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RESEARCH REPORT

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Plasma proteomic analysis on neuropathic pain in idiopathic peripheral neuropathy patients

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Abstract

Background and Aims: Why only half of the idiopathic peripheral neuropathy (IPN) patients develop neuropathic pain remains unknown. By conducting a proteomics analysis on IPN patients, we aimed to discover proteins and new pathways that are associated with neuropathic pain.

Methods: We conducted unbiased mass-spectrometry proteomics analysis on blood plasma from 31 IPN patients with severe neuropathic pain and 29 IPN patients with no pain, to investigate protein biomarkers and protein–protein interactions associated with neuropathic pain. Univariate modeling was done with linear mixed modeling (LMM) and corrected for multiple testing. Multivariate modeling was performed using elastic net analysis and validated with internal cross-validation and bootstrapping.

Results: In the univariate analysis, 73 proteins showed a *p*-value <.05 and 12 proteins showed a *p*-value <.01. None were significant after Benjamini–Hochberg adjustment for multiple testing. Elastic net analysis created a model containing 12 proteins with reasonable discriminatory power to differentiate between painful and painless IPN

(false-negative rate 0.10, false-positive rate 0.18, and an area under the curve 0.75). Eight of these 12 proteins were clustered into one interaction network, significantly enriched for the complement and coagulation pathway (Benjamini–Hochberg adjusted p-value = .0057), with complement component 3 (C3) as the central node. Bootstrap validation identified insulin-like growth factor-binding protein 2 (IGFBP2), complement factor H-related protein 4 (CFHR4), and ferritin light chain (FTL), as the most discriminatory proteins of the original 12 identified.

Interpretation: This proteomics analysis suggests a role for the complement system in neuropathic pain in IPN.

KEYWORDS
complement, neuropathy, pain, proteomics

1 | INTRODUCTION

Distal, symmetric, axonal polyneuropathy is one of the most common neurological disorders. Its prevalence is estimated at 5.5% in the general population and higher percentages in the elderly.[1] Although over 100 different etiologies are known to cause peripheral polyneuropathy, in about 40%–50% of patients no cause can be identified despite extensive laboratory testing.[1,2] The most common symptoms associated with polyneuropathy are paresthesias, dysesthesias, numbness, and neuropathic pain.[3] About half of all patients with idiopathic peripheral neuropathy (IPN) experience neuropathic pain[4,5] with different intensity levels. However, it remains unknown why only half of patients develop pain, and there are no known biomarkers associated with neuropathic pain in this population. We hypothesized that certain proteins in plasma could be associated with higher levels of neuropathic pain in IPN patients. We used exploratory proteomic analysis to evaluate if any difference in protein expression or interaction can be detected in patient cohorts with or without neuropathic pain.

2 | MATERIALS AND METHODS

2.1 | Research registry

The peripheral neuropathy research registry (PNRR) is a large repository of data and biospecimens from patients with either idiopathic, diabetic, chemotherapy, or HIV-induced sensory polyneuropathy.[6] The collected dataset includes a neurological examination, nerve conduction studies, relevant laboratory testing results, standardized symptom questionnaire, as well as the medical and family history of each participant at the time of enrollment.[6] In addition, serum, plasma, and DNA have been collected for each participant enrolled since 2015. All plasma samples utilized in this research were collected from patients who were enrolled in PNRR at these six consortia member sites: Johns Hopkins University School of Medicine, Icahn School of Medicine at Mount Sinai Medical Center, Beth Israel Deaconess Medical Center, Northwestern University Feinberg School of Medicine, University of Utah School of Medicine and University of Kansas Medical Center.

2.2 | Patient cohort

Patients with painful and non-painful IPN were included in this study. IPN was defined as the presence of an axonal sensory predominant peripheral polyneuropathy with negative family history, no alcohol abuse, and negative laboratory testing for the most common causes of PN, such as diabetes mellitus, kidney disease, vitamin B12 deficiency, and paraproteins. IPN patients that did not endorse pain symptoms in the patient questionnaire (0 on a 0–10 Likert scale) and had no prescription for pain medications were categorized as having non-painful IPN. Patients were characterized as having painful IPN if they reported a pain intensity of 6 or more on the 0–10 Likert scale and were taking at least one medication for neuropathic pain such as duloxetine, gabapentin, pregabalin, or opioids. Patients who reported high-intensity neuropathic pain without a prescription for pain medication were excluded, as were patients who did not endorse experiencing neuropathic pain but had prescriptions for neuropathic pain medications. These stringent criteria were applied to optimize the difference in pain intensity between the two groups and reduce the chance of false-negative results.

