Blood transcriptome and machine learning identified the crosstalk between COVID-19 and fibromyalgia: a preliminary study

Z. Zhang¹, Z. Zhu¹, D. Liu¹, Z. Mi¹, H. Tao², H. Fan¹

¹Department of Orthopaedics, Xi-Jing Hospital, The Fourth Military Medical University, Xi’an; ²Department of Orthopaedics, Shenzhen University General Hospital, Shenzhen, China.

Abstract

Objective

The COVID-19 pandemic caused by SARS-CoV-2 has seriously threatened the human health. Growing evidence shows that COVID-19 patients who recover will persist with symptoms of fibromyalgia (FM). However, the common molecular mechanism between COVID-19 and FM remains unclear.

Methods

We obtained blood transcriptome data of COVID-19 (GSE177477) and FM (GSE67311) patients from GEO database, respectively. Subsequently, we applied Limma, GSEA, Wikipathway, KEGG, GO, and machine learning analysis to confirm the common pathogenesis between COVID-19 and FM, and screened key genes for the diagnosis of COVID-19-related FM.

Results

A total of 2505 differentially expressed genes (DEGs) were identified in the FM dataset. Functional enrichment analysis revealed that the occurrence of FM was intimately associated with viral infection. Moreover, WGCNA analysis identified 243 genes firmly associated with the pathological process of COVID-19. Subsequently, 50 common genes were screened between COVID-19 and FM, and functional enrichment analysis of these common genes primarily involved in immune-related pathways. Among these common genes, 3 key genes were recognised by machine learning for the diagnosis of COVID-19-related FM. We also developed a diagnostic nomogram to predict the risk of FM occurrence which showed excellent predictive performance. Finally, we found that these 3 key genes were closely relevant to immune cells and screened potential drugs that interacted with the key genes.

Conclusion

Our study revealed the bridge role of immune dysregulation between COVID-19 and fibromyalgia, and screened underlying biomarkers to provide new clues for further clinical research.

Key words

fibromyalgia, COVID-19, molecular mechanism, immune, diagnosis
Abbreviations:
FM: fibromyalgia
COVID-19: Corona Virus Disease 2019
GEO: gene expression omnibus
WGCNA: Weighted gene co-expression network analysis
GO: Gene Ontology
KEGG: Kyoto Encyclopedia of Genes Genomes
GSEA: Gene Set Enrichment analysis
AUC: Area Under Curve
ROC: receiver operating characteristic
DCA: Decision Curve Analysis
HPA: hypothalamic-pituitary-adrenal
FIQR: Revised Fibromyalgia Impact Questionnaire.

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there are few studies focusing on the molecular mechanisms between FM and COVID-19. In this study, we first obtained blood transcriptome data of FM and COVID-19 from the GEO database and confirmed the common pathogenesis between FM and COVID-19 by Limma, WGCNA, and functional enrichment analysis. Subsequently, the key genes between FM and COVID-19 were screened by machine learning and developed a diagnostic nomogram for predicting the occurrence of COVID-19-related FM. In addition, we evaluated the relationship between key genes and immune cell infiltration. These findings provided new perspectives for understanding the molecular mechanisms between COVID-19 and FM.

Materials and methods

Data abstraction

The microarray dataset for illnesses was obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/), which contains a large collection of high-throughput sequencing and microarray data (26). The blood transcriptome data of FM were available from the GSE67311 dataset, including 67 FM patients and 75 healthy controls (HC) (27). The inclusion and exclusion criteria for FM patients are listed as follows. Inclusion criteria: 1) women >18 years of age; 2) patients with FM identified by ACR 1990 FM diagnosis and with symptoms lasting for more than 6 months. Exclusion criteria were all: 1) chronic inflammatory disease; 2) any major organ dysfunction; 3) autoimmune disease; 4) pregnancy or nursing; 5) Beck depression score ≥25; 6) chronic peripheral pain disease; 7) untreated malignancy; 8) surgery 6 weeks prior to blood collection; 9) long-term corticosteroid use. The details of clinical symptoms are shown in Supplementary Table S1. The blood transcriptome data for COVID-19 were available from the GSE177477 dataset, including 29 COVID-19 patients and 18 HC (28). The 29 COVID-19 patients included 11 symptomatic and 18 asymptomatic.

