


# Systemic gene expression profiles according to pain types in individuals with chronic spinal cord injury

Debra Morrison<sup>1</sup>, Anthony A Arcese<sup>1,\*</sup>, Janay Parrish<sup>2,\*</sup>, Katie Gibbs<sup>1,3</sup>, Andrew Beaufort<sup>1</sup>, Paige Herman<sup>1</sup>, Adam B Stein<sup>3</sup>, and Ona Bloom<sup>1,3,4</sup> 

Molecular Pain  
Volume 17: 1–13  
© The Author(s) 2021  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/17448069211007289  
journals.sagepub.com/home/mpx  


## Abstract

Pain affects most individuals with traumatic spinal cord injury (SCI). Major pain types after SCI are neuropathic or nociceptive, often experienced concurrently. Pain after SCI may be refractory to treatments and negatively affects quality of life. Previously, we analyzed whole blood gene expression in individuals with chronic SCI compared to able-bodied (AB) individuals. Most participants with SCI reported pain (N = 19/28). Here, we examined gene expression of participants with SCI by pain status. Compared to AB, participants with SCI with pain had 468 differentially expressed (DE) genes; participants without pain had 564 DE genes (FDR < 0.05). Among DE genes distinct to participants with SCI with pain, Gene Ontology Biological Process (GOBP) analysis showed upregulated genes were enriched in categories related to T cell activation or inflammation; downregulated genes were enriched in categories related to protein proteolysis and catabolism. Although most participants with pain reported multiple pain types concurrently, we performed a preliminary comparison of gene expression by worst pain problem type. Compared to AB, participants with SCI who ranked neuropathic (N = 9) as worst had one distinct DE gene (TMEM156); participants who ranked nociceptive (N = 10) as worst had 61 distinct DE genes (FDR < 0.05). In the nociceptive group, the GOBP category with the lowest P-value identified among upregulated genes was “positive regulation of T cell activation”; among downregulated genes it was “receptor tyrosine kinase binding”. An exploratory comparison of pain groups by principal components analysis also showed that the nociceptive group was enriched in T-cell related genes. A correlation analysis identified genes significantly correlated with pain intensity in the neuropathic or nociceptive groups (N = 145, 65, respectively, Pearson’s correlation  $r > 0.8$ ). While this pilot study highlights challenges of identifying gene expression profiles that correlate with specific types of pain in individuals with SCI, it suggests that T-cell signaling should be further investigated in this context.

## Keywords

Spinal cord injury, pain, neuropathic, nociceptive, gene expression

Date Received: 15 February 2021; Revised 15 February 2021; accepted: 9 March 2021

## Introduction/background

Traumatic spinal cord injury (SCI) affects more than 300,000 individuals in the United States.<sup>1</sup> Pain is one of the most common medical consequences of living with SCI.<sup>2–6</sup> Historically, pain in individuals with SCI was categorized according to the Bryce Ragnarsson scale as neuropathic (NP), nociceptive (NC) or other.<sup>7,8</sup> Neuropathic pain is caused by damage to nerves in the somatosensory system, resulting in symptoms such as paresthesia, allodynia, and hyperalgesia. Nociceptive

<sup>1</sup>Institute of Molecular Medicine, The Feinstein Institutes for Medical Research, Manhasset, NY, USA

<sup>2</sup>Donald and Barbara Zucker School of Medicine at Hofstra-Northwell, Hempstead, NY, USA

<sup>3</sup>Department of Physical Medicine and Rehabilitation, Zucker School of Medicine at Hofstra-Northwell, Hempstead, NY, USA

<sup>4</sup>Department of Molecular Medicine, Zucker School of Medicine at Hofstra-Northwell, Hempstead, NY, USA

\*These authors contributed equally.

### Corresponding Author:

Ona Bloom, The Feinstein Institutes for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA.  
Email: obloom@northwell.edu



pain is characterized by throbbing or aching as a result of damage to somatic structures such as tissues, tendons, and bone. Individuals with SCI often experience concurrent multiple types of pain problems, of different intensities, in several locations.<sup>4,5,9</sup> To facilitate a better understanding of pain in the SCI population, the International SCI Pain Basic Data Set (ISCIPBDS), based on the International SCI Pain Classification (ISCIP), was created to characterize pain by type (nociceptive, neuropathic, other or unknown), subtype (nociceptive: musculoskeletal, visceral or other, neuropathic: at- or below- level of injury, or other, unrelated to the injury), by pain location, as well as by pain intensity.<sup>10,11</sup> This enables a person with SCI to report and score the worst pain types, as well as their locations and intensity, of multiple pain types they are experiencing simultaneously.

To manage pain symptoms, many individuals with SCI are prescribed multiple medications simultaneously, including gabapentin/pregabalin, tricyclic antidepressants, and opioids for neuropathic pain, while physical therapy, exercise, and NSAIDs are most often prescribed for nociceptive pain.<sup>12,13</sup> As we and others have shown previously, unfortunately, pain symptoms in individuals with SCI are often refractory to treatment and may negatively impact quality of life.<sup>5,9,14</sup> Molecular mechanisms contributing to pain in persons with SCI are poorly understood, limiting development of more effective therapeutic options.

In pre-clinical models, blood-borne inflammatory mediators such as cytokines and chemokines, are increasingly considered to promote hyperexcitability of nociceptors, helping to drive chronic pain.<sup>15</sup> Cytokines and chemokines appear to exert sensitizing effects on pain behavior by acting on primary afferent neurons, particularly nociceptors.<sup>16</sup> Nociceptor cell bodies within dorsal root ganglia (DRGs) are not protected by a vascular permeability barrier, so they may be exposed to circulating inflammatory mediators that are elevated after SCI.<sup>17</sup> Inflammatory cytokines and chemokines may also contribute to the influx of immune cells such as macrophages into DRGs after SCI, thereby promoting a feed-forward cascade of inflammation.<sup>18,19</sup> In pre-clinical studies, exposure of dissociated nociceptors to low concentrations of the chemokine CCL2/MCP-1 dramatically upregulated the ion channels TRPV1 and Nav1.8, which are widely studied in the context of pain.<sup>15,20</sup> These channels are important for nociception, are upregulated in rodent models of neuropathic pain, in rodent DRG neurons after SCI, and their activation can promote pain responses in patients with SCI.<sup>15,21–24</sup> Other pathways that have been described as differentially expressed in rodent models of neuropathic pain after SCI include MAPK, CCL3 and mTOR.<sup>25</sup> Previously, we performed the largest systemic functional

genomics study of individuals living with chronic SCI and determined upregulation of the major pro-inflammatory Toll-like receptor (TLR) signaling pathway.<sup>26</sup> Subsequently, we used the ISCI-PBDS to characterize the experience of pain symptoms in some of the same participants, reporting that most participants experienced multiple types of pain, despite standard-of-care treatments.<sup>9</sup> Here, the objective was to leverage these studies to determine any differences in whole blood gene expression in individuals with chronic SCI with or without pain, compared to able-bodied persons. We also explored potential differences in gene expression among individuals with chronic SCI according to their worst reported pain type.

