In silico synergistic drug repurposing for combating novel coronavirus (COVID-19) outbreaks

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Research Article

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Abstract

As the number of novel coronavirus (COVID-19) cases continues to rise, there is a global need for rapid drug development. In this study, we propose a systems pharmacology approach to reposition FDA-approved drug candidates for coronavirus, identify targets and suggest a synergistic drug combination using network pharmacology. We collected 67 genes associated with coronavirus, performed an enrichment analysis to obtain coronavirus-associated disease- pathway and constructed protein-protein interaction (PPI) network based on 67 genes. Total 37 significant disease-pathways were retrieved, and associated FDA-approved drugs were listed for drug repurposing candidates. Our PPI network showed 51 targets from 67 genes and identified IL6 and TNF as potential targets for coronavirus. From the FDA drug list, we selected four drugs that are experimentally used or studied for coronavirus to construct two- drug combinations. From six drug-drug networks, we identified hydroxychloroquine + ribavirin combination had the highest number of overlapping targets (IL6, IL2, IL10, CASP3, IFNA1) from PPI network target list, suggesting a potent synergistic drug combination for coronavirus. With the aim to support the rapid drug development, we suggest a new approach using systems-level drug repurposing for COVID-19 treatment.

Introduction

Coronaviruses (CoVs) are enveloped, single-stranded RNA viruses that cause a pneumonia- like disease[1]. The last two decades have witnessed the emergence of two novel coronavirus (CoVs) epidemics—severe acute respiratory syndrome CoV (SARS-CoV, 2002–2003) and Middle East respiratory syndrome (MERS-COV, 2012)—that have caused high mortality and economic loss worldwide[2,3]. Since December 2019, an outbreak identified as a novel coronavirus (COVID–19) that started from a local seafood market in Wuhan, China, has grown to a pandemic that, as of March 13, 2020, has infected individuals worldwide, 11 countries in Africa, 22 in Americas, 15 in Eastern Mediterranean, 51 in Europe, 8 in South- East Asia, and 14 in Western Pacific (*https://www.cdc.gov/coronavirus/2019-ncov/locations-confirmed-cases.html#map)*.. One research team in Guangzhou, China, has reported that, at the whole-genome level, COVID–19 is 99% identical to coronavirus isolated from pangolins, ant-eating mammals often used in traditional Chinese medicine[4]. The infection, which exhibits clinical symptoms of fever, dry cough, dyspnea and headache, produces a progressive respiratory illness due to alveolar damage that is fatal in an uncertain percentage of cases[4]. To date, no specific antiviral or therapeutic agents are available to treat coronaviruses, with the only management strategy being supportive care[2,3]. As the number of COVID–19 cases continues to rise, there is a global need for candidate intervention approaches. In this study, we suggest strategies that might be options for resolving the current coronavirus outbreak.

Rapid virus evolution and the threat of antiviral resistance has motivated research investment in various drug-discovery strategies[5]. One strategy for more timely introducing novel antiviral drugs is ‘drug repurposing’. Drug repurposing (or repositioning) is the strategy of creating novel clinical opportunities for known approved or investigational drugs that are different from the original medical indication[6]. Drug repurposing offers three benefits for drug development. First, safety concerns are diminished because the

drug has already been found safe in clinical models[7]. Second, development time and costs can be reduced in preclinical, phase I and II studies[8]. Third, repositioning of drugs may elucidate new targets and pathways[7]. Faced with the threat of rapid worldwide transmission of novel coronaviruses, ongoing global needs for accelerated antiviral drug development have brought drug repurposing to the forefront as a promising strategy[5]. Therefore, encouraging *in vitro* and *in vivo* results from drug repurposing efforts to uncover novel antimicrobial agents may suggest new options for viral infection.

