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### Wired for pain, shaped by the mind

*Interactions between pain and psychopathology in pediatric and adult patient populations*

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# CHAPTER 8

PROTEOMICS BASED MARKERS OF CLINICAL PAIN SEVERITY IN  
JUVENILE IDIOPATHIC ARTHRITIS

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# PROTEOMICS BASED MARKERS OF CLINICAL PAIN SEVERITY IN JUVENILE IDIOPATHIC ARTHRITIS

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## **Abstract**

**Introduction:** Juvenile idiopathic arthritis (JIA) is a cluster of autoimmune rheumatic diseases occurring in children 16 years of age or less. While it is well-known that pain may be experienced during inflammatory and non-inflammatory states, much remains ambiguous regarding the molecular mechanisms that may drive JIA pain. Thus, in this pilot study, we explored the variability of the serum proteomes in relation to pain severity in a cohort of JIA patients.

**Methods:** Serum samples from 15 JIA patients (male and female,  $12.7 \pm 2.8$  years of age) were assessed using liquid chromatography/mass spectrometry (LC/MS). Correlation analyses were performed to determine the relationships among protein levels and self-reported clinical pain severity. Additionally, how the expression of pain proteins related to markers of inflammation (Erythrocyte Sedimentation Rate (ESR)) or morphological properties of the central nervous system (subcortical volume and cortical thickness) implicated in JIA were also evaluated.

**Results:** 306 proteins were identified in the JIA cohort of which 14 were significantly ( $p < 0.05$ ) associated with clinical pain severity. Functional properties of the identified pain proteins included but were not limited to humoral immunity (IGLV3.9), inflammatory response (PRG4) and angiogenesis (ANG). Associations among pain proteins and ESR (IGHV3.9, PRG4, CST3, VWF, ALB), as well as caudate nucleus volume (BTD, AGT, IGHV3.74) and insular cortex thickness (BTD, LGALS3BP) were also observed.

**Conclusions:** The current proteomic findings suggest both inflammatory- and non-inflammatory mediated mechanisms as potential factors associated with JIA pain. Validation of these preliminary observations using larger patient cohorts and a longitudinal study design may further point to novel serologic markers of pain in JIA.

**Keywords:** Proteomics, Mass spectrometry, Juvenile idiopathic arthritis, Pain, Inflammation

## Introduction

Juvenile idiopathic arthritis (JIA) is a common childhood rheumatic illness categorized into seven subtypes and characterized by musculoskeletal joint pathology present for greater than 6 weeks<sup>1,2</sup>. One of the most frequently occurring and debilitating symptoms of JIA is pain<sup>3,4</sup>, significantly impacting quality of life<sup>5</sup>. Pain in JIA appears to have multifactorial causes and can be driven by varying mechanisms<sup>6</sup>. Pain, particularly when induced by inflammation, may be ameliorated by pharmacological approaches such as disease modifying anti-rheumatic drugs (DMARDs) and biologics (e.g., tumor necrosis factor (TNF)- $\alpha$  inhibitors). Non-pharmacological modalities, including exercise or cognitive behavioral therapy, also may be prescribed in parallel<sup>4,7-13</sup>. Notwithstanding the availability of these therapeutic options, mitigating pain in JIA remains challenging, which may in part stem from unknown, non-inflammatory pain processes that are active in some JIA patients.

Cytokine products secreted by macrophages and T cells due to an activated immune system, are considered to mediate a chronic inflammatory status and joint pathology in JIA<sup>14,15</sup>. For example, levels of interleukin (IL) 18 in serum and synovial fluid of JIA patients have been identified as marker of disease severity, and TNF- $\alpha$ , macrophage inhibitory factor (MIF), IL-1, IL-6 and members of the CC chemokine family have been reported to contribute to inflammatory responses in JIA<sup>15-17</sup>. Moreover, several autoantibodies are currently used to distinguish JIA subtypes. Yet, no biomarkers have been validated for the purposes of guiding pain treatment in JIA populations<sup>18</sup>. To this end, we have embarked on a liquid-chromatography/mass spectrometry (LC/MS)-based serum proteomic approach, where protein expression in complex fluid samples is identified and accurately quantified<sup>19-21</sup>. A proteomic approach previously led to identification of proteins associated with ion channels, receptors and signaling pathways implicated with acute and chronic pain states<sup>22</sup>. Furthermore, MS has previously been utilized to decipher protein composition in cerebrospinal fluid in patients with fibromyalgia and rheumatoid arthritis (RA)<sup>23</sup>. Although the synovial proteome in JIA patients has been investigated in prior work<sup>24,25</sup>, to our knowledge, a serum proteomics approach in JIA in the context of pain has not been carried out as of now.

The aim of this preliminary study was to identify pain proteins in JIA to gain further insight into the biological underpinnings of pain in this rheumatic condition, which in turn may set the foundation for identifying novel therapeutic targets for pain treatment. In the current report, an LC/MS-based proteomics analysis of blood serum samples was performed to identify proteins that associate with clinical pain severity in a cohort of JIA patients. Subsequently, the association was examined between pain proteins and other aspects of JIA, such as erythrocyte sedimentation rate (ESR), clinical juvenile arthritis disease activity score (cJADAS)<sup>26</sup>, and CNS morphological properties previously implicated in JIA<sup>27</sup>.