Data processing and differential expression analysis

All microarray databases were first normalised by the R package “preprocessCore”. Subsequently, p-value <0.05 was set as a threshold for identifying
differentially expressed genes (DEGs) in FM. GSEA analysis was performed to characterise the different KEGG pathways between FM and HC, INESI >1 and NOM p<0.05 was set as the threshold value.

**Functional enrichment analysis**
To identify the biological functions and pathways involved in the genes, Wikipathway, GO and KEGG analyses were performed by the R package “clusterProfiler” and visualised via Metascape (https://metascape.org) (29).

**Weighted gene Co-expression network analysis and common gene screening**
To screen potential genes relevant to COVID-19, we established a weighted gene co-expression network based on the top 25% absolute median difference genes by the R package “WGCNA”(30). The appropriate soft threshold was determined by the picSoft-Treshold function to construct the scale-free network. The adjacency matrix was converted into a topological overlap matrix (TOM) and the modules were defined into branches of a hierarchical clustering tree. Different colours were used to distinguish the modules. Subsequently, the key modules of COVID-19 occurrence were identified based on correlation and the co-morbidity pattern of COVID-19 and FM was characterised by Wayne’s analysis.

**Machine learning**
To identify key diagnostic genes for COVID-related FM, we further filtered the common gene using two machine learning algorithms. LASSO algorithm is a regression algorithm which can adjust the model parameters, reduce the model complexity and avoid overfitting to improve the generalisation ability of the model. Random forest is a classifier containing multiple decision trees which allows random selection of feature subsets to improve the accuracy and generalisation of the model. In our study, LASSO algorithm was carried out by the R package “glmnet”, the minimum lambda value was set as a threshold value (31). Random forest algorithm was performed via the R package “randomForest” and the relative importance score ≥2 was set as a threshold (32). Finally, the intersection of the results of the two algorithms was considered as the key diagnostic gene.

**Diagnostic performance assessment and nomogram construction**
ROC curve was used to evaluate the combination diagnostic performance of these key genes for FM by R package “pROC”. Subsequently, R package “rms” was applied to construct a nomogram to predict the occurrence of COVID-19-related FM based on these key genes. The calibration curve was used to assess the robustness of the constructed nomogram. Decision curve analysis was performed to identify the clinical benefit of the constructed nomogram.

**Immune microenvironment characterisation**
The ssGSEA algorithm was used to calculated the expression of 28 immune cells in each sample in the FM cohort. Spearman correlation analysis was performed to assess the relationship between key genes and immune cells.

**Screening for potential drugs**
Drug Signatures database DSigDB tool of Enrichr was utilised to screen for drugs that interact with key genes (33). Combined score was used to filter the Top 10 drugs, and adjusted p-value <0.05 was considered statistically different

**Statistics**
Statistical analysis was conducted using the R 4.0.5 software and SPSS 21.0. All differences among and between groups were considered to be statistically significant at p<0.05 (*p<0.05, **p<0.01, and ***p<0.001).

**Results**
Identification of DEGs in FM and GSEA analysis
The workflow diagram of this study was shown in Figure 1. We found a to-