Computational research is a promising time-saving alternative to experimental research that may identify novel targets and pathways[9]. Here, we identified diseases and pathways associated with coronaviruses *in silico* based on coronavirus-associated genes that might suggest additional drug repurposing options. First, we propose a systems pharmacology approach to reposition FDA-approved drugs for coronavirus diseases by systemically incorporating associated pathway. Next, we construct a protein-protein interaction (PPI) network based on coronavirus-associated genes to identify potential targets. Lastly, we suggest a polypharmacology-based paradigm, such as compound combinations for designing multi-targeted therapy to achieve more effective clinical responses[10]. 'Synergistic effect' is the result of combining two or more compounds that produce a greater effect than 'additive effects'[11]. Despite the significance of compound combinations in therapeutics, compound combinations in computational research is currently limited[12]. Herein, we suggest systems- level drug-drug interaction networks that capture both on and off-target effects that may reveal functional links between coronavirus, and a drug combination that may synergistically inhibit coronavirus.

Methods

Drug repurposing candidate study: Coronavirus target gene search and associated disease search

Corona virus associated target genes (*H. sapiens)* were searched under keywords 'Corona virus', 'SARS', and 'MERS' on NCBI (https://www.ncbi.nlm.nih.gov/gene) and DisGeNET (*https://www.disgenet.org/)* databases. Based on gene target result, associated disease and

pathway search was conducted with Database for Annotation, Visualization and Integrated Discovery (DAVID ver. 6.8) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The list of drugs associated with diseases were retrieved from Food and Drug Administration (FDA).

Coronavirus target study: Protein-protein interaction (PPI)

Based on gene target result, PPI identification was conducted by STRING database (https://string-db.org/) with high confidence setting (0.7) and visualized using Cytoscape. The network view summarizes the network of predicted associations with other proteins. The nodes represent proteins and

the edges represent the predicted functional associations. Size of the nodes represent the degree of interaction in this network.

Synergistic drug study: Drug-Drug network

Four drugs were selected from retrieved FDA drug list. Two drug combination networks were obtained from STITCH (http://stitch.embl.de/) database. All networks were constructed within high confidence (0.7) minimum required interaction score.

Result And Discussion

A total of 161 genes were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/gene), and 109 genes were searched in DisGeNET (https://www.disgenet.org/). From these two databases, 67 overlapping genes were identified (S1). Based on this 67 gene list, pathological diseases associated with coronavirus were identified using DAVID (https://david.ncifcrf.gov/) and KEGG databases (Figure 1, Table 1).

FDA-approved treatments in accordance with coronavirus-associated diseases from KEGG database were obtained from FDA and Mayo clinic (*https://www.mayoclinic.org/).*. Diseases that had P-values over 0.05 were considered significant and listed in Table 1. Gene count represents the number of genes from 67 genes that are associated with the disease.

Table 1. Disease-Pathway enrichment and FDA approved treatments

KEGG ID: Term	Gene count	FDA approved treatments
hsa05164:Influenza A	14	Oseltamivir
hsa05142:Chagas disease (American	13	Benznidazole

trypanosomiasis)		
hsa05152:Tuberculosis	13	Isoniazid, Rifampin
hsa05168:Herpes simplex infection	12	Acyclovir, Famciclovir
hsa05166:HTLV-I infection	11	No specific treatment
hsa05321:Inflammatory bowel disease (IBD)	10	Mesalamine, Balsalzide
hsa05161:Hepatitis B	10	Tenofovir, Lamivudine, Entecavir
hsa05145:Toxoplasmosis	9	Pyrimethamine, Sulfadiazine
hsa05162:Measles	9	No specific treatment
hsa05323:Rheumatoid arthritis	8	Methotrexate, leflunomide, hydroxychloroquine, sulfasalazine, infliximab (TNF inhibitor)
hsa05200:Pathways in cancer	8	Cyclosporine, glucocorticoid
hsa05330:Allograft rejection	7	Depends on the type of rejection, corticosteroids
hsa05140:Leishmaniasis	7	Liposomal amphotericin B (IV), miltefosine
hsa05133:Pertussis	7	Erythromycin
hsa05169:Epstein-Barr virus infection	7	No specific treatment
hsa04940:Type I diabetes mellitus	6	Insulin
hsa05144:Malaria	6	Chloroquine, Hydroxychloroquine, Mefloquine, Proguanil
hsa05320:Autoimmune thyroid disease	6	Levothyroxine
hsa05146:Amoebiasis	6	Metronidazole
hsa05160:Hepatitis C	6	Interferon, Ribavirin
hsa05203:Viral carcinogenesis	6	Depends on viruses
hsa05020:Prion diseases	5	Anthracyclines, Amphotericin B
hsa05212:Pancreatic cancer	5	Capecitabine, Fluorouracil
hsa04932:Non-alcoholic fatty liver disease (NAFLD)	5	No treatment

