Progression of Structural Brain Changes in Patients With Chronic Pancreatitis and Its Association to Chronic Pain

A 7-Year Longitudinal Follow-up Study

Janusiya Muthulingam, MSc,† Søren Schou Olesen, MD, PhD,†‡ Tine Maria Hansen, PhD,*†*
David A. Seminowicz, PhD,§|| Shana Burrowes, MSc,§||
Asbjørn Mohr Drewes, MD, PhD, DMSc,†‡ and Jens Brøndum Frøkjær, MD, PhD†*

Objectives: Temporal information about the structural brain changes in chronic pancreatitis (CP) and its relation to the clinical manifestations is lacking. This study investigated changes in morphological brain parameters over 7 years in painful CP patients, compared with controls.

Methods: In this 7-year longitudinal magnetic resonance imaging study, we included 23 CP patients and 14 controls. Gray matter volume (GMV) and cortical thickness were examined using voxel-based and surface-based morphometry. In addition, patients completed pain questionnaires and diary.

Results: At baseline, patients had reduced GMV and cortical thickness in widespread brain areas compared with controls. After 7 years of follow-up, the GMV loss was more pronounced in patients compared with controls, particularly in precentral gyrus and putamen. Moreover, an increase in pain scores was associated with a less reduction of thalamic GMV ($P = 0.046$), whereas an increase in brief pain inventory score was associated with more reduction in cortical thickness of precentral ($P = 0.005$) and superior temporal gyri ($P = 0.019$), indicating that brain morphological alterations are associated with the pain.

Conclusions: Chronic pancreatitis pain is associated with morphological brain changes over time in several areas, reflecting that brain plasticity may be a consequence of repeated long-term nociceptive signaling.

Key Words: chronic pancreatitis, abdominal pain, brain imaging, brain/gut interaction

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Chronic pancreatitis (CP) is a multifactorial, progressive, fibro-inflammatory disease that is typically characterized by gradual fibrotic and ductal changes of the pancreatic gland, as well as development of exocrine and endocrine pancreatic insufficiency in many patients.[1] Chronic abdominal pain develops in most patients and is often the major debilitating symptom.[2] Unfortunately, the pain is often very difficult to treat, most likely owing to the complex interplay of various pain mechanisms that are only partially understood. Changes in processing of pain in the central nervous system (CNS) has been increasingly recognized in CP patients; these include central sensitization, impaired descending pain modulation, and altered resting brain activity. Various electroencephalography studies have demonstrated functional reorganization of brain networks in patients with painful CP,[3–5] specifically in the opercular/insular and *pain-matrix* networks. Besides the functional reorganization, structural reorganization has also been described in studies based on magnetic resonance imaging (MRI).[6] The structural brain reorganization in CP patients was expressed as regional differences in cortical thickness[7] as well microstructural alternations,[6] seen in brain areas involved in the visceral pain processing, indicating a central neuroplastic response to CP pain.[2] To date, only cross-sectional studies have shown both functional and structural reorganization in CP patients. However, exploration of changes over time is essential, aiming to determine more information of the causality between brain changes and the clinical course of the disease.

Hence, to better characterize the temporal evolution of the structural brain reorganization and its relation to the clinical characteristics, including pain, we performed a longitudinal 7-year brain MRI follow-up study. To quantify brain structural alterations, 2 standardized and automated whole-brain techniques were used. Voxel-based morphometry (VBM) provides estimates of regional gray matter volumes (GMVs). In contrast, surface-based morphometry (SBM) uses gyral and sulcal geometry providing direct and precise measures of cortical thickness.[8,9]

We hypothesized that CP patients with chronic abdominal pain as compared with healthy controls exhibited decreased GMV and cortical thickness in areas involved in visceral pain processing, and that the changes over a 7-year period were more pronounced in the CP patients, and lastly, that these changes were associated with clinical outcomes including pain. Hence, in a cohort of well-characterized CP patients with chronic abdominal pain, we aimed to (1) quantify and compare structural brain morphology including GMV and cortical thickness, (2) compare the longitudinal structural brain changes during a 7-year period, and (3) explore the associations to pain related parameters.

MATERIALS AND METHODS

Participants and Study Design

This was a 7-year prospective study conducted at Centre for Pancreatic Disease, Departments of Gastroenterology and Radiology, Aalborg University Hospital. Patients were seen on 2 occasions: (1) baseline visit (from 2009 to 2010) and (2) 7-year follow-up visit (from 2016 to 2017).

From the *Mech-Sense, Department of Radiology, Aalborg University Hospital; †Department of Clinical Medicine, Aalborg University; ‡Centre for Pancreatic Diseases, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Aalborg, Denmark; §Department of Neural and Pain Sciences, School of Dentistry; and ||Center to Advance Chronic Pain Research, University of Maryland, Baltimore, MD.

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Address correspondence to: Jens Brøndum Frøkjær, MD, PhD, Mech-Sense, Department of Radiology, Aalborg University Hospital, PO Box 365, DK-9100 Aalborg, Denmark (e-mail: jebf@rn.dk).

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Patients were recruited at baseline from our outpatient clinic as a part of another trial assessing the effect of pregabalin on CP pain[10]; at the follow-up study, only available patients who still maintained the inclusion/exclusion criteria were rescanned. Inclusion criteria were as follows: (1) a CP diagnosis based on the Mayo Clinic diagnostic criteria,[11] (2) chronic abdominal pain characteristic for CP including epigastric pain radiating to the back more the 3 days per week for at least 3 months, and (3) patients with stable opioid and/or nonopioid medication, meaning that patients did not switch between type of analgesics or significantly changed the dosage. Exclusion criteria were as follows: (1) inability to undergo MRI, (2) major illness such as cancer, (3) pain syndromes other than CP such as irritable bowel syndrome (IBS) or chronic low back pain, (4) continuous alcohol or drug abuse, and (5) had a history of major depression. Patients enrolled in the study continued their usual pharmacological treatment. Healthy controls were recruited from our imaging database and were age and sex matched with the CP patients. Healthy controls were free of any chronic pain conditions and did not have any gastrointestinal symptoms. They were allowed to take their ordinary medicine such as blood pressure medication but were excluded if they used CNS active medication. Inclusion and exclusion criteria were identical for both the baseline and follow-up visit. All subjects gave written informed consent before participating in the study, and the study was approved by the Ethics Committee of Northern Jutland (reference numbers N-20080028MCH and N-20090008). The study was conducted in accordance to the Declaration of Helsinki.

Pain Diary and Questionnaire

The pain diaries and questionnaires obtained at baseline and at follow-up were identical. Clinical pain scores were assessed using a pain diary based on a 0 to 10 visual analogue scale (VAS), in which patients rated the average and maximal daily pain intensity.[12] The diary was started 7 days before the MRI scan. In addition, patients were asked to complete the short form brief pain inventory (BPI) questionnaire.[13] Finally, we did review the electronic medical records in detail to evaluate the pharmacological treatment for patients.

