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ORIGINAL ARTICLE

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Identification of molecular mechanisms of association amongst comorbidities and COVID-19: An Interactome based Systems Biology Approach

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ABSTRACT

Background: Comorbidity has emerged as a major challenge in the last few decades that result from cascades of failures involving complex biochemical and physical interactions of genes, proteins, and metabolites responsible for cellular functions. Various epidemiological and demographic studies conducted since the emergence and global transmission of the SARS-CoV-2 virus have reported that patients with pre-existing medical conditions such as cardiovascular disease, diabetes, hepatitis, lung disease, and kidney disease are more prone to coronavirus infection.

Objective: The present study was undertaken to elucidate the molecular mechanisms that are common amongst COVID-19-associated comorbidities using an interactome-based network biology approach to identify the shared genes/proteins and biological pathways.
Methodology: Genes of COVID-19-associated comorbidity diseases retrieved from disease databases were analyzed using *in silico* bioinformatics and systems network biology tools STRING and Cytoscape plug-ins CytoHubba and CytoCluster.
Results: The shared hub proteins, namely *IL1B, ACTB, IL6, MMP2, ALB, AKT1, MAPK3, FN1, TNF, CCL2, VEGFA,* and *TP53*, among various pre-existing comorbidities, revealed their involvement in immunological pathways. All these proteins were also found to have significant associations with ACE2, TMPRSS2, and CD17/BSG, the entry receptors of the COVID-19 virus.
Conclusion: The higher risk factor for COVID-19 in patients with pre-existing comorbidities is due to immune dysfunction that results in their higher susceptibility to infection by SARS-CoV-2 via its entry receptors on the host cells. This study provides novel insights into the association between host genetics and the consequences of viral infection that is responsible for the severity of COVID-19 in patients suffering from pre-existing comorbidities.
Keywords: Comorbidity**,** SARS-CoV-2, systems network biology, interactome, COVID-19, *in silico,* hub genes, Cytokine storm (CS), Immune dysfunction

Introduction

A major challenge that has emerged in the last few decades is comorbidity, which refers to the presence of more than one disease in individuals. Disease comorbidity effects result from interactions between molecular components, which in turn may affect other cellular functions as well as mutated gene products.

Several epidemiological and clinical studies have reported that patients with pre-existing medical conditions have severe responses to the coronavirus infection. The highest risk factor for infection of COVID-19 resulting in extreme lung injury followed by death is in patients having comorbidities such as cardiovascular disease, diabetes, hepatitis, lung disease, and kidney disease, who may develop [1–3]. The strongest predictors of the prognosis of COVID-19 patients have been shown to be age, gender, and some pre-existing comorbidities. The higher risk group is ≥ 60 years, and those with certain hidden conditions, for example, cardiovascular and cerebrovascular sicknesses and diabetes [4].

Epidemiological studies on COVID-19-affected patients revealed that age factors along with comorbidities such as diabetes, lung infection, renal impairment, and liver damage

should be taken into consideration, as a substantial number of old patients showed a higher death rate with better survival rates for younger patients infected with COVID-19 [5, 6]. Earlier studies published in 2003 had reported that in patients infected with SARS, acute coronary syndrome and myocardial infarction cause 2 deaths per fifth patient [7, 8].
The principal risk factors for COVID-19 infection are acute respiratory failure and alveolar failure. The target site of SARS is the respiratory tract of an individual, causing mild upper respiratory illness followed by acute respiratory distress with septic shock and a higher infectious rate leading to pulmonary fibrosis by binding to the transmembrane receptor Angiotensin-Converting Enzyme 2 (ACE2) on the respiratory epithelium [9]. The presence of the viral genome of SARS in the hepatic tissue's endothelial cells causes multiple organ failures, including liver failure, which can be attributed to the widely distributed ACE2 receptors that are responsible for the entry of SARS-CoV [10]. The elevated levels of ALT and AST and pro-inflammatory cytokines were reported in liver-damaged patients, and patients with a history of hepatitis were at higher risk of COVID-19 exhibiting exaggerated replication during SARS-CoV infection [11].

