Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Research paper

Exploring the potential therapeutic effect of traditional Chinese medicine on coronavirus disease 2019 (COVID-19) through a combination of data mining and network pharmacology analysis

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ABSTRACT

Introduction: Historically traditional Chinese medicine (TCM) has been used as treatment during epidemics. During the recent COVID-19 pandemic patients evidence suggests that the use of TCM has provided health benefits and has been successfully used to control the spread of the disease in China. The aim of this study was to systematically explore the TCM formulae which have been used for the prevention and treatment of pneumonia or 'pestilence' to investigate their compatibility with the Chinese materia medica (CMM) and understand their potential mechanisms in the treatment of COVID-19.

Methods: Frequency analysis was performed to identify high-frequency CMM and CMM groups. Association rules analysis was applied to investigate the compatibility law of CMMs and generate the commonly used CMM groups.

Results: A total of 173 prescriptions were collected. The frequency analysis showed that seven out of ten high-frequency CMMs overlapped with *Lianhua Qingwen Capsules* (LHQWC), and five high-frequency pair-CMMs and four triple-CMMs were included in LHQWC, respectively. Then three groups of CMM were generated from association rules analysis, one of which is *Ma Xing Shi Gan Decoction* (MXSGD). The results of the protein-protein interaction network and enrichment analysis showed that the potential therapeutic mechanisms of the generated prescriptions were involved in the anti-inflammatory, anti-viral, and neuroprotective effects.

Conclusion: This study showed the importance of systematic research on TCM prescriptions and provided candidate CMM groups that have the potential to treat COVID-19. In vitro and in vivo experiments should be conducted to validate these network pharmacology results, which can provide more information for the development of potential antiviral drugs from TCM prescriptions. The combination of TCM treatment and modern medical approaches will benefit patients with COVID-19 and help to overcome the current epidemic.

1. Introduction

Coronavirus disease 2019 (COVID-19), a severe infectious disease caused by a newly discovered coronavirus (called SARS-CoV-2), was described as a pandemic by the World Health Organization (WHO) on 11 March 2020. The latest updated data shows that the spread of the new coronavirus has caused more than 34 million confirmed cases and more than 1 million deaths worldwide, and the epidemic is still ongoing globally. The people with COVID-19 have had a wide range of symptoms reported ranging from mild symptoms to severe illness. The mild symptoms include fever or chills, cough, fatigue, and muscle or body aches, etc., from which patients may recover without requiring special treatment. People presenting with severe illness such as acute respiratory distress syndrome (ARDS), refractory metabolic acidosis, septic shock, etc., will need to seek emergency medical care immediately.

Currently, there are no specific vaccines or treatments for COVID-19, but many ongoing clinical trials are evaluating potential treatments. Since the breakout of COVID-19 in Wuhan city in December 2019, China has taken unprecedented public health interventions to stop the spread of the virus within China with the principle of "early detection, early quarantine, and early treatment". With the long history practice and the proven efficacy in epidemic disease, traditional Chinese medicine (TCM) has recently been purposed for the clinical management of COVID-19. According to the Diagnosis and Treatment Protocol for Coronavirus

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Pneumonia (Version 7) issued by the National Health Commission, several TCM formulae were recommended for different stages of the disease. Besides, three herbal formulas and three Chinese patent medicines (called San Fang San Yao) have also been screened out for the effective COVID-19 treatment [1]. The good clinical outcomes have been achieved by using the TCM treatment, suggesting the importance of TCM in the fight against the COVID-19.

The COVID-19 belongs to the category of plague in TCM, caused by the epidemic pathogenic factors. TCM has accumulated abundant clinical experience and effective formulae for the prevention and treatment of epidemic diseases [2]. Thus, it is necessary to use modern research methods to conduct an in-depth exploration of TCM prescriptions and explore their compatibility laws and mutual connections, which helps to understand the effect of TCM against the epidemic disease. In this study, data mining and association networks were applied to mine the high-frequency Chinese materia medica (CMM) and their compatibility rules in ancient formulae. The CMM groups were highlighted by the association rules analysis, and subsequently, the network pharmacology approach was used to explore the potential therapeutic mechanism of the highlighted groups in COVID-19 treatment.

2. Methods

2.1. Data mining

2.1.1. Data sources

The TCM formulae in this study were obtained by searching the National Scientific Data Sharing Platform for Publication and Health (http://dbcenter.cintcm.com/) using pneumonia and pestilence as keywords. The data processing was conducted as follows: the prescriptions with clear prescription composition, dosage, and clinical indications were retained; then the deduplication was performed to eliminate recurring prescriptions; finally, the CMM name in prescription was standardized based on the record in the Chinese Pharmacopoeia.

2.1.2. Frequency and association rules analysis

The computer programming language Perl was used to conduct the frequency analysis of all CMM in formulae. The association rules of CMMs in prescriptions were analyzed by using the R software (version 3.6.2) with a priori algorithm in arules package. The resulting association rules were expressed in the form of X→Y, in which X represents antecedent items on the left-hand side (LHS) and Y represents consequent items on the right-hand side (RHS). The support degree and confidence level were used to evaluate each association rule. The support degree represents the probability that LHS and RHS were present in one prescription. The confidence level shows the probability of the appearance of RHS in a prescription based on the existence of LHS in the same prescription. These two parameters reflected the drug compatibility tendency statistically.

The support degree and confidence level were used to evaluate each association rule, which can be calculated as follows:

$$support(X \rightarrow Y) = P(XY) = (X,Y).count/T.count \quad (1)$$

$$confidence(X \rightarrow Y) = P(Y|X) = (X,Y).count/X.count \quad (2)$$

where *P(XY)* is the probability that X and Y were present in one TCM formula, *P (Y|X)* is the conditional probability of Y appearing under the premise of X, *(X,Y). count* is the number of TCM formulae containing both X and Y, *X.count* is the number of TCM formulae containing X, and *T.count* is the number of TCM formulae in T.