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**Fig. 3.** Functional enrichment analysis and key module screening. (A) Wikipathway analysis of DEGs. (B) KEGG pathway analysis of DEGs. (C) GO terms analysis of DEGs. (D) PPI analysis identification key module.
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Functional enrichment analysis
To further elucidate the potential pathogenesis of FM, we performed functional enrichment analysis on DEGs. Wikipathways analysis revealed that these DEGs were mainly involved in the ciliary landscape, focal adhesion: PI3K-Akt-mTOR-signaling pathway, network map of SARS-CoV-2 signalling pathway, VEGFA-VEGFR2 signalling pathway, DNA IR-double strand breaks and cellular response via ATM, glycosylation and related congenital defects, regulation of actin cytoskeleton, folate metabolism, biomarkers for urea cycle disorders, neuroinflammation and glutamatergic signalling, TCA cycle, haematopoietic stem cell differentiation, IL-3 signalling pathway, mevalonate arm of cholesterol biosynthesis pathway, DNA repair pathways full network, male in-
fertility, complement and coagulation cascades, MECP2 and associated Rett syndrome and development of ureteric collection system (Fig. 3A); KEGG analysis revealed that these DEGs were engaged mainly in Herpes simplex virus 1 infection, olfactory transduction, ECM-receptor interaction, protein processing in endoplasmic reticulum, asthma, nitrogen metabolism, AMPK signalling pathway, terpenoid backbone biosynthesis, neuroactive ligand-receptor interaction, Epstein-Barr virus infection, protein digestion and absorption, spliceosome, folate biosynthesis, Parkinson disease, ubiquitin mediated proteolysis, nicotine addiction, complement and coagulation cascades, spinocerebellar ataxia, butanoate metabolism, homologous recombination (Fig. 3B). GO analysis revealed that these DEGs were involved mainly in metabolic process, developmental process, multicellular organismal process, response to stimulus, immune system process, response to external biotic stimulus, response to other stimulus, response to external stimulus, innate immune effector process, innate immune effector response, olfactory receptor signaling pathway, olfactory transduction, olfactory receptor neuron signalling, olfactory receptor 3, olfactory receptor 1, olfactory receptor 2, regulation of kappa opioid receptor signalling, protein digestion and absorption, cell morphology, spermatid cell differentiation, and cellular metabolism (Fig. 3D). The above results suggest that dysregulation of multiple signalling pathways involved in the development of FM, providing a new understanding of the pathogenesis of FM.

WGCNA analysis

Subsequently, a scale-free network was constructed to screen the most relevant modules for COVID-19 pathological processes based on WGCNA analysis. When the soft threshold was equal to 10, the scale-free R² = 0.85, which ensured that the network was close to the scale-free network (Fig. 4A-B). Afterwards, β = 10 was considered as the most suitable soft threshold and six gene co-expression modules were constructed by average hierarchical clustering and dynamic tree clipping (Fig. 4C-D). Among these modules, we identified the black module as the most positively correlated with the occurrence of COVID-19 (Fig. 4E). A total of 243 genes in the black module, which were considered to be key genes involved in the progression of COVID-19 pathology were used in the subsequent analysis.

Confirmation of common biomarkers and biological functions between COVID-19 and FM

To reveal the underlying molecular mechanisms of COVID-19-related FM, 50 common genes were screened by the intersection of DEGs of FM and COVID-19-related key modules (Fig. 5A). Wikipathways analysis revealed that these common genes were mainly involved in Type II interferon signalling, IFNG, The human immune response to tuberculosis, Type I interferon induction and signalling during SARS-CoV-2 infection, hostpathogen interaction of human corona viruses interferon, induction, copper homeostasis, proteasome degradation, mitochondrial CII assembly, Type III interferon signalling, TFS regulate miRNAs related to cardiac hypertrophy, IL10 anti-inflammatory signalling pathway, ciliary landscape, COVID-19 adverse outcome pathway, pathways of nucleic acid metabolism and innate immune response.
Fig. 6. Screen for key genes. (A-B) LASSO algorithm. (C-D) Random Forest algorithm. (E) Wayne diagram to obtain the intersection of the two algorithms.
sensing and TCA cycle (Fig. 5B). GO analysis revealed that these common genes were primarily responsible for immune system process, immune effector process, defence response, response to other organism, response to external biotic stimulus, response to biotic stimulus, response to virus, defence response to virus, defence response to other organism, innate immune response, immune response, regulation of I-kappaB kinase/NF-kappaB signalling, Type I interferon signalling pathway, cellular response to type I interferon, Response to type I interferon (Fig. 5C). KEGG analysis showed that these common genes were involved in NOD-like receptor signalling pathway, hepatitis C, influenza A, human immunodeficiency virus1 infection, proteasome, C-type lectin receptor signalling pathway, lysosome, osteoclast differentiation, natural killer cell mediated cytotoxicity, measles, protein processing in endoplasmic reticulum, citrate cycle, galactose metabolism, SNARE interactions in vesicular transport and porphyrin and chlorophyll metabolism (Fig. 5D). These results suggested that SARS-CoV-2 infection might contribute to the development of FM through immune-related pathways.

