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Serum C-reactive protein levels predict regional brain responses to noxious cold stimulation of the hand in chronic whiplash associated disorders

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HIGHLIGHTS

- Patients with chronic whiplash show elevated levels of serum TNF-α and CRP.
- Levels of CRP correlated with cold pain activation levels in various brain regions.
- Levels of TNF-α were not related to noxious pressure or cold activation levels.

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ABSTRACT

Background and aims: Whiplash Associated Disorders (WAD) are a costly health burden. The condition is characterised by sensory disturbances such as widespread hyperalgesia likely indicative of central hyperexcitability. Recently elevated levels of pro-inflammatory biomarkers have also found in acute and chronic WAD. The aim of this cross-sectional study was to investigate the relationship between inflammatory biomarkers and pain processing in people with persistent whiplash associated disorders (WAD).

Methods: Twenty one participants with chronic whiplash (>3 months) were recruited. Venous blood samples were collected and assays performed for C-reactive protein (CRP) and TNF-α. Blood oxygen level-dependent (BOLD) contrast images of the brain were acquired with a Siemens 1.5T MRI scanner during repeated 24 s stimulus blocks of innocuous or painful stimuli (thumbnail pressure and cold stimulation of dorsum of hand) separated by 36 s inter-stimulus intervals. Stimulus intensities used during scanning were at the level of participants' thresholds for moderate pain. Parameter estimates representing BOLD signal increases during painful events from each participant were tested for associations with inflammatory biomarkers.

Results: Clinically relevant levels of CRP and TNF-α were found in 33% and 38% of participants. Levels of CRP showed a positive correlation with levels of cold pain activation in brain regions including the anterior insula, posterior parietal cortex, caudate and thalamus ($p_{corrected} < 0.05$). Levels of TNF-α were not related to activation levels during either noxious pressure or cold. Pressure pain activations also did not show a relationship with CRP levels.

Conclusions: Shared variance between inflammation and increased levels of regional pain-related activation in people with persistent whiplash symptoms is apparent for cold, but not pressure stimuli.

Implications: The results highlight cold pain processing as an important aspect of whiplash chronicity, although the implications of this modality-specific effect are not readily apparent.

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1. Introduction

Poor health outcomes following whiplash injury subsequent to a motor vehicle crash (MVC) are common, with up to 50% of those injured having persistent pain, disability and associated psychological distress [1,2]. The economic burden from health care costs and productivity loss as a result of poor recovery from the injury are substantial [3]. Current treatment approaches have only demonstrated modest effects [4] and as such better understanding of processes underlying persistent whiplash pain is required to guide the development of new approaches to management.

In the majority of patients, tissue damage in the form of a specific peripheral injury to the neck cannot be identified [5]. However data from cadaveric, experimental and clinical studies provides evidence supporting the likely presence of tissue damage, particularly involving the zygaophyseal joint [5,6] and that in some cases the tissue damage persists [5]. It would then seem logical that biomarkers such as pro-inflammatory biomarkers would be up-regulated in response to the tissue injury and damage. Few studies have investigated these processes in WAD. Kivioja et al. [7] showed elevated numbers of cytokine releasing immune cells at 3 days post whiplash injury which was not evident at 14 days, suggesting an initial but resolving inflammatory response. This study was small ($n = 11$) and likely lacked sufficient power to detect later differences. A recent longitudinal study conducted in our laboratory showed elevated serum levels of C-reactive protein (CRP) at the baseline acute injury stage (<3 weeks) that persisted in participants who did not recover but developed moderate pain and disability by 3 months [8]. Additionally CRP and IL-1β levels showed a moderate correlation with cold and mechanical hyperalgesia but no relationship with psychological factors of posttraumatic stress symptoms and pain catastrophising [8].

There is now strong evidence showing that sensory disturbances such as widespread hyperalgesia are present in individuals with chronic whiplash associated disorders (WAD) [9,10]. Specifically, cold hyperalgesia is predictive of poor recovery following the injury [11]. It is generally considered that these sensory phenomena represent augmented central nociceptive processing [9,10]. Our previous findings of relationships between inflammatory biomarkers and hyperalgesia suggest that persistent low grade systemic inflammation may contribute to the presence of sensory hypersensitivity in WAD. There is evidence that pro-inflammatory cytokines such as IL-1 β and TNF-α have effects on CNS processing [12]. Elevated levels of CRP are associated with higher pain sensitivity in healthy people [13] but the effects of CRP on the CNS are not well studied. The relationship between inflammatory processes and central nociceptive processing in WAD has not been investigated.

