated GM segments were further normalized to the Montreal Neurological Institute (MNI) space.
and smoothed using an 8 mm full width half maximum (FWHM) Gaussian kernel. An 8 mm smoothing kernel is optimal for detecting morphometric differences in both large and small neural structures (Honea et al., 2005; White et al., 2001).

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 effect on regional brain tissue volumes. Whole brain volume (calculated as the sum of gray and white matter) was entered as a global by means of proportional scaling. To examine the effect of age, an additional GLM was performed including only the participants aged below 30. Furthermore, the high genetic loading sibling group was compared with the low genetic loading control group. To examine the effect of schizotypy on GM, a full factorial model was created with two factors (HC/sibling and high/low schizotypy). Both the GM differences between high and low schizotypy as well as the schizotypy*group interaction (F-test, \( p<0.001 \), \( k>20 \)) were examined. All abovementioned analyses were performed twice, once for GMV and once for GMC. A GM majority optimal threshold mask, created based on the whole sample, was applied to all analyses to eliminate voxels of non-GM (Ridgeway et al., 2009). 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, a two-sample t-test was performed between the scanner sites in Groningen and Amsterdam in which whole brain volume was entered as a global by means of proportional scaling.

The threshold for all whole-brain analyses was set at \( p<0.05 \) Familial-Wise Error (FWE) corrected at the cluster level (corrected for non-stationary of smoothness characteristic for VBM data) with an initial voxel threshold of \( p<0.001 \) (Hayasaka et al., 2004; Woo et al., 2014). Furthermore, region of interest (ROI) analyses were performed in all abovementioned analyses. The ROIs were chosen based on previous reported regions in meta-analyses examining GM in relatives of patients with schizophrenia (Boos et al., 2007; Cooper et al., 2014; Fusar-Poli et al., 2014b; Palaniyappan et al., 2012). The selected ROIs were the amygdala, anterior cingulate, fusiform gyrus, hippocampus, inferior temporal gyrus, insula, lentiform nucleus (consisting of the putamen, pallidum and thalamus), medial frontal gyrus and parahippocampus. The mask were created based on the Automated Anatomical Labeling system implemented in the WFU pickatlas (http://fmri.wfubmc.edu/software/PickAtlas). Results from these ROI analyses had to meet \( p<0.009 \) FWE corrected for the spatial extent of the ROI to be considered significant. This \( p<0.009 \) was chosen to correct for the number of ROIs (i.e. 9), while taking into account their non-independency of the dependent measure [i.e. total GM volume of AAL masks: mean correlation \( r=0.23 \), corrected for total brain volume (http://www.quantativeskills.com/sisa/index.htm)].

|RESULTS|

Demographic and behavioral results

The healthy controls and siblings did not differ significantly on gender, age, education, and handedness (see Table 6.1). Furthermore, no differences were found between the two groups on subclinical psychotic symptoms as measured with the CAPE (see Table 6.1). No significant differences on gender, age, education and handedness were found between the subjects from Groningen and the subjects from Amsterdam (see Table 6.2).
VBM-results

Eight subjects (4 controls and 4 siblings) were excluded because of poor data quality. Furthermore, three subjects (2 controls and 1 sibling) were excluded because they were identified as outliers by 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 (for demographic details of this final sample see Table 6.1). No significant differences in total brain volume were found between the two groups (see Table 6.1) nor the two scanner sites (see Table 6.2).

The VBM results revealed no significant regional differences in GMV nor GMC 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 ACC volume in siblings compared to controls \([p=.012; k=429; Z=3.72; 8,33,15 (x,y,z)]\). However, this finding did not survive the multiple comparison threshold \((p<.009)\). No GMV nor GMC differences were found between the scans made in Groningen and the scans made in Amsterdam.

**Age < 30**

Selecting participants aged below 30 resulted in 33 controls and 40 siblings. The two groups did not differ on gender, age, education, handedness (EHI), scanner site and CAPE scores (lowest \(p=.21\)). The VBM results did not reveal any significant group differences on GMV nor GMC in the whole brain or the ROI analyses.

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| Table 6.1 | Mean, standard deviations and group differences between healthy controls and siblings on demographic variables, total brain volume and schizotypy |
|---|---|---|---|
| | HC (n=69) | Siblings (n=89) | Test statistic |
| Gender (% male) | 38 (55%) | 41 (46%) | \(\chi^2 = 1.26\) | \(p = .26\) |
| Age (in years) | 33.5 ± 10.2 | 32.1 ± 8.1 | \(t = .93\) | \(p = .36\) |
| Education* | 6.1 ± 0.8 | 5.9 ± 0.8 | \(t = 1.23\) | \(p = .22\) |
| Scan site (% Amsterdam) | 28 (41%) | 44 (50%) | \(\chi^2 = 1.23\) | \(p = .27\) |
| Handedness | | | |
| % right | 87% | 82% | |
| EHI | 65.3 ± 48.6 | 60.6 ± 52.8 | \(t = .57\) | \(p = .57\) |
| Total brain volume | 986 ± 65 | 982 ± 63 | \(t = .33\) | \(p = .74\) |