## Methods

### Study participants

Male and female JIA patients (N=15, 12.5 ± 2.8 years) were evaluated in this study (see also **Supplemental Figure 1**). This cohort was described in our prior report<sup>27</sup>. Patients were recruited from the Rheumatology Program at Boston Children's Hospital (BCH), following approval of the BCH Institutional Review Board. Written consent and assent were provided by the patients' parent or guardian and patient, respectively before participation in this investigation. Prior to blood sample collection (2 mL), patients completed the PROMIS® Numeric Rating Scale (0-10 scale), which is a self-assessment of clinical pain over the last 7 days (PROMIS; <http://www.healthmeasures.net>). In this scale, 0 corresponds to no pain and 10 is the worst pain imaginable. The cJADAS, specifically the cJADAS-10, a composite disease activity score for JIA, which does not include an acute phase reactant, was determined for each patient at the time of enrollment<sup>26</sup>. Blood sample collection and completion of the clinical pain rating questionnaire were accomplished during the same study visit. One patient did not provide a blood sample. All enrolled patients were on active treatment and underwent clinical examination to determine the presence of pain and other elements of arthritis, including joint inflammation, redness, stiffness, or tenderness. Furthermore, JIA patients were also evaluated with non-contrast MRI to detect possible bone erosion, cartilage degradation, joint space narrowing, bone marrow edema, soft-tissue edema, joint fluid, synovitis, and tenosynovitis. ESR values were available for 13 out of the

16 patients, while combined neuroimaging and musculoskeletal MRI analyses were performed as previously described in nine out of the 16 patients<sup>27</sup>.

### LC/MS analysis

A volume of 1ul serum was first diluted in 60µl of urea buffer (8M in 50mM ammonium bicarbonate buffer) and then 15µl of dithiothreitol (0.05M final concentration) was added and the samples were incubated 30 minutes at 800 rpm in room temperature. A volume of 10µl of iodoacetamide was added and an incubation was performed for 30 minutes at 800 rpm in room temperature in dark. A volume of 10ul of 0.05M DTT was added to quench the alkylation and the sample was incubated 15 minutes at room temperature. The samples were then transferred to SP3 beads mixture (1:10 protein to beads)<sup>28</sup>, previously washed twice with HPLC water. A volume of 150ul of absolute ethanol was added to the sample/bead mixture before incubation at 1,000 rpm at room temperature for 10 minutes. The mixture was then transferred to an automatic liquid handling robot instrument (Opentrons, NY) to wash the beads and remove the supernatant before adding 1µg of trypsin and incubating for 2 hours at 37°C at 1,000 rpm on thermomixer. After trypsin digestion, the samples were centrifuged 10 min at 3,220×g before acidification using 2% v/v formic acid.

A discovery-based proteomic workflow was performed with no depletion nor fractionation of the serum samples using the SP3 protocol followed by LC/MS analysis in data-dependent acquisition mode. An amount of 200ng was injected on the Evosep LC system equipped with a Pepsep column (15 cm length; ID = 150µm) connected to a timsTOF pro mass spectrometer instrument (Bruker Daltonics, Billerica, MA) using the 60 samples per day. The data were searched using MSFragger software v3.1.1 using the following parameters: a maximum of three missed cleavages, oxidation of methionine residues set as variable modification and carbamidomethylation of cysteine residues set as fixed modification (other parameters were set as default).

### Data analysis

The data were then searched to obtain the protein identification and quantification in all samples. RStudio software packages were utilized for all statistical analyses (<https://www.rstudio.com>). In the current report, our statistical analyses were focused on determining how levels of various proteins were associated with clinical pain severity, ESR, and clinical severity (as determined by cJADAS), and also, whether and how identified pain proteins were integrated with other elements of the JIA (i.e., inflammation, clinical severity and CNS properties). Proteins showing a significant correlation with clinical pain severity are subsequently referred to as 'pain proteins.' Spearman correlation coefficients were calculated to determine correlations between protein density and clinical pain severity, while Pearson's correlation coefficients were calculated to determine significant associations among continuous variables (e.g., protein levels, ESR values, and CNS morphological properties). Furthermore, as clinical pain severity is a subjective measure and clear cut off points are ill-defined, we aimed to investigate the serum proteome changes related to self-reports of pain using a more unbiased statistical approach by taking the extremes (i.e., the samples with the lowest and highest scores were sorted into two main groups generating "Low" and "High" pain JIA cohorts). Subsequently, a statistical comparison was performed using a student t-test comparing the mean difference between the High and Low pain cohorts. The results were shown by a Volcano plot with the log<sub>2</sub> mean difference on the x-axis and the -log<sub>10</sub> p-value on the y-axis.

## **Results**

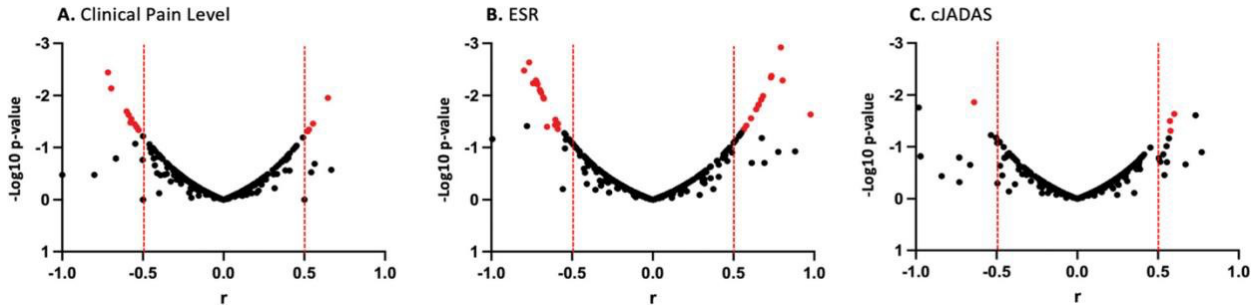
### Patient Overview

Clinical pain intensity ratings of the enrolled JIA patients ranged from 0 to 8 (mean pain intensity rating:  $3.00 \pm 2.56$ ). Furthermore, ESR values ranged from 1 to 26 (mean ESR:  $10.92 \pm 8.54$ ) and cJADAS scores were between 0 and 16 (mean score:  $5.17 \pm 5.78$ ).

