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Introducing Pain-Related Molecular Pathways in Painful Diabetic Neuropathy Via Protein Interaction Networks

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Abstract

Background: Painful diabetic neuropathy (PDN) is one of the most drastic complications of diabetes. Patients with PDN always reveal spontaneous and stimulus-evoked pain. However, the pathogenesis mechanisms of PDN are not entirely distinct.
Objectives: In the present study protein-protein interaction (PPI) network for PDN was constructed and analyzed to identify key proteins as potential biomarker candidates.
Methods: The transcriptomic (genes) and proteomic (proteins) data in articles that focused on PDN with differential expressions were collected. Protein networks were constructed and analyzed using STRING and Cytoscape software, respectively. Further PPI network analysis, gene ontology, and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis were performed using MCODE and DAVID tools.
Results: A total of 147 differentially regulated proteins/genes were identified in painful diabetic neuropathy, including 91 up-regulated and 56 down-regulated proteins/genes. A network analysis of genes/proteins related to PDN identified COX4I1, NDUFS8, UQCRC1, COX7C, and some other NADH dehydrogenases, including NDUFB7, NDUFS7, NDUFS3, NDUFB5, NDUFA2, and NDUFB4 as hub-bottleneck proteins. With functional enrichment analysis of network clustering, COX7C, HP, RPS12, KCNIP2, and CoL4A1 were established as distinct seed proteins in the obtained modules, which could lead to the discovery of biomarker candidates.
Conclusions: These results could provide new insights into pathology and molecular mechanisms, as well as the identification of pathways and proteins/genes involved in causing PDN in diabetic patients. COX7C, HP, RPS12, KCNIP2, and CoL4A1 are the top 5 seed nodes (hub proteins) and can serve as potential biomarker candidates and targets for PDN management. However, further investigations are needed to evaluate these proteins in detail.

Keywords: Pain, Painful Diabetes, Neuropathy, Protein Interaction Network, Hub Protein, Biomarker

1. Background

Diabetes mellitus (DM) is a serious public health problem and a devastating condition; its prevalence has increased over the past few decades. It is defined as a group of chronic metabolic dysregulation characterized by hyperglycemia resulting from the lack of insulin production, resistance to insulin action, or even both (1). According to the International Diabetes Federation, the number of diabetic people is expected to exceed 640 million by 2040 (2). Diabetic neuropathy is one of the complications of diabetes (2). Diabetic neuropathy is one of the complications of diabetes. Peripheral neuropathy is defined as peripheral nervous system disorders. Many etiological factors have been involved in the development of peripheral neuropathy, including cancer, drug toxicity, and vitamin deficiencies. The number of patients with DM is growing worldwide; it is one of the most common leading causes of neuropathy, resulting in high morbidity and mortality (3). Diabetic neuropathy includes various neuropathies, including mononeuropathy, polyneuropathy, plexopathy, and radiculopathy (4).

Diabetic neuropathy can produce both painful and non-painful forms. Painful diabetic neuropathy has been estimated to occur in 25% of patients with DM (5, 6). In this

regard, the most common form of neuropathic pain arises from type 2 diabetes mellitus (T2DM) (7). In addition, diabetic neuropathy pain (DNP) has been observed in 19% of insulin-dependent patients and 49% of those with non-insulin-dependent DM (8). As diabetes increases, DNP continues to rise with the global diabetes epidemic. Pain is the most common distressing symptom in diabetic neuropathy and mainly affects the lower limbs, including hands and feet. Also, there is a lack of safe and effective sedative drugs to control this chronic painful status. The risk factors causing painful diabetic neuropathy are not as well defined; however, the patient's age, duration of diabetes, nephropathy, peripheral vascular disease, and waist circumference are reported as possible predictors for painful neuropathy progression (9). Research on possible mechanisms involved in diabetic neuropathic pain is very complex because diabetes is a multifactorial disorder. According to the literature, diabetic peripheral neuropathy is associated with hyperglycemia and hyperlipidemia pathology (10). Additionally, diabetic peripheral neuropathy is related to demyelination and degeneration of axons, resulting in nerve dysfunction (7).