## Materials and methods

### Participants

This prospective, observational study was performed in an academic medical center in accordance with ethical standards of and with approval from the institutional IRB. Written informed consent was obtained prior to any study procedures. Additional data on whole blood gene expression and on pain symptoms reported by participants were published previously.<sup>9,26</sup> Briefly, participants included in this study were adults who had experienced a SCI at any level at least one year prior, and who had a SCI classified as American Spinal Injury Association Scale (AIS) grade of A-D. Participants were excluded if they had a concurrent infection such as frank urinary tract infection indicated by lab evidence and some clinical occurrence, or had pressure ulcers, cancer, chemotherapy, neutropenia, or autoimmune disease. A cohort of able-bodied individuals (N = 26) were recruited for comparison. As mentioned above, pain data was obtained prospectively from participants with SCI using the International SCI Pain Basic Data Set.<sup>9</sup>

### Gene expression profiling

As described previously, blood was collected from participants in PAXgene tubes and stored at  $-80^{\circ}\text{C}$  until RNA was isolated from whole blood, using standard methods (Qiagen QIAcube, Venlo, The Netherlands). RNA quality and quantity were measured using the Bioanalyzer (Agilent Technologies, Santa Clara, CA) and only RNA that met quality control criteria of RNA Integrity Number (RIN)  $>8$  were used. RNA was amplified using the Illumina RNA Total Prep Amplification Kit (Life Technologies, Carlsbad, CA) and analyzed on the HT-12v4 Expression BeadChips (Illumina, San Diego, CA).<sup>26</sup> Raw data were background subtracted, quantile normalized,  $\log_2$  transformed, and analyzed using Partek Genomics Suite

(Partek Inc., St. Louis, MO). After filtering for minimum detection thresholds and housekeeping genes, 11,209 genes were analyzed. Differentially expressed (DE) genes were identified by Gene Specific Analysis (GSA) algorithm (Benjamini-Hochberg corrected FDR  $\leq 0.05$ ) and 2-way hierarchical clustering analysis were performed in Partek. Principal components analysis (PCA) was performed reducing genes into 5 components, with all components contributing equally (Partek Genomics Flow). Component loadings for each comparison examined and all gene lists can be found in Supplemental Table 1. Venn diagrams of DE genes were created in Venny<sup>27</sup> to determine distinct or shared genes for comparisons, as indicated in Results. Functional analysis of DE genes was performed in the open bioinformatics platform Enrichr, as we have done previously, which enables analyses of a gene list by multiple independent bioinformatics platforms.<sup>26,28</sup> Here, we analyzed DE genes using the Gene Ontology (GO) Biological Process platform. In an independent analysis, we used the WikiPathways 2019 Human bioinformatics platform for analysis of PCA according to component loading.

## Results

### Participant characteristics

Participants were able-bodied (N = 26) or were individuals with chronic SCI (N = 28), were mostly male and of similar ages (Table 1). Participants with SCI were living with SCI for  $17 \pm 2.4$  years (average  $\pm$  sem). In SCI, injury severity is determined by a physical exam, the International Standards for the Neurological Classification of Spinal Cord Injury (ISNCSCI), which determines the severity of injury according to the American Spinal Injury Association Scale (AIS) grades of A, B, C, D, or E, where A is the most impaired (no motor or sensory function detectable in sacral segments S4-5), and E is normal motor and sensory function of all segments.<sup>29,30</sup> Among study participants, the most common AIS grades were A and D (N = 16, 6, respectively). Spinal cord injuries occurred mostly rostral to thoracic level T5 ( $\geq T5$ , 79%), the region where sympathetic nervous system (SNS) fibers exit the spinal cord and innervate immune and other organs.<sup>17,31</sup>

In a prior publication, we collected data with the International SCI Basic Pain Data Set from study participants with SCI on the ranking of three worst pain problems they experienced within the past 7 days.<sup>9</sup> As described above, the ISCI-PBDS asks people with SCI to score their worst pain problem according to a numerical rating scale from 0 to 10 where 0 is “no pain” and 10 is “pain as bad as you can imagine” as well as their worst pain type, categorized as nociceptive, neuropathic or

unknown. In summary, most participants reported pain (N = 19/28) and >70% of participants experienced multiple pain problems concurrently. Although more participants reported nociceptive as their worst pain type, the range of pain intensity scores for neuropathic pain was higher (see Table 1 for pain scores: NP 5–10, NC 4–8 range). There were no statistically significant differences in the group with or without pain by number of years after injury, or in neurological level of injury rostral or caudal to T5, or in AIS grade. This study did not require participants to discontinue medication use; according to combined sources of participant reports and medical chart data (as available), most participants were prescribed pharmacological pain treatments (see Table 1). Eight out of the nine participants with SCI who reported neuropathic pain as their worst pain problem were taking either opioids, anti-epileptic drugs (AEDs), NSAIDs or acetaminophen, alone or combination at the time of the study. Of the ten participants with SCI who reported nociceptive pain as their worst pain problem, six were taking either opioids, AEDs or acetaminophen, alone or in combination, with no reported NSAID use.<sup>9</sup> There were no significant differences between SCI participants who ranked neuropathic or nociceptive as their worst pain type with respect to medication use (yes/no) for opioids, AEDs, acetaminophen, or NSAIDs (Fisher’s exact test, two-tailed P value  $>0.05$  for each comparison respectively). Despite medication use, among participants who reported pain, approximately half ranked their worst pain problem as nociceptive (N = 10) or neuropathic (N = 9) (Figure 1a).<sup>9</sup>

### Gene expression analysis

To better understand molecular pathways activated in whole blood of individuals with chronic SCI in the presence of standard-of-care treatments, we used 2-way hierarchical clustering of profiles from participants with SCI who did (N = 19) or did not (N = 9) report pain, compared to AB participants (n = 26), (Figure 1b). For the SCI pain group, there were 468 DE genes compared to the AB group (FDR < 0.05) (Figure 1b), of which 181 were up- and 287 genes were down-regulated. For the SCI group without pain, there were 564 DE genes compared to the AB group (FDR < 0.05) (Figure 1b), of which 138 were up- and 426 were down-regulated. Somewhat surprisingly, hierarchical clustering illustrated that the most distinct separation of gene expression profiles was between samples from the AB group and the SCI participants without pain, with more mixed clustering of samples from the SCI participants with pain. We next compared differentially expressed genes in the SCI participant groups to determine genes that were shared or distinct according to pain status. As shown in Venn