2.2. Network pharmacology analysis

2.2.1. Collection of active compounds and their targets

The compounds in the highlighted CMMs were obtained from the online public database, including the traditional Chinese medicine systems pharmacology (TCMSP) platform [3], the Encyclopedia of Traditional Chinese Medicine (ETCM) database [4], and the BATMAN-TCM database [5]. The chemical components with the oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 in TCMSP and Drug likeness Grading was Good and moderate in ETCM were retrieved as the active ingredients in this study. Then the related drug targets of these active compounds were collected from the same database, where the screen rule in ETCM and BATMAN-TCM database are target ≥ 0.8 and target score ≥ 400, respectively.

2.2.2. Screening of disease target genes

The COVID-19 targets were obtained by searching the therapeutic target database (TTD) [6], in which the Target & Drug Data for Coronavirus section provided a comprehensive collection of anti-coronavirus drugs and their corresponding therapeutic targets data which are from previous and recent coronavirus research (http://db.idrblab.net/ttd/ttd-search/covid-19-profile). Since the COVID-19 is highly similar to SARS-CoV and MERS-CoV [7,8], especially the bat SARS-like coronavirus, the SARS and MERS were also used as keywords to search for the target that may be associated with the COVID-19 in our study.

2.3. Bioinformatics analysis

2.3.1. Protein-protein interaction background network construction

A protein-protein interaction (PPI) network was constructed to visualize the interaction between the targets related to the active CMM compounds and COVID-19-related targets. The PPI network of targets was constructed via the search tool for the retrieval of interacting genes/proteins (STRING, version 11.0) web tool [9]. STRING is a weighted interaction database with active interaction sources including text mining, experiments, databases, co-expression, neighborhood, gene fusion, and co-occurrence. STRING gives each interaction a confidence score to weigh the reliability of the interaction. In order to construct a background PPI network with high confidence edges, we set the threshold of confidence as 0.7. Only interactions with a confidence score above the threshold were selected. The visualization of the complex interaction networks was archived by using Cytoscape 3.7.0 software [10].

2.3.2. Protein-protein interaction sub-network construction

For each group of formulae, the common targets of CMM compounds and disease and their neighbor nodes with the interactions between them were extracted from the background network to form a new PPI sub-network.

2.3.3. Key protein selection

The degree is the number of the edge that connects with a specific protein, and the betweenness centrality ($C_B(v)$) measures the extent to which a node acts as a bridge between other nodes in the network.

$$C_B(v) = \sum_{s \neq t} \frac{\sigma_v(s,t)}{\sigma(s,t)}$$

Where $\sigma(s,t)$means the number of shortest paths from s to t; and $\sigma_v(s,t)$means the number of shortest paths from s to t with v as an inner vertex. Nodes of high degree and betweenness centrality could be bottlenecks of the network. The key proteins were considered as that possess higher values of degree and betweenness centrality in the sub-network.

2.3.4. Topological module analysis

To investigate the biological functions of the sub-network, a Cytoscape plugin called clusterMaker was applied to perform module analysis for each sub-network using Community cluster (GLay) mode, so that a sub-network could be separated into several topological modules according to the graph density and there are much more links within modules than between modules.

2.3.5. Gene ontology and pathway enrichment analysis

Gene Ontology (GO) annotation and KEGG pathway enrichment analysis were performed on the targets by using the Metascape web tool [11]. The p value was calculated and further corrected, and $p < 0.05$ was used as the cutoff criterion.

3. Results

3.1. Frequency analysis

A total of 318 CMMs were obtained which were contained in 179 TCM formulae. The 10 CMMs recorded which occurred with the highest frequency were screened out by frequency analysis method and are shown in Table 1. The most frequently used CMM was *Gancao* whose frequency in all formulae was close to 50%, followed by *Huangqin* and *Jiegeng* with a frequency of 29.48% and 26.01%, respectively. Most of high-frequency CMMs have the bitter flavor or cool or cold property, including *Huangqin, Jinyinhua, Shigao, Lianqiao, Dahuang*, and *Bohe*, which exert the effect of heat-clearing for the hot- or warm-syndrome. But there are some differences in their activities, *Shigao* has the strongest heat-clearing ability, *Huangqin* is always used to relieve lung heat, *Dahuang* could discharge damp-heat, and both *Jinyinhua* and *Lianqiao* exert the effect of heat-clearing and detoxifying, which can treat COVID-19 patients with the symptoms of fever, diarrhea, red eyes, and redness or swelling of the lips and tongue and so on. Besides, *Gancao* is a tonifying herb, and *Jiegeng, Kuxingren,* and *Mahuang* have the effect of relieving cough and asthma and expelling phlegm. Given all that, the CMMs can enhance the body resistance by regulating the overall functions of the human body and thus to cure disease.

3.2. CMM compatibility law analysis

The compatibility of CMM is very common in TCM practice, which can increase the therapeutic effect of CMMs and ensure medication safety. The pair-CMMs and triple-CMMs are the basic forms of CMM compatibility. The top ten pair-CMMs were shown in Table 2, in which the *Gancao* and *Huangqin* pair ranked first and present in almost 20% of the formulae. *Huangqin*, with the bitter flavor and cold property, has the effect of clearing away heat drying dampness, and heat-clearing and detoxicating. When coupled with *Gancao*, the clearing ability of *Huangqin* will be relieved but the detoxication effect will be exerted more effectively. For the triple-CMMs, *Huangqin, Jiegeng*, and *Gancao* group was the most frequently used in the prescription (Table 3), which increase the antitussive and expectorant effects based on the *Gancao* and *Huangqin* pair.

To further explore the compatibility of CMMs, the association rule analysis was performed to investigate the possible associations or connections among CMM. The overall scatter plot for all rules was shown in Fig. 1**A**, then 10 rules were obtained by setting the confidence level to 100%, as listed in **Table S1**. Taking rule of (*Gancao, Mahuang, Shigao*) → (*Kuxingren*) as an example, the support degree and confidence level of this rule is 8% and 100%, respectively, suggesting that the probability of all four medicines present in a prescription was 8%, but if there are *Gancao, Mahuang*, and *Shigao* in a formula, the probability of the existence of *Kuxingren* in this prescription is 100%. Then the association network plot was constructed to visualize the compatibility relations of CMMs. As shown in Fig. 1**B**, these CMMs were separated into three groups based on the association rules and listed in **Table S2**.

