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

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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 recovery 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

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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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Introduction

Fibromyalgia (FM) is an unspecified chronic syndrome characterised by widespread musculoskeletal pain, fatigue, sleep disturbances, cognitive impairment, and anxiety (1, 2). The average global prevalence of FM is 2.7%, with a significantly higher prevalence in women than in men. Meanwhile, the prevalence of FM increased significantly with older age (3). The study found that 91.6% of FM patients suffer from moderate to extremely severe symptoms, and up to 73.2% of patients can develop disability, which brings a heavy economic and mental burden to the whole society (4). The pathogenesis of FM remains enormously controversial, while central sensitisation is thought to be the key driver of the illness (5). However, it is commonly believed that central sensitisation in FM patients does not occur *de novo*, but secondary to the combination of genetic and environmental factors that render the patient susceptible to the illness, including infection, trauma, immune disturbance, endocrine dysfunction, and mental illness (6-8).Therefore, it is of great importance to elucidate the relationship between these triggers and FM to reveal the pathogenesis of FM.

Infectious viral diseases have been a serious challenge and threat to all mankind. COVID-19 is an acute respiratory infection caused by the novel coronavirus severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (9). Since its emergence in December 2019, COVID-19 has shown the devastating capacity to cause more than 500 million cases and more than 6 million deaths worldwide (10). Although the most common clinical symptoms of COVID-19 were fever, malaise and dry cough, myalgia (15–44%) and fatigue (38%) were also observed in many patients (11). Its self-limiting infection process and disease natural history suggest that viral clearance and pathological damage of COVID-19 is determined on the basis of immune response (12). SARS-CoV-2 infection can induce immune imbalance and release large amounts of pro-inflammatory cytokines, such as IL-6, IL-1β, IL-8, etc., resulting in cellular inflammatory factor storms which damage multiple organs and tissues throughout the body (13). Although most patients recover within a few weeks of acute infection, 10–30% of patients experience persistent symptoms after recovery 12 weeks, also defined as long COVID (14). Long COVID has a wide range of symptoms, most commonly reported physical symptoms include widespread musculoskeletal pain, fatigue and shortness of breath, which severely impact the life quality (15). The burden of FM is actually very controversial, since there is no consensus among studies about the incidence of chronic widespread musculoskeletal pain (up to full-blown FM) in recovered COVID-19 patients (16). Ursini *et al*. found that up to 30% of COVID-19 patients showed FM symptoms after recovery from infection in a cross-sectional study, and even more than six months after recovery from reinfection there were still few patients who showed symptoms of systemic musculoskeletal pain again (17). It was found that SARS-CoV-2 is not only confined to the respiratory tract, but also invades the nervous system, exacerbating neuroinflammation resulting in persistent central sensitisation symptoms and muscular system damage (18). Interestingly, among patients infected with SARS-COV-1 in 2003, a post-infection syndrome including chronic widespread musculoskeletal pain, fatigue, depression, and sleep disturbances, similar to FM, has been observed in some patients (19). The long-term persistence of similar clinical conditions has been likewise observed in other viral infections, suggesting a common pathogenesis of viral infections and FM (20).

Given the large absolute number of survivors of the pandemic in these years, there are increasing reports of long COVID (21). Bioinformatics analysis as an emerging technology can integrate and analyse biological data to facilitate the researcher to understand better the common mechanism between different illnesses and make effective countermeasures (22-24). For example, Wang *et al*. explored the interaction between COVID-19 and Alzheimer through bioinformatics analysis and revealed four targets for the development of COVID-19-related Alzheimer (25). However,