**Table 1.** Participant demographics and clinical characteristics.

ID	Age	Gender	Years post injury	ASIA impairment scale grade	Neurological level of injury	Worst pain type	Pain intensity score	Anti-epileptic drugs	Opioids	NSAIDs	Acetaminophen
1	69	Male	16	A	Thoracic	NP	7	Yes	Yes	No	No
2	71	Female	4	D	Cervical	NP	10	Yes	No	No	No
3	28	Male	12	C	Thoracic	NP	9	No	No	No	No
4	78	Male	1	D	Cervical	None	NA	Yes	No	Yes	No
5	53	Female	34	A	Cervical	NC	8	No	Yes	No	Yes
6	45	Male	17	A	Cervical	NC	7	No	No	No	No
7	64	Male	25	A	Thoracic	None	NA	No	No	No	No
9	62	Male	5	D	Cervical	None	NA	No	No	No	No
10	64	Male	2	A	Thoracic	None	NA	No	No	No	No
11	56	Male	10	D	Cervical	NC	6	Yes	No	No	No
12	57	Male	35	A	Thoracic	None	NA	No	Yes	No	No
14	80	Male	16	A	Cervical	NP	6	Yes	No	Yes	Yes
15	40	Male	23	B	Cervical	None	NA	No	No	No	No
16	34	Male	17	C	Cervical	None	NA	No	No	No	No
17	28	Male	2	C	Cervical	None	NA	No	No	No	No
18	63	Male	44	A	Cervical	NC	8	No	Yes	No	Yes
19	21	Female	2	C	Cervical	NC	6	Yes	No	No	No
20	60	Male	2	D	Cervical	NC	8	Yes	Yes	No	No
22	55	Male	16	A	Thoracic	NP	8	No	Yes	No	No
25	45	Male	27	A	Cervical	NC	5	No	No	No	No
26	79	Female	5	A	Cervical	NC	5	No	No	No	No
27	55	Male	36	B	Thoracic	NC	4	Yes	Yes	No	Yes
29	46	Female	10	D	Thoracic	NP	7	No	No	Yes	No
30	44	Male	23	A	Cervical	None	NA	No	No	No	No
31	52	Male	28	A	Thoracic	NP	5	No	No	Yes	Yes
42	72	Male	38	A	Thoracic	NC	4	No	No	No	No
49	59	Male	17	A	Cervical	NP	5	Yes	No	No	No
88	40	Male	2	A	Thoracic	NP	9	Yes	Yes	No	Yes

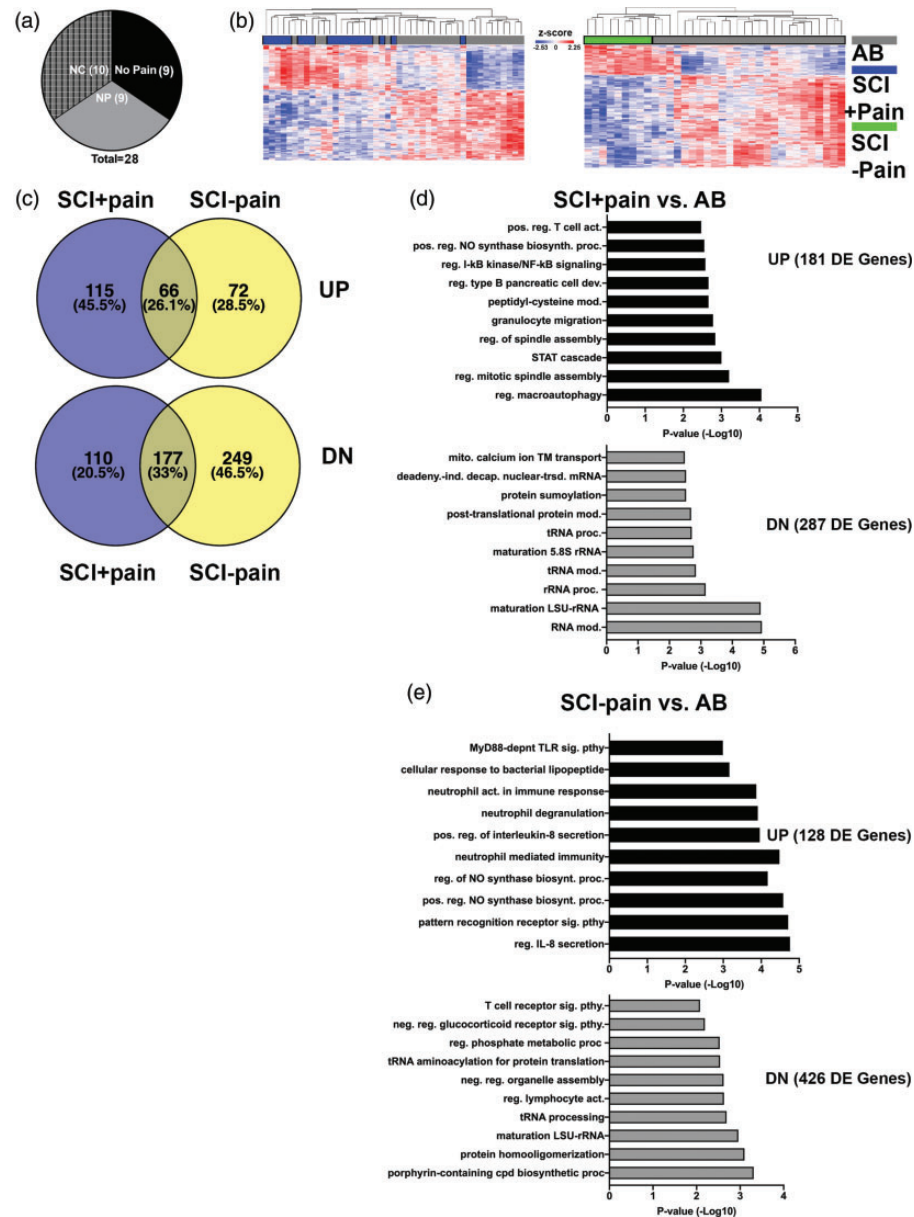
The last four columns refer to medication history. NA indicates data not applicable. Anti-epileptic drugs include pregabalin and gabapentin. NP: neuropathic; NC: nociceptive; NSAID: non-steroidal anti-inflammatory drug.

diagrams (Figure 1c), compared to the AB group, we identified 66 up- and 177 down-regulated DE genes that were shared across SCI participant groups, regardless of pain status. Compared to the AB group, we identified 115 DE genes that were up- and 110 DE genes that were down-regulated that were distinct to the group of SCI participants with pain.

We performed Gene Ontology (GO) analyses to determine functional categories that were enriched among DE genes for each of the group comparisons examined (Figure 1d and e). GO Biological Process analysis of the upregulated DE genes in the group of SCI participants with pain compared to AB participants included several significantly enriched categories related to inflammation and autophagy, as well as others (Figure 1d). GO Biological Process analysis of the down-regulated DE genes in the group of SCI participants with pain compared to AB participants included several significantly enriched categories related to RNA processing (Figure 1d). GO Biological Process analysis of the

upregulated DE genes in the group of SCI participants without pain compared to AB participants included several significantly enriched categories related to inflammation, such as pattern recognition/Toll like receptor (TLR) signaling, IL-8 and neutrophil activation (Figure 1e). GO Biological Process analysis of the down-regulated DE genes in the group of SCI participants without pain compared to AB participants included several significantly enriched categories related to RNA processing, but also included a steroid receptor signaling and T cell related pathways (Figure 1e).