The results of frequency analysis and association rule analysis indicated that *Gancao* was the key medicine of these formulae for the epidemic disease treatment, which was consistent with the previous data mining study [12]. In TCM practice, *Gancao* is described as “National Venerable Master” and is usually used in the combination with other CMMs in many formulae to harmonize the properties of other herbs, alleviate their toxicity and modulate the taste of the formulae due to its sweet flavor [13], which can support the high frequency of *Gancao* in these formulae.

3.3. Analysis of grouped CMMs prescription

The association network plot showed that the CMMs were divided into three groups based on the relations among them. The Group 1 contains *Mahuang, Kuxingren, Shigao*, and *Gancao*, which is a famous TCM formula called *Ma Xing Shi Gan Decoction* (MXSGD) recorded in *Shang Han Lun* edited by Zhang Zhongjing in the Han Dynasty. This formula has been widely applied for the treatment of colds, influenza, acute

Table 1
High-frequency CMMs in prescriptions (Top 10)

No.	Chinese name	Latin name	Frequency	Percentage (%)
1	*Gancao*	Glycyrrhizae Radix Et Rhizoma	84	49
2	*Huangqin*	Scutellariae Radix	51	29
3	*Jiegeng*	Platycodonis Radix	45	26
4	*Kuxingren*	Armeniacae Semen Amarum	37	21
5	*Jinyinhua*	Lonicerae Japonicae Flos	35	20
6	*Shigao*	Gypsum Fibrosum	34	20
7	*Lianqiao*	Forsythiae Fructus	33	19
8	*Mahuang*	Ephedrae Herba	32	18
9	*Dahuang*	Rhei Radix Et Rhizoma	30	17
10	*Bohe*	Menthae Haplocalycis Herba	28	16

Table 2
High-frequency pair-CMMs in prescriptions (Top 10)

No.	pair-CMMs	Frequency	Percentage (%)
1	*Huangqin* (Scutellariae Radix), *Gancao* (Glycyrrhizae Radix Et Rhizoma)	34	20%
2	*Gancao* (Glycyrrhizae Radix Et Rhizoma), *Jiegeng* (Platycodonis Radix)	28	16%
3	*Kuxingren* (Armeniacae Semen Amarum), *Gancao* (Glycyrrhizae Radix Et Rhizoma	26	15%
4	*Huangqin* (Scutellariae Radix), *Jiegeng* (Platycodonis Radix)	25	14%
5	*Shigao* (Gypsum Fibrosum), *Gancao* (Glycyrrhizae Radix Et Rhizoma)	22	13%
6	*Huangqin* (Scutellariae Radix), *Lianqiao* (Forsythiae Fructus)	21	12%
7	*Mahuang* (Ephedrae Herba), *Gancao* (Glycyrrhizae Radix Et Rhizoma)	20	12%
8	*Jiegeng* (Platycodonis Radix), *Lianqiao* (Forsythiae Fructus)	20	12%
9	*Lianqiao* (Forsythiae Fructus), *Gancao* (Glycyrrhizae Radix Et Rhizoma)	20	12%
10	*Gancao* (Glycyrrhizae Radix Et Rhizoma), *Jinyinhua* (Lonicerae Japonicae Flos)	20	12%

Table 3
High-frequency triple-CMMs in prescriptions

No.	triple-CMMs	Frequency	Percentage (%)
1	*Huangqin* (Scutellariae Radix), *Jiegeng* (Platycodonis Radix), and *Gancao* (Glycyrrhizae Radix Et Rhizoma)	17	10%
2	*Shigao* (Gypsum Fibrosum), *Kuxingren* (Armeniacae Semen Amarum), and *Mahuang* (Ephedrae Herba)	16	9%
3	*Shigao* (Gypsum Fibrosum), *Kuxingren* (Armeniacae Semen Amarum), and *Gancao* (Glycyrrhizae Radix Et Rhizoma)	15	9%
4	*Mahuang* (Ephedrae Herba), *Kuxingren* (Armeniacae Semen Amarum), and *Gancao* (Glycyrrhizae Radix Et Rhizoma	15	9%
5	*Jiegeng* (Platycodonis Radix), *Lianqiao* (Forsythiae Fructus) and *Gancao* (Glycyrrhizae Radix Et Rhizoma)	15	9%
6	*Huangqin* (Scutellariae Radix), *Lianqiao* (Forsythiae Fructus) and *Gancao* (Glycyrrhizae Radix Et Rhizoma)	14	8%
7	*Shigao* (Gypsum Fibrosum), *Gancao* (Glycyrrhizae Radix Et Rhizoma), and *Mahuang* (Ephedrae Herba)	13	8%
8	*Bohe* (Menthae Haplocalycis Herba), *Jiegeng* (Platycodonis Radix), and *Gancao* (Glycyrrhizae Radix Et Rhizoma)	13	8%
9	*Jiegeng* (Platycodonis Radix), *Lianqiao* (Forsythiae Fructus) and *Huangqin* (Scutellariae Radix)	13	8%

