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Default-mode network dysfunction and self-referential processing in healthy siblings of schizophrenia patients

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ABSTRACT

The default-mode network (DMN) of the brain shows highly coherent intrinsic activity in healthy subjects and is implicated in self-referential processing important for social cognitive functioning. Schizophrenia patients show abnormal resting-state connectivity within the DMN and this aberrant connectivity is thought to contribute to difficulties in self-referential and introspective processing. Subjects at increased genetic risk of developing schizophrenia, including unaffected siblings of patients, also exhibit brain abnormalities and impaired social cognitive processing. However, it is unclear whether resting-state connectivity within the DMN is abnormal in these subjects. Here, we investigate resting-state DMN connectivity in siblings and whether this is related to the functioning of the network during self-referential processing. Brain activity was measured using functional MRI in 25 unaffected siblings of patients with schizophrenia and 25 healthy controls during an 8-minute resting-state period and during a self-referential processing task in which the subjects had to indicate whether a trait adjective (e.g. "lazy") described their personality (self-referential condition) or whether the trait was socially desirable (non-referential condition). Compared with controls, siblings showed exaggerated connectivity during resting-state between the midline areas of the DMN. Moreover, they failed to adequately modulate connectivity between these areas during self-referential processing. No abnormalities in activation during self-referential processing were observed. These findings suggest that subjects at increased genetic risk of developing schizophrenia exhibit abnormal intrinsic connectivity within the midline DMN and that this is associated with aberrant interactions between these regions during self-referential processing.

1. Introduction

Functional MRI (fMRI) research on schizophrenia has increasingly focused on connectivity of neural networks during cognitive task performance and during task-free resting-state periods (Pettersson-Yeo et al., 2011). Functional connectivity during resting-state, or intrinsic connectivity, is thought to be important for normal cognitive functioning and behavior (Fox and Raichle, 2007) and is typically analyzed by calculating coherency of spontaneous activity between distant brain regions.

A network that consistently shows coherent intrinsic activity in healthy subjects is the default-mode network (DMN), which includes the medial prefrontal cortex (MPFC), the posterior cingulate cortex (PCC), extending into the precuneus, and the lateral posterior cortices (Gusnard and Raichle, 2001; Raichle et al., 2001; Greicius et al., 2003; Fox et al., 2005; Buckner et al., 2008). The DMN shows decreased activation during cognitive task performance relative to resting-state or internally focused tasks and is implicated in self-referential and introspective processes (Gusnard and Raichle, 2001; Mason et al., 2007; Buckner et al., 2008). Self-referential processing is important for social cognitive functioning, as interacting with others requires reflection on our own feelings and knowledge (Vogeley and Fink, 2003; Mitchell et al., 2005). Various studies have investigated DMN activity during tasks that evoke self-referential processing such as responding to statements and describing one's own personality, attitudes or preferences and reported increased activity, particularly within the midline areas (the MPFC and PCC/precuneus) (Craik et al., 1999; Gusnard et al., 2001; Johnson et al., 2002; Kelley et al., 2002; Fossati et al., 2003; Schmitz et al., 2004; Ochsner et al., 2005; van Buuren et al., 2010; Whitfield-Gabrieli et al., 2011). Furthermore, in a previous study we reported decreased connectivity between areas of the DMN during self-referential processing, indicating functional specialization within the network (van Buuren et al., 2010).

Studies in patients with schizophrenia have repeatedly reported abnormal resting-state connectivity within the DMN, particularly within the midline areas (Liu et al., 2006; Bluhm et al., 2007; Zhou et al., 2007; Lynall et al., 2010; Rotarska-Jagiela et al., 2010; Salvador et al., 2010; Camchong et al., 2011; Repovs et al., 2011). This aberrant

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intrinsic connectivity is thought to contribute to the difficulties in self-referential and introspective processing observed in schizophrenia (Bluhm et al., 2007; Zhou et al., 2007; Camchong et al., 2011; Liu et al., 2012). Recent support for this notion is provided by a study of Holt et al. (2011), which reported that aberrant DMN resting-state connectivity in patients was associated with abnormal activation within the network during a self-referential processing task.

