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Reduced cortical thickness and increased gyrification in generalized anxiety disorder: a 3 T MRI study

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Abstract

Background. Although the study of the neuroanatomical correlates of generalized anxiety disorder (GAD) is gaining increasing interest, up to now the cortical anatomy of GAD patients has been poorly investigated and still no data on cortical gyrification are available. The aim of the present study is to quantitatively examine the cortical morphology in patients with GAD compared with healthy controls (HC) using magnetic resonance imaging (MRI). To the best of our knowledge, this is the first study analyzing the gyrification patterns in GAD.

Methods. A total of 31 GAD patients and 31 HC underwent 3 T structural MRI. For each subject, cortical surface area (CSA), cortical thickness (CT), gray matter volume (GMV), and local gyrification index (LGI) were estimated in 19 regions of interest using the Freesurfer software. These parameters were then compared between the two groups using General Linear Model designs.

Results. Compared with HC, GAD patients showed: (1) reduced CT in right caudal middle frontal gyrus ($p < 0.05$, Bonferroni corrected), (2) hyper-gyrification in right fusiform, inferior temporal, superior parietal and supramarginal gyri and in left supramarginal and superior frontal gyri ($p < 0.05$, Bonferroni corrected). No significant alterations in CSA and GMV were observed.

Conclusions. Our findings support the hypothesis of a neuroanatomical basis for GAD, highlighting a possible key role of the right hemisphere. The alterations of CT and gyrification in GAD suggest a neurodevelopmental origin of the disorder. Further studies on GAD are needed to understand the evolution of the cerebral morphology with age and during the clinical course of the illness.

Introduction

Generalized anxiety disorder (GAD) is a condition characterized by excessive and persistent worry and anxiety accompanied by physical symptoms such as restlessness, fatigue, irritability, difficulty in concentrating, muscle tension, and sleep disturbance (American Psychiatric Association, 2013). Intolerance to uncertainty, a generalization of fear and alterations in emotional processing and regulation seem to characterize this disorder (Mochcovitch *et al.* 2014). This condition leads to significant distress and impairment in everyday life, as well as to increased risk for other mental and physical problems, including alcohol and drug abuse, being also frequently comorbid with major depression. Lifetime prevalence of GAD is estimated around 4.7% (Kessler *et al.* 2012), resulting more common among women, almost twice as high as men (McLean *et al.* 2011). Female sex, low socioeconomic status, and exposure to childhood adversity are considered as risk factors for the disease (Fonzo *et al.* 2016). The heritability of GAD has also been studied, suggesting a moderate contribution of additive genetics (estimated to be around 32%) in its pathogenesis (Hettema *et al.* 2001*a*).

Original Article

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Previous neuroimaging studies have suggested functional and structural brain abnormalities in patients suffering from GAD (see (Mochcovitch *et al.* 2014) and (Hilbert *et al.* 2014) for a review), involving both subcortical nuclei – especially the amygdala - and different cortical regions. In synthesis, GAD seems to be associated with altered functional connectivity in the amygdala (Etkin *et al.* 2009; Makovac *et al.* 2016*b*) and prefrontal cortex (Andreescu *et al.* 2014). These areas, together with portions of temporal and parietal cortices, were reported as abnormally activated during emotional tasks (Palm *et al.* 2011; Greenberg *et al.* 2013; Holzel *et al.* 2013; Fonzo *et al.* 2015; Moon & Jeong, 2015; Park *et al.* 2016). Structural studies reported neuroanatomical alterations in prefrontal (Abdallah *et al.* 2012; Strawn *et al.* 2013; Cha *et al.* 2014; Strawn *et al.* 2014; Canu *et al.* 2015), temporal (De Bellis *et al.* 2002; Abdallah *et al.* 2012; Strawn *et al.* 2014), parietal (Abdallah *et al.* 2012; Makovac *et al.* 2016*a*) and cingulate cortices (Strawn *et al.* 2014; Makovac *et al.* 2016*a*).

Considering the most recent magnetic resonance imaging (MRI) studies on adult GAD patients, prefrontal cortex (PFC) appears to be widely involved in the illness. Structural MRI (sMRI) studies found in GAD patients *v.* healthy controls (HC) increased gray matter volume (GMV) in dorsomedial PFC (Schienle *et al.* 2011), as well as reduced white matter volume (Hilbert *et al.* 2015; Moon & Jeong, 2016) and cortical thickness (CT) (Abdallah *et al.* 2012; Cha *et al.* 2014; Canu *et al.* 2015) in dorsolateral PFC. Functional connectivity studies have shed light on how, in GAD patients more than in HC, lateral PFC is richly connected to the amygdala (Etkin *et al.* 2009; Andreescu *et al.* 2014). Overall, the functional MRI (fMRI) studies on GAD showed an abnormal prefrontal activation in response to different tasks (Blair *et al.* 2008; Palm *et al.* 2011; Schlund *et al.* 2012; Greenberg *et al.* 2013; Holzel *et al.* 2013; Cha *et al.* 2014; Moon & Jeong, 2015; Park *et al.* 2016).

