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Frontoparietal network abnormalities of gray matter volume and functional connectivity in patients with generalized anxiety disorder

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ABSTRACT

We hypothesized that the frontoparietal region would exhibit differences in gray matter volume (GMV) and resting-state functional connectivity (rs-FC) in patients with generalized anxiety disorder (GAD) versus healthy controls (HCs). We also aimed to report on correlations between these neuroradiological findings and HAMA scores. We recruited 27 patients with GAD and 28 HCs, matched for gender, age and education. GMV was estimated using voxel-based morphometry (VBM). We found decreased GMV in the precentral gyrus (PrCG) and the superior frontal gyrus (SFG) in patients with GAD, which were used as regions of interest (ROI) for rs-FC analyses. We detected enhanced rs-FC in the inferior frontal gyrus (IFG) based on an increase in negative connections, and reduced rs-FC in the superior temporal gyrus (STG) based on a decrease in positive connections compared to HCs. The right PrCG may be a candidate biomarker in patients with GAD, as well as a potential stimulation target for improvement of anxiety symptoms. By combining GMV and rs-FC analyses, our findings help to understand the pathophysiology of GAD by combining GMV and rs-FC.

1. Introduction

GAD, defined as chronic, excessive and uncontrollable worrying and anxiety, is accompanied by muscle tension, difficulty concentrating, irritability, restlessness, fatigue and sleep disturbances (APA, 2013). Lifetime prevalence was 4.66‰ in mainland China, and about 5.3‰ in urban China (Guo et al., 2016; Yu et al., 2018). GAD diminishes one's quality of life and also significantly impacts healthcare systems (Hoffman et al., 2008).

Frontoparietal networks exhibit the most distinctive connectivity patterns in the brain, with high inter-subject variance (Finn et al., 2015; Mueller et al., 2013). In terms of GMV, studies have identified several alterations concentrated in the frontoparietal regions using VBM in patients with GAD. Varying results have been published, however, which may be related to differences among age groups studied. In adults, increased GMV is found in the dorsomedial prefrontal cortex (Schienle et al., 2011), while in children and adolescents, increased GMV is found in the precuneus and the PrCG (Strawn et al., 2013). When adolescent brains of patients with GAD were analyzed separately, decreased GMV was noted in the orbitofrontal gyrus (Strawn et al., 2013). Moreover, in geriatric patients with GAD, GMV was notably decreased in the IFG (Andreescu et al., 2017).

Previously, rs-FC has been used to reliably measure intrinsic functional architecture in the frontoparietal networks (Finn et al., 2015; Smith et al., 2009). Interestingly, patients with GAD showed aberrant functional connectivity in the frontoparietal networks. For instance, in adults with GAD, rs-FC studies have reported stronger rs-FC in structures of the frontoparietal executive control network (Etkin et al., 2009), such as the fusiform gyrus (Cui et al., 2016) and weaker rs-FC in the frontal cortex (Qiao et al., 2017). In terms of age effects on rs-FC connectivity, Liu et al. noted adolescents with GAD exhibited decreased rs-FC in the dorsolateral prefrontal cortex (Liu et al., 2015) whereas Andreescu and colleagues revealed a greater influence of effect on the functional connectivity between the posterior cingulate and the medial prefrontal cortex for the older participants relative to the younger participants (Andreescu et al., 2014).

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To date, several studies have suggested abnormalities of structure and function in frontoparietal regions of patients with GAD (Andreescu et al., 2014, 2017; Cui et al., 2016; Etkin et al., 2009; Liu et al., 2015; Qiao et al., 2017; Schienle et al., 2011; Senkowski et al., 2003; Strawn et al., 2013). However, it remains unclear whether structural abnormalities are associated with whole-brain rs-FC deficits. To address this question, we combined analyses of GMV and rs-FC analyses to examine neural correlates of patients with GAD.

The current study aimed to evaluate whole-brain differences in GMV, as well as differences in relative rs-FC, in patients with GAD versus HCs. We also aimed to determine correlations between GMV variations, rs-FC, and HAMA scores. Based on prior reports suggesting that frontoparietal networks are distinct and variable (Finn et al., 2015; Miranda-Dominguez et al., 2014; Mueller et al., 2013), we hypothesized that GMV and relative rs-FC in frontoparietal regions would differ between patients with GAD and HCs.