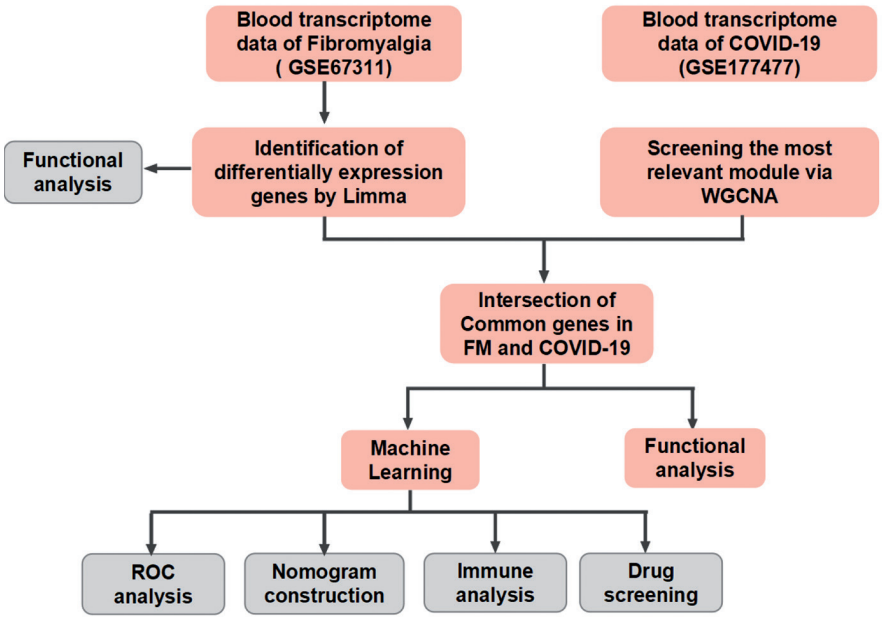

Fig. 1. Study flowchart.

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

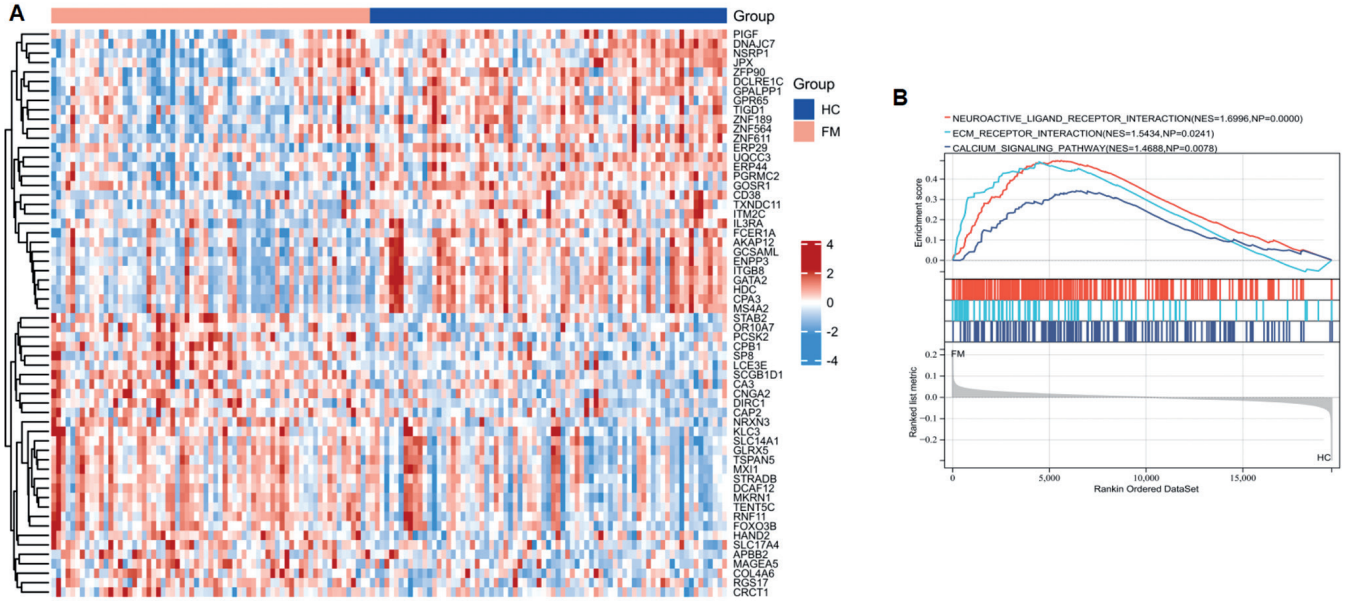

Fig. 2. Identification of DEGs and GSEA analysis in FM database. (**A**) Visualisation of heatmap for DEGs. (**B**) GSEA analysis.

