Phenotype Matters

The Absence of a Positive Association Between Cortical Thinning and Chronic Low Back Pain When Controlling for Salient Clinical Variables

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Aims/Objectives/Background: Studies have associated chronic low back pain (cLBP) with grey matter thinning. But these studies have not controlled for important clinical variables (such as a comorbid affective disorder, pain medication, age, or pain phenotype), which may reduce or eliminate these associations.

Methods: We conducted cortical thickness and voxel-based morphometry (VBM) analyses in 14 cLBP patients with a discogenic component to their pain, not taking opioids or benzodiazepines, and not depressed or anxious. They were age and gender matched to 14 pain-free controls (PFCs). An ROI-driven analysis (regions of interest) was conducted, using 18 clusters from a previous arterial spin labeling study demonstrating greater regional cerebral blood flow (rCBF) in these cLBP subjects than the PFCs. Cortical thickness and VBM-based gray matter volume measurements were obtained from a structural MRI scan and group contrasts were calculated.

Results: Multivariate analysis of variance showed a trend toward cortical thickening in the right paracentral lobule in cLBP subjects ($F_{1,17} = 3.667$, $P < 0.067$), and significant thickening in the right rostral middle frontal gyrus ($F_{1,17} = 6.880$, $P < 0.014$). These clusters were non-significant after including age as a covariate ($P < 0.891$; $P < 0.279$). A whole-brain cortical thickness and VBM analysis also did not identify significant clusters of thinning or thickening. Exploratory analyses identified group differences for correlations between age and cortical thickness of the right rostral middle frontal gyrus (cLBP: $R = -0.03$, $P = 0.9$; PFCs: $R = -0.81$, $P < 0.001$), that is, PFCs demonstrated age-related thinning while cLBP patients did not.

Conclusions: Our pilot results suggest that controlling for affect, age, and concurrent medications may reduce or eliminate some of the previously reported structural brain alterations in cLBP.

Key Words: chronic low back pain, cortical thickness, brain morphometry, clinical research methods

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A question under active investigation[1] is whether the experience of chronic pain (CP), such as chronic low back pain (cLBP), is associated with pathoplastic changes in the brain structure. Structural changes can be quantified with noninvasive imaging and morphometric measures, such as diminished cortical thickness or gray matter density. Previous studies in patients with CP have identified regions of cortical thinning localized to the dorsal lateral prefrontal cortex (DLPFC), thalamus, basal ganglia, primary somatosensory cortex, posterior parietal cortex, and brain stem structures.[2–7] These regions have also been associated with functional magnetic resonance imaging (MRI) signal changes during processing of acute and CP.[1] However, other structural MRI studies have examined the same brain regions and found no significant differences between participants with CP and pain-free controls (PFCs).[8,9]

Confounding factors, such as older age, severity of affective symptoms, or concurrent use of opioids, have themselves been shown to strongly correlate with cortical thinning, as have pain intensity and duration.[10–12] A recent meta-analysis of this body of work concluded that many of these highly prevalent clinical factors and potential confounders in CP populations were not adequately controlled for in prior studies, nor included as covariates in the statistical models.[13] These conclusions call into serious question whether there is indeed a relationship between chronic pain and cortical thickness.

In cLBP, these potential clinical confounders may in fact account for the previously reported associations between cLBP and cortical structural changes. Furthermore, although it is useful to account for the use of medications and depression/anxiety symptoms through covariate regression analyses, this does not necessarily fully or adequately control for these potential effects. This is because covariate analyses are *estimates* of the effect of the covariate on the model, which is potentially confounded by many factors, such as the degree of shared and unshared variances with other predictors in the regression model.[14] A better control for these factors would be to not include patients with an affective disorder, or those taking benzodiazepines and opioids in the study sample, for example, even though that will necessarily limit the generalizability of the results. In addition, in studying brain morphometry, it is important to use as homogenous a clinical sample as possible to prevent the subtle but noticeable effects of clinical factors/comorbidities influencing the disease state and brain structure. This is particularly true in such a heterogenous condition as cLBP.[15]

The aim of this present study was to use a homogenous population of patients with cLBP to study the possible associations of cLBP and brain morphometry versus an age-paired and sex-matched sample with no CP. We used 2 measures of gray matter morphometry—cortical thickness and gray matter volume—in areas we previously found to preferentially process the experience of exacerbated cLBP within the same study sample.[16] We carefully controlled for medication use, comorbid affective disorders, and age to ascertain if previously reported changes still hold true under careful sample selection and clinical characterization. We hypothesized that in a cLBP sample without psychiatric comorbidity, and not taking opioids or benzodiazepines, chronic pain would not be positively associated with cortical thinning.