Moreover, temporal and parietal cortices were shown to be more active in adult GAD patients than in HC during working memory tasks involving anxiety-inducing stimuli (Park *et al.* 2016) and during face emotion recognition tasks (Blair *et al.* 2008; Fonzo *et al.* 2015). The findings from the sMRI studies are less consistent: studies investigating CT found deficits in GAD compared with HC in right postcentral and left inferior parietal gyri (Abdallah *et al.* 2012) and left temporal pole and right fusiform gyrus (Canu *et al.* 2015), whereas GMV studies reported increased volume in right superior temporal pole (Hilbert *et al.* 2015) and reduced volume in portions of the parietal cortex, specifically supramarginal, precentral, and postcentral gyri (Makovac *et al.* 2016*a*).

The above heterogeneous findings could be explained in light of a recent theory, suggesting that symptoms and features of GAD reflect complex network alterations, in turn affecting the functionality of several cortical structures and deep brain nuclei (Etkin *et al.* 2009; Sylvester *et al.* 2012). The main circuits that are thought to be implicated in anxiety disorders are the fronto-parietal, the default mode, the cingulo-opercular and the ventral attention networks (Sylvester *et al.* 2012). Even if the reasons for these alterations are yet to be understood, the involvement of different perturbing factors during brain development is conceivable.

Nowadays, the sMRI studies on GAD mainly focused on gray and white matter volumes (De Bellis *et al.* 2002; Mohlman *et al.* 2009; Schienle *et al.* 2011; Strawn *et al.* 2013; Hilbert *et al.* 2015; Makovac *et al.* 2016*a*; Moon & Jeong, 2016), whereas only a few of them (Abdallah *et al.* 2012; Cha *et al.* 2014; Strawn *et al.* 2014; Canu *et al.* 2015) considered other cortical features, specifically thickness, reflecting the cellular organization of the brain and, indirectly, the brain development. To our knowledge, no data about the cortical surface area (CSA) and gyrification patterns in GAD are available.

The main objective of the present MRI study is to quantitatively compare cortical morphology between GAD patients and HC in terms of thickness, volume, curvature or gyrification, and surface area. The identification of specific morphological differences between GAD patients and HC can shed new light on the neurobiological determinants of GAD, with relevant implications for the management and treatment of anxiety disorders.

Methods and materials

Subjects

Thirty-one adult individuals (11 males, aged 21–70 years, 43.8 ± 14.9 years) who met the DSM-IV criteria for GAD underwent a sMRI session. The patients were recruited in the outpatient Psychiatric Clinic of the University Hospital of Udine, Italy. Patients with a history of traumatic head injury with loss of consciousness and neurological or medical illness were excluded from the study. The diagnosis of GAD was assessed using the Structured Clinical Interview for Axis I DSM-IV disorders (SCID-I) and confirmed by the clinical consensus of two expert psychiatrists, who discussed about any major symptom and psychopathological discrepancies emerged from interviews and SCID-I scores, finally agreeing on the presence of the illness.

Eighteen patients were taking one or more psychotropic drugs at the time of the MRI scanning. Fifteen were receiving regular antidepressants: four were taking escitalopram, three paroxetine, two sertraline, two mirtazapine, two citalopram, one venlafaxine and one duloxetine. One patient was taking an antipsychotic drug (amilsulpride). In addition, eight patients were taking benzodiazepines when needed. Given the variety of therapies administered to the patients, their possible contribution to brain morphology was not considered in the study design. Three patients met the criteria for a current comorbid anxiety or mood disorder, one major depressive disorder (MDD), one Specific Phobia (SP), and one SP and past MDD, whereas 16 patients had no history of other psychopathologic conditions, as determined by the SCID-I. Of the remaining twelve patients, four were diagnosed as having only past MDD, three only past panic disorder (PD), one only past eating disorder (ED), two past PD and MDD, one past MDD and ED and one past alcohol abuse and depressive episode induced by substance abuse. Since just a few patients presented a comorbidity at the time of the MRI acquisition and almost half of the sample had no history of other mental illnesses, in this work we chose not to consider the effect of an actual or past comorbid psychopathologic condition.

Thirty-one healthy subjects (14 males, aged 22–65 years, 39.7 ± 13.5 years) with no significant differences in terms of age, gender, handedness, socioeconomic status and ethnicity ($p < 0.05$) were also included in the study for reference. They were recruited from the local community and had no history of alcohol or substance abuse, head trauma or neurological or major medical illnesses, or psychiatric axis I disorders determined using a brief modified version of the SCID-I (non-patient version). They also had no history of psychiatric disorders among first-degree

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relatives. All the participants signed a written informed consent to the study protocol, which was approved by the competent ethical committee and was in accordance with the Declaration of Helsinki.

