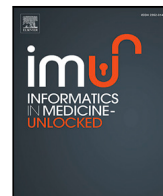




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# Severity of COVID-19 patients with coexistence of asthma and vitamin D deficiency

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## ABSTRACT

Coronavirus disease 2019 (COVID-19)-driven global pandemic triggered innumerable health complications, imposing great challenges in managing other respiratory diseases like asthma. Furthermore, increases in the underlying inflammation involved in the fatality of COVID-19 have been linked with lack of vitamin D. In this research work, we intend to investigate the possible genetic linkage of asthma and vitamin D deficiency with the severity and fatality of COVID-19 using a network-based approach. We identified and analysed 41 and 14 differentially expressed genes (DEGs) of COVID-19 being common with asthma and vitamin D deficiency, respectively, through the comparative differential gene expression analysis and their footprints on signalling pathways. Gene set enrichment analysis for GO terms and signalling pathways reveals key biological activities, including inflammatory response-related pathways (e.g., cytokine- and chemokine-mediated signalling pathways, IL-17, and TNF signalling pathways). Besides, the Protein-Protein Interaction network analysis of those DEGs reveals hub proteins, some of which are reported as inflammatory antiviral interferon-stimulated biomarkers that potentially drive the cytokine storm leading to COVID-19 severity and fatality, and contributes in the early stage of viral replication, respectively. Moreover, the regulatory network analysis found these DEGs associated with antiviral and tumour inhibitory transcription factors and micro-RNAs. Finally, drug-target enrichment analysis yields *tetradioxin*, *estradiol*, *arsenous acid*, and *zinc*, which have been reported to be effective in suppressing the pro-inflammatory cytokines production, and other respiratory tract infections. Our results yield shared biomarker-driven key hypotheses followed by network-based analytics, demystifying the mechanistic details of COVID-19 comorbidity of asthma and vitamin D deficiency with their potential therapeutic implications.

## 1. Introduction

The ongoing novel coronavirus disease 2019 (COVID-19) due to the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has come up amongst the most detrimental global health concerns in recent times [1–4]. Due to the high transmissibility of SARS-CoV-2, the number of confirmed COVID-19 cases have been reported as 612,724,171 globally, including 6,517,123 deaths as of September 27, 2022 [5]. The angiotensin-converting enzyme 2 (ACE2) receptor acts as the entrance for SARS-CoV-2 to penetrate through the surface of the host cells [6]. The spike (S) protein of coronaviruses binds to the ACE2 receptor and employs the cellular serine protease TMPRSS2 for priming S protein [7]. Even though several experiments have evidenced that ACE2 is considerably regulated in lung alveolar type 2 cells, it is

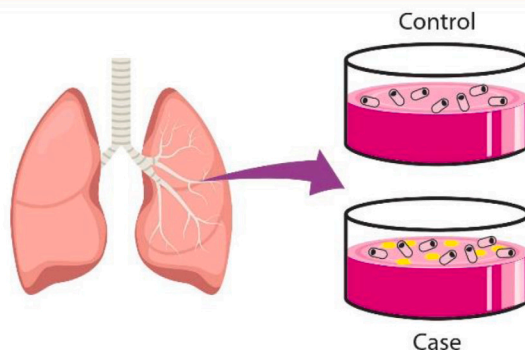
also associated with endothelial and smooth muscle cells in various organs [7,8]. Even after myriad research initiatives, the prognosis of this highly contagious coronavirus is yet poorly understood [9] that is mostly attributed to its varying characteristics and high mutation rate. Moreover, most of the COVID-19 cases show none or few lenient symptoms that include fever, sore throat, headache and dry cough. Although, the majority of cases have cured naturally, a notable portion of the patients have shown severe complications, including heart failure, acute kidney disease, sepsis and septic shock etc [10–13].