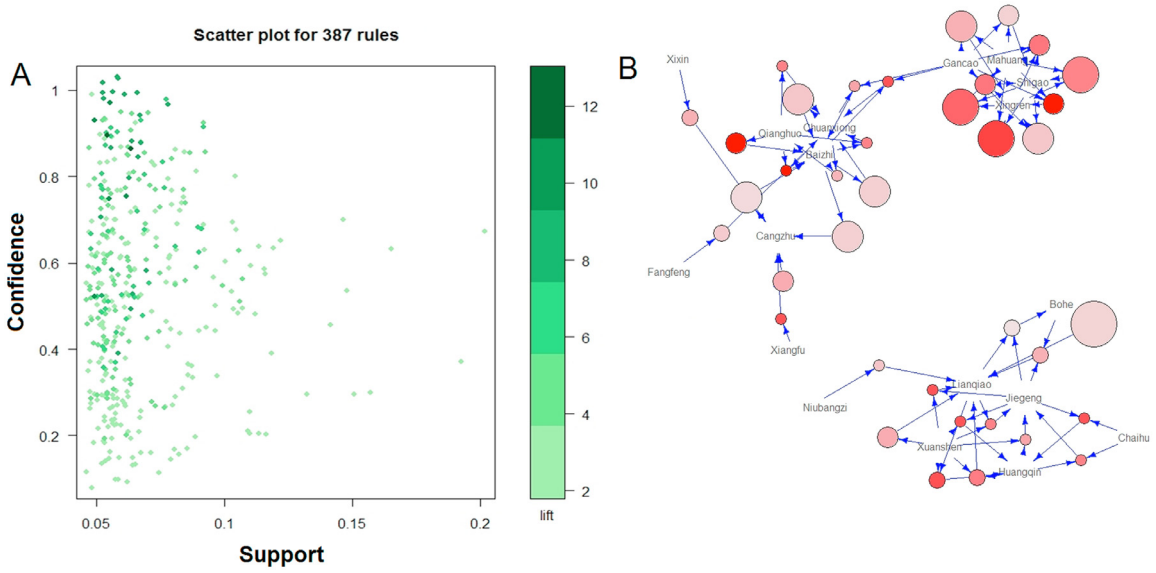

Fig. 1. The scatter plot for rules (A) and CMMs association network (B) of prescription.

bronchitis, bronchial asthma, and chronic obstructive pulmonary disease [14]. The clinical study also showed a positive effect for the patient with H1N1 influenza virus infection [15], and its ability to alleviate the virus-induced lung injury has attracted more and more research interest [16–17]. Therefore, this formula has been recommended as one of the prescriptions for the COVID-19 treatment and has been proved to be beneficial to cure respiratory tract diseases with symptoms of fever, cough, short breath, and body ache, and so on. Besides, based on the composition of the MXSGT, the *Qing Fei Pai Du Decoction* (QFPDD) and *Lianhua Qingwen Capsules* (LHQWC) were manufactured and issued by the National Health Commission and have been widely used in China to treat the COVID-19 patients. Data has suggested significant positive clinical outcomes [18–21].

Group 2 contains seven CMMs, and five of them overlap with *Yin Qiao Pulvis* (YQP). Six out of seven CMMs in Group 3 intersect with *Jiu Wei Qiang Huo Decoction* (JWQHD). The combined use of MXSGD and YQP could reduce the fever duration of H1N1 virus-infected patients [22]. The combination with modification has been recommended by the Health Commission of Gansu, Shaanxi, and Sichuan province for the treatment of COVID-19 [23]. The JWQHD has been used for the treatment of influenza and viral pneumonia for almost 900 years and has a protective effect against H1N1-induced pneumonia [24]. Clinically, the combination of MXSGD and JWQHD has a significant effect on the treatment of fever induced by acute viral upper respiratory tract infection [25].

Three core CMM combinations were obtained from the data mining of the TCM formulae, in which Group 1 is MXSGD, and Group 2 and Group 3 are similar to YQP and JWQHT. Since the MXSGD plays a vital role in the treatment of COVID-19, this prescription has been extensively investigated by using the network pharmacology strategy to reveal its underlying mechanisms in the disease treatment [26–29]. Therefore, we explored the mechanisms of Group 2 and Group 3 prescriptions for their treatment against COVID-19.

3.4. Construction of the compound-target network

A total of 114 and 204 eligible active compounds from Group 2 and Group 3 prescription with their 339 and 583 corresponding potential targets were screen out and collected from the database. Then the compound-target (C-T) network was constructed to show the relations among CMMs, active compounds, and targets. As shown in **Fig. 2A** (465 nodes and 2953 edges) and **Fig. 2B** (797 nodes and 3220 edges), the complex network showed that one compound can interact with several targets, and one target can be regulated by different compound, suggesting these two prescriptions exert the therapeutic function in multiple compounds and multiple target manner, which reflect the holistic thinking and comprehensive regulation of TCM. Several flavonoids such as norwogonin, baicalein, and wogonin, etc. in Group 2 prescription with the connectivity degree larger than 50, and tridecanoic acid, kumatakenin, and glycyrrhetinic acid, etc. in Group 3 have higher connectivity degree, which indicated that these compounds may play a crucial role in the whole regulation network, and could be the key effective compounds for these two formulae to treat disease.

3.4. Construction of protein-protein interaction network

The protein-protein interaction (PPI) network was established by using the STRING web tool to investigate the functional relations among