First-degree relatives of schizophrenia patients, including unaffected siblings, have an increased genetic risk to develop schizophrenia and exhibit brain abnormalities that are also observed in patients (MacDonald et al., 2009; Pettersson-Yeo et al., 2011; van Buuren et al., 2011). Furthermore, behavioral studies in unaffected first-degree relatives, including siblings, have reported deficits in social cognitive processing (Toomey et al., 1999; Irani et al., 2006; de Achaval et al., 2010). However, it is unclear whether siblings have abnormal resting-state DMN connectivity. So far, only a few studies have investigated intrinsic connectivity within the DMN in first-degree relatives and the findings of these studies are inconsistent (Jang et al., 2011; Repovs et al., 2011; Liu et al., 2012). Moreover, no study has yet investigated the functioning of the DMN during self-referential processing in these subjects. Investigating brain activity in unaffected relatives not only provides an indication whether brain abnormalities are associated to genetic risk factors but also avoids possible illness confounds such as medication use that may influence brain activity.

Here, we investigate resting-state DMN connectivity in siblings and whether this is related to the functioning of the network during self-referential processing. To this aim, brain activity was measured in unaffected siblings of schizophrenia patients and healthy control subjects during an 8-minute resting-state period and during a self-referential processing task. Based on studies in patients, we expected that the siblings would show abnormal resting-state connectivity within the DMN, particularly between the midline areas. Furthermore, we expected that this abnormal intrinsic connectivity would be related to aberrant changes in connectivity and activation within the network during self-referential processing.

2. Materials and methods

2.1. Participants

Twenty-five unaffected siblings of schizophrenia patients and twenty-five healthy control subjects participated in this study (see Table 1 for demographic characteristics). All subjects were right-handed and the groups did not differ in age, sex, level of own education or that of their father or mother (see Table 1). None of the participants received psychotropic medication (at the present or in the past), had any contraindications for MRI, suffered from alcohol or drug dependence, or had a history of a neurological or psychiatric disorder as verified by either the Mini International Neuropsychiatric Interview (7 subjects) or the World Health Organisation of Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1, 43 subjects). All participants were unrelated to each other and healthy control subjects who had a first-degree relative suffering from a psychotic disorder were excluded. Participants were recruited from the database of the Genetic Risk and Outcome of Psychosis (GROUP) study and received monetary compensation for participation. All gave written informed consent and the ethics committee of the University Medical Center Utrecht approved this study.

Data of the self-referential task of 7 controls were included in our recently published study (van Buuren et al., 2010). No resting-state data were acquired of these controls and of 7 siblings, thus resting-state analyses were performed on the data of 18 controls (7 male) and 18 siblings (7 male).

Table 1
Demographic characteristics of the unaffected siblings and healthy controls. Education was measured on a 9-point scale ranging from no education, 0, to university degree, 8.

	Healthy controls		Unaffected siblings		*p*-Value
	Mean	SD	Mean	SD	
Gender (male/female)	9/16		9/16		
Handedness (right/left)	25/0		25/0		
Age (years)	27.5	8.1	27.9	4.6	0.827
Own education	6.3	1.4	6.1	1.8	0.988
Paternal education	5.7	2.5	5.4	2.2	0.379
Maternal education	5.2	2.4	5.4	2.0	0.858

2.2. Resting-state

During a resting-state period of 8 min, subjects were instructed to lie still with their eyes closed and not to fall asleep. Afterwards, all subjects indicated that they stayed awake.