Note, it has been reported in some seminal works that the pre-existing health conditions could be fatal risk element for COVID-19 patients [14,15]. Many recent studies have confirmed hypertension as the most prevalent comorbidity for COVID-19 [16,17] and other

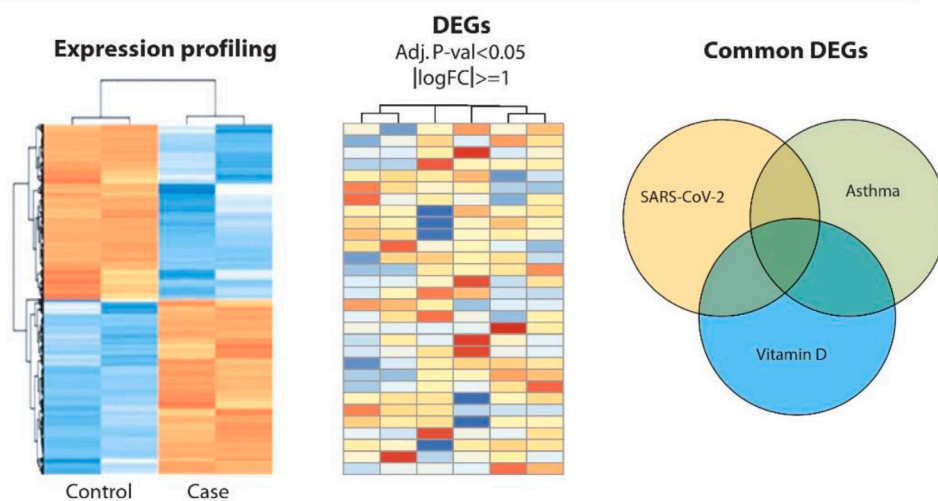
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## A. RNA-Seq data

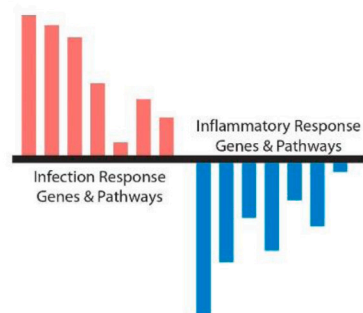
1. SARS-CoV-2 Infection (Case)  
Human lung epithelial cells
2. Asthma (Case)  
Bronchial epithelial cells
3. Vitamin D untreated (Case)  
Human ASM cells



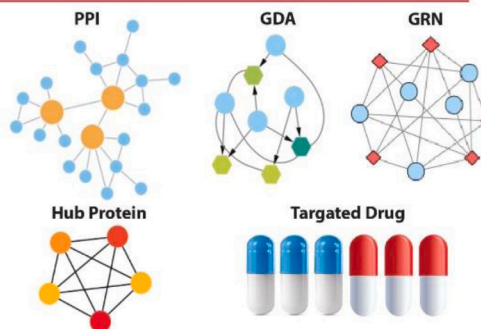
## B. RNA-Seq Data Analysis and Common DEGs Identification