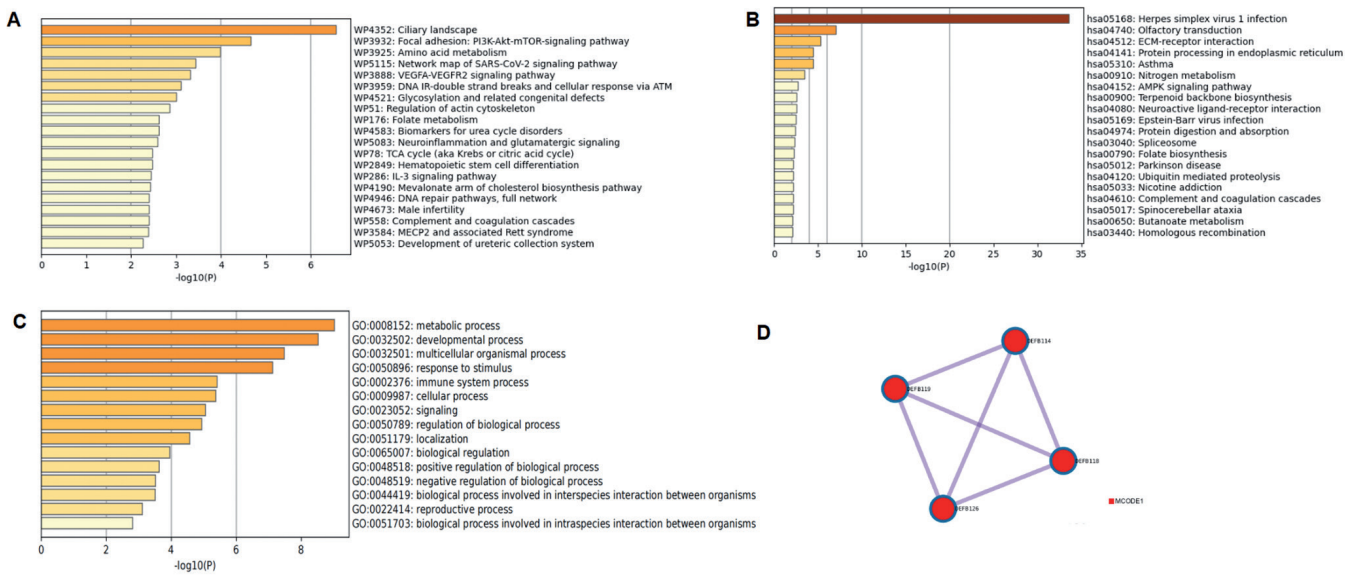

Fig. 3. Functional enrichment analysis and key module screening. (**A**) Wikipathway analysis of DEGs. (**B**) KEGG pathway analysis of DEGs. (**C**) GO terms analysis of DEGs. (**D**) PPI analysis identification key module.

differentially expressed genes (DEGs) in FM. GSEA analysis was performed to characterise the different KEGG pathways between FM and HC, |NES| >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 pickSoftTreshold 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 lamba 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-