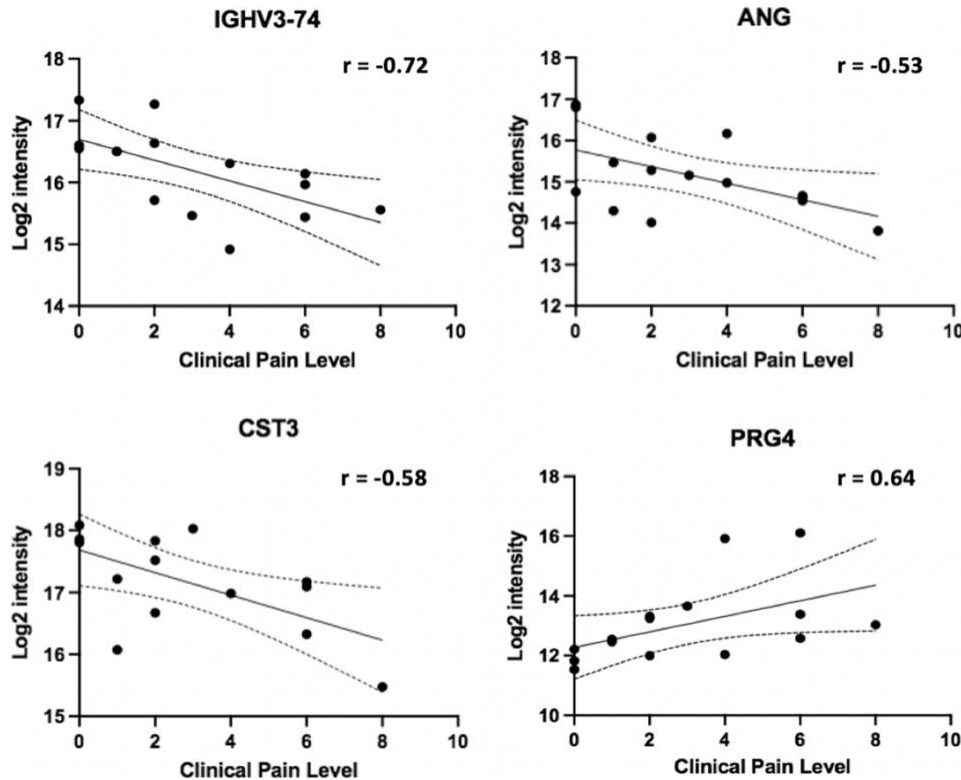
### Identification of Pain Proteins using Mass Spectrometry Analysis

High throughput serum proteomics of the samples collected from 15 JIA patients identified 306 proteins. Only proteins detected in 9 or more patients were used for further analyses. Spearman's correlation analyses between protein expression and clinical pain severity scores resulted in 14 protein that showed positive ( $r > 0.5$ ) or negative ( $r < -0.5$ ) correlations with pain levels (**Table 1, Figure 1A**). Functional roles of the 14 proteins ranged from humoral immunity,

angiogenesis, autoimmunity and enzymatic reactions (**Table 1**). Additionally, protein expression was evaluated using a grouping method based on the pain scale 0, 1, and 2 to define a 'Low' pain group and 4, 6, and 8 to define 'High' pain group (**Figure 2**). This grouping for 'Low' and 'High' demonstrated a number of 12 differentially expressed proteins (DEPs;  $p < 0.05$ ).

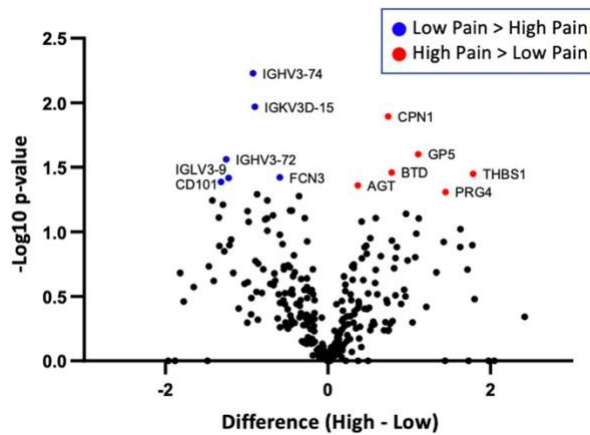


**Figure 1. Correlation Between Protein Expression and Clinical Benchmarks.** Volcano plots of protein intensity correlation to (A) pain severity, (B) erythrocyte sedimentation rate (ESR) values and (C) clinical juvenile disease activity score (cJADAS). Significantly ( $p < 0.05$ ) correlated proteins are plotted in red. Proteins passing an  $r$  value of 0.5, but not significant due to the small number of data points are shown in black. Further details on proteins significantly correlating with clinical pain levels are provided in **Table 1**.



**Figure 2. Association Between Protein Expression and Clinical Pain Severity.** Spearman correlation analyses were performed to determine the relationship between protein intensities and self-reported clinical pain severity. 95% confidence interval and Spearman correlation value ( $r$ ) are depicted in each

correlation plot. Proteins spanning distinct functional roles are depicted (i.e., IGHV3.74 (humoral immunity); ANG (angiogenesis); CST3 (inflammatory regulation); and PRG4 (articular joint, boundary lubrication)).



**Figure 3. Serum proteomics comparison between low and high pain JIA patients.** Sample grouping using scores of 0, 1, and 2 for the low pain cohort, and scores of 4, 6, and 8 for the high pain cohort was used. A comparison of protein expression was performed between low and high pain extremes or sub-cohorts of JIA patients. Blue data points represent proteins significantly ( $p < 0.05$ ) greater for the low pain group, while red data points represent proteins significantly greater for the high pain group.