## C. Enrichment Analysis



## D. PPI/GDA/GRN/Drug Target Analysis



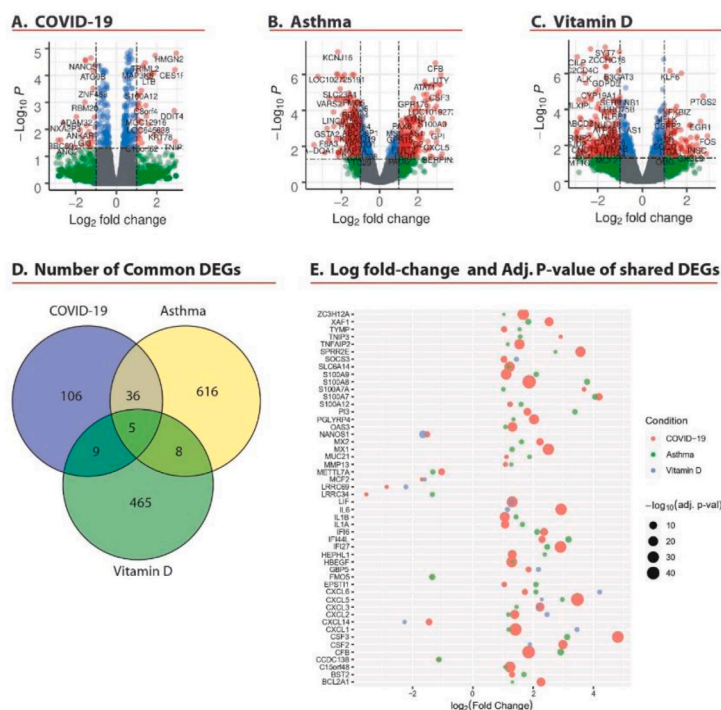
**Fig. 1.** Graphical workflow of the analytical pipeline adopted in this work. (A) RNA-Seq based gene expression profiles of lung epithelial cells for SARS-CoV-2 infection, bronchial epithelial cells for asthma and ASM cells for vitamin D deficiency were collected. (B) Differential expression analyses among all pair-wise conditions were conducted, which resulted shared Differentially Expressed Genes (DEGs) among all pair-wise conditions involving SARS-CoV-2 infection, asthma and vitamin D deficiency. (C) Functional enrichment analysis was conducted for the shared DEGs for Gene Ontology (GO) and pathway databases to assess the biological significance of those shared DEGs. (D) Protein-protein interaction (PPI) network-based analysis, gene-disease association and regulatory factors were conducted to identify potential therapeutic targets, possible comorbidities and prognostic molecular check-points, respectively.

consequences include diabetes, chronic obstructive pulmonary disease (COPD) and cardiac disorders [18–22]. Since COVID-19 is one of the most chronic respiratory contamination, it is anticipated that SARS-CoV-2 could have devastating influence on asthma exacerbations and vice-versa. In the United States, about 7.4% to 17% COVID-19 patients had comorbid asthma whereas, in China, it was <1% [22].

In many recent studies, vitamin D supplement has been suggested to have positive impact on the reduction of COVID-19 fatality [23–25]. Insufficiency of vitamin D contributes to decrease lung function and

accelerate asthma exacerbations [26]. Moreover, it has been demonstrated that vitamin D is involved in a wide spectrum of immunomodulatory activities [27,28]. It down-regulates ACE2 and activates the renin-angiotensin pathway, which in turn prevent the cytokine storm. Therefore, vitamin D might have preventive effect on COVID-19 severity [29–31]. In addition, as a supplementary therapy, vitamin D may ameliorate disease conditions of the patients with pre-existing asthma and other chronic lung infections [32,33].

This study primarily aims to study the genetic coherence of SARS-CoV-2 with asthma, and reveal any prospective link of vitamin D on



**Fig. 2.** Volcano plots depict the distribution of DEGs in  $\log_2(FC)$  and  $adj. p$ -value plane, where red dots indicate significant DEGs in SARS-CoV-2 infected lung epithelial cells (A), Asthma inflammatory bronchial epithelial cells (B), and vitamin D untreated human airway smooth muscle cells (C). Venn diagram in (D) shows the DEGs overlapping among COVID-19, asthma and vitamin D untreated cells. Bubble plot in (E) depicts all the overlapping DEGs among COVID-19 and each of the other two conditions as a function of  $\log_2(FC)$  and  $-\log_{10}(adj. p\text{-value})$ .

these respiratory infections. We employed a series of network-based bioinformatics approaches that incorporate analyses for gene expression, protein–protein interaction, functional enrichment, and regulatory analysis. The outcome of this study may advance the development of prospective therapeutic target to combat the ongoing pandemic engendered by the infection of SARS-CoV-2. The graphical workflow of the analytical pipeline used in this investigation is depicted in Fig. 1.

