Animal Trial

Using RNA-Seq to Explore the Hub Genes in the Trigeminal Root Entry Zone of Rats by Compression Injury

Ran Tao, BDS[1], Feng Huang, PhD[1], Kun Lin, MD[2], Shao-Wei Lin, PhD[3], De Wei, MD[2], and Dao-Shu Luo, PhD[1,4]

From: [1]Department of Human Anatomy, Laboratory of Clinical Applied Anatomy, School of Basic Medical Science, Fujian Medical University, Fuzhou, China; [2]Department of Neurosurgery, Fujian Provincial Hospital, Fuzhou, China; [3]Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment factors and Cancer, School of Public Health, Fujian Medical University, Fuzhou, China; [4]Key Laboratory of Brain Aging and Neurodegenerative Diseases of Fujian Province, Fuzhou, China

Address Correspondence:
Daoshu Luo, PhD
Department of Human Anatomy, School of Basic Medical Sciences, Fujian Medical University, No. 1 Xuefu North Road, University Town, Fuzhou, 350122, China
E-mail: luods2004@fjmu.edu.cn

Disclaimer: Ran Tao and Feng Huang contributed equally to this work. This work was funded by National Natural Science Foundation of China (Grant number: 81671100; Dr. Daoshu Luo), United Fujian Provincial Health and Education Project for Tackling the Key Research P.R.China (Grant number: 2019WJ26; Dr. Daoshu Luo), Joint Funds for the innovation of science and Technology, Fujian province (Grant number: 2017Y9111; Dr. Feng Huang).

Conflict of interest: Each author certifies that he or she, or a member of his or her immediate family, has no commercial association (i.e., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted manuscript.

Manuscript received: 09-28-2020
Revised manuscript received: 12-07-2020
Accepted for publication: 01-08-2021

Free full manuscript: www.painphysicianjournal.com

Background: Mechanical compression on the trigeminal root entry zone (TREZ) by microvascular is the main etiology of primary trigeminal neuralgia (TN).

Objectives: To study the pathogenesis of TN, hub genes screening in the TREZ of TN in an animal model was performed.

Study Design: A double blind, randomized study was designed in a controlled animal trial.

Setting: The research took place in the Laboratory of Clinical Applied Anatomy at the School of Basic Medical Science of Fujian Medical University.

Methods: Twelve male rats were randomly divided into a sham operation group and a TN animal model group. TN animal model was induced by chronic compression of trigeminal nerve root (CCT) operation. Gene expression in the TREZ were analyzed by RNA sequencing (RNA-Seq) technique. KEGG analysis, GO analysis, and PPI analysis were performed in the DEGs. Key signaling pathways analyzing by GSEA and the hub genes in the DEGs were also studied. Reverse transcription real-time polymerase chain reaction (RT-qPCR) was used to verify the RNA-Seq results.

Results: Transcriptome data showed that 352 genes up-regulated and 59 genes down-regulated in DEGs on post-operation day 21, after CCT operation in the TN group. KEGG analysis revealed that, "neuroactive ligand receptor interaction" and "cytokine cytokine receptor interaction" may be related to the pathogenesis of TN. GO analysis showed "regulation of signing receptor activity", "chemokine activity", and "carbohydrate binging" may be related to TN. The RT-qPCR results were consistent with the test results, indicating that the transcriptome sequencing results were reliable.

Limitations: Although the incidence of TN in female rats was higher than in male rats, we only used male SD rats to establish the TN animal model, to avoid the effect of estrogen on experimental results. This study only presents some respects of RNA-Seq technique and, therefore, did not identify the DEGs at the protein level. The relationship between the DEGs at different levels shoud be done in the future.

Conclusions: Based on the results of RNA-seq, this study discovered 6 hub genes in the TREZ that are closely related to the TN animal model, which provide a potential breakthrough point to explore the pathogenesis of TN.

Key words: Animal model, compression injury, hub gene, rat, RNA-seq, transcriptome, trigeminal neuralgia, trigeminal root entry zone

Pain Physician 2021: 24:E573-E581

Trigeminal neuralgia (TN) is a common neuropathic pain characterized by electric shock pain in the orofacial distribution area of the trigeminal nerve (1). The pathogenesis of TN is still undiscovered, which prevents humans from conquering this disease that seriously endangers the physical and mental health of patients (2,3). The trigeminal nerve root is the only way for orofacial sensory information to enter the central nervous system and microvascular compression on the trigeminal root entry zone (TREZ) is the main etiology for most primary TN patients (4). Due to the special anatomical structure and physiological characteristics in the TREZ, it would be an interesting potential research area for the pathogenesis of TN (5).

RNA sequencing (RNA-Seq) is a high-throughput sequencing technology used to study transcriptomes, enabling more accurate quantification of gene expression levels (6). Several studies showed strong correlations between transcriptional changes and nerve injury in the peripheral and central nervous system (7,8); however, there is still no report about the study of the transcriptome in the TN animal model.

To better understand the pathogenesis of the TN animal model, the transcriptome level of the TREZ was investigatd both in the TN group and a sham operation group. We used RNA-seq technology to detect the transcription map of the TREZ undergoing mechanical chronic compression in rats. The sequencing results were analyzed by bioinformatics and verified by reverse transcription polymerase chain reaction (RT-qPCR) (9,10).

Methods

Animals

Adult male Sprague-Dawley rats (150 ± 20 g) were used in this study. The experiments were done in accordance with the Fujian Medical University Institutional Animal Care and Use guidelines and were approved by the Experimental Animals Ethics Committee of Fujian Medical University. Rats were housed under a 12–12 hour light-dark cycle in a temperature-controlled room with food and water available ad libitum (11). Twelve Rats were randomly divided into a TN animal model group (n = 6) and a sham operation group (n = 6).

Surgery of TN Animal Model

The TN animal model was established by mechanical compression on the TREZ, modified from the previously described procedure (12). The main steps of the surgical procedure are as follows: rats were anesthetized with pentobarbital (40 mg/kg, ip), then the incision above edge of the right obit was made, then following the medial orbital wall to the right orbital bottom the right infraorbital nerve was exposed. A similar diameter (diameter 1 mm) hollow plastic catheter, instead of previous small plastic filament, was gently inserted into the cranial cavity from the infraorbital fissure following the path of infraorbital nerve to arrive at the TREZ. The hollow plastic catheter used for nerve compression in our study could also be a potential administration route for further pharmacological intervention. In the sham group, the right infraorbital nerve was exposed without TREZ compression.

Orofacial Mechanical Threshold Testing

The orofacial mechanical threshold in the TN group and sham group rats were tested as we previously described (13). von Frey filaments were used to determine the orofacial mechanical allodynia threshold on the vibrissal pad of the rats. The baseline mechanical threshold of rats were obtained by the mean value of the behavioral testing 3 days before operation.

RNA-Seq Library Preparation and Sequencing

RNA preparation, library construction, and sequencing were performed by BGI GENE using high-throughput genome sequencing platform BGISEQ-500 (10). In brief, the trigeminal nerve root tissue was harvested immediately after the rats were euthenized under excessive anesthesia. The samples were homogenized in Trizol (Invitrogen, California, USA). Total RNA was generated according to the protocol. Quantitative and qualitative analysis of RNA was performed by a Qubit Fluorometer (Agilent Technologies, California, USA). In order to determine whether the sequencing data were suitable for subsequent analysis, raw RNA sequencing data required quality control (QC) on the raw reads. The resulting clean data were aligned to the reference sequence and low-quality reads were discarded after QC.