Chronic WAD, similar to other musculoskeletal pain conditions, is also characterised by psychological factors including pain catastrophising, posttraumatic stress symptoms and fear of movement amongst others that play a role in the clinical presentation and outcome following whiplash injury [14]. Our previous study demonstrated little relationship between inflammatory biomarkers and psychological factors [8]. This was surprising considering that studies of other musculoskeletal conditions have shown such relationships, including associations between factors such pain catastrophising, depression, PTSD and various inflammatory biomarkers [15,16].

The aims of this study were to investigate in participants with chronic WAD: 1) relationships between inflammatory biomarkers and central pain processing using functional MRI and 2) relationships between inflammatory biomarkers, sensory measures and psychological factors.

2. Materials and methods

2.1. Participants

Twenty-one volunteers reporting persistent neck pain of at least 3 months as a result of a motor vehicle crash (MVC) participated in the cross-sectional study. Participants were recruited via primary care practices (medical and physiotherapy) and through radio and print media advertisements. They were eligible if they fulfilled the criteria of WAD II as defined by the Quebec Task Force (neck pain, decreased range of motion and point tenderness) [17]. Subjects were excluded if they experienced concussion, loss of consciousness or head injury as a result of the accident or were diagnosed with WAD III (neurological deficit) or WAD IV (fracture or dislocation) and if they reported a previous history of whiplash or neck pain. Additional exclusion criteria included a previous diagnosis of a neurological disorder (e.g. multiple sclerosis), inflammatory condition (e.g. rheumatoid arthritis), metabolic disorders (e.g. diabetes), recent illness (e.g. cold or flu), pregnancy or claustrophobia as well as any contra-indications to MRI as these factors may influence the measures to be collected. Twenty-one healthy asymptomatic age and gender matched controls were included for comparison of inflammatory biomarker data. The asymptomatic control groups was recruited from the general community from print media advertisement and were included provided they had never experienced any prior pain or trauma to the cervical spine, head or upper quadrant that required treatment.

Ethics approval for this study was granted by the Medical Research Ethics Committee of The University of Queensland, Australia. All participants provided written informed consent.

2.2. Questionnaires

2.2.1. Pain and disability

Participants were asked to rate their level of pain over the last 24 h on a 10 cm visual analogue (VAS) scale where 0 = no pain and 10 = worst pain imaginable. Pain related disability was measured with the Neck Disability Index (NDI) [18] a valid, reliable and responsive measure commonly used in studies of WAD.

2.2.2. Pain catastrophising

Pain catastrophising was measured using the Pain Catastrophising Scale (PCS) [19], a validated tool which measures catastrophic thinking related to pain across three domains: rumination, magnification and helplessness.

2.2.3. Fear of movement and re-injury

Fear of movement and (re)injury was measured using the Tampa Scale of Kinesiophobia (TSK), a 17-item measure [20].

2.2.4. Post-traumatic stress symptoms

Post-traumatic stress symptoms were measured using the Post-traumatic Stress Diagnostic Scale (PDS) [21], a reliable self-report measure with questions relating to the frequency of distressing and intrusive thoughts, post-traumatic avoidance and hyper-arousal experienced in the past 30 days. The total symptom severity score was used in all analyses.

2.3. Pain threshold measures

Pressure Pain Thresholds (PPTs) were measured over the C2 spinous process and bilaterally over the muscle bellies of Tibialis Anterior using a pressure algometer (Somedic, AB, Sweden). A probe size of 1 cm^2 and application rate of 40 kPa/s was used. Participants were required to press a button when the pressure

sensation was first perceived as painful. The test was performed in triplicate for each site, with the mean values used for analysis.

Cold and heat pain were measured over the C5 spinous process using a PATHWAY thermal sensor (PATHWAY, Medoc, Israel). The thermode was preset to 32 °C with the rate of temperature change being 1 °C/s. Participants were required to press a trigger when the thermal sensation (cold or heat) was first perceived as painful. Both heat and cold pain threshold tests were performed in triplicate, with the mean values used for analysis.

2.4. Inflammatory biomarkers

Venous blood samples were collected by a qualified person. Blood samples were taken to an off-site facility to be processed, with samples allowed to clot for 30 min and then centrifuged for 15 min (1000 g) before aliquoting and storage at −80 °C. ELISA assays for TNF-α and CRP were performed utilising commercially available kits (R&D System, Minneapolis, MN, USA: Quantikine® HS ELISA Human TNF-α–HSTA00D, Quantikine® ELISA Human C-Reactive Protein/CRP–DCRP00), with all samples assayed in duplicate according to the manufacturer's protocols.