It is related to other organ harm, for example, cardiovascular damage by means of an increased risk of hypertension through ACE2, gastrointestinal dysfunctionality, diabetes mellitus, liver brokenness, ongoing kidney illness, CNS damages, lung injury, visual dangers like conjunctival hyperemia, chemosis, conjunctivitis, and malignant growth hazard, venous thromboembolism, tuberculosis, maturing, and conceptive danger [12].

A recent study based on an integrated network biology approach reported cancers, pulmonary, hepatic, neurological, hypertensive, and cardiac disorders being linked to COVID-19 along with multiple organ damage [13]. A bioinformatics analysis revealed the genes and pathways were significantly enriched for immune system and cardiovascular-related phenotypes in COVID-19 comorbidities [14]. Another study based on computational methods reported 274 differentially expressed genes common in both COVID-19 patients and patients with comorbidities like cardiovascular disease, diabetes, and obesity, suggesting their role in the increased severity of COVID-19 [15].

Patients with certain pre-existing medical conditions have higher chances of being afflicted with the SARS-CoV-2 virus, followed by severe responses as reported following the emergence and global transmission of COVID-19. Therefore, there arises a need to study disease-disease relationships to better understand the human interactome and molecular causes of disease comorbidities. However, reports on the molecular factors responsible for the association of COVID-19 with pre-existing comorbidities are not well understood and

need to be deciphered for the design of preventive strategies. Therefore, this study was undertaken to identify the underlying common molecular mechanism of association of genes/proteins and biological pathways amongst various disease comorbidities and COVID-19 using a systems biology-based interactome approach. This study would give meaningful insights into understanding the etiopathology and the molecular association of disease comorbidity and COVID-19.

Material and methods

Data Mining

Genes reported to be associated with diabetes mellitus, cardiovascular diseases, pulmonary fibrosis, kidney diseases, hepatitis C, and hepatitis B were retrieved from the DisGeNet database (http://www.disgenet.org) [16], and genes common amongst these six diseases were obtained using PHP programming.

Construction and analysis of interactome

STRING database

A protein-protein interaction (PPI) network was constructed using the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins) database (https://string-db.org/) that performs analysis based on known and predicted PPIs, including physical and functional associations [17] for the genes found common amongst the six diseases.

Cytoscape

Network analysis was performed using Cytoscape, an open-source software project that integrates expression data with biomolecular interaction networks and molecular states to form a unified conceptual framework [18].

ClusterONE (Clustering with Overlapping Neighborhood Expansion) plugin of Cytoscape (http://apps.cytoscape.org/apps/clusterone) was used for the identification of highly connected regions in the form of clusters [19].

CytoHubba plugin of Cytoscape (http://apps.cytoscape.org/apps/cytohubba) was used for ranking the hub genes using the MCC (maximal clique centrality) scoring method [20], was used for the analysis of the interactome.

Reactome knowledgebase

The hub genes were mapped into their respective biomolecular pathways using Reactome (https://reactome.org), which is a peer-reviewed knowledge base that captures information about genes and molecules involved in pathways [21].

Results

Genes involved in diabetes mellitus (3134), cardiovascular diseases (1756), pulmonary fibrosis (924), kidney diseases (1180), hepatitis C (1768), and hepatitis B (1449) were retrieved from the DisGeNET database. One hundred and ten common genes among all six diseases were shortlisted.

Network construction of the 110 genes common amongst the diseases (Table 1) was done using the STRING database, and only 107 genes/proteins were present in *Homo sapiens,* which were used for the construction of an interaction network having 107 nodes and 1989 edges that represent genes and their interactions, respectively (Fig. 1).

On analysis of the network obtained from STRING using Cytoscape plug-in ClusterONE, four clusters were obtained, out of which only one cluster had a significant p-value (< 0.05) (Fig. 2 A & B). This network was analyzed with CytoHubba to identify hub nodes that represent highly connected nodes having a higher likelihood of being involved in an essential interaction [22, 23]. The top 50 ranked genes were obtained, where red, yellow, and gray nodes represent highly, least significant genes, and outliers, respectively (Fig. 3).

