



Original Article



Investigation of the potential molecular mechanisms of acupuncture in the treatment of long COVID: a bioinformatics approach

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Article Info

Abstract



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Long COVID is a poorly understood condition characterized by persistent symptoms following the acute phase of COVID-19, including fatigue, cognitive impairment, and joint pain. Acupuncture, a key component of traditional Chinese medicine treatment, has shown potential in alleviating long COVID symptoms. However, the molecular mechanisms underlying its therapeutic effects remain largely unknown. In this study, we employed bioinformatics approaches to explore the potential molecular mechanisms of acupuncture's therapeutic effects on long COVID symptoms. We screened protein targets of active ingredients produced by the body after acupuncture and identified potential therapeutic targets of long COVID. Protein-protein interaction networks were constructed, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to identify key targets and pathways. Our findings provide valuable insights into the potential molecular mechanisms of acupuncture's therapeutic effects on long COVID symptoms and may contribute to the development of targeted therapies for managing this challenging condition.

Keywords: Molecular mechanisms, Acupuncture, Long COVID, Bioinformatics approach.

1. Introduction

Long COVID, also known as post-acute sequelae of SARS-CoV-2 infection, is a complex and poorly understood condition characterized by a wide range of persistent symptoms following the resolution of the acute phase of COVID-19 [1-4]. These symptoms may include fatigue [5], cognitive impairment [6], joint pain [7], and dyspnea [4], among others. The pathophysiology of long COVID remains elusive, and the development of effective therapies for managing this condition is an urgent need [1].

Acupuncture, a key component of traditional Chinese medicine, has been widely used for various health conditions due to its therapeutic effects on pain [8], inflammation [9], and immune modulation [10]. Recent studies have suggested that acupuncture might be a promising treatment option for long COVID patients [11-15]. However, the molecular mechanisms underlying the therapeutic effects of acupuncture on long COVID symptoms remain largely unknown [11].

In this study, we utilized bioinformatics approaches to explore the potential molecular mechanisms of acupuncture's therapeutic effects on long COVID symptoms. By

screening protein targets of active ingredients produced by the body after acupuncture and identifying potential therapeutic targets of long COVID, we aimed to unveil the correlative targets of acupuncture against long COVID. Subsequently, we constructed protein-protein interaction networks and performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses to identify the key targets and pathways potentially involved in the therapeutic effects of acupuncture on long COVID symptoms [16-18]. Our findings provide valuable insights into the potential molecular mechanisms of acupuncture's therapeutic effects on long COVID symptoms and may help inform the development of targeted therapies for managing this challenging condition. Furthermore, our study contributes to the growing body of knowledge on acupuncture's potential benefits in the context of long COVID and may inspire further research in this area.

2. Materials and Methods

2.1. Screening of protein targets of active ingredients produced by the body after acupuncture

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To identify the protein targets with high binding affinity to the active ingredients produced by the body after acupuncture, we acquired target information from the STITCH [19] and SwissTargetPrediction databases [20]. STITCH is a comprehensive database of 430,000 chemicals linked to an easy-to-use resource that aims to facilitate the study of interactions between proteins and chemicals [19]. SwissTargetPrediction is a web server that combines 2D and 3D similarity measures with known ligands to accurately predict biologically active molecular targets [20].

2.2. Screening of potential therapeutic targets of long COVID

To enhance the translational significance of our research, we focused on human genes. GeneCards [21], a compendium of human genes, is a comprehensive database containing information on annotated and predicted genes, enabling researchers to effectively navigate and correlate the broad fields of human genes, variants, diseases, cells, proteins, and biological pathways. We searched the GeneCards database for key targets associated with long COVID, using keywords such as fatigue, cognitive impairment, joint pain, and dyspnea.

2.3. Screening of the correlative targets of acupuncture against long COVID

We analyzed the obtained protein targets using the Venn tool (<http://bioinfogp.cnb.csic.es/tools/venny>) [22]. The degree of overlap between targets associated with acupuncture-related active ingredients and long COVID-associated targets was visualized using a Venn diagram.

2.4. Construction of protein-protein interaction networks

To construct protein-protein interaction (PPI) networks, we used STRING11.0 (<http://string-db.org/cgi/input.pl>) [23]. Specifically, the possible therapeutic targets of acupuncture against long COVID were uploaded into the search tool (STRING11.0), selecting humans as the species. The PPI results were sorted by degree value, and the key targets with the top 20 connectivity were identified.