The study was approved by each site's institutional review board and all patients provided written informed consent.

2.3 | Proteomic profiling

Protein depletion: 85%–95% of the six most abundant plasma proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) were removed using the Multiple Affinity Removal Column (Hu-6, Human, 4.6 × 50 mm, Agilent), in conjunction with an Agilent 1200 high-performance liquid chromatography (HPLC). Flow-through fractions were combined and concentrated on a 3 kDa molecular weight spin cartridge (Amicon Ultra, Millipore). The protein amount was calculated with an AlphaSpec based on the absorbance at 280 nm.

2.3.1 | Trichloroacetic acid (TCA)/acetone precipitation

Proteins (50 μg) were reduced with 50 mM Dithiothreitol in 10 mM Triethylammonium bicarbonate (TEAB) at 60°C for 45 min followed by alkylating with 100 mM Iodoacetamide in 10 mM TEAB at room temperature in the dark for 15 min. Mass spectrometry (MS) interfering reagents were removed by precipitating 50 μg proteins by adding 8 volumes of 10% trichloroacetic acid in cold acetone at −20°C for 2 h. The pellet was centrifuged at 16000*g* for 10 min at 4°C. The TCA/Acetone supernatant was removed, and the protein pellet was washed with an equivalent 8 volumes of acetone at −20°C for 10 min prior to centrifuging at 16000*g* for 10 min at 4°C. The acetone supernatant was removed from the protein pellet.

2.3.2 | Isobaric mass tag labeling

The 10 protein pellets (50 μg) were resuspended and digested overnight at 37°C in 100 μL 100 mM TEAB with 5 μg Trypsin/Lys-C per sample. Each sample was labeled with a unique tandem mass tag (TMT) 10-plex reagent (Thermo Fisher, Lot # TK271715) according to the manufacturer's instructions. All 10 TMT-labeled peptides were combined and dried by vacuum centrifugation.

2.3.3 | Peptide fractionation

The combined TMT-labeled peptides (500 μg) were re-constituted in 100 μL 200 mM TEAB buffer and filtered through Pierce Detergent removal columns (Fisher Scientific PN 87777) to remove excess TMT label, small molecules, and lipids. Peptides in the flow through were diluted to 2 mL in 10 mM TEAB in water and loaded on a XBridge C18 Guard Column (5 μm, 2.1 × 10 mm, Waters) at 250 μL/min for 8 min prior to fractionation on a XBridge C18 Column (5 μm, 2.1 × 100 mm column (Waters) using a 0%–90% acetonitrile in 10 mM TEAB gradient over 85 min at 250 μL/min on an Agilent 1200 series capillary HPLC with a micro-fraction collector. Eighty-four 250 μL fractions were collected and concatenated into 24 fractions, as previously published by Wang et al in 2011,[7] and dried.

Mass spectrometry analysis: Peptides in each of the 24 fractions were analyzed on an Orbitrap-Fusion Lumos (Thermo Fisher Scientific) interfaced with an Easy-nLC1100 UPLC by reversed-phase chromatography using a 2%–90% acetonitrile in 0.1% formic acid gradient over 110 min at 300 nL/min on an in house packed 75 μm × 150 mm ReproSIL-Pur-120-C18-AQ column 3 μm, 120 Å (Dr. Albin Maisch, Germany). Eluting peptides were sprayed into the mass spectrometer through a 1 μm emitter tip (New Objective) at 2.4 kV. Survey scans (MS) of precursor ions were acquired from 350–1400 m/z at 120000 resolution at 200 m/z. Precursor ions were individually isolated within 0.7 m/z by data-dependent monitoring and 15 s dynamic exclusion and fragmented using a higher energy collisional dissociation (HCD) activation collision energy 35. Fragmentation spectra (MS/MS) were acquired using a 1e5 automatic gain control (AGC), 250 ms maximum injection time (IT) at 50000 resolution.