2. Methods

2.1. Participants

A total of 55 right-handed subjects took part in the case-control study by utilizing convenient sampling. 27 patients with GAD (20–56 years of age) were recruited from April 2014 to May 2018 from the Outpatient Department of Medical Psychology and Outpatient Department of Mood Disorders of Nanjing Brain Hospital. GAD diagnoses were made by an experienced psychiatrist based on the Diagnostic and Statistical Manual of Mental Disorders (fifth edition, DSM-5TM) (APA, 2013) and confirmed by another psychiatrist using the Mini-International Neuropsychiatric Interview (MINI). Inclusion criteria were: (1) primary diagnosis of GAD; (2) 20–60 years old; (3) free of psychiatric medications at least 6 months prior to study enrollment; (4) ability to read and write in Chinese at the 9th-grade level. Exclusion criteria were: (1) Any neurological disorders affecting the central and/or peripheral nervous systems; (2) Any comorbid psychiatric disorders, including depression, panic disorder, bipolar disorder, obsessive-compulsive disorder, schizophrenia, alcohol abuse and dependence, social phobia or eating disorder; (3)Severe physical illness, pregnancy and/or breastfeeding; (4) Suicide attempts in the past year; (5) Inability to complete MRI; and (6) Major life change in the last year as defined by death of spouse, unemployment, severe illness, serious injury, legal disputes, property loss, traffic accident, natural disasters or divorce. The vast majority of patients with GAD who were offered initial intake evaluation met all inclusion/exclusion criteria 88.89%, that is, 3 patients were excluded. One subject was unable to read/write in Chinese at the 9th grade level and two subjects were unable to complete the MRI scan and were therefore excluded from the study.

28 gender, age and education matched HCs (23–51 years of ages) were recruited from August 2014 to July 2018 from internet advertisements and posters in Nanjing. Inclusion criteria were: (1) Hamilton Anxiety Rating Scale (HAMA) (total score ≤ 7); (2) 20–60 years old; (3) ability to read and write in Chinese at a 9th grade level. Exclusion criteria were: (1) Comorbid neurological disorders; (2)History of any symptoms consistent with a psychiatric disorder; (3) Pregnancy and/or breastfeeding; (4)History of psychological consult within 3 months of study enrollment; (5) Inability to complete MRI(1 subject); (6) Major life change in the last year as defined by death of spouse, unemployment, severe illness, serious injury, legal disputes, property loss, accidents, natural disasters or divorce; (7) First degree relatives with history of psychiatric treatment. In order to improve the enrollment rate, participants with HAMA Scores greater than 7 but less than 14 were chosen for study inclusion at the discretion of an experienced psychiatrist based on the DSM-5TM criteria. One subject was excluded due to inability to read/write in Chinese at a 9th grade level, one subject was excluded due to inability to complete MRI scan, and two subjects were excluded due to having first degree relatives with history of psychiatric treatment.

This project was approved by the Ethics Committee of the Nanjing Brain Hospital, an affiliate of Nanjing Medical University. Each participant signed informed written consent and underwent f-MRI at the Imaging Research Center of Nanjing Brain Hospital.

2.2. Measures

A self-report questionnaire was used to collect subjects' demographic data including: gender, age, years of education, handedness, duration of illness (years), history of psychotropic substances and psychoactive substances, history of psychological counseling, history of physical illness, and so on. The clinician-rated HAMA, a well-established instrument with established reliability and validity, was used to assess severity of anxiety in each subject.

2.3. f-MRI data acquisition

f-MRI data were obtained with a Siemens 3.0 T scanner at the Department of Radiology, Nanjing Brain Hospital. During the scan, all subjects were asked to close their eyes but remain awake while keeping their heads motionless. To avoid head movement and noise, foam pads and earplugs were used. T1-weighted anatomical images were obtained using a 3D-GR/IR sequence according to the following scan parameters: matrix = 256 × 246, field of view (FOV) = 240 mm × 240 mm, repetition time (TR) = 1900 ms, echo time (TE) = 2.48 ms, flip angle (FA) = 9°, 176 slices, slice thickness = 1 mm, spacing between slices = 0 mm. Resting state data were acquired using the echo planar imaging (EPI) sequence according to the following scan parameters: acquisition matrix = 64 × 64, field of view (FOV) = 240 mm × 240 mm, repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, flip angle (FA) = 90°, 36 slices, slice thickness = 4 mm, spacing between slices = 4 mm.