Psychopathological and cognitive assessment

The Brief Psychiatric Rating Scale (BPRS, 24-item version) (Ventura *et al.* 2000), the Hamilton Rating Scale for Anxiety (HARS) and Depression (HDRS) (Hamilton, 1959; Hamilton, 1960) and the Global Assessment Functioning (GAF) scales (Endicott *et al.* 1976) were administered to all participants. Taken together, these gold standard instruments allowed to comprehensively investigate the variety of symptoms experienced by GAD patients. The socioeconomic status and level of education were also assessed. Handedness was determined by the Edinburgh Handedness Inventory (Oldfield, 1971). The subjects' cognitive performances were evaluated by administering the Raven Matrices Test (RMT) (Raven, 2000).

MRI data acquisition

MRI data were acquired using a Philips Achieva 3.0 T scanner (Best, The Netherlands). An 8-channel head coil was used for radiofrequency transmission and reception. The images were obtained with a magnetization prepared rapid acquisition gradient echo T1-weighted three-dimensional (3D) turbo field echo sequence using the following parameters: echo time = 3.7 ms, repetition time = 8.1 ms, field of view = 240 × 240 mm^2, matrix size = 240 × 240, 190 axial slices with no gap, isotropic voxel size = 1 mm^3. Scan parameters were consistent across all the imaging sessions of this study.

MRI data processing

All the MRI scans were processed on the same workstation using the Freesurfer (FS) image analysis suite, v5.3.0 (http://surfer.nmr.mgh.harvard.edu/). Technical details regarding the use of FS are described elsewhere (Fischl, 2012). Briefly, T1-weighted images were used to segment brain tissues and to estimate the gray matter–white matter interface, which was used as the starting point for the 3D reconstruction of the cortical surfaces. The segmentation output and reconstructed surfaces were visually inspected and manually corrected when necessary. Each subject's cortical model was parceled into regions of interest (ROIs) according to the Desikan–Killiany atlas (Desikan *et al.* 2006), and surface-based measures, specifically CT, CSA, GMV and local gyrification index (LGI), were estimated at the ROI level for all participants. More details on their computation are in the Supplementary section. Based on the clinical knowledge of anxiety disorders, 19 cortical ROIs of the Desikan–Killiany atlas (i.e. caudal and rostral anterior cingulate, isthmus cingulate, posterior cingulate, caudal and rostral middle frontal, lateral and medial orbitofrontal, superior frontal, entorhinal, inferior parietal, superior parietal, supramarginal, lateral occipital, superior, middle and inferior temporal, fusiform, and insula) were selected for morphological comparison between GAD patients and HC.

Statistical analyses

The demographic, socioeconomic and clinical data were analyzed using the Statistical Package for the Social Sciences (SPSS) (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA), whereas the FS cortical measures were compared among patients and HC using Matlab R2015b in-house scripts (The Mathworks, Inc. Natick, MA, USA).

Group differences in sociodemographic and clinical variables were examined using the chi-squared test or the Mann–Whitney *U* test for independent samples, as appropriate. The level of significance was placed at 0.05. The choice to use the Mann–Whitney *U* test was motivated by the fact that most variables were not normally distributed in either one or both groups, based on the Shapiro–Wilk normality test.

The neuroimaging data were analyzed using General Linear Model (GLM) designs, which compared one by one the regional morphological measures between GAD patients and HC while accounting for possible confounding factors. A total of 152 GLM analyses (one for each cortical parameter of each ROI) were performed. Age and gender were included as covariates in all the GLM analyses. No additional regressors were added in the analyses of gyrification and thickness, whereas the total intracranial volume and total hemispheric surface area were added as covariates in the analyses focusing on GMV and CSA, respectively. To limit the false positive rates, a correction for multiple comparisons was applied throughout the regional analyses by using the Bonferroni method (considering the 19 different regions within one hemisphere).

Within the GAD group, the relationship between neuroanatomical and clinical variables was also investigated. To this end, we conducted Pearson linear partial correlation analyses between each regional parameter (GMV, CSA, CT, and LGI) and the score of the clinical tests (GAF, BPRS, HDRS, and HARS) while accounting for the contribution of age and gender.

Results

Sample characteristics

The sample characteristics are reported in Table 1. There were no significant differences in age, gender, handedness, socioeconomic status and ethnicity between the two groups. Compared to HC, GAD patients showed lower intelligence quotient (IQ) as calculated with the RMT ($p < 0.05$), as well as higher scores in all the clinical tests that measured anxiety or depressive symptoms ($p < 0.001$).

Surface area

Although no significant CSA differences emerged between GAD patients and HC after the Bonferroni correction, some tendencies were observed, as GAD patients presented reduced CSA in the left inferior parietal, fusiform and lateral orbitofrontal regions ($p < 0.05$, uncorrected). The statistical details are reported in Table 2.