Magnetic Resonance Imaging

Images at both visits were acquired with the same 3 T magnetic resonance scanner (Signa HDxt; General Electrics, Milwaukee, Wis) equipped with an 8-channel standard head coil. The high-resolution 3-dimensional T1-weighted anatomical scans (BRAVO) lasting 5½ minutes were acquired with the following parameters: 150 slices; field of view, 250 mm; echo time, 3.6 ms; repetition time, 9.0 ms; flip angle, 14 degrees; resolution, 0.78 × 0.78 mm; matrix size, 320 × 320 mm; slice thickness, 1 mm; full head coverage; no gap).

Structural MRI Data Analysis — Cross-sectional at Baseline

To determine whether CP patients differed in GMV and cortical thickness from healthy controls, both VBM and SBM analyses were performed using the Computational Anatomy Toolbox (CAT12, r1203) implemented through Statistical Parametric Mapping software (Wellcome Trust Centre for Neuroimaging, London, England) (SPM12, http://www.fil.ion.ucl.ac.uk/spm/). The default settings described in detail in the manual of the CAT12 toolbox were used.[14]

For VBM analysis, the preprocessing steps were briefly described as follows: T1-weighted structural images were normalized with diffeomorphomic anatomical registration through exponentiated lie algebra (DARTEL) to the Montreal Neurological Institute (MNI) template and then segmented into gray matter (GM), white matter, and cerebrospinal fluid.[14] Next, bias correction was performed to remove intensity nonuniformities. Then, the images were normalized with a voxel size of 1.5 × 1.5 × 1.5 mm, and the segmented maps were modulated by Jacobian determinants.[14] Finally, the normalized GM images were smoothed with an 8-mm Gaussian kernel. For each subject, whole-brain GMV was calculated. For the statistical analysis, we used the smoothed, modulated, DARTEL-warped, normalized GM images and applied an absolute threshold mask of 0.1 in the analyses. Gray matter volume was assessed by a voxel-wise independent samples *t*-test with total intracranial volume and age as covariates to correct for different brain sizes among subjects. Group comparisons were assessed with the use of a family-wise error (FWE) at a threshold of $P < 0.05$ and a cluster extent of 100 voxels, and corrected for multiple comparisons using random field theory at a cluster level.

The SBM preprocessing pipeline was nearly identical to the VBM preprocessing pipeline, except that a projection-based thickness method was added to estimate cortical thickness and to create the central cortical surface for both hemispheres. This is a fully automated method that allows for measurement of cortical thickness. To analyze surface parameters, all the images were resampled into template space and then smoothed with a 15-mm full-width-at-half-maximum Gaussian kernel. The cortical thickness of the hemispheres was statically analyzed separately using unpaired 2-sample *t*-tests. Group comparisons were performed at $P < 0.005$ uncorrected threshold.

Structural MRI Data Analysis — Longitudinal Analysis

For VBM analysis, we quantified longitudinal GMV changes using SPM12 software. The preprocessing pipeline was the same as the baseline preprocessing pipeline, with the exception that the longitudinal preprocessing pipeline did not include modulation, as normalization estimates for one subject are the same for both timepoints and thus modulation will also be the same for both time points. Briefly, T1-weighted images were segmented into GM, white matter, and cerebrospinal fluid, and then spatially normalized to MNI template with DARTEL. Next, the normalized images were resampled to a voxel size of 1.5 × 1.5 × 1.5 mm and then smoothed with an 8-mm Gaussian kernel. An absolute threshold of 0.15 was used. The smoothed, DARTEL-warped, and normalized GM images were used for the statistical analysis. Voxel-wise comparisons in GMV were performed using repeated measures analysis of variance (RM-ANOVA) (group × time), flexible factorial model with total intracranial volume as covariate.

For SBM analysis, longitudinal cortical thickness changes were quantified using CAT12 toolbox. The preprocessing pipeline for longitudinal SBM analysis was identical to baseline SBM analysis. The cortical thickness of the 2 hemispheres were analyzed separately using RM-ANOVA (group × time), flexible-factorial model.

Both longitudinal VBM and SBM analysis, and group comparisons were assessed with the use of an FWE at a threshold of $P < 0.05$ and a cluster extent of 100 voxels, and corrected for multiple comparisons using random field theory at a cluster level.

Statistical Analyses

For baseline, unpaired *t*-tests and χ^2 test were used to compare demographic characteristics between the groups. For follow-up, paired *t*-tests and Wilcoxon rank test were performed to compare demographic characteristics within both groups. In addition, RM-ANOVA was used to compare the total GMV differences between the groups. Moreover, to ensure that there were no significant differences in total GMV between drop-out patients and patients who completed both visits, an independent *t*-test was

performed. Finally, putative associations between total GMV and confounding factors (including alcoholic etiology, sex, opioid use, analgesic use, diabetes, and CP duration) were analyzed using multivariate regression analysis with backward stepwise elimination. For this purpose, analgesic use were categorized according to the World Health Organization classification (none, weak analgesics, and opioids).

To test whether the results from group comparisons for GMV and cortical thickness at baseline, and within-subject differences in patients at follow-up, were related to clinical variables, we created a 5-mm sphere based on peak MNI coordinates and extracted GMV data from those spheres. Next, we performed Spearman correlation analyses with the VAS and BPI scores. Cortical thickness values were extracted directly from the clusters.

IBM SPSS Statistic version 24.0 (IBM Corp., Armonk, NY) was used to perform the statistical tests and correlation analyses. P values <0.05 were considered significant.

RESULTS

Demographic and Clinical Characteristics

Twenty-three CP patients with a mean age of 53.1 (standard deviation [SD], 11.3) years and 14 healthy controls with a mean age of 46.8 (SD, 11.1) years underwent brain MRI at the baseline visit. The patient and control groups were comparable in sex and age both at baseline and follow-up (all $P < 0.05$) (Table 1). At follow-up, 11 CP patients and 12 healthy controls were rescanned. None of the patients had any psychiatric disorders during the follow-up period. The average observation time for CP patients was 7.1 years (range, 6.3–7.4 years), whereas, for healthy controls, the average observation time was 6.3 years (range, 5.3–7.3 years) ($P = 0.192$). Reasons for discontinuation of the follow-up period are listed in Figure 1. Table 1 presents the characteristics of the enrolled patient and controls. Patients completing the 7-year follow-up had stationary diary pain ratings and BPI scores at baseline and follow-up (all $P > 0.05$).

Baseline

Whole Brain GMV

At the baseline visit, CP patients had a smaller total GMV compared with healthy controls (mean [SD]) (598 [49] mL vs 656 [62] mL; $P = 0.004$). There was no significant difference in total GMV between patients who completed both visits (n = 11) and drop-out patients (n = 12) ($P = 0.564$).