Total RNA Extraction from the TREZ

Twenty-one days after operation, the right trigeminal nerve roots of every 2 rats were aggregated to extract RNA as a sample. Three biological repeats were performed in each group (n = 6). Trizol was used to extract RNA, and the integrity of the extracted RNA was determined by agarose gel electrophoresis. The integrity of the extracted RNA was detected under

the ultraviolet lamp. The concentration and purity of RNA were determined by sample absorbance. The absorbance of the sample at 260 nm and 280 nm were measured and expressed by optical density (OD). RNA concentration was calculated by the following formula: RNA concentration (UG / UL) = OD260 * RNA dilution multiple "40 / 1000". The purity of RNA was evaluated by OD260 / OD280 (ratio, R), and the normal range was 1.8 to 2.0.

Quantitative Reverse Transcription PCR

Total RNA samples were isolated with Trizol (Invitrogen, California, USA) and reverse transcribed into cDNA with the HiScript® III RT SuperMix for qPCR (+gDNA wiper) following the manufacturers' guidelines (Vazyme, Nanjing, China). RNA were reverse transcribed into cDNA at 37°C for 15 minutes and the reaction was stopped at 85°C for 5 seconds. ChamQ SYBR qPCR Master Mix (Vazyme, Nanjing, China) was used to perform qPCR on a CFX96 System (Bio-Rad, California, USA). Relative gene expression was calculated with the 2ΔΔCt method using glyceraldehyde-3-phosphate dehydrogenase (Gapdh) as the reference housekeeping gene. The Quantitative Reverse Transcription PCR (RT-qPCR primers) are listed in Table 1.

Statistical Analysis

All data were expressed as mean ± SEM. Two-way ANOVA and Sidak's multiple comparisons test were used to analyze the mechanical threshold data. Student's t test was used to analyze the RT-qPCR validation data. P value < 0.05 was considered statistically significant.

Results

Analysis Results of behaviors test

Compared with the baseline orofacial mechanical threshold and the sham group, rats in the TN group showed significant mechanical allodynia (Fig. 1) from postoperation day 14 to day 21 ($P < 0.05$). While rats in the sham group showed no obvious changes after sur-

Table 1. *RT-qPCR primers*

Gene	Forward primer (5'--3')	Reverse primer (5'--3')
Gpnmb	TGCCAACGGCAATATCGTCT	TCCATTTCTTCCGTCCGTGG
Cxcl14	TGAAGCCAAAGTACCCACACT	GACCCTGCGTTTCTCGTTCC
Ccl2	GCATCAACCCTAAGGACTTCAG	TTCTCTGTCATACTGGTCACTTCT
Cd68	TTCGGGCCATGCTTCTCTTG	GGGCTGGTAGGTTGATTGT
Lgals3	CCTACGATATGCCCTTGCCT	AGGCGATGTCGTTCCCTTTC
Hspb1	GTGGAGATCACTGGCAAGCAC	CGCTGATTGTGTGACTGCTTT
Gapdh	ACGGCAAGTTCAACGGCACAG	GAAGACGCCAGTAGACTCCACGAC

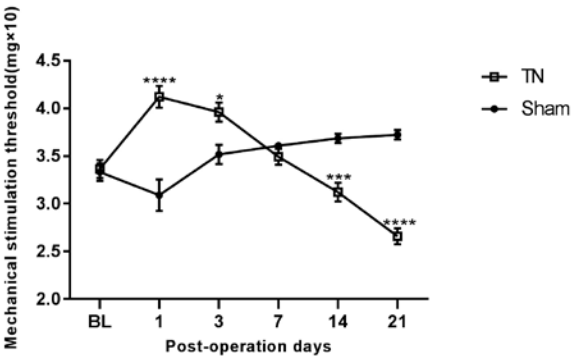


Fig. 1. *Results of animal behaviors. von Frey filaments were used to measure the threshold of mechanical stimulation at 3 days before operation and 1, 3, 7, 14, and 21 days after operation. The results showed that the 2 groups had similar baseline pain threshold 3 days before operation ($P > 0.05$). One day after operation, the behavior of rats between the TN group and sham group was significantly different ($P < 0.0001$). Three days after operation, mechanical stimulation threshold of rats in the operation group began to decrease significantly. On the 14th day after operation, the mechanical pain threshold of the TN group was significantly lower than that of sham group ($P < 0.001$). At the same time, the mechanical stimulation threshold of the TN group decreased continuously at 21 days after operation. The mechanical pain threshold of the sham group returned to the baseline value from 7 days after operation until 21 days.*

gery. These results suggest that the TN animal model, induced by chronic mechanical compression on the TREZ of rats, was successfully established.

Analysis Results of Quality Control and DEGs

Transcriptome data were obtained from TREZ samples of rats on postoperation day 21. The average number of readings per sample was about 46.18 million; the Q20 of all samples ranged from 96.06 to 97.12, Q30 ranged from 86.95% to 89.23%, and 86.59% to 89.52% of the samples could be mapped to the reference genome (14) indicating that the sequencing results were reliable (Table 2). Figure 2 shows the correlation of gene expression levels among samples. The representative distribution of up-regulated or down-regulated genes in a volcano map is shown in Fig. 3. Our data show that 352 genes were up-regulated and 59 genes were down-regulated in TG, 21 days after CCT (|log2FC| > = 2; Q value < = 0.001; [Fig. 4]).

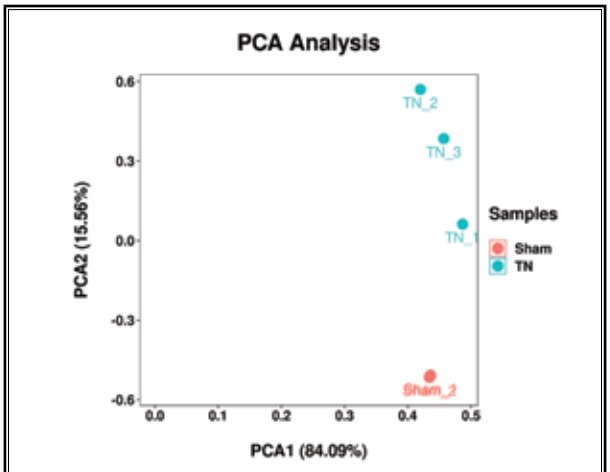


Fig. 2. *The result of PCA Analysis. The coordinate axis represents the new data set corresponding to the principal component after dimensionality reduction, which is used to represent the difference between samples; the value in bracket of coordinate axis label represents the percentage of variance of corresponding principal component interpretation population. Blue represents TN group, Red represents sham group.*

Table 2. *Quality control and sequencing information for samples.*

Sample	raw_ reads	clean_ reads	Q20	Q30	reads_ ratio
Sham_1	50.83	45.12	96.89	88.66	88.77
Sham_2	50.83	45.51	97.12	89.33	89.52
TN_1	50.83	45.05	97.08	89.23	88.62
TN_2	56.09	48.91	96.06	86.95	87.19
TN_3	52.59	45.53	96.24	87.47	86.59