2.5. Functional-MRI (fMRI) measures and analysis

2.5.1. Provocation of moderate-intensity experimental pain

Pain sensitivity was evaluated by subjective scaling of multiple pain sensations during a baseline assessment to determine stimulus intensities sufficient to elicit moderate pain (5 out of 10 on the VAS). This was performed separately for pressure pain and cold pain. These graded pain stimuli were used to ensure similar pain ratings were produced in all participants during the functional scans. In both situations the stimulus was applied to the fixated left thumbnail, with previous studies showing that these 'neutral' regions, such as the thumb, accurately reflect an individual's overall pain sensitivity [22].

Pressure was applied using a hydraulic pressure device in a predictable ascending sequence. Starting at 0.75 g/cm^2, pressure was increased in 0.25 kg/cm^2 increments until the participant reported 'moderate pain' or to a maximum of 10 kg/cm^2, with a 15 s inter-stimulus interval included after each incremental increase to prevent sensitisation. When the participant's perceived pain reached 5/10 or greater on the VAS, the stimulus was reduced by 1 kg/cm^2 and increased again by increments of 0.25 kg/cm^2 to confirm the perceived rating of 5/10. This process was repeated three times and an average of the three trials will be used to determine the stimulus for the MRI testing.

Cold stimuli were delivered using the PATHWAY thermal sensory (PATHWAY, Medoc, Israel). Temperature was lowered at a rate of 1 °C/s starting at a baseline of 32 °C until the participant reported 'moderate pain' (5/10 on the VAS) by pressing a trigger. This process was repeated three times, with the average temperature then used to determine the stimulus for the MRI testing. A lower limit of 5 °C was set if the participant failed to reach a target level of VAS 5/10 pain, with the subjective rating of pain recorded for the 5 °C stimuli.

2.5.2. Experimental protocol

MRI and fMRI scans were performed on a 1.5T MR scanner using a conventional sequence. A T1-weighted anatomical scan (192 coronal slices, 1.2 mm thickness, 1.2 × 1.2 mm^2 in-plane resolution, TE = 3.91 ms, TR = 1700 ms, FA = 15°) was followed by functional scanning during which either pressure pain and cold pain was provoked. Three functional scans (blood oxygen level-dependent (BOLD) contrast) of 387.5 s durations were acquired for each stimulus modality (42 axial slices, 3.2 mm thickness, 3.2 × 3.2 mm^2 in-plane resolution, TE = 40 ms, TR = 2500 ms, FA = 80°). Participants were randomly assigned to receive pressure or cold stimuli in a randomised order.

During the functional scans, a sequence of 24 s intervals of moderate pain (cold or pressure stimuli) or innocuous pressure (sensation of probe on thumb only) were interspersed with 36 s blocks of no sensation, as shown in Fig. 1. Each 24 s interval of stimulus (moderate pain or innocuous pressure) was delivered as 4 intermittent blocks of stimuli lasting 5 s each and released for 1 s [Fig. 1]. This intermittent stimulus delivery was designed to prevent sensitisation of the local tissue and avoid any associated temporal summation of pain. While stimulus intensity was based on that found to elicit 'moderate pain' in the pre-MRI session (provocation of moderate-intensity experimental pain), between each run of the MRI testing the patient was asked to rate the pain of the stimulus, either cold or pressure, on a VAS, with the intensity of the stimulus then adjusted accordingly to correspond to a VAS of 5/10. The pain sequence was repeated three times for the pressure stimulus and three times for the cold stimulus.

2.6. Procedure

The study was conducted over two sessions. At the initial consultation informed consent was obtained, questionnaires completed and the physical examination conducted. The physical examination included the determination of pain threshold measures and the provocation of moderate-intensity experimental pain procedure. Blood samples were also drawn at the initial session prior to the physical examination, with samples processed and stored at an off-site facility prior to being analysed. Within one week of the initial consultation participants were required to attend a second session, during which MRI scans were taken.

The healthy asymptomatic controls attended once where blood samples were drawn.

2.7. Data analysis

2.7.1. Analysis of stimulus-evoked fMRI signal changes

Image analysis was performed on each subject's data to reveal significant brain activation based on changes in BOLD signal. Reprocessing of individual participants' functional brain images included realignment of all volumes in a scanning run to the image acquired hallway through the run, removal of non-brain voxels, high pass filtering and spatial smoothing with a Gaussian kernel of 6 mm FWHM. Transformations of functional data to a standard template were calculated in a two-step process involving participants' high-resolution T1 images as implemented in FMRIB's Linear Image Registration Tool (FLIRT) [23–25].