We next analyzed the functional categories enriched among the 115 up- and 110 down-regulated DE genes that were distinct to the group of SCI participants with pain (Figure 2a). Upregulated genes were enriched in GO Biological Process categories related to T cell activation/signaling and inflammation. Downregulated GO Biological Process categories were more general and related to RNA processing. Finally, the functional categories of genes up- and down-regulated that were shared

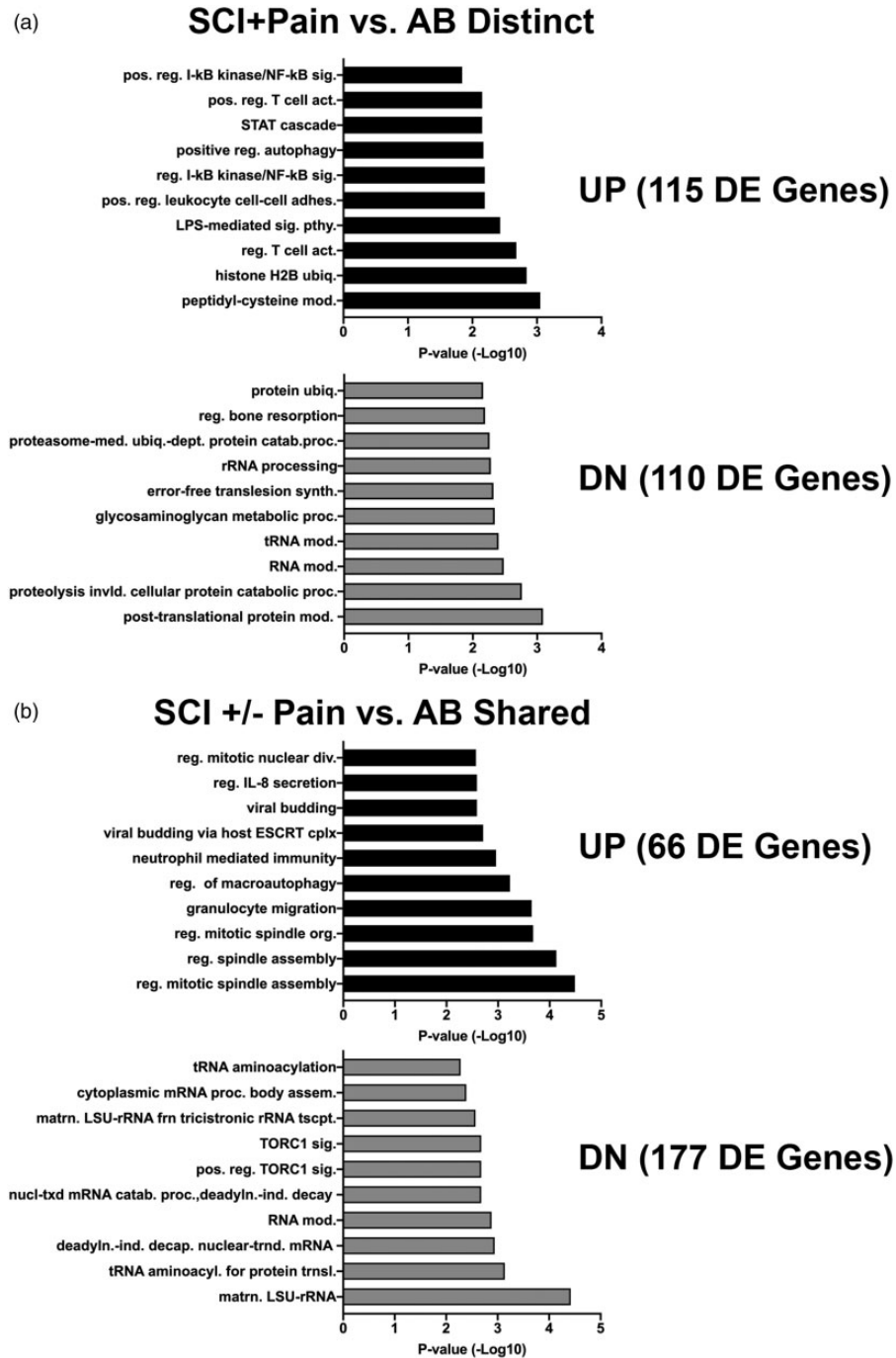


**Figure 1.** Gene expression profiles differ between individuals with chronic SCI with or without reported pain and able-bodied persons. (a). Summary of pain symptoms reported by individuals with SCI, presented fully in Gibbs et al.<sup>9</sup> (b) Dendrograms from two-way hierarchical clustering shows gene expression profiles from individuals with SCI with (left, blue) or without (right, green) reported pain compared to AB participants (left and right, gray). Heat map shows pattern of expression for genes that are up- (blue) or down-regulated (red) in participants with SCI compared to AB participants. (c) Venn diagrams showing numbers of shared and distinct regulated differentially expressed genes (FDR < 0.05) in participants with SCI according to pain status compared to AB participants. (d) and (e) For up- or down-regulated differentially expressed genes in each comparison, 10 of the top 20 categories (by smallest P-value) identified by GO Biological Process analysis are shown, with comparison and number of genes indicated for each set of panels. GO Category numbers are provided in Supplemental Table 1.

across SCI groups were analyzed (Figure 2b). The up-regulated genes were related to mitosis, autophagy, viral budding, Il-8, neutrophil biology and Il-8. The down-regulated genes were related to RNA processing.

While most participants with pain (N = 19) reported multiple pain problems of varying types (neuropathic

and nociceptive), we performed an exploratory analysis of the gene expression profiles according to the worst type of pain problem reported. Compared to AB, participants with SCI who ranked neuropathic (N = 9) as worst pain type had only one distinct DE gene with an FDR < 0.05, transmembrane protein 156 (TMEM156).



**Figure 2.** Functional enrichment categories of genes distinct to or shared by individuals with chronic SCI with or without reported pain. (a) and (b) For up- or down-regulated differentially expressed genes in each comparison, 10 of the top 20 categories (by smallest P-value) identified by GO Biological Process analysis are shown, with comparison and number of genes indicated for each set of panels. GO Category numbers are provided in Supplemental Table 1.

However, compared to AB, participants with SCI who ranked nociceptive ( $N = 10$ ) as their worst pain type had 61 distinct DE genes with an  $FDR < 0.05$ . In the nociceptive group, GO Biological Process analysis of the 24 upregulated genes identified “positive regulation of T cell activation” as the category with the lowest P-value,

while analysis of the 37 downregulated genes identified “receptor tyrosine kinase binding” as the category with the lowest P-value.