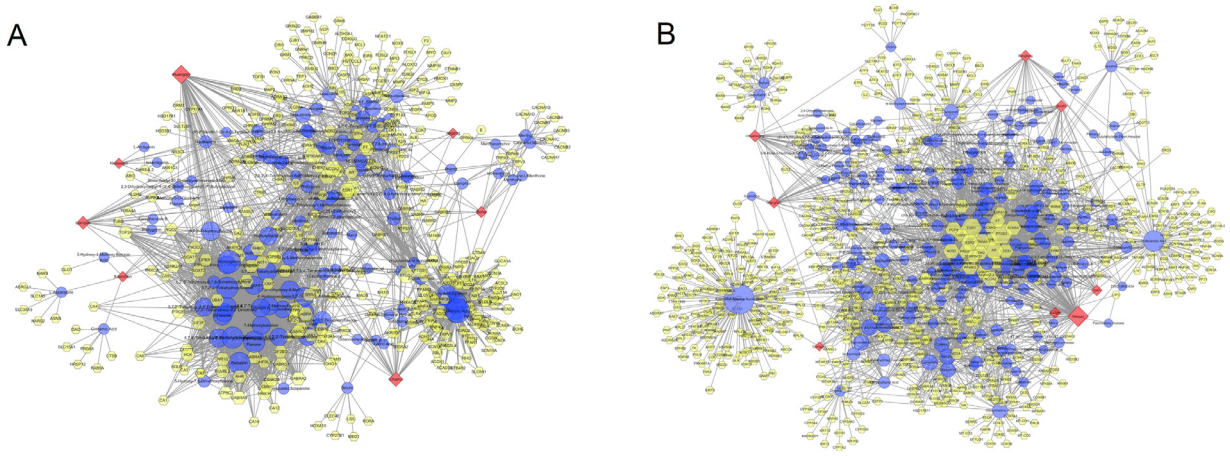

Fig. 2. The CMMs-compound-target network of Group 2 (A) and Group 3 (B) prescriptions (◆: CMM, ●: compound, and ⬢: target; the larger the node, the higher the connectivity degree).

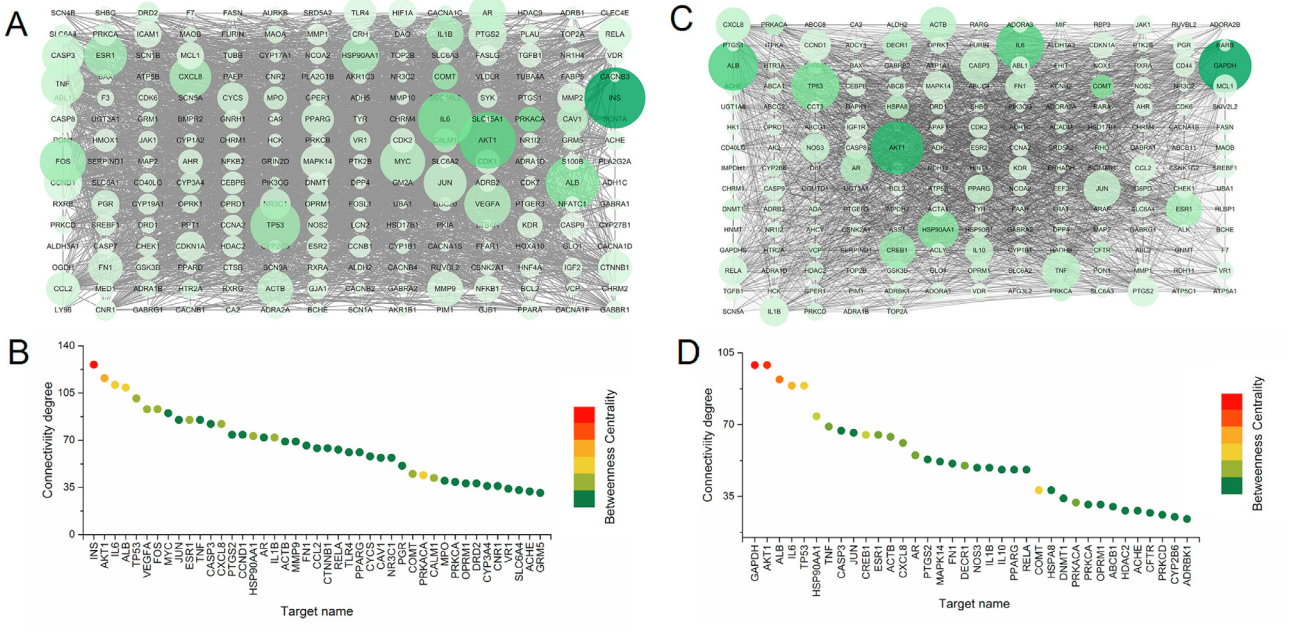

Fig. 3. The PPI network of the target related to Group 2 (A) and Group 3 (C) prescriptions. The larger the node, the higher the connectivity degree, and the darker the color, the larger the betweenness centrality. The distribution of target proteins with the connectivity degree and betweenness centrality higher than the average value in Group 2 (B) and Group 3 (D) prescriptions.

different targets. For the Group 2 formulae, the 399 potential targets and 106 disease targets were used to construct the background PPI network, then the PPI network containing 14 overlapping targets and their neighboring target was extracted from the background network using Cytoscape software. As shown in **Fig. 3A**, the PPI network contains 210 nodes and 3227 edges, in which the nodes represent target proteins, and the edges are the associations between proteins. The degree is the number of the edge that connects with a specific protein, and the betweenness centrality measures the extent to which a node acts as a bridge between other nodes in the network. Both parameters were applied to find the key proteins that possess higher values of degree and betweenness centrality in the network. The topology analysis shows that the average degree and betweenness centrality of this network are 30 and 0.005, respectively. There are 43 nodes with both parameters higher than the averaged values and depicted in **Fig. 3B**. The INS, AKT1, IL6, ALB have a relatively higher connectivity degree and betweenness centrality, which indicated the key role of these proteins in the PPI network.

Through the same procedures, the PPI network of Group 3 prescription containing 20 overlapping targets and their adjacent targets was constructed in **Fig. 3C** with the node number of 187 and edge number of 2189. Then 36 proteins with the degree and betweenness centrality higher than 23 and 0.006 were selected and exhibited in **Fig. 3D**, in which GAPDH, AKT1, ALB, and IL6 possess the higher value of these parameters, suggesting that this protein may be the key target for the disease treatment of the prescription.

A Cytoscape plugin called clusterMaker was applied to perform module analysis for each PPI network using GLay cluster mode, and three main clusters were recognized from the PPI network of Group 2 (**Fig. 4A**) and Group 3 prescriptions (**Fig. 5A**), respectively. For Group 2 formulae, Cluster I contained 59 nodes and 251 interactions, Cluster II had 48 nodes and 272 edges, and Cluster III included 80 nodes and 935 interactional pairs. A total of 66, 43, and 96 nodes, and 519, 198, and 1570 edges were included in Cluster I, II, and III networks of Group 3 prescription, respectively. Then the enrichment analysis of each

sub-network was conducted to explore the therapeutic mechanism of two prescriptions.