2.5. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis

To further investigate the correlative targets of acupuncture against long COVID and potential signaling pathways, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. The effector targets were plotted and visualized using the DAVID database (<https://david.ncifcrf.gov/home.jsp>) [16-18].

2.6. Statistical analysis

Various bioinformatics tools were employed to screen, analyze, and visualize the data. Protein-protein interaction networks were constructed to identify key targets, while Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were utilized to pinpoint significant pathways and molecular functions involved. The overlap of targets associated with acupuncture and Long COVID was determined through Venn diagrams, highlighting potential therapeutic targets. Statistical significance in the enrichment analyses was assessed, providing insight into the molecular mechanisms by which

acupuncture may exert therapeutic effects on Long COVID symptoms.

3. Results

3.1. Identification of protein targets of active ingredients after acupuncture

After querying the STITCH and SwissTargetPrediction databases, we identified a list of protein targets with high binding affinity to the active ingredients produced by the body after acupuncture (Table 1). These targets are believed to be involved in various biological processes and signaling pathways related to the therapeutic effects of acupuncture. Our results indicated that acupuncture may influence key targets related to various neurotransmitters, cytokines, and growth factors, which play crucial roles in the regulation of immune response, inflammation, and cellular stress (Figure 1). The substances associated with acupuncture include the following:

(1) Neurotransmitters

Dopamine: a neurotransmitter involved in reward, motivation, and motor control. It also plays a role in modulating immune cell function [24].

β -endorphin: an endogenous opioid peptide that regulates pain perception and contributes to the immune response [25].

Serotonin: a neurotransmitter involved in mood, appetite, and sleep regulation, and it has immunomodulatory effects on various immune cells [26].

γ -aminobutyric acid (GABA): the primary inhibitory neurotransmitter in the central nervous system, which also has immunomodulatory properties [27].

Table 1. Identification of protein targets of active ingredients after acupuncture

Substances	References (PMID)
Neurotransmitter	
dopamine	31139074
β -endorphin	15135942
serotonin	35422904
γ -aminobutyric acid (GABA)	28600329
acetylcholine	29520557
glutamic acid	28889839
glycine	33788436
Cytokines	
Interleukins (IL)	27912957
tumor necrosis factor (TNF)	32420752
Interferon (IFN)	28610421
Growth factors	
vascular endothelial growth factor (VEGF)	34114405
nerve growth factor (NGF)	21701996
basic fibroblast growth factor (bFGF)	34114405
brain-derived neurotrophic factor (BDNF)	33752446
glial cell line-derived neurotrophic factor (GDNF)	15925097

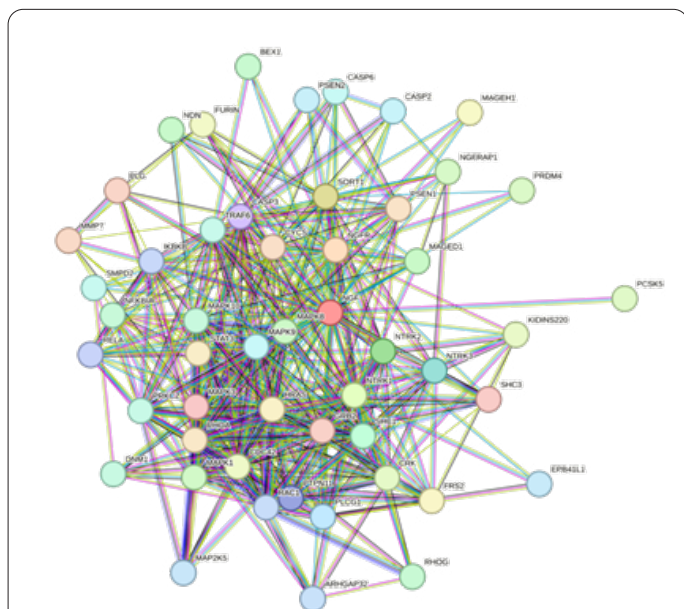


Fig. 1. Protein-protein interaction network analysis of active ingredients after acupuncture. This network diagram depicts the complex interplay between protein targets and active substances produced post-acupuncture. Nodes represent protein targets, their sizes indicating the degree of connectivity - larger nodes signify greater interactions. The thickness of the lines, or edges, shows interaction strength, with thicker lines pointing to stronger interactions.