Data analysis: Fragmentation spectra were processed by Proteome Discoverer v2.4 (PD2.4, ThermoFisher Scientific) and searched with Mascot v.2.8.0 (Matrix Science, London, UK) against RefSeq human database. Search criteria included trypsin enzyme, one missed cleavage, 3 ppm precursor mass tolerance, 0.01 Da fragment mass tolerance, with TMT 6Plex on N-terminus and carbamidomethylation on C as fixed and TMT 6Plex on K, oxidation on M, deamidation on N or Q as variable modifications. Peptide identifications from the Mascot searches were processed within PD2.4 using Percolator at a 5% false discovery rate confidence threshold, based on an auto-concatenated decoy database search. Peptide spectral matches (PSMs) were filtered for isolation interference <30%. Relative protein abundances of identified proteins were determined in PD2.4 from the normalized median ratio of TMT reporter ions, having signal-to-noise ratios >1.5, from all PSMs from the same protein. Technical variation in ratios from our mass spectrometry analysis is less than 10%.[8]

2.4 | Data preparation

The proteomic analysis was performed in six batches, each matched for type of IPN (5 painful and 5 non-painful), sex, age, and body mass index. In total, 2276 different proteins were analyzed.

There was a difference between the six batches in absolute values of many of the proteins. Therefore, all data were normalized in Proteome Discoverer using the Reporter and Precursor Ions Quantifier nodes for all proteins in the result file that have any protein abundance. The normalization factor is the factor of the sum of a sample and the maximum sum in all files. These data were also corrected for batch effects by scaling the abundance values of each sample so that the average of all samples is 100 using the Scaling Mode parameter in Proteome Discoverer. Also due to batch effect, multiple proteins were not detected in one or multiple complete batches. Missing proteins were consequently absent in at least 17% of cases (one batch of 10 subjects) or a multiplication of that. Because of this high percentage and this biased reason for missingness, imputation was not appropriate.

2.5 | Univariate regression

All variables were individually checked for association with pain, adjusted for age, sex, and batch. Linear mixed modeling (LMM) was used to account for the non-independence of protein levels, due to batch effect of the mass spectrometry. Proteins missing in 50% of the subjects or more, were excluded (1023 proteins included). LMM was performed with batch number as a random effect. Coefficients were calculated from the estimates. Correction for multiple testing was performed by Bonferroni correction and Benjamini–Hochberg correction.

2.6 | Elastic net analysis

In correlated datasets, a multivariable penalized regression analysis results in a more favorable tradeoff between sensitivity and false-discovery proportion compared to univariate regression methods.[9] Therefore, we applied the elastic net analysis, a variable selection method that is particularly helpful in datasets with more predictors than subjects.[10] Elastic net analysis was conducted with R-package glmnet version 4.0–2.[11] All variables were standardized to a mean of zero and a standard deviation of 1. Minimal lambda was obtained by calculating the area under the receiver operator curve (ROC) using cross-validation. Alpha was chosen as the value with the lowest mean square error. Age, sex, and batch were forced in the model by decreasing their lambda penalty factors to 0.

2.7 | Bootstrap internal validation

The elastic net analysis was validated by bootstrapping with 2000 iterations. The data were bootstrapped based on the different batches. Elastic net analysis was repeated in each iteration as described in the previous section. Proteins that were present in at least 80% of the elastic net analyses were selected as the most relevant biomarkers.

2.8 | Cross-validation

Internal validation of the elastic net model was further assessed using k-fold cross-validation. The six different batches of the mass spectrometry analysis were used as the basis for the cross-validations, where each batch was alternately left out. The remaining batches were used for training the model, the batch that was left out was used as the test set for the cross-validation ($k = 6$). Training of the model was performed by logistic regression, using the proteins from the elastic net analysis as independent variables and pain status as a dependent variable. Applying this model to the test set, pain status was predicted for the patients in this set. This was done for each batch as test set. By comparing the predicted pain status with the true pain status, the average false-positive rate and average false-negative rate of our model were determined.

2.9 | Protein network and pathway enrichment analysis

For the construction of the protein network based on protein–protein interactions, proteins from the elastic net analysis were analyzed in STRING v11.[12] This is an online accessible database that uses multiple sources of protein–protein interactions for the construction of protein networks. It calculates whether there is a significant enrichment of interactions compared to a random protein list of the same number, with comparable protein sizes and degree distribution. A minimal required interaction score was set at 0.4.

The database for annotation, visualization, and integrated discovery (DAVID v2021)[13] was used for KEGG pathway enrichment analysis. In the functional annotation chart, pathways with a Benjamini–Hochberg adjusted p-value below .05 were considered significant.