Gray Matter Volume

Compared with healthy controls, CP patients had a smaller GMV in the right angular gyrus, right superior temporal pole, left inferior temporal gyrus, left middle occipital gyrus, and left precuneus (all FWE-corrected $P < 0.05$, at cluster level) (Fig. 2A, Table 2). Chronic pancreatitis patients had no significant regional increases in GMV compared with healthy controls.

Cortical Thickness

Compared with healthy controls, CP patients had cortical thinning in the right middle temporal gyrus, right superior frontal gyrus, and right inferior frontal gyrus (all uncorrected $P < 0.005$ at cluster level) (Fig. 2C, Table 2). Chronic pancreatitis patients had no areas of increased cortical thickness compared with healthy controls.

Association Between Structural Brain Changes and Pain Variables

A negative correlation between the GMV for the inferior temporal gyrus and the BPI interference score ($r = -0.549$, $P = 0.007$) was seen (Fig. 2B), meaning that patients with reduced brain volume in this region reported increased pain interference scores. No significant correlations were found between clinical pain parameters and cortical thickness in CP patients (all $P > 0.05$). Likewise, total GMV was not correlated with pain variables or disease duration (all $P > 0.05$).

Confounding Factors for Reduced Total GMV in CP Patients

None of the potential confounding factors were associated with total GMV at baseline in our multivariate model: CP duration ($P = 0.30$), opioid use ($P = 0.92$), diabetes ($P = 0.27$), alcoholic etiology ($P = 0.81$), and analgesics use ($P = 0.67$), except for age (coefficient, −2.71 mL, $P = 0.002$), which was significantly and independently associated with the total GMV.

Follow-up After 7 Years

Whole Brain GMV

At the follow-up visit, CP patients had reduced total GMV compared with healthy controls (mean [SD]) (563 [71] mL vs 643 [64] mL; $P = 0.010$) (Fig. 3A). Significant decrease in total GMV between baseline (patients, 592 [56] mL; controls, 660 [59] mL) and follow-up (patients, 563 [72] mL; controls, 643 [65] mL) was found for both CP patients ($P = 0.013$) and healthy controls ($P = 0.002$). The total GMV reduction for patients over time was 28.7 (SD, 31.6) mL, which is a reduction of 4.9%, whereas the GMV reduction for healthy controls was 16.8 (SD, 14.9) mL, which is a reduction of 2.5%. No significant differences in the total GMV decrease were found between patients and controls ($P = 0.26$). When testing for interaction (visit*group interaction), there was no significant GMV reduction over the time in patients compared with controls ($F = 2.4$, $P = 0.135$) (Fig. 3A).

Gray Matter Volume

Significant changes in GMV were observed over time in several cortical regions for both CP patients and healthy controls (Table 3). Particularly, significant decreased GMV over time was observed in CP patients at follow-up relative to baseline in the left putamen, bilateral precentral gyrus, right pallidum, and bilateral thalamus posterior cingulate cortex (Fig. 4A) (all $P < 0.05$). However, the decrease in GMV over time only differed significantly between groups in the left precentral gyrus ($P = 0.021$) and left putamen ($P = 0.035$), with more cortical atrophy observed in the patient group (Table 3; Figs. 3B, C). In addition, patients had increased GMV in the right inferior temporal gyrus.

Cortical Thickness

Significant changes in cortical thickness were observed over time in several cortical regions for both CP patients and healthy controls (Table 3). Particularly, CP patients developed cortical thinning in the postcentral gyrus, superior frontal gyrus, inferior parietal angular gyrus, superior occipital gyrus, precentral gyrus, and superior parietal gyrus over time in the left hemisphere (all $P < 0.05$) (Fig. 4B). In the right hemisphere, cortical thinning was observed in the superior frontalis gyrus, postcentral gyrus, inferior frontalis gyrus, supramarginal gyrus, lateral occipital cortex, middle frontal gyrus, precentral gyrus, and superior temporal gyrus (all $P < 0.05$) over time (Fig. 4B). There were no brain regions

TABLE 1. Demographic and Clinical Characteristics of CP Patients and Healthy Controls at Baseline and at 7-Year Follow-up

Baseline	CP Patients (n = 23)	Healthy Controls (n = 14)	*P*
Age, mean (SD), y	53.1 (11.3)	46.8 (11.1)	0.1
Sex, n (%)			0.6
Male	16 (70)	8 (57)	
Female	7 (30)	6 (43)	
Etiology, n (%)			
Toxic-metabolic (alcoholic)	12 (52)		
Idiopathic	7 (31)		
Genetic	2 (9)		
Recurrent and severe acute pancreatitis	1 (4)		
Obstructive	1 (4)		
Diary pain score, mean (SD), (VAS, 0–10)			
Average pain	3.3 (2.0)		
Maximal pain	4.7 (2.2)		
Pain pattern, n (%)			
Constant pain	17 (74)		
Intermittent pain	6 (26)		
BPI short form, mean (SD)			
Pain score	3.2 (1.7)		
Interference	4.2 (2.0)		
Duration of CP, mean (SD), y	9.3 (6.5)		
Analgesic group, n (%)			
None	4 (17.4)		
Weak analgesics	6 (26.1)		
Opioids	13 (56.5)		
Use of opioids, mean (SD), (morphine equivalent, mg)	81.0 (142.4)		
Diabetes mellitus, n (%)	7 (30)	0 (0)	

Follow-up	CP Patients Baseline (n = 11)	CP Patients Follow-up (n = 11)	Healthy Controls Baseline (n = 12)	Healthy Controls Follow-up (n = 12)	*P*
Age, mean (SD), y	54.6 (10.6)	61.4 (10.3)	47.8 (10.3)	54.6 (10.8)	0.1*
Diary pain score, mean (SD), (VAS, 0–10)					
Average pain	3.2 (1.9)	3.6 (2.8)			0.5
Maximal pain	4.7 (2.0)	5.4 (3.3)			0.4
Pain pattern, n (%)					
Constant pain	6 (55)	5 (45)			
Intermittent pain	5 (45)	5 (46)			
Constant pain with exacerbation		1 (9)			
BPI short form, mean (SD)					
Pain score	3.4 (1.7)	3.6 (3.2)			0.9
Interference	3.3 (2.0)	2.5 (2.4)			0.4
Duration of CP, mean (SD), y	10.4 (9.5)	17.4 (9.0)			
Diabetes mellitus, n (%)	1 (9)	4 (36)	0 (0)	1 (8)	
Opioids, mean (SD), (morphine equivalent, mg)	62.2 (90.6)	41.1 (77.4)			0.344

Descriptive statistical values are represented as percentages (%) or mean (SD).

Baseline comparisons included 23 patients and 14 controls, whereas follow-up comparisons included 11 patients and 12 controls. Paired *t*-tests were performed for diary pain score and for BPI.

*Between patients and controls at follow-up visit. Wilcoxon rank test was performed for opioids.

where CP patients had significantly more cortical thinning as compared with healthy control during the 7-year period.