Acetylcholine: a neurotransmitter involved in muscle contraction, learning, and memory. It also modulates inflammation through the cholinergic anti-inflammatory pathway [28].

Glutamic acid: an excitatory neurotransmitter involved in learning, memory, and synaptic plasticity. It also influences the immune system, particularly in the context of neuroinflammation [29].

Glycine: an inhibitory neurotransmitter with additional roles in immune system regulation and anti-inflammatory effects [30].

(2) Cytokines

Interleukins (IL): a group of cytokines that regulate cell growth, differentiation, and motility, playing essential roles in immune response and inflammation [31].

Tumor necrosis factor (TNF): a cytokine that promotes inflammation and is involved in the regulation of immune cells, apoptosis, and cell survival [32].

Interferon (IFN): a group of cytokines that play crucial roles in the innate immune response to viral infections and modulate adaptive immunity [33].

(3) Growth factors

Vascular endothelial growth factor (VEGF): a growth factor that stimulates angiogenesis and is involved in wound healing and tissue regeneration [34].

Nerve growth factor (NGF): a neurotrophic factor that promotes the survival, growth, and differentiation of neurons, and plays a role in the regulation of pain and inflammation [35].

Basic fibroblast growth factor (bFGF): a growth factor involved in wound healing, tissue repair, and angiogenesis [34].

Brain-derived neurotrophic factor (BDNF): a neurotrophic factor that supports neuronal survival, growth, and synaptic plasticity, and has immunomodulatory effects [36].

Glial cell line-derived neurotrophic factor (GDNF): a neurotrophic factor that supports neuronal survival and regeneration and has anti-inflammatory properties [37].

3.2. Screening of potential therapeutic targets of long COVID

Using GeneCards, we identified several key targets associated with long COVID symptoms, such as fatigue, cognitive impairment, joint pain, and dyspnea (Supplementary Table S1). These targets may play a crucial role in the pathological processes of long COVID and are therefore potential therapeutic targets for intervention.

3.3. Overlap analysis of acupuncture-related and long COVID-associated targets

Using the Venn tool, we found an overlap (84 genes) between the protein targets associated with the active ingredients produced by the body after acupuncture and the long COVID-associated targets (Figure 2). This overlap suggests that acupuncture may exert its therapeutic effects on long COVID through its influence on these common targets.

3.4. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis

The GO and KEGG enrichment analyses showed that the correlative targets of acupuncture against long COVID were significantly enriched in biological processes and signaling pathways related to immune response, inflammation, and cellular stress. This suggests that acupuncture may ameliorate long COVID symptoms by modulating these processes and pathways.

The GO enrichment analysis revealed that the correlative targets of acupuncture against long COVID were significantly enriched in the following biological processes and molecular functions: cytokine receptor binding, growth factor receptor binding, GPCR (G-protein coupled receptor) signaling and MAP (mitogen-activated protein) kinase activity (Figure 3). These findings indicate that acupuncture may modulate immune response, inflammation, and cellular stress by targeting these specific pathways and molecular functions. Our GO enrichment analyses revealed specific signaling pathways and biological processes that are potentially modulated by acupuncture in the context of long COVID. These pathways and processes play cru-