Shows the summary of RNA-sequencing data of 5 samples, including raw reads number, clean reads number, clean data rate, mapped rate, and percentage of clean reads, as well as Q20 and Q30

Analysis of Important KEGG Pathways and GO Analysis

We used the BGI online platform (https://biosys.bgi.com/) to analyze KEGG pathway of different genes. Differential genes were significantly enriched in the classification of "neuroactive ligand receptor interaction" and "cytokine cytokine receptor interaction" (Fig. 5). It was suggested that nerve active ligand receptor signal transduction and inflammatory reaction occur in TREZ after mechanical compression of trigeminal nerve. We further analyzed the function of the 2 signaling pathways, as shown in Supplemental Figs. 1-2. The results show that the differentially expressed genes were involved in pain response, inflammatory cytokines, and transcription factors. In addition, we also analyzed the important mutual pathways of interest by GSEA (Fig. 6). Similarly, we analyzed Gene Ontology of different genes from the cellular component (CC), molecular function (MF), and biological process (BP) aspects (Fig. 7). By the result of GO analysis, "regulation of signing receptor activity", "chemokine activity", and "carbohydrate binging" may

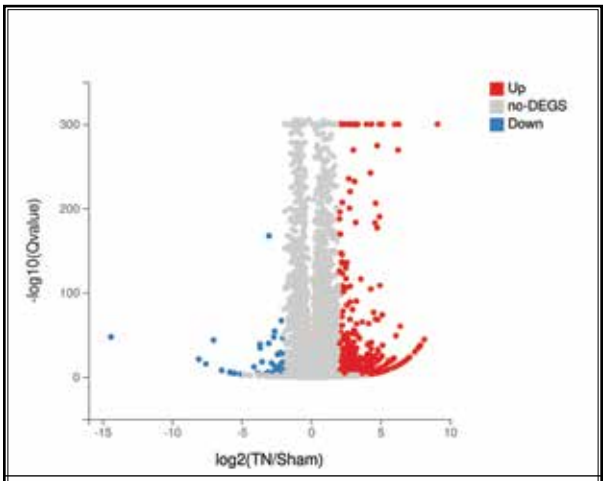


Fig. 3. *Differently expressed genes between TN and sham group. Red represents DEG up-regulated, blue represents DEG down-regulated, and gray represents non-DEG. Our data showed that 352 genes up-regulated and 59 genes down-regulated in DEG 21 days after CCT (|log2FC| > = 2; Q value < = 0.001).*

be related with pathogenesis of trigeminal neuralgia (Supplementary Fig. 3).

Analysis Results of Protein Protein Interaction Network (PPI)

In order to further explore the mechanism of differential genes involved in the pathogenesis of trigeminal nerve root compression (352 up-regulated genes and 59 down-regulated genes) and to explore the interaction between differential genes, a PPI network based on 411 genes was established on a string database for functional association analysis (Supplementary Fig. 4). The sequential visualization was performed on Cytoscape. The plug-in "cytohubba" was used to find the top 20 hub genes (Supplementary Fig. 5) and the differential genes directly related to them, according to the association grade difference between different genes (Fig. 8). Meanwhile, the interactive density region in PPI network by "mcode" plug-in was also discovered. Figure 9 shows the top 4 dense regions.

Results of Real-Time qPCR

In order to verify the transcriptome sequencing results and further analyze the pathogenesis of microvascular compression of trigeminal nerve, 6 representative genes were selected for real-time PCR verification. The results showed that the relative expression of pain related genes Cd68, Ccl2, Gmnpb, Lgala3, Cxcl14 and Hspb-1 increased in the TN group. The expression trend of these genes was consistent with the results of RNA-seq, which indicated that RNA sequencing data could reliably reflect the change of gene expression. Among them, Ccl2 and Cxcl14 were important components of cytokine cytokine receptor signaling pathway (Fig. 10).

Discussion

At present, most people believe that primary TN is mainly caused by microvascular compression. Microvascular decompression and microballoon compression are commonly used in clinical treatment for primary TN (15,16); however, surgical strategy for TN patients also exhibited treatment failure or recurrence (17). Therefore, it is necessary to uncover the pathogenesis of TN at different levels to better treat this disease, including at the transcriptome level. Although there were some studies on the pathogenesis of chronic pain in the animal model of sciatica by using RNA-Seq technique (18,19), it is rarely reported in the TN studied using this technique.

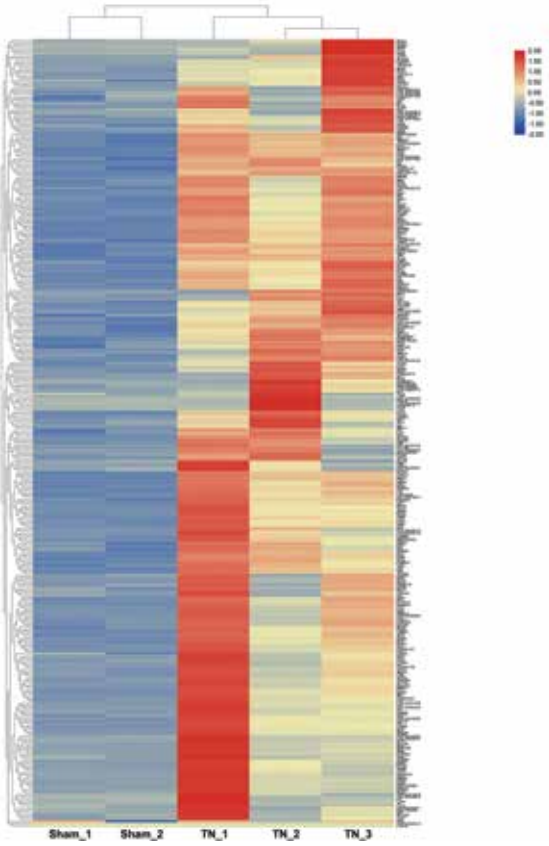


Fig. 4. *Genetic clustering of differential expression genes (|log2FC| >= 2; Q value <= 0.001). Red color represents high fold-change, blue color represents low fold-change.*

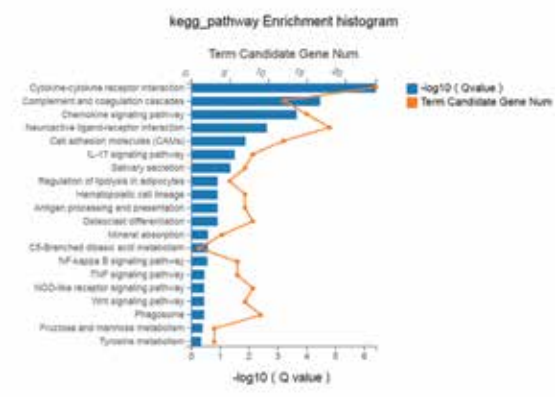


Fig. 5. *Analysis results of KEGG(S-Fig. 3). The possible signaling pathways were arranged from high to low according to Q value, the broken line shows the number of differential genes involved in the pathway.*