#### Relationship Between Pain Proteins and ESR values and cJADAS scores

Amongst all 306 proteins identified in the serum of JIA patients, various proteins were associated with ESR (**Figure 1B**, see also **Supplemental Figure 1**). **Table 2** denotes the level of association among pain proteins and ESR levels. IGLV3.9, PRG4, CST3, VWF and ALB in particular showed significant correlation with ESR with most proteins having roles in immune-mediated responses in addition to other cellular functions (e.g., vascular remodeling and cell migration (CST3) or boundary lubrication within articulating joints (PRG4)). Significant correlation between cJADAS scores and protein levels were found for IGHV6-1 ( $r = -0.64$ ,  $P = 0.014$ ), IGHV2-70 ( $r = 0.58$ ,  $P = 0.05$ ), IGHD ( $r = 0.60$ ,  $P = 0.023$ ), and IGLC3 ( $r = 0.57$ ,  $P = 0.03$ ) (**Figure 1C**), which were primarily related to humoral immunity<sup>29</sup>. These correlation values listed above were derived based on 14 data points. A significant association between pain proteins and cJADAS scores was absent throughout all pain proteins (**Table 2**).

#### Relationship Between Pain Proteins and Central Morphological Properties

Our recent investigation utilizing the same JIA patient sample identified a significant association between caudate volume and clinical pain intensity ratings as well as a significant correlation

between cortical thickness of the insula cortex and ESR values<sup>27</sup>. Therefore, the relationship, or lack thereof, between pain proteins described above and morphological properties of the caudate nucleus and insula were explored.

The left hemisphere caudate nucleus volume was not significantly correlated with any of the pain proteins (**Table 3**). The right hemisphere caudate nucleus volume negatively correlated with BTD and AGT, while positively correlated with IGHV3.74. The left insular cortical thickness was not significantly correlated to any of the pain proteins. However, significant ( $P=0.05$ ) associations were revealed between right insular thickness values and two of the pain proteins (BTD and LGALS3BP).

## **Discussion**

There is a clear need for a deeper understanding of the complex mechanisms that underlie pain in JIA and other rheumatic conditions impacting pediatric populations. The current study revealed a number of pain proteins by characterizing the serum proteome derived from JIA patients. The identified proteins ranged in their biological functions from humoral immunity, angiogenesis, autoimmunity and enzymatic reactions. Furthermore, these preliminary results further suggest the close interactions between immune and nervous systems, and the critical role immune cells and their mediators play in regulating acute and chronic pain states<sup>30</sup>.

Many of the identified pain proteins are involved in immune processes. LGALS3BP is a heavily glycosylated secreted molecule with an immunoinhibitory function<sup>31</sup> often found to be upregulated in cancer, but has also been implicated in various other diseases (e.g., RA)<sup>32</sup>. Furthermore, the subvariants IGLV3.9, IGHG4, IGKV1.5, IGKV3D.15, IGHV3.72 and IGHV3.74 are all members of the immunoglobulin family, binding to particular antigens as part of the immune response, but specific targets of these immunoglobulins are unknown<sup>33</sup>. The identification of immunoglobulins whose abundance levels show positive correlations with pain severity is consistent with current experimental therapies, as clinical trials on binding immunoglobulin protein (BiP), targeting immunoglobulins, have been performed for treatment of RA<sup>34</sup>. Amongst an abundance of processes, CST3 is also considered to be involved with immune responses<sup>35</sup>. ANG, or angiogenin, is most often implicated in tumor-associated angiogenesis, but has been suggested to inhibit inflammatory processes<sup>36,37</sup> and to mediate local inflammation in arthritis<sup>38</sup>.

Moreover, VWF is thought to reflect vascular damage and thrombosis<sup>39</sup>. Lastly, PRG4 has been shown to play a protective factor within articular joints<sup>40</sup>, but its role in mediating anti-inflammatory processes has also been confirmed<sup>41</sup>. The association that is found between the density of these predominantly immune related proteins in serum and clinical pain severity in JIA patients, could reflect inflammatory and immune components of pain in JIA. Furthermore, five proteins (IGHV3.9, PRG4, VWF, ALB and CST3) were significantly associated with ESR values, with CST3, a protein active in neurodegenerative (Alzheimer's Disease) and demyelinating (multiple sclerosis) neurological conditions<sup>42</sup>. ESR values obtained from blood samples reflect overall inflammatory status, and do not capture isolated joint inflammation. Thus, biological samples derived from the synovial compartment of inflamed joints may better identify proteomic markers that are associated with joint inflammation and inflammatory pain.

The current sample consisted of patients who were in remission, reported pain without joint inflammation or reported pain with joint inflammation, where inflammatory status and other joint-related pathology was determined via musculoskeletal MRI. Moreover, our study confirms that pain in JIA is not always commensurate with the amount of inflammation, and also, subsets of proteins were solely associated with inflammation, but not pain. While confirmation in larger studies is necessary, we hypothesize that expression of some proteins is driven by pain and likely associated with peripheral or central sensitization, and other proteins will track more with the inflammatory status of JIA patient or JIA sub-type.

A finer assessment of pain proteins in JIA is arguably obtained by differentiating patients based on additional diagnostic criteria, which include, JIA subtype (e.g., polyarticular JIA vs. systemic JIA), rheumatoid factor (RF) status, or antinuclear antibody (ANA) status as each can be associated with distinct JIA disease trajectories or severity<sup>43</sup>. A more specific, phenotypic differentiation of JIA patients may elucidate differential proteins expression of proteins in conjunction with distinct sets of pain proteins. Likewise, comparison of patients based on treatment regimen (e.g., methotrexate vs. methotrexate + TNF- $\alpha$  inhibitor) may also yield unique proteomic signatures. Although this pilot cohort varied in terms of JIA subtype, RF and ANA status, or treatment type the limited sample size did not allow for a robust comparison across these domains. Future work will investigate the contribution of these JIA subtypes and phenotypes in a broader population and importantly include clinical control groups. The

incorporation of control cohorts is essential in order to decipher whether proteomic alterations are disease specific for JIA or pain specific.