Data was statistically analysed with FEAT (fMRI Expert Analysis Tool) Version 5.4 [26,27].

Regressors representing the timing of innocuous and painful stimulus blocks were convolved with a hemodynamic response function and included in General Linear Models to explain variance in BOLD signal changes for each scanning run for each participant. Motion parameters were also included as regressors in the modelling of BOLD signal changes. Parameter estimates were calculated for the fit of regressors to observed BOLD signals for each voxel in the space of participants' functional brain images. Statistical parametric maps (SPM) of contrasts of parameters estimates (i.e. pain > innocuous) for each scanning run were transformed to MNI space and averaged across the three scans of like-stimulus modality (cold or pressure) for each participant. Averaged SPM were used to perform group analyses using mixed effects (FMRIB's Local Analysis of Mixed Effects (FLAME) [28]. Regions of activation were considered statistically significant when the constituent voxels had values exceeding $z = 2.3$, and a cluster corrected threshold of $p < 0.05$ to

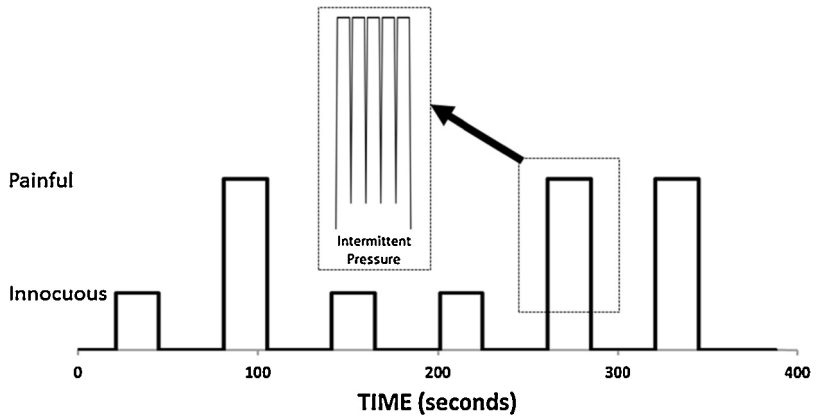

Fig. 1. Participants were stimulated with innocuous and noxious levels of either cold or pressure during the acquisition of functional brain images. The order of the two stimulus intensities was varied across the course of three scanning runs for each modality. Stimuli were applied during 24 s blocks interspersed with 36 s no-stimulus periods. Cold stimuli were applied with a Peltier thermode placed on the dorsum of the hand. Pressure was applied to the thumbnail with a hydraulic device, and was released intermittently (inset) to avoid temporal summation of pain that can occur during sustained pressure.

take account of the spatial smoothness of the images and the effects of multiple comparisons on inferences of significance [29].

Thresholded SPMs were rendered onto a structural image using MRIcron (Version 6.6), allowing visualisation and identification of voxel clusters. Peak voxels were determined for each cluster, with voxel co-ordinates and corresponding *z*-scores recorded. To determine the neuroanatomy of each cluster, peak voxel co-ordinates were analysed using the Harvard-Oxford atlases [30].

2.7.2. Correlation analyses—fMRI and inflammatory biomarkers

Mixed effects analyses were performed to test for relationships between participants' levels of CRP and TNF-α (inflammatory biomarkers) and regional pain activation. Demeaned values of CRP and TNF-α were incorporated as explanatory variables in the modelling of group variance in pain activation, and contrasts were generated to identify regions with either a positive or negative relationship to the inflammatory biomarkers. The *z* statistic images from the correlation analyses were thresholded to include voxels with *z*-scores greater than 2.3 and a cluster corrected threshold of $p < 0.05$ [29]. Thresholded SPMs were then placed onto a structural image using MRIcron (Version 6.6), allowing visualisation and identification of voxel clusters. Peak voxels were determined for each cluster, with voxel co-ordinates and corresponding *z*-scores recorded. To determine the neuroanatomy of each cluster, peak voxel co-ordinates were analysed using the Harvard–Oxford atlases.

2.7.3. Analysis of clinical and psychological data

Data was analysed using SPPS (SPSS 21.0 for Windows, Chicago, Illinois, USA) software package. Inter-relationships between neck pain and disability, psychological measures, pain threshold measures and inflammatory biomarkers were evaluated using Pearson correlations. Independent sample *t*-tests were used to compare the inflammatory biomarker data between patients and controls. For all analyses the significance level was set at $p < 0.05$.

