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Acute low back pain: Differential somatosensory function and gene expression compared to healthy no-pain controls

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Abstract

Objectives—Low back pain is the second most frequently diagnosed pain condition in the United States' and although a majority of individuals have resolution of pain during the acute period, an estimated 40% of individuals will experience persistent pain. Given the heterogenous nature of low back pain, this study sought to describe and compare somatosensory and molecular (gene expression) profiles between individuals with acute low back pain and healthy no-pain controls

Methods—Using a previously established protocol, we comprehensively assessed somatosensory parameters among 31 no-pain control participants and 31 participants with acute low back pain. Samples of whole blood were drawn to examine mRNA expression of candidate genes involved in the transduction, maintenance, and modulation of pain.

Results—The acute low back pain group exhibited increased pain sensitivity to cold stimuli, mechanical stimuli, including mechanical temporal summation at both the painful back area and remote location suggesting a mechanism of enhanced central nervous system excitability. In addition, deep tissue specific peripheral sensitization was suggested due to significant differences in pressure pain threshold of the painful back area, but not the remote body site. Several genes that were differentially expressed were significantly associated with somatosensory alterations identified in the acute low back pain group.

Discussion—Acute low back pain participants showed selective pain sensitivity enhancement and differential gene expression profiles compared to pain-free controls. Further research to

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characterize pain-associated somatosensory changes in the context of altered mRNA expression levels may provide insight on the molecular underpinnings of maladaptive chronic pain.

Keywords

gene expression; low back pain; quantitative sensory testing; sensitization; somatosensory

INTRODUCTION

Low back pain (LBP) is a musculoskeletal symptom defined as discomfort in the region between the thoraco-lumbar and lumbo-sacral junctions and is considered to be “acute” up to the first six weeks after onset [1]. It is the second most frequently diagnosed pain condition in the United States’ and although a majority of individuals have resolution of pain during the acute period, an estimated 40% of individuals will experience persistent LBP, lasting for ≥12 weeks [2–6]. Given the heterogeneous and multidimensional nature of LBP, researchers have focused on psychosocial and environmental risk factors as a means of identifying individuals at risk for persistent LBP [7–11]. Abundant evidence demonstrates that these factors contribute to pain, however, interventions designed to reduce psychosocial and environmental risks factors have only been marginally successful in decreasing the incidence of long-term pain and disability [12–15]. Less attention has been focused on characterizing the somatosensory alterations associated with acute LBP, which may provide a deeper understanding of the biologically-relevant pain mechanisms involved in this condition as well as the transition from acute to persistent LBP.

One method of characterizing the pain experience is through quantitative sensory testing (QST) which was primarily introduced to detect and differentiate between neuropathic syndromes, but also represents a standardized and comprehensive tool for somatosensory testing. The advantages of QST are that it is non-invasive and measures different modalities of pain (mechanical, heat and cold) with reproducibly controlled protocols [16]. While there have been several studies demonstrating increased peripheral and central sensitivity in individuals with chronic LBP [17, 18] few studies have used comprehensive QST protocols to assess for somatosensory alterations during the acute phase. A study that compared only pressure pain threshold in acute and chronic LBP participants found that the chronic group had localized and generalized pressure hyperalgesia, which was not present in the acute group [19]. LeResche and colleagues [20] used pressure pain thresholds, cold pressor pain ratings, conditioned pain modulation and mechanical temporal summation in patients with acute LBP to assess whether test responses predicted clinically significant back pain four months later. Of the psychophysical tests used, lower pressure pain thresholds significantly predicted back pain at four months but the associations were no longer significant after controlling for participant age and sex. More recently, Hübsher and colleagues [21] used thermal thresholds and tolerance as well as temporal summation to evaluate differences between individuals with acute LBP, chronic LBP and healthy (no-pain) controls. They found that chronic LBP participants had significantly more sensitive cold pain threshold in the painful area of the back as well as a remote site compared with no-pain controls, however, participants with acute LBP did not show elevated pain sensitivity in response to the selected tests. Whereas these studies suggest efficient pain modulation in individuals

with acute LBP [22], to our knowledge, there have been no studies to systematically evaluate peripheral and central sensory alterations using QST during an acute episode of LBP.

