Regional Gray Matter Density Changes in Brains of Patients With Irritable Bowel Syndrome

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BACKGROUND & AIMS: Several studies have examined structural brain changes associated with chronic pain syndromes, including irritable bowel syndrome (IBS), but study sample sizes have been small and heterogeneous. **METHODS:** We used magnetic resonance imaging–based techniques, voxel-based morphometry, and cortical thickness analysis to examine brain anatomical differences in a relatively large, tightly screened sample of IBS patients (n = 55); we compared data with that from healthy persons (controls; n = 48). **RESULTS:** IBS was associated with decreased gray matter density (GMD) in widespread areas of the brain, including medial prefrontal and ventrolateral prefrontal cortex, posterior parietal cortex, ventral striatum, and thalamus. Compared with controls, we observed increased GMD in patients with IBS in the pregenual anterior cingulate cortex and the orbitofrontal cortex, as well as trends in the posterior insula/secondary somatosensory cortex, (para)hippocampus, and left dorsolateral prefrontal cortex. In accounting for anxiety and depression, we found that several of the regions involved in affective processing no longer differed between patients with IBS and controls, whereas the differences in prefrontal and posterior parietal cortices remained. The areas of decreased GMD associated with IBS were largely consistent across clinical subgroups, based on predominant bowel habit and pain predominance of symptoms. No overall or regional differences were observed in cortical thickness between patients with IBS and controls. **CONCLUSIONS: Changes in density of gray matter among regions involved in cognitive/evaluative functions are specifically observed in patients with IBS, whereas changes in other areas of the brain can be explained by levels of anxiety and depression.**

Keywords: Neuroimaging; Voxel-Based Morphometry; VBM; Neural.

Irritable bowel syndrome (IBS) is a complex, symptom-based disorder defined by recurrent abdominal pain or discomfort associated with alterations in bowel habits.[1] The syndrome is heterogeneous, with subgroups based on predominant bowel habits and most bothersome symptoms. Increased anxiety (or anxiety disorders) is observed in most patients.[2] As would be predicted with such a heterogeneous syndrome, results of functional neuroimaging studies in patients with IBS show variable results; nevertheless, increased regional activity in insula (INS) and anterior midcingulate cortex (aMCC) have been most commonly reported.[3]

IBS has been categorized as a "functional" pain syndrome, along with a number of other syndromes that have as a characteristic pain of unknown origin, including fibromyalgia, chronic low back pain, headache, and chronic vulvar pain. Although increased afferent drive may contribute to the hypersensitivity of these syndromes, accumulating evidence suggests that an alteration in descending pain modulation, and an associated alteration in cortico-limbic-pontine brain circuits may make an important contribution to the hypersensitivity.[4] Structural abnormalities in these regions have been identified in small populations of patients experiencing different persistent pain disorders (including fibromyalgia,[5–8] chronic low back pain,[9,10] headache/migraine,[11–13] and chronic vulvar pain[14]). Although decreases in gray matter size predominate, in some cases, increases in regional gray matter are observed and interpreted as a possible use-dependent hypertrophy.[14] The relationship of changes in gray matter density (GMD)

Abbreviations used in this paper: ACC, anterior cingulate cortex; aMCC, anterior midcingulate cortex; BA, Brodmann area; CSF, cerebrospinal fluid; CT, cortical thickness; CTA, cortical thickness analysis; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders 4th edition; GLM, general linear model; GM, gray matter; GMD, gray matter density; IBS, irritable bowel syndrome; INS, insula; MFG, middle frontal gyrus; MRI, magnetic resonance imaging; OFC, orbitofrontal cortex; PAG, periaqueductal gray; PFC, prefrontal cortex; S2, secondary somatosensory cortex; UCLA, University of California Los Angeles; VBM, voxel-based morphometry.

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with functional alterations and symptoms in pain disorders, and the underlying mechanisms remain incompletely understood. Regional GMD changes may be a trait of patients or could be a consequence of disease through mechanisms such as neurotoxicity or neuroinflammation (in the case of GMD decreases) or from use-related increases.[15–18] Two studies have also reported morphometric differences between small samples of patients with IBS and healthy controls, which included a reduction in GMD and cortical thickness (CT) in aMCC.[19,20] However, these studies did not determine which characteristics of patients with IBS (eg, pain, other IBS symptoms, anxiety, depression) contributed to the anatomical abnormalities.

In the current study, we attempted to dissect possible components of neuroanatomical abnormalities in patients with IBS by evaluating a large tightly screened sample of female patients with IBS, for which we had information about multiple IBS symptoms, as well as measures of anxiety and depression. We hypothesized that (1) IBS is associated with decreased gray matter in regions shown to have increased responsiveness to rectal distension and its expectation, including INS and anterior cingulate cortex (ACC), possibly because of overuse excitotoxicity; (2) IBS is associated with an increase in gray matter in some regions, particularly in regions involved in cognitive and attentional modulation of interoceptive information, consistent with a role of these regions in central pain amplification; and (3) depression, anxiety, and predominant symptom explain part of the anatomical differences between patients with IBS and controls.

Materials and Methods

Subjects

The sample consisted of 106 women of whom 56 had IBS and 49 were age-matched female controls from 3 different functional magnetic resonance imaging (MRI) study protocols performed on the same MRI scanner. These studies were approved by the Office for the Protection of Research Subjects at the University of California, Los Angeles. Subjects for these studies were recruited by advertisement and from specialty clinics of the University of California Los Angeles (UCLA) Division of Digestive Diseases. All patients were evaluated by 1 of 2 gastroenterologists at the UCLA Center for Neurobiology of Stress, experienced in the diagnosis of functional gastrointestinal disorders. Patients with IBS met ROME II criteria and were screened by a psychologist using a structured psychiatric interview for DSM-IV (Diagnostic and Statistical Manual of Mental Disorders 4th edition) to exclude any patient with a recent DSM-IV diagnosis of mood and affect disorders. Patients were also excluded if they were taking any centrally acting medications, such as antidepressants (tricyclic antidepressants, selective serotonin reuptake inhibitors, norepinephrine serotonin reuptake inhibitors), anxiolytics, or pain medications. Three subjects were excluded because of missing behavioral data (n = 2) and poor MRI data (n = 1).