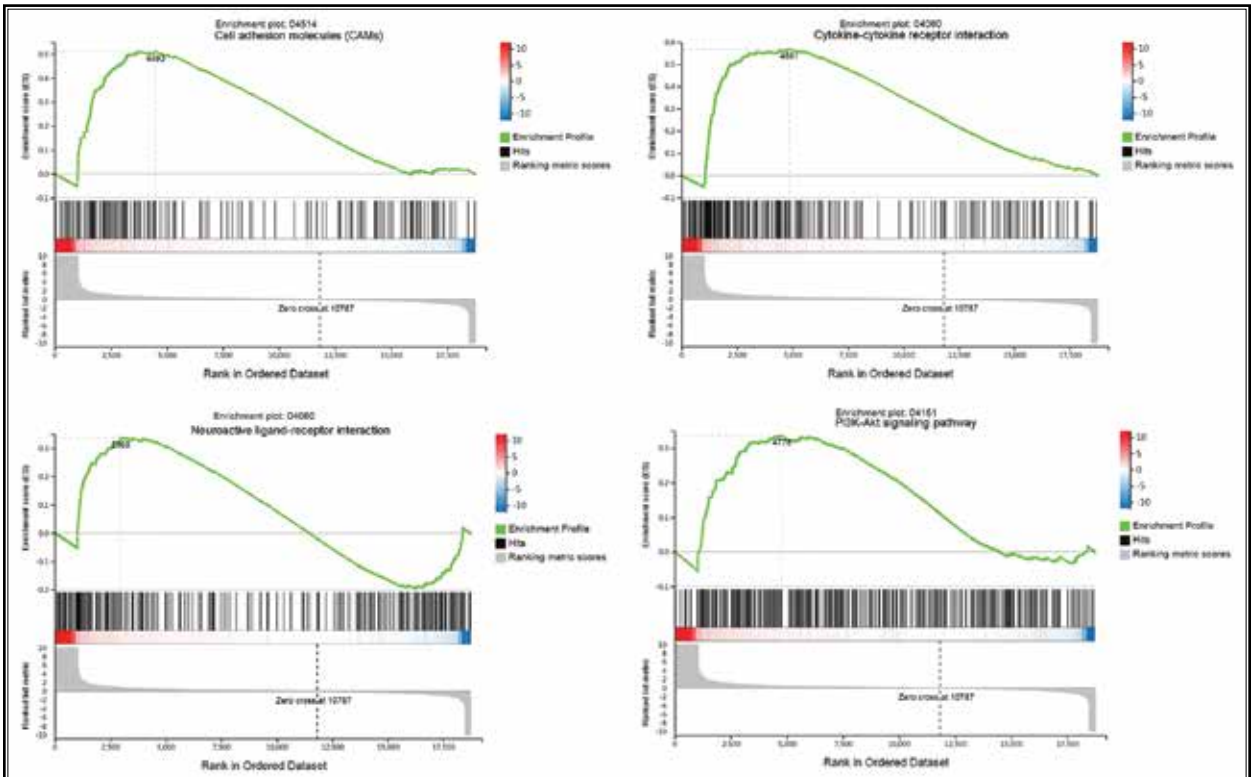


Fig. 6. *The results of GSEA enrichment analysis on 4 major signaling pathways were carried out. The results of differential genes participating in 2 important signaling pathways("neuroactive ligand receptor interaction" and "cytokine cytokine receptor interaction").*

In this study, we sequenced the TREZ transcriptome of rats undergoing CCT or sham surgery. Our data show that there were 352 up-regulated genes and 59 down-regulated genes in rat TREZ 21 days after CCT injury. We performed KEGG and GO analysis of differential genes. These genes were mainly enriched and these pathways were considered to be related to the occurrence and development of neural pain. We found the hub gene related to TN through data analysis from different algorithms and plotted the network relationship between the hub gene and surrounding genes (8,19). We found 6 of the 411 differential genes were closely related to chronic pain, which may be very important in signaling pathways and gene interaction, including Cd68, Cxcl14, Ccl2, Gpnmb, Hspb1, and Lgals3. As a marker of microglia, Cd68 was closely related to neuroinflammatory pain (20). Cxcl14 is a new member of the CXC family of chemokines. Cxcl14 was significantly up-regulated in the spinal nerve ligation model and was found to be closely related to peripheral neuralgia (21,22). Ccl2 acts on the central and peripheral nervous system and participates in the process of pain perception after inflammation or nerve injury (23). Constitutively expressed small heat shock protein Hspb1 regulated many basic cellular processes; it also plays a major role in many human pathological diseases (24). In addition, dorsal root ganglion transcriptome sequencing suggest that Hspb1 is related to peripheral nerve injury and pain (7). Recent studies show that Gpnmb played a key role in neuron survival and neuroprotection (25,26); Galectin-3 (gal3) is a multifunctional protein belonging to the carbohydrate-ligand lectin family. Emerging studies show that gal3 induces a pro-inflammatory response by recruiting and activating lymphocytes, macrophages, and microglia (27,28). Besides, Galectin-3 inhibition is associated with the reduction of neuropathic pain after peripheral nerve injury (29). It was reported by Di Stefano and colleagues that there are rare variants of genes encoding voltage-gated channels and transient receptor potential channels by the genomic screen in systematic clinical study of familial TN, with a focus on genes relating to neuronal excitability in the trigeminal ganglia tissues.(30) While in

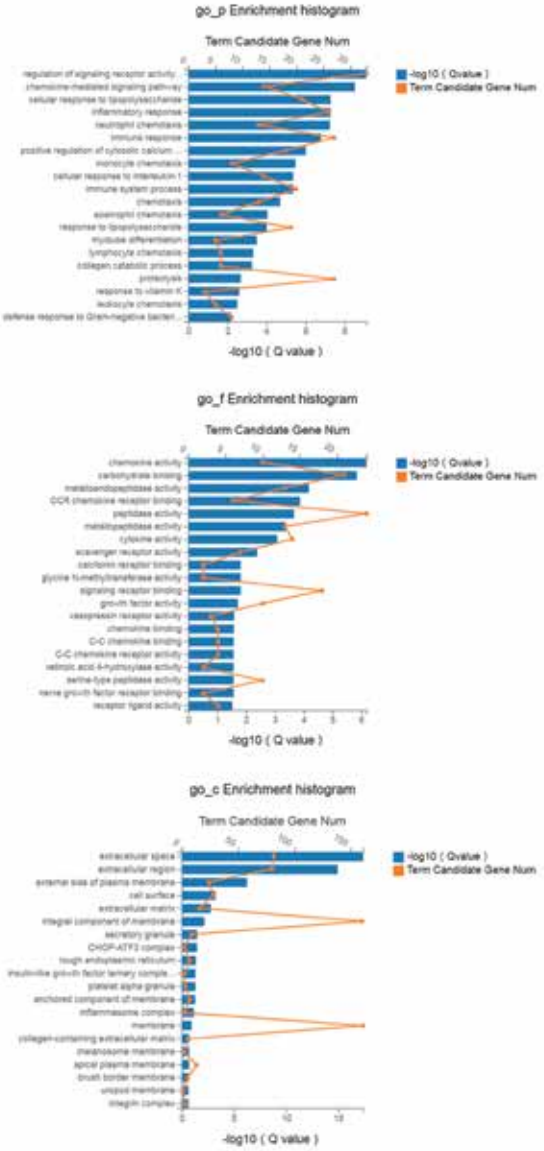


Fig. 7. *They are the results of GO analysis in cellular component, biological process, and molecular function. The functions of differential genes are arranged from high to low according to Q value and the broken line shows the number of differential genes involved in the GO term.*