2.4. f-MRI data preprocessing

First, files in Digital Imaging and Communications in Medicine (DICOM) format were segmented using REST 1.8 software (http://restfmri.net/forum/) based on the Matlab 2008b. After segmenting T1 data with the Cat12 Toolbox runs within SPM12 (http://www.fil.ion.ucl.ac.uk/spm/), GMV images of individual participants were normalized to the Montreal Neurological Institute (MNI) template space and smoothed with a 6 mm full-width at a half-maximum Gaussian kernel to enhance the signal-to-noise ratio. The images were then preprocessed by eliminating the first 10 functional images and nuisance regression with DPARSF (http://rfmri.org/DPARSF) removed covariates including 6 head motion parameters, global mean signal, cerebrospinal fluid signal, white matter signal and white matter signal. Standardization of rs-FC images was performed in the same fashion. We excluded more than 2.5 mm or 2.5° of head motion in both groups. Additionally, we calculated translation and rotation of head motion between-groups with the following formula: Head motion/rotation $= \frac{1}{L-1}\sum_{i=2}^{L}\sqrt{|x_i - x_{i-1}|^2 + |y_i - y_{i-1}|^2 + |z_i - z_{i-1}|^2}$ (L (230): the length of the time series; x_i, y_i and z_i: translations/rotations at the ith time point in the x, y and z directions). There were no significant differences in translational motion ($t = -0.2148$, $p = 0.8310$) and rotational motion ($t = 0.6160$, $p = 0.5414$) of head motion between patients with GAD and HCs.

2.5. Statistical analyses

2.5.1. Demographic and clinical characteristics

Clinical and demographic characteristics were evaluated using chi-square test for gender and two-sample t-test for age, years of education, and HAMA scores, using SPSS21 (https://www.ibm.com/analytics/spss-statistics-software). Also, K-S test was performed for age and education. The significance level was set at $p < 0.05$.

2.5.2. GMV

GMV was compared between GAD patients and HCs using the two-sample *t*-test with multiple covariates (total intracranial volume (TIV), age, sex and the years of education) with the SPM statistical package. Results are reported with AlphaSim and corrected using a threshold of $p < 0.001$ and a minimum cluster size (cluster $p = 0.05$) (Song et al., 2011). Box-plots and receiver operating characteristic (ROC) curves are also used to discriminate GMV differences between groups.

2.5.3. The rs-FC analysis

In order to identify rs-FC alterations between GAD patients and HCs, we created rs-FC maps of the PrCG and SFG that could be analyzed with xjView (http://people.hnl.bcm.tmc.edu/cuixu/xjView/). First, voxel-wise rs-FC maps at the individual-subject level were constructed after re-slicing masks with a resolution of 3 mm × 3 mm × 3 mm with the REST toolbox (Song et al., 2011). Next, Fisher's r-to-Z was applied to each map in order to transform data into Z scores. Then, rs-FC was assessed with the one-sample *t*-test and two-sample *t*-test using multiple covariates (age, sex and the years of education) to report at a significance threshold of $p < 0.001$ (AlphaSim corrected, cluster $p = 0.05$) with a minimum cluster size (Song et al., 2011). Box-plots and ROC curves were then used to discriminate abnormal rs-FC in GAD patients compared to HCs.

2.5.4. Correlation analyses

In each group, Pearson correlations were conducted to examine the correlation between GMV alterations and HAMA scores, as well as rs-FC of GMV alterations and HAMA scores. The correlation coefficients were assessed with 95% confidence interval in SPSS21.

3. Results

3.1. Demographic and clinical characteristics

We recruited 27 patients with GAD and 28HCs matched for gender, age and education. During the course of the study, 7 subjects (GAD: 3; HCs: 4) dropped out due to conflicts with the experimental schedule. After excluding for head motion exceeding 2.5 mm or 2.5° (GAD: 3; HCs: 4), we were left with 21 patients with GAD and 20 HCs. Table 1 shows the demographic and clinical characteristics of participants (27GAD vs. 28HCs, 21GAD vs. 20HCs). Moreover, HAMA-T scores were significantly different between groups (21GAD vs. 20HCs).

