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ORIGINAL RESEARCH

Decreased grey matter volume in mTBI patients with post-traumatic headache compared to headache-free mTBI patients and healthy controls: a longitudinal MRI study

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Abstract

Traumatic brain injury (TBI) occurs in 1.7 million people annually and many patients go on to develop persistent disorders including post-traumatic headache (PTH). PTH is considered chronic if it continues past 3 months. In this study we aimed to identify changes in cerebral grey matter volume (GMV) associated with PTH in mild TBI patients. 50 mTBI patients (31 Non-PTH; 19 PTH) underwent MRI scans: within 10 days post-injury, 1 month, 6 months and 18 months. PTH was assessed at visit 4 by a post-TBI headache questionnaire. Healthy controls ($n = 21$) were scanned twice 6 months apart. Compared to non-PTH, PTH patients had decreased GMV across two large clusters described as the right anterior-parietal ($p = 0.012$) and left temporal-opercular ($p = 0.027$). Compared to healthy controls non-PTH patients had decreased GMV in the left thalamus ($p = 0.047$); PTH patients had decreased GMV in several extensive clusters: left temporal-opercular ($p = 0.003$), temporal-parietal ($p = 0.041$), superior frontal gyrus ($p = 0.008$) and right middle frontal/superior frontal gyrus (0.004) and anterior-parietal ($p = 0.003$). Differences between PTH and non-PTH patients were most striking at early time points. These early changes may be associated with an increased risk of PTH. Patients with these changes should be monitored for chronic PTH.

Keywords Persistent post-traumatic headache · Post-traumatic headache · Mild traumatic brain injury · Gray matter volume · Brain

Introduction

Mild traumatic brain injuries (mTBI) account for 75% of all brain injuries sustained ranging from 131 to 640 per 100,000 population, although many go unreported (Obermann et al. 2010, 2009). A subset of mTBI patients develop persistent, sometimes disabling disorders, with one of the most common being post-traumatic headache (PTH) (Anderson et al. 2015; Defrin 2014; Lucas et al. 2012). PTH usually arises within seven days of the injury and is considered chronic if it persists three months after injury (Defrin 2014; Lucas et al. 2012). The prevalence of PTH has been reported as high as 79% at 3 months and 65% at 12 months following mTBI (Lucas et al. 2012).

Several MRI studies have characterized the structural and functional changes associated with mTBI and its symptoms (Eierud et al. 2014). Wide spread white and grey matter reductions have been reported. In particular decreases in GMV have been reported in the thalamus, right precentral and postcentral gyri and supplementary motor area (Bendlin et al. 2008). Other studies have found that the GMV atrophy one year after TBI was located in the right precuneus, suggesting vulnerabilities in particular areas of the brain (Zhou et al. 2013).

Despite the common occurrence of PTH, its pathogenesis is unknown. The spectrum of contributing factors both biological and behavioral are likely to vary by person (Lucas et al.

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2012). In chronic PTH damage to the pain modulatory system after mTBI has been implicated in the development of the headache disorder (Defrin 2014). Obermann et al. (2009) reported that PTH in patients post-whiplash was associated with significantly decreased GMV in the anterior cingulate cortex and dorsolateral prefrontal cortex (DLPFC) at two weeks and three months post-injury; however, these changes completely resolved along with the headache after a year. Studies have reported mixed results on resolution of PTH in various TBI populations and have yet to define a core set of predictors or pathophysiological changes associated with PTH (Obermann et al. 2009). Given the difficulty in assessing these predictors and changes associated with PTH in TBI, we assessed patients both with and without headache and explored GMV changes that are unique to mTBI and those specific to patients with headache post-mTBI. In this analysis we aimed to compare changes in GMV in mTBI patients with and without PTH and healthy controls over four time points occurring up to 18 months after injury.

Materials and methods

Participants and study design

Fifty-one mTBI patients (15 females, 36 males) seen at the R. Adam Cowley Shock Trauma Center at the University of Maryland Medical Center were prospectively recruited and enrolled between March 2010 and May 2012. These patients were a subset of a larger imaging protocol using a combination of advanced MR imaging and neuropsychological assessments including the Automated Neuropsychological Assessment Metrics (ANAM) and the Modified Rivermead Post-Concussion Symptom Questionnaire (RPQ) which was used to assess the level of post-concussive symptoms (Kane et al. 2007; King et al. 1995). The current study is a re-use of data from a previous study, in which the main objective of the larger study was to investigate the longitudinal effects of mTBI on resting state functional connectivity (Sours et al. 2015). Methods have been described in detail in that paper. In brief, patients were screened and excluded if they had a history of the following: neurological and psychiatric illness, stroke, brain tumors or seizures and contraindications to MR. All patients were classified as having a mTBI based on their Glasgow Coma Scale (GCS) score (range 13–15) and mechanism of injury consistent with trauma. Additionally, 21 healthy controls, age 18 or older, free of mTBI and no previous head injury resulting in hospitalization, were enrolled. Exclusion criteria for patients also applied to controls.