Questionnaires

Each subject completed the Brief Symptom Inventory,[21] the Hospital Anxiety and Depression Scale,[22] and the UCLA Digestive Disease Center Symptom Questionnaire[23] and reported his or her most bothersome symptom and bowel habits (constipation, diarrhea, or alternating). Symptom severity was assessed on a 5-point scale from 0 (none: no symptoms) to 4 (very severe: markedly affects my lifestyle).

MRI

Brain images were acquired on a 3T MRI scanner (Siemens Allegra). First, a sagittal scout was used to position the head. Then each subject underwent a high-resolution 3-dimensional T1-weighted, sagittal, magnetization prepared rapid gradient echo (TR = 23 ms, TE = 2.85 ms, flip angle 9, final resolution 1 × 1 × 1 mm).

VBM and CTA Preprocessing

We used the CIVET pipeline (v. 1.1.9) for all preprocessing of voxel-based morphometry (VBM) and CT analysis (CTA) data (http://wiki.bic.mni.mcgill.ca/index.php/CIVET). Details (including references) of these methods are provided in Supplementary Methods. For VBM, tissues were classified as gray matter (GM), white matter, or cerebrospinal fluid (CSF), and smoothed 8 mm. CT in millimeters was calculated at 81,924 vertices for each brain and smoothed 20 mm. Cerebellum was excluded from analyses.

Analysis

For whole brain tissue analyses, we extracted the mean GMD, white matter density, and CSF density, as well at the mean CT for each person. We plotted these values against age and performed a 1-way analysis of variance (ANOVA) to compare between groups (SPSS 16.0, SPSS Inc, Chicago, IL).

We used SurfStat (http://www.stat.uchicago.edu/~worsley/surfstat/) for VBM and CTA. We applied a general linear model (GLM) comparing groups, with age as a covariate of no interest. Separate GLMs comparing controls and patients with IBS were run for CTA and VBM with the covariates: age, age + anxiety; age + depression, age + anxiety + depression. In addition for VBM, we performed separate analyses comparing subgroups of patients with IBS with age as a covariate. The subgroups were based on bowel habits (constipation [n =

Table 1. Subject Characteristics

	IBS (n = 55)	IBS-C (n = 15)	IBS-D (n = 17)	IBS-A (n = 19)	Controls (n = 48)	F value (*P* value)
Age (*y*)	32.2 (12.3)	35.0 (13.4)	32.0 (5.46)	31.2 (10.9)	31.1 (12.3)	0.243 (.62)
Anxiety score[a]	5.89 (3.50)	6.67 (3.67)	5.24 (2.68)	5.21 (3.16)	3.49 (2.38)	16.5 (<.0001)
Depression score[a]	2.98 (2.77)	3.27 (2.40)	3.06 (2.81)	2.21 (2.15)	0.80 (0.922)	27.4 (<.00001)
IBS severity score[b]	2.00 (0.544)	2.07 (0.704)	2.00 (0.612)	1.95 (0.405)		
IBS duration (*y*)	11.1 (7.73)	16.5 (9.78)	9.06 (6.06)	8.47 (5.98)		

NOTE: Values are presented as mean (standard deviation). F and *P* values are from 1-way analysis of variance between patients with IBS (n = 55) and controls.
IBS, irritable bowel syndrome; IBS-C, IBS with bowel habit of constipation; IBS-D, IBS with bowel habit of diarrhea, and IBS-A, IBS with bowel habit of alternating.
[a]Depression and anxiety were measured on the Hospital Anxiety and Depression Scale; possible scores for anxiety and depression range from 0 to 21.
[b]On a scale of 0 (none: no symptoms) to 4 (very severe: markedly affects my lifestyle).

15], diarrhea [n = 17], alternating [n = 19], or unspecified [n = 5], or whether the most bothersome symptom was pain [pain predominant; n = 17] or something else [non–pain predominant; n = 38]). However, all patients reported abdominal pain as one of their symptoms and met the Rome II criteria.[24] For all analyses, corrections for multiple comparisons were performed with the random field theory-based cluster analysis.[25] We set a threshold so that only contiguous voxels with a t value > 2.5 could be considered in the cluster analysis. Results from the GLM analyses are thus reported as corrected at the cluster level. For the CTA, we set an initial threshold of $P < .05$ for the voxels to be included in the cluster analysis.

For brain regions known to process nociceptive information and interoception, including primary and secondary somatosensory (S2) cortices, INS, ACC, and prefrontal cortex (PFC), brain stem, and thalamus, regions which are also most commonly observed to have different GMD between controls and chronic pain populations (for a list of such studies see reference 14), we applied a liberal corrected threshold ($P < .1$; note that the cluster contains voxels showing a minimum t value of 2.5, so the uncorrected threshold at the voxel level is about $P < .01$).

For each significant cluster from the GLM analysis between the IBS and control groups removing age, we extracted the mean GMD for each subject for plotting results and for the analyses below.

To compare the pain predominant (n = 17) and non–pain predominant (n = 38) groups, we computed GLMs between groups with age as a covariate for the average GMD in each significant cluster in the overall group comparison between patients with IBS and controls.