2.3. Self-referential task

In the self-referential task, subjects were instructed to make judgments about trait adjectives, as described previously (van Buuren et al., 2010). In short, depending on the condition, the subjects were asked to indicate whether a trait adjective (e.g. “lazy”) described their own personality (self-referential, Self condition), the Dutch prime-minister's personality (other-referential, Other condition) or whether the trait was socially desirable (non-referential, Control condition) by pressing the left “yes” or the right “no” button.

2.4. Behavioral data analyses

Using SPSS 15.0 (Statistical Package for the Social Sciences), two-sample t-tests were performed to test for group differences in accuracy on Control trials as well as in the number of negative and positive traits of the Self condition rated as self-descriptive. Also, a repeated-measures ANOVA analysis was performed to test for effects of condition and group on reaction time (RT).

2.5. Cardiorespiratory confounds

Cardiorespiratory (CR) processes may affect the fMRI signal independently of neuronal activity (Glover et al., 2000; Wise et al., 2004) and influence measures of connectivity (Birn et al., 2006; Shmueli et al., 2007; van Buuren et al., 2009). To correct for these CR processes, we measured heart beat and respiration during scanning and corrected the fMRI data using the method described in our previous studies (van Buuren et al., 2009, 2010).

2.6. Functional magnetic resonance imaging

2.6.1. Measurements

All imaging was performed on a Philips 3.0 T Achieva whole-body MRI scanner (Philips Medical Systems, Best, The Netherlands). A total of 800 functional images were obtained during resting-state using a 3D PRESTO-SENSE pulse sequence (Neggers et al., 2008) with the following parameters: voxel size 4 mm isotropic, TR = 21.75 ms; TE = 32.4 ms; flip angle = 10°; matrix 56 × 64 × 40; field of view 224 × 256 × 160; and scan duration 609 ms per 40-slice volume. A reference image of the same volume of brain tissue was acquired with a high flip-angle (25°) for image co-registration. Subsequently, a total of 395 functional images were acquired during the self-referential task using a 2D-EPI-SENSE sequence and a T1-weighted structural image was acquired with scan parameters identical to those described in our previous study (van Buuren et al., 2010).

2.6.2. Image preprocessing

Image preprocessing and analyses were carried out with SPM5 (http://www.fil.ion.ucl.ac.uk/spm/). Resting-state functional images were realigned to the reference image, and the structural image was co-registered to the reference image. Next, using unified segmentation the structural scan was segmented and normalization parameters were estimated. All scans were then registered to a MNI T1-standard brain using these normalization parameters and the functional images were spatially smoothed using a 3D Gaussian filter (8-mm full width at half maximum).

Functional images of the self-referential task were preprocessed using a similar approach as described above with the addition of slice-time correction prior to realignment. Also, the structural image was co-registered to the mean functional image.

2.6.3. Resting-state analyses

The preprocessed functional images were corrected for CR effects (see Section 2.5) and corrected images were temporally band-pass filtered (0.01 Hz<f>0.1 Hz). Next, time series were extracted from two seed regions. These regions were 8-mm radius spheres centered in midline regions of the DMN, the ventral MPFC (vMPFC) [−2 58 −8], and PCC [−2 −50 36], based on a previous study (Buckner et al., 2009). Subsequently, for each region, a whole-brain multiple linear regression analysis was performed with the average time series of the seed region as a regressor of interest to calculate correlations between the seed region and every other voxel in the brain. To reduce spurious correlations, realignment parameters and the average time series of the whole brain were included as regressors of no interest. The resulting correlation coefficient images were then converted to a normal distribution by Fisher's *z* transformation.

Fisher's *z* correlation maps were entered into one-sample t-tests to reveal positive connectivity patterns of each seed region and group. Subsequently, two-sample t-tests were performed to test whether siblings showed significant aberrant connectivity. Group connectivity maps were tested for significance using cluster-inference with a cluster-defining threshold of $p<0.001$ and a cluster-probability of $p<0.05$ family-wise error (FWE) corrected for multiple comparisons.