3.2. GMV alterations

Patients with GAD showed significant reduction in GMV of the right PrCG and the right SFG at $p < 0.001$, Alphasim corrected for multiple comparisons with minimal cluster size (cluster $p = 0.05$; Fig. 1). Patients with GAD showed no significant enlargement in GMV expansions throughout the brain (Table 2). ROC curves of PrCG and SFG discriminated reliably between GMV alterations of patients with GAD and HCs (Fig. 2).

3.3. The rs-FC of GMV alterations

In both groups (GAD vs. HCs), analysis of rs-FC maps of the SFG shows positive connectivity with the cerebellum posterior lobe and frontal gyrus (middle frontal gyrus and the IFG), and negative connectivity with the precuneus – parietal lobule – temporal gyrus network (Fig. 3). Analysis of rs-FC maps of the PrCG shows in both groups positive connectivity in postcentral gyrus, and negative connectivity in cerebellum posterior lobe, precuneus, inferior parietal lobule and the STG (Fig. 4). Results are displayed at $p < 0.001$, Alphasim corrected for multiple comparisons with minimal cluster size (cluster $p = 0.05$) in MNI space.

The rs-FC maps of the right PrCG ROI contains enhanced rs-FC in the right IFG based on an increase in negative connections, and reduced rs-FC in the STG based on a decrease in positive connections compared to HCs (Table 3; Fig. 5). Results were examined at $p < 0.001$, AlphaSim corrected with cluster size > 24 (cluster $p = 0.05$) in MNI space. Using the same parameters, no clusters survived for analysis of the right SFG ROI.

3.4. Correlation analyses

GMV of the right PrCG correlated positively with the HAMA scores ($r = 0.453$, $p = 0.039$) in patients with GAD and negatively in HCs ($r = -0.459$, $p = 0.042$) (Fig. 6). In contrast, no such correlation between brain structure and clinical finding was found in the right SFG. In terms of functional data, the rs-FC of PrCG - STG correlated negatively with HAMA scores ($r = -0.634$, $p = 0.02$) in patients with GAD (HCs: $r = -0.033$, $p = 0.891$) (Fig. 7). There were no significant correlations between rs-FC of PrCG - IFG and HAMA scores in GAD patients ($p < 0.05$).

4. Discussion

This study used ROIs identified from whole-brain GMV alterations to explore rs-FC differences in patients with GAD versus HCs. We hypothesized that in patients with GAD versus HCs, reduced GMV in the frontoparietal areas would correlate with relative differences in rs-FC of affected GMV regions. Our results support this hypothesis and help to explain how the frontoparietal networks mediated cognition and predict behavior (Finn et al., 2015).

Our findings are consistent with many other studies noting pronounced GMV reductions in the right cerebral hemisphere (Andreescu

Table 1
Comparison of demographic and clinical variables among GAD and HCs.

Variable	GAD	HCs	t/χ^2	*p*	K-S test
27GAD vs. 28HCs	***n* = 27**	***n* = 28**			
Age (years), mean ± SD[a]	34.47 ± 9.473	36.63 ± 8.934	−0.87	0.388	0.746
Sex, males (%)[b]	14 (51.9%)	14 (50%)	0.019	0.891	0.061
Duration of illness (years)	2.327 ± 3.143	13.46 ± 4.872	−0.049	0.961	
Years of education[a]	13.41 ± 3.765				
21GAD vs. 20HCs	***n* = 21**	***n* = 20**			
Age (years), mean ± SD[a]	34.92 ± 9.485	35.96 ± 8.708	−0.368	0.715	0.679
Sex, males (%)[b]	12(57.1%)	10(50%)	0.21	0.647	
Duration of illness (years)	2.393 ± 3.440				
Years of education[a]	12.381 ± 3.584	14.250 ± 4.303	−1.507	0.14	0.051
TIV[a]	1529.092 ± 136.993	1513.293 ± 155.277	0.345	0.732	
HAMA Scores[a]	17.095 ± 5.319	2.350 ± 1.872	11.951	0.000*	

Note: SD: standard deviations; [a] Both *t* and *P*-value were obtained from two-sample *t*-tests; [b] Both χ^2 and *P*-value were obtained from Chi square test; * Statistically significant. TIV: total intracranial volume; HAMA: Hamilton Anxiety Rating Scale.