3.5. Enrichment analysis

The enrichment analysis was performed using the Metascape web tool, which incorporates a core set of ontologies, including the GO process, KEGG pathways, Reactome gene sets, etc. The results of GO enrichment and KEGG pathway enrichment of two prescriptions were shown in **Fig. 4B and C** and **Fig. 5B and C**, respectively. For Group 2 prescription, the GO terms for Cluster I were mainly related to the synaptic signaling, regulation of ion transport, as well as G-protein-coupled receptor pathway; and the mechanism of Cluster II was mainly associated with sterol and steroid metabolic process, and the hormone metabolic process; and the response to toxic substance, oxygen levels and regulation of cell death were significantly enriched GO term for Cluster III. The KEGG pathway enrichment showed that the neurotransmitter related pathway including serotonergic synapse, neuroactive ligand-receptor interaction, GABAergic synapse, and dopaminergic synapse were associated with the mechanisms of Cluster I; and Cluster II had a high degree of enrichment in the hormone-related pathways; and Cluster III is mainly involved in the regulation of inflammation/cytokine related pathways such as TNF signaling pathway, IL-17 signaling pathway, PI3K-Akt signaling pathway.

The GO enrichment of Group 3 prescription showed that the Cluster I were associated with steroid metabolic process, ribose metabolic process, and purine nucleotide metabolic process; and Cluster II was significantly associated with synaptic signaling and synaptic transmission; and the Cluster III was greatly enriched in response to toxic substance, response to steroid hormone, and response to lipopolysaccharide. The most significant KEGG pathway of the Cluster I include valine, leucine and isoleucine degradation, steroid hormone biosynthesis, purine metabolism, and so on; and Cluster II was mainly involved in the regulation of vascular smooth muscles contraction, salivary secretion, and neurotransmitter related pathway including serotonergic synapse, neuroactive ligand-receptor interaction, GABAergic synapse, and dopaminergic synapse; and the pathway associated with the viral and microbial infections and inflammation responses were enriched in Cluster III.

In summary, the three main clusters of the PPI network of each prescription play synergistic roles in processes like regulating inflammatory response, chemokine production, response to oxygen radicals,

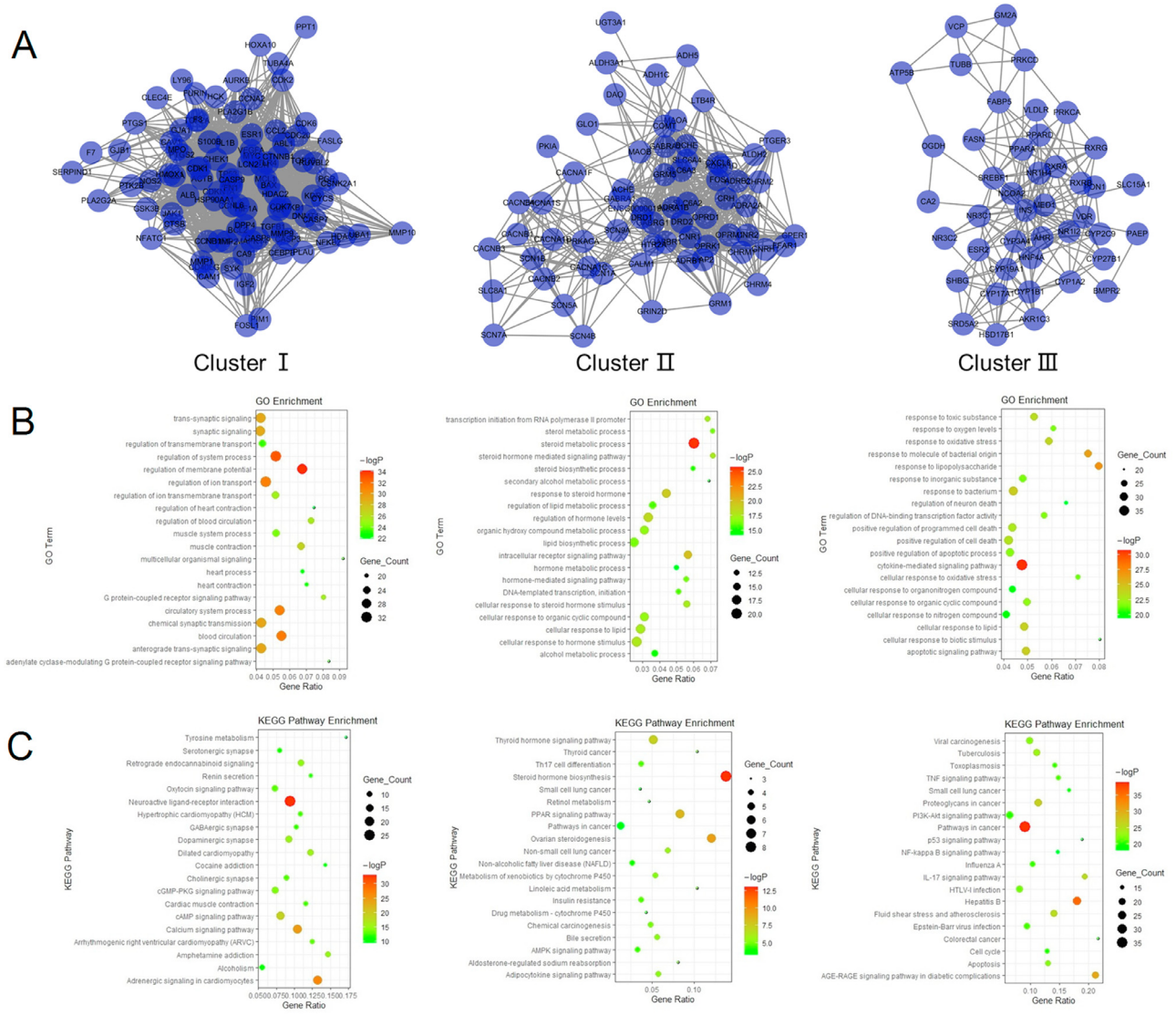

Fig. 4. The PPI cluster of Group 2 prescription and their related GO enrichment (B) and KEGG enrichment (C) analysis results.