Previous work aimed to gain more insight into the proteomic underpinnings of pain by performing a meta-analysis of 535 pain related genes in the human cerebrospinal fluid (CSF)<sup>44</sup>. These genes are typically responsible for proteins related to synaptic transmission, inflammatory responses, neuropeptide signaling, and hormonal activity. Furthermore, this study pointed to ten proteins that were potential factors for distinguishing dysfunctional (fibromyalgia) from inflammatory pain (rheumatoid arthritis) disorders. Although proteomic composition in the CSF will differ from blood serum, both tissues can be used to identify pain proteins. Considering the age of the cohort of JIA patients in this study, retrieving CSF samples was not feasible for ethical reasons. Serum protease network behavior in complex regional pain syndrome (CRPS) has also been recently investigated<sup>45,46</sup>. Here, relative to controls and other clinical pain populations, CRPS patients showed distinct degradation activity of inflammatory mediators that likely play a role in the development of post-traumatic pain. König and colleagues have specifically suggested that degradation of dabsyl-bradykinin is particularly compromised in CRPS, which subsequently drives an inflammatory process.

In prior work, JIA patients were shown to harbor altered CNS properties. Specifically, caudate nucleus volume was negatively correlated to clinical pain intensity and decreased cortical thickness of the insula was not only observed to be lower in JIA patients relative to controls, but also showed a negative correlation with ESR values<sup>27</sup>. Besides a role of the caudate nucleus in the motor system, it has also been proposed to play an important part in modulation of pain experience<sup>47</sup>. The insula, a key hub of the salience networks, plays an essential role in the regulation of emotional aspects of pain<sup>48</sup>. Correlations between both left and right caudate nucleus volume and some of the proteins were observed, and HRG was associated with caudate nucleus volume bilaterally and with right insular thickness. Left insular thickness did not correlate with any of the identified pain related protein densities, whilst right insular thickness was associated with three of the proteins, BTDR, LGALS3BP and HRG. The associations of morphological properties and pain related proteins points to an interplay between peripheral mechanisms and the CNS. Pain in JIA is multifaceted and could be underpinned by altered

proteomic composition contributing to pain and altered neurobiological properties to the emotional mediation of this pain.

With the novelty of this investigation into proteomic alterations concerning pain in JIA, limitations of this exploratory study are noted. All patients were on active treatment during study evaluation, and treatment plans varied from single immune-modulatory agents (i.e., methotrexate or adalimumab) to combination therapies (i.e., methotrexate and adalimumab). Moreover, some patients also noted intermittent use of non-steroidal anti-inflammatory drugs (e.g., naproxen). The type of therapy could have impacted the serum proteomic composition, particularly in specific immune-related proteins in the collected serum. In order to determine the influence of various therapies on the JIA proteome, future studies should characterize protein expression before and after treatment or before and after the onset of a new therapeutic regimen. Characterization of the proteome at multiple points post-treatment induction may also provide new biological insights towards treatment response vs. non-response. Patients presented with different joints with pain and arthritis, and the number of indicated joints varied prior to and during participation in the study. Additionally, the small sample size is also a limiting factor in this investigation yet provides a basis for extending this work into a larger patient sample.

## **Conclusions**

In summary, LC/MS-based serum proteomic analysis identified 14 pain proteins in the serum of JIA patients. A number of the identified proteins had functional roles in immune or inflammatory processes. This investigation provides novel insights into deviations in the proteome in relation to clinical pain in JIA patients and could contribute to the ongoing search for prognostic markers and treatment targets for JIA pain.

**Table 1.** Proteins vs. Clinical Pain Level. Proteins identified in serum of JIA patients demonstrating significant ( $p < 0.05$ ) association to clinical pain levels with corresponding function. Samples sizes of  $N = 14^*$  or  $N = 15^{**}$  were present for all proteins.

Proteins Related to Pain	Function	Spearman Correlation Coefficient (r)	P-value (two-tailed)	95% confidence interval
IGLV3.9*	Immunoglobulins: Humoral immunity	-0.70	0.007	-0.90 to -0.25
IGHG4**	Immunoglobulins: Humoral immunity	-0.60	0.020	-0.86 to -0.11
IGKV1.5**	Immunoglobulins: Humoral immunity	0.54	0.040	-0.83 to -0.023
IGKV3D.15**	Immunoglobulins: Humoral immunity	0.59	0.024	-0.85 to -0.091
IGHV3.72**	Immunoglobulins: Humoral immunity	-0.54	0.041	-0.83 to -0.018
IGHV3.74	Immunoglobulins: Humoral immunity	-0.72	0.004	-0.902 to -0.31
LGALS3BP**	Promotes integrin-mediated cell adhesion: Immune response	0.52	0.049	-0.005 to 0.82
ANG**	Role in angiogenesis and autoimmune response, suggested to play a role in immune-mediated inflammatory response	-0.53	0.046	-0.82 to -0.002
PRG4**	Role in boundary lubrication within articulating joints, involved in vesicle-mediated transport in immune responses	0.65	0.011	0.183 to 0.874
CST3*	Cystatin C: Inhibition of cysteine proteases, involved in inflammation and immune regulation, vascular remodeling and cell migration	-0.58	0.033	-0.85 to -0.050
VWF**	Hemostasis, thrombosis and vascular inflammation	-0.55	0.035	-0.84 to -0.038
ALB**	Regulate osmotic pressure	0.57	0.028	-0.84 to -0.067
AGT**	Angiotensinogen: Blood pressure and fluid and salt regulation	0.55	0.035	0.041 to 0.84
BTD**	Enzyme biotinidase: Biotin removal from food	0.53	0.045	0.005 to 0.824

**Table 2.** Pain-associated Proteins vs. ESR and cJADAS. Pain protein (see Table 1) correlation with erythrocyte sedimentation rate (ESR) and clinical juvenile disease activity score (cJADAS) in JIA patients. Correlation (r) and P-values (two-tailed) are displayed. Sample sizes (N) are denoted for each correlation analyses.