3. Results

3.1. Participants

Twenty-one participants with WAD were included in the final analysis. Over 70% of participants were female (71.4%, $n = 15$), with an average age of 44.4 years (±11.1 years). On average participants reported an NDI of 34.7% (±13.9%), with an average resting pain intensity of VAS 3.9 (±2.4). The control sample comprised 15 (71.4%) females with the average being 44 (±11) years.

3.2. Inflammatory biomarkers

3.2.1. TNF-α

Based on the analysis premise used by Wang et al. [31], in which TNF-α serum concentrations greater than 2.0 pg/mL are considered 'positive', 38.1% ($n = 8$) of WAD participants in this study were TNF-α positive. The geometric mean of TNF-α was 2.3 pg/mL, with a standard deviation of 1.7 pg/mL. None of the control participants were TNF-α positive with a geometric mean of 1.07 (0.56) pg/mL. There was a significant difference between the groups on this marker ($p = 0.046$).

3.2.2. C-reactive protein (CRP)

Clinically important levels of hs-CRP (>3.0 mg/L) were found in 33.3% ($n = 7$) of WAD individuals. The geometric mean of hs-CRP was 2.1 mg/L, with a standard deviation of 1.7 mg/L. Comparison of these groups revealed no significant difference in age, gender distribution, pain intensity or disability levels but the group with hs-CRP levels >3.0 mg/L demonstrated lower pressure pain thresholds at Tibialis Anterior (mean (SD): 283 (75) vs 384 (73) kPa; $p = 0.05$); the cervical spine (144 (50) vs 316 (63) kPa; $p = 0.047$) and lower cold pain thresholds (21.8 (7) vs 14.2 (7) °C; $p = 0.05$) compared to the groups with hs-CRP levels <3.0 mg/L.

Two (9.5%) of the control participants had hs-CRP levels >3.0 mg/L with a geometric mean (SD) of 1.4 (0.25) mg/L which was significantly different from the WAD group ($p = 0.04$).

3.3. Main effect of pressure pain

Provocation of pressure pain in patients with chronic WAD resulted in activation of brain regions commonly observed in pain imaging experiments of healthy individuals [32,33]. The activations resulting from the noxious pressure stimuli are listed in Supplementary Table 1.

3.4. Main effect of cold pain

Provocation of cold pain in the patients activated typical pain processing regions, such as insula, thalamus and dorsolateral prefrontal cortex. Activation was also found in posterior cingulate gyrus, Broca's area, posterior parietal cortex, secondary motor