It is demonstrated that the soma of the primary afferent neurons that innervate the feet are reported to be present in the lumbar dorsal root ganglia. A dysregulated peripheral nociceptor is involved in promoting pain hypersensitivity in patients with diabetic peripheral neuropathy (11). The proposed causes of dysfunction of nociceptive neurons in the dorsal root ganglia are still being investigated. Studying and modeling complex biological systems to describe various human diseases has attracted much attention in recent years (12). Major biological processes and disease pathogenesis are mediated through physical interactions of proteins; hence, there is a requirement to discover the protein interaction network that forms these processes that leads to understanding human diseases (13). The applications of protein interaction networks allow the identification of genes and proteins related to diseases. Several omics-based investigations were performed on differentially expressed genes (DEGs) in painful diabetic peripheral neuropathy models to identify DEGs contributing to pathological processes and neuropathic pain (14-16). Additionally, network-based analysis can explain the critical genes associated with different diseases (13, 17, 18). Since the full mechanism of painful diabetic neuropathy is not clear, the protein interaction network analysis could be a promising way to manage this problem.

2. Objectives

In the present study, we collected known genes/or proteins related to painful diabetic neuropathy and constructed a network. The important proteins are highlighted as critically involved proteins in pain among patients with diabetic neuropathy.

3. Methods

3.1. Collection of Expression Data Associated with Painful Diabetic Neuropathy

The transcriptomic (genes) and proteomic (proteins) data associated with PDN (painful diabetic neuropathy) were extracted from Web of Sciences, PubMed, Google Scholar, and ScienceDirect using "Painful Diabetic Neuropathy "AND" Differential Protein OR Genes and "Expression Profiling" keywords. The differentially expressed proteins or genes were collected after a literature review and selection of related papers (14, 15, 19, 20).

3.2. Functional Annotation and Pathway Enrichment Analysis

Gene ontology (GO) categories were analyzed to identify the function of genes related to PDN. The GO analysis includes biological processes, molecular function, cellular components, and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis performed using DAVID tools.

3.3. Construction and Analysis of Protein-Protein Interaction Network Related to Painful Diabetic Neuropathy

The Uniporter accession numbers of the collected data were extracted (https://www.uniprot.org). The construction and analysis of the protein-protein interaction (PPI) network were performed using the STRING online web resource and Cytoscape software platform, respectively. The STRING database contains protein interaction data from different sources, including experimental information, computational prediction methods, and public text collections. Cytoscape is an open-source software project for visualization and integrating biomolecular interaction networks with high-throughput expression data (21). The current study analyzed the network characteristics by the Molecular Complex Detection (MCODE) plugin in Cytoscape.

4. Results

4.1. Identification of Differentially Expressed Genes

After the literature review, 4 studies were selected for this analysis. The characteristics of the included studies are shown in Table 1. According to the systematic search, 147 candidate proteins/genes were identified, which include 91 up-regulated and 56 down-regulated proteins/genes (Table 2). For those studies that were performed on rat and mouse models, only the proteins that were common in humans were selected. The threshold of a P-value < 0.05 and FC ≥ 1.5 were considered significantly differentially expressed proteins (Table 2).

4.2. Functional and Pathway Enrichment Analyses

According to Table 3, in the biological processes-associated category, the DRGs significantly enriched mitochondrial respiratory chain Complex I assembly (GO: 0032981, P-value = 2.1E-4), mitochondrial ATP synthesis coupled proton transport (GO: 0042776, P-value = 2.1E-4), aerobic respiration (GO: 0009060, P-value = 2.1E-4), etc. The molecular function annotation results of DEGs included NADH dehydrogenase (ubiquinone) activity (GO: 0008137, P-value = 1.2E-2), antigen binding (GO: 0003823, P-value = 1.2E-2), receptor binding (GO: 0005102, P-value = 5.9e-01), cytochrome-c oxidase activity (GO: 0004129, P-value = 2.8E-2), etc. In addition, the cellular component annotation indicated that the mitochondrial inner membrane (GO: 0005743, P-value = 3.0E-6), mitochondrial respiratory chain Complex I (GO: 0005747, P-value = 3.6E-6), and blood microparticle (GO: 0072562, P-value = 3.6E-6) were major enriched categories in DEGs.

The KEGG pathway analysis showed that oxidative phosphorylation (P-value = 2.7E-9), diabetic cardiomyopathy (P-value = 3.5E-8), and non-alcoholic fatty liver disease (P-value = 9.1E-7) were the mainly enriched pathways associated with DRGs in painful diabetic neuropathy, as presented in Table 4.