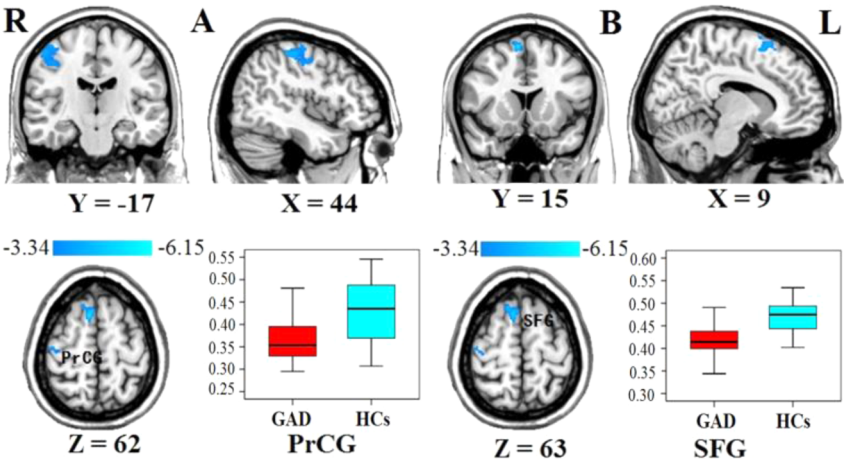

Fig. 1. A) GAD patients showed reduced (cool colors) GMV in the right PrCG compared to HCs. B) GAD patients showed reduced GMV in the right SFG compared to HCs. Results of the two-sample *t*-test are shown at $p < 0.001$with AlphaSim correction, cluster size > 279. Peak voxel coordinates (*x, y, z*) are in MNI space. The color bar represents *t*-values. R: right; L: left. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
GMV alterations in patients with GAD ($n = 21$) versus HCs ($n = 20$).

Lobe	Laterality	Brain areas	MNI coordinates			Voxels in cluster	*t*	BA
			x	*y*	*z*			
GAD > HC								
None								
GAD < HC								
Frontal	Right	Precentral gyrus	44	−17	62	453	−3.99	BA6
Frontal	Right	Superior frontal gyrus	9	15	63	362	−5.29	BA6

Note: BA: Brodmann Area. Results of the two-sample *t*-test are shown at $p < 0.001$ with AlphaSim correction, cluster size > 279. Peak voxel coordinates (*x, y, z*) are in MNI space.

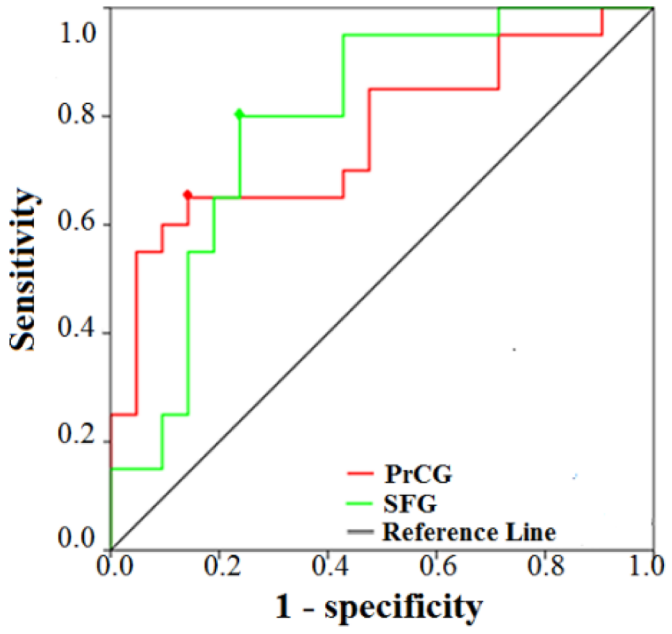

Fig. 2. ROC curves discriminated reliably between GMV alterations of patients with GAD versus HCs. Larger areas under the curve (AUC) improved separation between groups. ♦: The best diagnostic point. PrCG: area under the curve, 0.764; cutoff: sensitivity, 65%; specificity, 85.7%; SFG: area under the curve, 0793; cutoff: sensitivity, 80%; specificity, 76.2%.