Head motion may influence functional connectivity results (Power et al., 2012; Satterthwaite et al., 2012; Van Dijk et al., 2012). To test for group differences in head motion, mean displacement of each brain volume as compared to the previous volume was calculated as described in Van Dijk et al. (2012), and a two-sample t-test was conducted.

2.6.4. Self-referential task — activation analyses

The preprocessed functional images were submitted to a general linear model regression analysis after correcting for CR effects. The design specification and first-level analyses were conducted as described in our previous study (van Buuren et al., 2010).

Next, first-level images of the contrast self-referential versus non-referential processing were entered into one-sample t-tests. In addition, a two-sample t-test was performed to investigate whether the siblings showed abnormal activation. Group activation maps were tested for significance using cluster-inference with a cluster-defining threshold of $p<0.001$ and a cluster-probability of $p<0.05$ FWE-corrected.

Furthermore, region of interest (ROI) analyses were performed using the seed regions of the resting-state analyses to ensure that these regions were involved in self-referential processing and to test for group differences within these areas specifically at a less stringent threshold. These analyses are described in the Supplementary Materials section.

2.6.5. Self-referential task — psychophysiological interaction analyses

Using psychophysiological interaction (PPI) analyses (Friston et al., 1997), we examined changes in functional coupling between a seed region and all other brain areas during self-referential processing relative to non-referential processing. The seed region was defined as an 8 mm-radius sphere around the peak voxel within the DMN in a conjunction map of activation of all subjects during self-referential processing. First-level analysis was conducted as described previously (van Buuren et al., 2010) and these contrast images were entered in second-level analyses. One-sample t-tests were performed to test for each group which areas showed increased coupling (positive PPI) and which areas showed decreased coupling (negative PPI) with the seed region during self-referential processing relative to non-referential processing. Furthermore, a two-sample t-test analysis was performed to test for group differences in these connectivity changes. Significance was assessed using cluster-inference with a cluster-defining threshold of $p<0.001$ and a cluster-probability of $p<0.05$ FWE-corrected.

Next, PPI analyses were performed using the results of the between-group resting-state analyses, to test whether abnormal resting-state connectivity was related to altered task-related changes in connectivity. First-level analyses were conducted with the resting-state seed region that revealed significant group differences in connectivity. Subsequently, one-sample and two-sample t-tests were performed and were limited to 8 mm-radius spheres around the peak voxels of areas exhibiting significant group differences in resting-state connectivity with the seed region using the WFU Pickatlas toolbox (Maldjian et al., 2003). Significance within the spheres was assessed using a threshold of $p<0.05$ FWE-corrected.

3. Results

3.1. Behavioral results

Response accuracy on Control trials was above 90% (mean ± SD, 92.8 ± 4.2%), and did not differ between siblings and controls ($p=0.739$). Also, no group differences were observed in the percentage of positive ($p=0.856$) and negative traits ($p=0.544$) rated as self-descriptive.

RT were within the maximum response time (mean ± SD, 1.45 ± 0.19 s) and siblings and controls did not differ in overall RT (no main effect of group, $p=0.560$). Similar to our previous study (van Buuren et al., 2010), RT differed per condition (main effect of condition, $F(2,47)=56.80$, $p<.0005$), but this pattern did not differ between the groups (no group by condition interaction, $p=0.306$).

3.2. Resting-state connectivity

Connectivity maps of the two seed regions showed significant positive correlations between midline and lateral regions of the DMN in both groups (see Tables 2 and 3).

No significant group differences were observed when comparing the connectivity maps of the PCC. However, comparison of the maps of the vMPFC did reveal enhanced connectivity between the vMPFC and the precuneus in siblings relative to controls (see Table 3 and Fig. 1). No areas showed reduced connectivity in siblings relative to controls.

Head motion did not differ between the two groups (mean; siblings, 0.087 mm; controls, 0.085 mm; $p=0.856$) making it unlikely that the group difference in connectivity is due to the effects of motion.