Although a precise structural etiology of persistent LBP is rarely present, recent studies document functional alterations that reflect peripheral and possibly central nervous system (CNS) sensitization [23,24]. Peripheral sensitization may be triggered by inflammatory processes resulting from tissue or nerve injury and manifests as increased sensitivity to experimental pain restricted to the site of injury. In contrast, central sensitization can manifest as allodynia, hyperalgesia and/or enhanced temporal summation in non-injured regions of the body. Enhanced sensitivity to experimental pain may be a trait characteristic, possibly conferred through genetic polymorphisms that influence pain signaling [25]. However, accumulating evidence indicates that sensitivity to pain can also develop over time in response to modifications in the expression of genes that encode pain signaling molecules and their receptors, particularly genes associated with neurotrophins [26–29], inflammatory mediators [30] and catecholamines [31]. Altered levels of these pain signaling molecules and their receptors are thought to be important for the early steps of nervous system sensitization. Given that levels of gene expression associated with enhanced pain sensitivity are potentially modifiable [32], further elucidation of the differences in somatosensory function and gene expression at the onset of LBP, during the acute phase, may provide foundational knowledge to evaluate and guide the development of predictive markers and preventative interventions to reduce the incidence of persistent LBP.

Therefore, we sought to describe and compare somatosensory responses to experimental pain and gene expression data between individuals with acute LBP and healthy (no-pain) controls. Using a previously established protocol [16], we comprehensively compared QST parameters between no-pain control participants and those with acute LBP. As this study was exploratory we did not select specific parameters to compare. However, our hypothesis was that there would be significant differences in somatosensory function and gene expression profiles between the acute LBP group and the no-pain control group.

MATERIALS & METHODS

Participants

Men and women between the ages of 18–50 years of age diagnosed with an acute nonspecific LBP episode and able to read and write in English were invited to participate from primary healthcare clinics through advertisements. An acute nonspecific LBP episode was defined as pain anywhere in the region of the low back bound superiorly by the thoraco-lumbar junction and inferiorly by the lumbo-sacral junction, which had been present for >24 hours but <4 weeks duration and was preceded by at least 1 pain-free month [33]. This age range was selected to provide a more homogeneous sample in terms of general health, work status and contributing factors of persistent LBP. Recruitment took place at an urban university health system in the mid-Atlantic region after approval from the Institution Review Board. All participants provided written consent prior to study participation.

Patients were excluded for the following conditions: (a) pain at another site or associated with a painful condition (eg., degenerative disc disease, herniated lumbar disc, fibromyalgia,

neuropathy, rheumatoid arthritis, sciatica); (b) previous spinal surgery; (c) presence of neurological deficits; (d) history of comorbidities that affect sensorimotor function (eg., multiple sclerosis, spinal cord injury, diabetes); (e) pregnant or within 3-months postpartum; (f) taking opioid, antidepressants or anticonvulsant medication; and, (g) history of psychological disorders (major depression, bipolar disorder, schizophrenia) because of a possible associations with biological markers [34–36]. Eligibility for the healthy no-pain control group included men and women (a) between 18–50 years of age; (b) could read and write in English; (c) with no known medical, psychological problems or prescribed medication; (d) not pregnant or breastfeeding; and, (e) no recent history of pain at any location.

We estimated the sample size based on a previous study that compared LBP patients with healthy controls and reported the effect size of several QST parameters (with Cohen's d ranging from 0.80–1.12) [18]. Using the more conservative effect size of 0.8 to achieve a power of 80% at probability level of 0.05 the minimum sample size using a one-tailed hypothesis was estimated to be 21 per group [37]. Due to the exploratory nature of the study, it was deemed most appropriate to increase the size to 31 per group in order to ensure adequate power to detect significant differences between groups.

Procedures

After obtaining informed consent, participants were scheduled to undergo baseline data collection as soon as possible but no longer than one week from the time of consent. Data collection took place in a private research suite to complete questions about age, gender, socioeconomic status, educational attainment, lifestyle behaviors (smoking, exercise), comorbidities, and past episodes of LBP. Following completion of the questionnaires, participants underwent venipuncture for collection of blood samples and quantitative sensory testing (QST). The sequence of data collection was followed for all participants.