Next, we used PCA to compare potential differences in gene expression profiles of participants with SCI who ranked nociceptive or neuropathic pain as their worst

pain type (Figure 3a, left). With dividing total variation in gene expression into five components, the first component explained 25.3%, while the second component explained 11.5% of the total variation in gene expression. Profiles from participants who ranked neuropathic as their worst pain type had a higher correlation coefficient in PC1 (Figure 3a, middle), while participants who ranked nociceptive pain had a higher correlation coefficient in PC2 (Figure 3a, right). Functional analysis of the top 500 genes ranked by component loading in PC1 (Supplemental Table 1) using WikiPathways showed an enrichment in inflammation-associated genes, indicated by IL-1 and TNF-alpha associated signaling pathways (Figure 3b). Functional analysis of the top 500 genes in PC2 using WikiPathways showed an enrichment in T-cell related signaling, indicated by TCR and co-stimulatory signaling, TCR pathway signaling, IL-2 signaling and other pathways, extending the analysis of this group compared to AB profiles (Figure 3b). The top 20 genes common to the most highly enriched categories are shown for PC1 and PC2 (Figure 3c). A complete list of transcript component loadings for each of the five principal components is included in Supplemental Table 1.

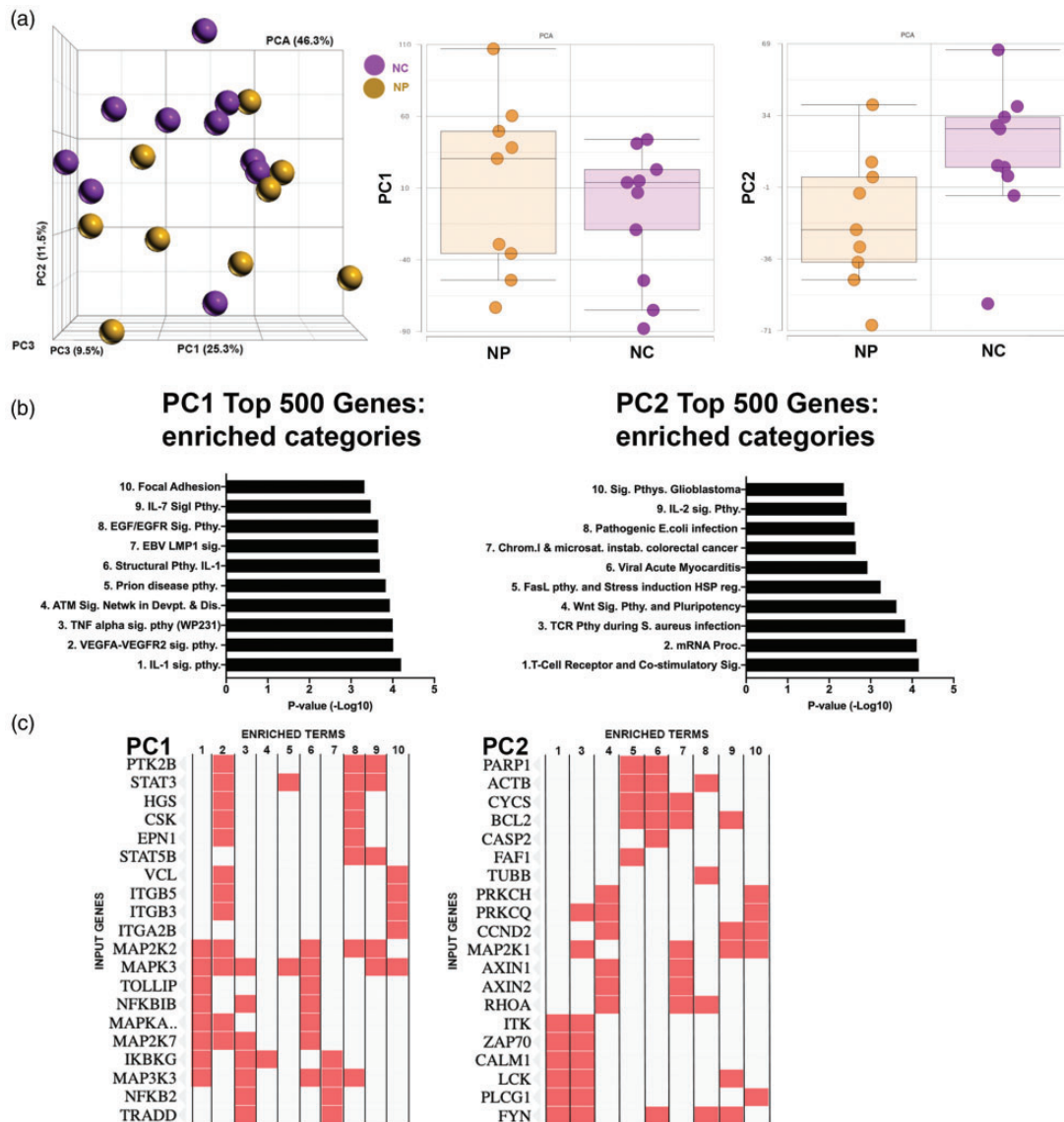
Next, we determined the correlation of individual gene expression with pain intensity, using Pearson's correlation coefficient (Figure 4; Supplemental Table 1). For the participants who ranked neuropathic pain as their worst type, 145 genes displayed a strong correlation with pain intensity (Pearson's correlation coefficient  $r \geq 0.8$  or  $< -0.8$ , P value  $< 0.01$ ) while 65 genes correlated significantly with pain intensity in participants who ranked nociceptive pain as their worst pain type. Only two genes were significantly correlated with intensity of both pain types, KLHL36 (Kelch like family member 36) and GPN2 (GPN-loop GTPase 2). Among the genes positively correlated with neuropathic pain intensity were PTPRC (protein tyrosine phosphatase receptor type C, aka CD45/LCA), BCL7B (BAF chromatin remodeling complex subunit), IRF1 (interferon regulatory factor 1), IFITM1 (Interferon induced Transmembrane protein 1), and MAPK1 (Mitogen Activated protein kinase 1). Genes highly correlated with nociceptive pain intensity included CD44 (GP90 Lymphocyte Homing/Adhesion Receptor), HSPA1B (Heat Shock Protein Family A Member 1B), MAPK1IP1L (Mitogen-Activated Protein Kinase 1 Interacting Protein 1 Like), and TXNRD2 (thioredoxin reductase 2).

## Discussion

Clinical and demographic characteristics of participants with SCI were consistent with national data, as most participants were male with neurologically motor complete (AIS A) injuries.<sup>1</sup> A majority of individuals with

SCI reported more than one pain problem, of both nociceptive and neuropathic pain types. As is typical, participants with SCI reported pain symptoms despite concurrent pharmacological therapies. Since pain is a common unresolved medical consequence of SCI and inflammation is increasingly proposed as a contributor to pain, here we examined differences in whole blood gene expression between AB participants and participants with chronic SCI according to their worst reported pain type.