hsa05143:African trypanosomiasis	4	Pentamidine, Suramin, Melarsoprol, Eflornithine, Nifurtimox
hsa05014:Amyotrophic lateral sclerosis (ALS)	4	Edaravone, Riluzole
hsa05150:Staphylococcus aureus infection	4	Cefazolin, Cefuroxime, Oxacillin, Vancomycin, Rifampin
hsa05416:Viral myocarditis	4	Enalapril, Lisinopril
hsa05210:Colorectal cancer	4	5-Fluorouracil (5-FU) Capecitabine (Xeloda)
hsa05120:Epithelial cell signaling in Helicobacter pylori infection	4	Omeprazole, Amoxicillin, Clarithromycin
hsa05220:Chronic myeloid leukemia	4	Imatinib
hsa05410:Hypertrophic cardiomyopathy (HCM)	4	beta blockers, diuretics, Disopyramide
hsa05132:Salmonella infection	4	Ciprofloxacin, Azithromycin, Ceftriaxone
hsa05322:Systemic lupus erythematosus	4	Belimumab
hsa04614:Renin-angiotensin system	3	angiotensin-converting enzyme (ACE) inhibitors, selective AT1 receptor blockers (ARBs)
hsa05310:Asthma	3	Albuterol, Levalbuterol
hsa04930:Type II diabetes mellitus	3	Metformin, Sulfonylureas, DPP4 inhibitors

Interestingly, several drugs, including oseltamivir, interferon and corticosteroids, have been used in patients with coronavirus (SARS or MERS), a finding in accord with our *in silico* results[13]. Moreover, a recent study on COVID−19 reported that chloroquine or hydroxychloroquine (for malaria), which is ranked in our disease results, showed antiviral efficiency *in vitro*[14]. In the case of recent studies on biomarkers, angiotensin-converting enzyme 2 (ACE2) has received attention as a new biomarker of SARS and COVID−19, a finding in agreement with treatments for viral myocarditis and Renin-angiotensin system both of which use ACE inhibitors, such as enalapril and lisinopril as treatments. Therefore, coronavirus-

associated disease-pathways and FDA-approved drug list were proposed for drug repurposing candidates and further examined to uncover molecular mechanisms in host infection against COVID–19[4].

Based on retrieved 67 genes, protein-protein interaction (PPI) network was retrieved from STRING database and visualized using Cytoscape (Figure 2, Figure 3). The node size reflects the number of interactions (degree) between proteins, and proteins with high number of degree were considered potent targets in our study.

From our *in silico* PPI network, 51 proteins from 67 genes lists were identified to be connected to form a network (S2). The proteins that showed the highest degree of interaction among all proteins was IL–6, followed by TNF, which can be considered potential targets for future coronavirus studies. Among these targets, DDP4 was recently reported to be a functional receptor for the MERS virus, a finding in agreement with our *in silico* results[3]. We also identified ACE2 that has also received attention as a new biomarker of SARS and COVID–19, as confirmed by our search [4].

Lastly, we applied the systematic dynamics that can be investigated through drug-drug network to observe coronavirus targets associated within the predicted drug combinations.

Based on the disease and FDA-approved drug list (Table 1), we selected four diseases (influenza A, Malaria, Hepatitis C, and viral myocarditis), and matching drugs (oseltamivir, hydroxychloroquine, ribavirin, and lisinopril) that are currently used in clinic or within study for COVID–19 to construct drug-drug networks (Figure 4, Figure 5).