Association Between Structural Brain Changes and Pain Variables

Among others, thalamus GMV was reduced significantly in patients over the 7-year period. This region was significantly associated with VAS mean ($r = 0.61$, $P = 0.046$), meaning that patients who increased in VAS over the 7-year period had less GMV loss in the thalamus (Fig. 4C). In addition, cortical thickness was significantly reduced in superior temporal gyrus and precentral gyrus in patients over the 7-year period. These regions were significantly correlated with BPI interference, meaning that patients with increased BPI interference had more cortical

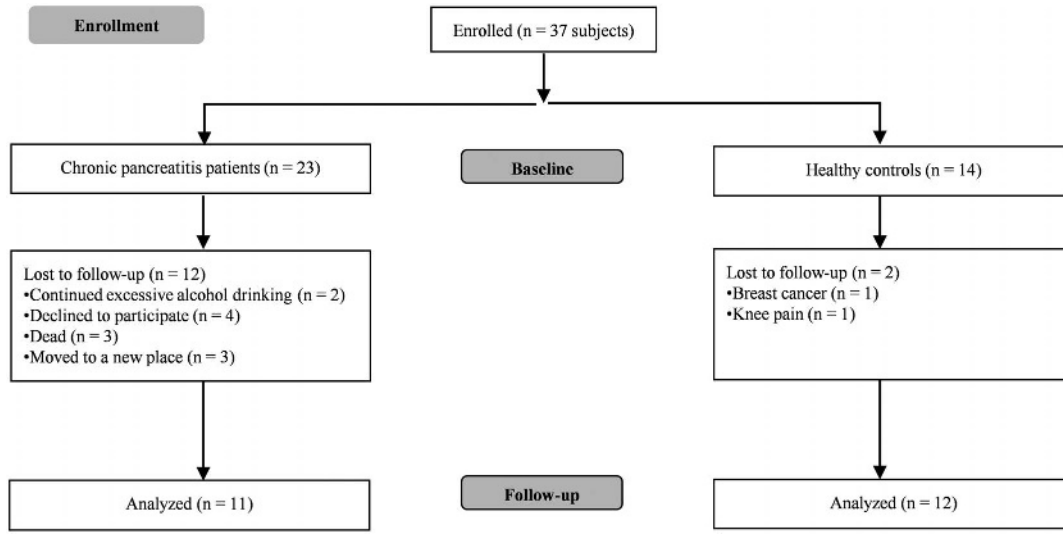

FIGURE 1. Consolidated standards of reporting trials diagram.

thinning in precentral ($r = -0.78$, $P = 0.005$) and superior temporal gyrus ($r = -0.69$, $P = 0.019$) (Fig. 4C).

DISCUSSION

The current study characterizes structural brain changes over a 7-year period between well-characterized CP patients with chronic abdominal pain and healthy controls. The main findings were as follows: (1) reduced GMV and cortical thickness in CP patients at baseline compared with healthy controls; (2) GMV reduction in CP patients over a 7-year period that was more pronounced in the CP patients compared with healthy controls, particularly in the precentral gyrus and putamen; and finally (3) increased VAS

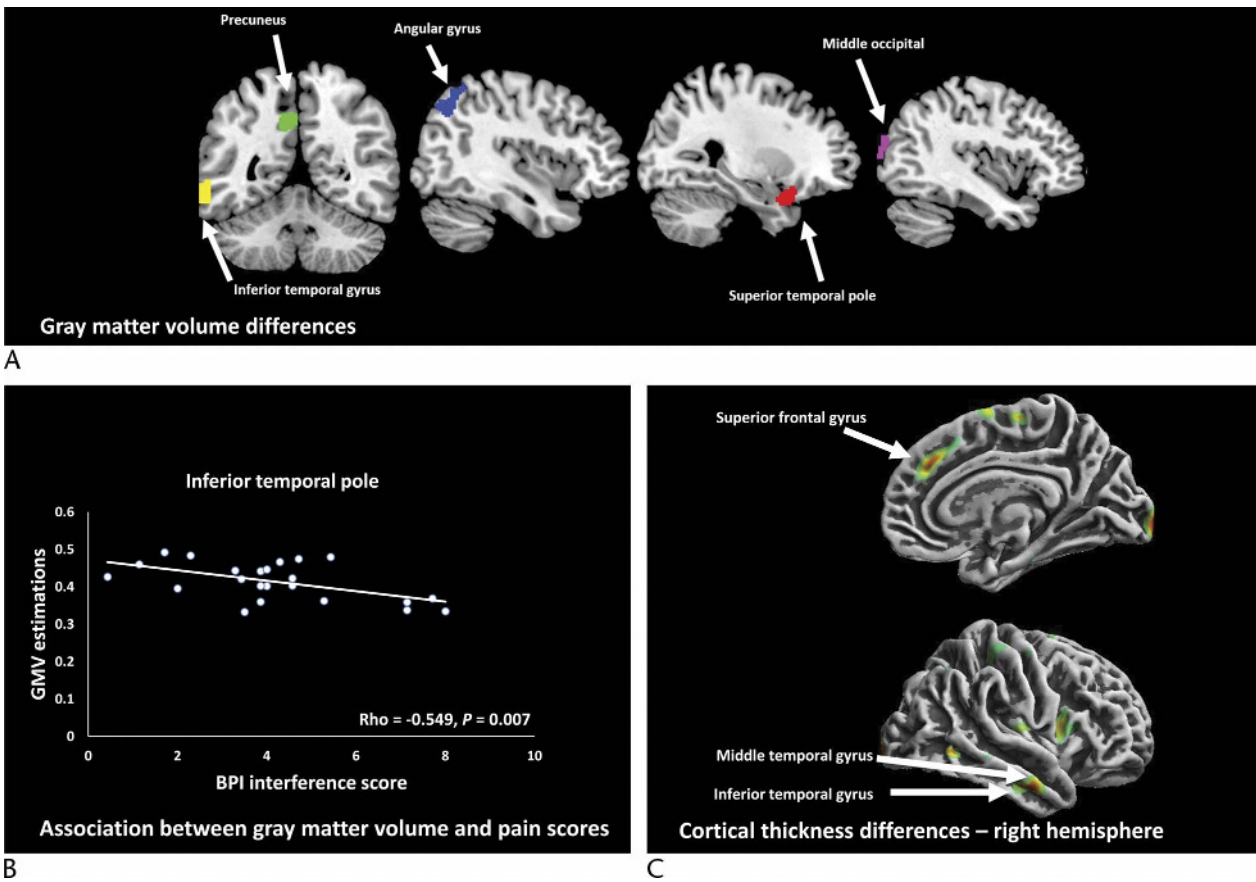

FIGURE 2. A, C, Significant reductions in GMV and cortical thickness in patients (n = 23) compared with healthy controls (n = 14). B, Area of where GMV was negatively correlated with BPI interference scores, meaning that patients with reduced brain volume in this region reported increased pain interference scores. **Editor's note:** A color image accompanies the online version of this article.