Cortical thickness

Compared to HC, GAD patients showed significantly reduced CT in the right caudal middle frontal gyrus ($p < 0.05$, Bonferroni corrected). An additional tendency was observed in the superior parietal region of the right hemisphere, whose CT was lower in GAD compared with HC ($p < 0.05$, uncorrected). The statistical details are reported in Table 3.

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Table 1. Characteristics of the sample

Characteristic[a]	GAD ($n = 31$)	HC ($n = 31$)	Test	p
Gender (M/F)	11/20	14/17	$\chi^2 = 0.603$ (df = 1)	n.s.
Age	43.77 ± 14.61	39.68 ± 13.48	$Z = -1.275$	n.s.
Language (Italian/other)	29/2	30/1	$\chi^2 = 0.350$ (df = 1)	n.s.
Handedness (EHI)	15.79 ± 7.74	14.03 ± 11.48	$Z = -0.009$	n.s.
SES TOT	33.23 ± 13.04	38.50 ± 14.15	$Z = 1.198$	n.s.
SES EDU	11.00 ± 4.65	11.67 ± 4.54	$Z = 0.79$	n.s.
SES OCC	22.23 ± 9.54	26.83 ± 10.28	$Z = 1.650$	n.s.
RM TOT	45.00 ± 9.67	50.87 ± 6.85	$Z = 2.490$	0.013
RM CENT	87.07 ± 16.16	95.03 ± 6.55	$Z = 1.448$	n.s.
RM IQ	120.21 ± 10.60	125.97 ± 3.97	$Z = 2.444$	0.015
GAF	75.68 ± 7.41	87.57 ± 4.62	$Z = 5.460$	<0.001
BPRS	30.36 ± 3.26	24.97 ± 1.30	$Z = -5.877$	<0.001
HARS	10.82 ± 7.34	3.33 ± 4.65	$Z = -4.037$	<0.001
HDRS	7.93 ± 5.13	1.90 ± 3.04	$Z = -5.028$	<0.001

M, male; F, female; GAD, General Anxiety Disorder; HC, healthy controls; χ^2, chi-squared test value; Z, z-statistic value from the Mann–Whitney U test; P, p value of the test; df, degrees of freedom; EHI, Edinburgh handedness inventory; SES EDU, socioeconomic status, level of education; SES OCC, socioeconomic status, occupation level; SES TOT, socioeconomic status, total; RM TOT, Raven Matrices, total score; RM CENT, Raven Matrices, percent score; RM IQ, Raven Matrices, Intelligence Quotient; BPRS, Brief Psychiatric Rating Scale; GAF, Global assessment of functioning; BPRS, Brief Psychiatric Rating Scale; HDRS, Hamilton Depression Rating Scale; HARS, Hamilton Anxiety Rating Scale.
[a]For continuous variables, mean ± s.d. is given.

Table 2. Results of the surface area comparison

Brain region	Hemisphere	Result	GAD[a]	HC[a]	T stat[b]	p[c]
Inferior parietal	Left	GAD < HC	4208.26 ± 534.76	4307.16 ± 608.74	2.01	0.049
Fusiform	Left	GAD < HC	2832.65 ± 426.46	2935.16 ± 414.50	2.62	0.011
Lateral orbitofrontal	Left	GAD < HC	2719.87 ± 390.92	2753.29 ± 348.48	2.02	0.048

GAD, General Anxiety Disorder; HC, Healthy Controls; ROI, region of interest.
[a]in mm^2; mean ± s.d. is given.
[b]General Linear Model: ROI area ~ 1 + Diagnosis + Gender + Age + Total Hemispheric Area.
[c]Level of significance after Bonferroni correction: $p < 0.0026$.

Gray matter volume

No significant GMV differences between patients and HC were found.

Gyrification

The significant results of the LGI comparison are shown in Fig. 1. Compared with HC, GAD patients were characterized by significantly higher LGI in the fusiform, inferior temporal and supramarginal gyri and in the superior parietal cortex of the right hemisphere, as well as in the supramarginal and superior frontal gyri of the left hemisphere ($p < 0.05$, Bonferroni corrected). The analysis details are reported in Table 4.

Correlation analyses

Within the GAD group, the neuroanatomical parameters were not significantly correlated to the clinical scores ($p < 0.05$, Bonferroni corrected). However, a few tendencies were observed ($p < 0.01$). Specifically, we found positive correlations between (1) BPRS score and CSA in the right anterior cingulate cortex (rostral

Table 3. Results of the cortical thickness comparison

Brain region	Hemisphere	Result	GAD[a]	HC[a]	T stat[b]	p[c]
Caudal middle frontal	Right	GAD < HC	2.34 ± 0.11	2.46 ± 0.15	3.44	0.001
Superior parietal	Right	GAD < HC	2.03 ± 0.11	2.11 ± 0.08	2.89	0.005

GAD, General Anxiety Disorder; HC, Healthy Controls; ROI, region of interest.
[a]in mm; mean ± s.d. is given.
[b]General Linear Model: ROI thickness ~ 1 + Diagnosis + Gender + Age.
[c]Level of significance after Bonferroni correction: $p < 0.0026$.