mTBI patients were seen at four time points starting approximately within 10 days post-injury, one (1) month, six (6) months, and eighteen (18) months post-TBI. mTBI patients were classified as post-traumatic headache (PTH) based on self-reported headache post-TBI using an in-house developed post-TBI headache questionnaire. The questionnaire was administered at the final visit (approx. 18 months post injury) and was modified from the Theeler et al. (2010) headache questionnaire which assessed PTH in post-concussive soldiers. The questionnaire included, but was not limited to, the start date of headache post-TBI (within one week or within one month post-injury), headache pain intensity, quality of the headache pain (throbbing or not) and location of the headache pain (one sided or not). Controls were seen twice approximately six months apart. Each visit included an MRI scan. For the purposes of this study, only structural MRI data was utilized. This study was approved by the Internal Review Board at the University of Maryland and all participants provided written informed consent and HIPAA compliance.

MRI data acquisition

Imaging was performed on a Siemens Tim-Trio 3 T MRI scanner using a 12 channel receiver head coil. A high resolution image T1-MPRAGE was acquired in either axial (TE = 3.44 ms, TR = 2250 ms, flip angle = 9°, resolution = 256 × 256 × 96, FOV = 22 cm, slice thickness = 1.5 mm) or sagittal (TE = 2.91 ms, TR = 2300 ms, flip angle 9°, resolution 256 × 256 × 176, FOV = 256 mm, slice thickness = 1 mm, with parallel imaging factor of 2). Controls received scans with the axial protocol, but some patients had scans with the sagittal sequence (See below for details).

Data analysis

Voxel based morphometry (VBM)

We used longitudinal VBM to compare whole-brain GMV between mTBI patients with and without PTH and healthy controls. After realignment all T1 images were preprocessed using the Computational Anatomy Toolbox (CAT12) (http://www.neuro.uni-jena.de/cat12/) in SPM12. The preprocessing pipeline consists of several steps in which the images are spatially normalized to MNI space (resampled to a voxel size of 1.5mmX1.5mmX1.5 mm), segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF), and smoothed with an 8 mm Gaussian Kernel. This process has been customized for longitudinal analysis and takes into account the features of intra-subject analysis. Images are registered to the mean image of each subject by an inverse consistent alignment. This also included a bias correction between the different time points and the mean image of realigned images is calculated. Furthermore spatial normalization is only estimated for the mean image of all time points and applied to all images. Whole-brain GMV changes were calculated in both the TBI patients and the healthy controls, and an absolute threshold mask of 0.1 was employed for

analyses. This threshold excludes voxels with less than 10% probability of being grey matter occurring because of partial volume and smoothing effects.

Patient scans were acquired in both axial and sagittal sequences over time; the sagittal sequence which was conducted on the same scanner, was introduced later in the study during data acquisition as it produced better quality scans. Initial analyses attempted inclusion of all patient scans regardless of acquisition sequence, but preprocessing failed. Therefore patients with scans acquired in both the axial and sagittal sequences at different visits, had their visits with the most consistent scan sequence included in the data analysis. For example, if a patient was seen at all four time points and had 3 axial scans and one sagittal, we excluded the sagittal scan. Patients with equal numbers of axial and sagittal scans had their sagittal scans utilized in the analysis. This resulted in the exclusion of 25 scans from 19 patients across all four time points in the non-PTH patients and 10 scans from 8 patients in the PTH group. Given that the missingness was at random (the timing of the upgrade and the scans impacted was not systematic) and that the excluded scans spanned across all visits and both groups (affecting equal numbers in both PTH and non-PTH), the resulting sample of images was likely representative of the entire sample.

Quality control of all scans was conducted before and after preprocessing. This included visual inspection and assessing the quality of pre-processing using the CheckReg function in SPM12. Post preprocessing each image was assessed for quality using quality control tools in CAT12 (Gaser and Kurth 2018). Though there is no clear cut off for a bad image we decided that images which were below more than two standard deviations were further assessed in CheckReg. Due to abnormal brain morphology one participant from the PTH group was excluded from analysis, resulting in a final sample size of 50 mTBI patients.