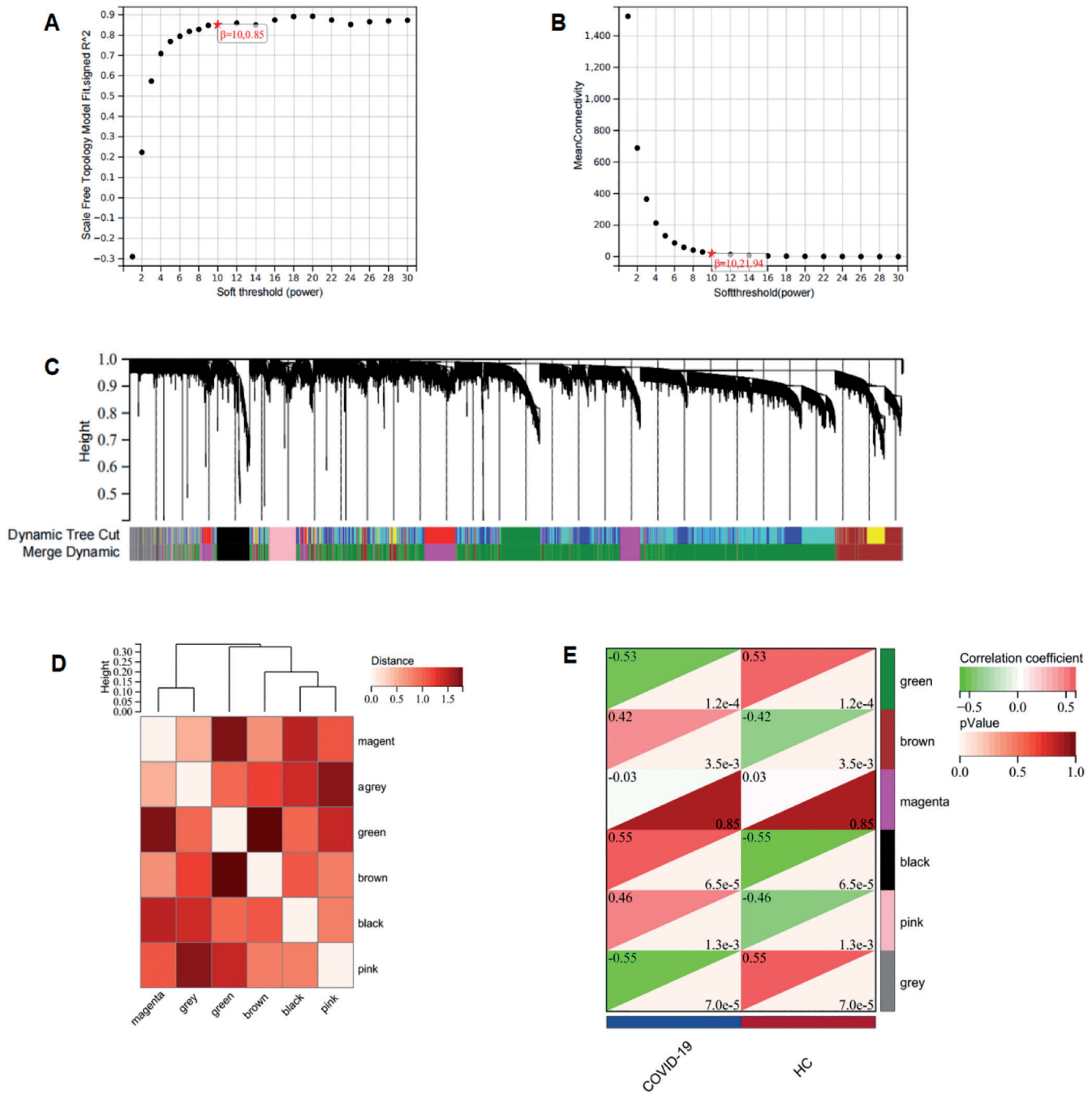

Fig. 4. WGCNA analysis in COVID-19 database. (**A-B**) β=10 was considered as the optimal soft threshold for scale independence and average connectivity analysis. (**C**). The cluster dendrogram of co-expression genes in COVID-19. (**D**) Co-relationship of different modules and characteristics.

tal of 2505 DEGs between the FM and HC groups, of which 1242 genes were up-regulated and 1263 genes were down-regulated in expression (Fig. 2A). GSEA analysis revealed that Neuroactive ligand receptor interaction, ECM receptor interaction, and Calcium signalling pathways were significantly activated in FM (Fig. 2B). These findings improve new evidence for the central sensitisation hypothesis.