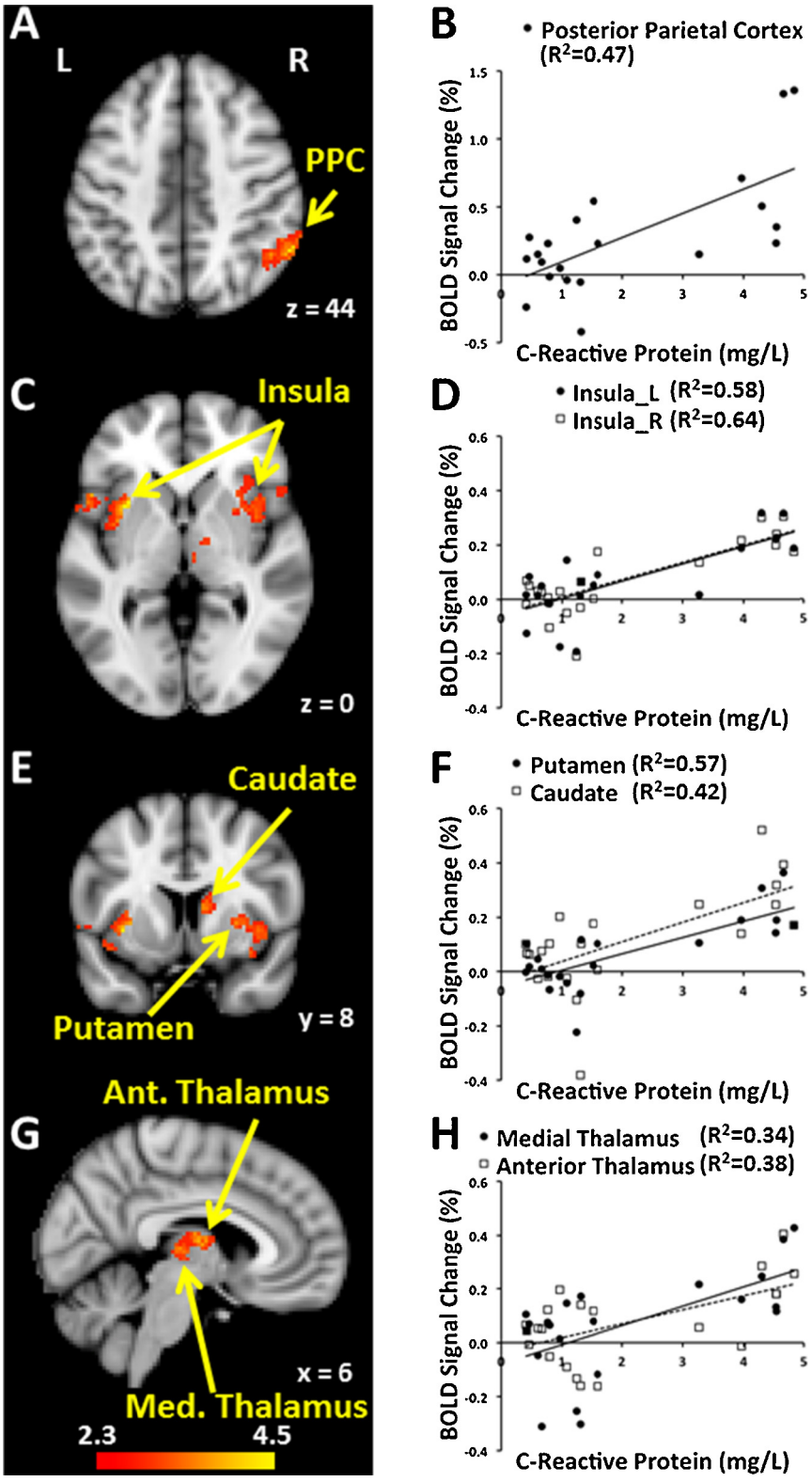

Fig. 2. Regional pain activation was tested for associations with levels of CRP in the whiplash patients. (A) The posterior parietal cortex (PPC) in the right hemisphere had pain activation levels that correlated with CRP. (B) Whiplash patients with the highest levels of CRP had increased levels of pain activation in the posterior parietal cortex. (C) CRP-related variance in pain activation was also seen in the mid insula in both hemispheres, (D) and demonstrated a positive relationship between the parameters. (E) Both the right caudate and putamen had pain activations related to CRP levels. F. Both the striatal regions had positive correlations with CRP. (G) Two loci of CRP-related variance in pain actviations were seen in the right thalamus–medial and anterior. (H) Pain activations in both thalamic regions were positively related to CRP.

Table 1
Cold pain activations correlated with levels of CRP.

Region	BA	Side				*Z* stat
			x	*y*	*z*	
Posterior parietal cortex	40	R	58	−52	44	4.30
Insula	13	R	40	6	−2	3.34
	13	L	−32	10	0	4.73
Thalamus						
Anterior		R	6	−10	10	3.78
Medial		R	6	−20	6	3.48
Putamen		R	28	8	2	3.47
Caudate		R	10	8	10	3.44

cortex, frontopolar area, caudate, and cerebellum (Supplementary Table 2).

3.5. Correlation analyses (WAD group only)

Whole brain correlation analysis revealed that during provoked cold pain, clusters in bilateral anterior insula, posterior parietal cortex, caudate and thalamus (Fig. 2) correlated positively with the concentration of serum inflammatory biomarker CRP (Table 1). No region correlated positively with TNF-alpha concentrations, and no regions correlated negatively with either inflammatory biomarker. For experimental pain pressure, no significant correlation with either inflammatory biomarker was observed.

3.6. Psychological measures

The median pain catastrophising score was 13 (IQR 6-22) with 5/21 (23.8%) of participants exceeding the threshold score of >24 [34]. The median (IQR) of the TSK scores was 36.5 (24–48). Thirteen (62%) participants recorded scores above 37, the value which differentiates between low and high scores [35]. On the PDS, 14 participants (66%) reported mild post-traumatic stress symptoms (total symptom severity score between 1 and 10), with 6 (28.5%) reporting moderate levels (total symptom severity score between 11 and 20 and one (1.3%) falling within the moderate to severe category (total symptom severity score between 21 and 35) [36].

3.7. Pain thresholds

For PPTs performed at the cervical spine a mean pressure of 192.4 kPa was recorded, with a standard deviation of 92.8 kPa. Results from bilateral Tibialis Anterior PPTs were averaged for each participant and the sample mean was found to be 350.6 kPa (±172.8 kPa).