Pain Proteins	Correlation with ESR (N = 13)	Correlation with cJADAS (N = 14)
IGLV3.9	<b>r = -0.59, P = 0.04</b>	r = -0.34, P = 0.26 (N = 13)
IGHG4	r = -0.10, P = 0.76	r = -0.49, P = 0.079
IGKV1.5	r = -0.27, P = 0.37	r = -0.16, P = 0.60
IGKV3D.15	r = 0.001, P = 0.99	r = 0.011, P = 0.97
IGHV3.72	r = -0.49, P = 0.09	r = 0.12, P = 0.685
IGHV3.74	r = -0.30, P = 0.31	r = -0.02, P = 0.95
LGALS3BP	r = 0.20, P = 0.51	r = 0.31, P = 0.28
ANG	r = 0.02, P = 0.95	r = -0.36, P = 0.21
PRG4	<b>r = 0.65, P = 0.02</b>	r = -0.27, P = 0.35
CST3	<b>r = -0.74, P = 0.01</b>	r = -0.18, P = 0.56 (N = 13)
VWF	<b>r = -0.72, P = 0.01</b>	r = 0.10, P = 0.73
ALB	<b>r = -0.70, P = 0.01</b>	r = 0.09, P = 0.75
AGT	r = 0.16, P = 0.59	r = -0.02, P = 0.95
BTD	r = 0.13, P = 0.66	r = 0.05, P = 0.86

**Table 3.** Pain-associated Proteins vs. Central Morphological Properties. Pain protein (see Table 1) correlation with caudate nucleus volume and insular thickness values in JIA patients. Pearson correlation  $r$  values and corresponding  $P$ -values (two-tailed) are displayed. Sample size is indicated for each correlation pair.

Pain Proteins	Left Caudate Nucleus Volume (N = 9)	Right Caudate Nucleus Volume (N = 9)	Left Insular Thickness (N = 9)	Right Insular Thickness (N = 9)
IGLV3.9	$r = 0.56, P = 0.12$	$r = 0.64, P = 0.06$	$r = 0.11, P = 0.77$	$r = 0.54, P = 0.14$
IGHG4	$r = 0.41, P = 0.27$	$r = 0.30, P = 0.43$	$r = 0.05, P = 0.90$	$r = 0.11, P = 0.77$
IGKV1.5	$r = 0.13, P = 0.75$	$r = 0.27, P = 0.49$	$r = 0.27, P = 0.48$	$r = 0.43, P = 0.24$
IGKV3D.15	$r = 0.58, P = 0.10$	$r = 0.64, P = 0.06$	$r = -0.25, P = 0.51$	$r = 0.31, P = 0.42$
IGHV3.72	$r = 0.41, P = 0.28$	$r = 0.435, P = 0.242$	$r = 0.15, P = 0.70$	$r = 0.14, P = 0.72$
IGHV3.74	$r = 0.63, P = 0.07$	<b><math>r = 0.70, P = 0.04</math></b>	$r = -0.13, P = 0.73$	$r = 0.52, P = 0.15$
LGALS3BP	$r = -0.63, P = 0.07$	$r = -0.64, P = 0.07$	$r = -0.61, P = 0.09$	<b><math>r = -0.75, P = 0.02</math></b>
ANG	$r = 0.44, P = 0.24$	$r = 0.44, P = 0.23$	$r = 0.47, P = 0.19$	$r = 0.54, P = 0.14$
PRG4	$r = -0.53, P = 0.14$	$r = -0.52, P = 0.15$	$r = -0.25, P = 0.51$	$r = -0.24, P = 0.53$
CST3	$r = -0.02, P = 0.95$	$r = 0.03, P = 0.95$	$r = 0.65, P = 0.06$	$r = 0.28, P = 0.47$
VWF	$r = 0.30, P = 0.43$	$r = 0.33, P = 0.39$	$r = 0.36, P = 0.34$	$r = 0.17, P = 0.67$
ALB	$r = 0.57, P = 0.11$	$r = 0.62, P = 0.07$	$r = 0.13, P = 0.74$	$r = 0.26, P = 0.50$
BTD	$r = -0.67, P = 0.051$	<b><math>r = -0.78, P = 0.01</math></b>	$r = -0.17, P = 0.66$	<b><math>r = -0.69, P = 0.04</math></b>
AGT	$r = -0.61, P = 0.082$	<b><math>r = -0.73, P = 0.03</math></b>	$r = 0.15, P = 0.70$	$r = -0.45, P = 0.22$