## 2. Materials and methods

### 2.1. RNA-sequencing gene expression datasets

In this research work, we collected three RNA-seq gene expression profiling datasets from the publicly available genomics data repository NCBI-GEO (Gene Expression Omnibus) having GEO accession numbers GSE147507, GSE146532, and GSE58434. The characteristics of these datasets are depicted in Table 1. The dataset for SARS-CoV-2 infection (GSE147507) was derived by gene expression profiling of lung epithelium (NHBE) tissues from SARS-CoV-2 infected humans and mock-treated ferrets using high throughput sequencing [34]. The corresponding study provided a comprehensive investigation of the host's transcriptional activity for SARS-CoV-2 infection. In our experiment, we considered the cell replicates from three SARS-CoV-2 infected humans as *Case* condition and 3 mock-treated ferrets as *Control* condition. For asthma, an RNA-seq dataset with GEO Accession number: GSE146532, has experimented on bronchial epithelial cells from an adult cohort ( $n = 120$ ) including asthmatics, Chronic obstructive pulmonary disease (COPD) and healthy controls, cultured with and without Rhinovirus 1 A using Illumina HiSeq 2000 platform [35]. From this dataset, we compared virus uninfected samples with asthma ( $n = 20$ ) and healthy individuals ( $n = 20$ ). Finally, the dataset GSE58434 was derived by comparing mRNA profiles by RNA-seq of airway smooth muscle (ASM) tissues from white human donors, 6 of them had fatal asthma and 12 control donors had treatment using vitamin D, albuterol, or were left untreated [36]. To conduct differential expression analysis, we

considered the 12 vitamin D treated samples as *Control* condition, and 12 untreated samples as the *Case* condition.

### 2.2. Identification of differentially expressed genes

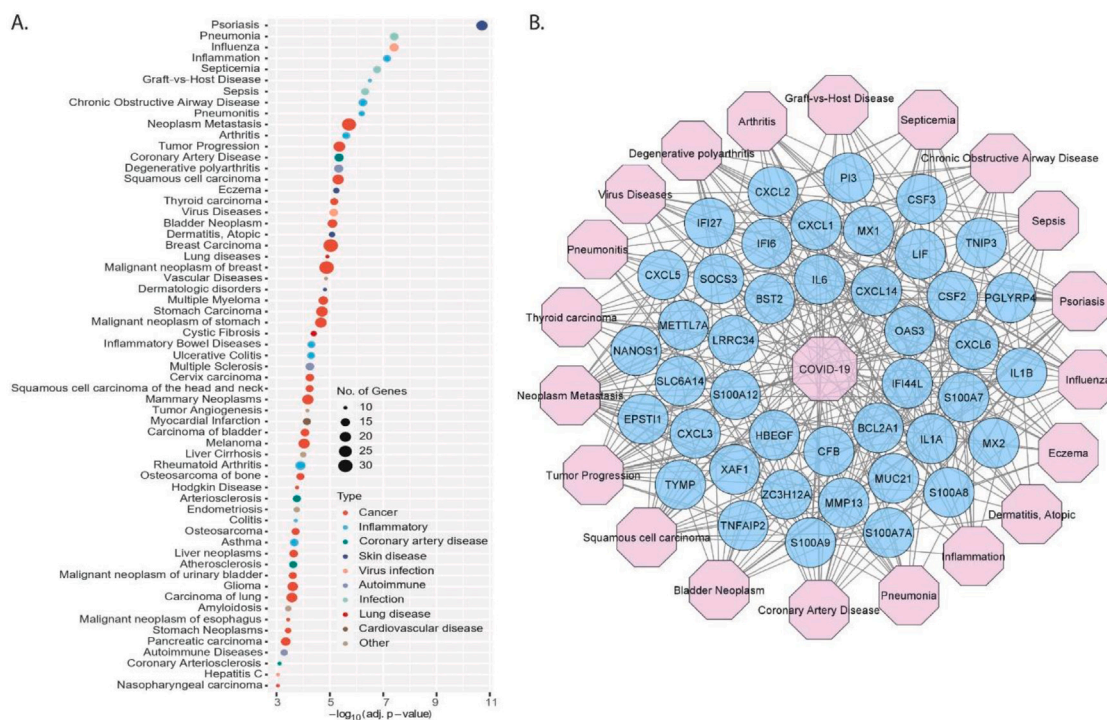
With the Case-vs-Control conditions for all the experiments, we used R Bioconductor package, DESeq2 [37] for analysing RNA-seq data and for determining the DEGs based on the negative binomial distribution model. After obtaining the DEGs for each of three conditions, i.e., COVID-19, asthma, and vitamin D deficiency, we filtered them considering FDR adjusted  $p$ -value (FDR) not more than 0.05 and the absolute  $\log_2(\text{Fold Change})$  as at least 1 to identify the most significant DEGs.