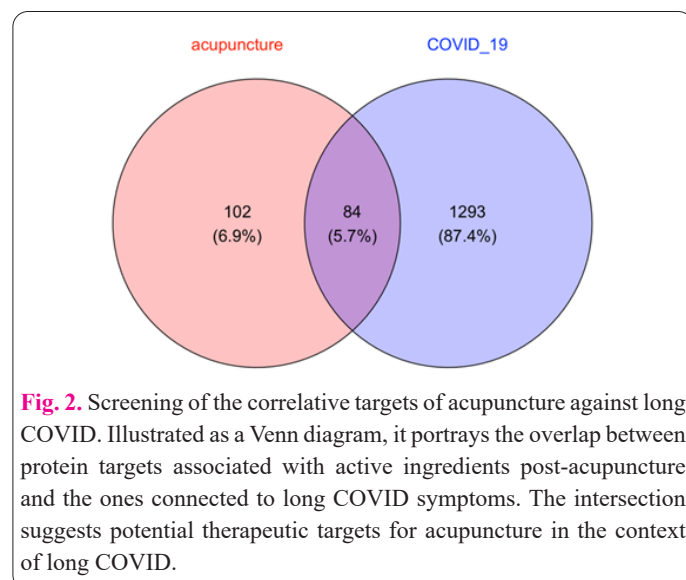


Fig. 2. Screening of the correlative targets of acupuncture against long COVID. Illustrated as a Venn diagram, it portrays the overlap between protein targets associated with active ingredients post-acupuncture and the ones connected to long COVID symptoms. The intersection suggests potential therapeutic targets for acupuncture in the context of long COVID.

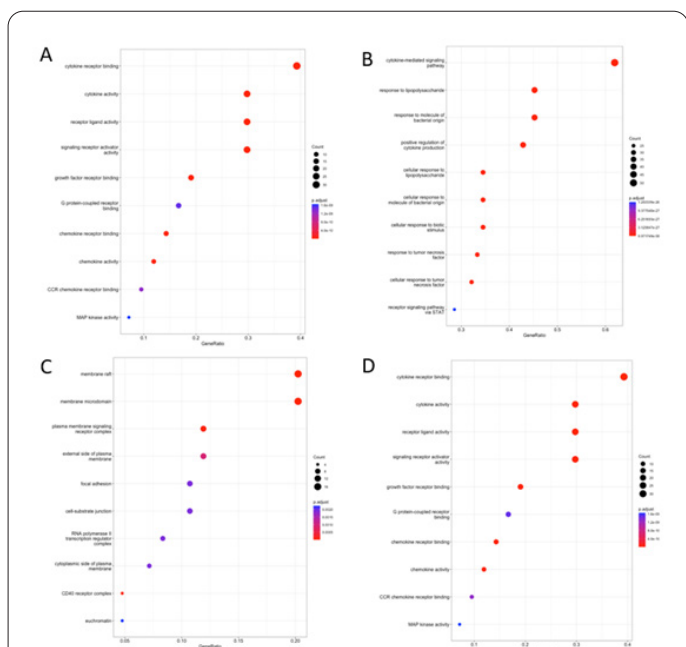


Fig. 3. Comprehensive Gene Ontology Analysis of Key Intersection Genes. (A) Gene Ontology – Molecular Feature (GO-MF) analysis of key intersection genes. This dot plot showcases the most significantly enriched molecular features, presented as functional characteristics of the overlapping genes between acupuncture and long COVID targets. (B) Gene Ontology – Biological Process (GO-BP) analysis of key intersection genes. This dot plot represents the top biological processes, considered as specific, ordered events of the overlapping genes, offering insight into their potential roles in the biological system. (C) Gene Ontology – Cellular Component (GO-CC) analysis of key intersection genes. This dot plot provides a glimpse into the cellular locations or structures where the overlapping genes may function. (D) Gene Ontology total analysis of key intersection genes. This combined plot integrates the results of GO-MF, GO-BP, and GO-CC, illustrating a broad view of the potential roles and interactions of the overlapping genes.

cial roles in immune regulation, inflammation, and cellular stress response, and their modulation may contribute to the therapeutic effects of acupuncture on long COVID symptoms. The GO enrichment key pathways and processes identified in our study include:

Cytokine receptor binding [38]: This GO term refers to the interaction between cytokines and their specific cell surface receptors, which plays a vital role in regulating immune and inflammatory responses. Acupuncture may modulate cytokine receptor binding, thereby influencing the immune system and inflammation in long COVID patients.

Growth factor receptor binding [39]: Growth factors are signaling molecules that regulate various cellular processes, such as cell proliferation, differentiation, and survival. Acupuncture may modulate growth factor receptor binding, potentially affecting cell growth and tissue repair in long COVID patients.

GPCR signaling [40]: G protein-coupled receptors (GPCRs) are cell surface receptors that play crucial roles in signal transduction, modulating various physiological processes. Acupuncture may influence GPCR signaling, thereby affecting a wide range of cellular responses in long COVID patients.

MAP kinase activity [41]: Mitogen-activated protein (MAP) kinases are serine/threonine-specific protein ki-

nases involved in various cellular processes, such as cell proliferation, differentiation, and apoptosis. Acupuncture may influence MAP kinase activity, potentially affecting cellular stress responses and inflammation in long COVID patients.