MATERIALS AND METHODS

Participants/Patients

Twenty-eight (28) participants were studied, which included 14 cLBP patients (46.9 ± 14.6 y) and 14 age-paired and sex-matched PFCs (45.9 ± 12.9 y). cLBP patients were selected consecutively from 2009 to 2011, as they responded to advertisements for the study. Patients with cLBP were included if they were: (1) between the ages of 21 to 65 year, (2) had ongoing chronic pain that averaged at least 3 on a 0 to 10 scale of pain intensity, (3) had no back surgery within the past year, (4) were not taking opioids or benzodiazepines, (5) had low back pain with intermittent radicular pain of at least 6 months' duration, (6) did not have sensory or motor deficits that precluded participation in the study procedures (ie, Quebec Task Force Classification Grade III[17]), (7) were right handed, (8) had a significant discogenic component to their pain syndrome, confirmed by a clinical evaluation and a lumbar MRI, and (9) did not have a current comorbid affective disorder, as assessed by the Hospital Anxiety and Depression Scale (HADS).

The lead investigator (A.D.W.) determined eligibility at the first visit through a review of history and physical examination and MRI findings confirming disk disease. Patients were included if this evaluation found that the source of pain was at least 1 degenerated, herniated, or torn lumbar disk with either a minimum grade III disk degeneration, abnormal morphology, or a hyperintense zone.[18–20] These criteria, used by the authors in previous studies,[16,21,22] narrows the heterogeneity of patients with cLBP. They exclude those with pain resulting from purely nonspecific or myofascial causes and include those with the commonly presenting mixed syndrome of low back pain with underlying disk pathology and possibly spinal stenosis or facet disease, and no sensory or motor deficits. Studies indicate that 40% of all cLBP may be discogenic in nature.[23]

Age-paired and sex-matched PFCs were also enrolled and evaluated with the same procedures as cLBP patients to exclude CP. Other inclusion and exclusion criteria were the same as noted above for cLBP patients. All study protocols were approved by the Partners Human Research Committee and informed consent was obtained from all participants.

Data Acquisition

Participants completed clinical measures of their pain and affect including the Brief Pain Inventory (BPI),[24] Neuropathic Pain Questionnaire (NPQ),[25] Oswestry Disability Index (ODI),[26] and HADS.[27] Average pain was calculated by averaging the baseline BPI average pain score obtained at the screening visit with the second study visit BPI average pain score 1 week later.

Description of Measures

The BPI

The BPI is a 15-item questionnaire assessing pain location, intensity, relief, and quality, as well as pain-interference, and has been validated in cancer and non-cancer pain studies.[28]

The HADS

The HADS is a self-report measure that does not include somatic items that may be attributable to medical illness, and thus it is more appropriate for screening in a CP patient population. On each subscale a high score is >8.[29] Scores above these levels are highly correlated with a comorbid major depression or generalized anxiety disorder in patients with cLBP.[29,30] Anxiety and depression subscale scores each had to be <9 for study inclusion.[27] The HADS best functions as a unidimensional measure of negative effect, while retaining excellent case-finding ability.[31,32]

The ODI

The ODI is an extensively used 10-item scale to describe the level of physical function in patients with cLBP.[33]

The NPQ

The NPQ is a validated, 12-item questionnaire that asks patients to rate the neuropathic qualities of their pain.[34] It was used to classify the pain syndrome as having a neuropathic symptom component or not.