Quantitative sensory testing

QST was used to evaluate responses to experimental pain and uses standardized stimuli to test both nociceptive and non-nociceptive systems [38]. Quantitative sensory testing was performed in the lumbar region (at the location of pain for the acute LBP group) and on the dominant forearm (remote area). A standardized protocol of administration, including examination room conditions and instructions provided for the participant, were strictly followed. Participants were given a practice run on the non-dominant forearm in order to verify the participant's understanding of the protocol.

Mechanical pain threshold and sensitivity were measured with a standard set of von Frey hairs (Optihair$_2$-Set, Marstock Nervtest, Germany) that exert forces between 0.25 and 512 mN with a rounded tip that is 0.5 mm in diameter. The final threshold is calculated as the geometric mean of five series of ascending and descending stimuli intensities. Wind-up ratio (WUR) was determined from this series with the mean pain rating of trains divided by the mean pain rating to a single stimuli. Dynamic mechanical allodynia (ALL) was tested using a standardized brush applied five times with a single stroke; the pain rating to each stroke was recorded.

Thermal and pressure testing was performed using the Medoc Pathway System™ (Ramat Yishai, Israel). The Medoc thermode, with contact area of 7.84 cm^2, was placed in contact with the participant's skin in the area to be tested. The Medoc software guided the examiner through a series of thermal testing procedures in the following order: cold detection threshold, warm detection threshold, cold pain threshold, and heat pain threshold. The mean threshold temperature of three consecutive measurements were calculated and used for analysis. All thresholds were obtained with ramped stimuli (1°C/second) that were terminated when the participant pressed a button attached to the Medoc device. Cut-off temperatures were 0° and 50° C with a baseline temperature of 32°C. For pressure pain threshold, the examiner used an algometer (range from 50–600 kPa) attached to the Medoc Pathways system to increase the pressure at a steady rate (30 kPa/s) until the participant indicated first pain sensation by pressing the button. The pressure pain threshold (PPT) was determined by repeating the procedure at the same site until either: (1) Two values were recorded within 20 kPa of one another or (2) Three trials were administered. In either case, the mean of the two closest values were recorded as the threshold estimate. During the testing, the computer screen was positioned so that the participant was not able to watch temperature and pressure fluctuations.

Gene Expression Profiles

Whole blood was collected by venipuncture into one 5-mL EDTA vacutainer and one 10-mL cell preparation tube with sodium citrate, labeled with a unique study identification label, and transported directly to the laboratory for processing. RNA isolation was performed using the PAXgene™ total RNA isolation system (Qiagen, Valencia, CA) according to the manufacturer's protocol and was reverse transcribed using iScript cDNA synthesis kit (Invitrogen, Valencia, CA). The mRNA expression of 84 genes involved in the transduction, maintenance, and modulation of pain was determined (Neuropathic & Inflammatory RT2 Profiler PCR Array; Sabio Sciences, Valencia, CA; BioRad, Hercules, CA) using qPCR performed on the BioRad CFX96®. After an initial incubation step, 35 cycles (95°C for 15 seconds and 1 minute at 60°C) of PCR were performed. Expression levels were quantified using the $^{\Delta\Delta}$CT method which normalizes data of the genes of interest to β-actin (controls are included in array). The BioRadCFX software was used to determine optimal baseline and threshold settings of the assay C_t values.

Statistical Analysis

Normality of the data was tested using the Kolmogorov-Smirnov test. Student t-tests were used to test for group differences in demographic and QST variables that were normally distributed, whereas the Kruskal-Wallis test followed by Bonferroni-Holm adjusted Mann-Whitney *U* test for post hoc analyses were used for variables that were not normally distributed. Categorical variables were compared using χ^2 tests. Post-hoc analyses were conducted as necessary to account for multiple testing. For gene expression analyses, each LBP participant was randomly matched to healthy no-pain control participants by age within three years and gender. Five male LBP participants who could not be matched based on the criteria were matched with the remaining participants in the same group with the closest age. Missing Cq values were imputed by 35, a maximum cycle of PCR performed. For each housekeeping gene (HKG) included in the assay (*ACTB, B2M, GAPDH, HPRT1*, and