3.3. Self-referential task

3.3.1. Activation results

Both the controls and siblings showed significant activation within the midline areas of the DMN during self-referential processing (Supplementary Table 1). In addition, activation was observed within the left superior temporal gyrus in controls. However, the two-sample t-test failed to show significant group differences (cluster-defining threshold of $p<0.001$, $p=0.05$ FWE-corrected critical cluster size of

Table 2
Clusters showing significant positive correlations with the PCC seed region during resting-state. MNI coordinates represent the location of the peak voxels. Cluster-defining threshold of $p<0.0005$ and a $p=0.05$ FWE-corrected critical cluster size of 27 voxels. L = left, R = right.

Brain region	MNI coordinates			Z score	voxels
	x	y	z		
Controls					
L precuneus	−4	−52	32	Inf	2426
R angular gyrus	48	−68	36	5.60	150
L superior frontal gyrus	0	56	12	4.91	146
R middle temporal gyrus	60	−12	−16	4.60	73
Cerebellum	48	−64	−36	4.38	57
L middle temporal gyrus	−64	−24	−8	3.81	28
Siblings					
L cingulate gyrus	−4	−48	32	Inf	2360
R angular gyrus	48	−60	36	5.39	203
Cerebellum	44	−60	−40	4.74	141
L superior frontal gyrus	0	56	8	4.28	76

23 voxels). Even when lowering the statistical threshold ($p<0.0001$ uncorrected) or when looking at the ROIs specifically (see Supplementary Materials) no group differences were observed.

3.3.2. Connectivity results

Conjunction analysis of activation during self-referential processing revealed a peak locus of activation within the MPFC (x, y, z = 4, 48, 20), which was taken as seed region for the PPI analysis (see Section 2.6.5). Both groups showed an increase in connectivity between this region and regions mainly outside the DMN during self-referential relative to non-referential processing (see Fig. 2 and Supplementary Table 2). Furthermore, controls showed a reduction in connectivity between the MPFC and precuneus during self-referential processing (see Fig. 2 and Supplementary Table 2). In contrast, no decrease in connectivity was observed in the siblings (see Fig. 2). Direct comparison of the two groups did however not reveal any significant group differences in connectivity changes.

Additional PPI analyses based on the result of abnormal resting-state connectivity between the vMPFC (seed region) and precuneus revealed a decrease in connectivity between these areas in controls (Fig. 3). Siblings did not show this change in connectivity within the precuneus and direct comparison between the groups revealed that this group difference was significant (Fig. 3).

Table 3
Clusters showing significant positive correlations with the vMPFC seed region during resting-state. MNI coordinates represent the location of the peak voxels. Cluster-defining threshold of $p<0.0005$ and a $p=0.05$ FWE-corrected critical cluster size. Critical cluster size was 27 voxels for the within-group analyses and 34 voxels for the comparison between the controls and siblings. L = left, R = right, NS = non-significant.

Brain region	MNI coordinates			Z score	Voxels
	x	y	z		
Controls					
L superior frontal gyrus	−8	56	−8	Inf	2565
L precuneus	0	−60	24	5.66	182
L angular gyrus	−52	−64	32	5.38	40
R angular gyrus	52	−64	28	5.01	80
R middle temporal gyrus	60	−12	−16	4.54	86
Siblings					
L superior frontal gyrus	−4	60	−8	7.80	793
R middle temporal gyrus	60	−8	−12	5.48	61
R angular gyrus	52	−64	20	5.07	107
L angular gyrus	−48	−68	28	4.87	69
L precuneus	−12	−52	8	4.77	278
L middle temporal gyrus	−56	−12	−16	4.54	51
L orbital gyrus	−36	20	−12	4.21	31
R fusiform gyrus	36	−44	−12	4.00	45
Siblings > Controls					
L precuneus	−8	−68	20	4.23	54
Controls > Siblings					
NS					