Cold pain threshold at the cervical spine was, on average, 18.1 °C (±7.6 °C), while the average temperature at heat pain threshold was 42.3 °C (±3.8 °C).

Relationships between inflammatory biomarkers, sensory measures and psychological factors

Significant moderate correlations were found between TNF-α serum levels and pain catastrophising scores, as measured by the PCS ($r = 0.47$, $p = 0.032$). No other significant correlations were found between serum biomarkers and symptoms of PTSD (PDS total symptom score), pressure or thermal pain thresholds or fear of movement (TSK). Three correlations came close to significance, including relationships between TNF-α serum levels and fear of movement ($r = 0.45$, $p = 0.075$), and symptoms of PTSD ($r = 0.391$, $p = 0.080$) as well as the relationship between CRP serum levels and PPT at the cervical spine ($r = 0.371$, $p = 0.098$).

4. Discussion

The pathophysiology of chronic WAD is not clear but there is strong evidence to indicate that altered central nociceptive processing [9,10] and psychological factors including pain catastrophising and PTSD symptoms [2,37] are features of the condition. More recently it has been shown that both local and systemic inflammatory processes may also be involved in the presentation of chronic WAD [8,38]. The present study sought to explore relationships between these processes. The results indicate that levels of the inflammatory biomarker CRP, but not TNF, were related to the cerebral processing of provoked cold pain in individuals with chronic WAD. Neither blood parameter (TNF or CRP) was associated with pressure pain activation. Only TNF levels were associated with any psychological parameter where a moderate positive correlation between TNF and pain catastrophising was demonstrated.

Our WAD sample reported, on average, moderate levels of disability, neck pain and psychological distress, and their presentation was very similar to other reports of chronic WAD samples [6,39]. Thus we are confident that our sample is representative of chronic WAD. Our findings in regard to inflammatory biomarker levels are also consistent with our previous work with elevated levels of CRP and TNF found in a proportion of participants [8]. The cause of elevated inflammatory biomarker levels in patients with chronic WAD is not clear. It may be as a result of unresolved peripheral tissue damage with evidence pointing to the cervical zygaophyseal joint as the most likely candidate for ongoing pathology [5,6]. As with our earlier study, we excluded patients with known systemic diseases including cardiovascular disease, metabolic disease and diabetes as these factors have shown associations with inflammatory biomarker levels [40]. The elevated levels of CRP would appear to be associated with the clinical presentation of WAD, as those with clinically important levels (>3.0 mg/L) were more hyperalgesic to both pressure and cold at local and remote sites. Although there was no difference in levels of pain and disability. The mechanisms for increased CRP levels are not clear. However, there is increasing evidence that local musculoskeletal injury/disorders result in a subclinical systemic inflammatory response including release of CRP [41,42]. It is possible that ongoing local inflammation in the cervical spine may maintain elevated CRP levels to some extent. Other potential factors could be related to psychological processes such as depression which can be a feature of chronic WAD [43] as well as levels of CRP [44] and these could also potentially be involved.

Cold hyperalgesia is a common feature of both acute and chronic WAD [11,14]. There is moderate evidence indicating that when present in the acute injury stage, cold hyperalgesia is prognostic for poor functional recovery [11] and some data suggest that it may also be an indicator for non-responsiveness to physical rehabilitation in patients with chronic WAD [45]. Potential mechanisms underlying cold hyperalgesia in WAD have not been elucidated but in view of its relationship to non-recovery, gaining insight into the underlying mechanisms may provide an avenue for future interventions. We have previously shown moderate correlations between serum CRP and cold pain thresholds in both acute and chronic WAD [8]. In the present study we found a similar moderate correlation, although this did not quite reach significance likely due to a smaller sample size. Together these findings would suggest that CRP may be a contributing factor to cold hyperalgesia in WAD. There is also evidence available to indicate that elevated levels of CRP in generally healthy people are associated with higher pain sensitivity [13].