Twenty common genes were found to be significant, namely *IL6, TNF, IL1B, AKT1, ACTB, CXCL8, CCL2, VEGFA, ALB, TP53, IL10, MAPK3, FN1, PTGS2, STAT3, PPARG, IL4, TLR4, IFNG,* and *MMP2,* amongst the six diseases, *i.e.,* cardiovascular disease, diabetes mellitus, hepatitis, pulmonary fibrosis, and kidney disease. A network of these 20 hub genes was constructed in STRING, and the resulting network had 190 edges and an average node degree of 19, which indicates the number of interactions (at the score threshold) that a protein has on average in the network (Fig. 4).

The hub genes/proteins common among the comorbidities were found to be enriched in 500 biological pathways (FDR ≤ 0.05) using the Reactome knowledgebase, with most of them being associated with the immune system, signal transduction, disease, and transcription-associated pathways (Table 2).

Infection of host cells by SARS-CoV-2 has been reported to require the presence of entry receptors, namely transmembrane receptors ACE2 (Angiotensin-Converting Enzyme 2), TMPRSS2 (Type II Transmembrane Serine Protease), and CD147 (cluster of differentiation

147)/EMMPRIN (Extracellular matrix metalloproteinase inducer)/BSG (Basigin) [24–26]. A study proposed that CD147 and ACE2 allow entry of the virus into the cell cytoplasm by sequential activation of NLRP3 inflammasome, resulting in cleavage of interleukins IL-1β and IL-18 [24] followed by binding of SARS-CoV-2 to ACE2. Subsequently, TMPRSS2 primes the SARS-CoV-2 spike protein that allows membrane fusion and its entry into the host cell [24, 27].

To identify the association of pre-existing disease comorbidities with SARS-CoV-2, an interaction network of the 20 hub proteins identified with the SARS-CoV-2 entry receptors, namely ACE2, TMPRSS2, and CD147/BSG, was constructed in STRING (Fig. 5).

In the present study, SARS-CoV-2 entry receptors TMPRSS2, ACE2, and CD147/BSG showed interactions with 2 (excluding ACE2), 8 (excluding TMPRSS2), and 7 hub proteins, respectively, that were common amongst the comorbidities (Table 3).

From the analysis of the network, it is observed that out of the 20 hub proteins only 12 proteins, *i.e.*, IL1B, ACTB, IL6, MMP2, ALB, AKT1, MAPK3, FN1, TNF, CCL2, VEGFA, and TP53, have significant interactions with the SARS-CoV-2 entry receptors. Each of these 12 proteins was studied using literature mining to understand their association with SARS-CoV-2 infection, given their significant number of interactions with SARS-CoV-2 entry receptors TMPRSS2, ACE2, and CD147/BSG [24-26].

Discussion

Protein kinase B alpha (PKBalpha/Akt-1) was found to interact with all the 11 proteins previously shortlisted and has significant interactions with all the SARS-CoV-2 entry receptors. AKT1 is reported to be involved in the regulation of cell proliferation and transformation thereby playing an essential role in immune cell modulation, resulting in diabetes and cardiovascular diseases, among others [28–30]. Through its downstream targets GSK3 and GLUT4, AKT increases cellular metabolism, and alterations in AKT signaling affect various cardiovascular pathological processes such as atherosclerosis [31]. During the progression of acute kidney injury to chronic kidney disease, the renal fibrosis and tubular dedifferentiation observed have been attributed to AKT, along with its role in increasing the oncogenic potential of the Hepatitis B virus X protein by phosphorylation [32, 33]. AKT has been reported to affect the progression and severity of COVID-19 along with IL10, TNF, and other proteins in non-alcoholic fatty liver disease patients [34]. In COVID-19/asthma comorbidity, AKT1 along with TP53, ALB, IL-6, TNF, and VEGFA have been reported to be hub proteins [35].

Levels of proinflammatory cytokines and chemokines such as TNF-α, IL-6, and IL-1β have been found to be elevated in cytokine storm (CS), which is a hallmark of COVID-19 pathogenesis [36, 37]. TNF can induce cells to release cytokines and evoke various intracellular signaling pathways, such as inflammation, apoptosis, programmed cell necrosis, and immunity [38, 39]. IL-6 has been reported to be associated with the host's response to infection, which affects the viral load and severity of the disease in a patient [40]. Therefore, CS is responsible for the escalation of disease severity resulting in septic shock and multi-organ failure in COVID-19 patients with comorbidities [41].