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-

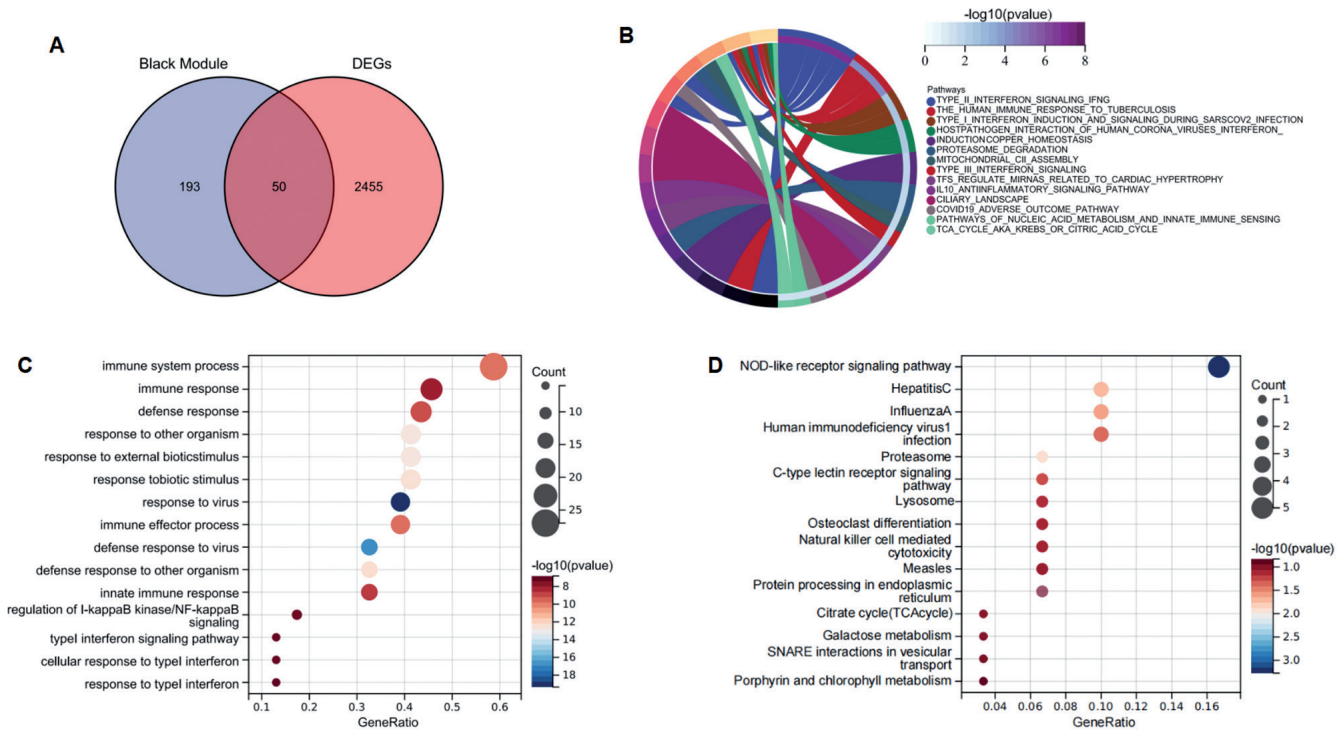

Fig. 5. Recognition of common genes between FM and COVID-19 and enrichment analysis. (**A**) Venn diagram showing 50 common genes. (**B**) Wikipathway analysis of common genes. (**C**) GO terms analysis of common genes. (**D**) KEGG pathway analysis of common genes.

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, cellular process, signalling biological process, regulation of biological process, localisation, biological regulation, positive regulation of biological process, negative regulation of biological process, biological process involved in interspecies interaction between organisms, reproductive process and biological process involved in intraspecies interaction between organisms (Fig. 3C). PPI analysis showed that hub mode in these DEGs was strongly associated with beta-Defensins (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 R^2= 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, inductioncopper 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