4.3. Identification of Hub Genes via PPI Network Analysis

After removing disconnected nodes, the STRING database yielded a PPI network with 117 nodes. The network was then analyzed in Cytoscape software, shown in Figure 1. Hub proteins were selected according to CytoHubba, and 6 distinct clusters were extracted from the network using the MCODE plugin (Figure 2A - F). The results showed that the most important hub proteins in the network included COX4I1, NDUFS8, UQCRC1, COX7C, and some other NADH dehydrogenases, including NDUFB7, NDUFS7, NDUFS3, NDUFB5, NDUFA2, and NDUFB4. The results of the functional enrichment analysis of the clusters showed that the most important pathways that the clusters (clusters 1 - 5) were involved in oxidative phosphorylation, cell cycle, complement, and coagulation cascades, ribosome, and GnRH secretion (Table 5), while no significant KEGG pathways detected for Cluster 6.

5. Discussion

Since the proteins interact with each other in the cellular pathways, many disorders result from the deregulation of proteins. The protein interaction network-based analysis is beneficial for systematically studying complex and multifactorial diseases such as cancer and DM (22). Protein-protein interactions contribute to all vital biological activities in living organisms. Identifying protein interactions in the cells is essential to reveal the function and cellular and molecular mechanisms in cells. Commonly, PPI can provide a valuable overview for a great comprehension of the functional organization of the proteome. This modern approach is now used as an efficient method to identify potential drug, therapeutic, diagnostic, and prognostic targets in various diseases (17, 23). An important advantage of network analysis is the identification of hub nodes in the protein interaction network. In the present study, the protein interaction network associated with painful diabetic neuropathy was constructed and evaluated. We extracted 147 proteins and genes with differential expression from literature and predicted the main proteins as potential biomarkers related to peripheral PDN. The top 10 nodes (hub proteins), which mostly interact with the other nodes, are represented in the result section, include COX4I1, NDUFS8, UQCRC1, COX7C, NDUFB7, NDUFS7, NDUFS3, NDUFB5, NDUFA2, and NDUFB4. These proteins were identified as the essential proteins that play critical roles in pathophysiology and cellular pathways related to pain in diabetic neuropathy.

In this study, COX4I1 and COX7C were identified as hub proteins with the highest degree. Cytochrome c oxidase (COX) is an indispensable part of mitochondrial machinery needed for ATP production in mammalian cells. In addition to 3 mitochondria-encoded subunits necessary for COX catalytic function, 11 nuclear-encoded subunits build up the COX enzyme and regulate COX enzyme activity. Cytochrome c oxidase is regulated via tissue-, development- or environment-controlled expression of subunit isoforms. The COX4 subunit is thought to optimize respiratory chain function based on the

Table 1. Characteristics of Included Studies for Expression Data of Painful Diabetic Neuropathy

Study Title	Samples	References
Integrative multi-omic analyses of dorsal root ganglia in diabetic neuropathic pain using proteomics, phosphor-proteomics, and metabolomics	Dorsal root ganglia (DRG) (L4, L5, and S1) from human	Doty et al. 2022 (15)
Transcriptomic analysis of human sensory neurons in painful diabetic neuropathy reveals inflammation and neuronal loss.	L4 and L5 ganglia from human	Hall et al. 2022 (19)
Proteomics analysis of the spinal dorsal horn in diabetic painful neuropathy rats with electroacupuncture treatment	Spinal dorsal horn sample from a rat model	Yu et al. 2021 (20)
Diabetic neuropathic pain induced by streptozotocin alters the expression profile of non-coding RNAs in the spinal cord of mice as determined by sequencing analysis.	L4-5 spinal cord tissues from mice model	He et al. 2021 (14)

Table 3. Functional Annotation of Differential Genes Associated with Painful Diabetic Neuropathy