3 | RESULTS

In total, 60 patients were selected for analysis, 30 with painful and 30 with nonpainful IPN. After examination of the clinical data, one patient in the painless group was misclassified and fulfilled the criteria for the painful IPN group, resulting in 31 painful and 29 painless IPN patients. Baseline characteristics are described in Table 1.

The proteomic profiling resulted in 2276 found proteins (Table S1). Proteins with less than 50% missing ($n = 1023$ proteins) were included in the analysis. LMM was performed due to the batch-specific protein levels. LMM was corrected for age and sex, with batch number as random effect. In total, 73 proteins showed a p-value <.05 and 12 proteins showed a p-value <.01 (Table 2). None were significant after Bonferroni correction (Figure 1) or Benjamini–Hochberg correction for multiple testing. Pathway analysis on the 73 proteins showed no significant enriched pathways.

Since there was not a single protein that was associated with the occurrence of neuropathic pain, we used multivariate modeling by elastic net analysis to assess if there was a combination of proteins with a collective association with the presence of neuropathic pain. Only proteins without missing data (548 proteins) can be used in this analysis. The elastic net analysis created a model containing 12 proteins (Table 3). Internal cross-validation of these 12 proteins showed a good discriminatory power of the model to differentiate between painful and painless IPN, with a false-negative rate (FNR) of 0.10, a false-positive rate (FPR) of 0.18, and an area under the curve (AUC) of 0.75.

Network analysis of the 12 proteins showed that eight clustered in one interaction network with complement component 3 (C3) as the central node (Figure 2). This constructed network had a significant enrichment of protein–protein interactions, compared to what is expected from an equal number of random proteins ($p = 9.2 \times 10^{-11}$). Pathway enrichment analysis on the associated proteins showed the highest significant enrichment in the KEGG pathway "complement and coagulation cascades" (Benjamini–Hochberg adjusted p-value = .0057).

Bootstrap validation was used to find the most discriminatory proteins out of these 12 proteins. After 2000 bootstrap iterations of elastic net modeling, three out of the 548 proteins were found in more than 80% of the models. These three proteins, insulin-like growth factor-binding protein 2 (IGFBP2), complement factor H-related protein 4 (CFHR4), and ferritin light chain (FTL), were all present in the 12 proteins from the initial elastic net analysis. Internal cross-validation on these three proteins showed a slightly improved discriminatory power compared to the 12 proteins (FNR = 0.07, FPR = 0.18, AUC = 0.77). Based on these AUC discriminatory properties, the combination of these three proteins have a discriminatory

TABLE 1 Baseline characteristics.

	All	Painful	Painless	*p*-value
n (%)	60	31 (52)	29 (48)	
Female (%)	22 (36.7)	12 (38.7)	10 (34.5)	.73
Age in years, mean (SD)	65.0 (11.7)	63.8 (10.9)	66.3 (12.5)	.40
Disease duration, mean (SD)	8.4 (9.2)	7.4 (6.9)	9.5 (11.3)	.38
Missing (%)	1 (1.7)	0 (0)	1 (3.4)	
Height in cm, mean (SD)	176.8 (9.9)	177.5 (10.6)	176.0 (9.2)	.55
Weight in kg, mean (SD)	86.9 (18.8)	91.3 (20.5)	82.2 (15.8)	.061
BMI, mean (SD)	27.6 (4.6)	28.7 (4.9)	26.4 (4.0)	.052
Vitamin B12 level, mean (SD)	717.7 (456)	766.8 (470)	665.2 (442)	.39
HgA1C level, mean (SD)	5.4 (0.41)	5.4 (0.47)	5.4 (0.34)	.86
Missing (%)	6 (10)	2 (6.5)	4 (13.8)	
Sural nerve SNAP, mean (SD)	2.5 (1.9)	2.9 (1.8))	2.0 (2.2)	.10
Missing (%)	8 (13)	4 (13)	4 (14)	

Abbreviations: BMI, body mass index; SD, standard deviation; SNAP, sensory nerve action potential.

TABLE 2 Proteins with univariate linear mixed modeling (LMM) unadjusted *p*-value below .01 (no *p*-value below .05 after adjusting for multiple testing).