RPLP0), the *GAPDH* was the most stable and suitable HKG used for normalization of the Cq values as it exhibited the lowest variance and highest abundance. Results are based on normalization using the *GAPDH* and the three most stable HKGs (*GAPDH, ACTB*, and *B2M*). For each of the 86 non-housekeeping genes considered "genes of interest" (GOI), the ΔCq value were calculated as $\Delta Cq = Cq_{,GOI} - Cq_{,GAPDH}$. Thereafter, for each subject, the relative fold change in expression was calculated as $2^{-\Delta(\Delta Cq)}$ where i.e. $\Delta(\Delta Cq) = \Delta Cq_{,LBP} - \Delta Cq_{,Normal}$, or $\Delta Cq_{,LBPvisit5} - \Delta Cq_{,LBPvisit1}$. For each comparison, a linear model was fit for each gene with sample group as the independent variable. Empirical Bayes method was applied to obtain robust estimators [39]. Benjamini and Hochberg's method was used to control the false discovery rate (FDR) [40]. The moderated unpaired and paired t-statistics were computed for the two sample comparisons. Non-parametric tests were applied as appropriate. The differentially expressed (DE) genes were defined with FDR less than 5%.

RESULTS

Study Participant Characteristics

Demographic and clinical information of the participants is seen in Table 1. Of note, there were more African-American participants in the acute LBP group compared to the healthy no-pain control group ($p<0.01$). There were more participants in the acute LBP group earning an annual income less than $60,000 compared with the control group ($p<0.01$) and more participants were working full- or part-time in the control group compared to the acute LBP group ($p=0.01$). In addition, more participants in the control group had started college or were currently pursuing advanced education compared to the acute LBP group ($p<0.01$). Although there were no significant differences in exercise frequency, a significantly higher number of participants in the acute LBP group were current smokers and had common comorbidities such as hypertension compared to the control group ($p<0.01$; $p<0.05$ respectively). As expected, the acute LBP group had significantly more prior episodes of LBP compared to the control group ($p<0.01$) but none reported a prior episode lasting >3 months in the previous 6 months.

Subjects with acute LBP have lower pain threshold and higher pain scores compared to no-pain controls

Compared to the no-pain control group, participants in the acute LBP group had lower threshold for cold at both the remote and back area ($p<0.05$ for both), meaning that pain was elicited at a higher temperature in the acute LBP group. The pressure pain threshold was significantly lower only in the back area of the acute LBP group compared to the control group ($p<0.01$) meaning that less pressure stimulus was required to elicit pain. Mechanical sensitivity and the wind-up ratio were significantly higher in both the remote and back area in the acute LBP group compared to the control group ($p<0.01$ for both) meaning that the acute LBP group reported higher pain scores to a set mechanical stimulus. Finally, pain scores were significantly elevated in response to a standard brush applied to the painful back region in the acute LBP compared to controls ($p<0.01$) suggesting mechanical allodynia (Table 2).

Subjects with acute LBP have differential gene expression compared to no-pain controls

When *GAPDH* was used for normalization, ten genes were differentially expressed in the acute LBP group as compared to the control group (3 upregulated and 7 dysregulated). The three upregulated genes were *CCL2, PNOC*, and *CNR2*. The seven dysregulated genes were *GCH1, CSF1, CALCA, PTGES, GDNF*, and *KCNQ2*. The mean Δ(ΔCq) and fold change expression values can be found in Table 3. Results of gene expression when using *ATCB, B2M, GAPDH* as reference genes in normalization are also provided in Table 4. Upregulation of *PNOC* was significantly associated with mechanical sensitivity of the back region (Spearman r=0.359; p=0.047). Dysregulation of *CALCA* and *GDNF* were significantly associated with cold pain threshold at the remote site (r=0.541, p=0.002; r=0.431; p=0.015 respectively) while *CSF1* was associated with wind-up ratio of the painful back region (r=0.368; p=0.050).

DISCUSSION

The primary aim of this study was to describe and compare somatosensory/pain sensitivity measures and gene expression data between individuals with acute LBP and healthy (no-pain) controls. This study indicates a unique profile of somatosensory alterations and differential gene expression present at the initial episode of acute LBP and is the first, to our knowledge, to report these differences in comparison to no-pain controls. Significantly, we found that acute LBP participants showed selective pain sensitivity enhancement and differential gene expression profiles compared to pain-free controls. The acute LBP group exhibited increased pain sensitivity to cold stimuli, mechanical stimuli, including mechanical temporal summation at both the painful back area and remote location. Along with mechanical allodynia of the painful back region, these findings suggest a mechanism of enhanced central nervous system excitability in participants with acute LBP. In addition, deep tissue specific peripheral sensitization was suggested in the acute LBP group due to significant differences in pressure pain threshold of the painful back area, but not the remote body site.