Sandwich estimator analysis (SwE)

Since all patients were not seen at every time point and some scans had to be dropped due to inconsistencies in scan type, SwE was used to account for the unbalanced nature of the data (Guillaume et al. 2014). It uses an unstructured covariance correlation structure and accounts for all the random effects possible in the model and has been shown to be particularly robust for longitudinal neuroimaging data (Guillaume et al. 2014). Using the non-parametric SwE model with 999 bootstraps, we conducted two different analyses. We first compared how GMV changed over time between the two mTBI patient groups (those with and without PTH). To examine this we used group-by-time interactions (PTH x visit vs non-PTH x visit). This model was adjusted for sex to account for unbalance over time due to loss to follow up and scans included in the analysis (Supplementary Table 1). We also examined the difference between those included and excluded from the analysis on sex and age to assess potential bias (Supplementary Tables 1 and 2). Additionally we examined change in GMV over time in all mTBI patients compared to healthy controls. This also utilized group by time interactions (PTH and non-PTH x time vs Control x visit). Given that healthy controls were only scanned at two time points, this analysis only compared patients to healthy controls at visit 1 and visit 3. A cluster forming threshold of $p = 0.005$ was applied and FWE (estimated from the wild bootstrap distribution) was set to 0.05 (Guillaume et al. 2014).

Cluster and volume extraction for brain regions

Results are written in a -log10 cluster-wise and FWE-corrected non-parametric p value image. To obtain the clusters with a corrected p value ≤ 0.05, the images were thresholded using the following approach in FSL cluster tool (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Cluster): $-\log10(0.05) = 1.301$. Whereas traditional MRI clusters results are given with coordinates to identify a peak voxel, results from SwE cluster analyses are written to produce clusters where all voxels within any given cluster have the same intensity value. Each cluster has one peak value which indicates where the intensity value is first located. For visualization of the results, clusters were displayed on a brain surface using SurfIce (https://www.nitrc.org/projects/surfice/).

Marsbar was used (https://www.nitrc.org/projects/marsbar/) to extract data for significant GM clusters identified in the above analyses. Values were converted to mm^3 using the following equation: cluster size x voxel size x beta value. The beta value extracted represents the proportion of the voxel attributed to GM. Differences between patient groups at each time point and patient groups and controls were assessed using 2-sample T-tests and Wilcoxon tests. Mean volume over time by group was plotted and the coefficient of the interaction between group and time was analyzed using linear mixed models. Whole brain differences in GM and WM were also assessed. Using the total intracranial volume (TIV) estimation module in CAT12 the volumes for GM, WM and CSF and TIV were extracted. All statistical analyses including plots were conducted in SAS (v. 9.4, SAS Institute Inc. Cary, NC). Testing was two-sided and done at the 0.05 level of significance.

Comparison of GMV and demographic variables by PTH and control status

Linear and logistic regression analyses provided comparisons of clinical and demographic variables between mTBI patient groups and controls. Additionally differences in overall RPQ as well as anxiety and depression subscales of the RPQ were examined between the PTH and non-PTH groups. We also

assessed the relationship between RPQ total scores and subscale scores with GMV, in all significant clusters in the group-by-time interaction SwE analysis. This analysis was conducted using linear mixed models in SAS (v.9.4, SAS Institute Inc. Cary, NC). Testing was two-sided and done at the 0.05 level of significance.

Results

Sample description

Thirty-one non-PTH and 19 PTH patients were included in the final analysis. PTH and non-PTH mTBI patients were of similar age ($p = 0.41$). Controls were on average 40 years old and were seen at two time points 205 ± 42 days apart (Table 1). We found that sex varied over time due to scan inclusion, with a significant difference at visit 4 where the number of men in the PTH group dropped to 7 (from a max of 10) and increased to 22 in the Non-PTH group (from 16 at visit 1). Furthermore the number of women in the non-PTH group was reduced to 3 from a total 7 at visit 1 (Supplementary Table 1). Total Rivermead scores were higher in PTH than non-PTH at both visits 1 ($p = 0.032$) and 4 ($p = 0.002$) (Table 1). In longitudinal models there was no significant relationship between GMV and overall RPQ scores or anxiety and depression subscales. Eighteen mTBI patients had evidence of head injury with positive CT results (12 non-PTH; 6 PTH). Eighteen PTH patients completed the headache questionnaire with an average headache pain of 5 on a 0–10 scale. Sixteen reported headaches within one week of injury, with all but 2 reporting headaches at the end of follow-up.