Mitogen-activated protein kinase (MAPK3) plays an essential role in viral infections and other important cellular physiological and pathological processes as it regulates cell growth, differentiation, and inflammatory response. Studies have also shown reprogramming caused by tumor therapy is due to a link between MAPK signals and control of the inflammatory network [42, 43].

Matrix metalloproteinases (MMPs) are involved in the release of substrates such as growth factors that are anchored at the extracellular matrix or cell membrane and also contribute to injurious processes in lung pathologies. Following SARS-CoV-2 infection, clinical studies report an increase in plasma MMPs, suggesting their release by the host's immune system and target cells [44]. Fibronectin (FN) is a glycoprotein that is present in high concentrations in blood and is a component of the extracellular matrix. In critically ill COVID-19 patients, it has been reported as a disease marker of severity and also found to be associated with different kinds of inflammatory diseases [45]. Actin cytoplasmic 1 (ACTB) is a highly conserved protein found in the cytoplasm and a component of the cytoskeleton. It is involved in the proper functioning of the immune system, cell motility, and division, and in COVID-19 patients it had a low expression, resulting in immune cell infiltration and altered immune function [46].

In the present study, association of shared hub genes/proteins, namely *IL1B, ACTB, IL6, MMP2, ALB, AKT1, MAPK3, FN1, TNF, CCL2, VEGFA,* and *TP53,* among various pre-existing comorbidities (cardiovascular disease, diabetes mellitus, hepatitis, pulmonary fibrosis, and kidney disease) with COVID-19 have been identified, which were found to be directly involved in immunological responses. Moreover, each of these hub proteins was found to have significant associations with ACE2, TMPRSS2, and CD17/BSG, which are the entry receptors of the COVID-19 virus. People suffering from pre-existing comorbidities have impaired immune functions, making them more likely to contract viral infections, including SARS-CoV-2. The genes identified can be further explored to develop preventive

therapeutic interventions for patients with pre-existing comorbidities to contain the severity of SARS-CoV-2 and decrease morbidity and mortality rates.

Conclusions

This study provides novel insights into the associated molecular mechanisms in pre-existing comorbidities with COVID-19 using a robust interactome-based systems biology approach. Controlling the inflammatory response proteins is a common critical factor amongst the six disease co-morbidities studied and the cytokine storm, which is the hallmark of COVID-19. The higher risk factors for COVID-19 in patients with pre-existing comorbidities are associated with immune dysfunction, and two proteins, AKT1 and TNF, identified in the present study having multiple interactions with the entry receptors of SARS-CoV-2, can be used as biomarkers and explored further for the design of potential therapeutic strategies. A synergistic approach is needed to block pathologies at multiple levels using therapeutic interventions to inhibit viral infection and regulate the dysfunctional immune responses to alleviate the severity of COVID-19 in patients having pre-existing comorbidities.

Article information

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Conflict of interest: *The author declares there is no conflict of interest.*

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Table 1. Genes common amongst the diseases Diabetes mellitus, Cardiovascular diseases, Pulmonary fibrosis, Kidney diseases, Hepatitis C and B

CDKN2A	*AHSA1*	*MTCO2P12**	*PPARGC1A*	*CHI3L1*
NLRP3	*CNR1*	*RBM45*	*SLCO6A1*	*COX8A*
CRK	*MAPK14*	*CCN2*	*CTNNB1*	*ACE*
AGT	*EGFR*	*AKT1*	*ESR1*	*ALB*
F2	*FN1*	*SIRT1*	*BRD4*	*FOS*
MTOR	*GABPA*	*RNF19A*	*POLDIP2*	*PTPN22*
IL37	*CXCR3*	*ANGPT2*	*GPT*	*ANXA1*
HLA-DRB1	*HMGB1*	*HMOX1*	*HSPA1B*	*HSPA4*
APOA1	*ICAM1*	*IFNG*	*IGF1*	*APRT*
IL1A	*IL1B*	*IL1RN*	*IL4*	*IL6*
CXCL8	*IL10*	*IL17A*	*IL18*	*GSTK1*
LEP	*LGALS3*	*MIR145*	*MIR146A**	*MIR155**
MIR21	*MFAP1*	*MMP2*	*MMP14*	*COX2*
NFE2L2	*NFKB1*	*NOTCH1*	*SERPINE1*	*ABCB1*
PIK3CG	*PPARG*	*PRKAA1*	*MAPK1*	*MAPK3*
MAPK8	*PTGS2*	*RELA*	*ACTB*	*CCL2*
CCL5	*GORASP1*	*WNK1*	*SOAT1*	*SPP1*
STAT3	*STAT4*	*SYT1*	*ADAM17*	*TERT*
TGFB1	*THBS1*	*TLR2*	*TLR4*	*TNF*
TNFRSF1A	*TP53*	*C3*	*VDR*	*VEGFA*
VTN	*VWF*	*CXCR4*	*AIMP2*	*CAT*
CAV1	*SOCS1*	*TNFSF10*	*GRAP2*	*GDF15*
*Genes in grey boxes are not present in *Homo sapiens*				