Our findings that serum CRP levels were associated with greater brain activation patterns in response to provoked cold pain further support a role of this inflammatory biomarker in the presence of cold hyperalgesia. Areas of brain activation that showed greater activation with higher CRP levels included bilateral anterior insula, posterior parietal cortex, caudate nucleus and the thalamus. The

activation of these areas in response to noxious stimuli is not surprising, as each of these areas have been identified as playing a role in the 'pain matrix'. In a recent review [32], the anterior insula and posterior parietal cortex were described as part of the second order perceptual matrix. The anterior insula is known to play an important role in affective pain processing [46], and together with the posterior parietal cortex, which directs attention towards the painful stimuli [47], may be important in cognitive, memory related stimulus evaluation [46]. The thalamus is considered part of both the discriminative and attentional networks involved in pain processes, attentional processes, vigilance and general arousal reactions to pain [33]. The caudate, as part of the basal ganglia network is involved in encoding noxious stimuli intensity in order to minimise bodily harm and has been linked to processes involved in avoidance behaviour to pain [48]. In overall terms, these brain areas are those ascribed with attention and perception related functions suggesting that patients with chronic WAD and increased serum levels of CRP experience the noxious cold stimuli as more worthy of attention with ensuing avoidance behaviour. Importantly our results suggest that CRP may have influences on the central processing of cold nociception. Interestingly we found no relationship between CRP levels and the psychological factors measured including PTSD symptoms, pain catastrophising and fear of movement suggesting that these factors are not mediators of the relationship between CRP and brain activation responses.

There is a wealth of data demonstrating lowered pain thresholds to pressure both local and remote to the cervical spine in patients with chronic WAD [9]. This widespread hypersensitivity to pressure has been interpreted to reflect augmented central nociceptive processing. Our results showed that pressure stimulation activated brain areas commonly observed in pain imaging experiments of healthy individuals [32,33]. In contrast to our findings for cold pain, we found no relationship between inflammatory biomarker levels and brain activation patterns with pressure stimulation. This would suggest that different mechanisms underlie hyperalgesic responses to pressure compared to cold stimulation in chronic WAD.

Few studies have investigated relationships between inflammatory processes and brain activation in chronic pain conditions. In a recent review, Linman et al. [49] conclude that preliminary data indicate a potential role for neuroimmune and cytokine induced changes that affect brain systems but emphasise that this is an under investigated area of research and that the majority of studies did not include patients with pain. However, one study found that administration of TNF- blocker in patients with rheumatoid arthritis led to diminished activation in the somatosensory cortex, the parietal cortex, the posterior cingulate cortex and the medial prefrontal cortex with joint pressure. To our knowledge no studies have investigated relationships between CRP and brain activation. Thus, our findings are novel and suggest a potential role of CRP influencing CNS processing and the clinical presentation of cold sensitivity in chronic WAD.

We found a significant moderate correlation between TNF-α and pain catastrophising and trends toward significant correlations between this biomarker and PTSD symptoms and fear of movement. Whilst few in number, previous studies have shown relationships between inflammatory biomarkers and pain behaviour. Experimental pain induced levels of IL-6 were significantly correlated to pain catastrophising in healthy volunteers [50]. In patients with chronic pain, focusing on the negative aspects of their pain condition led to elevated levels of IL-6, with this association being more pronounced in women indicating an increased and delayed inflammatory responses following negative emotional expression [51]. Thus preliminary studies in this area suggest a relationship between inflammatory levels and psychological factors but that the relationship between inflammation and pain variables would appear to be stronger [8].

Although the prescription of anti-inflammatory medication is advocated for musculoskeletal pain [52] and is commonly used in the management of WAD [53], there are few available trials demonstrating evidence for such medications. The results of our study would suggest that non-steroidal anti-inflammatories may be beneficial for this condition. The early presence of hyperalgesia, particularly cold hyperalgesia is a consistent adverse prognostic indicator for poor recovery [11]. Interventions that decrease levels of CRP may attenuate processes underlying hyperalgesia and potentially improve recovery following whiplash injury. Physical treatments such as exercise have shown capacity to modulate inflammatory processes [54]. To date various exercise approaches have shown only modest effects on pain and disability in patients with chronic WAD [39,45,55], but these studies have focussed on specific neck exercises rather than general whole body exercise which has demonstrated anti-inflammatory effects [56]. This could be an area for further research in WAD.

There are limitations to our study. Participants were requested to refrain from taking anti-inflammatory medications 7 days prior to testing. Whilst most reported adherence with this request, some (10%) failed to do so and this may have influenced our results. We did not collect data on obesity such as Body Mass Index which can influence levels of inflammatory biomarkers [57]. The number of subjects included in the study is small. In the study there are only patients and no controls. The results are preliminary and will need replication in another cohort to increase the impact of the results.

In summary, levels of the inflammatory biomarker CRP were related to the cerebral processing of provoked cold pain in individuals with chronic WAD. TNF-α levels were associated with the psychological parameter of pain catastrophising.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sjpain.2015.11.003.

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