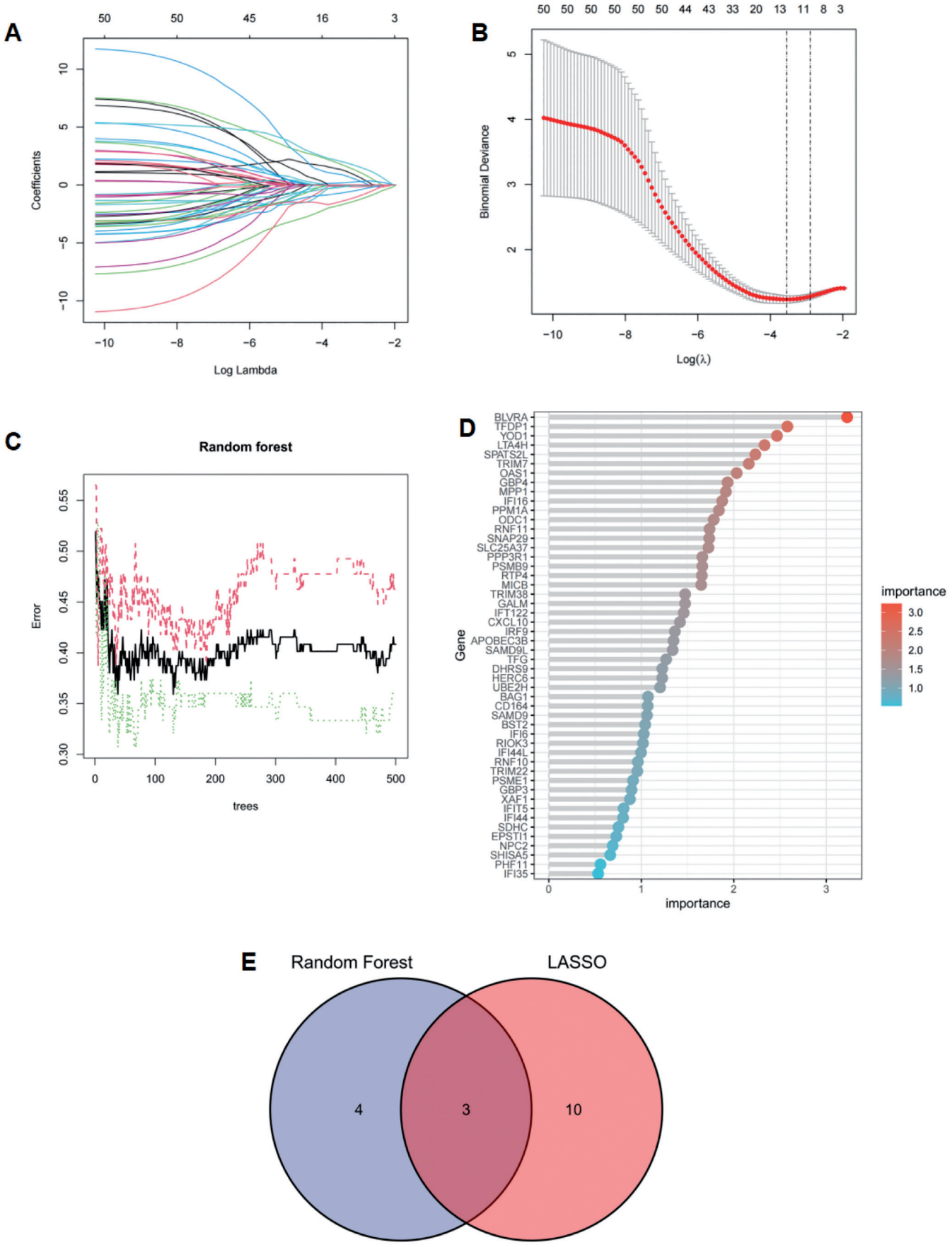

Fig. 6. Screen for key genes. (**A-B**) LASSO algorithm. (**C-D**) Random Forest algorithm. (**E**) Wayne diagram to obtain the intersection of the two algorithms.

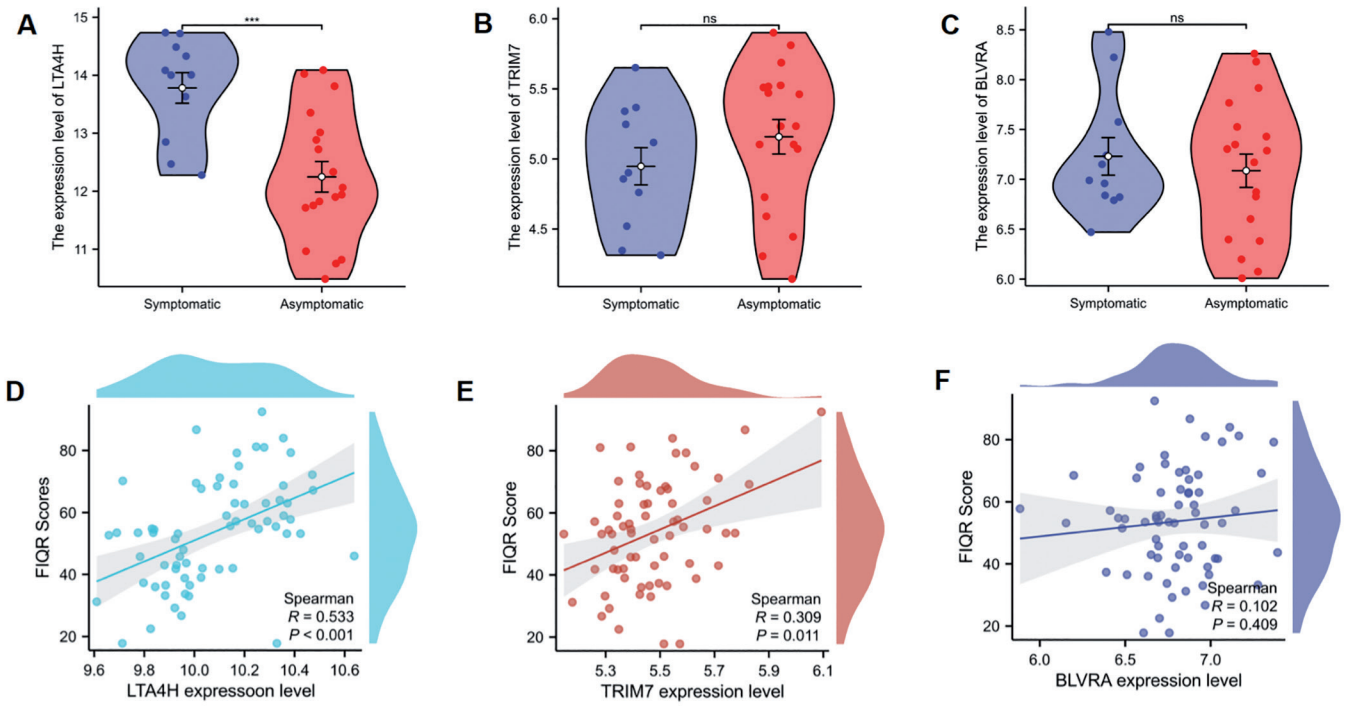

Fig. 7. Relationship between key gene and clinical characteristics. (**A-C**). Differential expression of key genes in clinical symptoms of COVID-19. (**D-E**). Correlation between the expression levels of key genes and the FIQR scores of FM patients.