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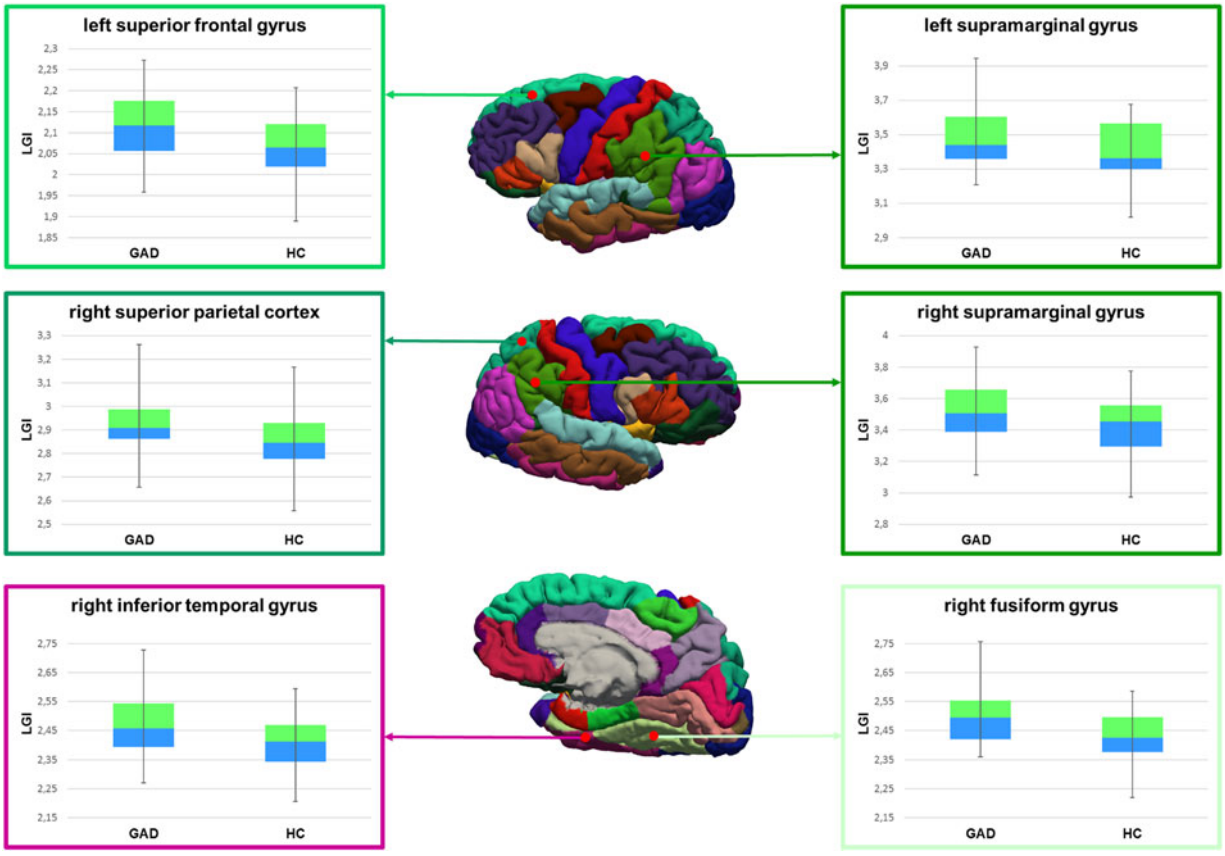


Fig. 1. Results of local gyrification index (LGI) comparison. The regions with significant differences between generalized anxiety disorder (GAD) patients and healthy controls (HC) are highlighted with red dots on the cortical surface parceled according to the Desikan–Killiany atlas. The diagrams show the LGI minimum, maximum, median, and 25th and 75th percentiles in the two groups.

section) (rho = 0.554, $p = 0.004$), (2) HDRS score and CT in the left isthmus cingulate cortex (rho = 0.564, $p = 0.003$), (3) HARS score and CT in the left middle temporal cortex (rho = 0.563, $p = 0.003$).

Discussion

The aim of the present work was to comprehensively investigate the cortical anatomy of patients affected by GAD, providing a robust basis for understanding the pathophysiology of this disabling illness. To this end, regional cerebral morphological parameters (CT, CSA, GMV, and LGI) were estimated and compared between GAD and HC. Overall, our results suggest the presence of neuroanatomic underpinnings of GAD, affecting specific morphological parameters. Indeed, we observed significant CT deficits and hyper-gyrification mainly in the parietal, frontal and temporal cortices of the right hemisphere, but no differences in surface area and volume of the cortex.