Although previous studies have reported sensory alterations among participants with chronic LBP, this study is the first to report indications of selective peripheral and central sensitization in participants with acute LBP compared to normal controls. For instance, O'Neill and colleagues showed that chronic but not acute LBP patients have reduced pressure pain threshold [19, 41]. Similarly, Hübsher and colleagues reported significantly lower cold pain threshold in the painful area of the back as well as a remote site in chronic LBP compared with no-pain controls [21]. The authors note only a non-significant trend of cold sensitivity in the acute LBP compared with chronic LBP group, however, the small sample size of the acute LBP group (n=20) may have influenced the ability to detect significant differences. Collectively, the findings from the present study complement and extend the results from previous investigations measuring somatosensory changes in participants with acute and chronic LBP [20–21]. A common finding among these studies is that participants with acute and chronic LBP display more sensitive pain thresholds at the lumbar site, albeit the QST endpoints that were altered in acute versus chronic LBP are different. For example, in a chronic LBP sample Puta et al. [18] found increased cold and

warm detection thresholds, which were unaltered in our acute LBP participants. The differences in these endpoints could be due to the stage at which LBP was tested suggesting that the presentation of pain changes over time. Alternately, it could point to the heterogeneous nature of the condition, thus making pain management modalities complex.

In addition to differences in somatosensory function, we found ten genes that were differentially expressed (three upregulated and seven dysregulated) in the acute LBP group compared to no-pain controls. Of the upregulated genes, chemokine (C-C motif) ligand 2 (*CCL2*) upregulation has previously been shown in the oral surgery model of tissue injury and acute pain, with upregulation associated with pain intensity at three-hours post-surgery along with increased levels of proinflammatory cytokines [42] Prepro-nociceptin (*PNOC*), the precursor of nociceptin, appears to induce upregulation of cytokines and interleukin (IL)-10 decreases the expression of *PNOC* [43]. Upregulation of *PNOC* was associated with mechanical sensitivity of the painful back region in the acute LBP group, suggesting a role in contributing to peripheral sensitization. We also found upregulated cannabinoid type-2 (CB2) receptor (*CNR2*) gene expression consistent with the findings in a post-mortem study and vast preclinical literature that the CB2 receptor and endocannabinoid system play an important role in modulating pain sensitivity [44, 45]. As increased expression of the *CNR2* gene has been associated with reduced pain sensitivity, it remains unclear why higher expression levels were found in the acute LBP group. However, in the presence of multiple dysregulated genes *GCH1, CSF1, TRPV1, CALCA, PTGES, GDNF*, and *KCNQ2* found in participants with acute LBP, there may be interactions that contribute to the sensory alterations observed. Noting that expression does not imply transcription of proteins and due to the design of the study we cannot assume causality. Differential gene expression may be directly related to activation of nociceptive pathways as has been described in skin, muscle and dorsal root ganglion after plantar incision in a rat model of acute pain [46]. In that study, few genes changed in the DRG, however, there were several dysregulated neurotrophin genes in the skin and muscle. Further research to characterize pain-associated somatosensory changes in the context of altered mRNA expression levels may provide insight on the molecular underpinnings of maladaptive chronic pain. For instance, in patients with complex regional pain syndrome, genome-wide expression profiling of whole blood revealed differential expression of 80 genes involved in signal transduction, cell structure and motility, and immunity [47]. Given the small sample size, it is beyond the scope of this study to predict definitive genetic markers of the somatosensory changes that occur in acute LBP. Nonetheless, these preliminary findings provide some evidence of potential candidate genetic markers that may influence pain signaling and inflammatory processes during the acute stage.