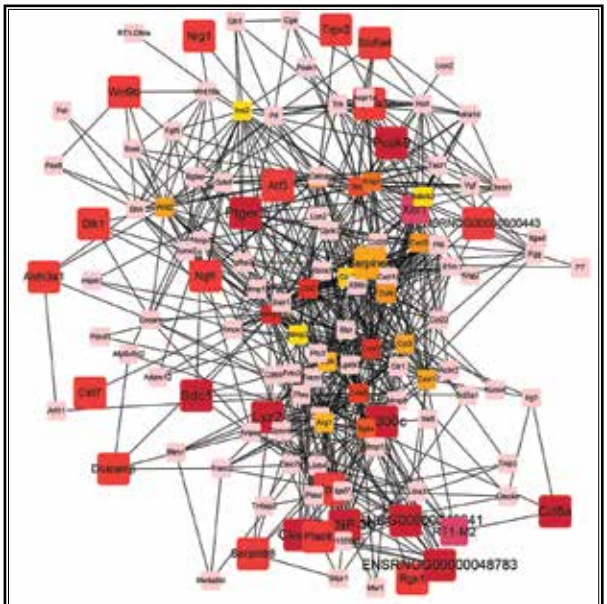


Fig. 8. *Analysis Results of PPI. The figure shows the network interaction of the top 20 hub genes and the differential genes directly related to them. The redder the color, the more important it is in the network relationship.*

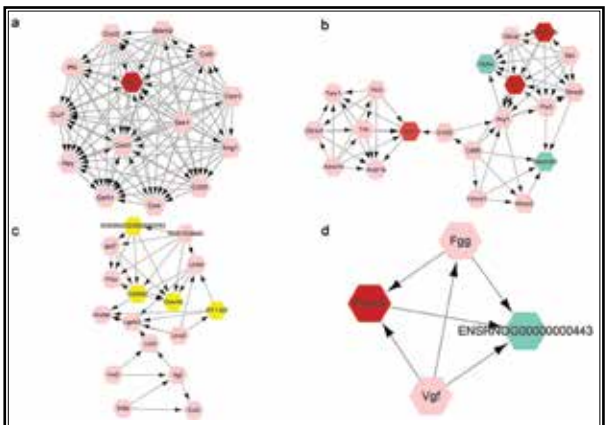


Fig. 9. *Analysis Results of PPI. The top 4 dense regions of the interactive density region in PPI network.*

our study, we screened the RNA-Seq in the TREZ tissues based on the TN animal model induced by mechanical compression injury of the TREZ, showing that DEGs were mainly enriched in neuroactive ligand receptor interaction and cytokine receptor interaction. Therefore, the genes identified in our results are not consistent with those in systematic clinical study by Di Stefano, et al (30). The inconsistency may be because the TN animal model in rodents is not exactly equivalent to human disease of TN as well as TREZ tissue only containing axons and neuroglia cells and is different from the trigeminal ganglion containing neurons.

In our study, RT-qPCR experiments confirmed that these 6 genes were significantly up-regulated in TREZ

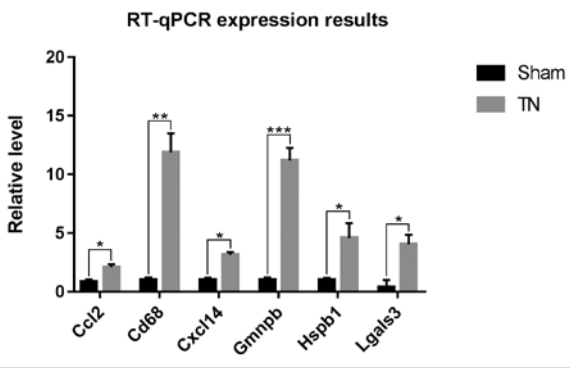


Fig. 10. *RT-qPCR relative expression results. The abscissa is the gene name and the ordinate is the relative expression. The 6 hub genes were significantly up-regulated at 21 days after CCT, which is consistent with RNA-Seq results.*

in CCT-operated rats. According to our previous study (12), the mechanical hyperalgesia of rats after CCT was significantly different from the sham group. The difference was most obvious 21days after CCT; therefore, the transcriptome level in the TREZ of TN animal model was investigated only on post-operation day 21 to verify the RNA-Seq results. The hub genes may be used as a research focus to further explore the pathogenesis of TN.

Limitations

Although the incidence of TN was higher in female rats than male, we only used male SD rats to establish the TN animal model, to avoid the effect of estrogen on experimental results. We only focused on transcriptome level changes; however, noncoding RNAs have been found to play an important role, which were not involved in our experiment (31,32). Besides the previously mentioned, we also did not identify the DEGs at the protein level.

Conclusions

In summary, we used RNA-Seq analysis of transcriptome changes in the TREZ after compression injury on post-operation day 21 to determine that there were 352 genes up-regulated and 59 genes down-regulated as well as the finding that Cd68, Cxcl14, Ccl2, Gpnmb, Hspb1, and Lgals3 are closely related to the TN animal model, which provide a potential breakthrough point to explore the pathogenesis of TN. Additionally, Cxcl14 and Ccl2 are 2 important chemokines from CXC and CC families respectively, which are closely related to neuroinflammation and neuroimmunity. In future studies, we will try to explore the neural signaling pathways related to Cxcl14 and Ccl2 in the TN animal model. If these chemokines might contribute to pathogenesis of TN, chemokine targeted drugs could be developed to block the progress of TN disease in the early stage, or even prevent the occurrence of TN.