Term	Count	Benjamini-Corrected P-Value	Fold Enrichment
GOTERM: Biological process			
GO:0032981~mitochondrial respiratory chain complex I assembly	8	2.1E-4	17.76
GO:0042776~ mitochondrial ATP synthesis coupled proton transport	8	2.1E-4	17.22
GO:0009060~ aerobic respiration	8	2.1E-4	15.99
GO:0006123~ mitochondrial electron transport, cytochrome c to oxygen	6	2.1E-4	33.57
GO:0006120~ mitochondrial electron transport, NADH to ubiquinone	7	2.1E-4	20.83
GO:0045907~ positive regulation of vasoconstriction	5	2.7E-2	18.40
GO:0045333~ cellular respiration	5	3.5E-2	16.65
GO:0071805~ potassium ion transmembrane transport	7	4.6E-2	7.53
GOTERM: Molecular function			
GO:0008137~ NADH dehydrogenase (ubiquinone) activity	8	6.0E-6	26.19
GO:0003823~ antigen binding	8	1.2E-2	7.76
GO:0005102~ receptor binding	12	1.2E-2	4.25
GO:0004129~ cytochrome-c oxidase activity	4	2.8E-2	28.16
GO:0034987~ immunoglobulin receptor binding	6	3.7E-2	8.80
GO:0004111~ creatine kinase activity	3	3.8E-2	70.40
GOTERM: Cellular component			
GO:0005743~ mitochondrial inner membrane	18	3.0E-6	3.4
GO:0005747~ mitochondrial respiratory chain complex I	8	3.6E-6	2.9
GO:0072562~ blood microparticle	11	3.6E-6	2.4
GO:0005751~ mitochondrial respiratory chain Complex IV	6	4.2E-5	1.6
GO:0045202~ synapse	14	9.2E-4	1.4
GO:0005739~ mitochondrion	25	9.2E-4	1.0
GO:0031966~ mitochondrial membrane	8	3.2E-3	1.1
GO:0070062~ extracellular exosome	30	1.1E-2	1.1
GO:0043025~ neuronal cell body	11	2.1E-2	0.8

oxygen-controlled expression of its isoforms COX4I1 and COX4I2 (24). Studies show low COX4I1 links mitochondrial dysfunction to obesity and T2DM in humans and mice (25). Dysregulation of the COX complex is related to mitochondrial oxidative stress (26). In addition, the oxidative stress condition in mitochondria is associated with obesity, metabolic syndrome, and T2DM (27). COX4I1 is suggested to be the most important regulatory subunit of COX (28). Van der Schueren et al. (25) conducted a study to investigate the association of mitochondrial

Table 4. KEGG Pathway Enrichment Analysis of Differentially Expressed Genes by the DAVID Tool [a]

KEGG Pathway	Count	Benjamini-Corrected P-Value	Fold Enrichment
Oxidative phosphorylation	15	2.7E-9	11.71
Diabetic cardiomyopathy	16	3.5E-8	8.24
Non-alcoholic fatty liver disease	13	9.1E-7	8.77
Amyotrophic lateral sclerosis	15	9.7E-7	6.76
Thermogenesis	16	3.3E-6	5.47
Huntington disease	14	3.3E-6	6.57
Chemical carcinogenesis - reactive oxygen species	17	4.3E-6	4.88
Parkinson's disease	10	2.2E-5	5.36
Prion disease	12	3.4E-5	4.36
Alzheimer's disease	10	1.1E-5	3.51
Pathways of neurodegeneration - multiple diseases	17	4.1E-4	6.36
Metabolic pathways	25	6.8E-2	1.69

Abbreviation: KEEG, Kyoto Encyclopedia of Genes and Genomes.
[a] The count threshold was $\geq$ 10.