The KEGG enrichment analysis further demonstrated that the potential signaling pathways influenced by acupuncture in the context of long COVID mainly include IL-17 signaling, TNF signaling, NOD-like receptor signaling, COVID-19-related signaling, and TLR (Toll-like receptor) signaling pathways (Figure 4). These pathways are known to be involved in immune regulation, inflammation, and the host response to viral infections, which are relevant to long COVID pathogenesis. These pathways play crucial roles in immune regulation, inflammation, and cellular stress response, and their modulation may contribute to the therapeutic effects of acupuncture on long COVID symptoms. The key pathways identified in our study include:

IL-17 signaling [42]: Interleukin-17 (IL-17) is a pro-inflammatory cytokine primarily produced by Th17 cells. It plays a critical role in host defense against various pathogens, as well as in autoimmune and inflammatory diseases. Acupuncture may modulate the IL-17 signaling pathway, thereby regulating inflammation and immune response in long COVID patients.

TNF signaling [43]: Tumor necrosis factor (TNF) is a potent pro-inflammatory cytokine involved in the regulation of immune cells, apoptosis, and cell survival. Dysregulation of TNF signaling has been implicated in various inflammatory and autoimmune diseases. Acupuncture may influence the TNF signaling pathway, contributing to the modulation of inflammation and immune cell function in long COVID patients.

NOD-like receptor signaling [44]: Nucleotide-binding

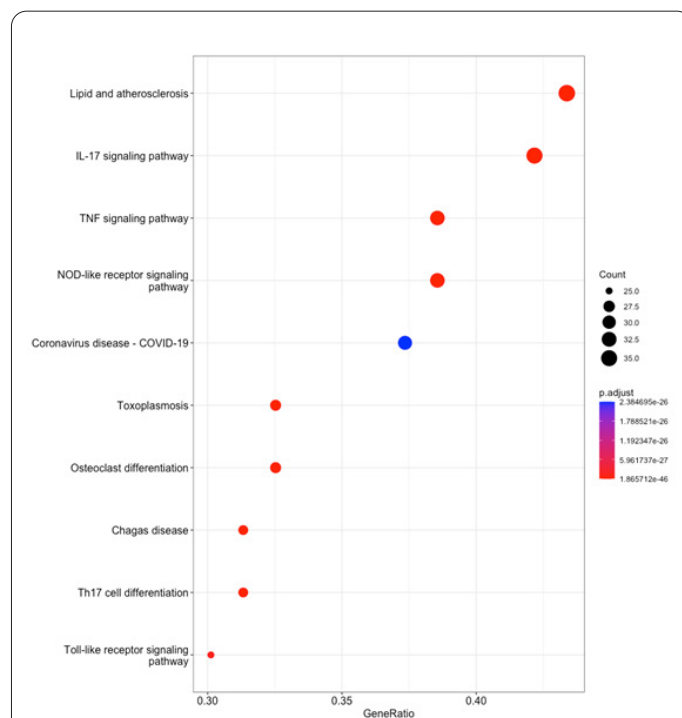


Fig. 4. KEGG enrichment analysis of key intersection genes. Displayed as a dot plot, it reveals the top enriched KEGG pathways of the overlapping genes, helping understand the complex biological behaviors these genes may be involved in. The size of the dots represents the gene ratio, and the color signifies the adjusted p-value, with darker colors indicating higher significance.

oligomerization domain (NOD)-like receptors (NLRs) are intracellular pattern recognition receptors that play crucial roles in innate immunity and inflammation. They can recognize microbial components and endogenous danger signals, initiating inflammatory responses and activating the inflammasome. Acupuncture may target the NOD-like receptor signaling pathway, thus influencing the innate immune response and inflammation in long COVID patients.

COVID-19-related signaling [13]: This category encompasses various signaling pathways that are directly or indirectly involved in the host response to SARS-CoV-2 infection, such as viral entry, replication, immune evasion, and cytokine storm. Acupuncture may modulate these pathways, potentially mitigating the adverse effects of viral infection and improving the clinical outcome in long COVID patients.