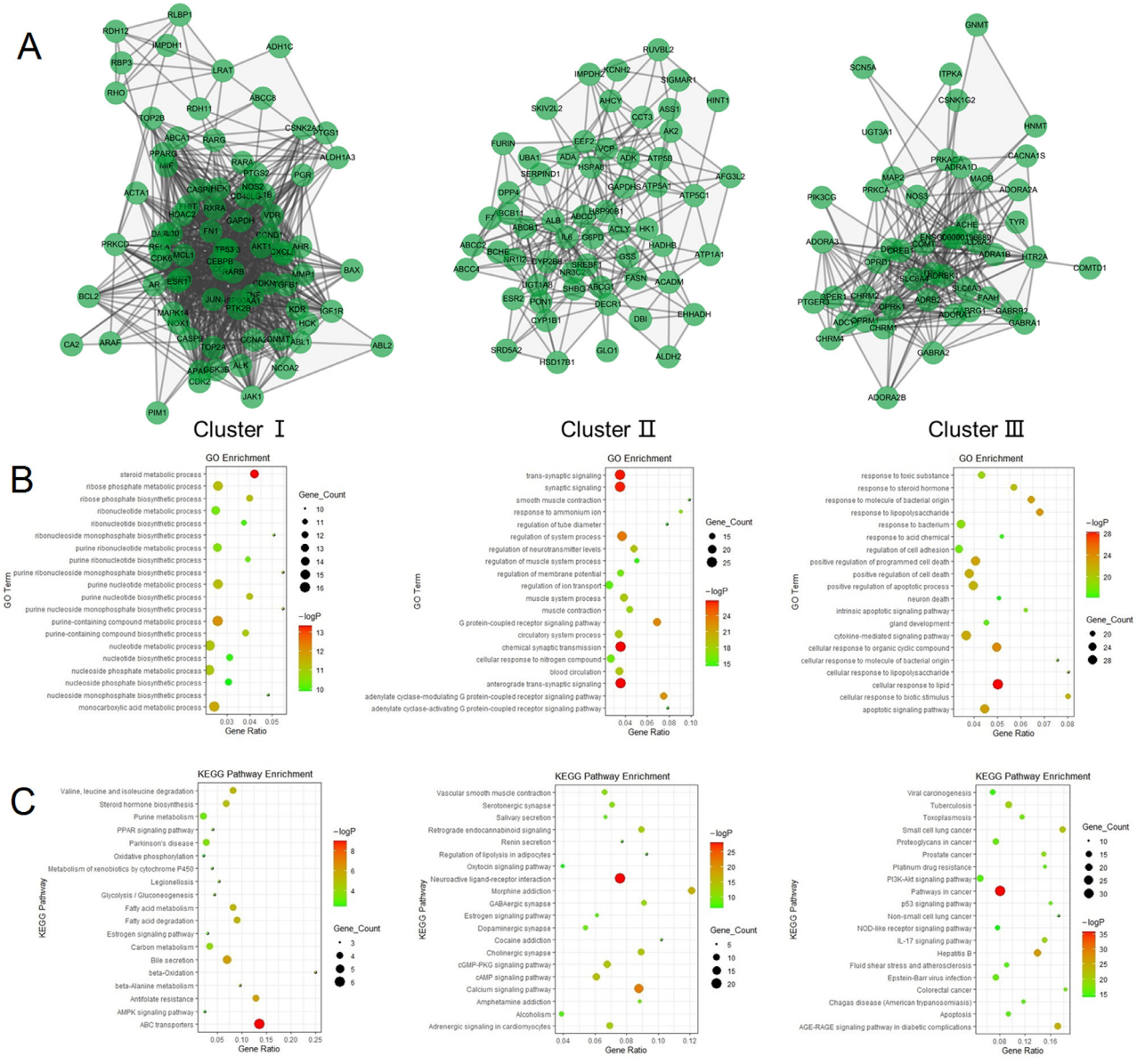

Fig. 5. The PPI cluster of Group 3 prescription and their related GO enrichment (B) and KEGG enrichment (C) analysis result.

apoptosis, and nerve system dysfunction. Understanding the functional modules of each prescription was beneficial for exploring their underlying therapeutic mechanism from different perspectives.

4. Discussion

There are no specific vaccines and effective antiviral agents for preventing and treating COVID-19, and the development of these medications may require months or years, thus, there is an urgent to find a more immediate and effective treatment for COVID-19. Since the COVID-19 outbreak, the Chinese government has encouraged the use of CMMs medication to fight this new viral pneumonia, which has achieved positive clinical outcomes. Historical records show that China has gone through at least 300 epidemics in the past, giving rise to an abundance of experience and literature on combating contagious diseases that are worth exploring. In this study, we collected 173 prescriptions that related to pneumonia and pestilence, then high-frequency CMMs, pair-CMMs, and triple-CMMs were selected by frequency analysis. Compared with the recommended prescriptions in Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia, seven out of ten high-frequency CMMs overlapped with LHQWC, and five pair-CMMs and four triple-CMMs highlighted in this study were included in LHQWC. Besides, three groups of CMMs as new prescriptions were generated based on the association rule analysis. Group 1 is MXSGD, which has shown a pivotal role in treating pneumonia and is also the fundamental part of several TCM prescriptions for the COVID-19 treatment.l