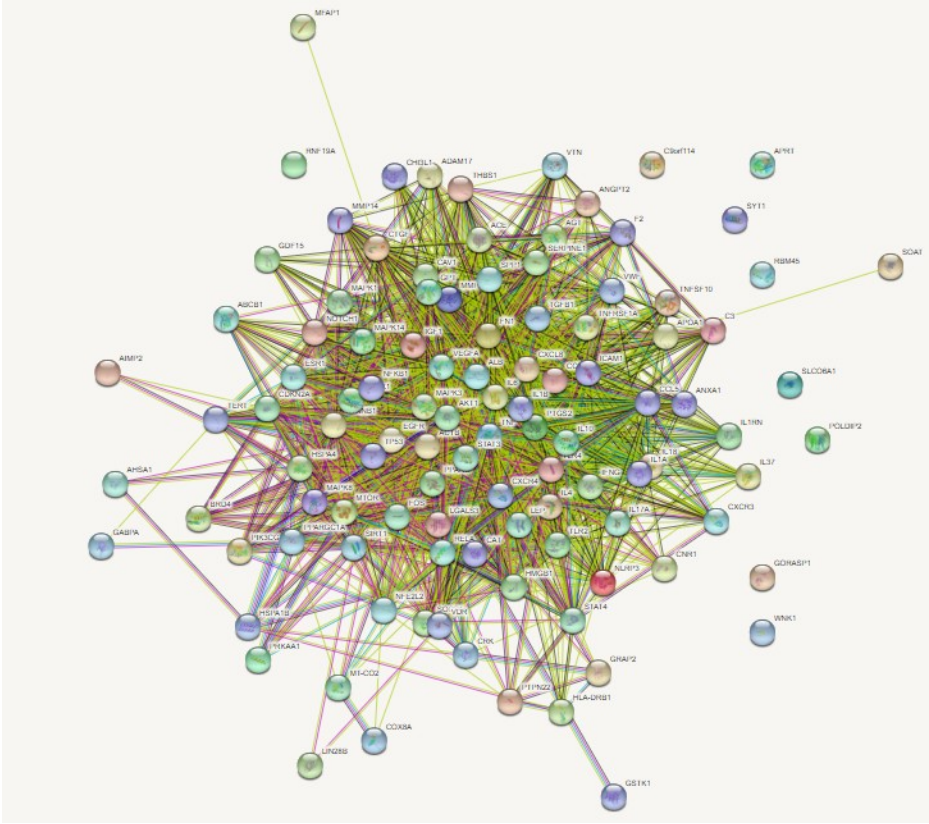
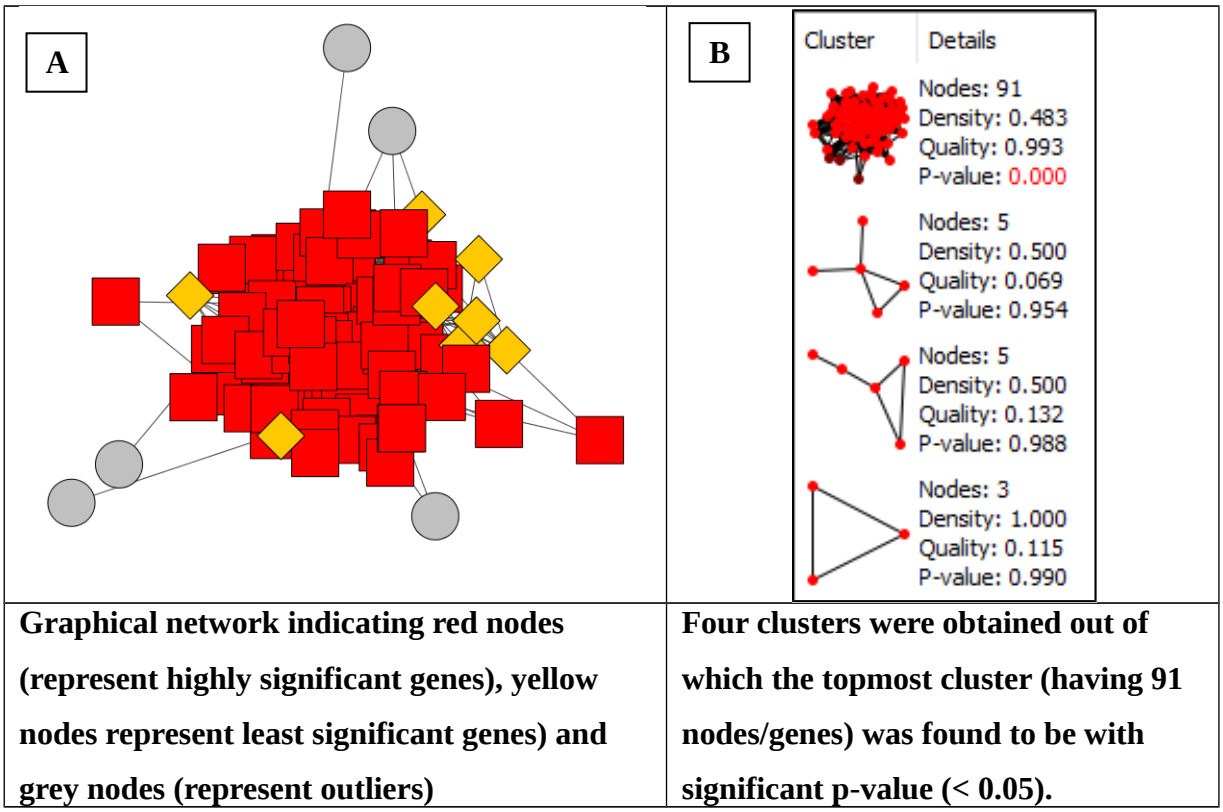