To test for effects of disease duration and symptom severity, we performed bivariate correlation analyses between each of the variables IBS duration and IBS symptom severity and the GMD for each significant cluster from the GLM analysis between IBS and control groups removing age.

Results

Patient Characteristics

Subject characteristics (age) and behavioral variables (depression and anxiety) for patients with IBS and controls are shown in Table 1. Mean (±SD) ages for patients with IBS and controls were 31.0 ± 12.3 years (range, 19–57 years) and 32.2 ± 10.1 years (range, 19–63 years), respectively. Of those 39 controls and 53 patients with IBS who provided menses stage information, 5 controls and 4 patients with IBS were postmenopausal. Patients had a wide range of disease duration (11.1 + 7.7 years; median, 9.0 years; range, 1–34 years) and moderate IBS symptom severity (2.0 + 0.5; median, 2.0; range, 1–3 on a scale from 0 to 4).

Although patients with IBS had statistically significantly higher anxiety (5.9 + 3.5 vs 3.5 + 2.4; on a scale from 0 to 21; $P < .001$) and depression scores (3.0 + 2.8 vs 0.8 + 0.9; on a scale from 0 to 21; $P < .001$) than controls, both individual values and means for both groups were at subclinical levels for anxiety and depression. Further characteristics of the sample subgroups are given in Table 1.

Whole Brain Tissue Densities

Regardless of group, we found age-related decreases in overall GMD and CT and age-related increases in CSF, as expected (Figure 1). No significant differences between groups were seen for these whole brain age-related changes ($P > .5$ for each tissue type, 1-way analysis of variance).

VBM

VBM results are summarized in Figure 2 and Table 2. Both decreases and increases in GMD in the IBS group compared with the control group were observed.

Decreased GMD in IBS. The group GLM with age as a covariate of no interest showed decreased GMD clusters in patients with IBS relative to controls in the left

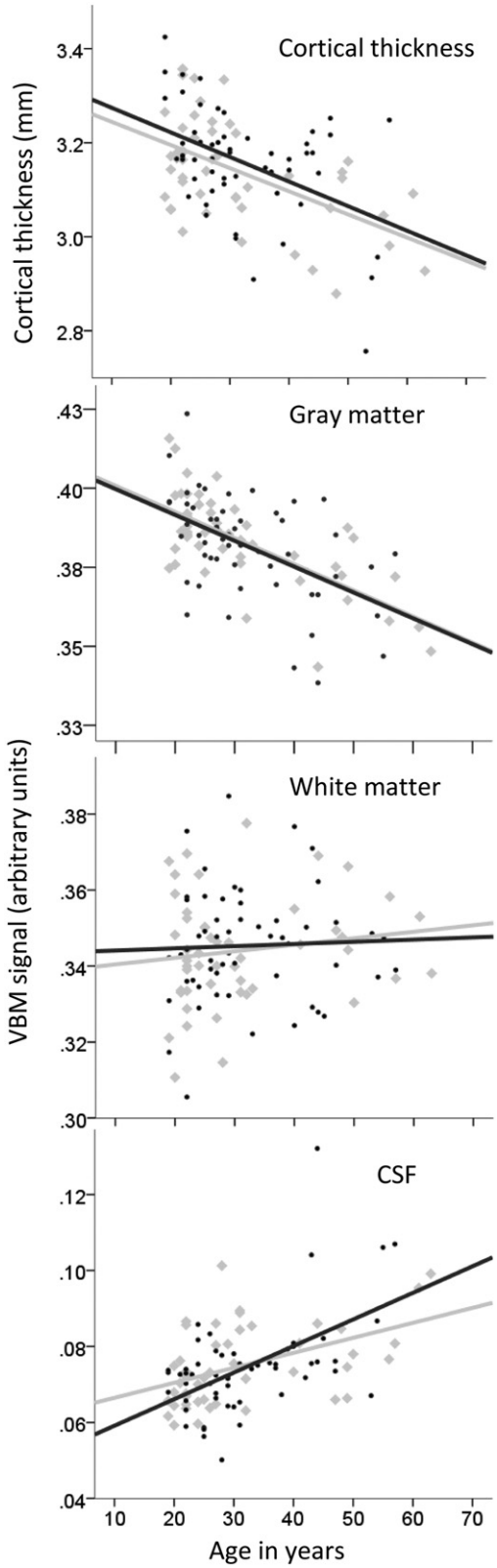

Figure 1. Age-related brain changes. Scatter plots for patients with IBS (black symbols and regression lines) and healthy controls (gray symbols and regression lines) are shown for average cortical thickness (CT), gray matter density (GMD), white matter density, and cerebrospinal fluid (CSF) density across the whole brain. Age-related decreases in GMD and CT are as expected, with no apparent differences between patients with IBS and controls.