4. Discussion

Here, we investigated whether unaffected siblings of schizophrenia patients show abnormal resting-state connectivity within the DMN. Furthermore, we studied whether such abnormal intrinsic connectivity is related to the functioning of the network during self-referential processing. Compared with controls, siblings showed exaggerated connectivity during resting-state between the vMPFC and precuneus. Moreover, they failed to show adequate task-related modulation of connectivity between these areas during self-referential processing. No abnormalities in activation during self-referential processing were observed. These findings suggest that the intrinsic connectivity within the midline DMN is abnormal in subjects genetically at increased risk of developing schizophrenia and that this dysfunction is associated with aberrant interactions between the midline regions of the network during self-referential processing.

Our finding of exaggerated connectivity in siblings between the vMPFC and precuneus during resting-state is in line with the results of two previous studies in subjects at increased risk of developing schizophrenia. In a study by Whitfield-Gabrieli et al. (2009) enhanced connectivity between the vMPFC and PCC was reported during rest periods of a cognitive task in first-degree relatives. Furthermore, a study (Shim et al., 2010) in young adults who were at ultra-high risk of developing psychosis revealed increased connectivity between these midline areas during resting-state. Our finding, however, is not consistent with three other studies in first-degree relatives that reported reduced connectivity (Jang et al., 2011) within the PCC or failed to find abnormal connectivity within the midline areas of the DMN (Repovs et al., 2011; Liu et al., 2012). This divergence in findings may be due to the differences in the choice of seed region and analyses. That is, in contrast to our study, the studies of Liu et al. (2012) and Jang et al. (2011) did not include the vMPFC as seed region, and the study of Repovs et al. (2011) limited the analyses to specified regions of interest. By including a seed region in the vMPFC in addition to the PCC and by analyzing connectivity with all other brain areas, our analyses may have revealed connectivity differences that might not have been observed when restricting the analyses to regions of interest or using one seed region. However, our results are still dependent on the definition of the seed regions. Future studies may consider using data-driven approaches such as Independent Component Analyses that do not require seed regions.

The observed aberrant intrinsic functional connectivity within the midline DMN in siblings could be related to abnormalities in self-referential processing. Indeed, siblings failed to show a reduction in connectivity between these areas during such processing. This decrease in connectivity was observed in healthy controls in the current study as well as in our previous study (van Buuren et al., 2010) and may suggest functional specialization within the network. That is, various studies have suggested that the DMN consists of separate components (Buckner et al., 2008) that are involved in different subfunctions of self-referential processing (Northoff and Bermpohl, 2004; Northoff et al., 2006). For example, previous studies have associated the vMPFC with identifying stimuli as self-relevant by integrating cognitive, emotional and sensory information (Gusnard et al., 2001; Northoff and Bermpohl, 2004; Northoff et al., 2006; Schmitz and Johnson, 2007). The posterior part of the DMN, including the PCC, has been implicated in emotion processing, in the representation of the external