Neuroimaging Methods and Cortical Thickness Analysis

A high-resolution MPRAGE scan was obtained for each participant using 3 T Siemens TIM Trio MRI System (Siemens Medical, Erlangen, Germany), equipped with a 32-channel head coil (TR/TE 2300/3.39 ms, voxel size $1 \times 1 \times 1.33$ mm). Arterial Spin Labeling data were also used to identify significant clusters where exacerbated back pain caused greater cortical activation and increased regional cerebral blood flow in cLBP patients than HCs. For a review of these methods and scan parameters, please see Wasan and Loggia, et al.[16]

Cortical thickness measurements were obtained by first processing the structural T-1 weighted data using the FreeSurfer version 5.1 (http://surfer.nmr.mgh.harvard.edu) cortical reconstruction pipeline.[35,36] Any inaccuracies in the reconstruction of white and pial surfaces of individual participants were manually corrected before calculating cortical thickness following the instructions provided by the software developers. The cortical thickness measure was computed as the averaged distances between the pial and white matter surfaces at each point across the cortical mantle.

A region of interest (ROI) analysis was used to contrast changes in morphometric indices between cLBP patients and HCs in regions previously identified to preferentially activate in cLBP patients undergoing a measured low back pain exacerbation during an fMRI scan session.

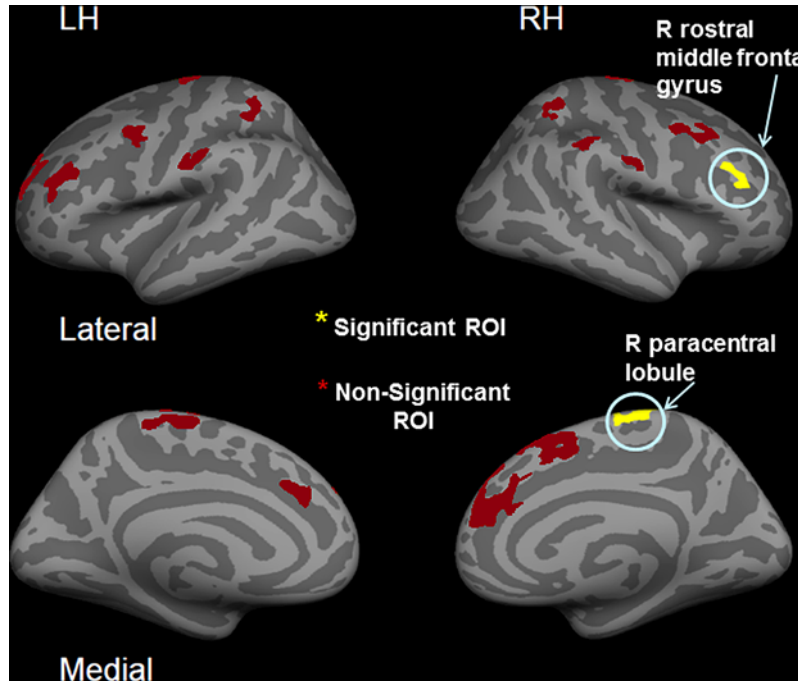

FIGURE 1. Regions of interest (ROI) from a previous ASL study where cluster activation in cLBP patients versus pain-free controls (cLBP > PFCs) after exacerbated low back pain was significant. Two regions showing cortical thickness, which was more in cLBP patients than in PFC, are labeled. cLBP indicates chronic low back pain; PFC, pain-free control.

A total of 18 clusters were used for comparison (Fig. 1, for a list of ROI coordinates see Wasan et al[16]; Table 2). Cortical thickness measurements for each participant were extracted from the ROIs and analyzed in SPSS version 20 (Chicago, IL). Multivariate analysis of variance was used to contrast cortical thickness measurements from each ROI by group and multivariate analysis of covariance was used to control for age and HADS scores as covariates in the model of ROI thickness and group (to control for between-subject variability). For each ROI, from the cortical thickness measurement differences between groups, we also calculated the required sample size to determine if there are significant differences between groups, should the magnitude of those differences exist.

A follow-up whole-brain cortical thickness analysis was also conducted between cLBP patients and PFCs. Group analyses were performed by resampling each participant's data to the FreeSurfer average atlas, distributed as a part of FreeSurfer. Cortical thickness maps were smoothed using a Gaussian kernel with a full-width half maximum of 8 mm. Vertex-wise analyses of cortical thickness between groups were performed with an initial vertex-wise threshold set at $P < 0.001$ (uncorrected). Montecarlo simulations (5000 sets) were run on the vertex-wise results to control for multiple comparisons using a vertex-level threshold of $P < 0.001$ and cluster-level threshold of $P < 0.05$.