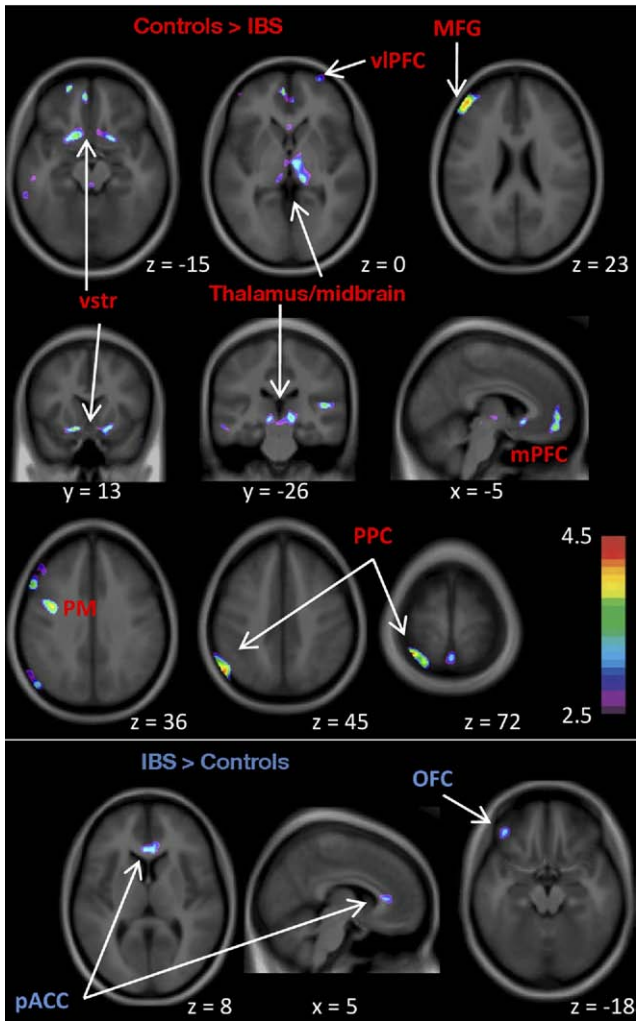

Figure 2. VBM results. Significant GMD clusters from GLM comparing patients with IBS and controls, with age as a covariate. Peak coordinates and correlations with behavioral variables are shown in Table 2. Results are displayed on a group average brain in stereotaxic (MNI) space. See Table 2 for abbreviations. Color bar shows *t* value. Left side of image is left side of brain.

posterior parietal cortex (Brodmann area [BA] 40/7) and precuneus, bilateral temporal lobe (right ventral temporal pole, left middle and inferior temporal gyri); bilateral ventrolateral PFC (BA 10) and medial PFC (BA 10), left middle frontal gyrus (MFG; BA 46), bilateral thalamus/midbrain (this cluster consisted primarily of bilateral ventral medial thalamus, which extended ventrally to the posterior medial midbrain, which contains the periaqueductal gray [PAG]), bilateral ventral striatum, premotor cortex (BA 6), and a small cluster in the occipital cortex.

Increased GMD in IBS. Clusters with greater GMD in patients with IBS than in controls included midline pregenual ACC (BA 24/32) and left orbitofrontal cortex (OFC; BA 11). At a lower threshold ($P < .1$ corrected), other areas with increased GMD included bilateral hippocampus/parahippocampus, right S2/posterior INS, and left dorsolateral PFC (BA 9). The increased

Table 2. Cluster-corrected Results from GLMs

					Age		Age and anxiety		Age and depression		Age, anxiety, and depression		IBS-C	IBS-D	IBS-A	Pain	Non-pain
Cluster, side	Peak x,y,z			*t* value	Volume	*P* value	Volume, mm^3	*P* value	Volume	*P* value	Volume	*P* value	*P* value	*P* value	*P* value	*P* value	*P* value
control>IBS																	
PPC, left	−38	−53	69	4.45	6550	.00002	11,046	.00000	19,935[a]	.00000	4006	.00037		.02250	.00018		.03706
MFG, left	−47	46	18	4.32	5407	.00007	8291	.00000	14,158[a]	.00000	1368	.01989	.03333	.02720		.01029	.00014
Thalamus bilat,					4901	.00012											
Left	−12	−29	−4	3.66			205	.32757	1637	.01218				.06771	.00189		.12237
Right	13	−29	−2	3.36			1399	.01876						.00028			.00141
Temporal, right	30	26	−36	3.72	3668	.00057	5001	.00011	7259	.00001	3144	.00116					
vStr bilat					3042	.00134											
Left	−14	14	−13	3.94												.00203	.24716
Right	22	12	−12	3.47												.00097	.00081
mPFC, left	−7	55	9	4.56	2244	.00442	2003	.00653	14,158[a]	.00000			.02640				.00153
Precun, right	8	−77	64	3.72	2227	.00454	7479	.00001	19,935[a]	.00000	3887	.00043	.02692				
Temporal, left	−59	−30	−11	3.39	1905	.00768					4707	.00015			.00605		.00089
PM6, left	−38	0	37	4.02	1363	.02008	1417	.01814	1344	.02081				.01538			.01825
vlPFC, right	27	67	−4	3.56	1303	.02250			2651	.00237						.00354	
Occipital, left	−21	−79	11	4.70	1095	.03392	1272	.02389	969	.04409							.04818
sTG, right	46	−27	11	3.52	1001	.04120	1671	.01147	1924	.00744							
Fr pole, left	−21	60	−15	3.73	982	.04289			14,158[a]	.00000	4162	.00030				.00209	.00153
Putamen, left[b]	−31	−11	11	2.95	888	.05250					1396	.01887					
vlPFC, left[b]	−39	40	−9	3.68	768	.06873			14,158[a]	.00000						.04337	
vlPFC, right[b]	55	38	26	3.09	703	.08001	4921	.00012									
IBS > control																	
pACC, left	−3	30	9	3.14	1156	.03000	1078	.03512						.01838			.00027
OFC, left	−41	38	−22	3.53	983	.04280										.00064	
dLPFC, left[b]	−42	19	34	3.91	745	.07249											.02648
Hc/pHc, left[b]	−38	−59	−14	4.02	740	.07334			923	.04865	2390	.00352	.03333				.00960
Hc/pHc, right[b]	31	−42	−19	3.44	641	.09291					926	.04834					
Hc/pHc, left[b]	−30	−32	−22	3.40	639	.09337					2390	.00352					.00960
S2/pINS, right[b]	46	−26	23	3.27	621	.09761											.01308