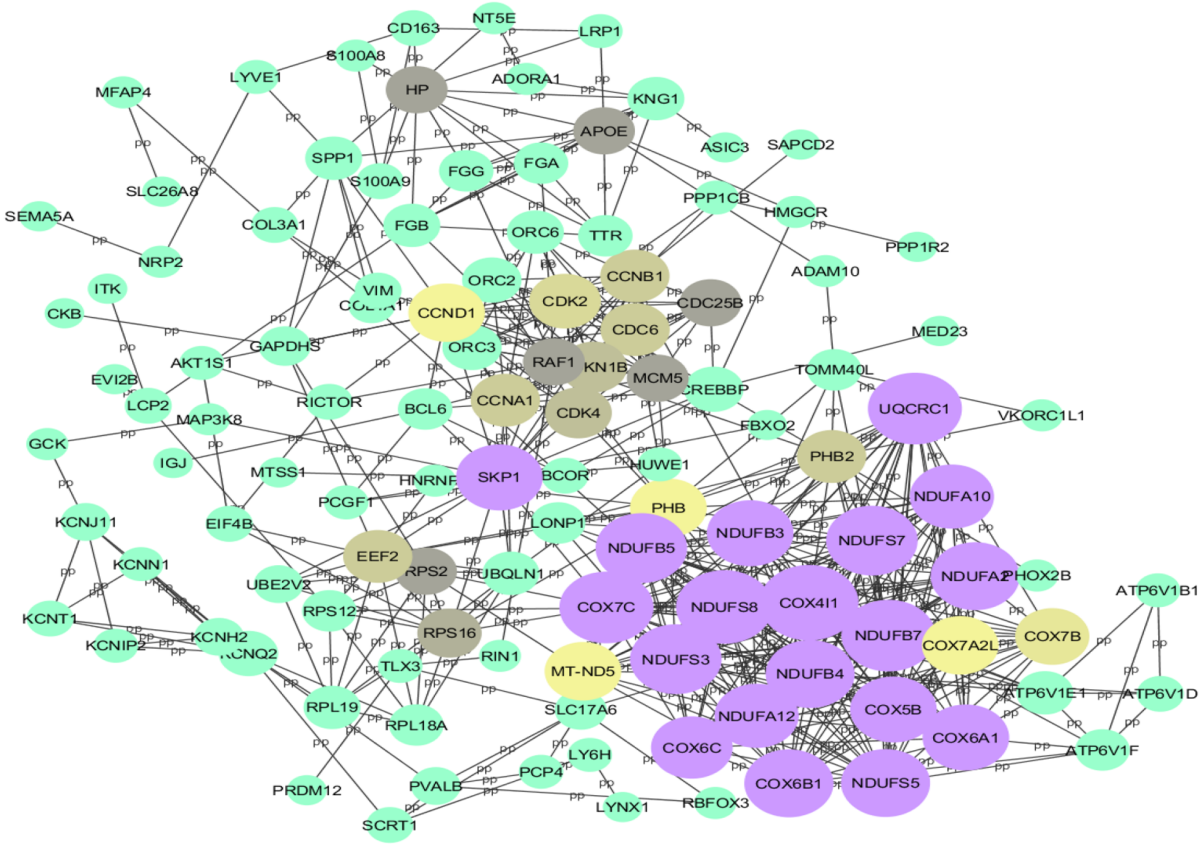

Figure 1. The protein-protein interaction network of painful diabetic neuropathy. Larger circles show nodes with higher degrees.

oxidative stress with obesity, metabolic syndrome, and T2DM and evaluate COX4I1 in peripheral blood monocytes as well as a potential biomarker for harmful metabolic development in obesity patients. They reported that COX4I1 depression is associated with insulin resistance and T2DM in obesity. Moreover, it is perhaps a helpful diagnostic biomarker in peripheral blood monocytes (25). Another study reported that low cytochrome oxidase1