### 2.3. Profiling comorbidities associated with shared DEGs

To assess the potential diseases connected with COVID-19, we foreseen the comorbidity connections for the shared DEGs by means of Enrichr web-platform [38], utilizing the DisGeNET database [39]. For this, we only selected health complications having at least 10 enriched genes and  $p$ -value  $\leq 0.001$ . For better representation of the gene-disease association (GDA), we reconstructed a bipartite network using the Cytoscape tool [40].

### 2.4. Functional enrichment analysis

We performed functional enrichment analysis for the common DEGs to have a deeper insight about the molecular pathways involved in SARS-CoV-2. In this analysis, we utilized the web-based platform Enrichr [38] considering gene ontology (GO) and signalling pathways. In this investigation, we considered KEGG [41], WikiPathways [42], and Reactome [43] databases as annotation source of signalling pathways. For ontology analysis we included GO biological process (BP), molecular function (MF), and cellular component (CC). For statistical importance of the enrichment results, we set the upper threshold for the adjusted  $p$ -value as 0.05.



**Fig. 3.** Genetic relationship between COVID-19 and its comorbidities associated with shared DEGs. (A) The bubble plot presents the 61 significantly enriched diseases as comorbid of COVID-19 based on the enrichment  $-\log_{10}(adj. p\text{-value})$ . The sizes of the bubbles proportionate to the number of genes related to each disease. (B) The gene disease association network shows the interaction between 20 top-most diseases and their associated genes related to COVID-19. Here, the circular nodes indicate the common DEGs and the octagonal nodes specify COVID-19 and other diseases.

**Table 1**  
Description of the datasets used in this study. The 4th column represents the number of samples where the values in the parentheses indicate the number of subjects considered as case and controls.

Accession No.	Disease name	Tissues	Samples	Platform
GSE147507	SARS-CoV-2	Lung Epithelium (NHBE)	6 (3+3)	Illumina NextSeq 500
GSE146532	Asthma	Bronchial Epithelial	40 (20+20)	Illumina HiSeq 2000
GSE58434	Vitamin D deficiency	Airway smooth muscle (ASM)	24 (12+12)	Illumina HiSeq 2000

2.5. Protein-protein interaction analysis

The interactions that proteins take part, usually known as PPIs, are crucial to perceive the cell physiology in normal and complicated health conditions. PPIs provide the functional and structural insight about the cellular protein networks [44]. In this analysis, we obtained and visualized the PPI network for the identified shared DEGs by utilizing the protein interaction database STRING for medium confidence level (0.4) [45]. In this analysis, we applied the Markov clustering (MCL) technique to the PPI network to predict functional modules and protein complexes. Furthermore, we employed the Cytoscape plugin MCODE [46] and Cytohubba [47] to recognize the functional sub-network modules and the hub proteins anticipated by their degree of connectivity in the PPI network, respectively.

2.6. Regulatory analysis

Transcription factors (TFs) and their correspondence with genes play crucial role in their functional inference [48]. We investigated the association of the TFs with the DEGs using the NetworkAnalyst [49] platform. In this analysis, we choose the curated database Encyclopedia of DNA Elements (ENCODE) [50] in NetworkAnalyst. Likewise, microRNAs (miRNAs) are non-coding RNAs that participate in the biological process through post-transcriptional regulation. We utilized the curated database TarBase 8.0 [51] to obtain the gene-miRNA interactome. TarBase is a repository of imperially validated data of miRNA-gene interaction.