Machine learning to screen for key genes
Two different machine learning algorithms, LASSO and random forest, were used to identify key genes for COVID-19-related FM. A total of 13 genes were identified by the LASSO algorithm, and 7 genes were identified by the random forest algorithm (Fig. 6A-D). To ascertain the reliability of the results, Wayne’s analysis screened 3 key genes between the two algorithms, including TRIM7, LTA4H and BLVRA (Fig. 6E).

Relationship between key gene and clinical characteristics
To increase the clinical significance of key genes, we further analysed the relationship between key genes and the clinical features. The results revealed that LTA4H was significantly higher expressed in symptomatic COVID-19 patients than in asymptomatic patients, while the other two biomarkers were not significantly different (Fig. 7A-D). Additionally, we found that the expression levels of LTA4H and TRIM7 were positively correlated with the FIQR scores of FM patients (Fig. 7D-E). As the expression of LTA4H and TRIM7 increased, the FIQR scores of patients also increased. Whereas BLVRA expression levels were not statistically related to FIQR scores (Fig. 7F). Besides, there was no statistical difference between the expression levels of key genes and diverse clinical symptoms, including chronic fatigue syndrome, irritable bowel syndrome, major depression and migraine (Supplementary Fig. S1). This might be due to the relatively small number of cases and the wide variation in clinical symptoms presented by each patient, making the statistics slightly biased.

ROC curve and constructing diagnostic nomogram
The logistics analysis revealed that the AUC value of the ROC curve was 0.746 when these key genes were integrated as one variable, indicating that these key genes hold an elevated value for the diagnosis of COVID-19-related FM (Fig. 8A). To further aid clinicians in the diagnosis of COVID-19-related FM, we constructed diagnostic nomograms to predict the occurrence of COVID-19-related FM based on the expression profiles of key genes. As shown in Figure 8B, the risk of FM in patients increased significantly as the total score increased. Calibration curve found that nomogram could accurately predict the
occurrence of FM (Fig. 8C). DCA curve showed that FM patients could benefit from nomogram (Fig. 8D).

**Immune microenvironment analysis**
Considering the vital role of immune dysregulation in COVID-19-related FM, we further analysed the relationship between key gene and immune cells. As shown in Figure 9, the expression of CD56bright natural killer cell, mast cell, memory B cell, and Type 2 T helper cell in FM was obviously lower than that in HC group (Fig. 9A). Correlation analysis revealed that LTA4H was positively correlated with effector memory CD4 T cell and regulatory T cell; TRIM7 was positively correlated with CD56bright natural killer cell and Monocyte, and negatively correlated with Activated B cell, Effector memory CD4 T cell, and Immature dendritic cell; BLVRA was positively correlated with Central memory CD4 T cell, Effector memory CD8 T cell; gamma delta T cell, MDSC, monocyte, regulatory T cell, T follicular helper cell, and Type 2 T helper cell (Fig. 9B).

**Identification of candidate drugs**
To identify potential drugs for the treatment of COVID-19-related FM, we screened for drugs that intervene in these key genes via Enrichr database. As shown in Table I, a total of top 10 drugs were identified based on combined score, of which 5 drugs interacted with LTA4H, 4 drugs interacted with BLVRA and 1 drug interacted with both LTA4H and BLVRA. Unfortunately, no drugs were found to interact with TRIM7. These findings could provide new insights into the treatment of COVID-19-related FM.