## References

1. Petty RE, Southwood TR, Manners P, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol*. 2004;31(2):390-392.
2. Prakken B, Albani S, Martini A. Juvenile idiopathic arthritis. *Lancet Lond Engl*. 2011;377(9783):2138-2149. doi:10.1016/S0140-6736(11)60244-4
3. Tollisen A, Selvaag AM, Aulie HA, et al. Physical Functioning, Pain, and Health-Related Quality of Life in Adults With Juvenile Idiopathic Arthritis: A Longitudinal 30-Year Followup Study. *Arthritis Care Res*. 2018;70(5):741-749. doi:10.1002/acr.23327
4. Barth S, Haas JP, Schlichtiger J, et al. Long-Term Health-Related Quality of Life in German Patients with Juvenile Idiopathic Arthritis in Comparison to German General Population. *PLoS One*. 2016;11(4):e0153267. doi:10.1371/journal.pone.0153267
5. Rebane K, Orenius T, Ristolainen L, et al. Pain interference and associated factors in young adults with juvenile idiopathic arthritis. *Scand J Rheumatol*. 2019;48(5):408-414. doi:10.1080/03009742.2019.1596308
6. La Hausse de Lalouvière L, Ioannou Y, Fitzgerald M. Neural mechanisms underlying the pain of juvenile idiopathic arthritis. *Nat Rev Rheumatol*. 2014;10(4):205-211. doi:10.1038/nrrheum.2014.4
7. Schanberg LE, Anthony KK, Gil KM, Maurin EC. Daily pain and symptoms in children with polyarticular arthritis. *Arthritis Rheum*. 2003;48(5):1390-1397. doi:10.1002/art.10986
8. Vanoni F, Minoia F, Malattia C. Biologics in juvenile idiopathic arthritis: a narrative review. *Eur J Pediatr*. 2017;176(9):1147-1153. doi:10.1007/s00431-017-2960-6
9. Giancane G, Consolaro A, Lanni S, Davi S, Schiappapietra B, Ravelli A. Juvenile Idiopathic Arthritis: Diagnosis and Treatment. *Rheumatol Ther*. 2016;3(2):187-207. doi:10.1007/s40744-016-0040-4
10. Weiss JE, Luca NJC, Boneparth A, Stinson J. Assessment and management of pain in juvenile idiopathic arthritis. *Paediatr Drugs*. 2014;16(6):473-481. doi:10.1007/s40272-014-0094-0
11. Rashid A, Cordingley L, Carrasco R, et al. Patterns of pain over time among children with juvenile idiopathic arthritis. *Arch Dis Child*. 2018;103(5):437-443. doi:10.1136/archdischild-2017-313337
12. Tarakci E, Yeldan I, Kaya Mutlu E, Baydogan SN, Kasapcopur O. The relationship between physical activity level, anxiety, depression, and functional ability in children and adolescents with juvenile idiopathic arthritis. *Clin Rheumatol*. 2011;30(11):1415-1420. doi:10.1007/s10067-011-1832-0
13. Morgan EM, Mara CA, Huang B, et al. Establishing clinical meaning and defining important differences for Patient-Reported Outcomes Measurement Information System (PROMIS®) measures in juvenile idiopathic arthritis using standard setting with patients, parents, and providers. *Qual Life Res Int J Qual Life Asp Treat Care Rehabil*. 2017;26(3):565-586. doi:10.1007/s11136-016-1468-2
14. Rochette E, Duché P, Merlin E. Juvenile idiopathic arthritis and physical activity: possible inflammatory and immune modulation and tracks for interventions in young populations. *Autoimmun Rev*. 2015;14(8):726-734. doi:10.1016/j.autrev.2015.04.007
15. Lotito APN, Campa A, Silva CAA, Kiss MHB, Mello SBV. Interleukin 18 as a marker of disease activity and severity in patients with juvenile idiopathic arthritis. *J Rheumatol*. 2007;34(4):823-830.
16. de Jager W, Hoppenreijns EPAH, Wulfraat NM, Wedderburn LR, Kuis W, Prakken BJ. Blood and synovial fluid cytokine signatures in patients with juvenile idiopathic arthritis: a cross-sectional study. *Ann Rheum Dis*. 2007;66(5):589-598. doi:10.1136/ard.2006.061853
17. Lin YT, Wang CT, Gershwin ME, Chiang BL. The pathogenesis of oligoarticular/polyarticular vs systemic juvenile idiopathic arthritis. *Autoimmun Rev*. 2011;10(8):482-489. doi:10.1016/j.autrev.2011.02.001
18. Durland CL, Wedderburn LR. Current developments in the use of biomarkers for juvenile idiopathic arthritis. *Curr Rheumatol Rep*. 2014;16(3):406. doi:10.1007/s11926-013-0406-3
19. Husi H, Albalat, A. *Handbook of Pharmacogenomics and Stratified Medicine*. Vol 147–179.; 2014.
20. Aebersold R, Mann M. Mass spectrometry-based proteomics. *Nature*. 2003;422(6928):198-207. doi:10.1038/nature01511