TABLE 2. Baseline: VBM Analysis of GMV and SBM Analysis of Cortical Thickness (CP Patients, n = 23; Healthy Controls, n = 14)

Group Comparisons	MNI Coordinates	Left/Right	Region	Cluster Size, μL	*P*
GMV differences					
Decreased GMV in patients compared with healthy controls	39 −68 44	Right	Angular gyrus	1422	0.002*
	27 10 −26	Right	Superior temporal pole	3180	0.001*
	−62 −50 −4	Left	Inferior temporal gyrus	741	0.038*†
	−34 −82 26	Left	Middle occipital gyrus	837	0.025*
	−8 −51 39	Left	Precuneus	1108	0.008*
Cortical thickness differences					
Decreased cortical thickness in patients compared with healthy controls	58 −10 −27	Right	Middle temporal gyrus	558	0.039‡
	7 36 36	Right	Superior frontal gyrus	582	0.034‡
	51 9 10	Right	Inferior frontal gyrus	609	0.029‡

Only significant clusters are presented.

*FWE-corrected *P* values at cluster level.

†Correlated with clinical scores (Fig. 2B).

‡Uncorrected *P* values at cluster level.

score over time was associated with less degree of thalamus GMV loss, whereas increased BPI interference score was associated with greater degree of cortical thinning in superior temporal gyrus and precentral gyrus over time in patients.

Traditionally, the mainstay of pain treatment in CP has focused on the pancreatic gland based on the assumption that pain is generated by increased pressure in the pancreatic duct or in the pancreatic parenchyma. Our findings suggest that long-term CP pain results in measurable macrostructural changes over time within specific areas of the brain involved in processing and integration of sensory information. Such knowledge from longitudinal studies are clinically very meaningful, as it gives more causal support to the hypothesis that central pain processing is abnormal in CP owing to sustained pain input involving CNS neuroplasticity as part of the pain pathogenesis.

Accelerated Total GMV Decrease in CP Patients

Consistent with previous chronic pain studies,[15,16] CP patients had significant reduced total GMV compared with healthy controls. Furthermore, during the 7-year follow-up, the patients also had a trend, even though not significant, toward greater total GMV loss compared with healthy controls, indicating that CP patients could have accelerated general GM loss compared with normal aging. A possible explanation for accelerated GM loss in CP patients might be atrophy due to excitotoxicity[17] and/or exposure to inflammation-related agents, such as neuropeptides, cytokines including TRPV1 (transient receptor potential cation channel subfamily V, member 1).[16,18] On the other hand, it is ambiguous whether excitotoxicity has an impact because we found no significant difference in total GMV between patients with alcohol etiology and patients without alcohol etiology at the baseline visit; hence, the effect of excitotoxicity may be limited. Another explanation for reduced total GMV could be reduction in cell size, extracellular fluids, synaptogenesis, angiogenesis, neural or glial cell apoptosis, or blood volume changes.[19] Although we showed that opioid use has no significant impact on total GMV, larger studies are needed to confirm this finding because the literature indicates that use of opioids could also partially explain the GMV loss. Accumulating evidence indicates that chronic pain disorders are characterized by GMV loss[15,20]; however, in this study, it is uncertain whether the GMV loss is occurring owing to visceral pain because we found no association between total GMV and pain variables nor any association between total GMV and disease duration. However, the inability to show any significant correlations could potentially be owing to the low statistical power.

Morphological Brain Changes in CP Patients at Baseline

We revealed significant decreased GMV in various regions in CP patients. Interestingly, a strong negative correlation between BPI interference and the GMV in inferior temporal gyrus was observed in CP patients, meaning that patients with reduced brain volume in this region reported increased pain interference scores (Fig. 2). Thus, patients with reduced GMV in this region deeply affect the patient's daily activity. Functionally, the temporal pole is involved in the processing of auditory perception, speech, language comprehension, and emotion.[21] Although the role of inferior temporal gyrus has received little attention until recently, a previous pain imaging study provides strong support for inferior temporal gyrus involvement in chronic pain conditions,[21] possibly involving in mnemonic processes related to the affective component of pain.[21] Inferior temporal gyrus is known to be connected to the amygdala and hippocampus, involving in emotional processing. Thus, our findings suggest that reduced GMV in the inferior temporal gyrus is associated with BPI interference, a measure of pain-related quality of life, and could be relevant to pain-related dysfunction. Furthermore, reduced GMV in superior temporal gyrus has also been detected in heavy drinking adolescents.[17] Hence, it is undistinguishable whether the reduced GMV in the temporal lobe is exclusively owing to pain, previous alcohol abuse, or whether it is a combination of both factors. Moreover, we also found evidence of decreased GMV in the precuneus. The precuneus is shown to be part of the default mode network and has widespread connections with the thalamus, caudate nucleus, and putamen.[22] Altered default mode network is a well-known phenomenon in chronic pain conditions.[23–25] Thus, we suspect that functional connectivity between precuneus and other pain regions may be altered, which further could contribute to the observed GM abnormalities in the precuneus.

We revealed significant cortical thinning in the middle temporal gyrus, superior frontal gyrus, and inferior frontal gyrus in CP patients compared with healthy controls. Because of the lack of correlation between the altered regions and pain scores, it could be questionable whether cortical thinning in these regions may be related to abdominal pain. However, in our previous cortical thickness study, we found cortical thinning of brain areas involved in pain processing, which were further correlated with pain variables,[7]