A sensitivity analysis was conducted that included investigating the interaction of age and group on cortical thickness in the whole-brain analysis as well. ROI thickness was correlated with age within each group, identifying Pearson correlations and P-values. The age × group interaction was also examined among all ROIs and significant interactions were identified ($P < 0.05$).

Voxel-based Morphometry (VBM) Analysis

VBM is a measure of cortical density and volume.[37] VBM is comprised of a voxel-wise comparison of the local concentration of gray matter between 2 groups of participants.[37] VBM analysis was conducted in SPM8[38] (http://www.fil.ion.ucl.ac.uk/spm) running in Matlab version 7.7 (The MathWorks, Natick, MA). First, structural T-1 weighted images were skull-stripped using Brain Extraction Tool[39] and spatially normalized into the MNI standard space. Next all images were segmented into gray matter, white matter, cerebrospinal fluid, and non–brain volumes. Gray matter images were then averaged to produce the initial template brain. An iterative process of applying the inverse deformation from this initial template to individual brains was completed to create a new template—that is, the final DARTEL template brain (Diffeomorphic Anatomical Registration using Exponentiated Lie algebra: DARTEL toolbox8[40]). Data were smoothed with a Gaussian kernel and full-width half maximum of 8 mm.

Voxel-wise general linear model analysis was performed contrasting gray matter volumes from cLBP and HC participants. Statistical maps were thresholded at an uncorrected voxel level ($P < 0.001$) and then corrected for multiple comparisons by false discovery rate ($P < 0.05$).

RESULTS

Clinical Characteristics

Demographic and clinical characteristics of 14 cLBP patients and 14 age-paired and sex-matched PFCs are displayed in Table 1. No significant differences were identified between groups in age as determined by an independent samples t test ($t_{26} = 0.206$, $P < 0.838$). Significant

TABLE 1. Demographics and Clinical Measures

Clinical Measures	cLBP Patients (n = 14)	Pain-Free Controls (n = 14)
Age	46.93 (± 14.56)	45.86 (± 12.88)
Sex (F)	9 (64.29%)	9 (64.29%)
HADS (total score)	12.07 (± 5.3)	1.17 (± 2.73)*
Avg pain (0-10)	5.21 (± 1.77)	—
Pain duration (y)	8.21 (± 2.1)	—
NPQ (% neuropathic pain)	35.71%	—
ODI (% disability)	16.8% (± 5.13)	0

Values are represented as mean± 1 SD.

Avg pain, average pain on a 11-point numerical rating scale (0-10); cLBP, chronic low back pain; F, female; HADS, Hospital Anxiety and Depression Scale; NPQ, Neuropathic Pain Questionnaire; ODI, Oswestry Disability Index.

*$P < 0.01$

differences were identified in HADS scores between groups ($P < 0.01$). However, all cLBP patients and PFCs scored below the threshold for likely having a comorbid affective disorder ($HADS_{anxiety} < 9$ and $HADS_{depression} < 9$).

Neuroimaging Findings

Multivariate analysis of variance of ROIs between cLBP patients and HCs (Fig. 1 and Table 2) showed cortical *thickening* trend in the right paracentral lobule, part of S1—the primary somatosensory area ($F_{1,17} = 3.667$, $P < 0.067$), and significant *thickening* in the right rostral middle frontal gyrus, part of the DLPFC in a cLBP patient ($F_{1,17} = 6.880$, $P < 0.014$). However, these areas did not hold significance after including age as a covariate in the model (S1, $P < 0.891$; DLPFC, $P < 0.279$). The follow-up whole-brain analysis as well did not identify significant differences in cortical thickness between groups after correcting for multiple comparisons. VBM also did not identify any significant differences in gray matter volume after controlling for multiple comparisons. Using the cortical thickness measurements, Table 2 also displays the sample sizes needed to find statistically significant differences in cortical thickness between groups for each ROI, should they exist for the magnitude of differences we found between groups. The numbers needed per group range from 17 to 1616. These statistics illustrate the extent to which our sample size of 14 per group may have failed to identify significant differences between groups. However, they also convey how similar the cortical thickness measurements are between groups.