Acknowledgment

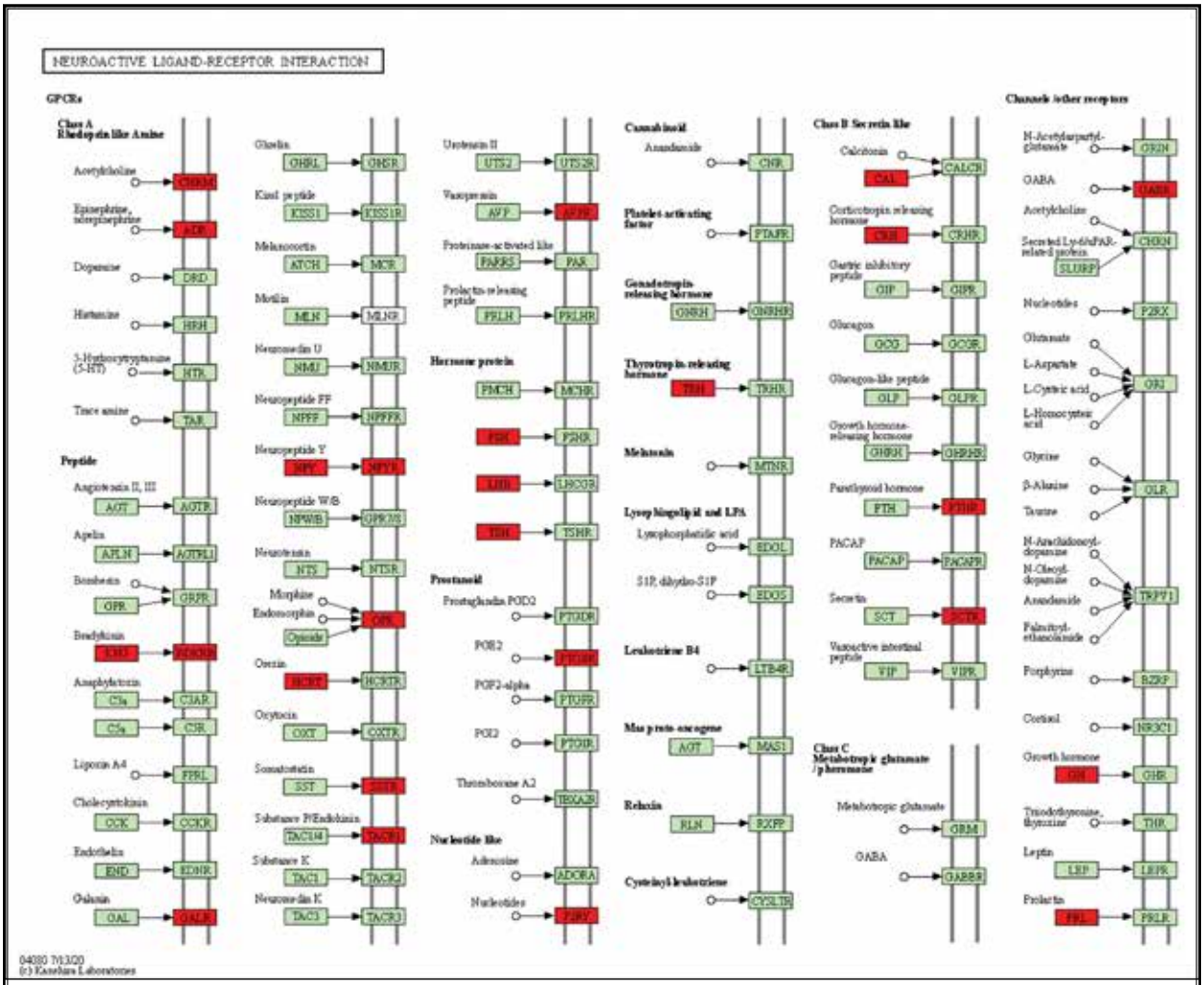
The authors ensure that all authors meet the criteria listed in the author guidelines. Each author had participated sufficiently in the work to take public responsibility for appropriate portions of the content.

References

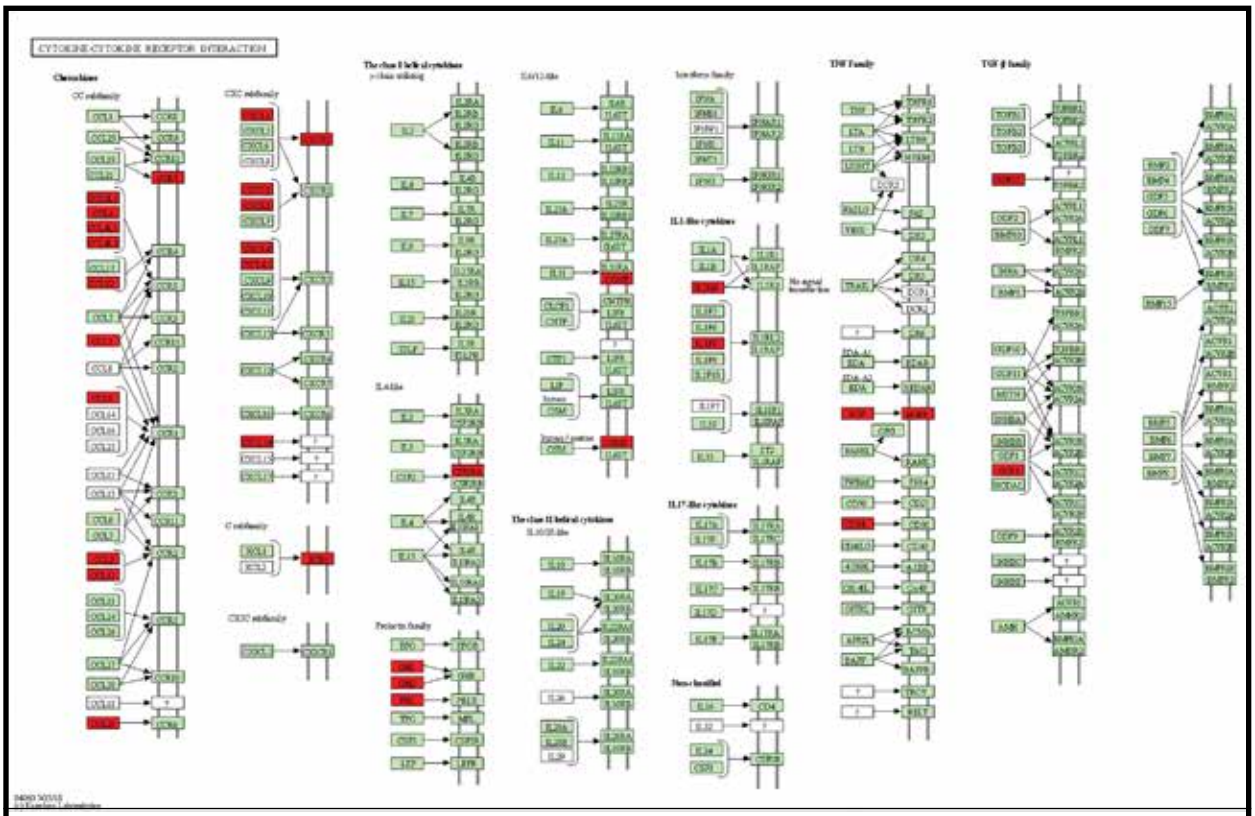
1. Zakrzewska JM, Linskey ME. Trigeminal neuralgia. *BMJ (Clinical research ed)* 2015; 350:h1238.
2. Cruccu G, Di Stefano G, Truini A. Trigeminal Neuralgia. *The New England journal of medicine* 2020; 383:754-762.
3. Megat S, Ray PR, Tavares-Ferreira D, et al. Differences between Dorsal Root and Trigeminal Ganglion Nociceptors in Mice Revealed by Translational Profiling. *J Neurosci* 2019; 39(35):6829-6847.
4. Lin R, Luo L, Gong Y, et al. Immunohistochemical analysis of histone H3 acetylation in the trigeminal root entry zone in an animal model of trigeminal neuralgia. *J Neurosurg* 2018; 131:828-838.
5. Bendtsen L, Zakrzewska JM, Heinskou TB, et al. Advances in diagnosis, classification, pathophysiology, and management of trigeminal neuralgia. The Lancet Neurology 2020; 19:784-796.
6. Patterson J, Carpenter EJ, Zhu Z, et al. Impact of sequencing depth and technology on de novo RNA-Seq assembly. *BMC Genomics* 2019; 20:604.
7. Sun W, Kou D, Yu Z, et al. A Transcriptomic Analysis of Neuropathic Pain in Rat Dorsal Root Ganglia Following Peripheral Nerve Injury. *Neuromolecular Med* 2020; 22:250-263.
8. Wang K, Yi D, Yu Z, et al. Identification of the Hub Genes Related to Nerve Injury-Induced Neuropathic Pain. *Front Neurosci* 2020; 14:488.
9. Bustin SA, Benes V, Nolan T, et al. Quantitative real-time RT-PCR--A perspective. *Journal of molecular endocrinology* 2005; 34:597-601.
10. Fang C, Zhong H, Lin Y, et al. Assessment of the cPAS-based BGISEQ-500 platform for metagenomic sequencing. *Gigascience* 2018; 7:1-8.
11. Islam J, Kc E, Oh BH, et al. Optogenetic stimulation of the motor cortex alleviates neuropathic pain in rats of infraorbital nerve injury with/without CGRP knock-down. *The journal of headache and pain* 2020; 21:106.
12. Luo DS, Zhang T, Zuo CX, et al. An animal model for trigeminal neuralgia by compression of the trigeminal nerve root. *Pain physician* 2012; 15:187-196.
13. Luo D, Lin R, Luo L, et al. Glial Plasticity in the Trigeminal Root Entry Zone of a Rat Trigeminal Neuralgia Animal Model. *Neurochemical* research 2019;