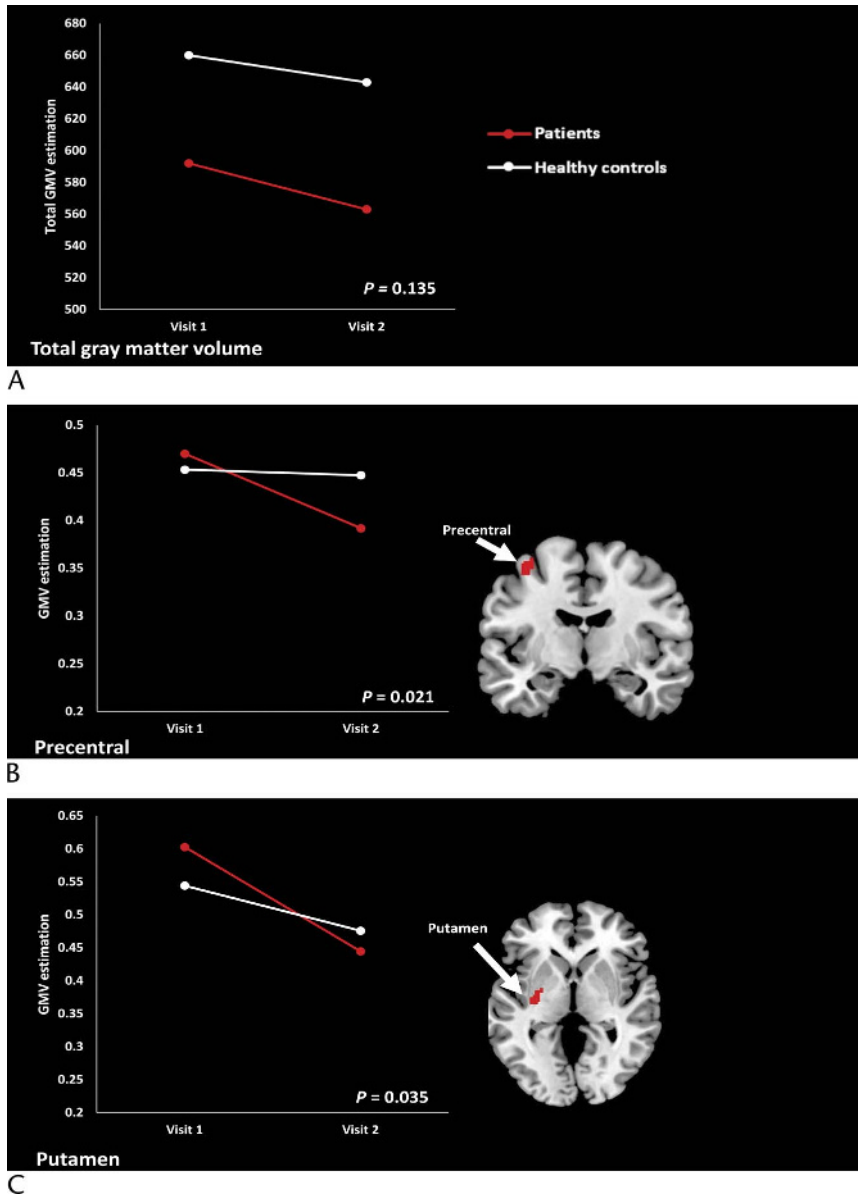

FIGURE 3. A, Whole brain GMV changes over time. B, C, Patients had more decreased precentral and putamen GMV than healthy controls over time. **Editor's note:** A color image accompanies the online version of this article.

suggesting that cortical thinning is related to the CP pain. On the other hand, previous alcohol abuse may explain these findings, as MRI studies regarding alcoholism showed cortical thinning in the same regions: superior frontal gyrus, middle temporal gyrus, and inferior frontal gyrus.[26] Then again, it is ambiguous whether these alterations are a consequence of previous alcohol use, as we did not find any significant reduction in total GMV in CP patients with alcohol etiology comparing with CP patients without alcohol etiology. Conversely, the latter analysis was based on 5 and 6 patients respectively; hence, the results could possibly be a type 2 error owing to small sample size.

Morphological Brain Changes in CP Patients Over Time

Consistent with other functional gastrointestinal disease,[27] we found reduced GMV of the thalamus over the 7-year period in the patient group, which was not seen in the control group. We demonstrated that the thalamus GMV decreased more in patients who developed less pain over the 7-year period than in patients who developed more pain over the 7-year period (Fig. 3C). One of the most consistently described areas of GMV alterations associated with chronic pain is the thalamus.[28] The thalamus serves both sensory and motor mechanisms and has an essential role in pain processing mediating nociceptive inputs to the cortex. Our findings may reflect the thalamic neuronal dysfunction in CP patients, which could possibly be explained by the excessive afferent input, as a result of pancreatic fibroinflammation and ductal changes followed by secondary peripheral/spinal neuropathic changes.[29] However, it is unclear whether the fibrosis of the pancreas produces primary plastic changes within the thalamus or whether thalamus changes are secondary to neuropatic-like changes of the CNS.[16,29]

TABLE 3. Follow-up After 7 Years: VBM Analysis of GMV and SBM Analysis of Cortical Thickness (CP Patients, n = 11; Healthy Controls, n = 12)

Contrast	MNI Coordinates	Left/ Right	Regions	Cluster Size, μL	*P*
GMV changes					
Follow-up less than baseline — volume loss (patients)	−30 −12 −4	Left	Putamen	1201	<0.001
	−27 −21 63	Left	Precentral gyrus	3342	<0.001
	28 −21 63	Right	Precentral gyrus	4385	<0.001
	28 −10 −4	Right	Pallidum	1194	<0.001
	−10 −26 4	Left	Thalamus	550	<0.001*
	12 −26 2	Right	Thalamus	361	<0.001
	6 −39 27	Right	Posterior cingulate gyrus	176	<0.001
Follow-up greater than baseline — volume increase (patients)	68 −30 −18	Right	Inferior temporal gyrus	298	<0.001
Follow-up less than baseline — volume loss (controls)	39 −27 58	Right	Precentral	1222	<0.001
	−24 −22 63	Left	Precentral	1110	<0.001
	−30 −10 −8	Left	Putamen	356	<0.001
	−10 3 62	Left	Superior motor area	151	<0.001
	16 10 57	Right	Superior frontal region	268	<0.001
Patients decrease over time more than controls — volume loss relative to controls	−38 −6 60	Left	Precentral	189	0.021
	−33 −15 2	Left	Putamen	153	0.035
Cortical thickness changes					
Follow-up less than baseline — cortical thinning (patients)	−37 −34 66	Left	Postcentral gyrus	2476	<0.001
	−13 60 21	Left	Superior frontal gyrus	1240	<0.001
	−31 −78 37	Left	Inferior parietal angular gyrus	1205	<0.001
	−22 −80 33	Left	Superior occipital gyrus	971	<0.001
	−53 −1 46	Left	Precentral gyrus	823	<0.001*
	−20 −45 69	Left	Parietal superior gyrus	759	<0.001
Follow-up less than baseline — cortical thinning (controls)	−36 −34 67	Left	Postcentral gyrus	1737	<0.001
	−26 −17 70	Left	Precentral gyrus	1134	<0.001
	−22 −62 64	Left	Superior parietal gyrus	785	<0.001
	−27 −88 −17	Left	Inferior sulcus occipital	504	<0.001
	−9 −36 65	Left	Sulcus centralis	208	<0.001
Follow-up less than baseline — cortical thinning (patients)	15 59 18	Right	Superior frontalis gyrus	799	<0.001
	33 −34 68	Right	Postcentral gyrus	2832	<0.001
	41 52 −10	Right	Inferior frontalis gyrus	568	<0.001
	58 −35 44	Right	Supramarginal gyrus	612	<0.001
	26 −76 37	Right	Lateral occipital cortex	700	<0.001
	49 27 30	Right	Middle frontal gyrus	411	<0.001
	49 −60 44	Right	Lateral occipital cortex	658	<0.001
	63 −9 31	Right	Postcentral gyrus	425	<0.001
	59 8 29	Right	Precentral gyrus	404	<0.001
	68 −29 2	Right	Superior temporal gyrus	332	0.001*
Follow-up greater than baseline — cortical thickening (patients)	4 −68 6	Right	Lingual gyrus	131	0.010
Follow-up less than baseline — cortical thinning (controls)	33 −32 69	Right	Postcentral gyrus	1121	<0.001
	15 −51 66	Right	Superior parietal gyrus	2031	<0.001
	37 −88 −15	Right	Lateral occipital cortex	620	<0.001
	63 −9 31	Right	Postcentral gyrus	636	<0.001
	40 −7 60	Right	Precentral gyrus	324	0.001
	65 −21 −1	Right	Superior temporal gyrus	222	0.003
	21 54 25	Right	Superior frontalis	346	0.001
	48 −61 44	Right	Lateral occipital cortex	118	0.012
Follow-up greater than baseline — cortical thickening (controls)	46 −42 6	Right	Middle temporal gyrus	442	<0.001

Only significant clusters are shown. All *P* values are FWE corrected.