Total six networks were constructed, and only four networks were interconnected to each other (Figure 5 a-d). Figure 5-a, Hydroxychloroquine + Ribavirin combination had the highest number of overlapping targets compared to PPI target lists (5 targets: IL6, IL2, IL10,

CASP3, IFNA1), followed by Figure 5-b Hydroxychloroquine + Lisinopril (4 targets: IL6, CASP3, ACE, ACE2), Figure 5-c Hydroxychloroquine + Oseltamivir (3 targets: IL6, TNF, CASP3), and Figure 5-d Oseltamivir+ Lisinopril (2 targets: ACE, ACE2). From these four networks, we identified two targets, IL6 and CASP3 were found in common. Overall, our result indicates that drug combination of Hydroxychloroquine + Ribavirin (Figure 5-a) is most favorable combination among six networks, and two targets IL6 and CASP3 can be potent targets for coronavirus.

Collectively, our study provides new insights in drug screening, which can be applied to further coronavirus drug development. Through *in silico* study, we suggest drug repurposing, uncover molecular mechanism of drugs and targets involved with coronavirus, and synergistic combination through network pharmacology. To our knowledge, this concept of drug repurposing and network pharmacology-based drug combination strategy has not yet been established elsewhere. Because of its prospective advantages of time- and cost-savings, *in silico* screening and drug combinations for drug repurposing can be considered a viable approach for discovering novel coronavirus treatment.

Declarations

Acknowledgements

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Competing interests

No competing interest was reported by the author(s).