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-C). 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 IFQR 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

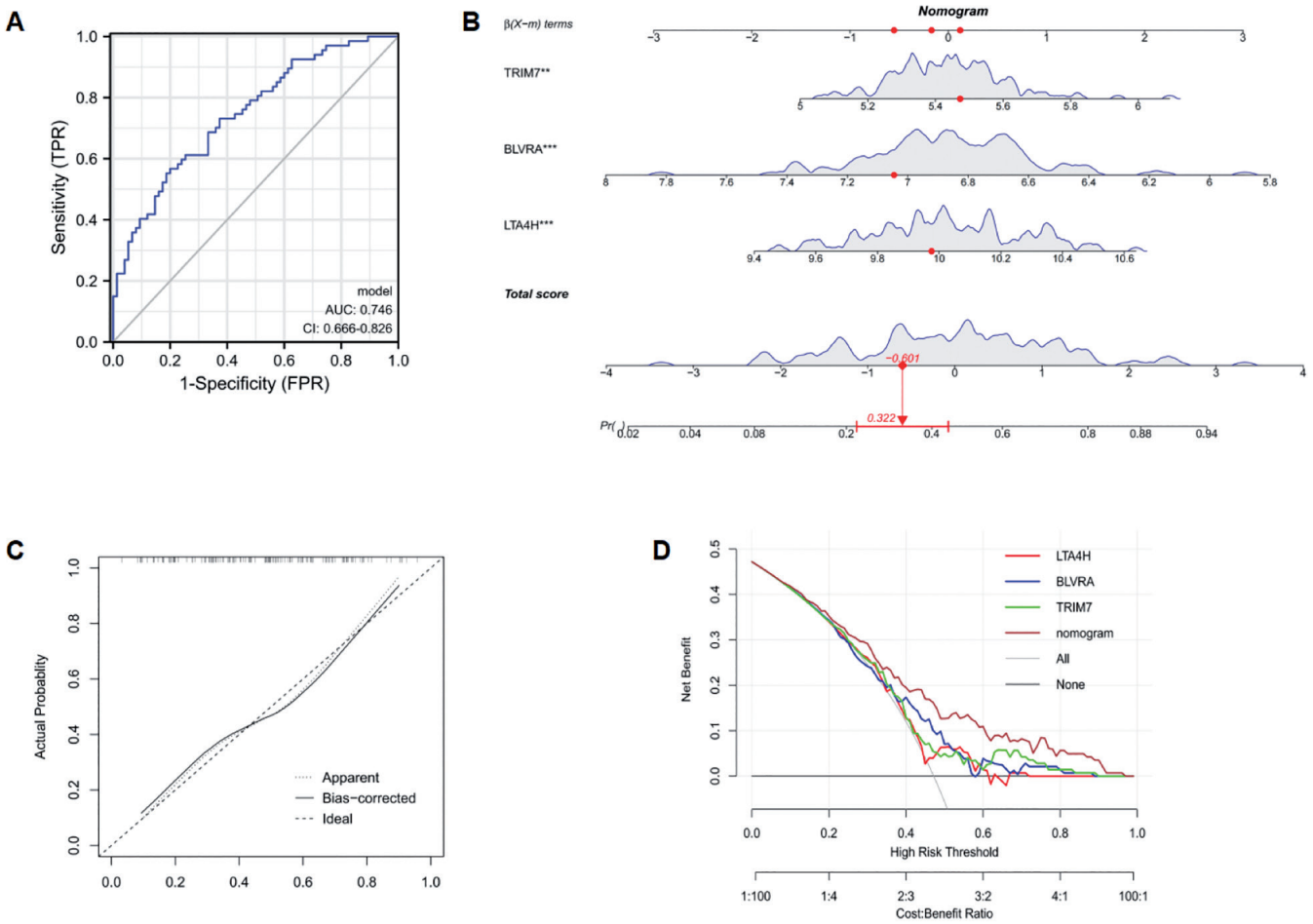

Fig. 8. Diagnostic performance assessment and nomogram construction. (**A**) ROC curve for the Key genes. (**B**) Diagnostic nomogram to predict COVID-19 related FM. (**C**) Calibration curve for diagnostic nomogram. (**D**) Decision curve analysis for diagnostic nomogram.