Table 1 Demographic and clinical characteristics of healthy controls and mTBI patients by posttraumatic headache status

Variable	Non-PTH	PTH	Healthy Controls
Age/years	42 ± 16	48 ± 21	40 ± 19
Male (n)	24	11	11
Female (n)	7	8	10
Clinical Characteristics in mTBI patients			
Time	Non-PTH	PTH	*p* value*
Days post-TBI (n) when MRI scan occurred			
Visit 1	6.0 ± 3(23)	7 ± 3(17)	0.27
Visit 2	37 ± 10(22)	34 ± 7(18)	0.40
Visit 3	193 ± 26(29)	206 ± 29(17)	0.18
Visit 4	600 ± 92(25)	538 ± 72(14)	**0.037**
Total Rivermead Score			
Visit 1	12 ± 16	21 ± 17	**0.032**
Visit 2	15 ± 15	17 ± 15	0.61
Visit 3	9 ± 12	18 ± 20	0.16
Visit 4	8 ± 10	29 ± 22	**0.002**

*p values based on T-tests, *p* values <0.05 are in bold. Chi-Square and Wilcoxon tests. Values are Mean ± S.D. mTBI mild traumatic brain injury, PTH post traumatic headache

PTH vs. Non-PTH

Total GMV over time differed between PTH and non-PTH, with PTH having an overall decrease over time (Please refer to supplementary Figure 1 in the online resource). SwE analysis revealed no areas of increased GMV in PTH patients over time in the interaction model, but there was a significant group by time interaction between the two mTBI groups resulting in two extensive clusters of decreased GMV (Table 2). Figure 1a shows the region described as the left temporal-opercular cluster ($p = 0.027$) (1905 voxels), which includes left middle temporal gyrus (MTG), superior temporal gyrus (STG) and parietal operculum, and the second cluster labeled the right anterior-parietal cluster ($p = 0.012$). This latter cluster was even more extensive (3166 voxels) spanning areas of frontal, temporal and parietal lobes including right inferior temporal gyrus (ITG), middle temporal gyrus (MTG), angular gyrus (Ang), supramarginal gyrus, superior temporal gyrus (STG), primary somatosensory cortex (S1) and primary motor cortex (M1). Table 3 shows the difference in GMV between PTH and non-PTH at each of the four time points and Fig. 1a also shows plots of the change in GMV in these regions over time in PTH and non-PTH. In the left temporal-opercular cluster the difference between the two groups is significant only at visit 1 ($p = 0.022$), while in the right anterior-parietal cluster differences were most evident at visits 2 and 4. However, PTH consistently show negative slopes over time, starting with higher GM volumes compared to non-PTH and decreasing through to visit 4 in both areas. This change in GMV was significant in PTH in both clusters ($p < 0.0001$) as indicated by the interaction *p* value whereas in non-PTH the change over time was relatively constant and not significant.

mTBI vs. Healthy controls

PTH had decreased GMV compared to healthy controls in five clusters (Fig. 1b, Table 3), two of which were similar to those observed between PTH vs. non-PTH. These clusters included left temporal-opercular cluster ($p = 0.003$), temporal-parietal cluster ($p = 0.041$) and the SFG ($p = 0.008$). The left temporal-opercular cluster covered the parietal and frontal operculum, STG, and MTG. The temporal-parietal cluster included the angular gyrus and the parietal junction. There was also decreased volume in two clusters on the right: a middle/superior frontal gyrus cluster (MFG/SFG) ($p = 0.004$) and an anterior-parietal cluster (p = 0.003). The MFG/SFG cluster extends posteriorly to M1. The right anterior-parietal cluster includes the STG, supramarginal gyrus, M1 and S1 (Fig. 1b).