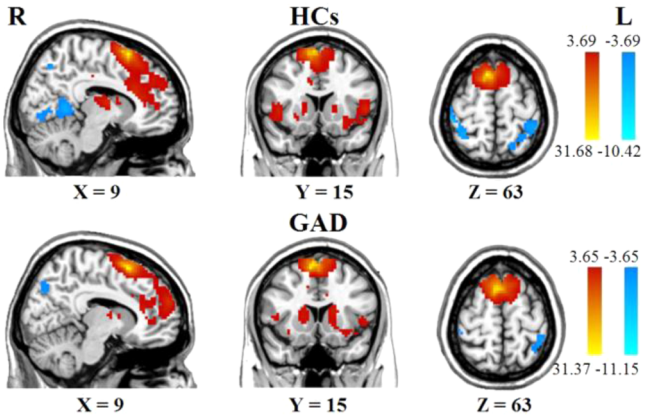

Fig. 3. The rs-FC maps are in the right SFG in patients with GAD and HCs. Patterns of both positive and negative connectivity were generally conserved between groups. Results are shown at $p < 0.001$, AlphaSim corrected for multiple comparisons with minimum cluster size of 30 (GAD) or 32 (HCs) (cluster: $p = 0.05$) in MNI space. The color-bar represents *t*-values. R: right; L: left. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

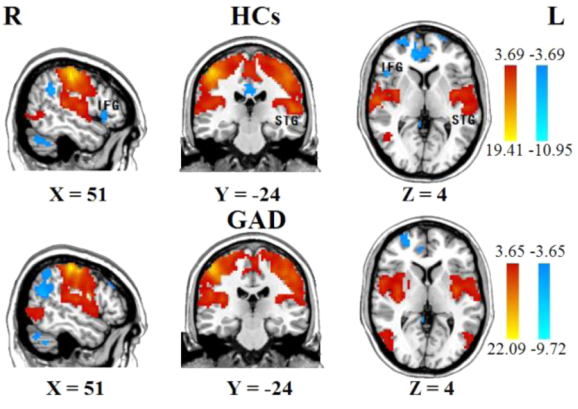

Fig. 4. The rs-FC maps are in the right PrCG in patients with GAD and HCs. Overall patterns of both positive and negative connectivity were conserved between groups. Results are shown at $p < 0.001$, AlphaSim corrected for multiple comparisons with minimum cluster size of 32 (GAD) or 34 (HCs) (cluster $p = 0.05$) in MNI space. The color-bar represents *t*-values. R: right; L: left. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Differences in rs-FC of the PrCG between GAD (n = 21) and HCs (n = 20).

Lobe	Laterality	Brain areas	MNI coordinates			Voxels in cluster	t	BA
			x	y	z			
GAD > HC								
Frontal	Right	Inferior frontal gyrus	51	18	0	31	4.24	BA47
GAD < HC								
Temporal	Left	Superior temporal gyrus	−54	−24	6	25	−5.15	BA41

Note: BA: Brodmann Area. Results of the two-sample t-test are shown at $p < 0.001$ with AlphaSim correction, cluster size > 24. Peak voxel coordinates (x, y, z) are in MNI space.

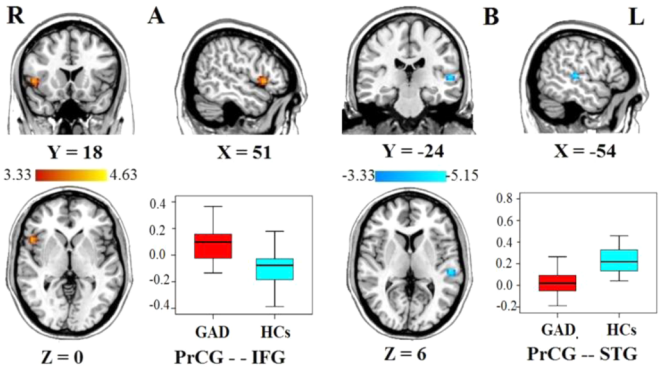

Fig. 5. A: GAD patients showed enhanced (warm colors) rs-FC between the right PrCG and the IFG compared to HC. B: GAD patients showed reduced (cool colors) rs-FC between the right PrCG and the left STG compared to HC. Results of the two-sample t-test are shown at $p < 0.001$, AlphaSim corrected, cluster size > 24. Peak voxel coordinates (x, y, z) are in MNI space. The color bar represents t-values. R: right; L: left. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

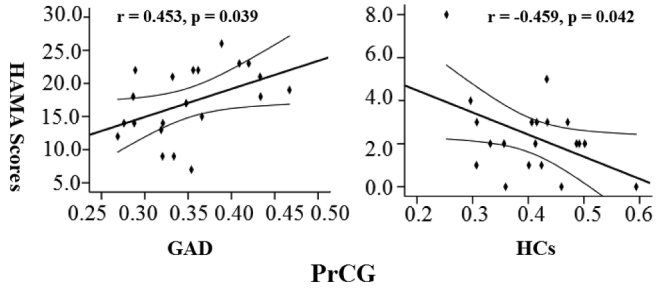

Fig. 6. Relationship between PrCG GMV and HAMA scores in both groups.