Previously, there have been two studies of whole blood systemic gene expression in persons with chronic SCI.<sup>26,32</sup> Battaglino, Morse and colleagues reported on the upregulation of the autoimmune-promoting signaling pathways in persons with chronic SCI.<sup>32</sup> Subsequently, we reported that persons with chronic SCI had a marked induction of Toll-like receptor (TLR) signaling pathways, as well as a downregulation of Natural Killer (NK) cell signaling and a reduction in adaptive immune cell related signaling, which was most pronounced in persons with injuries rostral to T5, where SNS fibers exit the spinal cord and innervate immune (and other) organs.<sup>26</sup> For some of the participants in that study, we then analyzed pain symptoms using the new International SCI Basic Pain Data Set, which was designed to be used by clinical and research professionals to facilitate a deeper understanding of pain symptoms in the SCI population.<sup>9</sup> In order to broaden our understanding of potential systemic gene expression changes related to pain in persons with chronic SCI, here we combined these data sets. We first compared gene expression profiles of participants with chronic SCI with or without pain symptoms to able-bodied participants. Individuals with SCI who reported pain (of any type) had an enrichment of differentially expressed upregulated genes related to both inflammation and T-cell activation. Participants with SCI who did not report pain had an enrichment of differentially expressed upregulated genes related to inflammation, specifically nitric oxide signaling, as well as IL-8/CXCL8, a pro-inflammatory chemokine. Interestingly, compared to able-bodied participants, participants with SCI who did not report pain had an enrichment of differentially expressed downregulated genes related to T cell signaling. Previously, studies of animal models of pain reported an influx of T cells into dorsal root ganglia and T cells have been implicated in inflammation-related pain.<sup>15,33</sup> Compared to able-bodied persons, upregulated differentially expressed genes that were distinct to participants with pain compared to those without pain included pro-inflammatory pathways such as NF-kB, LPS signaling, STAT signaling. While most molecular signaling studies in pain have focused on dorsal root ganglia or the spinal cord itself, these and other inflammatory pathways have been implicated in



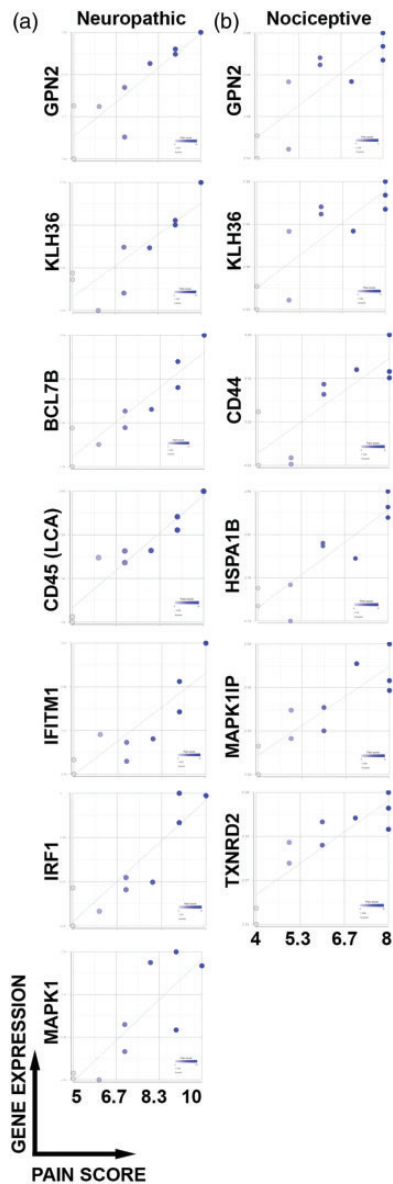
**Figure 3.** Whole blood gene expression differences in individuals with SCI who rank neuropathic or nociceptive as their worst pain problem. (a, left) Principal Component Analysis (PCA) shows patterns of gene expression. Purple symbols represent data obtained from participants with SCI who ranked nociceptive as their worst pain type. Yellow symbols represent participants with SCI who ranked neuropathic as their worst pain type. PCA gene expression differs along the Y-axis (PC2) and X-axis (PC1). (a, middle) Box and whisker plots show that participants with SCI who ranked neuropathic as their worst pain type had a higher correlation coefficient in PC1. (NP: median= 30.5, Q1= -35, and Q3= 49.6; NC: median= 10.3, Q1= -36.8, Q3= 18.9.) (a, right) Box and whisker plots show that participants with SCI who ranked nociceptive as their worst pain type had a higher correlation coefficient in PC2. (NP: median= -21.8, Q1= -37.6, Q3= 3.9; NC: median= 18.5, Q1= 6.5, Q3= 31.1.) (b) For the top 500 genes loading PC1 (left) or PC2 (right), top categories (by smallest P-value) identified by WikiPathways platform are shown. (c) Clustergrams were generated showing the top 20 genes with common expression (gene symbols shown in rows) in categories enriched in PC1 (left) or PC2 (right), are indicated. Category numbers in (c) correspond to those in B. for PC1 or PC2.

diverse animal models of pain.<sup>15</sup> For example, recent GO analysis of genes that were consistently differentially expressed in dorsal root ganglia in multiple rodent studies of nerve injury identified categories related to inflammation.<sup>34</sup> IL-1 receptor antagonist and IL-16, which contribute to T-cell division and maturation, were up-regulated at 1 week post nerve injury, along with genes

related to the pro-inflammatory transcription factor NF- $\kappa$ B.<sup>34</sup>

We next used principal component analysis to explore broad differences in gene expression profiles between the SCI participant groups and found that individuals who identified neuropathic pain as their worst type of pain problem had gene expression profiles that skewed into





**Figure 4.** Correlation analysis of pain intensity with gene expression. A subset of genes displaying a strong correlation with pain intensity is shown for (a) neuropathic and (b) nociceptive worst pain groups. Two genes, KLHL36 and GPN2, are correlated with pain intensity in both groups. All other genes are unique to one particular group. Intensity of blue symbol color correlates with pain intensity.

PC1. Functional analysis of the top 500 genes that loaded PC1 revealed that there were many pro-inflammatory pathways enriched, consistent with the growing body of literature implicating inflammation in neuropathic pain. Participants who identified nociceptive pain as their worst type of pain had gene expression profiles that skewed into PC2. Consistent with the differential expression analysis of this group compared to able-bodied participants described here, functional

analysis of the top 500 genes that loaded PC2 were enriched in T-cell related pathways, further supporting an exploration of T cells in the context of pain after SCI. Intriguingly, analysis of microarray data previously deposited in the GEO database from human peripheral mononuclear blood cells obtained from individuals with SCI who reported neuropathic pain also showed significant enrichment in T-cell receptor signaling.<sup>35</sup>

Many of the genes that correlated significantly with either neuropathic or nociceptive pain intensity have been previously studied in the context of pain. For example, CD44, a negative regulator of TLR receptor activation and a receptor for hyaluronan, is a mediator of hyperalgesia that can be prevented by antisense oligodeoxynucleotides to CD44 mRNA in a preclinical model of neuropathic pain.<sup>36</sup> In a study of more than 1300 patients with head and neck squamous cell carcinoma, MAPK1 expression was highly correlated with severe pre-treatment pain.<sup>37</sup> BCL7B and HSPA1B were both elevated in a rat chronic constriction model of neuropathic pain.<sup>38,39</sup> It was previously suggested in several publications that PTPRC/CD45, a gene identified as significantly differentially expressed following SCI in pre-clinical models, may be important in the development of neuropathic pain and a possible target of pain intervention.<sup>40-42</sup> Other genes, IFITM1 and IRF1, both of which are regulated by the pro-inflammatory cytokine interferon gamma, were significantly upregulated in preclinical models of SCI models. IFITM1 was expressed in intraspinal leukocytes and activated microglia, suggesting possible involvement in the pathological process of pain.<sup>43</sup> In a preclinical SCI model, IRF1 colocalized in the spinal cord with activated caspase-3, and thus may play a role in neuronal apoptosis.<sup>44</sup> Some of the other genes that were highly correlated with pain intensity (e.g.: TXNRD2, KLHL36, GPN2, MAPK1IP1L) have not been previously described in pain-related publications in the context of pain pathways and thus may be worthy of further inquiry.