Figure 1. Protein-protein interaction network of common genes amongst six diseases obtained from STRING containing 107 nodes and 1989 edges



Graphical network indicating red nodes (represent highly significant genes), yellow nodes represent least significant genes) and grey nodes (represent outliers)	**Four clusters were obtained out of which the topmost cluster (having 91 nodes/genes) was found to be with significant p-value (< 0.05).**

Figure 2. ClusterONE results obtained by analyzing the PPI Network

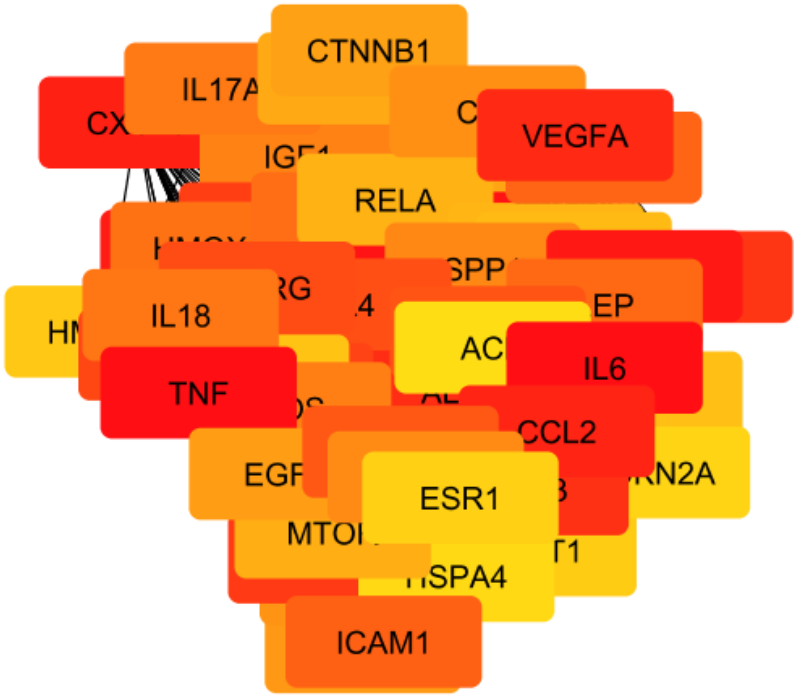


Figure 3. Graphical view of ranked hub nodes obtained from Cytoscape plug-in Cytohubba with color coding