44:1893-1902.

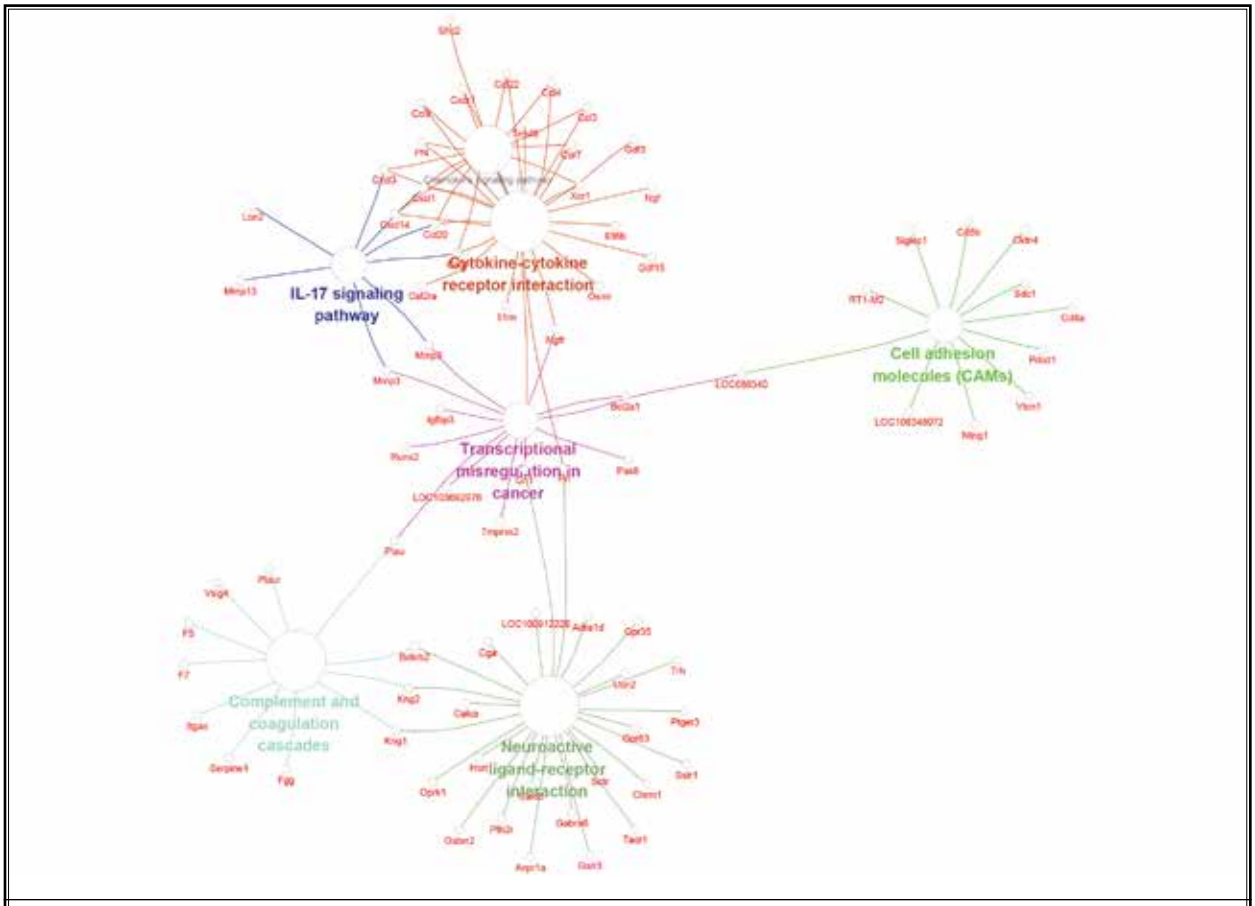
14. Zhou Y, Liu C, Zhou R, et al. SEQdata-BEACON: A comprehensive database of sequencing performance and statistical tools for performance evaluation and yield simulation in BGISEQ-500. *BioData Min* 2019; 12:21.
15. Sun Z, Wang Y, Cai X, et al. Endoscopic Vascular Decompression for the Treatment of Trigeminal Neuralgia: Clinical Outcomes and Technical Note. *J Pain Res* 2020:13: 2205-2211.
16. Bendtsen L, Zakrzewska J, Heinskou T, et al. Advances in diagnosis, classification, pathophysiology, and management of trigeminal neuralgia. *Lancet Neurol* 2020; 19:784-796.
17. Nova C, Zakrzewska J, Baker S, et al. Treatment Outcomes in Trigeminal Neuralgia-A Systematic Review of Domains, Dimensions and Measures. *World Neurosurg X* 2020; 6:100070.
18. Yu H, Zhang P, Chen YR, et al. Temporal Changes of Spinal Transcriptomic Profiles in Mice With Spinal Nerve Ligation. *Frontiers in neuroscience* 2019; 13:1357.
19. Athie MCP, Vieira AS, Teixeira JM, et al. Transcriptome analysis of dorsal root ganglia's diabetic neuropathy reveals mechanisms involved in pain and regeneration. *Life sciences* 2018; 205:54-62.
20. Zhao H, Alam A, Chen Q, et al. The role of microglia in the pathobiology of neuropathic pain development: what do we know?. *British journal of anaesthesia* 2017; 118:504-516.
21. Strong JA, Xie W, Coyle DE, et al. Microarray analysis of rat sensory ganglia after local inflammation implicates novel cytokines in pain. *PloS one* 2012; 7:e40779.
22. Cereijo R, Gavaldà-Navarro A, Cairó M, et al. CXCL14, a Brown Adipokine that Mediates Brown-Fat-to-Macrophage Communication in Thermogenic Adaptation. *Cell Metab* 2018; 28:750-763. e756.
23. Van Steenwinckel J, Reaux-Le Goazigo A, Pommier B, et al. CCL2 released from neuronal synaptic vesicles in the spinal cord is a major mediator of local inflammation and pain after peripheral nerve injury. *J Neurosci* 2011; 31:5865-5875.
24. Haidar M, Asselbergh B, Adriaenssens E, et al. Neuropathy-causing mutations in HSPB1 impair autophagy by disturbing the formation of SQSTM1/p62 bodies. *Autophagy* 2019; 15:1051-1068.
25. Hou L, Zhang Y, Yang Y, et al. Intrathecal siRNA against GPNMB attenuates nociception in a rat model of neuropathic pain. *J Mol Neurosci* 2015; 55:533-540.
26. Neal ML, Boyle AM, Budge KM, et al. The glycoprotein GPNMB attenuates astrocyte inflammatory responses through the CD44 receptor. *J Neuroinflammation* 2018; 15:73.
27. Ma Z, Han Q, Wang X, et al. Galectin-3 Inhibition Is Associated with Neuropathic Pain Attenuation after Peripheral Nerve Injury. *PloS one* 2016; 11:e0148792.
28. Gilson RC, Gunasinghe SD, Johannes L, et al. Galectin-3 modulation of T-cell activation: Mechanisms of membrane remodelling. *Prog Lipid Res* 2019; 76:101010.
29. Wu ZS, Lo JJ, Wu SH, et al. Early Hyperbaric Oxygen Treatment Attenuates Burn-Induced Neuroinflammation by Inhibiting the Galectin-3-Dependent Toll-Like Receptor-4 Pathway in a Rat Model. *International journal of molecular sciences* 2018; 19(8):2195.
30. Di Stefano G, Yuan JH, Cruccu G, et al. Familial trigeminal neuralgia - A systematic clinical study with a genomic screen of the neuronal electrogenisome. *Cephalalgia : an international journal of headache* 2020; 40:767-777.
31. Wu S, Bono J, Tao YX. Long noncoding RNA (lncRNA): A target in neuropathic pain. *Expert Opin Ther Targets* 2019; 23:15-20.
32. Wang C, Xu X, Chen J, et al. The Construction and Analysis of lncRNA-miRNA-mRNA Competing Endogenous RNA Network of Schwann Cells in Diabetic Peripheral Neuropathy. *Frontiers in bioengineering and biotechnology* 2020; 8:490.