This study has several limitations. First, the study enrolled volunteers and blinding of the examiner to group status was not possible due to the requirement of assessing the region of pain in the acute LBP group. Second, the sample size is small to detect generalizable differences in gene expression and further research in this area should be conducted to confirm the upregulated and dysregulated genes identified. Third, our sample did not match on all demographic characteristics such as race and education, however, the demographics of the acute LBP group were consistent with previous literature [1, 2]. A prospective blinded study with matched controls based on age, sex, race, socioeconomic level, educational

attainment and smoking status may assist in clarifying the study findings. In addition, because many participants in the acute LBP group had previous episodes of low back pain one could argue that this was a heterogeneous sample of participants with acute (first-episode and recurrent) low back pain. However, contrary to traditional thought that recurrent pain leads to persistent sensitization of peripheral and central pain processing, Slade and colleagues [47] found that participants with intermittent painful temporomandibular joint disorder (TMD) did not exhibit increased sensitization to pressure stimuli once the pain resolved. Whether the same phenomenon occurs during the continuum of intermittent/recurrent low back pain, in which flare-ups frequently occur, remains to be systematically evaluated.

This study is the first to characterize the manifestations of nervous system sensitization through rigorous quantitative pain sensitivity testing at the onset of an acute episode of nonspecific LBP with results compared with no-pain controls. In addition, this study is among the first to examine gene expression profiles in whole blood of participants with acute LBP. We have systematically identified a unique profile of somatosensory parameters and differential gene expression in an acute LBP sample compared to healthy no-pain controls. Future studies will examine these parameters in a longitudinal cohort to determine whether there are specific indicators of somatosensory function and gene expression that contribute to persistent LBP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Sample Demographics

		Low Back Pain (N=31)	No-Pain Control (N=31)	T-test/χ^2 P-value
Gender	Female	17 (54.84%)	13 (41.94%)	0.31
	Male	14 (45.16%)	18 (58.06%	
Age [Years; Mean (SE)]		35.77 (1.83)	37.06 (2.04)	0.64
Race	African American	19 (61.29%)	6 (19.35%)	**0.0008** *
	Caucasian	6 (19.35%)	20 (64.52%)	
	Other	6 (19.35%)	5 (16.13%)	
Income	<$60K	23 (74.19%)	11 (40.74%)	**0.009** *
>/=$60K		8 (25.81%)	16 (59.26%)	
Employment	Full or Part-time	18 (58.06%)	27 (87.10%)	**0.010** *
	Unemployed	13 (41.94%)	4 (12.90%)	
Education	High School or lower	15 (48.39%)	3 (9.68%)	**0.0008** *
	College	16 (51.61%)	28 (90.32%)	
Exercise	None	5 (16.13%)	3 (9.68%)	0.72
	1–3 days/week	14 (45.16%)	14 (45.16%)	
	4 or more days/week	12 (38.71%)	14 (45.16%)	
Smoking	Current smoker	15 (48.39%)	3 (10%)	**0.001** *
	Not smoking	16 (51.61%)	28 (90%)	
Comorbidities	Present	12 (41.38%)	4 (12.90%)	**0.013** *
	Not present	17 (58.62%)	27 (87.10%)	
Prior episodes None		4 (12.9%)	31 (100%)	**0.0001** *
<6 months ago		10 (32.26%)		
>6 months ago		17 (54.84%)		

* reflects $p<0.05$; not all columns in each row add up to total N due to missing values

Table 2

Quantitative Sensory Testing

Test	Low Back Pain (N=31)	No-Pain Control (N=31)	P-value
Cold Detection Threshold [°C; Mean (SE)]			
Remote area	27.96 (0.40)	27.58 (0.98)	0.72
Back area	28.30 (0.30)	28.11 (0.97)	0.85
Warm Detection Threshold [°C; Mean (SE)]			
Remote area	35.89 (0.45)	34.54 (1.23)	0.3
Back area	35.91 (0.33)	33.97 (1.21)	0.13
Cold Pain Threshold [°C; Mean (SE)]			
Remote area	19.47 (1.20)	14.24 (2.13)	**0.04** *
Back area	21.12 (1.28)	15.43 (2.16)	**0.03** *
Heat Pain Threshold [°C; Mean (SE)]			
Remote area	39.58 (0.71)	37.37 (1.9)	0.29
Back area	38.5 (0.61)	36.56 (1.9)	0.33
Pressure Pain Threshold [KPa; Mean (SE)]			
Remote area	190.52 (18.15)	215.15 (24.81)	0.43
Back area	174.28 (25.24)	310.68 (38.76)	**0.005** *
Mechanical Pain Threshold [mN; Mean (SE)]			
Remote area	6.01 (0.15)	6.17 (0.30)	0.64
Back area	5.59 (0.20)	6.02 (0.30)	0.24
Mechanical Sensitivity [NRS 0–10; Mean (SE)]			
Remote area	2.27 (0.35)	0.53 (0.14)	**0.0001** *
Back area	3.60 (0.45)	0.97 (0.22)	**0.0001** *
Wind-up Ratio [Mean (SE)]			
Remote area	1.26 (0.10)	0.38 (0.10)	**0.0001** *
Back area	1.44 (0.13)	0.52 (0.14)	**0.0001** *
Dynamic Mechanical Allodynia [Mean (SE)]			
Remote area	0.38 (0.14)	0.25 (0.09)	0.42
Back area	1.60 (0.42)	0.42 (0.12)	**0.01** *