**Discussion**
Since the outbreak of COVID-19 causing from SARS-CoV-2, the spread of the infection has spread globally with a large number of people, causing an extremely serious risk to human life and health (10). The severe clinical manifestations and sequelae of COVID-19 are closely associated with immune-mediated cytokine storm (12). With the increase in the number of cures, the wave of recovery has brought a substantial number of patients with the long COVID syndrome, in which respiratory dysfunction, somatic dysfunction, and psychological dysfunction are the predominant symptoms (34, 35). Growing evidence showed that the majority of patients with Long COVID developed the symptoms of FM, including widespread pain, which dramatically affected their routine life (16, 17, 36). Therefore, it is of great importance to exploit the common mechanisms of COVID-19 and FM. In the present study, we identified 2505 DEGs between FM and HC. Func-
tional enrichment analysis revealed that the pathogenesis of FM correlated with abnormalities in multiple signalling pathways, including Neuroactive ligand receptor interaction, ECM receptor interaction and viral infection-related pathways. Notably, we detected that Network map of SARS-CoV-2 signalling pathway was intimately associated with the development of FM, suggesting that SARS-CoV-2 infection might cause the development of FM. Subsequently, a total of 50 commonly genes were identified between FM and COVID-19. Interestingly, both Wikipathways and GO analysis demonstrated that these common genes were mainly involved in immune-related responses, especially in the interferon-related pathway. Interferons are the key effector molecules of the innate immune signalling pathway and constitute the first barrier of defence against viral immunity (37). Depending on the receptor, structure and source, interferon can be classified into type I, II and III, which not only disrupt various stages of viral replication to exert antiviral effects, but also serve an integral role in immune regulation (38). The main pathological damage of COVID-19 is not the directly killing cells, but the excessive inflammatory response and tissue damage caused by the virus multiplication (39). It was found that the early stage of COVID-19 can lead to suppression or late onset of interferon response, resulting in an imbalance of the innate and adaptive immune responses in the body, exacerbating a large number of pro-inflammatory cytokines and ultimately causing the inflammatory factor storm that contributes to the progression of the disease (40). In contrast, an elevated interferon response was clearly observed in patients with late or mild stage of COVID-19 (41). FM as an idiopathic chronic illness and its pathogenesis is still controversial. Currently, autoimmunity and neuroinflammation are thought to exert an essential role in

Fig. 9. Immune microenvironment analysis. (A) Differences in immune microenvironment between FM and HC. (B) Heat map showing the correlation between key genes and immune cells.
the pathophysiology of FM (42). Fine-schi et al. revealed that interferons were markedly increased in FM patients and that inflammatory serum protein levels were tightly correlated with the severity of FM (43). Dolcino et al. identified the immune pathway associated with the type I interferon signature as playing an important role in the pathogenesis of FM through the gene expression profile of FM (42). Hence, we suggested that the occurrence of FM in patients with SARS-CoV-2 infection might be due to the abnormal activation of interferon signalling resulting in the disruption of autoimmune and inflammatory responses. Indeed, FM is also considered to be a condition linked to chronic stress, hypothalamic-pituitary-adrenal (HPA) axis, and autonomic nervous system dysregulation (8). It has been reported that the development of COVID-19-related FM might be related also to stress and traumas coming from social isolation and COVID-19-related treatments (2, 16). These points indicate that apart from immune dysregulation, the critical role of stress, HPA axis, and sympathetic nervous system hyperactivity must also be considered in the pathogenesis of COVID-19-related FM. Overall, our findings provided new ideas for a deeper understanding of the molecular mechanisms underlying the occurrence of COVID-19-related FM. Later, three key genes (LTA4H, TRIM7, and BLVRA) involved in COVID-19-related FM occurrence were identified between these common genes based on two machine learning algorithms. LTA4H can encode an enzyme that contains both hydrolase and aminopeptidase activities, which are essential for the synthesis of the pro-inflammatory mediator leukotriene B4 (44). Leukotriene B4 expression levels have been found to be significantly elevated in FM patients and further elevated with increasing injury (45, 46). Leukotriene B4 is involved in the initiation of inflammation and immune response regulation, which is closely related to the excessive systemic inflammatory response after SARS-CoV-2 infection (47, 48). Kiyoyuki et al. identified that the increased leukotriene B4 could augment NMDA receptor sensitivity in dorsal horn neurons leading to central sensitisation and eventually mechanical pain hypersensitivity (49). In addition, Snelgrove et al. found that LTA4H was able to chemotactic neutrophils to accumulate continuously, thus exacerbating the inflammatory response (50). TRIM7 as a member of the tripartite motif (TRIM) family considered to be a key regulator of innate immunity and antiviral responses (51). Previous studies have shown that upregulated expression of triplet motif genes was disturbed in SARS-CoV-2-positive patients (52). Liang et al. found that TRIM7 could complex with multiple peptides of SARS-CoV-2 proteins to trigger ubiquitination and degradation of the substrates to exert antiviral effects (53). Lu et al. identified that TRIM7 can facilitate TLR4-mediated innate immune response through the E3 ligase structural domain in macrophages (54). BLVRA encodes a biliverdin reductase family protein, of which mutations are associated with hypercholesteraemia. BLVRA was found to significantly inhibit oxidative stress and prevent hippocampal neuron death through MAPK signalling pathway (55). Zhang et al. found that BLVRA was able to trigger the eNOS/NO/TLR4 signalling cascade to suppress inflammation (56). Furthermore, BLVRA was shown to be a key player in Alzheimer’s pathogenesis and could be used for the early diagnosis of Alzheimer (57).