*Correlated with clinical scores (Fig. 3C).

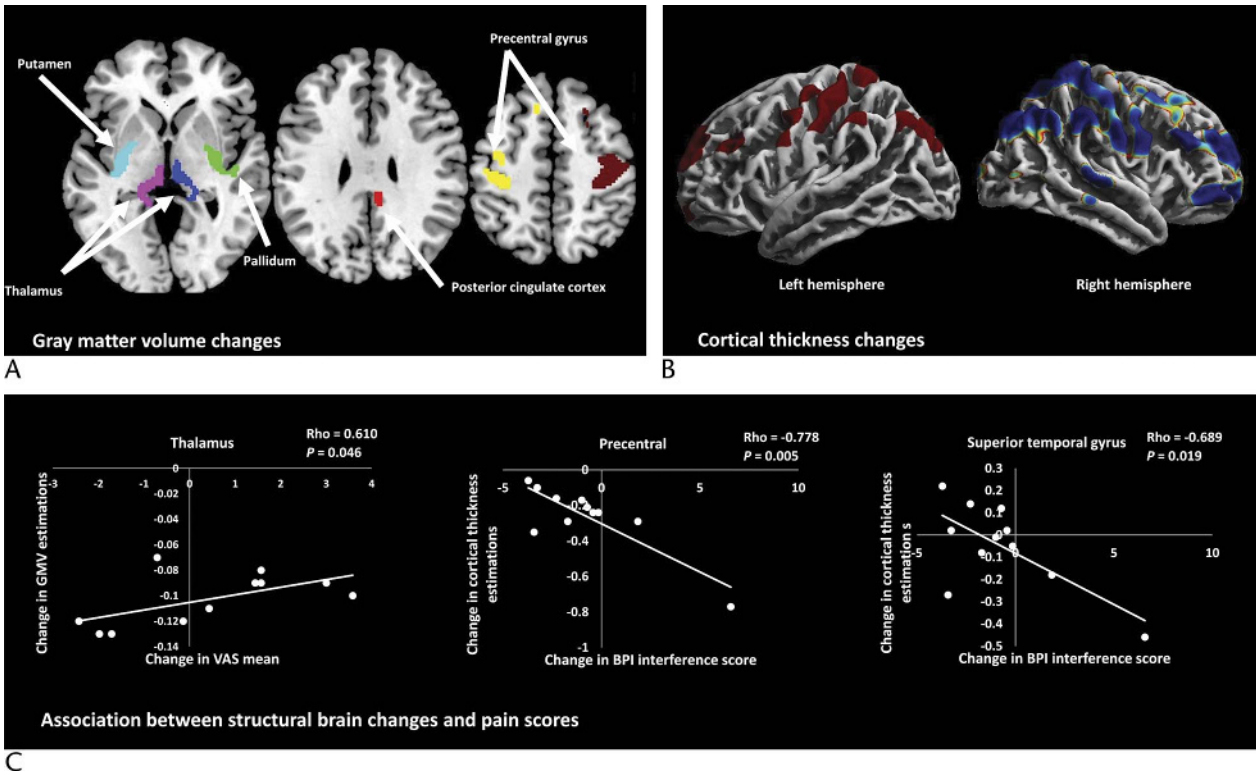

FIGURE 4. A, B, Significant GMV and cortical thickness reductions in patients over time. C, Association between changes in GMV and cortical thickness over time and changes in pain scores over time. **Editor's note:** A color image accompanies the online version of this article.

In the current study, CP patients had more decreased GMV in the putamen and precentral gyrus. The putamen, as part of the dorsal striatum and the basal ganglia, plays a unique role in movement regulation, motor function, coordination, and cognition including learning and memory tasks.[30] Reduced GMV in the putamen and thalamus has been observed in IBS patients.[27] It is likely that both pain syndromes, IBS and CP, share some common underlying pathophysiological mechanisms, such as reorganization of the thalamus, allowing normally "silent" visceral signals to be processed differently, gaining access to higher cortical structures, and be perceived as painful.[31]

Both patients and healthy controls developed significant cortical thinning over time. However, it is noteworthy that CP patients, relative to controls during the 7 years, had no significant cortical thinning. The regional cortical thinning in patients was in general similar that in to healthy controls, indicating that the cortical alterations in patients are probably owing to normal aging. Interestingly, we showed negative correlations between change in BPI interference and changes of cortical thinning in both precentral gyrus and superior temporal gyrus (Fig. 3C), suggesting that patients with most pronounced cortical thinning in the frontal lobe are those who also are affected in terms of daily activity.

Methodological Considerations

This study is unique in the length of follow-up, the quantitative assessment of structural brain alterations, and the availability of follow-up data for healthy controls so that brain changes in the CP patient can be studied relative to ongoing normal aging. Furthermore, the strength of this study is acquiring MRI data from the same scanner and using the same MRI protocol. However, there are also a number of limitations in this study. First, our sample size was relatively small, and there was a considerable drop-out rate (12 patients, 52%; 2 healthy controls, 14%). The fact that about half of the patients discontinued may have contributed to the MRI results at the 7-year follow-up, as these patients could potentially have been those where regional brain atrophy and cortical thinning progressed the most, leading to selection bias. Although there were no significant differences in terms of age and sex, patients included in this study were on average older than healthy controls. To account for this issue, we used age as covariate in the MRI analyses. Second, our sample of CP patients was clinically heterogeneous. Nevertheless, all patients reported pain, 5 patients had constant pain, 5 patients had intermittent pain, whereas 1 patient had constant pain with exacerbation, indicating that pain progression is individual and there is vast intersubject variability in the general response to CP pain as well as coping strategies. Furthermore, factors such as diabetes, previous alcohol misuse, malnutrition, and other comorbidities could potentially have an impact on the MRI findings. Another factor, which may bias our interpretation of the results, is the fact the nearly all patients were on medication, that is, analgesic drugs and other CNS active drugs (eg, hypnotic drugs). One could argue that nonsteroidal anti-inflammatory drugs could have some effects on the neural systems and that the impact of analgesics on morphometric findings should not be underestimated.[15] Although we found no significant impact of opioids and analgesics on total GMV, pain medication could have a specific impact on our findings. Lastly, it is worthwhile to report that only 5 patients of 23 used other types of CNS active drugs; thus, we believe that our findings are not driven by those patients. Finally, baseline SBM analysis was reported using uncorrected *P* values at 0.005 due to low statistical power, which is in line with similar explorative studies.[32] Thus, baseline SBM results should be interpreted with caution.