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-

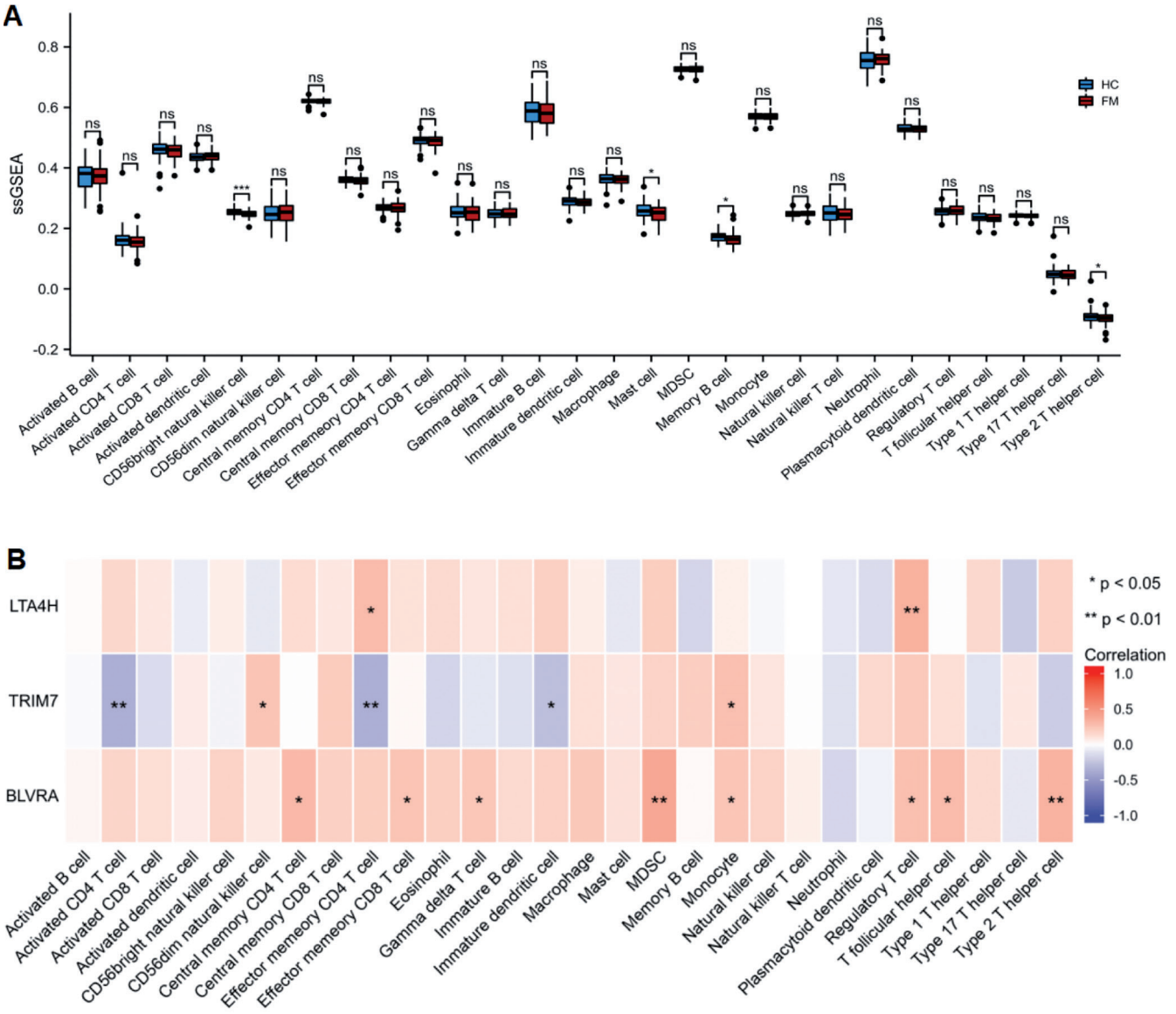

Fig. 9. Immune microenvironment analysis. (**A**) Differences in immune microenvironment between FM and HC. (**B**) Heat map showing the correlation between key genes and immune cells.

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

Table I. Drug candidates combined with key genes.

Drug Term	*p*-value	Adjusted *p*-value	Combined score	Genes
AC1NRCGS	0.002098597	0.028832745	4739.656184	BLVRA
73151-67-4	0.002398159	0.028832745	4018.416231	LTA4H
acetate	0.003146801	0.028832745	2877.37169	LTA4H
LXB4	0.003446153	0.028832745	2574.276639	LTA4H
montelukast	0.003745445	0.028832745	2324.863325	LTA4H
TAUROCHOLIC ACID	0.003895069	0.028832745	2216.110625	BLVRA
succinylsulfathiazole	0.004493415	0.028832745	1860.860041	BLVRA
amantadine	0.006287015	0.037238475	1233.68694	BLVRA
2-CHLOROETHYL ETHYL SULFIDE	0.006884403	0.037864219	1103.677423	LTA4H
valproic acid	0.000618282	0.028832745	1014.833651	LTA4H; BLVRA

the pathophysiology of FM (42). Fineschi *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 importance 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 indicated 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

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 feature, 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.

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