2.7. Drug candidates identification

To identify the prospective drug candidates, we enquired the Drug Signatures Database (DSigDB) using the overlapping DEGs among COVID-19 and each of the conditions under investigation using the web-based tool Enrichr [38]. The DSigDB contains 22,527 genes associated with different drugs and small compounds based on the dysregulated expression patterns of the genes for those elements [52]. This database is very useful for drug repurposing and translational research. The significant drug molecules were identified by applying an enrichment threshold of the FDR adjusted  $p$ -value < 0.05.

3. Results

3.1. Differential expression analyses revealed shared DEGs among SARS-CoV-2, asthma and vitamin D deficiency

We performed the differential expression analyses with three gene expression datasets including SARS-CoV-2 infection, asthma, and vitamin D untreated individuals with corresponding control cases. We selected the most significant DEGs with the criteria mentioned in Section 2.2. With these criteria, we found 156, 665, and 487 potential DEGs for COVID-19, asthma, and vitamin D deficiency, respectively. The volcano plots in Fig. 2 show all the DEGs and highlight the most significant DEGs with red dots. In a cross-matching process presented as a Venn diagram in Fig. 2D, we found 41 concordant genes for SARS-CoV-2 and asthma, while the number drops down to 14 for SARS-CoV-2

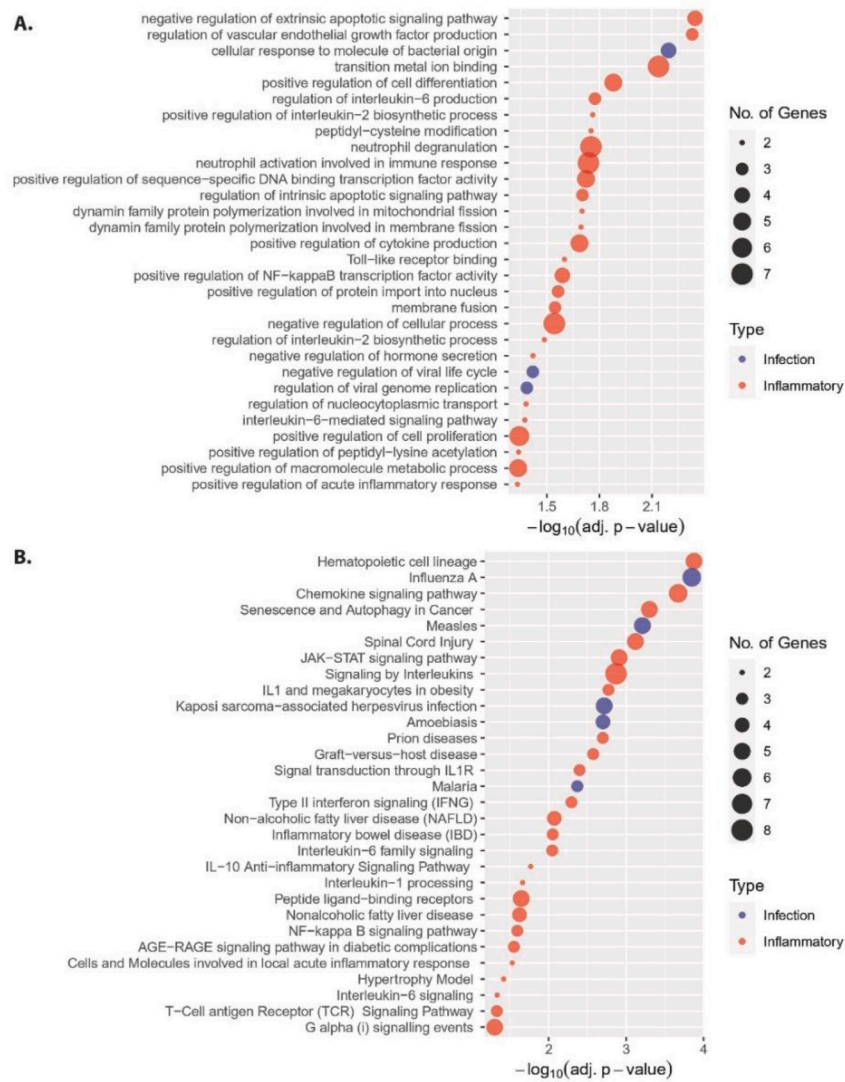


Fig. 4. Functional analyses using web-based platform Enrichr [38] for the shared DEGs considering GO (BP, MF and CC) and cell signalling pathways (KEGG, WikiPathways and Reactome) revealed that inflammatory and infection responses were enriched for SARS-CoV-2 infection. The top 30 notably enriched (A) GO terms and (B) Cell signalling pathways.

and vitamin D untreated subjects. In our cross-comparative analysis, we revealed 5 DEGs from the subfamily of chemokines, namely CXCL1, CXCL2, CXCL3, CXCL5, and CXCL6 that were common among COVID-19, asthma, and vitamin D deficient conditions. Therefore, we found 50 distinct DEGs shared by COVID-19 with asthma and lack of vitamin D. These 50 genes are presented in Fig. 2E from the perspective of  $\log_2(FC)$  and negative value of  $\log_{10}(adj. p\text{-value})$ . The subsequent analysis were performed on these 50 common DEGs.

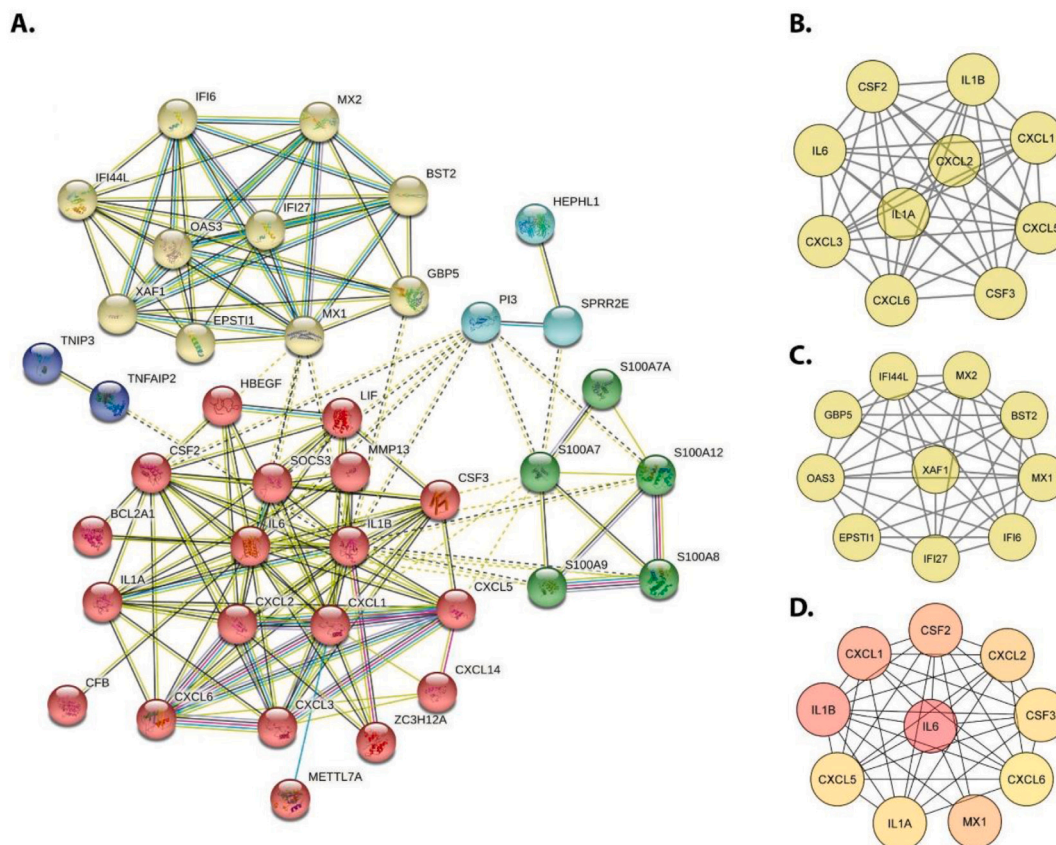
### 3.2. Genetic association of COVID-19 with other diseases