Accession	Protein	Gene	Coefficient	*p*-value
NP_001121070.1	Insulin-like growth factor II	IGF2	.50	.00035
NP_000588.2	Insulin-like growth factor-binding protein 2	IGFBP2	.31	.00054
NP_001269122.1	Ribonuclease 4	RNASE4	.70	.00085
NP_001305754.1	Beta-hexosaminidase subunit alpha	HEXA	2.06	.00086
NP_002075.2	Glutathione peroxidase 3	GPX3	.59	.0020
NP_000590.1	Insulin-like growth factor-binding protein 5	IGFBP5	.68	.0023
NP_001072993.1	Multifunctional protein ADE2	PAICS	1.51	.0034
NP_066934.1	Reversion-inducing cysteine-rich protein with Kazal motifs	RECK	.23	.0042
NP_065833.1	Kelch-like protein 42	KLHL42	1.53	.0048
NP_003869.1	Gamma-glutamyl hydrolase precursor	GGH	1.37	.0050
XP_016863203.1	Predicted: alcohol dehydrogenase 4	ADH4	1.92	.0062
NP_000886.1	Leukotriene A-4 hydrolase	LTA4H	1.19	.0094

power that is comparable to that of the total model, and thus seem to be the principal discriminatory proteins.

4 | DISCUSSION

In this proteomic study on neuropathic pain in IPN patients, we were unable to find proteins that were independently associated with severe neuropathic pain. However, we were able to create a multivariate model, containing 12 proteins that collectively exhibit a reasonable discriminatory power in distinguishing patients with painless IPN from those with severely painful IPN. Several of these proteins play a role in the immune system and the model is significantly enriched for the complement and coagulation cascade pathway. Furthermore, complement factor C3 is the central hub in the model, and one of the three principal discriminatory proteins (CFHR4) in the multivariate bootstrap analysis serves as a complement activator by binding with C-reactive protein and C3b.[14] In the univariate LMM analysis, C3 was associated with neuropathic pain (coefficient 1.50, $p = .037$, Table 2). This suggests a role for the complement system in neuropathic pain in IPN.

There is already evolving preclinical evidence that the complement system plays a pivotal role in the development of neuropathic pain in multiple forms of peripheral neuropathy. In a chemotherapy-induced peripheral neuropathy (CIPN) study on rats, paclitaxel caused complement activation, but in C3 knockout rats, mechanical allodynia after paclitaxel administration was significantly lowered compared to the wild-type rats.[15] Another study showed that the complement system, and specifically C3, was upregulated in a rat model for neuropathic pain. After plasma depletion of C3 using cobra venom factor, there was a significant decrease in pain behavior.[16] In a proteomic study of pain in mice, multiple inflammatory pathways were altered after partial sciatic nerve injury. On day one, the complement and coagulation cascade pathway was the most significant altered

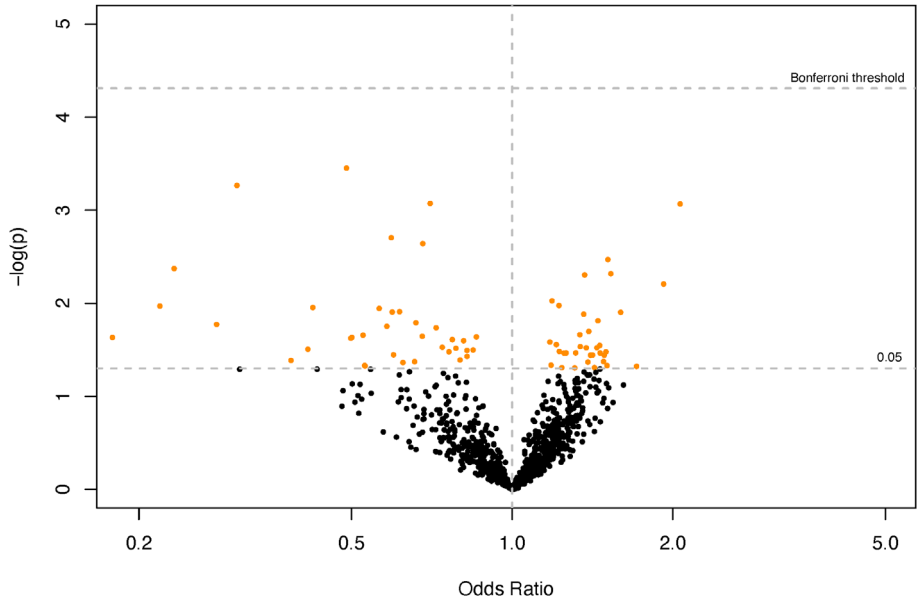

FIGURE 1 Volcano plot of the univariate LMM analysis of 1023 proteins with pain status. In total, 73 proteins had a *p*-value below .05 (orange dots above lower dotted line), but no *p*-value surpassed the Bonferroni threshold for multiple testing (upper dotted line).