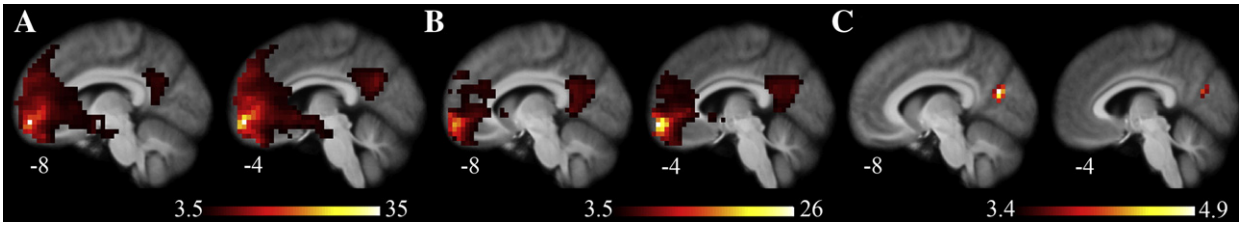

Fig. 1. Resting-state connectivity with the vMPFC. Areas showing significant positive correlations with the vMPFC seed region during resting-state A) in healthy controls and B) in siblings. C) Significant enhanced connectivity was revealed between the vMPFC and precuneus in the siblings relative to the healthy controls. No areas showed less connectivity in the siblings relative to the controls. Colorbar represents t-values, left = left. Cluster-defining threshold of $p<0.0005$ and a $p=0.05$ FWE-corrected critical cluster size of 27 voxels (A, B) and 34 voxels (C).

environment as well as in autobiographic memory through dense connections with the hippocampus formation (Kobayashi and Amaral, 2003, 2007). Interactions between the posterior DMN and the anterior DMN may serve to process self-referential information by integration of autobiographic information, external information and internal information about stimuli. The absence of a decrease in connectivity during self-referential processing together with exaggerated resting-state connectivity may suggest abnormal functional interactions between the posterior and anterior midline DMN. This in turn might possibly lead to difficulties in self-referential processing. However, this interpretation is highly speculative and requires further research investigating the functional relevance of aberrant interactions within the DMN. These future studies may benefit from the use of more elaborate tasks that encompass various components of self-referential processing such as identifying, evaluating and integrating of self-referential stimuli.

Surprisingly, we did not observe aberrant activation during self-referential processing in the siblings. We did expect to find such abnormalities, as Holt et al. (2011) showed abnormal activation in schizophrenia patients using a task comparable to the task used here. However, the absence of abnormal activation in combination with the finding of abnormal connectivity might be viewed in the context of the notion that the core deficits of schizophrenia can be attributed to abnormal connectivity or integration of brain regions (Friston, 1999; Stephan et al., 2006). Local brain abnormalities could then follow these aberrant interactions but are secondary. In line with this notion, our findings may tentatively suggest that brain interactions are disrupted in subjects who are genetically at risk for schizophrenia while local brain activation is still intact. Longitudinal imaging studies in at risk subjects may provide more insight in the development of brain abnormalities in schizophrenia.

A possible caveat of the present study is that we did not observe differences in task performance between the siblings and controls, suggesting that the siblings did not suffer from behavioral abnormalities. However, although well-validated, the task used in this study might not have been suitable to detect subtle behavioral abnormalities as it was relatively easy to perform. Subjects had to indicate the self-descriptiveness of traits with either "yes" or "no" instead of on a scale of self-descriptiveness. Also, the subjects were not instructed to respond as fast as possible. Of note, using a similar task, Holt et al. (2011) also failed to observe behavioral abnormalities in schizophrenia patients. The use of a more complex task with a response scale might be more suitable to reveal behavioral abnormalities in future studies. A second possible caveat of our study is that we included unaffected siblings who were, on average, past the age of greatest risk of developing schizophrenia and did not suffer from other psychiatric diagnoses. As a result, we may have selected only siblings who will never develop schizophrenia and do not have (sub)clinical symptoms. While these characteristics did enable us to asses functioning of the DMN in genetically at risk subjects without having to take into account possible influences of (sub)clinical factors, future research may benefit from the inclusion of younger subjects at high risk of developing schizophrenia based on clinical features as well as genetic relatedness. These studies could then investigate associations between (sub)clinical factors, such as schizotypal symptoms, and activity and connectivity within the DMN.