There are many limitations to this pilot study that may influence, confound, or limit its interpretations. One limitation is that while individuals in the able-bodied group were asked general questions about their health, they were not asked in-depth questions about their pain history or pain medication use. Also, it is important to note that the assignment of individuals with SCI by worst pain type (neuropathic or nociceptive), was based on the participant-reported ranking of their *worst* pain type, which was not their *only* pain type.<sup>9</sup> As is typical for persons living with chronic SCI, many participants reported more than one type of pain concurrently.<sup>9</sup> Another limitation is that participants with SCI were not asked to discontinue their concurrent pain medications, which were directed against different types of pain during this study, thus pharmacological influences

on gene expression are expected. Although the International SCI Basic Pain Data Set is a validated tool for the SCI population,<sup>10,11,14,45</sup> pain type ranking is subjective for each individual and not based on an objective evoked measurement. Furthermore, the sample size of this pilot study was not large enough to make generalizations about the broader SCI population. With respect to other factors that can influence systemic gene expression, there were more participants over the age of 65 in the SCI group, which may be relevant, as increased age may contribute to chronic systemic inflammation.<sup>46</sup> Also, in any participant group, we did not collect data on additional factors known to influence whole blood gene expression, such as body composition or physical activity.<sup>17</sup> Despite these limitations, to our knowledge this pilot study provides a unique human data set that supports further exploration of the role of inflammation and of T-cells in promoting different types of pain in individuals with chronic SCI. In the future, larger prospective studies addressing some of the limitations highlighted above should be performed to determine if the observations made here are consistent and to promote discovery of novel therapeutic targets to reduce pain after SCI.

### Acknowledgements

We thank the participants for making this study possible.

### Author Contributions

JP, AA, DM, PH analyzed data. JP, DM, and OB wrote the manuscript. KG and AS recruited participants, analyzed data and edited the manuscript. AB analyzed clinical and demographic pain data. OB designed the study, analyzed data, and wrote the manuscript.

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Support for this study was provided to OB by the Craig H. Neilsen Foundation, the NY State Empire Clinical Research Program, the NY State Spinal Cord Injury Research Board and Northwell Health.

### ORCID iD

Ona Bloom  <https://orcid.org/0000-0002-8340-2392>

### Supplemental Material

Supplementary material for this article is available online.

### References

1. National Spinal Cord Injury Statistical Center. *Facts and figures at a glance*. Birmingham, AL: University of Alabama at Birmingham, 2020.
2. Burke D, Fullen BM, Stokes D and Lennon O. Neuropathic pain prevalence following spinal cord injury: a systematic review and meta-analysis. *Eur J Pain (United Kingdom)* 2017; 21: 29–44.
3. Burke D, Fullen BM and Lennon O. Pain profiles in a community dwelling population following spinal cord injury: a national survey. *J Spinal Cord Med* 2019; 42: 201–211.
4. Jensen MP, Hoffman AJ and Cardenas DD. Chronic pain in individuals with spinal cord injury: a survey and longitudinal study. *Spinal Cord* 2005; 43: 704–712.
5. Müller R, Brinkhof MWG, Arnet U, Hinrichs T, Landmann G, Jordan X and Béchir M; for the SwiSCI Study Group. Prevalence and associated factors of pain in the swiss spinal cord injury population. *Spinal Cord* 2017; 55: 346–354.
6. Hoffman JM, Bombardier CH, Graves DE, Kalpakjian CZ and Krause JS. A longitudinal study of depression from 1 to 5 years after spinal cord injury. *Arch Phys Med Rehabil* 2011; 92: 441–448.
7. Bryce TN and Ragnarsson KT. Pain after spinal cord injury. *Phys Med Rehabil Clin N Am* 2000; 11: 157–168.
8. Bryce TN, Budh CN, Cardenas DD, Dijkers M, Felix ER, Finnerup NB, Kennedy P, Lundeberg T, Richards JS, Rintala DH, Siddall P and Widerstrom-Noga E. Pain after spinal cord injury: an evidence-based review for clinical practice and research – report of the National Institute on Disability and Rehabilitation Research Spinal Cord Injury Measures Meeting. *J Spinal Cord Med*. 2007; 30: 421–440.
9. Gibbs K, Beaufort A, Stein A, Leung TM, Sison C and Bloom O. Assessment of pain symptoms and quality of life using the international spinal cord injury data sets in persons with chronic spinal cord injury. *Spinal Cord Ser Cases* 2019; 5: 32.
10. Widerström-Noga E, Biering-Sørensen F, Bryce TN, Cardenas DD, Finnerup NB, Jensen MP, Richards JS and Siddall PJ. The International Spinal Cord Injury Pain Basic Data Set (version 2.0). *Spinal Cord*. 2014; 52: 282–286.
11. Bryce TN, Biering-Sørensen F, Finnerup NB, Cardenas DD, Defrin R, Lundeberg T, Norrbrink C, Richards JS, Siddall P, Stripling T, Treede R-D, Waxman SG, Widerström-Noga E, Yeziarski RP and Dijkers M. International spinal cord injury pain classification: Part I. Background and description. *Spinal Cord* 2012; 50: 413–417.
12. Bastrup C and Finnerup N. Pharmacological management of neuropathic pain following spinal cord injury. *CNS Drugs* 2008; 22: 455–475.
13. Siddall PJ and Middleton JW. Spinal cord injury-induced pain: mechanisms and treatments. *Pain Manag* 2015; 5: 493–507.
14. Stampacchia G, Massone A, Gerini A, Battini E and Mazzoleni S; Research Partners. Reliability of the Italian