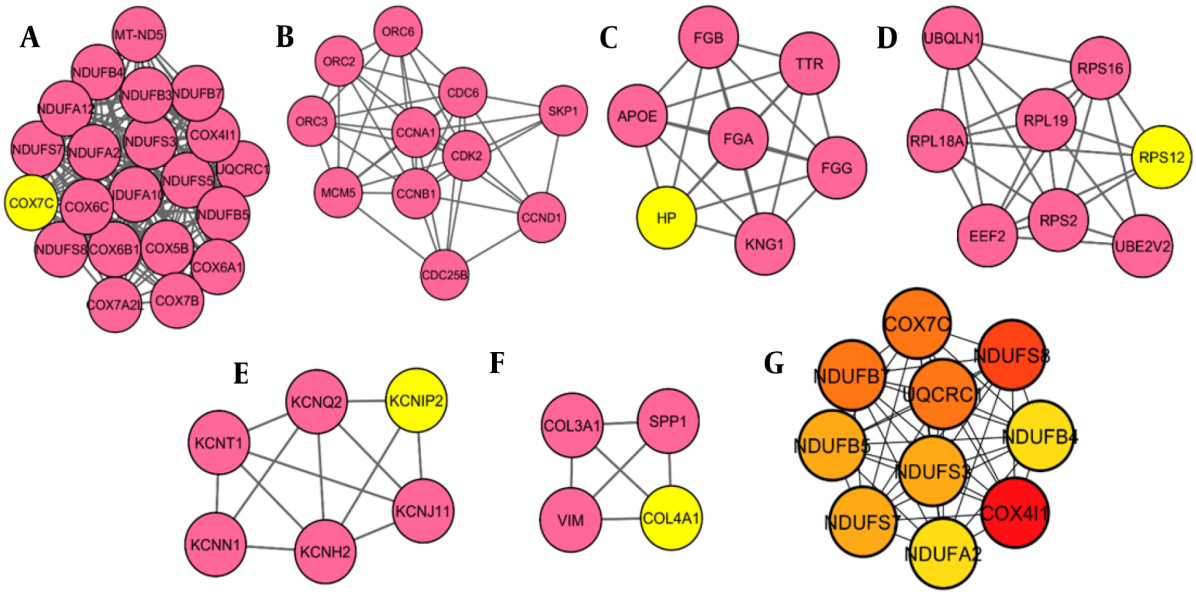

Figure 2. Network clusters extracted from MCODE. Seed nodes in each cluster are colored yellow (A-F). G: the top 10 hub nodes of the network

Table 5. Results of the Pathway Enrichment Analysis of the Network Clusters

KEGG Pathway	Count	Benjamini-Corrected P-Value
Oxidative phosphorylation (cluster 1)	21	6.5E-36
Cell cycle (cluster 2)	11	3.3E-17
Complement and coagulation cascades (cluster 3)	4	1.7E-4
Ribosome (cluster 4)	5	3.2E-5
GnRH secretion (cluster 5)	2	4.2E-2

Abbreviation: KEEG, Kyoto Encyclopedia of Genes and Genomes.

links mitochondrial dysfunction to atherosclerosis (29). Recently, a study analyzed the proteomics of the spinal dorsal horn in diabetic painful neuropathy rats, and their results indicated that COX (COX, Complex IV) factors, including COX4I1, COX5B, COX6C2, COX7B, and COX7C, were significantly up-regulated in spinal dorsal horn during PDN (20). Besides, the COX7C is not only a hub but also recognized as a seed node, which shows its importance in the pathogenesis of neuropathy as well as a potential drug target.

In our study, NDUFS8 is detected as another hub protein. The NDUFS8 protein is a subunit of NADH dehydrogenase (ubiquinone), also called Complex I, that is located in the inner membrane of mitochondria. Mutations in NDUFS8 have been associated with clinical features, including ptosis, external ophthalmoplegia, proximal myopathy, cardiomyopathy, pigmentary retinopathy, encephalopathy, and neurodegenerative disorders. Type 1 diabetes mellitus (T1DM) is an endocrine disorder characterized by destroying pancreatic β cells. This is attributed to the development of chronic diabetic complications: neurovascular and macrovascular. The development of complications is associated with various risk factors, mainly insulin resistance (30, 31) and hyperglycemia. Flotynska et al. conducted a study to evaluate NDUFS8 serum concentration as a Complex I marker and the relationship with insulin resistance in T1DM. It has been found that a higher serum concentration of NDUFS8 protein is associated with higher insulin sensitivity among adult patients with T1DM (32). In addition, the NDUFS8 gene was expressed at a high level in the skeletal muscle tissue of T2DM patients, which might indicate that increased expression of NDUFS8 can affect the glucose metabolism in the skeletal muscle

tissue, causing insulin resistance and then diabetes development. Furthermore, based on bioinformatics analysis, NDUFS8 is a potential therapeutic target (33).

Another hub protein in our analysis is UQCRC1, characterized as a subunit of Complex III in the mitochondrial respiratory chain. The functional effect of UQCRC1 mutations was investigated in several study models to assess their potential pathogenicity in the disease process. In this regard, it is demonstrated that the mitochondrial UQCRC1 mutations cause autosomal dominant Parkinsonism with polyneuropathy (34). Although the important role of mitochondria in the development of diabetes and its complications, especially neuropathy, is evident, there have been fewer studies on the physiology of this organelle in diabetic neuropathy than in other complications such as cardiomyopathy. According to this, in one study of alterations in mitochondrial physiology, the mRNA level of UQCRC1 decreased in the Diabetic Akita mouse model (35).