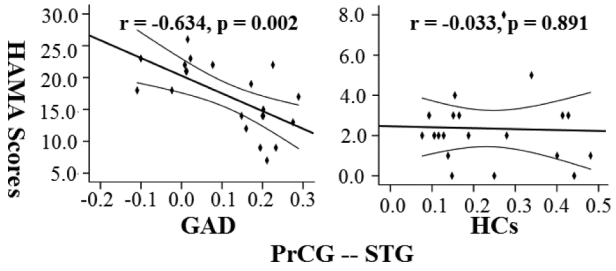

Fig. 7. Relationship between rs-FC of PrCG - STG and HAMA scores in both groups.

et al., 2017; Hilbert et al., 2015; Liao et al., 2013; Moon et al., 2014), however, to our knowledge, this study is the first to describe abnormally reduced GMV in both the right PrCG and SFG based on VBM in patients with GAD. Given the differences in developmental stages between adults and adolescents, Strawn et al. (2013) found increased GMV in the PrCG of adolescents with GAD, while Moon et al. found reduced white matter volume in the PrCG of adults with GAD (Moon et al., 2015). Since rumination is an early symptom of GAD, the findings loosely suggest that rumination may be causing reduced GMV in the PrCG in some adults (Blair et al., 2012). Importantly, the PrCG, associated with interoception processing of motor and somatosensory information, may also be central to anxiogenesis (Pollatos et al., 2007; Strawn et al., 2013). Atrophy of PrCG may induce abnormalities in integration of networks and mutual adjustment between cognitive function, modulation of anticipatory threat, and support of motor avoidance (Di Bono et al., 2017; Drabant et al., 2011; Ely et al., 2016). On the other hand, in adults with GAD, a PET study reporting increased functional activity in the PrCG measured is accordant with the findings of a task-based f-MRI study using anxiety-inducing words (Moon et al., 2015; Wu et al., 1991). This could reflect improvements in regulation of cognitive function, anticipatory threat and motor avoidance in GAD (Di Bono et al., 2017; Drabant et al., 2011; Ely et al., 2016). While it is challenging to fully explain alterations in the PrCG in terms of manifestation of GAD symptoms, the ROC curves (AUC = 0.764) provided potential practical significance regarding prediction of a good clinical response to pharmacological treatment.

In terms of the right SFG, our results showed a significant reduction in GMV relative to HCs. Because the right SFG is implicated in inhibitory control, self-awareness and coordination with the sensory system, particularly in the “top-down” process (Goldberg et al., 2006), its reduced GMV could impair function of the sensory system. Few studies find reduced GMV in the SFG in patients with GAD, and only one shows lower white matter volume in children and adolescents with GAD (Strawn et al., 2013). Shang and colleagues did however discover decreased GMV in the dorsolateral prefrontal cortex which may increase the vulnerability for pathological anxiety (Shang et al., 2014), as it includes a part of the SFG. Thus, disruption of the right SFG may dampen ability to inhibit behaviors, manifesting as a loss of control.

The IFG, intimately connected with the amygdala, is one of the main structure involved in the processing of anxiety as it plays a critical role in emotional regulation of anxiety and fear (Etkin et al., 2011). Notably, we found that the PrCG demonstrated enhanced rs-FC with the right IFG based on an increase in negative connections when compared to HCs. One explanation for this increase in negative connections could be the GMV reductions found in the IFG in adults with anxiety and late-life patients and GAD (Andreescu et al., 2017; Shang et al., 2014).The increase in negative connections is thought to impact the IFG's inability to appropriately gauge riskiness of a situation, leading to increased risk aversion and worry or anxiety (Etkin et al., 2011). Previous studies corroborated the correlation between increased activity of IFG and higher risk aversion (Christopoulos et al., 2009; Shang et al., 2014), even in situations provoking social anxiety (Kilts et al., 2006). Moreover, reduced GMV in IFG was one reason for an increase in negative connections with the PrCG found in adults.