21. Domon B, Aebersold R. Mass spectrometry and protein analysis. *Science*. 2006;312(5771):212-217. doi:10.1126/science.1124619
22. Gomez-Varela D, Barry AM, Schmidt M. Proteome-based systems biology in chronic pain. *J Proteomics*. 2019;190:1-11. doi:10.1016/j.jprot.2018.04.004
23. Khoonsari PE, Ossipova E, Lenggqvist J, et al. The human CSF pain proteome. *J Proteomics*. 2019;190:67-76. doi:10.1016/j.jprot.2018.05.012
24. Ling XB, Park JL, Carroll T, et al. Plasma profiles in active systemic juvenile idiopathic arthritis: Biomarkers and biological implications. *Proteomics*. 2010;10(24):4415-4430. doi:10.1002/pmic.201000298
25. Brescia AC, Simonds MM, Sullivan KE, Rose CD. Secretion of pro-inflammatory cytokines and chemokines and loss of regulatory signals by fibroblast-like synoviocytes in juvenile idiopathic arthritis. *Proteomics Clin Appl*. 2017;11(5-6). doi:10.1002/prca.201600088
26. Consolaro A, Ruperto N, Bazso A, et al. Development and validation of a composite disease activity score for juvenile idiopathic arthritis. *Arthritis Rheum*. 2009;61(5):658-666. doi:10.1002/art.24516
27. Upadhyay J, Lemme J, Cay M, et al. A multidisciplinary assessment of pain in juvenile idiopathic arthritis. *Semin Arthritis Rheum*. 2021;51(4):700-711. doi:10.1016/j.semarthrit.2021.05.011
28. Hughes CS, Foehr S, Garfield DA, Furlong EE, Steinmetz LM, Krijgsveld J. Ultrasensitive proteome analysis using paramagnetic bead technology. *Mol Syst Biol*. 2014;10:757. doi:10.15252/msb.20145625
29. Struyf S, Burdick MD, Proost P, Van Damme J, Strieter RM. Platelets release CXCL4L1, a nonallelic variant of the chemokine platelet factor-4/CXCL4 and potent inhibitor of angiogenesis. *Circ Res*. 2004;95(9):855-857. doi:10.1161/01.RES.0000146674.38319.07
30. Raof R, Willems HLDM, Eijkelkamp N. Divergent roles of immune cells and their mediators in pain. *Rheumatol Oxf Engl*. 2018;57(3):429-440. doi:10.1093/rheumatology/kex308
31. Läubli H, Alisson-Silva F, Stanczak MA, et al. Lectin galactoside-binding soluble 3 binding protein (LGALS3BP) is a tumor-associated immunomodulatory ligand for CD33-related Siglecs. *J Biol Chem*. 2014;289(48):33481-33491. doi:10.1074/jbc.M114.593129
32. Ohshima S, Kuchen S, Seemayer CA, et al. Galectin 3 and its binding protein in rheumatoid arthritis. *Arthritis Rheum*. 2003;48(10):2788-2795. doi:10.1002/art.11287
33. Kazeeva TN, Shevelev AB. Unknown functions of immunoglobulins A. *Biochem Biokhimiia*. 2007;72(5):485-494. doi:10.1134/s0006297907050045
34. Kirkham B, Chaabo K, Hall C, et al. Safety and patient response as indicated by biomarker changes to binding immunoglobulin protein in the phase I/IIA RAGULA clinical trial in rheumatoid arthritis. *Rheumatol Oxf Engl*. 2016;55(11):1993-2000. doi:10.1093/rheumatology/kew287
35. Staun-Ram E, Miller A. Cathepsins (S and B) and their inhibitor Cystatin C in immune cells: modulation by interferon- $\beta$  and role played in cell migration. *J Neuroimmunol*. 2011;232(1-2):200-206. doi:10.1016/j.jneuroim.2010.10.015
36. Hooper LV, Stappenbeck TS, Hong CV, Gordon JI. Angiogenins: a new class of microbicidal proteins involved in innate immunity. *Nat Immunol*. 2003;4(3):269-273. doi:10.1038/ni888
37. Lee SH, Kim KW, Min KM, Kim KW, Chang SI, Kim JC. Angiogenin reduces immune inflammation via inhibition of TANK-binding kinase 1 expression in human corneal fibroblast cells. *Mediators Inflamm*. 2014;2014:861435. doi:10.1155/2014/861435
38. Lioté F, Champy R, Moenner M, Boval-Boizard B, Badet J. Elevated angiogenin levels in synovial fluid from patients with inflammatory arthritis and secretion of angiogenin by cultured synovial fibroblasts. *Clin Exp Immunol*. 2003;132(1):163-168. doi:10.1046/j.1365-2249.2003.02117.x
39. Gagnano F, Sperlongano S, Golia E, et al. The Role of von Willebrand Factor in Vascular Inflammation: From Pathogenesis to Targeted Therapy. *Mediators Inflamm*. 2017;2017:5620314. doi:10.1155/2017/5620314

40. Novince CM, Koh AJ, Michalski MN, et al. Proteoglycan 4, a novel immunomodulatory factor, regulates parathyroid hormone actions on hematopoietic cells. *Am J Pathol.* 2011;179(5):2431-2442. doi:10.1016/j.ajpath.2011.07.032
41. Alquraini A, Garguilo S, D'Souza G, et al. The interaction of lubricin/proteoglycan 4 (PRG4) with toll-like receptors 2 and 4: an anti-inflammatory role of PRG4 in synovial fluid. *Arthritis Res Ther.* 2015;17:353. doi:10.1186/s13075-015-0877-x
42. Maniwa K, Yano S, Sheikh AM, et al. Association between cystatin C gene polymorphism and the prevalence of white matter lesion in elderly healthy subjects. *Sci Rep.* 2020;10(1):4688. doi:10.1038/s41598-020-61383-7
43. Hügler B, Hinze C, Lainka E, Fischer N, Haas JP. Development of positive antinuclear antibodies and rheumatoid factor in systemic juvenile idiopathic arthritis points toward an autoimmune phenotype later in the disease course. *Pediatr Rheumatol Online J.* 2014;12:28. doi:10.1186/1546-0096-12-28
44. Ultsch A, Kringel D, Kalso E, Mogil JS, Lötsch J. A data science approach to candidate gene selection of pain regarded as a process of learning and neural plasticity. *Pain.* 2016;157(12):2747-2757. doi:10.1097/j.pain.0000000000000694
45. König S, Bayer M, Dimova V, et al. The serum protease network-one key to understand complex regional pain syndrome pathophysiology. *Pain.* 2019;160(6):1402-1409. doi:10.1097/j.pain.0000000000001503
46. König S, Schlereth T, Birklein F. Molecular signature of complex regional pain syndrome (CRPS) and its analysis. *Expert Rev Proteomics.* 2017;14(10):857-867. doi:10.1080/14789450.2017.1366859
47. Wunderlich AP, Klug R, Stuber G, Landwehrmeyer B, Weber F, Freund W. Caudate nucleus and insular activation during a pain suppression paradigm comparing thermal and electrical stimulation. *Open Neuroimaging J.* 2011;5:1-8. doi:10.2174/1874440001105010001
48. Craig ADB. How do you feel--now? The anterior insula and human awareness. *Nat Rev Neurosci.* 2009;10(1):59-70. doi:10.1038/nrn2555