Table 2 Significant clusters from whole brain time by group analysis

Brain Region	Cluster Size	FWE *p* value	Peak Voxel MNI Coordinates (x, y, z)
PTH vs. Non-PTH			
Left Temporal-Opercular Cluster	1905	0.027	−66, −20 -30
Right Anterior-Parietal Cluster	3166	0.012	62, −48, −17
Non-PTH vs. Controls			
Left Thalamus	1292	0.047	−10.5, −33, −11
PTH vs. Controls			
Right Anterior-Parietal Cluster	4393	0.003	71, −18, 9
Left Temporal-Opercular Cluster	3990	0.003	−71, −21, −20
Right MFG-SFG	3120	0.004	45, 47, 21
Left SFG	2512	0.008	−15, 62, 23
Left Temporal-Parietal Cluster	1218	0.041	−56, −75, −3

MNI Montreal Neurological Institute, *mTBI* mild traumatic brain injury, *PTH* post traumatic headache, *SFG* superior frontal gyrus, *MFG* middle frontal gyrus

Non-PTH had decreased GMV compared to controls in one cluster, which covered the bilateral thalamus and extended to the left and right parahippocampal gyrus ($p = 0.047$; Table 3, Fig. 1c). The decrease over time was also noted in PTH, but it was not significantly different from controls as PTH started with lower thalamus volume than non-PTH (Please refer to supplementary Figure 2 in the online resource).

Figure 1b and c show GMV change over time in each mTBI group compared to controls. PTH show a consistently negative slope in all regions. Thalamic GMV was consistent in controls over time ($p = 0.27$) but is initially higher in the non-PTH group and decreases significantly over time ($p < 0.0001$). PTH and controls start with comparable GMV in the left temporal-parietal and SFG clusters, while controls increase over time, PTH patients decrease between visits 1 and 3 ($p < 0.0001$) (plots not shown). In the left temporal-opercular cluster, right anterior-parietal, and MFG/SFG clusters, PTH initially had larger volumes which decrease over time compared to controls (who have an increase in volume). Though differences between PTH patients and controls were not significant except for the right anterior-parietal cluster at visit 3 (Please refer to supplementary Table 4 in the online resource), the change within groups over time was significant. mTBI patients regardless of headache status did not show increased GMV compared to healthy controls.