* Scoring according to Verhage, 1964; Abbreviations: CAPE: Community Assessment of Psychic Experiences; EHI: Edinburgh handedness inventory; HC: healthy controls

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**HC (n=69) Siblings (n=89)**

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<th>Test statistic</th>
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<td>(\chi^2 = 1.26)</td>
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<td>(t = .93)</td>
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Genetic loading

Selecting siblings with at least one additional family member with a psychotic disorder in first- or second-degree family members, resulted in 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 on gender, age, education, EHI, and scanner site (lowest p=.11). However, there was a small, but not significant, difference in the CAPE positive symptoms scores (p=.08) with higher scores in the sibling group (Mcontrols=.27; Msiblings=.61). The VBM results did not show significant GMV or GMC differences between the high genetic risk siblings and the low genetic risk controls in the whole brain or the ROI analyses.

Schizotypy

Due to missing or incomplete CAPE data, six controls and seven siblings had to be excluded from this analysis, leaving 63 controls and 82 siblings not differing on demographic variables (lowest p=.25). Furthermore, siblings and healthy controls did not differ in CAPE scores (see Table 6.1). To divide the groups in high and low schizotypy, a median-split based on total CAPE scores was performed per group (Mediancontrols=2.4; Mediansiblings=2.4). The resulting high schizotypy group (n=72) did not differ from the low schizotypy group (n=73) on gender, age, education, EHI, and scanner site (lowest p=.43). As provided, these two groups did differ on CAPE positive symptoms (Mhigh=.59; Mlow=.13; p<.001), negative symptoms (Mhigh=1.7; Mlow=4.9; p<.001), depressive symptoms (Mhigh=1.9; Mlow=.67; p<.001), and CAPE total scores (Mhigh=4.2; Mlow=1.3; p<.001). The full-factorial analysis revealed that high schizotypal individuals had larger right precuneus/posterior cingulate volume compared to low schizotypal individuals [p=.03; k=730; Z=4.45; 6,-40,48 (x,y,z)], but no differences were found in GMC. Furthermore, the interaction analysis did not reveal a schizotypy*group interaction on GMV nor GMC in the whole brain or the ROI analyses (F-test, k<20 on p<.001).

Repeating abovementioned analyses without including the covariates (sex, scanner site, age and handedness) did not significantly alter the findings.

|Table 6.2 Mean, standard deviations and group differences between the subjects from the two scanner sites on demographic variables and total brain volume|

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<th>HC (n=69)</th>
<th>Siblings (n=89)</th>
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<tr>
<td></td>
<td>Groningen (n=41)</td>
<td>Amsterdam (n=28)</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>21 (51%)</td>
<td>17 (61%)</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>33.7 ± 10.7</td>
<td>33.2 ± 9.6</td>
</tr>
<tr>
<td>Education*</td>
<td>6.0 ± .7</td>
<td>6.1 ± .8</td>
</tr>
<tr>
<td>Handedness EHI</td>
<td>68.0 ± 44.9</td>
<td>61.3 ± 54.2</td>
</tr>
<tr>
<td>TBV</td>
<td>986 ± 59</td>
<td>984 ± 75</td>
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* Scoring according to Verhage, 1964; Abbreviations: EHI: Edinburgh Handedness Inventory; HC: healthy controls; TBV: total brain volume
DISCUSSION

The aim of the current study was to examine putative GM differences between siblings of patients with schizophrenia and controls and to investigate whether previously suggested factors as age, genetic loading and schizotypy influence these GM differences. The results revealed no significant differences in GMV nor GMC between siblings and controls. Furthermore, selecting specifically on age, genetic loading or schizotypy did not alter these findings, although there was an effect for schizotypy across groups.

The finding of non-significant GM differences between siblings and controls is in accordance with three previous large studies on GM in relatives of patients with schizophrenia (Boos et al., 2012; Honea et al., 2008; Job et al., 2003). This suggests that enhanced genetic risk for developing schizophrenia might not be related to substantial differences in GM. Although large studies are unable to find GM alterations in relatives of patients with schizophrenia, smaller studies with lower thresholds often do report GM differences between relatives and controls (e.g. Lui et al., 2009; Oertel-Knöchel et al., 2012) which may explain the positive (albeit inconsistent) effects found in previous meta-analyses (Boos et al., 2007; Fusar-Poli et al., 2014b; Palaniyappan et al., 2012). These discrepancies were previously proposed to be due to differences in age, genetic loading, and schizotypy (Moran et al., 2013). However, the current results indicate that these factors might not explain these discrepancies.

The results revealed that selecting young participants, below the critical ages of developing schizophrenia (<30), did not result in GM differences between controls and siblings, indicating that the null finding in the total sample was not caused by including siblings above the age of 30. This finding is consistent with a previous study in which including subjects ages below 30 also did not result in significant GM differences between controls and relatives of schizophrenia (Job et al., 2003).