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Figures

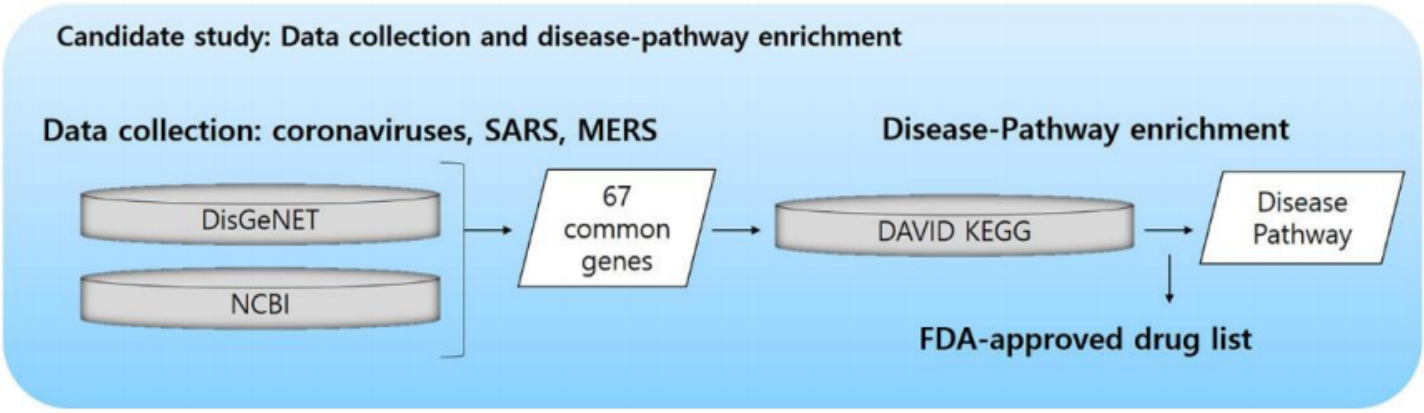

Figure 1

Candidate study: data collection and disease-pathway enrichment

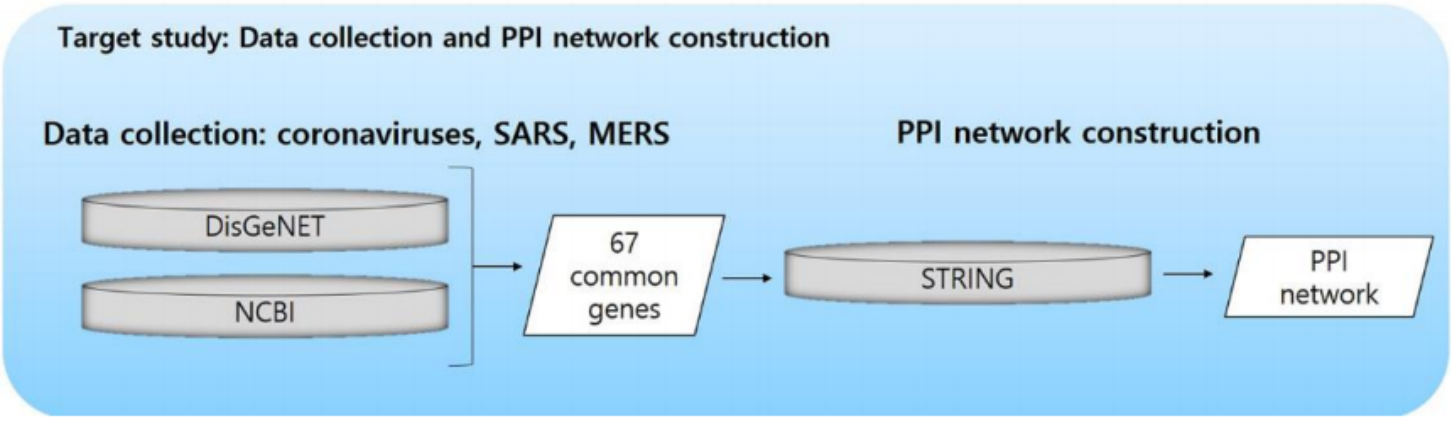

Figure 2

Target study: Data collection and PPI network construction