TABLE 3 Proteins identified in the elastic net model, and their univariate linear mixed modeling (LMM) results.

Accession	Protein	Gene	LMM Coefficient	LMM *p*-value
NP_001290430.1	Calponin-2	CNN2	1.46	.034
NP_000055.2	Complement C3	C3	1.50	.037
NP_001188479.1	Complement factor H-related protein 4	CFHR4	.42	.011
NP_001104026.1	Filamin-A	FLNA	1.45	.067
NP_000137.2	Ferritin light chain	FTL	.62	.043
NP_001191236.1	Vitamin D-binding protein	GC	.50	.024
NP_000549.1	Hemoglobin subunit alpha 2	HBA2	1.49	.036
NP_000509.1	Hemoglobin subunit beta	HBB	1.50	.033
NP_001121070.1	Insulin-like growth factor II	IGF2	.49	.00035
NP_000588.2	Insulin-like growth factor-binding protein 2	IGFBP2	.31	.00054
NP_002611.1	Platelet factor 4 variant 1	PF4V1	1.40	.036
NP_000292.1	Plasminogen	PLG	.53	.022

pathway and C3 was one of the most significantly upregulated proteins. Transfer experiments of mouse sera to naïve mice lowered pain threshold and induced cold hypersensitivity.[17] These rodent studies support our findings that the complement system (with upregulation of C3) is associated with neuropathic pain. These studies also might indicate that complement activation is not only associated with but also is a causal factor for the development of neuropathic pain in rodents.

We found IGFBP2 as another central protein in our model. In the univariate regression analysis, it was associated with a decreased risk for neuropathic pain (coefficient 0.31, $p = .00054$), although not significant after correction for multiple testing. IGFBP2 is a potent binder of insulin-like growth factors I and II (IGF-I and IGF-II). It prevents the binding of IGFs to their receptor, IGF1R, limiting their biological effects. Apart from the regulation and growth of axons, both IGF forms play a role in the upregulation of neuropathic pain and neuroinflammatory responses by promoting the release of interleukins and tumor necrosis factor alpha (TNF-α).[18,19] IGFBP2 is capable of decreasing this neuroinflammatory response and could thereby potentially reduce the development of neuropathic pain.

Low IGFBP2 levels are reported as an independent predictor for future development of prediabetes and type 2 diabetes.[20,21] Diabetes is an important risk factor for the development of peripheral neuropathy. Although we cannot exclude the possibility that some IPN patients might develop diabetes in the future, the patients did not fulfill the criteria for diabetes or prediabetes at the time of the plasma withdrawal (based on HgA1C and/or fasting glucose). Furthermore, the mean disease duration at time of this study was 8.4 years, which makes it unlikely that patients will develop diabetes as a cause for their neuropathy.

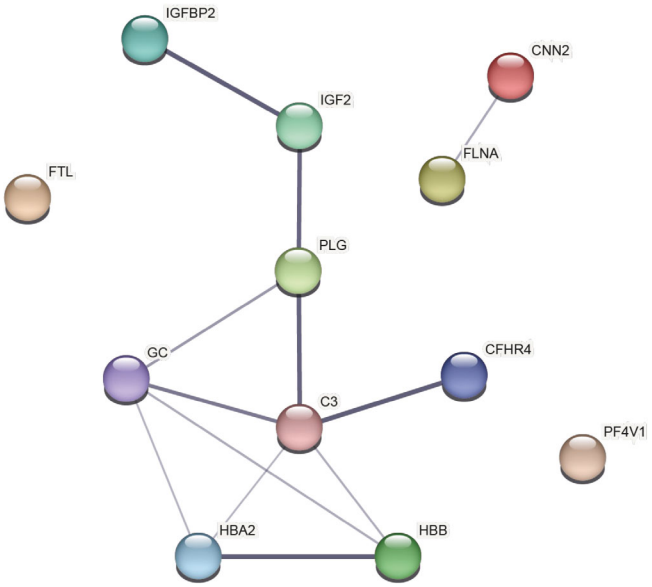

FIGURE 2 Network analysis of the proteins from the elastic net analysis, based on protein–protein interactions. Line thickness indicates strength of interaction evidence. Eight proteins formed an interaction network with C3 as the central node, having the most interactions in the model. The total network has a significant interaction enrichment ($p = 9.2 \times 10^{-11}$).