To examine the relationship between clinical factors and cortical thickness, a linear regression analysis was used. Sex, pain intensity, the duration of pain, the presence of neuropathic pain, HADS scores, or functional ratings (ODI scores) were not significant univariate predictors of cortical thickness among the ROIs or the whole-brain measurements. However, a dichotomous trend in the correlation of age and cortical thickness of the right rostral middle frontal gyrus (DLPFC) between cLBP patients ($R = -0.027$, $P < 0.928$) and PFCs ($R = -0.805$, $P < 0.001$) was identified (Fig. 2). This indicates that the PFCs had thinning with age in this area, whereas the cLBP patients did not.

DISCUSSION

This study investigated the impact of simultaneously controlling for known predictive factors of morphometric change in a cLBP patient sample with a discogenic pain component and comparing them to PFCs. We used multiple methods of gray matter quantification to identify potential differences in regions we previously found to be

TABLE 2. ROI Cortical Thickness Values

ROI Brain Region	Average (SD) (mm) cLBP Cortical Thickness	PFC Cortical Thickness	*F*	Significant *P*	# Needed per Group for 80% Power†
Left superior parietal lobule	2.50 (0.26)	2.39 (0.26)	1.34	0.26	90
Left secondary somatosensory cortex	2.60 (0.19)	2.50 (0.34)	0.99	0.33	121
Left superior frontal gyrus	2.85 (0.39)	2.75 (0.24)	0.66	0.42	163
Left rostral middle frontal gyrus	2.53 (0.23)	2.51 (0.15)	0.08	0.79	1098
Left superior parietal lobule	2.27 (0.30)	2.32 (0.31)	0.19	0.67	586
Left paracentral gyrus	2.39 (0.20)	2.42 (0.28)	0.08	0.79	1467
Left rostral middle frontal gyrus	2.55 (0.30)	2.59 (0.18)	0.17	0.69	607
Left precentral gyrus	2.71 (0.27)	2.57 (0.42)	1.04	0.32	106
Left caudal middle frontal gyrus	2.53 (0.27)	2.43 (0.38)	0.62	0.44	177
Right insula	3.37 (0.40)	3.26 (0.24)	0.77	0.39	147
Right superior frontal gyrus	2.77 (0.18)	2.72 (0.23)	0.36	0.56	324
Right superior parietal lobule	2.34 (0.28)	2.25 (0.25)	0.81	0.38	138
Right caudal middle frontal gyrus	2.69 (0.12)	2.62 (0.19)	1.20	0.28	95
Right superior frontal gyrus	2.95 (0.28)	2.88 (0.23)	0.60	0.45	185
Right paracentral gyrus	2.36 (0.20)	2.22 (0.16)	3.67	0.07	31
Right rostral middle frontal gyrus*	2.46 (0.29)	2.21 (0.22)	6.88	0.01	17
Right postcentral gyrus	2.42 (0.31)	2.39 (0.34)	0.07	0.80	1616
Right supramarginal gyrus	2.68 (0.30)	2.74 (0.28)	0.34	0.57	324

†Number needed to find significant differences between groups, should they exist for these magnitude of differences between groups at each ROI, assuming 80% power, $P = 0.05$, and a 2-tailed independent samples *t* test.

cLBP, chronic low back pain; PFC, pain-free control; ROI, region of interest.

*$P < 0.01$.