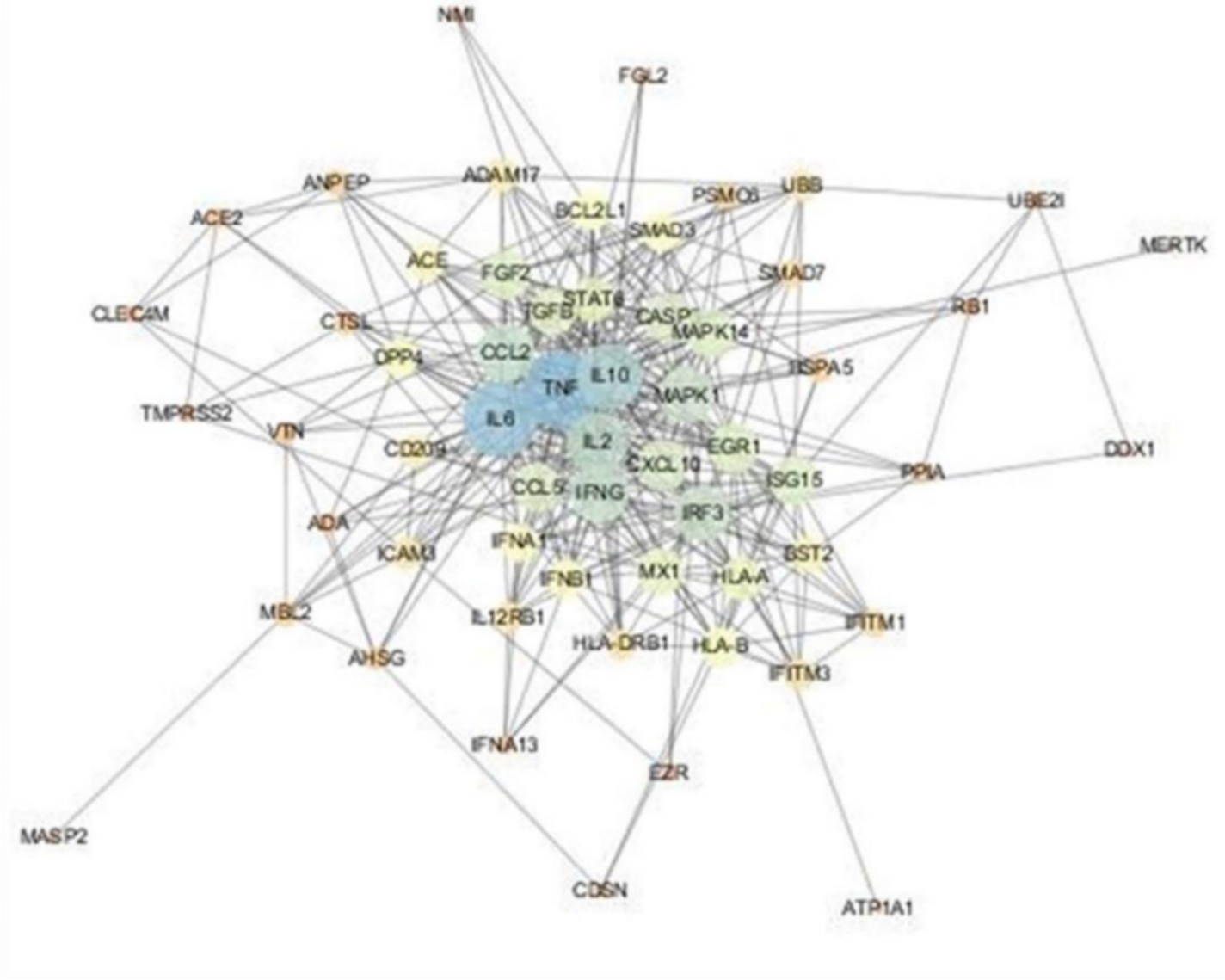

Figure 3

Protein-protein interaction network

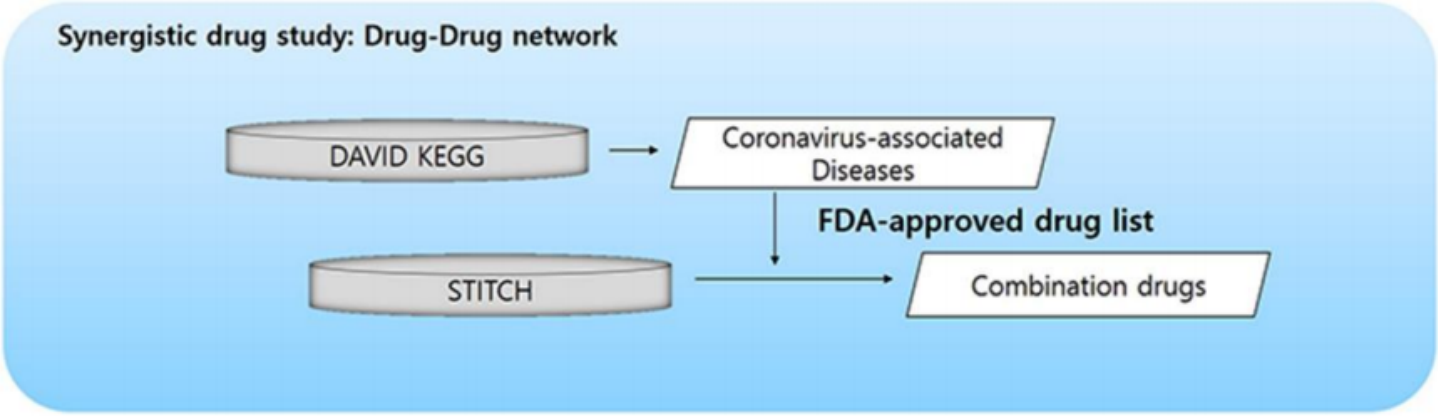

Figure 4

Drug combination approach for drug repurposing

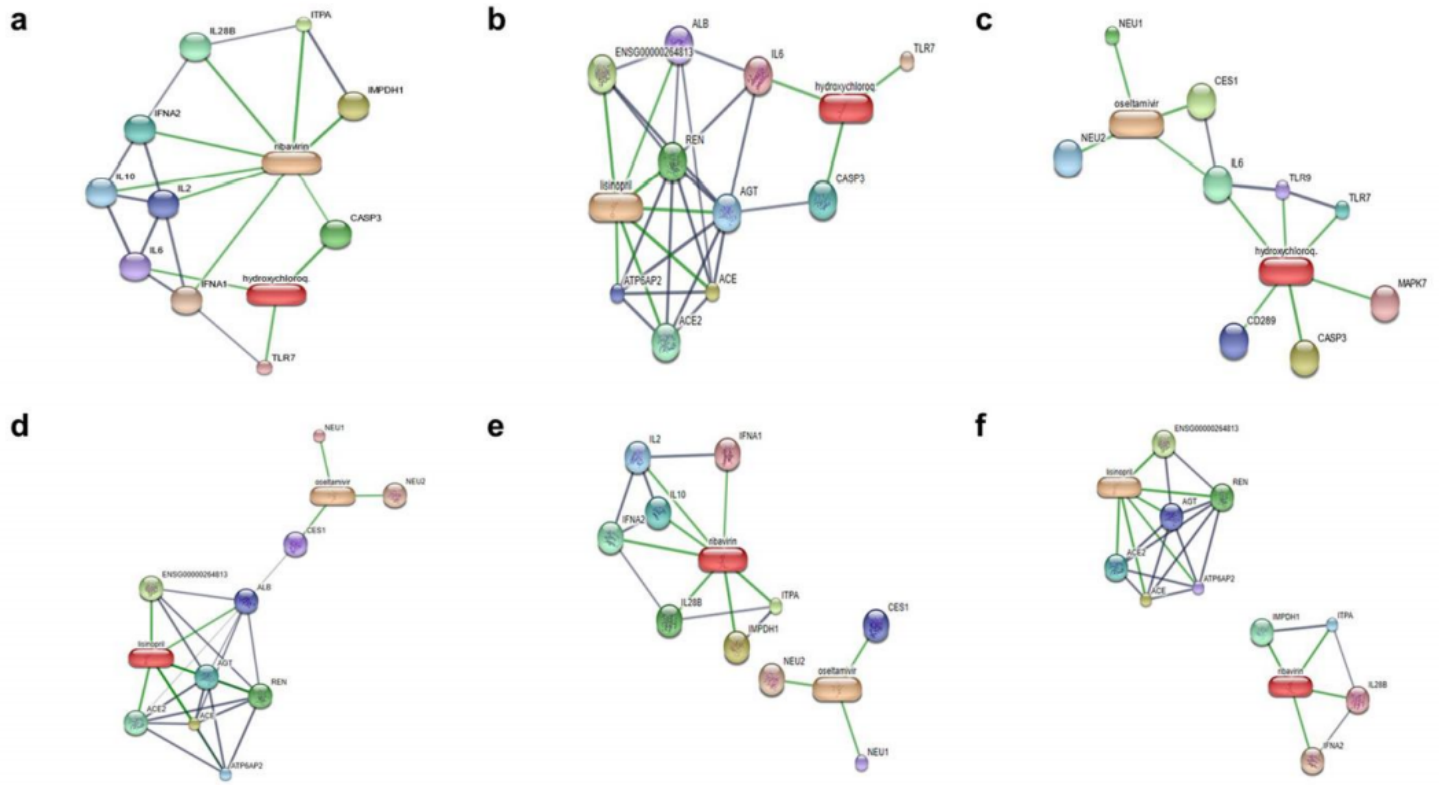

Figure 5

Drug-Drug network a: Hydroxychloroquine + Ribavirin; b: Hydroxychloroquine + Lisinopril; c: Hydroxychloroquine + Oseltamivir; d: Oseltamivir + Lisinopril; e: Oseltamivir + Ribavirin; f: Ribavirin + Lisinopril

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