The right PrCG demonstrated reduced rs-FC with the left STG based on a decrease in positive connections in GAD patients versus HCs. The STG receives the neurotransmitters of the amygdala, which plays a critical role in emotional response and interoceptive processing,

including the anxiety response, and is purported to have a timely regulatory function (van Tol et al., 2010). Also, GMV of the STG in adults with GAD has been reported to be atrophied relative to controls (Hilbert et al., 2015; Moon et al., 2014), while it is expanded in children and adolescents with GAD (Bellis et al., 2002). Thus, the morphological alterations in STG could be associated with abnormal functional connections with the PrCG.

The analysis of clinical characteristics revealed that GMV in the PrCG correlated positively with HAMA scores ($r = 0.453$, $p = 0.039$) in GAD and negatively in HCs ($r = -0.459$, $p = 0.042$). In HCs rumination could be induced if the subject had moderate anxiety, thus rumination may be causing reduced GMV in the PrCG (Blair et al., 2012; Teigen, 1994). In GAD, due to severity of anxiety, muscle tension and difficulty concentrating are more likely to blame for impaired performance and rumination as adults displayed increased GMV in the PrCG (APA, 2013; Blair et al., 2012; Teigen, 1994). This makes sense, as different levels of anxiety are known to manifest differently in the brain such as in a study conducted by Warnell and colleagues in which developing children (HCs) who reported higher anxiety levels had smaller amygdalae (Warnell et al., 2018).In terms of functional connectivity, rs-FC of PrCG - STG correlated negatively with HAMA scores ($r = -0.634$, $p = 0.02$) in patients with GAD. One possible explanation for this is that the patients' inability to regulate timely negative stimuli, which was evident from the impaired connectivity between the PrCG and STG in GAD (van Tol et al., 2010). Taken together, the PrCG may be a potential biomarker for GAD to discriminate patients from HCs.

Several limitations should be considered in our study. First, the generalizability of our results is limited given a relatively small sample size of 21 patients with GAD and 20 HCs in an ethnically homogeneous Chinese population of non-geriatric adults. We didn't have the statistical power to explore differences in specific aspects of anxiety symptoms and severity versus developmental stages. Still, our study size was reasonable given that 16–32 subjects per group had been described as the optimal sample size for between-subject comparisons in neuroimaging studies (Friston, 2012). Moreover, there is selection bias/sample bias by convenient sampling, and we didn't record the number of patients approached, nor did we record any details about the non-responders. In the future, it would be important that we submit our data to the brainnetome program for use in meta-analyses to study changes in different aspects of anxiety and age or developmental stages within a large sample. Second, although we explored the relationship between these neuroradiological findings and HAMA scores, it remains possible that the frontoparietal network in GAD patients contributed to those findings. In the future, we would like to investigate behavioral and cognitive impairments in GAD using this framework. Third, our study was limited in that it is a cross-sectional, correlative study, such that we are unable to conclude causal mechanisms of GAD. Future longitudinal studies may further our understanding of disease progression and parse out neuroanatomical and neurophysiological differences among age groups.

In conclusion, this study analyzed whole-brain GMV alterations in order to investigate rs-FC differences in patients with GAD relative to HCs. We also examined for correlations between neuroradiological findings (GMV alterations; relative rs-FC differences) and clinical findings (HAMA score). The results showed decreased GMV in the right PrCG and SFG, which were selected as ROIs for rs-FC. Within the PrCG, we found increased rs-FC in the right IFG and reduced rs-FC in the STG in patients with GAD relative to HCs. In addition, we found a positive correlation between the PrCG volume and HAMA scores, together with a negative correlation between the rs-FC of PrCG - STG and HAMA scores. These findings suggest that the right PrCG may be a candidate biomarker in patients with GAD. Furthermore, the right PrCG may even serve as a stimulation target for improvement of anxiety symptoms. The PrCG is an attractive potential stimulation target for transcranial magnetic stimulation (TMS) given its relatively superficial location in brain. In total, the findings in this report contributed to our understanding of GAD's pathophysiology by combining morphological changes with functional connectivity.

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Conflicts of interest

All co-authors listed have approved the manuscript and declare that he/she has no conflict of interest. The views expressed in this paper do not necessarily reflect the position or policy of the U.S. Department of Veterans Affairs.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pscychresns.2019.03.001.

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