The average BMI level in the painful IPN group is somewhat higher than in the painless group, and is near-significant (Table 1, $p = .052$). An elevated BMI is one of the components of the metabolic syndrome, a condition associated with the development of peripheral neuropathy.[22] This might also play a role in the IGFBP2 association from this study, since low IGFBP2 levels are inversely associated with BMI.[23] Serum complement C3 levels have previously been associated with insulin resistance,[24] body fat percentage,[25] metabolic syndrome components and BMI.[26] It is possible that a proportion of the found associations with C3 and IGFBP2 levels are caused by potential BMI differences, although the direction of causality between BMI, C3, IGFBP2, and neuropathy remains unknown, as is whether inflammation or neuropathic pain plays a role in this association.

Ferritine light chain (FTL), the third central protein of our model, is a subunit of ferritin and is traditionally linked to iron storage. However, it was subsequently shown to have broader effects including immunomodulation.[27] Ferritin is upregulated during infection, inflammation, and malignancy. It reduces the extracellular iron levels and limits the availability for microbes.[27,28] This effect is macrophage-induced,[28] the complement system does not seem to play a role in its inflammatory effect.[29] Ferritin can also have other effects on the immune system, for instance by suppressing antibody production of B-lymphocytes and decrease phagocytosis of granulocytes.[29] The impact of FTL or ferritin on the peripheral nervous system and peripheral neuropathy remains unknown.

The mean sural sensory nerve action potential (SNAP) is higher in the painful neuropathy group compared to the painless group (respectively 2.0 and 2.9 μV) although the difference is not significant ($p = .10$). This difference is probably caused by a higher portion of small fiber neuropathy involvement in the painful neuropathy group. Small fiber damage causes neuropathic pain but does not alter the nerve conduction studies, since these measure large fiber involvement. We can expect the nerve conduction studies to be less affected (and thus higher) in the painful neuropathy group, although in this study only a non-significant trend toward a difference was found.

One important limitation of this study is the relatively small sample size ($n = 60$). An increased sample size has improved power to identify more associated proteins, especially in the univariate analysis. In this univariate analysis, no protein was significantly associated with pain status after correction for multiple testing. To avoid false-positive associations due to the many proteins tested (1023) compared to the relative low number of patients (60), we used Bonferroni adjustment, a stringent correction for multiple testing, for the univariate analysis. However, also when using more lenient adjustment methods, like the Benjamini–Hochberg correction for multiple testing, none of the individual proteins reached significance. It is possible that in a larger cohort, some of these proteins might show a significant association on univariate analysis that withstands correction for multiple testing. It might also improve the AUC, which with 0.75–0.77 is reasonable but not high enough for clinical application. A larger cohort size may also lead to the discovery of additional pathways with smaller effect sizes, providing more opportunities for potential insight into mechanisms of neuropathic pain in patients with IPN.

Another limitation is the lack of an independent replication cohort. Although we show robust internal validation with cross-validation and bootstrapping, an external validation by an independent cohort could further verify our findings.

Therapeutic development of agents that target-specific components involved with neuroinflammation is rapidly evolving.[30,31] For instance, monoclonal antibodies and receptor antagonist proteins directed at components of the complement system are currently being investigated for multiple different indications, such as chronic inflammatory demyelinating polyneuropathy (CIDP), systemic lupus erythematosus, and Guillain Barre syndrome.[31] Recently, antibodies directed at complement C3 and C5, have been approved for paroxysmal nocturnal hemoglobinuria, myasthenia gravis, and neuromyelitis optica.[32,33] Whether such complement therapies are of use for neuropathic pain remains uncertain. A follow-up proteomics study on a larger patient group would be the next step toward verifying the association with the complement system and identifying a potential optimal antibody target.

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Mamatha Pasnoor, Constantine Farmakidis, Omar Jawdat; Washington University in St. Louis School of Medicine: Sarah Yang.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[34] partner repository with the dataset identifier PXD046464.

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SUPPORTING INFORMATION

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