NOTE: The column labels indicate the factors used as covariates. IBS-C (n = 15), IBS-D (n = 17), IBS-A (n = 19), pain predominant (n = 17), and non–pain predominant (n = 38) refer to GLMs in which these subgroups are compared with controls, with age as a covariate. Peak x,y,z are coordinates in MNI/ICBM standard for analysis with age as covariate; *t* value refers to that peak. *P* values were corrected at cluster level.

dlPFC, dorsolateral prefrontal cortex; Fr. Pole, front pole Brodmann area 10; Hc/pHc, hippocampus/parahippocampal gyrus; IBS, irritable bowel syndrome; IBS-A, IBS with bothersome symptom and bowel habit of alternating; IBS-C, IBS with bothersome symptom and bowel habit of constipation; IBS-D, IBS with bothersome symptom and bowel habit of diarrhea; MFG, middle frontal gyrus; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; pACC, pregenual anterior cingulate cortex; PM6, premotor Brodmann area 6; PPC, posterior parietal cortex; precun, precuneus; S2/pINS, secondary somatosensory cortex/posterior insula; sTG, superior temporal gyrus; thalamus bilat, bilateral thalamus, midbrain; vlPFC, ventrolateral prefrontal cortex; vStr bilat, bilateral ventral striatum.

[a]Cluster contains multiple regions.

[b]Area met corrected significance level of $P < .1$ for regions selected a priori.

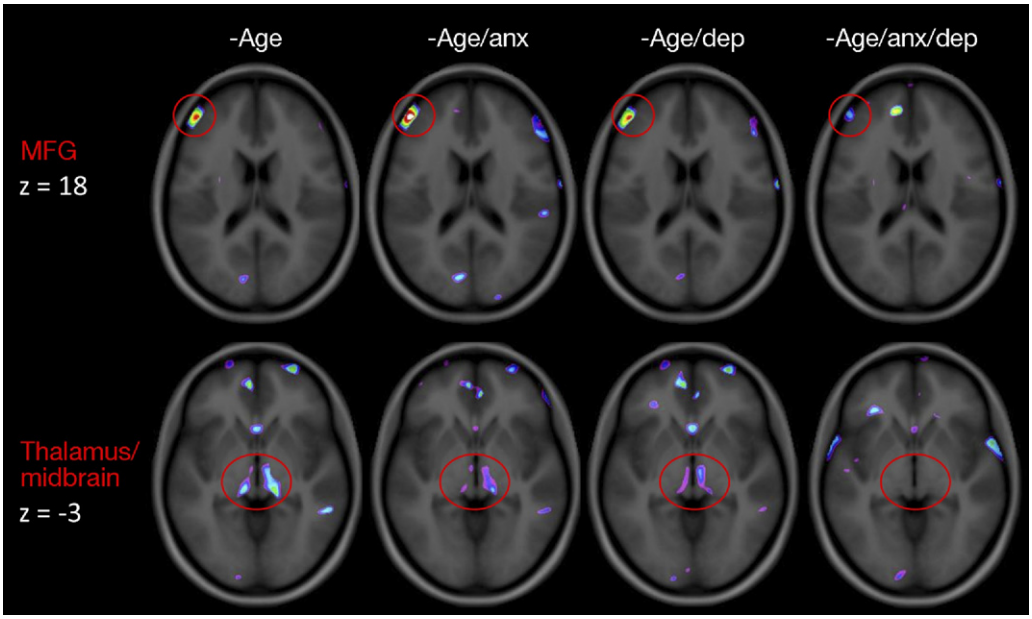

Figure 3. Covariates with anxiety and depression. Examples of regions affected by adding covariates for anxiety and depression to the GLM. The MFG cluster shown on the *top row* was significant in all GLMs, regardless of the covariate(s). The *bottom row* compares the thalamus cluster, which is eliminated when anxiety and depression are included as covariates. The image is thresholded the same way as Figure 2 (ie, $t > 2.5$ up to $t = 4.5$). No masking has been applied to the images (ie, whole brain results shown).

S2/posterior INS and hippocampus/parahippocampus GMD are shown in Supplementary Figure 1 as T-maps masked by clusters.

Covariate with anxiety and depression. Results of analyses that included the addition of anxiety, depression, or anxiety and depression as covariates (along with age in each case) for patients with IBS versus controls are summarized in Table 2. Inclusion of either anxiety or depression as covariates abolished the group differences in bilateral ventral striatum and left OFC. Inclusion of anxiety and depression together as covariates additionally removed the group differences in bilateral thalamus/midbrain and left pregenual ACC. However, even after controlling for depression and anxiety, group effects between patients with IBS and controls remained in the left posterior parietal cortex, bilateral temporal cortices, and left MFG (Figure 3).

IBS symptom-based subgroups. Comparing pain predominant (n = 17) and non–pain predominant groups showed lower GMD in the pain predominant subgroup ($F_{1,32} = 7.11$; $P = .010$) in the dorsolateral PFC cluster that was found to have greater GMD in patients with IBS than in controls. A comparison of the dorsolateral PFC decrease compared with controls in Figure 4 (*far right column*) indicates that only the non–pain predominant group showed this GMD increase. Also shown in Figure 4 are areas of reduced GMD in all patients with IBS compared with controls that are apparently driven by either the pain predominant group (ventrolateral PFC, ventral striatum) or the non–pain predominant group (thalamus/midbrain, medial PFC).

The results for GLM and cluster analyses for the subgroups of patients with IBS based on bowel habits are summarized in Table 2. Of note is that the IBS constipation subgroup does not appear to contribute to the decrease in thalamic GMD relative to controls. In general, the findings of decreased or increased GMD relative to controls is consistent across all bowel habit subgroups, with the exception of the thalamus, the OFC, and pregenual ACC, regions in which the constipation-predominant group does not differ from controls.