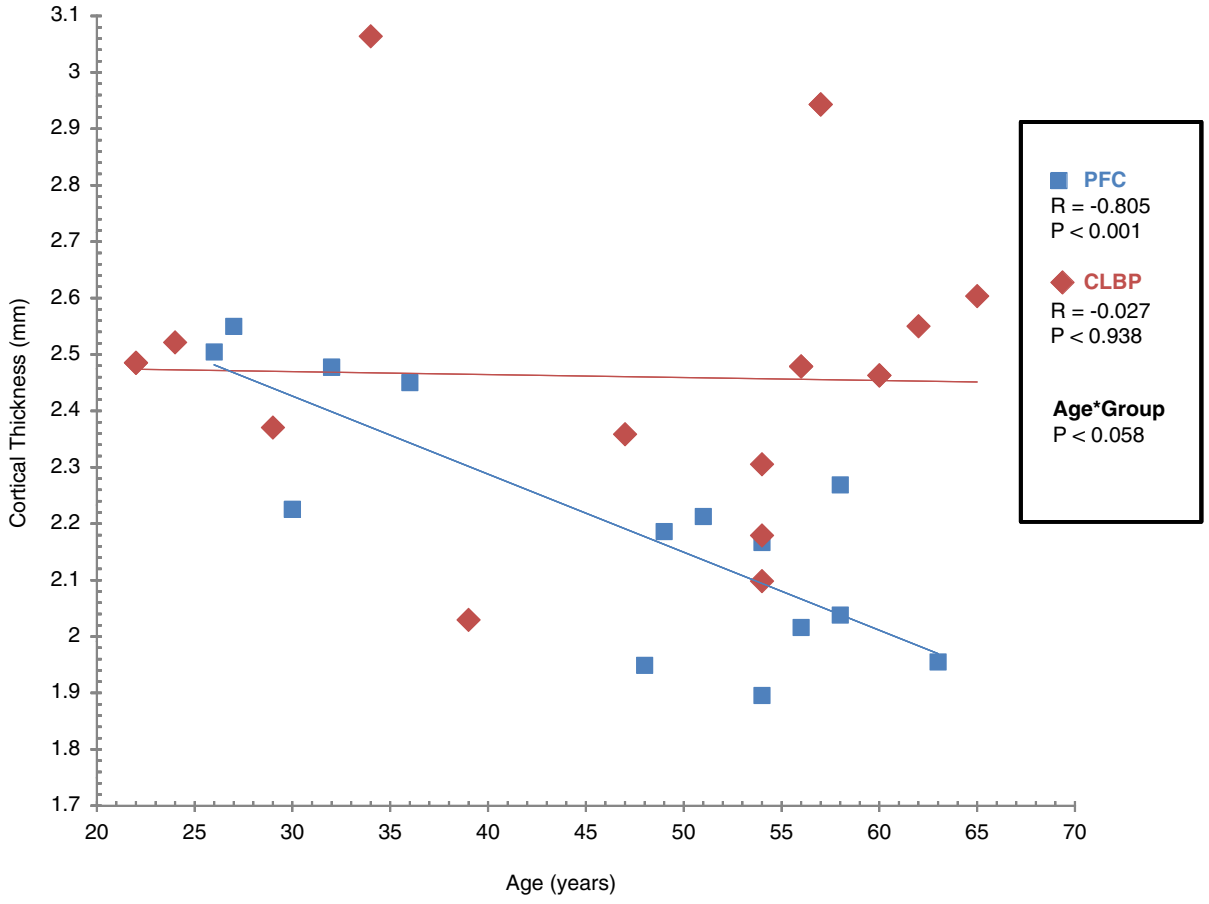

FIGURE 2. Correlations of age and right rostral middle frontal gyrus thickness are plotted by group. A trending interaction between group and age is observed. Pain-free controls (PFCs) have a strong negative correlation between thickness and age, whereas chronic low back pain (cLBP) patients have no correlation.

more active in cLBP, when LBP was worsened. These include ROI, whole brain, and VBM approaches. In contrast to most studies of cLBP cortical thickness, the present study found that cLBP patients demonstrated cortical *thickening* in pain processing regions—significant at the level of the DLPFC and trending in S1 (leg somatotopic region, paracentral lobule). These regions did not retain significance when age was included as a covariate of no-interest in the statistical model. However, a dichotomous trend was found in the DLFPC, indicating that the cLBP cohort did not have expected age-related thinning as seen in the PFCs. VBM analysis did not show state-related changes (either thinning or thickening). Thus, in a carefully phenotyped population with cLBP, our *primary result* was that we could not replicate previously reported findings of cortical thinning found in more diverse clinical cohorts,[2–7] despite the use of multiple methods to investigate these findings.