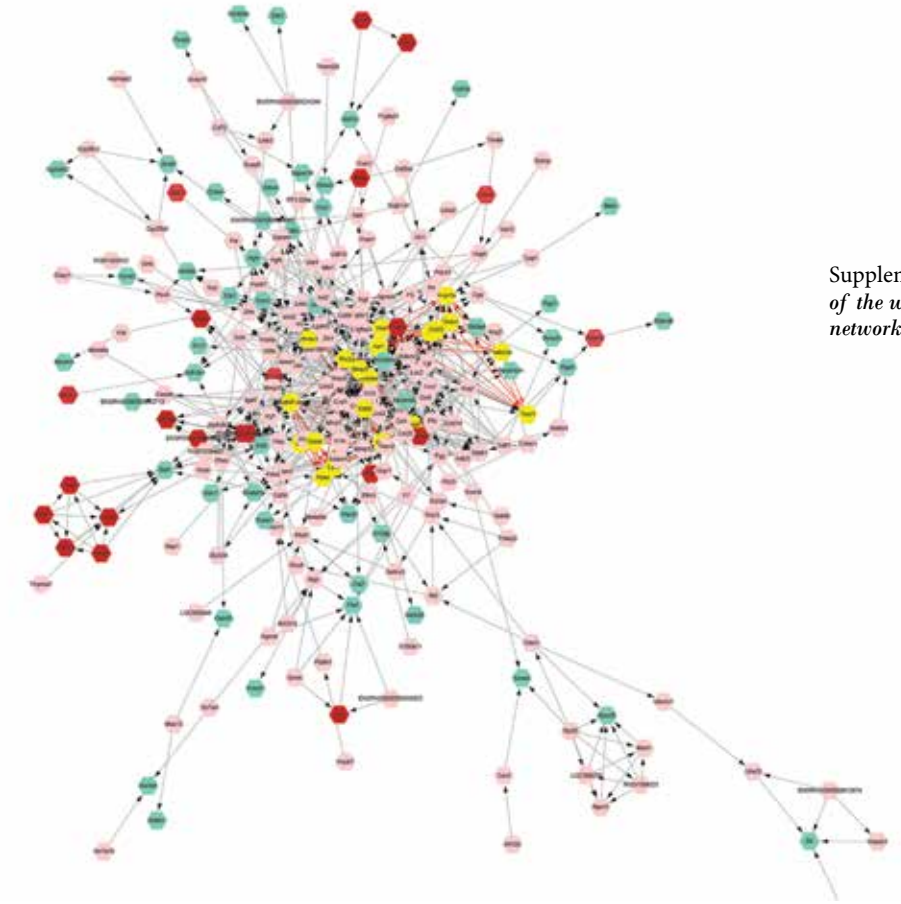
Supplemental Fig. 1. ***The results of differential genes participating in 2 important signaling pathways ("neuroactive ligand receptor interaction" and "cytokine cytokine receptor interaction").***



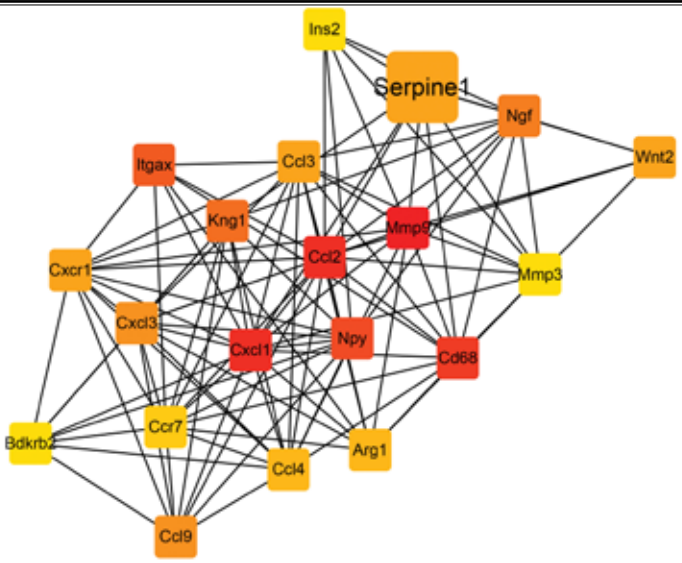
Supplemental Fig. 2. *The results of differential genes participating in 2 important signaling pathways ("neuroactive ligand receptor interaction" and "cytokine cytokine receptor interaction").*



Supplemental Fig. 3. *Analysis of Important KEGG Pathways*.



Supplemental Fig. 4. ***Analysis Results of the whole protein protein interaction network.***



Supplemental Fig. 5. ***Top 20 DEGs in PPI***