Previous studies have examined subjects at high genetic risk for schizophrenia. However, this is as far as we know, the first VBM study comparing these high genetic risk individuals (e.g. one affected sibling and at least one other affected first- or second degree family member) to low risk controls (i.e. no reported psychiatric problems in first- or second degree family members). The results showed that even when comparing a high genetic risk group of siblings and a low genetic risk group of controls, no GMV nor GMC differences were found. This finding is in line with several studies unable to find GM differences in unaffected monozygotic twins of patients with schizophrenia (Borgwardt et al., 2010; van Haren et al., 2004), which are at the highest possible genetic risk for developing schizophrenia.

Examining GM differences between high and low schizotypal individuals (irrespective of group) revealed larger GMV (but not GMC) in the right precuneus which is in accordance with a previous study on schizotypy in healthy undergraduates (Modinos et al., 2010a). This larger precuneus volume might be underlying the problems in the reallocation of attention as suggested in a recent fMRI study in which less deactivation of the right precuneus was found in schizotypy (Fink et al., 2013). As suggested by the authors, this process could result in overinclusive thinking which is a characteristic of schizophrenia (Cutting et al., 1987). Although larger precuneus volume was found in the high schizotypy group, no interaction between schizotypy and group (HC or sibling) on GM was found. These results indicate that previous reports on larger precuneus volume in relatives of patients with schizophrenia (Honea et al., 2008) (after lowering threshold for exploratory purposes) might be caused by differences on schizotypy scores rather than genetic risk differences between groups. The fact that GMV differences are associated with subclinical psychotic symptoms but not to
genetic risk indicates that volume differences indeed might be more related to psychotic symptoms, as previous proposed by Boos et al. (2012).

We are not certain whether the affected siblings of our genetic risk group had GM abnormalities, as this was not investigated. However, previous reports reliably documented GM abnormalities in patients with schizophrenia (Fornito et al., 2009; Glahn et al., 2008). Furthermore, in a study of Boos et al. (2007) GM abnormalities in patients were reported, while their non-affected siblings did not show these abnormalities.

This study indicates that GM 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 current study combined with previous reports (Boos et al., 2012; Honea et al., 2008; Job et al., 2003) questions the idea that gray matter abnormalities are present in relatives of patients with schizophrenia. However, the current results did reveal marginal significant higher anterior cingulate cortex (ACC) volume in siblings of patients with schizophrenia through a ROI-analysis. This finding is inconsistent with some previous reports on lower ACC volume in relatives (Honea et al., 2008; Job et al., 2003). However, these studies also only found lower ACC volume when lowering the threshold or when performing ROI analyses. Furthermore, others have failed to show any volumetric differences in the ACC (e.g. Hu et al., 2013; McIntosh et al., 2004). The lack of reproducibility of these and other previous reported findings, as indicated by the different results in four separate meta-analyses (Boos et al., 2007; Fusar-Poli et al., 2014; Palaniyappan et al., 2012), rises further doubts to the value of GM as an endophenotype for schizophrenia. For example, only one of these meta-analyses reported lower ACC volume in siblings of patients with schizophrenia (Fusar-Poli et al., 2014b). These divergent findings may not be fully explained by differences in age, schizotypy and genetic loading, because specifically selecting participants on these factors did not reveal any GM differences between siblings and controls. One possibility could be that only specific schizophrenia related genes are associated with GM abnormalities, such as aberrant ACC volume. For example, previous research has shown that DISC-1 risk allele carriers have lower GMV in the ACC (Szeszko et al., 2008), while CNNM2 risk allele carriers have higher ACC volume (Rose et al., 2014). Future research should examine whether these genetic variations can explain the divergent findings on GM abnormalities in relatives of patients with schizophrenia.

Limitations

Several limitations of the current study need to be addressed. First, by subdividing the current sample, the group sizes for these sub-analyses became smaller. Especially, the analysis regarding the genetic loading has lower power compared to the other analyses. However, the power of this analysis is still sufficient to detect medium to small effects (Friston et al., 1996). Furthermore, selecting on high genetic loading versus low genetic loading increased the sensitivity to detect differences related to genetic risk. Hence, smaller sample sizes would be sufficient to detect GM differences between these groups. Second, the group was too small to select subjects on two factors combined (e.g. aged below 30 and high genetic risk). Future research should consider selecting subjects specifically on the combination of these factors to examine whether this has an effect on gray matter. Third, participants were scanned using two different scanners. Although the reliability of multi-scanner VBM has proven to be good when adding scanner as a covariate (Focke et al., 2011; Stonnington et al., 2008), 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 significantly change the results. Fourth, all participants with an axis-I mood, anxiety or psychotic disorder were excluded from this study. This exclusion method may have resulted in excluding the most vulnerable siblings. However, this method was chosen to make sure that possible gray matter abnormalities were not due to comorbid psychiatric disorders. The current findings show that the differences between previous reported findings might not be explained by differences in age, genetic loading or schizotypy between studies, nor by examining either GMV or GMC. However, it is still possible that methodological differences (e.g. differences in T1 acquisition, the applied VBM method or 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 current study provides further support for the hypothesis that GM 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 (MacDonald et al., 2009; Pettersson-Yeo et al., 2011). Furthermore, research should examine the role of specific genetic variations on gray matter, specifically on the anterior cingulate cortex.

| ACKNOWLEDGEMENTS |

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