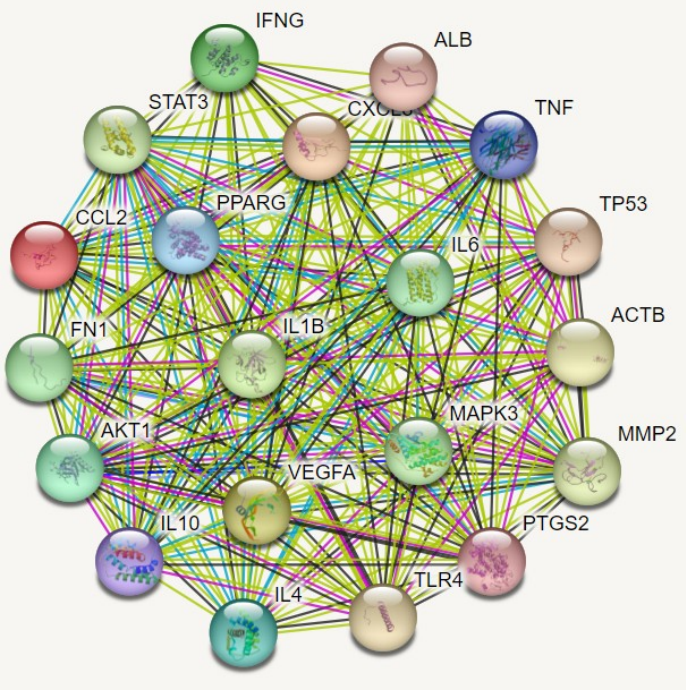


Figure 4. Protein-Protein interaction network of 20 hub genes (nodes) from STRING having 190 edges responsible for co-morbidity of the six diseases

Table 2. The top 15 most significantly enriched pathways obtained from Reactome

Pathway name	p-value	FDR	Gene count	Genes in pathway
Interleukin-10 signaling	1.11E-16	1.28E-14	8	*IL10*; *IL6*; *CXCL8*; *IL1B*; *STAT3*; *CCL2*; *PTGS2*; *TNF*
Interleukin-4 and interleukin-13 signaling	1.11E-16	1.28E-14	14	*IL10*; *CXCL8*; *MMP2*; *STAT3*; *FN1*; *PTGS2*; *TNF*; *VEGFA*; *IL4*; *IL6*; *IL1B*; *CCL2*; *AKT1*; *TP53*
Signaling by interleukins	1.11E-16	1.28E-14	16	*IL10*; *CXCL8*; *MMP2*; *STAT3*; *FN1*; *PTGS2*; *TNF*; *VEGFA*; *IL4*; *IL6*; *IFNG*; *IL1B*; *CCL2*; *AKT1*; *TP53*; *MAPK3*
Cytokine signaling in the immune system	1.11E-16	1.28E-14	16	*IL10*; *CXCL8*; *MMP2*; *STAT3*; *FN1*; *PTGS2*; *TNF*; *VEGFA*; *IL4*; *IL6*; *IFNG*; *IL1B*; *CCL2*; *AKT1*; *TP53*; *MAPK3*
Immune system	1.11E-16	1.28E-14	18	*IL10*; *CXCL8*; *MMP2*; *STAT3*; *FN1*; *PTGS2*; *TNF*; *ACTB*; *VEGFA*; *IL4*; *IL6*; *IFNG*; *IL1B*; *CCL2*; *AKT1*; *TLR4*; *TP53*; *MAPK3*
Senescence-associated secretory phenotype (SASP)	8.17E-09	7.84E-07	5	*IL6*; *CXCL8*; *IL1B*; *STAT3*; *MAPK3*
Cellular responses to stress	7.68E-08	6.29E-06	11	*IL6*; *CXCL8*; *IL1B*; *ALB*; *STAT3*; *CCL2*; *AKT1*; *TLR4*; *TP53*; *MAPK3*; *VEGFA*
Cellular responses to stimuli	9.68E-08	6.52E-06	11	*IL6*; *CXCL8*; *IL1B*; *ALB*; *STAT3*; *CCL2*; *AKT1*; *TLR4*; *TP53*; *MAPK3*; *VEGFA*
Cellular senescence	1.02E-07	6.52E-06	6	*IL6*; *CXCL8*; *IL1B*; *STAT3*; *TP53*; *MAPK3*
Signaling by receptor tyrosine kinases	6.35E-05	0.0012	6	*STAT3*; *FN1*; *AKT1*; *ACTB*; *MAPK3*; *VEGFA*