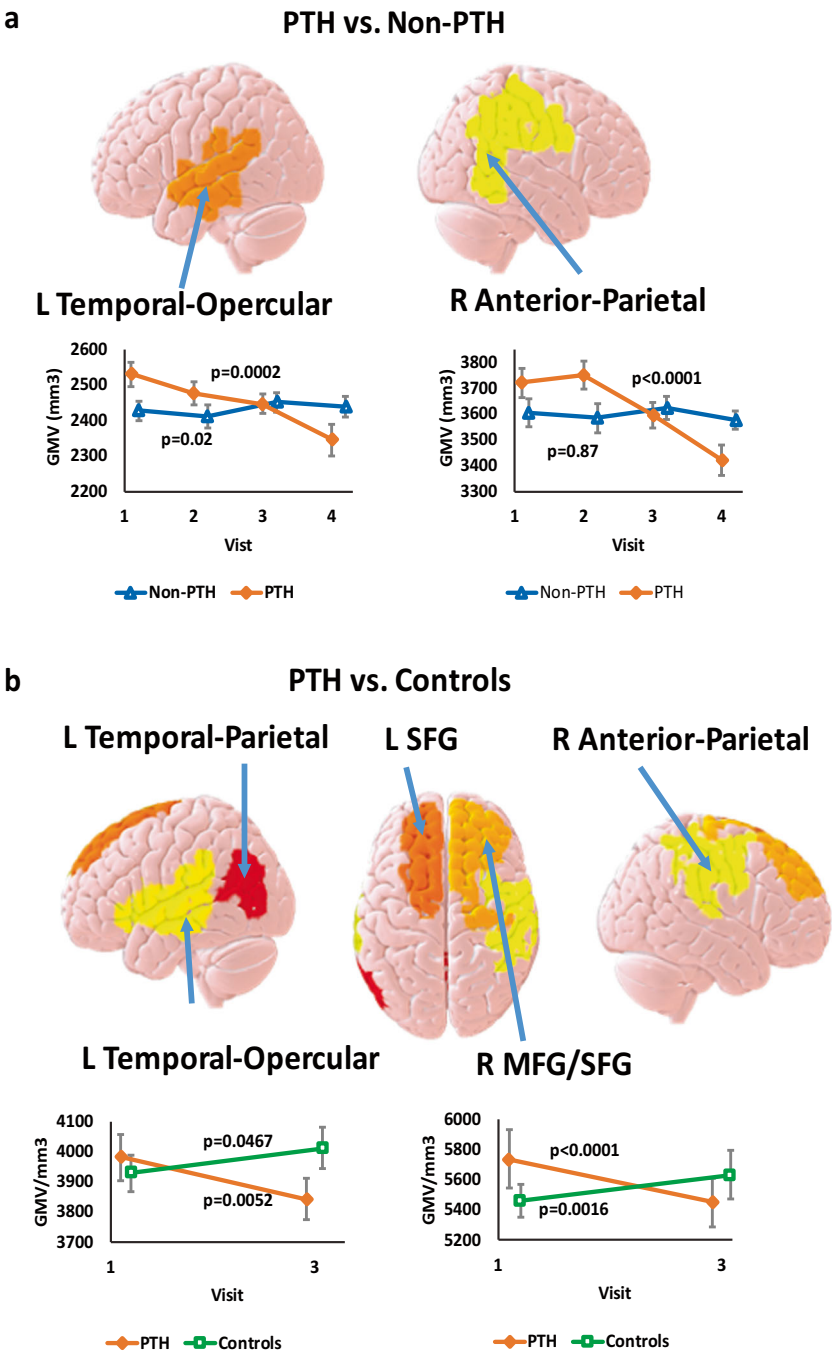
Discussion

We found that mTBI patients with evidence of PTH approximately 18 months post-injury had decreased GMV in two main regions of the brain compared to mTBI patients who do not develop headache. These were defined as the left temporal-opercular cluster and the right anterior-parietal cluster, and in both there was a steady GMV decline over time in PTH, but relatively stable GMV in the non-PTH group. Differences between the two groups were apparent at visit 4 in the left temporal-opercular cluster and at visit two in the right anterior-parietal cluster.

Compared to controls, both mTBI groups showed a general decrease in GMV over time. Controls were only scanned at two time points, so comparisons to patients could only be made between visits 1 and 3. Therefore the observed decrease in PTH patients in the R MFG/SFG may be attributed to patients having initially higher GMV at visit 1 which declined to comparable levels to controls at visit 3. Conversely in the left temporal-opercular cluster, PTH patients and controls have comparable volumes at visit 1 and diverge by visit 3. The anterior-parietal cluster, which is decreased in PTH compared to both non-PTH and controls, extends into the somatosensory cortex, which is often implicated in headache disorders (DaSilva et al. 2007).

Non-PTH patients compared to controls had decreased thalamic GMV over time. PTH patients showed a similar, non-significant trend. Thalamic GMV loss post-TBI up to one year after injury has been reported, and this trajectory differs from healthy controls (Eierud et al. 2014; Lucas et al. 2014), consistent with our findings in non-PTH patients.

Research shows that persistent PTH patients compared to healthy controls had decreased cortical thickness in several regions including bilateral SFG, caudal MFG, and precentral gyrus, right supramarginal, superior and inferior parietal and precuneus regions (Chong et al. 2018). Persistent PTH was also associated with decreased cortical thickness compared to migraine patients and healthy controls in the right orbitofrontal cortex, right supramarginal gyrus and left superior frontal lobe (Schwedt et al. 2017). These are consistent with our finding where PTH had decreased GMV over an extensive area, including MFG and bilateral SFG, compared to controls and in the right anterior-parietal cluster, including supramarginal gyrus, compared to controls and non-PTH.


a
PTH vs. Non-PTH
L Temporal-Opercular
R Anterior-Parietal
GMV (mm3)
2600
2500
2400
2300
2200
p=0.0002
p=0.02
1
2
3
4
Vist
Non-PTH
PTH
3800
3700
3600
3500
3400
3300
p<0.0001
p=0.87
Visit
b
PTH vs. Controls
L Temporal-Parietal
L SFG
R Anterior-Parietal
L Temporal-Opercular
R MFG/SFG
GMV/mm3
4100
4000
3900
3800
3700
p=0.0467
p=0.0052
Visit
PTH
Controls
6000
5800
5600
5400
5200
p<0.0001
p=0.0016
c
Non-PTH vs. Controls
L Thalamus
GM/mm3
1850
1800
1750
1700
1650
1600
p<0.0001
p=0.273

◀ **Fig. 1** Decreased GMV in mTBI patients over time. Results based on SwE analyses. **a** decreased GMV over time in mTBI patients with PTH compared to non-PTH in the left temporal-parietal and right anterior parietal regions. **b** decreased GMV over time in mTBI patients with PTH compared to healthy controls in the left temporal-opercular, temporal-parietal cluster, superior frontal gyrus (SFG) and right middle/superior frontal gyrus (MFG/SFG) and anterior parietal. Plots are shown for the Temporal-Opercular and MFG/SFG clusters. **c** decreased GMV over time in mTBI patients with non-PTH compared to healthy controls in the bilateral thalamus. A cluster forming threshold of $p < 0.005$ was applied and FWE (estimated from the wild bootstrap distribution) was set to 0.05. In all plots PTH patients are represented by an orange line with filled diamond markers. Non-PTH are represented by a blue line with triangle markers and controls are represented by a green line with square markers. Error bars in plots represent the standard error and are centered at the mean for each group at each time point. *P* values represent the interaction term for group*time from a linear mixed model. Brain images were made with SurfIce