Interestingly, some other NADH dehydrogenases, including NDUFB7, NDUFS7, NDUFS3, NDUFB5, NDUFA2, and NDUFB4, are detected as hub proteins in our network analysis. Diabetic neuropathy is a main complication of DM that causes significant morbidity among patients with diabetes. Meanwhile, mitochondrial dysfunction and oxidative stress have been suggested as important mediators of neurodegeneration in diabetes (36). It is suggested that high glucose in tissues triggers extreme electron donation to the electron transport chain and an elevated supply of NADH in the mitochondria, leading to increased reactive oxygen species and degeneration of target tissues (37). Considering the importance of mitochondria and their cellular processes in diabetes and its complications, we found that oxidative phosphorylation is a significant pathway involved in painful diabetic neuropathy through KEGG pathway analysis. In agreement with the results obtained from our study, several studies also demonstrated that mitochondrial dysfunction occurred in neuropathy (38, 39). In the current study, 6 clusters and 5 seed nodes were also determined through protein network analysis, including COX7C, HP, RPS12, KCNIP2, and CoL4A1, which can serve as candidate biomarkers for painful diabetic neuropathy. However, further investigations are needed to evaluate these proteins in detail.

5.1. Conclusions

Our study intended to detect the main proteins and genes involved in painful diabetic neuropathy progression and identify potential biomarkers using comprehensive bioinformatics analyses. Collectively, 147 differentially regulated (91 up- and 56 down-regulated) proteins/genes were identified in painful diabetic neuropathy. Our research has provided new points into PDN pathogenesis by analyzing the DEG proteins/genes and their interactions with each other and presenting their hub proteins, pathways, and functional annotation. These proteins, including COX7C, HP, RPS12, KCNIP2, and CoL4A1, can be candidate biomarkers and targets for PDN management and potential treatment.

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Footnotes

Authors' Contribution: All of the authors participated in the design, performing the project, writing, review, editing and reading of the manuscript, and agreed to the published version of the manuscript.

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Table 2. List of the Identified Differentially Regulated Proteins/Genes in Painful Diabetic Neuropathy [a]

N	UniProt AC. No.	Gene Symbol	Direction of Regulation	N	UniProt AC. No.	Gene Symbol	Direction of Regulation
1	P02766	TTR	Up	**75**	P34910	EVI2B	Up
2	P62140	PPP1CB	Up	**76**	P41236	PPP1R2	Up
3	P31213	SRD5A2	Up	**77**	E1BB50	CDK12	Up
4	Q969M1	TOMM40L	Up	**78**	P08670	VIM	Up
5	Q96RN1	SLC26A8	Up	**79**	Q2TB10	ZNF800	Up
6	Q86Y07	VRK2	Up	**80**	P10451	SPP1	Up
7	P35557	GCK	Up	**81**	Q9C0I1	MTMR12	Up
8	P01042	KNG1	Up	**82**	Q13671	RIN1	Up
9	P00738	HP	Up	**83**	P23588	EIF4B	Up
10	Q8IYT4	KATNAL2	Up	**84**	O14672	ADAM10	Up
11	P0DOY2	IGLC2	Up	**85**	P13639	EEF2	Up
12	Q9H7M9	VSIR	Up	**86**	Q7Z4Q2	HEATR3	Up
13	O14556	GAPDHS	Up	**87**	P10915	HAPLN1	Up
14	P02671	FGA	Up	**88**	Q92752	TNR	Up
15	P34982	OR1D2	Up	**89**	P48539	PCP4	Up
16	P0DP58	LYNX1	Up	**90**	Q7Z4Q2	HEATR3	Up
17	Q99623	PHB2	Up	**91**	O43312	MTSS1	Up
18	P02679	FGG	Up	**92**	Q9UK22	FBXO2	Down
19	P02675	FGB	Up	**93**	Q9UBY5	LPAR3	Down
20	P35232	PHB1	Up	**94**	A5PKU2	TUSC5	Down
21	Q6R327	RICTOR	Up	**95**	Q5JUK3	KCNT1	Down
22	O94772	LY6H	Up	**96**	Q9BWW7	SCRT1	Down
23	P21589	NT5E	Up	**97**	Q8N398	VWA5B2	Down
24	P09669	COX6C	Up	**98**	Q53GA4	PHLDA2	Down
25	Q9P2U8	SLC17A6	Up	**99**	O43711	TLX3	Down
26	P24311	COX7B	Up	**100**	Q9UHG2	PCSK1N	Down
27	O00217	NDUFS8	Up	**101**	Q9BQ87	TBL1Y	Down
28	P01834	IGKC	Up	**102**	Q99453	PHOX2B	Down
29	P13073	COX4I1	Up	**103**	Q9UHR6	ZNHIT2	Down
30	P03915	MT-ND5	Up	**104**	Q96A47	ISL2	Down
31	P15954	COX7C	Up	**105**	Q9H4Q4	PRDM12	Down
32	P17568	NDUFB7	Up	**106**	Q02575	NHLH1	Down
33	O43674	NDUFB5	Up	**107**	Q9NQ03	SCRT2	Down
34	Q9UI09	NDUFA12	Up	**108**	Q9UIU6	SIX4	Down
35	O14548	COX7A2L	Up	**109**	P12277	CKB	Down
36	Q8NA47	CCDC63	Up	**110**	P12532	CKMT1A	Down
37	Q6ZR08	DNAH12	Up	**111**	C9JSQ1	CKMT1B	Down
38	Q16864	ATP6V1F	Up	**112**	Q12809	KCNH2	Down
39	Q96JX3	SERAC1	Up	**113**	O43526	KCNQ2	Down
40	Q6ZTW0	TPGS1	Up	**114**	Q14654	KCNJ11	Down
41	O75489	NDUFS3	Up	**115**	Q92952	KCNN1	Down
42	Q96ID5	IGSF21	Up	**116**	Q9NS61	KCNIP2	Down
43	Q9ULK4	MED23	Up	**117**	Q9Y2W7	KCNIP3	Down
44	Q9UGC6	RGS17	Up	**118**	Q9UHC3	ASIC3	Down
45	P10606	COX5B	Up	**119**	P30542	ADORA1	Down