During our study, the results identified that LTA4H was related to the severity of COVID-19. The expression level of LTA4H was significantly increased in symptomatic COVID-19 patients than in asymptomatic. Additionally, we found that the expression levels of LTA4H and TRIM7 were positive related to the FIQR scores of FM patients. The FIQR score is a comprehensive assessment of FM condition, including the evaluation of daily life functions, overall life impact and the severity of the illness (58). As the score gets higher, the patient’s condition gets more severe. Thus, we believe that the expression levels of LTA4H and TRIM7 were important predictors of the severity of FM, which further contributed to the clinical importance of key genes. Subsequently, ROC analysis showed that these three key genes showed satisfactory results for the diagnosis of FM. Moreover, the constructed nomogram also exhibited excellent predictive performance for the occurrence of FM. These results suggest that these key genes may be important diagnostic biomarkers for the occurrence of COVID-19-related FM and could be used to forecast the early development. Immune analysis revealed that these key genes were closely associated with a variety of immune cells. A increasing evidence showed that immune cells perform an essential role in the maintenance of musculoskeletal pain and central sensitisation (42). Immune cells from FM patients

<table>
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<tr>
<th>Drug Term</th>
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<th>Adjusted p-value</th>
<th>Combined score</th>
<th>Genes</th>
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<td>LTA4H</td>
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</table>
can release more inflammatory factors and chemokines than those from HC patients, driving a systemic immune response resulting in widespread pain and autonomic dysfunction (59). Thus, we suggested that these key genes might mediate the pathological progression of FM by regulating immune cells. In addition, based on the combined score of genes and drugs we identified 10 potential drugs for the treatment of COVID-19-related FM. These predicted drugs might be able to interact with key genes to exert therapeutic effects, providing a novel reference for future clinical work in COVID-19-related FM. Collectively, these findings provided novel biomarkers for the early diagnosis and intervention of COVID-19-related FM.

There were some limitations in this study. First, the present study is a retrospective bioinformatics analysis based on a public database, without available some important clinical features, such as the viral infection history, which may increase the bias of our results. Collecting more information on FM patients to explore the relationship between viral infection and the occurrence of FM is imperative in the coming work. Second, the study included a small sample and lacked an independent validation cohort, so the constructed diagnostic model might have some bias. Finally, the underlying mechanisms of biomarkers and the effectiveness of drugs still need to be confirmed by basic experiments or clinical trials. Considering that shared transcriptional data might not fully describe the mechanism of FM occurrence in COVID-19 patients, further study of transcriptomic data on the occurrence of FM after COVID-19 patient recovery will be the focus of future studies.

Conclusion
In conclusion, our study identified 50 common genes between COVID-19 and FM and revealed an essential function of immune dysregulation in the pathogenesis of COVID-19-related FM. Moreover, 3 key genes were screened by machine learning for early diagnosis and treatment of COVID-19-related FM. Our study furnished a further basis and insight into the molecular mechanisms of co-morbidity of COVID-19 with FM which will contribute to the push for early intervention in the post-COVID-19 era of complications.

References
25. WANG F, XI J, XU SI, GUO H, WANG F, WANG Z.
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Acetylcholine, fatty acids, and lipid mediators are linked to COVID-19 severity. J Immunol 2022; 209(2): 250-61. https://doi.org/10.4049/jimmunol.2200079