The holistic thinking of TCM has a lot in common with the key ideas of network pharmacology, which makes network pharmacology an ideal strategy to understand the TCM syndrome (ZHENG) and investigate the effect of TCM prescription to overcome complex diseases systematically [30–32]. Thus, the network pharmacology analysis was performed to investigate the underlying mechanism of Group 2 and Group 3 prescriptions for disease treatment. The results showed that the proteins associated with two prescriptions were widely involved in the process of inflammation modulation, including immune response, cytokine production, apoptosis, and oxygen-free radical accumulation. The basic pathology of COVID-19 is viral-caused abnormal immune activation and further result in a cytokine storm that could be a key factor associated with the severity and mortality of COVID-19 [33]. The evidence showed that the concentrations of several cytokines were changed in the COVID-19

patients, confirming the relations between cytokine and diseases. As depicted in **Fig. 3B and D**, two prescriptions were greatly involved in the production of inflammatory factors, including TNF, IL-6, IL-1B, IL-10, and CXCL8, which are well-known pro-inflammatory cytokines. As described by a previous study, the MXSGD, which is Group 1 in the present study, is also widely involved in mediating such processes as inflammation, the immune response, cytokine production, cellular structural integrity, vascular permeability, hypoxia, and oxygen-free radical accumulation. The therapeutic mechanisms of MXSGD on COVID-19 were closely related to the inflammation reduction, cytokine storm suppression, protection of the pulmonary alveolar-capillary barrier, regulation of immune response, and decreasing fever [26].

IL-6 actively participates in inflammation and immune regulation mechanisms. In the lungs, IL-6 is produced by epithelial cells, interstitial fibroblasts, macrophages and other inflammatory cells in response to various stimuli, including allergens, respiratory viruses, exercise, environmental particles, and inhaled toxic particles [34]. A meta-analysis based on nine studies including 1426 patients showed that IL-6 is an important marker of inflammation, which can guide the clinicians to identify patients with severe COVID-19 early in the disease progress [35]. The increased level of IL-6 has been considered as an indicator for the patients with extensive lung lesions and severe cases in Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Version 7).

Reactive oxygen species (ROS) are key signaling molecules that have a crucial role in the inflammatory response. In vitro and in vivo studies showed that SARS-CoV-1 is associated with increased ROS production, and severe lung injury and pro-inflammatory host response depend on activation of the oxidative stress mechanism in monkeys infected by SARS-CoV-1 [36,37]. Besides, while the viral-derived proteins inhibit innate sensing machinery, the inflammasome can independently trigger a stress-induced ROS production via the redox-homeostatic sensing mechanisms and mitochondrial mechanisms, leading to cytokine production [38]. The decrease of the ability to main redox homeostasis with age will increase the risk of excessive immune activation and viral infections in the lung, which may explain that the young healthy individuals have a better ability to respond to coronavirus infections [39].

As reported previously, glycyrrhizin, 18β-glycyrrhetinic acid, and other flavonoids isolated from *Gancao* have anti-inflammatory activity. And the *Gancao* extracts exhibited beneficial effects in both acute and chronic inflammatory conditions and have significant antiviral activities against for example SARS-CoV, influenza virus, and respiratory syncytial virus [40,41]. Moreover, the evidence has confirmed that the flavonoids, the most abundant constituents in *Huangqin*, possess anti-inflammatory and anti-oxidative activities, which have great potential in the treatment of inflammation, cancer, and virus infection [42]. For example, Baicalin exerts an anti-inflammatory effect by inhibiting the binding of chemokines to human leukocytes and limiting their biological functions [43]. The *Huangqin* extract can also inhibit the replication of the influenza virus in mice [44]. Various compounds in *Gancao, Huangqin, Chaihu*, have been proved to have the potential to directly inhibit papain-like protease and 3C-like protease, which are essential for the replication of the pathogen SARS-CoV-2 that causes COVID-19 [12,45].

The COVID-19 are not always limited to the respiratory tract, but they have the potential for targeting the central nervous system (CNS), and several neurological symptoms have been described in patients with severe respiratory distress. Coronaviruses with such potential are the beta-coronaviruses, including SARS-CoV and MERS-CoV [46]. The previous study indicated that neural invasion propensity was a common feature of coronaviruses, and coronaviruses may initially attack peripheral nerves and enter the CNS through synaptic pathways. This trans-synaptic transfer has been recorded in HEV67 and the avian bronchitis virus [47]. As the neural attack of SARS-CoV-2 is accompanied by respiratory failure in COVID-19 patients, it is necessary to prevent the virus from entering the CNS. As an emerging virus, the knowledge that SARS-CoV-2 may enter the CNS is of great significance for the disease prevention and treatment. It is also important to find effective antiviral drugs that can cross the blood-brain barrier. The enrichment analysis showed that both prescriptions were involved in the synaptic and trans-synaptic signaling, as well as the pathway involve in serotonergic synapse, GABAergic synapse, and dopaminergic synapse, which indicated that these prescriptions may exert the therapeutic effect through preventing the virus from entering the CNS.

5. Conclusion

In this study, a data mining strategy was applied to explore the TCM prescriptions, the high-frequency CMMs were selected, then the compatibility rule of the CMMs was studied using the association rules analysis, the pair CMMs and triple CMMs were highlighted and three groups prescriptions were generated. The network pharmacology analysis was applied to investigate the underlying mechanism of the generated prescriptions in treating COVID-19. The results showed that CMMs exert potential therapeutic effect via comprehensively modulating the target proteins, mainly focus on the anti-inflammatory, anti-viral, immune regulation and, neuroprotective effects, which reflect the multi-compound, multi-target, and multi-pathway synergistic mechanism of CMMs. However, given the limited number of the prescriptions collected in this study, the CMMs and prescriptions are only for the mild and moderate disease stage. More prescriptions associated with the antivirus effect will be included to explore the CMM effect for the severe and critical disease stage. Besides, the network pharmacology result should be further validated by in vitro and in vivo experiments, which can provide more information for the development of the antiviral drugs from CMM.

Author contributions

Xiuli Sun: Methodology, Software, Visualization; **Jinhe Jiang**: Investigation, Data curation; **Yang Wang**: Conceptualization, Investigation, Writing - Original draft preparation; **Shuying Liu**: Writing - Reviewing and Editing. All research done by the authors.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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Data availability

The data in this study is available from the corresponding author upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version at doi:10.1016/j.eujim.2020.101242.

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