Future Perspective

Although our findings are consistent with morphometric studies conducted in chronic pain patients, further work is needed in a larger sample of CP patients to generalize the current findings to a broader clinical population and to investigate whether our findings are specific to CP pain. In addition, an interventional study would be highly relevant to assess whether structural and functional abnormalities are reversible by pain treatment.

CONCLUSIONS

Our findings suggest that structural brain changes over time may be related to the complex pain syndrome seen in CP patients. Furthermore, this study indicates that CP and other visceral pain syndromes share resemblances with structural brain alterations in similar pain-related regions. This longitudinal information is relevant for further understanding the pain mechanisms involved in painful CP including the causality between brain changes and the clinical course of the disease.

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REFERENCES

1. Muniraj T, Aslanian HR, Farrell J, et al. Chronic pancreatitis, a comprehensive review and update. Part I: Epidemiology, etiology, risk factors, genetics, pathophysiology, and clinical features. *Dis Mon*. 2014;60: 530–550.
2. Poulsen JL, Olesen SS, Malver LP, et al. Pain and chronic pancreatitis: a complex interplay of multiple mechanisms. *World J Gastroenterol*. 2013; 19:7282–7291.
3. Lelic D, Olesen SS, Graversen C, et al. Electrophysiology as a tool to unravel the origin of pancreatic pain. *World J Gastrointest Pathophysiol*. 2014;5:33–39.
4. Dimcevski G, Sami SA, Funch-Jensen P, et al. Pain in chronic pancreatitis: the role of reorganization in the central nervous system. *Gastroenterology*. 2007;132:1546–1556.
5. Lelic D, Olesen SS, Hansen TM, et al. Functional reorganization of brain networks in patients with painful chronic pancreatitis. *Eur J Pain*. 2014;18: 968–977.
6. Frøkjær JB, Olesen SS, Gram M, et al. Altered brain microstructure assessed by diffusion tensor imaging in patients with chronic pancreatitis. *Gut*. 2011;60:1554–1562.
7. Frøkjær JB, Bouwense SA, Olesen SS, et al. Reduced cortical thickness of brain areas involved in pain processing in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol*. 2012;10:434–438.e1.
8. Ashburner J, Friston KJ. Voxel-based morphometry—the methods. *Neuroimage*. 2000;11:805–821.
9. Dahnke R, Yotter RA, Gaser C. Cortical thickness and central surface estimation. *Neuroimage*. 2013;65:336–348.
10. Olesen SS, Graversen C, Olesen AE, et al. Randomised clinical trial: pregabalin attenuates experimental visceral pain through sub-cortical mechanisms in patients with painful chronic pancreatitis. *Aliment Pharmacol Ther*. 2011;34:878–887.
11. Layer P, Yamamoto H, Kalthoff L, et al. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. *Gastroenterology*. 1994;107:1481–1487.
12. Jensen MP, Turner JA, Romano JM, et al. Comparative reliability and validity of chronic pain intensity measures. *Pain*. 1999;83:157–162.
13. Cleeland CS. Brief Pain Inventory (BPI). *MD Anderson Cancer Cent*. 1982;1100:6.
14. Gaser C, Kurth F. *Manual Computational Anatomy Toolbox - CAT12*. June 28, 2017. Available at: http://www.neuro.uni-jena.de/cat12/CAT12-Manual.pdf. Accessed May 1, 2017.
15. Rodriguez-Raecke R, Niemeier A, Ihle K, et al. Brain gray matter decrease in chronic pain is the consequence and not the cause of pain. *J Neurosci*. 2009;29:13746–13750.
16. Apkarian AV, Sosa Y, Sonty S, et al. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *J Neurosci*. 2004;24: 10410–10415.
17. Heikkinen N, Niskanen E, Könönen M, et al. Alcohol consumption during adolescence is associated with reduced grey matter volumes. *Addiction*. 2017;112:604–613.
18. Demir IE, Friess H, Ceyhan GO. Neural plasticity in pancreatitis and pancreatic cancer. *Nat Rev Gastroenterol Hepatol*. 2015;12:649–659.
19. May A. Chronic pain may change the structure of the brain. *Pain*. 2008; 137:7–15.
20. Apkarian AV, Hashmi JA, Baliki MN. Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. *Pain*. 2011;152(3 Suppl): S49–S64.
21. Wang Y, Cao DY, Remeniuk B, et al. Altered brain structure and function associated with sensory and affective components of classic trigeminal neuralgia. *Pain*. 2017;158:1561–1570.
22. Baliki MN, Geha PY, Apkarian AV, et al. Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. *J Neurosci*. 2008;28:1398–1403.
23. Čeko M, Shir Y, Ouellet JA, et al. Partial recovery of abnormal insula and dorsolateral prefrontal connectivity to cognitive networks in chronic low back pain after treatment. *Hum Brain Mapp*. 2015;36:2075–2092.
24. Baliki MN, Schnitzer TJ, Bauer WR, et al. Brain morphological signatures for chronic pain. *PLoS One*. 2011;6:e26010.
25. Kucyi A, Moayedi M, Weissman-Fogel I, et al. Enhanced medial prefrontal-default mode network functional connectivity in chronic pain and its association with pain rumination. *J Neurosci*. 2014;34: 3969–3975.
26. Fortier CB, Leritz EC, Salat DH, et al. Reduced cortical thickness in abstinent alcoholics and association with alcoholic behavior. *Alcohol Clin Exp Res*. 2011;35:2193–2201.
27. Seminowicz DA, Labus JS, Bueller JA, et al. Regional gray matter density changes in brains of patients with irritable bowel syndrome. *Gastroenterology*. 2010;139:48–57.e2.
28. Gwilym SE, Filippini N, Douaud G, et al. Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study. *Arthritis Rheum*. 2010;62: 2930–2940.
29. Agostini A, Benuzzi F, Filippini N, et al. New insights into the brain involvement in patients with Crohn's disease: a voxel-based morphometry study. *Neurogastroenterol Motil*. 2013;25:147–154.
30. Starr CJ, Sawaki L, Wittenberg GF, et al. The contribution of the putamen to sensory aspects of pain: insights from structural connectivity and brain lesions. *Brain*. 2011;134:1987–2004.
31. As-Sanie S, Harris RE, Napadow V, et al. Changes in regional gray matter volume in women with chronic pelvic pain: a voxel-based morphometry study. *Pain*. 2012;153:1006–1014.
32. Woo CW, Krishnan A, Wager TD. Cluster-extent based thresholding in fMRI analyses: pitfalls and recommendations. *Neuroimage*. 2014;91: 412–419.