Furthermore the decreased GMV in our PTH patients in the parietal region is consistent with the cortical thickness results found in those with persistent PTH (Chong et al. 2018). Results from the current study and others suggest that GMV changes in some brain areas might be specific to PTH and not migraine or headache disorders more generally, but further work is required to clarify this.

Though PTH is one of the most common and debilitating effects of mTBI, there is still much we do not understand about how PTH arises in patients. This is due in part to the fact that mTBI are quite heterogeneous in nature, and the progression of injury as well as subsequent sequelae depends on several biological and sociodemographic factors (Xiong et al. 2013). Because of this, the current animal models for mTBI and PTH struggle to fully explain the structural brain changes we observe, as there is not a direct translation to human populations (Bree and Levy 2018; Xiong et al. 2013). This is due in part to the manner in which mTBI is initiated in these models and the methods available to ascertain PTH in animals (Bree and Levy 2018; Xiong et al. 2013). While these difficulties exist there is general agreement that GMV in several areas is reduced post injury and researchers think this may indicate particular vulnerabilities in these areas (Bendlin et al. 2008; Zhou et al. 2013). Of note reductions have been in observed in the thalamus, right precentral and postcentral gyri, the supplementary motor area and the right precuneus (Bendlin et al. 2008; Zhou et al. 2013).

Table 3 Grey matter volumes extracted from significant clusters in mTBI patients by posttraumatic headache status

Time	Non-PTH	PTH	Difference	*p* value*
Left Temporal-Opercular Cluster				
Visit 1	2426.7 ± 128.6	2529.9 ± 143.3	−103.2	**0.022**
Visit 2	2411.1 ± 156.3	2476.1 ± 139.2	−64.9	0.18
Visit 3	2451.1 ± 146.0	2447.0 ± 112.0	4.1	0.92
Visit 4	2439.9 ± 145.0	2344.8 ± 164.9	95.1	0.069
Right Anterior-Parietal Cluster				
Visit 1	3605.6 ± 255.1	3721.9 ± 234.9	−116.2	0.15
Visit 2	3584.9 ± 258.8	3752.4 ± 230.4	−167.5	**0.039**
Visit 3	3623.9 ± 253.0	3594.8 ± 204.7	29.1	0.69
Visit 4	3575.3 ± 180.8	3419.7 ± 221.0	155.7	**0.022**

*p values obtained from post-hoc T-test, *p* values <0.05 are in bold. Grey matter volumes (mm^3) based on cluster result from whole brain time by group analysis from SwE. Values are Mean ± S.D. mTBI mild traumatic brain injury, PTH post traumatic headache

In human research these reductions in GMV are evident on MRI after injury, but their presence does not convey the full picture. Below the resolution that MRI can detect there are a series of complex changes which occur at the neuronal level, including disruption to ionic balance, neurotransmitters and electrical signals (Mayer et al. 2013). Furthermore these neuronal changes are accompanied by the activation of the innate immune system which some hypothesize is the start of many of the pain conditions such as PTH which plague patients post TBI (Mayer et al. 2013).

Though there are few studies reporting changes in GMV in PTH, there are many on migraine and other headache disorders. While PTH is a secondary headache disorder and migraine is a primary headache disorder, the phenotypic similarities between PTH and migraine may provide some insight into the changes we observed (Schwedt et al. 2017). A recent meta-analysis in migraine reported decreased GMV in the bilateral inferior frontal gyrus (IFG), left MFG, cingulate gyrus and right precentral gyrus (Jia and Yu 2017). Interestingly the changes we saw across extensive clusters in our PTH patients that covered portions of the somatosensory cortex are quite similar to those that observed in migraine patients (Sprenger and Borsook 2012). Furthermore these regions such as the prefrontal cortex are important in maintaining and updating internal representations of pain (Obermann et al. 2009; Schwedt et al. 2017) and the DLPFC and ACC have shown to have decreased GMV with the development of chronic pain (Schwedt et al. 2017).