Among the available literature, the present work investigated the neuroanatomy of GAD in one of the largest samples of patients. Besides confirming the most consistent literature knowledge on brain structure in GAD, our work identified its neuronal

Table 4. Results of the local gyrification index comparison

Brain region	Hemisphere	Result	GAD[a]	HC[a]	*T* stat[b]	*p*[c]
Superior frontal	Left	GAD > HC	2.114 ± 0.083	2.071 ± 0.078	3.41	0.001
Supramarginal	Left	GAD > HC	3.483 ± 0.170	3.394 ± 0.171	3.50	0.001
Fusiform	Right	GAD > HC	2.505 ± 0.104	2.432 ± 0.081	4.37	<0.001
Inferior temporal	Right	GAD > HC	2.466 ± 0.108	2.406 ± 0.098	3.62	0.001
Superior parietal	Right	GAD > HC	2.925 ± 0.126	2.838 ± 0.141	3.54	0.001
Supramarginal	Right	GAD > HC	3.540 ± 0.188	3.409 ± 0.210	4.44	<0.001

GAD, general anxiety disorder; HC, healthy controls; ROI, region of interest.
[a]mean ± S.D. is given.
[b]General Linear Model: ROI LGI ~ 1 + Diagnosis + Gender + Age.
cLevel of significance after Bonferroni correction: $p < 0.0026$.

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bases with improved morphological specificity compared to previous ones, as it considered different cortical determinants in several regions. The sample characteristics, methods, and findings of previous anatomical and functional MRI studies on GAD and other anxiety or depressive disorders are summarized in online Supplementary Table S1. Among the fMRI studies, only the ones whose results are related to our structural findings are reported. It should be noticed that only in a couple of studies the patients' sample size was similar to ours (Greenberg *et al.* 2013; Cha *et al.* 2014), while the majority of previous works was conducted on smaller samples. Two out of four studies investigating CT were based on less than 15 patients (Abdallah *et al.* 2012; Strawn *et al.* 2014).

Cortical thickness

The present study found CT deficits in the right caudal middle frontal gyrus of GAD patients compared with HC. Since CT is thought to reflect phenomena taking place in cortical maturation during intrauterine life (asymmetric division of founder cells) (Rakic, 2009) and during childhood and adolescence (synaptic pruning, myelination and apoptosis) (Bourgeois & Rakic, 1993; Morrison & Hof, 1997; Sowell *et al.* 2004; Shaw *et al.* 2008; Vanderhaeghen & Cheng, 2010), the CT alterations in GAD may result from neurodevelopmental factors.

As summarized in online Supplementary Table S1, just a few studies quantitatively investigated CT in anxiety, and fewer of them focused specifically on GAD (Abdallah *et al.* 2012; Cha *et al.* 2014; Strawn *et al.* 2014). Among them, (Abdallah *et al.* 2012) and (Canu *et al.* 2015) observed in GAD patients CT deficits in the rostral middle frontal cortex, adjacent to the area emerged from our study. Frontal neuroanatomical changes emerged also from studies on social anxiety (Kuhn *et al.* 2011; Syal *et al.* 2012; Frick *et al.* 2013).

The just mentioned neuroanatomical deficits may underlie intolerance to uncertainty and emotional dysregulation characterizing GAD patients (Ladouceur *et al.* 2000). Indeed, fMRI studies reported altered activity of the middle frontal gyrus in GAD patients during face emotion processing tasks. Taken together, these results strongly support the role of the dorsolateral PFC for the neurobiological underpinnings of GAD.

It should be noticed that a study from Strawn and colleagues on a pediatric population (Strawn *et al.* 2014) also identified regions with higher CT in GAD than in HC, mainly in frontal, temporal, and occipital cortices. However, CT seems to be widely affected by brain maturation: for example, the relationship between prefrontal CT and anxiety/depressive symptoms has been shown to change throughout the course of adolescence (Ducharme *et al.* 2014). Therefore, the discrepancy between our findings and the ones of Strawn *et al.* may be due to the younger sample of patients characterizing the latter study.

Future longitudinal studies are needed to shed light on the CT developmental changes in GAD patients in comparison with normally developing subjects.

Gyrification

One of the main novelties of our study concerns the investigation of cortical gyrification in GAD. We found significantly altered gyrification in GAD patients compared to HC in regions belonging to frontal, temporal and parietal lobes of both hemispheres.

Gyrification is believed to reflect the cortical connectivity during neurodevelopment, as the patterns of cortical folding are thought to result from different growth mechanisms of cell layers during brain maturation (Van Essen, 1997). Recent works put forward the hypothesis that abnormalities in cortical folding could be linked to altered connectivity (Schaer *et al.* 2013; Nixon *et al.* 2014).