We found only one significant ($P < .05$, uncorrected) correlation between either IBS severity or IBS symptom duration and any of the average gray matter values for the clusters from the group (removing age) GLM. This was a small negative correlation between IBS duration and the dorsolateral PFC cluster GMD ($r = -0.28$, $P < .05$). This correlation was found to only be in the non–pain predominant group ($r = -0.35$, $P < .05$) and not in the pain group ($r = -0.22$, $P = .40$).

CTA

We found no significant differences between groups regardless of the covariates entered.

Discussion

Here, we report morphometric brain differences between patients with IBS and controls, in terms of regional increases and decreases in GMD, whereas no differences in total GMD or CT were observed. Even though some similarities with previously published re-

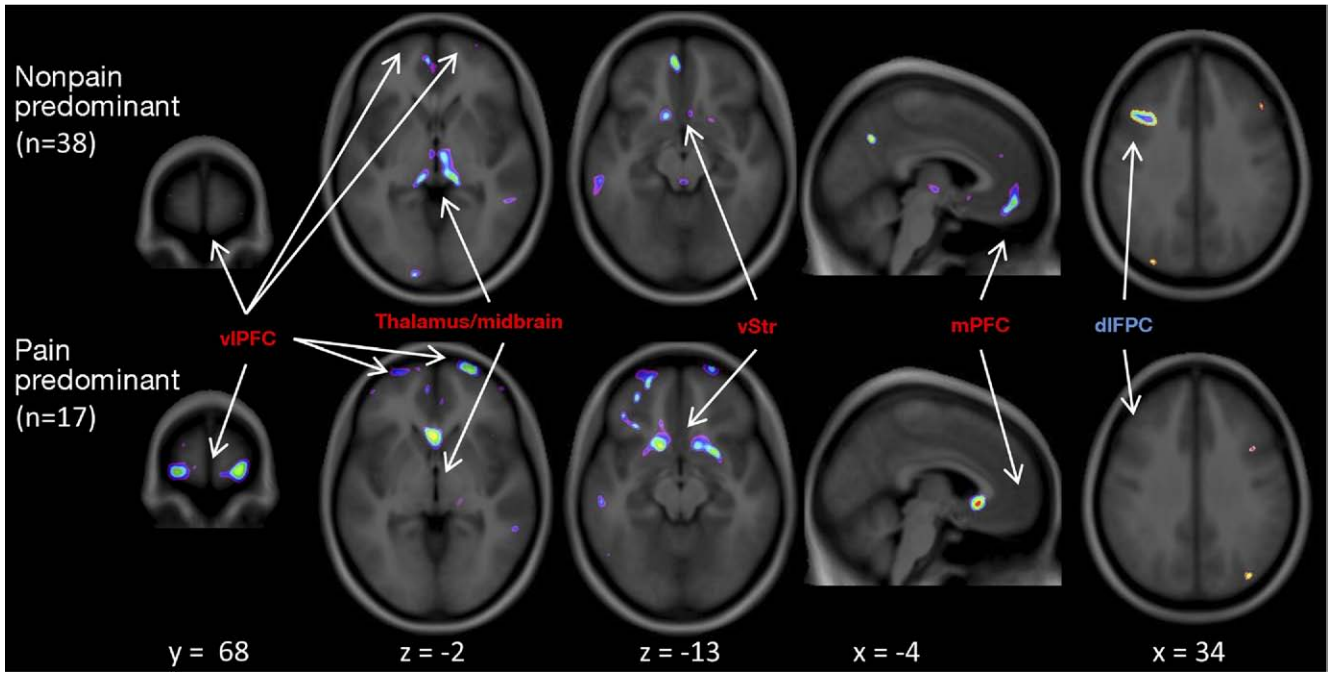

Figure 4. GMD differences between the pain predominant and non–pain predominant groups. Examples of regions that differ between IBS subgroups based on most bothersome symptom (pain or nonpain, which includes any other symptom). The clusters in *red font* are those that showed decreased GMD overall in patients with IBS vs controls, whereas the dlPFC is where patients with IBS had increased GMD relative to controls. The figure clearly shows that the pain predominant group contributed to the ventrolateral PFC and ventral striatum differences, whereas the non–pain predominant group contributed the differences in the thalamus/midbrain, medial PFC, and dorsolateral PFC. The image is thresholded the same way as Figures 2 and 3 (ie, $t > 2.5$ up to $t = 4.5$). No masking has been applied to the images (ie, whole brain results shown).

sults from other patient populations were observed, many of the involved regions were different, and a number of regions with increased GMD were observed. Results from both CT and VBM analyses indicated that whole brain GMD and CT decreased in both patients with IBS and controls with age, but there were no differences between groups. Highly significant regional differences were seen with VBM but not with CTA.

Even though significant changes in GMD were observed in multiple regions, we limit our discussion to the regions implicated in our 2 main a priori hypotheses and compare them to published results in the literature from patient populations with various persistent pain disorders.