Similar results have been reported in other CP populations. In a matched study of fibromyalgia patients with and without affective disorder and PFCs, cortical thickness measurements were obtained with negative affect scores as a covariate.[8] It was found that patients with high negative affect showed thinning in the right insula. However, those without an affective disorder did not significantly differ from PFCs, suggesting that an affective disorder and not a pain condition may be associated with cortical thinning. In addition, age is another strong predictor of cortical thickness that is associated with decreases in brain volume.[12] One study has identified interactions between age and group (patients with temporomandibular disorder vs. PFCs) in the premotor area and dorsal striatum, where chronic pain patients showed increased cortical density with age rather than the expected cortical thinning that is observed in PFCs.[41] These findings comport with our results and highlight the importance of including age in the statistical model. If age is not included as a covariate, the interaction between disease state and thickness cannot be well characterized.

Of note, our sample size (n = 28) was similar to many previous studies of cortical thickness in patients with CP.[13] Using the cortical thickness measurement differences between groups, we also performed a power analysis to address issues of being under powered and to illustrate the similarities in thickness measurements between groups (ie, noninferiority, not equivalence). We have demonstrated that for the vast majority of the ROI's (except S1 and the DLPFC, which were not significant when controlling for age), the required sample sizes are quite large (> 100 per group) to determine that the magnitude of cortical thickness differences we found between groups are statistically significant, should they indeed exist.

Although the cortical thickening results in the cLBP patients are subtle findings, they are useful to discuss. The exact mechanisms of possible cortical thickening are not well understood in pain conditions, and one possible explanation for this thickening is activation and proliferation of glial cells, including astrocytes and microglia, in brain areas showing increased activation and regional cerebral blood flow in patients with cLBP. Reactive gliosis can occur in association with a variety of pathologic events, including excitotoxocity resulting from excess release of glutamate.[42] In cLBP, S1 is repeatedly stimulated due to nerve inflammation or nerve injury located at the site of disk degeneration. Nociceptive afference to S1 results from potential tissue damage even during benign behaviors. Overtime, S1 neurons may undergo damage due to the increased glutamatergic signaling coming from ascending sensory inputs. Although neuronal damage is often associated with cortical thinning, glial cells may proliferate in areas of inflammation (particularly in S1 for CP conditions), leading to glial scarring, astrocytosis, and increased cortical thickness.

This reactive gliosis hypothesis may also hold true for the thickening findings related to the DLPFC. This area is implicated in working memory and attention.[43] Studies have associated cLBP with decreased performance on attention demanding and working memory tasks through detailed neuropsychological testing when compared with age and education-matched PFCs.[44] The constant pain signaling is a highly salient event that directs attention to the experience of pain. Consequently, the DLPFC area may also become hyperactivated with resultant excitotoxicity and increased astrocyte growth. This may also explain our findings in cLBP, where, in contrast to PFCs, age-related thinning was not found in DLPFC. With possible glial cell proliferation in cLBP, the DLPFC may hold a stable thickness rather than thinning over time.

Although this study was careful in its characterization and selection of participants, it still holds some important limitations. The power of the study would be greatly enhanced if the sample size were increased. Hence, our results are pilot findings, as a larger sample size would be required to dispel definitively the notion that cLBP is intrinsically associated with cortical thinning. Our findings suggest that there is more to the morphometry of cLBP than cortical thinning, but carefully planned follow-up studies will be needed to test this theory of cortical thickening. It would also be important to include a sample of patients who have cLBP and depression/anxiety as another arm in a future study and compare the differences between cortical thickness in depressed and nondepressed cLBP patients and to then collapse these 2 groups into 1 cohort and compare against PFCs. When the pain sample is more heterogenous we may expect to find cortical thinning; however, this thinning may be better attributed to a comorbid affective disorder rather than cLBP in and of itself. Another follow-up study could examine reactive gliosis and neuroinflammation through the effects of anti-inflammatory medications and novel glial cell inhibitors on cortical thickness. Such a study may provide molecular evidence for gliosis in vivo.

The present study provides groundwork for future studies to be conducted investigating morphometric changes in a homogenous pain population. In addition to the reported methodological advances in phenotyping patients with CP, morphometric brain change in cLBP may include more nuanced changes than cortical thinning as reported by previous studies. Cortical thickening may have important implications regarding neuroinflammation and provide a new avenue to pursue novel treatment therapies for chronic pain. Our pilot results suggest that controlling for affect, age, and concurrent medications may reduce or eliminate some of the previously reported structural brain alterations in cLBP.

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