Continued on next page

Table 2. List of the Identified Differentially Regulated Proteins/Genes in Painful Diabetic Neuropathy [a] (Continued)

46	Q92793	CREBBP	Up	**120**	P18825	ADRA2C	Down
47	Q86UD0	SAPCD2	Up	**121**	P41145	OPRK1	Down
48	O43920	NDUFS5	Up	**122**	A6NFN3	RBFOX3	Down
49	O95168	NDUFB4	Up	**123**	O76070	SNCG	Down
50	Q9UKT6	FBXL21	Up	**124**	P20472	PVALB	Down
51	Q96RD9	FCRL5	Up	**125**	Q8N9F0	NAT8L	Down
52	A0A0C4DH67	IGKV1-8	Up	**126**	Q8N7H5	PAF1	Down
53	A0A0B4J1U7	IGHV6-1	Up	**127**	Q96B36	AKT1S1	Down
54	P01599	IGKV1-17	Up	**128**	P04035	HMGCR	Down
55	A0A0C4DH29	IGHV1-3	Up	**129**	Q9C005	DPY30	Down
56	A0A0C4DH69	IGKV1-9	Up	**130**	Q9UMX0	UBQLN1	Down
57	P15018	LIF	Up	**131**	Q0D2I5	IFFO1	Down
58	P01591	JCHAIN	Up	**132**	Q15819	UBE2V2	Down
59	P01857	IGHG1	Up	**133**	Q68D86	CCDC102B	Down
60	P06702	S100A9	Up	**134**	P07910	HNRNPC	Down
61	P05109	S100A8	Up	**135**	P36776	LONP1	Down
62	Q9Y5Y7	LYVE1	Up	**136**	P02461	COL3A1	Down
63	Q86VB7	CD163	Up	**137**	Q13591	SEMA5A	Down
64	Q16649	NFIL3	Up	**138**	Q9Y5H1	PCDHGA2	Down
65	Q16666	IFI16	Up	**139**	P55083	MFAP4	Down
66	P41182	BCL6	Up	**140**	Q8N0U8	VKORC1L1	Down
67	Q6W2J9	BCOR	Up	**141**	Q8WUX1	SLC38A5	Down
68	Q08881	ITK	Up	**142**	P22692	IGFBP4	Down
69	P41279	MAP3K8	Up	**143**	P02462	COL4A1	Down
70	P24941	CDK2	Up	**144**	Q9BX70	BTBD2	Down
71	O60462	NRP2	Up	**145**	P15880	RPS2	Down
72	P23588	EIF4B	Up	**146**	Q7Z6Z7	HUWE1	Down
73	O14672	ADAM10	Up	**147**	P02649	APOE	Down
74	P13639	EEF2	Up				

[a] P-value < 0.05 and fold change > 1.5.