Signal transduction	0.0012	0.0107	12	*CXCL8*; *MMP2*; *STAT3*; *FN1*; *TNF*; *ACTB*; *VEGFA*; *IL6*; *AKT1*; *CCL2*; *PPARG*; *TP53*; *MAPK3*
Disease	0.0013	0.0118	11	*IL10*; *IL6*; *IL1B*; *STAT3*; *FN1*; *AKT1*; *TLR4*; *TP53*; *ACTB*; *MAPK3*; *VEGFA*
Infectious disease	0.0023	0.0172	7	*IL10*; *IL6*; *IL1B*; *AKT1*; *ACTB*; *MAPK3*; *VEGFA*
Gene expression (transcription)	0.0024	0.0172	8	*IL6*; *IFNG*; *AKT1*; *PPARG*; *TP53*; *ACTB*; *MAPK3*; *VEGFA*

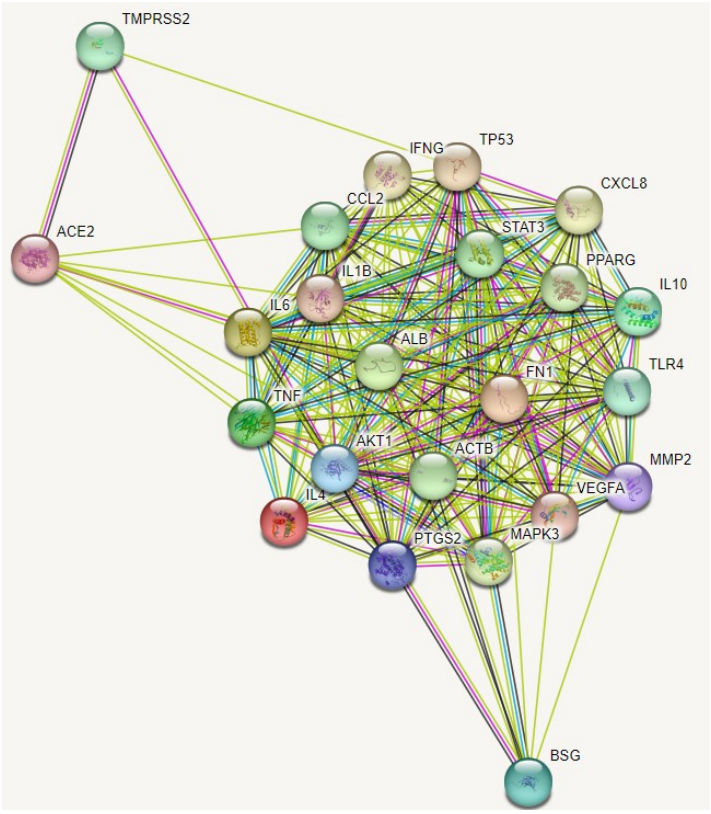


Figure 5. Interaction network of hub proteins common amongst comorbidities with SARS-CoV-2 entry receptors constructed in STRING

Table 3. SARS-CoV-2 entry receptors having direct interactions with hub proteins of comorbidities

SARS-CoV-2 entry receptors	TMPRSS 2	ACE2	CD147/BSG
Hub proteins having significant interactions with the SARS-CoV-2 entry receptors	AKT-1	AKT-1	AKT-1
		ACTB	ACTB
		FN1	FN1
		ALB	ALB
	TP53	TNF	VEGFA
		IL6	MAPK3
		ILIB	MMP2
		CCL2	