In conclusion, our data show exaggerated intrinsic connectivity between midline areas of the DMN, the vMPFC and precuneus, in unaffected siblings of schizophrenia patients. In addition, we report that siblings fail to adequately modulate connectivity between these areas during self-referential processing. Together, these findings suggest that functional interactions within the midline DMN are disrupted in subjects

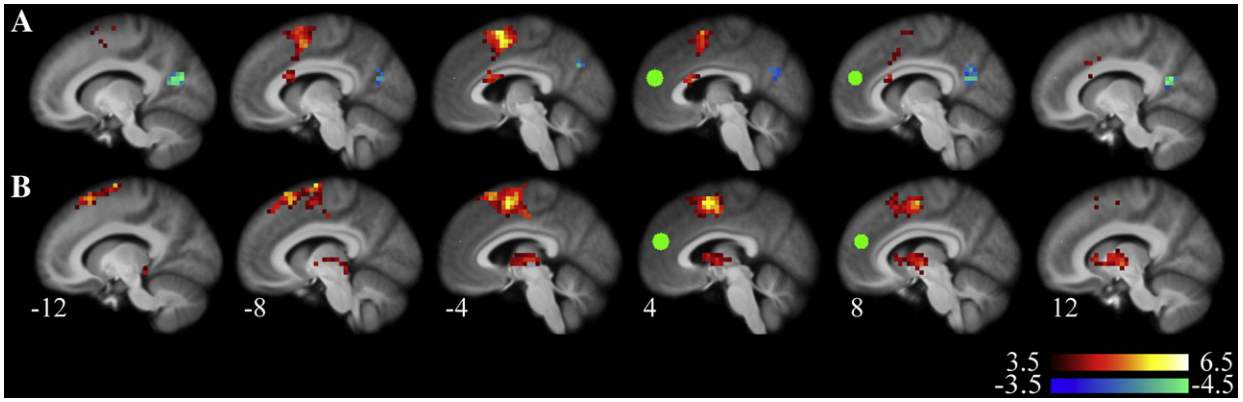

Fig. 2. Areas showing significant connectivity changes during the self-referential task. Areas showing decreased (blue) or increased (red) connectivity with the seed region MPFC (green circle) during self-referential processing relative to non-referential processing in healthy controls (upper row) and in the siblings (lower row). Note that the siblings do not show reductions in connectivity. Colorbar represents t-values, left = left. Cluster-defining threshold of $p<0.0005$ and a $p=0.05$ FWE-corrected critical cluster size of 24 voxels.

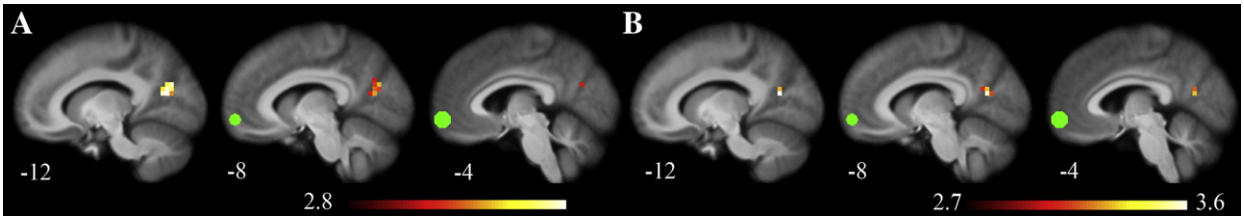

Fig. 3. Connectivity changes during the self-referential task with the resting-state seed region. Precuneus shows a significant decrease in connectivity with the seed region vMPFC during self-referential processing relative to non-referential processing in A) healthy controls and B) this decrease in connectivity was significantly greater in healthy controls than in siblings, peak voxel; x, y, z = −12 −64 16, Z = 3.33. Colorbar represents t-values, left = left, $p = 0.05$ FWE-corrected within search volume.

genetically at increased risk of developing schizophrenia. This dysfunction of the midline regions of the network may contribute to difficulties in self-referential processing.

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Funding has played no role in the realization of this study.

Contributors

M. van Buuren designed the study, contributed to writing of the study protocol, performed the literature searches, data acquisition and data analyses and wrote the first draft of the manuscript. M. Vink supervised the data acquisition and analyses, and contributed to the writing of the manuscript and the study protocol. R.S. Kahn, end revision, supervised the analyses and writing of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

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