TLR signaling [45]: Toll-like receptors (TLRs) are a family of pattern recognition receptors that play essential roles in innate immunity by recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Activation of TLR signaling leads to the production of pro-inflammatory cytokines, chemokines, and type I interferons, which regulate immune and inflammatory responses. Acupuncture may influence the TLR signaling pathway, modulating innate immune response and inflammation in long COVID patients.

Taken together, the involvement of these pathways and molecular functions in immune regulation, inflammation, and cellular stress response further supports the hypothesis that acupuncture has the potential to be an effective therapeutic intervention for long COVID patients.

3.5. Protein-protein interaction network analysis

The PPI network analysis using STRING11.0 revealed the key targets with the top 20 connectivity within the network, indicating their central roles in the interactions between acupuncture-related active ingredients and long COVID-associated targets (Figure 5). These key targets could be critical for understanding the molecular mechanisms underlying acupuncture's potential therapeutic effects on long COVID.

3.6. Gene set enrichment analysis (GSEA)

Our gene set enrichment analysis (GSEA) revealed specific signaling pathways that are potentially modulated by acupuncture in the context of long COVID (in particular, the COVID-related pathways, Figure 6). Collectively, our results suggest that acupuncture may exert its therapeutic effects on long COVID by modulating the levels of key neurotransmitters, cytokines, and growth factors, and by influencing the associated signaling pathways. The involvement of these pathways and molecular functions further supports the hypothesis that acupuncture may ameliorate long COVID symptoms by regulating immune response, inflammation, and cellular stress.

In summary, our results provide a comprehensive overview of the potential molecular targets and signaling pathways involved in the therapeutic effects of acupuncture on long COVID. These findings pave the way for further experimental validation and may help develop new acupuncture-based treatment strategies for long COVID patients.

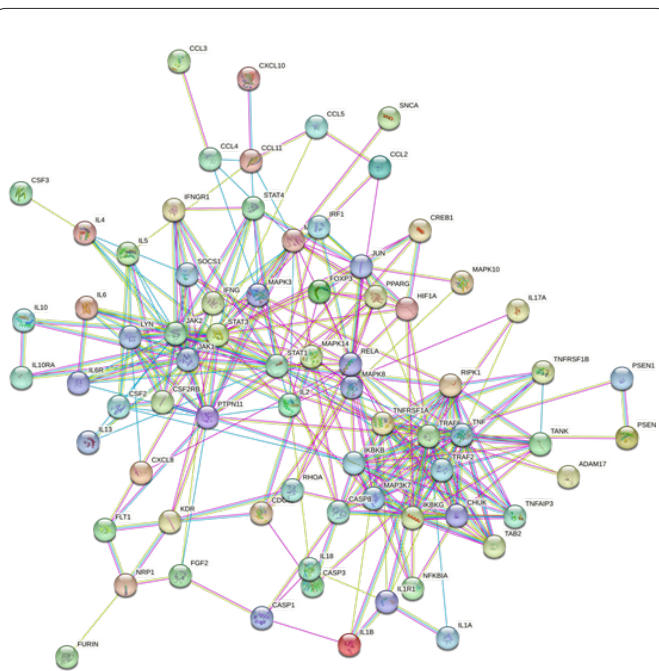


Fig. 5. Protein-protein interaction network analysis of GO enriched genes. This network diagram provides a visual representation of the potential interactions among the GO-enriched genes. The nodes represent genes, their size indicating the degree of connectivity, and the lines represent interactions, with their thickness implying interaction strength.

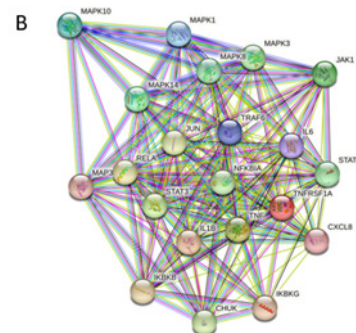
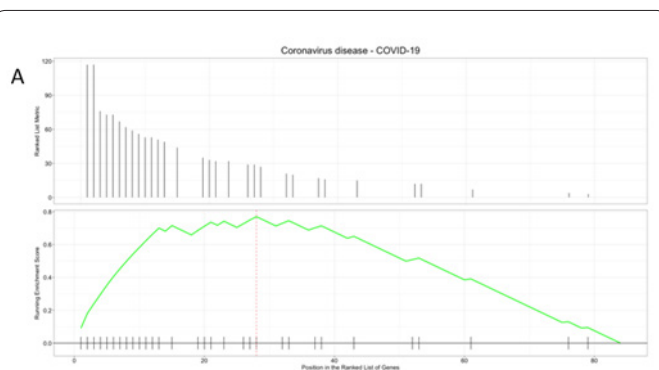