The abnormalities emerged from our study may be interpreted in terms of neuronal development. Indeed, although the development of the cerebral cortex takes mainly place during life in utero and early childhood, structural modifications, especially in gyrification patterns, have been observed also during adolescence and young adulthood, suggesting that cortical structure encounters different levels of change during lifetime (White *et al.* 2010; Raznahan *et al.* 2011; Hogstrom *et al.* 2013; Mutlu *et al.* 2013; Su *et al.* 2013). Since anxiety disorders often emerge during adolescence and early adulthood, the presence of cortical folding abnormalities in GAD suggests a link between late brain maturation and neurobiological bases of anxiety.

Specifically, our results suggest that GAD is characterized by hyper-gyrification in bilateral supramarginal, left superior frontal and right fusiform, inferior temporal and superior parietal gyri. The absence of gyrification studies on GAD prevents us from making any direct comparison between our and previous findings. Nonetheless, structural and functional alterations in these regions have previously been observed in GAD. Literature evidence suggests abnormal activity of superior frontal, superior parietal, supramarginal, fusiform and inferior temporal gyri during emotional processing in GAD patients (Blair *et al.* 2008; Strawn *et al.* 2012; Fonzo *et al.* 2015), which may explain the poorer performances in working memory tasks with emotional distracters (Moon & Jeong, 2015; Park *et al.* 2016).

The involvement of bilateral supramarginal gyri in GAD is further supported by recent evidence of (1) GMV deficits in this region (Makovac *et al.* 2016*a*) and (2) decreased functional connectivity between right supramarginal gyrus and right amygdala (Makovac *et al.* 2016*b*).

Interestingly, the hyper-gyrification in right supramarginal gyrus that we observed in GAD *v.* HC already emerged in patients with MDD (Peng *et al.* 2015). In this respect, it is worth mentioning that GAD and MDD have a shared genetic risk, a high grade of comorbidity and common clinical features, to the point that some theories have hypothesized an overlap between the two conditions (Hettema *et al.* 2001*b*; Watson, 2005; Mennin *et al.* 2008). Such an overlap was also observed in our study, where one-third of GAD patients had a past or current comorbidity with MDD. The hypothesis of shared neuroanatomic bases for the two conditions is fascinating and deserves further investigations.

Moreover, we still need to understand whether the hyper-gyrification that emerged from our study is the primary alteration or represents a compensatory, local, response of a single brain region to a disrupted network, as an effort to keep an efficient cumulative function. An interesting insight could be given from longitudinal studies of GAD, following the evolution of cortex through age, in order to: (1) identify the brain structural peculiarities in drug naïve first episode subjects, (2) check if the evolution of illness can modify (increase or reduce) its original cortical determinants.

Cortical volume and surface area

Our study suggests no significant differences in volume and superficial area of the cortex in GAD patients compared to HC.

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However, GAD patients had a slightly reduced CSA – not surviving after multiple comparison corrections – in left fusiform, inferior parietal and orbitofrontal cortices.

It is worth remarking that our study is the first in the literature combining the common analysis of GMV with the investigation of other morphological parameters. As for gyrification, no studies before the current investigated CSA in GAD. Therefore, the tendencies observed here should be verified by future CSA studies.

Regarding GMV, in contrast with our findings, previous volumetric studies agreed on the presence of alterations in GAD. Overall, they suggested GMV deficits in the parietal region (Strawn *et al.* 2013; Makovac *et al.* 2016*a*) and increased GMV in the temporal region (De Bellis *et al.* 2002; Hilbert *et al.* 2015). The discrepancy between our and previous results can be attributed, at least in part, to a methodological issue, as all previous works used voxel-based morphometry (VBM) instead of the surface-based approach employed in the current study. First, while we reported evidence of GMV abnormalities at the regional level, using the Desikan–Killiany atlas as a reference, previous studies investigated GMV abnormalities at the voxel level. Moreover, the calculation of GMV in our study was performed as the product of CT and CSA on the region of interest, whereas in VBM studies it was based on tissue probability maps. Due to these and other methodological differences, voxel-based, and surface-based techniques may provide different results even when applied on the same sample, as recently shown in a study on Parkinson's disease (Gerrits *et al.* 2016). Future parallel investigations are encouraged to obtain a complete understanding of GMV correlates of GAD.

Considerations about hemispheric asymmetry in GAD

In accordance to previous structural and functional studies (Davidson & Irwin, 1999; De Bellis *et al.* 2002; Zhang *et al.* 2013), we detected a predominant role of the right side of the brain in GAD, as already suggested by our group in an independent population (Brambilla *et al.* 2012).