Hypothesis 1. IBS Is Associated With Decreased Gray Matter in Regions Shown to Have Increased Responsiveness to Rectal Distension and Its Expectation

This hypothesis was based on the concept that morphometric changes observed in key regions of the so-called "pain matrix" in various patient populations with persistent visceral or somatic pain (including IBS) may be a consequence of chronic nociceptive input from the periphery,[9,11,13,26] possibly involving glutamate-induced excitotoxicity, or other neurodegenerative processes. Increased visceral afferent signaling to the brain as a consequence of peripheral or central sensitization in IBS pathophysiology has been suggested, and some, but not all studies have shown that patients with IBS indeed show greater activation in INS and cingulate cortices in response to an acute visceral stimulus[3] or to expectation of visceral stimuli.[27] However, in the current study, no significant GMD reduction was observed in either cingulate or INS cortices. Our results are in contrast to findings of Davis et al[19] in a smaller sample of patients with IBS, which included decreased CT in the right aMCC and bilateral anterior INS, and decreased GMD in aMCC and thalamus. In a recent study of a different sample, the same group reported cortical thinning in the aMCC. In the aINS, CT was related to symptom duration, with thinning only seen in patients with short duration of symptoms.[20] In contrast, but similar to our findings, Schweinhardt et al[14] did not observe morphometric differences in INS and ACC in a study of female patients with provoked vestibulodynia, a form of chronic vulvar pain. They speculated that the absence of GMD reductions in these regions may have been unique to their study population, in which pain is not constant but only experienced during stimulation, eg, during intercourse. In this sense lower abdominal pain in IBS is analogous to pain in provoked vestibulodynia, because it is not constant (in contrast to functional abdominal pain syn-

drome[28]) but only occurs in association with bowel movements and/or food intake.

In summary, we did not confirm our hypothesis of GMD decreases within the interoceptive network in areas related to pain processing.

Hypothesis 2. IBS Is Associated With Increased Gray Matter in Some Regions, Particularly in Regions Involved in Cognitive and Attentional Modulation of Interoceptive Information

This hypothesis was based on the concept that regional GMD changes may be a genetically determined or acquired trait of patients with IBS which is positively correlated with function of involved brain circuits. Evidence for such a positive correlation between cortical volume and function has been provided from different lines of investigation.[18,29] On the basis of this hypothesis, GMD reductions would be expected to be associated with reduced functionality and vice versa.

We observed a nonsignificant increase (IBS > control) of GMD in right S2/posterior INS (Supplementary Figure 1). S2/posterior INS is considered the primary interoceptive cortex and is activated during noxious and thermal stimulation.[30] Because of its role in interoceptive processing, persons with increased pregenual INS GMD may be more vulnerable to central amplification of normal interoceptive input. It should be noted that for these regions about which we had a priori hypotheses, we applied a lower statistical threshold. This increases the chance of type I statistical error, although our use of cluster analysis still gives us much confidence in the results. We found GMD reductions in several prefrontal regions, in particular bilateral ventrolateral PFC. The right ventrolateral PFC plays a role in cognitive modulation of pain[31] and in placebo analgesia.[32] Previous studies reported compromised engagement of right ventrolateral PFC in patients with IBS,[27,33] and it is intriguing to speculate that reduced GMD in this area provides a neuroanatomical basis for compromised control of emotion and pain perception. In support of this hypothesis is our finding that VBM reduction in the ventrolateral PFC was contributed exclusively by the subgroup of patients who identified pain as their most bothersome symptom (see below).

Similar to findings in several other populations with chronic pain,[5,26] we found reduced GMD in medial PFC. Medial PFC activity has been associated with antihyperalgesia,[34] and release of endogenous opioids in the medial PFC has been shown during tonic pain.[35] The reduced GMD in medial PFC might therefore be related to a reduced ability to engage this pain inhibition system. Inhibitory interactions between medial PFC and dorsolateral PFC have been reported, and an alteration in these interactions has been implicated in patients with low back pain.[36] Decreases in dorsolateral PFC GMD have been reported in patients with low back pain,[9] arthritic hip pain,[37] and migraine.[38] We found a GMD increase in a small area of the dorsolateral PFC in the patient group (in the non–pain predominant group), as well as decreased GMD in the premotor cortex and MFG.

We also found highly significant reductions in thalamus/midbrain GMD (including PAG), which were independent of anxiety and depression symptoms. Because the PAG plays a prominent role in descending pain modulation, and alterations in the engagement of endogenous pain modulation systems has been suggested as a possible mechanism contributing to central pain amplification in IBS,[33,39,40] these GMD reductions in the PAG region may be related to compromised descending modulation of pain.

Hypothesis 3. Depression, Anxiety, and Predominant Symptom Explain Part of the Anatomical Differences Between Patients With IBS and Controls

Emotional modulatory regions and the effects of depression and anxiety. Emotional factors, in particular anxiety and increased stress responsiveness, play an important role in the modulation of chronic pain.[2,41] An increased prevalence of anxiety disorders and depression has been reported both in patients and in non–health care-seeking persons who meet symptom criteria for IBS.[2] We showed that controlling for anxiety and depression eliminated many of the regions considered to be part of an emotional arousal circuitry, including ventral striatum, medial thalamus/midbrain, medial PFC, pregenual ACC, and OFC. In a previous study in patients with IBS, the decreased GMD in the anterior/medial thalamus in patients with IBS may in fact have been related to subclinical levels of anxiety or depression.[19]

Pain as the most bothersome symptom. To explore if morphometric brain changes in IBS may be related to clinical characteristics of these patients, we looked at subgroups of patients according to symptom duration, IBS symptom severity, pain predominance (patients who described pain as the most bothersome of their symptoms), and bowel habits. Visceral sensitivity, which is not a constant trait of patients with IBS and was only evaluated in a small subset of the current sample, was not used in these analyses. Only patients with predominant pain showed reductions in ventral prefrontal pain inhibitory regions (ventrolateral PFC), as well as in the ventral striatum. In contrast, those who identified a symptom other than pain as most bothersome showed GMD reductions in MFG, medial PFC, and thalamus/midbrain, the latter including the PAG. On the basis of the implications of all these regions in different corticolimbic-pontine modulatory systems,[33,36] we suggest that differential alterations in these systems may result in

different predominant symptoms. However, this hypothesis will have to be tested in larger samples. The fact that no major correlations with symptom duration were observed argues against the concept that prolonged altered signaling from the gut may induce secondary reductions in regional brain volumes. The question of whether the observed volumetric changes are related to primary alterations in brain activity, or if they are a consequence of altered visceral signaling to the brain, could be addressed by studies looking at GMD changes in patients undergoing successful cognitive behavioral approaches, or in studies of asymptomatic relatives of patients with IBS.