Fig. 6. Enrichment and Interaction Analysis of Key Intersection Genes. (A) Gene set enriched analysis (GSEA) of key intersection genes. This enrichment plot demonstrates the distribution and enrichment score of the overlapping genes in a predefined gene set, indicating the collective behavior of these genes. (B) Protein-protein interaction network analysis of enriched genes in COVID-related pathway of GSEA. This network diagram highlights the potential interactions among the enriched genes in the COVID-related pathway. The nodes denote individual genes, with their size reflecting the degree of connectivity, and the lines represent possible interactions, with their thickness showing the strength of these interactions.

4. Discussion

The present study leveraged bioinformatic tools to explore the potential molecular mechanisms by which acupuncture might provide therapeutic benefits for long COVID patients. Our comprehensive analysis identified multiple key protein targets and signaling pathways that are implicated both in the therapeutic effects of acupuncture and in the pathogenesis of long COVID.

In the wake of acupuncture, the body produces various active ingredients that target a multitude of proteins related to neurotransmitters, cytokines, and growth factors. These substances are known to play pivotal roles in the regulation of immune response, inflammation, and cellular stress. Among them, neurotransmitters such as dopamine, serotonin, and acetylcholine, and cytokines like interleukins and tumor necrosis factor, are well-documented for their immunomodulatory effects. Growth factors like vascular endothelial growth factor and nerve growth factor are crucial for wound healing, tissue repair, and angiogenesis, which might have implications for recovery from long COVID.

The Gene Ontology (GO) enrichment analysis revealed that the correlative targets of acupuncture against long COVID were significantly enriched in biological processes and molecular functions like cytokine receptor binding, growth factor receptor binding, GPCR signaling, and MAP kinase activity. These findings suggest that acupuncture might modulate immune response, inflammation, and cellular stress by targeting these specific pathways and molecular functions. Meanwhile, the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis further identified key pathways influenced by acupuncture in the context of long COVID, such as IL-17 signaling, TNF signaling, NOD-like receptor signaling, COVID-19-related signaling, and TLR signaling pathways.

The protein-protein interaction network analysis and gene set enrichment analysis (GSEA) further highlighted the central roles and collective behavior of these targets and pathways, providing a system-level view of the potential molecular mechanisms underlying acupuncture's therapeutic effects on long COVID.

In summary, our study suggests that acupuncture may exert its therapeutic effects on long COVID through modulating the levels of key neurotransmitters, cytokines, and growth factors, and influencing the associated signaling pathways. The involvement of these pathways and molecular functions in the pathogenesis of long COVID further supports the hypothesis that acupuncture may be a potential therapeutic intervention for long COVID patients.

However, it should be noted that our study has its limitations. Although bioinformatics analysis can provide valuable insights into the potential mechanisms of acupuncture against long COVID, these findings need to be further validated by experimental studies. Moreover, the complexity of acupuncture's effects and the heterogeneity of long COVID symptoms necessitate personalized and comprehensive treatment strategies. Future research should also explore other potential mechanisms, such as the influence of acupuncture on the gut microbiota and the nervous system.

Despite these limitations, our study provides a promising starting point for the development of new acupuncture-based treatment strategies for long COVID patients. Further investigations are warranted to validate our find-

ings and translate them into clinical practice.

In conclusion, our study provides a comprehensive overview of the potential molecular targets and signaling pathways involved in the therapeutic effects of acupuncture on long COVID. These findings pave the way for further experimental validation and may help develop new acupuncture-based treatment strategies for long COVID patients. Understanding the molecular basis of acupuncture's effects on long COVID can not only contribute to the optimization of acupuncture protocols but also shed light on the complex interplay between acupuncture and the human body, leading to a deeper appreciation of this ancient medical practice in modern healthcare.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Ying Xu and Yuequn Xie designed the study and performed the experiments, Jing Zhong collected the data, Yang Yang analyzed the data, Ying Xu and Yuequn Xie prepared the manuscript. All authors read and approved the final manuscript.

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