Even if the topic is still controversial, previous works tried to understand how each hemisphere is involved in the pathogenesis of anxiety: already in 1997, Heller *et al.* linked anxious arousal in anxious subjects to the activation of the right parietal region. However, the same subjects at rest were characterized by a more predominant role of the left hemisphere compared to reference subjects, suggesting different patterns of activation between anxious apprehension and anxious arousal (Heller *et al.* 1997). Recently, other authors investigated the issue of laterality in emotional processing, suggesting that the right hemisphere: (1) is more sensitive to perception and processing of frightening stimuli (Noesselt *et al.* 2005) and (2) is implicated in the physical response to stress, by modulating the hypothalamus–pituitary–adrenal axis through cortisol secretion (Lueken *et al.* 2009). Interestingly, a relationship between anxiety symptoms and gliomas only in the right hemisphere was reported, with decreased symptomatology after surgical removal (Mainio *et al.* 2003).

Taken together, our and previous evidence supports the idea that the two hemispheres contribute to the pathogenesis of anxiety in different ways, with a key role of the right brain in determining stress response and anxiety symptoms. The increased gyrification in right temporoparietal cortex and the CT deficits in right prefrontal cortex could be the neurobiological substrate of the aberrant processing of stress occurring in GAD, taking place especially in right brain structures.

Link between neuroanatomy and functional connectivity in GAD

Overall, the regions presenting cortical abnormalities in our work belong to the functional networks that have already been associated with anxiety disorders, especially the default mode network (DMN), including lateral parietal cortex, inferior temporal gyrus and superior frontal cortex, and the lateral frontoparietal executive network (FPN), whose key regions include the dorsolateral PFC and the posterior parietal cortices (Sylvester *et al.* 2012).

The DMN, whose activity is suppressed during cognitive tasks, is thought to be responsible for self-referential activities such as introspection, planning of future, and emotion regulation (Greicius *et al.* 2003). On the other hand, the FPN processes strategy adjustments when receiving feedbacks on behavioral errors (Dosenbach *et al.* 2008). The interaction between these two networks is essential for an adequate cognitive regulation of emotion. A recent study has hypothesized that decreased functioning of DMN and FPN may lead to some features of anxiety disorders (Sylvester *et al.* 2012), such as emotional dysregulation and deficits in cognitive control respectively. Our findings support this hypothesis by providing a structural basis for the DMN and FPN alterations, contributing in turn to emotive–cognitive dysfunction typical of GAD.

Methodological issues and limitations

The present study suffers from some limitations. First, the modest sample size may prevent from detecting small anatomical differences between GAD and HC, besides limiting the statistical power of the results due to second type errors. It should also be mentioned that a number of GAD patients presented past or current comorbidities with other psychiatric conditions, which may have affected the reliability and specificity of our findings. Possible inaccuracies may also derive from the estimation of the morphological parameters at the regional level, which assumes the structural homogeneity within each ROI. In this respect, the parallel use of a voxel-wise approach should be considered for future studies. Finally, age and gender are known to affect cortical morphology and may partially contribute to group differences; however, we did adjust for age and gender in all models, in order to reduce the effect of these confounding factors. The application of Bonferroni correction also allowed us to limit the rate of false positive findings, thereby increasing the reliability of our results.

Future perspectives

The results of neuroimaging studies have potential to give new insights on the clinical management of GAD patients. Our results let us speculate that brain morphology alterations could serve as a substrate for cognitive and emotional features of GAD. In this respect, future research works should investigate in depth the link between functional and structural characteristics in GAD patients.

Future morphological studies should also explore whether innovative therapeutic interventions can influence the cortical gyrification and thickness trajectories in the regions involved in GAD symptomatology. Among the new interventional strategies, a technique that deserves mention is transcranial direct current stimulation (tDCS), which was successfully used in the treatment of MDD (Kalu *et al.* 2012) and obsessive–compulsive disorder (D'Urso *et al.* 2016), showing encouraging results in a single

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case pilot study on GAD (Shiozawa *et al.* 2014). Since tDCS effects largely depend on the electrode placement, the treatment efficacy can be maximized by improving the localization of brain morphological alterations in GAD patients.

Conclusions

The present work deals with the comprehensive investigation of the neuroanatomical correlates of GAD. For the first time, the cortical morphology of GAD patients has been inspected in terms of thickness, area, volume and gyrification of the cerebral cortex. Our results suggest that GAD is characterized by specific structural abnormalities that mainly involve the right hemisphere. GAD patients showed CT deficits in the right caudal middle frontal gyrus and hyper-gyrification in the superior frontal and supramarginal gyri of the left hemisphere and in the fusiform, inferior temporal, superior parietal and supramarginal regions of the right hemisphere.

The above regions are part of the networks implicated in cognitive-emotion regulation and processing, therefore necessary for an adequate interaction between cognition and emotions. Thence, the structural alterations emerged from our study may represent the substrate and/or the consequence of more widespread network alterations. Since thickness and gyrification reflect lifetime changes in the microscopic anatomy of the cerebral cortex, further longitudinal studies are needed in order to establish the chronology of these alterations over the course of the illness.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S003329171700352X

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Declaration of interest. None.

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

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