Conclusions

The findings in this large sample of female patients with IBS with moderate symptom severity suggest that morphometric alterations occur primarily in brain networks concerned with attention and emotion modulation, as well as in cortico-limbic-pontine pain modulatory systems and, to a smaller degree, in networks processing interoceptive information (as suggested by the increased GMD of the pregenual INS). Future studies in much larger samples of patients are required to correlate cognitive (attention, hypervigilance, catastrophizing), affective (trait anxiety), and pain measures with the observed morphmetric brain changes. The finding that changes in the attentional and emotional networks as well as in the primary interoceptive cortex were not related to disease duration or severity suggests the possibility that these structural changes are endophenotypes of IBS, which may also be detectable in asymptomatic relatives.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2010.03.049.

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Conflicts of interest

E.A.M. has received research support from Avera Pharmaceuticals and GlaxoSmithKline. The remaining authors disclose no conflicts.

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Supplementary Methods

CIVET

We used the CIVET pipeline (v. 1.1.9) for all preprocessing of VBM and CTA data (http://wiki.bic.mni.mcgill.ca/index.php/CIVET).[1,2] The detailed description of the steps can be found on the website or see Fahim et al[3] for a step-by-step description. In brief, the steps included nonuniformity correction to correct for RF inhomogeneity,[4] normalization (nonlinear and linear steps) to the MNI/ICBM 152 template,[5,6] classification of tissue, labeling each voxel as gray matter (GM), white matter (WM), or cerebrospinal fluid (CSF), and partial volume estimation,[7] which labels voxels as partially GM, WM, and/or CSF (eg, a voxel covering the pial boundary could be labeled 50% GM, 50% CSF). A cortical fitting stage registers the brain surfaces to a model that calculates 81924 vertices, which are then backtransformed to the original brains to calculate thickness in millimeters at each vertex for each brain,[8–10] and a final surface registration step was performed.[11] We applied an isotropic Gaussian kernel of 8 mm FWHM to the VBM data, and we used a diffusion-smoothing kernel of 20 mm for the cortical thickness surface.[12] A mask was created from the average smoothed GM images of all subjects, excluding the cerebellum, and areas not included in this mask were excluded from further analyses.

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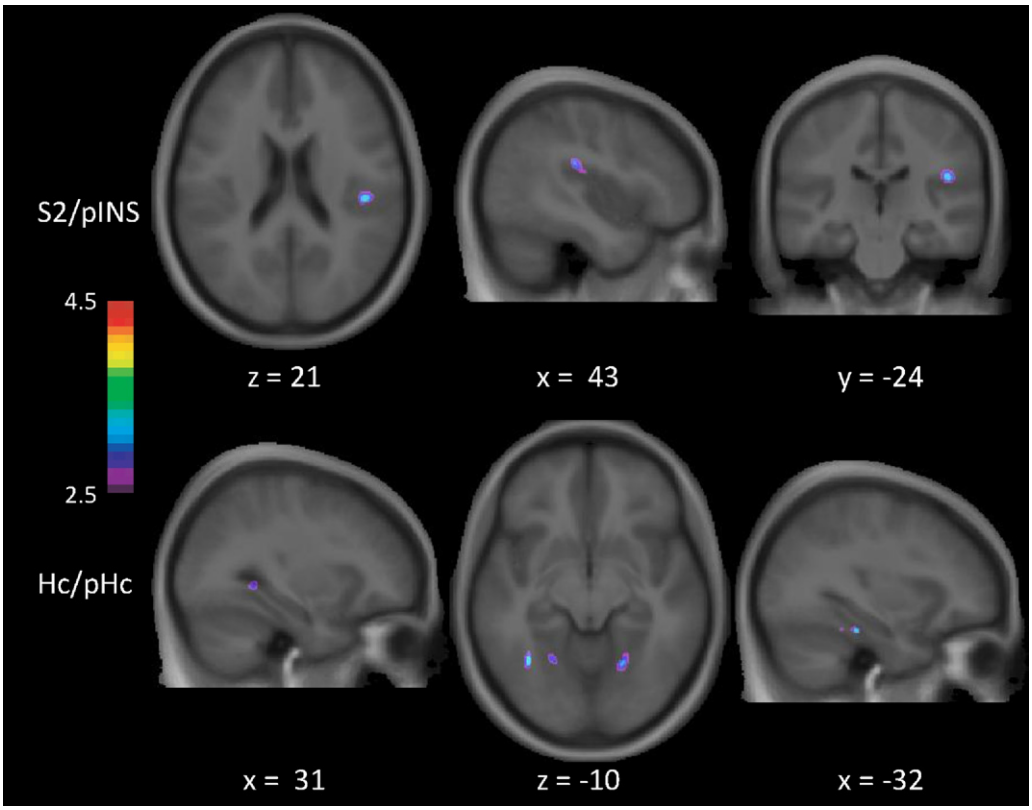

Supplementary Figure 1. Secondary somatosensory/posterior insula cortex (S2/pINS) and hippocampus/parahippocampus (Hc/pHc) clusters that had increased GMD in patients with IBS compared with healthy controls (HCs). These clusters met a corrected cluster threshold of $P < .10$. The images are T-maps (*color bar shows t value*), masked by clusters (ie, voxels that did not meet a cluster-level corrected value of $p < .10$ were excluded). Left side of image is left side of brain.