- version of the International Spinal Cord Injury Pain Basic Data Set. *Spinal Cord* 2019; 57: 128–133.
15. Walters ET. Neuroinflammatory contributions to pain after SCI: roles for central glial mechanisms and nociceptor-mediated host defense. *Exp Neurol* 2014; 258: 48–61.
  16. Cook AD, Christensen AD, Tewari D, McMahan SB and Hamilton JA. Immune cytokines and their receptors in inflammatory pain. *Trends Immunol* 2018; 39: 240–255.
  17. Bloom O, Herman PE and Spungen AM. Systemic inflammation in traumatic spinal cord injury. *Exp Neurol* 2020; 325: 1131–1143.
  18. Detloff MR, Fisher LC, McGaughy V, Longbrake EE, Popovich PG and Basso DM. Activation of microglia and pro-inflammatory cytokines predict the onset and severity of below-level neuropathic pain after spinal cord injury in rats. *Exp Neurol* 2008; 212: 337–347.
  19. Chhaya SJ, Quiros-Molina D, Tamashiro-Orrego AD, Houlié JD and Detloff MR. Exercise-induced changes to the macrophage response in the dorsal root ganglia prevent neuropathic pain after spinal cord injury. *J Neurotrauma* 2019; 36: 877–890.
  20. Belkouch M, Dansereau M-A, Réaux-Le Goazigo A, Van Steenwinckel J, Beaudet N, Chraïbi A, Melik-Parsadaniantz S and Sarret P. The chemokine CCL2 increases Nav1.8 sodium channel activity in primary sensory neurons through a  $\text{g}\beta\gamma$ -dependent mechanism. *J Neurosci* 2011; 31: 18381–18390.
  21. Wu Z, Yang Q, Crook RJ, O'Neil RG and Walters ET. TRPV1 channels make major contributions to behavioral hypersensitivity and spontaneous activity in nociceptors after spinal cord injury. *Pain* 2013; 154: 2130–2141.
  22. Hameed S. Nav1.7 and Nav1.8: role in the pathophysiology of pain. *Mol Pain* 2019; 15: 1744806919858801.
  23. Ramer LM, Peter van Stolk A, Inskip JA, Ramer MS and Krassioukov AV. Plasticity of trpv1-expressing sensory neurons mediating autonomic dysreflexia following spinal cord injury. *Front Physiol* 2012; 3: 1–16.
  24. Finnerup NB, Pedersen LH, Terkelsen AJ, Johannesen IL and Jensen TS. Reaction to topical capsaicin in spinal cord injury patients with and without central pain. *Exp Neurol* 2007; 205: 190–200.
  25. Zhang G and Yang P. Bioinformatics genes and pathway analysis for chronic neuropathic pain after spinal cord injury. *Biomed Res Int* 2017; 2017: 1–11.
  26. Herman P, Stein A, Gibbs K, Korsunsky I, Gregersen P and Bloom O. Persons with chronic spinal cord injury have decreased natural killer cell and increased toll-Like receptor/inflammatory gene expression. *J Neurotrauma* 2018; 35: 1819–1829.
  27. Oliveros JC. Venny: an interactive tool for comparing lists with Venn's diagrams, <https://bioinfogp.cnb.csic.es/tools/venny/index.html> (accessed 28 March 2021).
  28. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR and Ma'ayan A. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinform* 2013; 14: 128.
  29. Kirshblum SC, Burns SP, Biering-Sorensen F, Donovan W, Graves DE, Jha A, Johansen M, Jones L, Krassioukov A, Mulcahey MJ, Schmidt-Read M and Waring W. International standards for neurological classification of spinal cord injury (revised 2011). *J Spinal Cord Med* 2011; 34: 535–546.
  30. Kirshblum S, Snider B, Rupp R and Read MS. Updates of the international standards for neurologic classification of spinal cord injury: 2015 and 2019. *Phys Med Rehabil Clin N Am* 2020; 31: 319–330.
  31. Herman PE and Bloom O. Altered leukocyte gene expression after traumatic spinal cord injury: clinical implications. *Neural Regen Res* 2018/08/22. 2018; 13: 1524–1529. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/30127106>
  32. Saltzman JW, Battaglino RA, Salles L, Jha P, Sudhakar S, Garshick E, Stott HL, Zafonte R and Morse LR. B-cell maturation antigen, a proliferation-inducing ligand, and B-cell activating factor are candidate mediators of spinal cord injury-induced autoimmunity. *J Neurotrauma* 2013; 30: 434–440.
  33. McKay SM and McLachlan EM. Inflammation of rat dorsal root ganglia below a mid-thoracic spinal transection. *Neuroreport* 2004; 15: 1783–1786.
  34. Pokhilko A, Nash A and Cader MZ. Common transcriptional signatures of neuropathic pain. *Pain* 2020; 161: 1542–1554.
  35. He X, Fan L, Wu Z, He J and Cheng B. Gene expression profiles reveal key pathways and genes associated with neuropathic pain in patients with spinal cord injury. *Mol Med Rep* 2017; 15: 2120–2128.
  36. Ferrari LF, Khomula EV, Araldi D and Levine JD. CD44 signaling mediates high molecular weight hyaluronan-induced antihyperalgesia. *J Neurosci* 2018; 38: 308–321.
  37. Reyes-Gibby CC, Wang J, Silvas MRT, Yu R, Yeung S-CJ and Shete S. MAPK1/ERK2 as novel target genes for pain in head and neck cancer patients. *BMC Genet* 2016; 17: 40.
  38. Zhou J, Fan Y and Chen H. Analyses of long non-coding RNA and mRNA profiles in the spinal cord of rats using RNA sequencing during the progression of neuropathic pain in an SNI model. *RNA Biol* 2017; 14: 1810–1826.
  39. Cao S, Yuan J, Zhang D, Wen S, Wang J, Li Y and Deng W. Transcriptome changes in dorsal spinal cord of rats with neuropathic pain. *J Pain Res* 2019; 12: 3013–3023.
  40. Yang YK, Lu XB, Wang YH, Yang MM and Jiang DM. Identification crucial genes in peripheral neuropathic pain induced by spared nerve injury. *Eur Rev Med Pharmacol Sci* 2014; 18: 2152–2159.
  41. Wei L, He F, Zhang W, Chen W and Yu B. Identification of critical genes associated with spinal cord injury based on the gene expression profile of spinal cord tissues from trkB.T1 knockout mice. *Mol Med Rep* 2019; 19: 2013–2020.
  42. Yu H, Liu Y, Li C, Wang J, Yu B and Wu Q. Bioinformatic analysis of neuroimmune mechanism of neuropathic pain. *Biomed Res Int* 2020; 2020: 1–10.
  43. Wang Y, Lin Y-H, Wu Y, Yao Z-F, Tang J, Shen L, Wang R, Ding S-Q, Hu J-G and Lü H-Z. Expression and cellular localization of IFITM1 in normal and injured rat spinal cords. *J Histochem Cytochem* 2018; 66: 175–187.

44. Zhao J, Chen C, Xiao J-R, Wei H-F, Zhou X-h, Mao X-X, Zhang W-d, Qian R, Chen X-l, He M-q, Yu X-W and Zhao J. An up-regulation of IRF-1 after a spinal cord injury: Implications for neuronal apoptosis. *J Mol Neurosci* 2015; 57: 595–604.
45. Jensen MP, Widerström-Noga E, Richards JS, Finnerup NB, Biering-Sørensen F and Cardenas DD. Reliability and validity of the international spinal cord injury basic pain data set items as self-report measures. *Spinal Cord* 2010; 48: 230–238.
46. Francheschi C, Bonafe M, Valensin S, Olivieri F, De Luca M and Ottaviani E. Inflamm-aging: an evolutionary perspective on immunosenescence. *Ann N Y Acad Sci* 2006; 908: 244–254.