In migraine the structural reorganization across these pain centers of the brain is attributed to the repeated attacks that are common to the headache disorder (Sprenger and Borsook 2012). This has been extended to PTH where this reorganization is also thought to be present and suggests that the continued GMV changes we see goes beyond the initial injury and is related to the ongoing presence of headaches (Schwedt et al. 2017). Given that all of these observed changes occur post injury the issue of the origin of PTH is still unclear. Nevertheless current research suggests that there may exist, shared origins between PTH and migraine, involving activation and sensitization of the trigemoinovascular pathway (Bree and Levy 2018).

A strength of our study compared to recent research is the longitudinal follow-up of our patients. Not only do we report

decreased GMV over time when compared to healthy controls and non-PTH patients, but our results show that these differences are present in PTH patients as early as one week post-TBI. Though, as stated previously, the pathology of PTH is still unclear research suggests it may involve peripheral and central mechanisms (Defrin 2014). In mice following a mild closed injury, enhanced peripheral cranial nociception is observed which may be associated with the initiation of PTH (Benromano et al. 2015). The initial increased GMV that we observed, particularly in regions of the somatosensory cortex in PTH patents, may represent damage to the pain processing areas or pathways, increasing the vulnerability to the development of PTH. Conversely, decreased GMV over time is consistent with an effect of ongoing pain experienced by patients in our sample. It is of course important to note that what we draw from this study utilizes GMV, and like all biological markers there are inherent limitations in interpreting these findings. Many studies in PTH have used cortical thickness as the preferred measurement, as it is thought be more sensitive to small changes over time (Schwedt et al. 2017). Though VBM as a means to assess GMV has traditionally been thought to be more adept at detecting large changes over a long period of time, the development of the CAT12 toolbox has allowed for the implementation of VBM methods to detect smaller changes over short time spans (Gaser and Kurth 2018). A further criticism often leveled at VBM methods is the uncertainty around the underlying cause of biological changes we observe as they can be due to changes at the cellular level or in tissue water content (Pomares et al. 2017). However studies which utilize both cortical thickness and GMV show that these measures are not only complementary but are also correlated with each other (Gennatas et al. 2017; Schwedt et al. 2017).

While our study provides some important insights into PTH, there were some limitations. The sample consisted of 19 PTH patients, some of whom were not seen at all four time points. However, by comparing PTH and non-PTH mTBI patients as well as healthy controls we were able to assess both the impact of mTBI and PTH on GMV. The use of the SwE toolbox allowed us to account for the unbalanced nature of the sample across visits and perform non-parametric inference. We further examined the distribution in age and sex in those with missing data as well as the difference between those included and excluded at each time point and found that there was no significant difference (Supplemental Tables 2 and 3). Furthermore, the original study was not developed to study PTH, so several methodological considerations that would have improved the conclusions drawn were not implemented. By assessing headache at 18 months via a patient-completed questionnaire there was possible misclassification of persons by headache group. We were also unable to capture patients as they transitioned from acute to no headache or to chronic headache. While this co-mingling of headache status likely biases the result towards a null finding as patients with resolved headache likely recovered GMV, it is an important nuance in the interpretation of the results. By including patients with both acute and chronic PTH in the study, we were able to capture those patients who had greater morbidity due to PTH and examine the effects on their GMV. While this may make it difficult to compare our results to other PTH studies, it highlights the fact that many mTBI patients experience PTH longer than 3 months post-injury. Future studies will therefore be aimed at recruiting larger numbers of mTBI patients and recording PTH status at multiple time points (as well as ascertain pre-existing headache status), including at one week and 3 months, as a means to capture progression of PTH and its relationship with GMV (Lucas et al. 2012; Stacey et al. 2016).

The original study did not assess participant headache status prior to injury. If there were patients with pre-existing migraine or any other headache disorder prior to injury this could bias the results. Since the questionnaire to ascertain headache status was administered at the final visit, there is a possible recall and selection bias as only those seen at that visit would have received the questionnaire and those who had PTH at that visit are likely to have differential recall of headache over the study period.

The literature on the impact of mTBI and PTH on brain structure is growing, and our findings are consistent with cortical thickness studies in persistent PTH. By following patients for 18 months, we were able to capture GMV differences at the first visit post-injury and changes over time. We found brain regions in PTH with decreased GMV compared to both healthy controls and non-PTH patients. Some of these differences were evident as early as one week post-injury, suggesting that early brain changes following mTBI might predict risk for PTH.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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