* reflects $p<0.05$

Table 3

Mean (ΔCq), mean Δ(ΔCq) and fold-change expression values of 10 differentially expressed genes in comparison between acute low back pain and healthy no-pain participants (**when GAPDH used for normalization**).

Genes	Acute low back pain (ΔCq)	No-pain controls (ΔCq)	Δ(ΔCq)	Fold-Change*	FDR
CCL2	10.367 (±1.593)	11.251 (±1.627)	−0.884 (±1.593)	1.845 (±0.331)	0.042
PNOC	9.694 (±1.060)	10.188 (±1.438)	−0.495 (±1.060)	1.409 (±0.480)	0.031
CNR2	6.840 (±0.944)	7.276 (±1.530)	−0.436 (±0.944)	1.353 (±0.520)	0.031
GCH1	4.735 (±0.855)	4.277 (±1.277)	0.458 (±0.855)	0.728 (±0.553)	0.011
CSF1	7.144 (±0.855)	6.636 (±1.109)	0.508 (±0.855)	0.703 (±0.553)	0.031
CALCA	11.451 (±1.04)	10.862 (±1.215)	0.589 (±1.040)	0.665 (±0.486)	0.031
PTGES	11.465 (±1.317)	10.490 (±1.594)	0.974 (±1.317)	0.509 (±0.401)	0.011
GDNF	12.674 (±1.472)	11.687 (±1.336)	0.986 (±1.472)	0.505 (±0.360)	0.033
KCNQ2	13.418 (±1.473)	12.409 (±1.216)	1.009 (±1.473)	0.497 (±0.360)	0.049
HTR2A	12.255 (±1.202)	11.048 (±1.356)	1.207 (±1.202)	0.433 (±0.435)	0.011

*Fold change: $2^{-\Delta(\Delta Cq)}$ where $\Delta(\Delta Cq) = \Delta Cq_{,LBP} - \Delta Cq_{,Normal}$, and FDR: False discovery rate.

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Table 4

Mean (ΔCq), mean Δ(ΔCq) and fold-change expression values of 7 differentially expressed genes in comparison between acute low back pain and healthy no-pain participants (**when ACTB, B2M, and GAPDH used for normalization**).

Genes	Acute low back pain (ΔCq)	No-pain controls$^{(\Delta Cq)}$	Δ(ΔCq)	Fold-Change*	FDR
CCL2	12.175 (±1.594)	13.17 (±1.579)	−0.996 (±1.594)	1.994 (±0.33)	0.018
PNOC	11.501 (±1.062)	12.108 (±1.38)	−0.607 (±1.062)	1.523 (±0.47)	0.011
TRPV1	10.587 (±1.134)	11.137 (±1.76)	−0.549 (±1.134)	1.464 (±0.456)	0.049
CNR2	8.648 (±0.946)	9.196 (±1.479)	−0.548 (±0.946)	1.462 (±0.519)	0.011
GCH1	6.542 (±0.857)	6.197 (±1.216)	0.346 (±0.857)	0.787 (±0.552)	0.018
PTGES	13.272 (±1.319)	12.41 (±1.546)	0.862 (±1.319)	0.550 (±0.401)	0.018
HTR2A	14.063 (±1.203)	12.968 (±1.298)	1.095 (±1.203)	0.468 (±0.434)	0.015

*Fold change: $2^{-\Delta(\Delta Cq)}$ where $\Delta(\Delta Cq) = \Delta Cq_{,LBP} - \Delta Cq_{,Normal}$ and FDR: False discovery rate.