We identified 61 most significant diseases associated with at most 31 genes and at least 10 genes in COVID-19 with adjusted  $p$ -value ranges from  $1.98 \times 10^{-11}$  to  $8.91 \times 10^{-4}$ . A bubble chart for all of these comorbid diseases is presented in Fig. 3A as a function of  $-\log_{10}(adj. p\text{-value})$  and the number of genes involved. Among these 61 diseases, we selected 20 top-most enriched diseases with their corresponding adjusted  $p$ -value of enrichment, and created gene-disease association (GDA) network as shown in Fig. 3B. In this GDA analysis, we found that most of the comorbidities are different forms of cancers followed by inflammatory diseases, coronary artery disease, and so on. The maximum 31 DEGs were obtained to be related to breast carcinoma

and malignant neoplasm of the breast. Some other crucial comorbidities found in our GDA analysis are neoplasm metastasis, stomach carcinoma, lung carcinoma, glioma, rheumatoid arthritis, pancreatic carcinoma, coronary artery disease, pneumonia, chronic obstructive airway disease, and inflammatory bowel diseases etc. The number of DEGs involved with these diseases ranges from 14 to 29. The resulted DEGs and associated diseases could reveal how the progression of COVID-19 may engender long-term health complications.

### 3.3. Enrichment of gene ontology and cell signalling pathway for SARS-CoV-2 infection

We performed extensive functional analysis through the enrichment tests of the shared DEGs using Gene Ontology terms (GO terms) and cell signalling pathways. Based on adjusted  $p$ -value, we found a total of 81 significant GO terms (Biological Process, BP; Molecular Functions, MF; and Cellular Components, CC), where cytokine and chemotaxis related functional terms were the most predominant to the infection profile of SARS-CoV-2, as shown in Fig. 4A. Among these 81 terms, we found most of the enriched ones were inflammatory-related, whereas very few terms were associated with viral or other infections. Likewise, the enrichment of the shared DEGs with the cell signalling pathways from three pathway annotation database, KEGG [41], Reactome [43], and



**Fig. 5.** (A) The PPI network, obtained from STRING database [45], depicts the interactions among the shared DEGs with confidence score 0.4. The node colours represent MCL clustering with granularity parameter as 3. Two sub-networks that were recognized by the MCODE plugin [46] of Cytoscape as shown in (B) and (C), respectively. Interactions among the ten most significant hub proteins considering degree measurement, are shown in (D). The colour ranging from red to yellow indicates the degree from high to low.

WikiPathways [42] revealed a total of 48 significant pathways in which inflammatory interleukin, cytokines, TNF signalling, and interferon signalling were found most significantly enriched, as shown in Fig. 4B.

### 3.4. Protein–protein interaction network analysis revealed significant sub-networks and potential hub proteins

We constructed the PPI network for the common DEGs between COVID-19 and other two conditions (asthma and vitamin D untreated subjects) as depicted in Fig. 5. In the network, a protein is represented by a node and an edge corresponds to the functional interaction among a pair of proteins, based on the annotations in STRING database [45]. While constructing the PPI network, we used Markov clustering (MCL) procedure with the granularity parameter set to 3 in order to discern the similar groups (clusters) of the proteins. Fig. 5A shows the involvement of proteins in the PPI network. Further analysis using MCODE plugin [46] recognized the top two sub-networks as displayed in Fig. 5B and 5C, respectively. The first sub-network comprises 10 DEGs including IL1A, IL1B, IL6, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CSF2, and CSF3. The second sub-network consists of XAF1, IFI6, IFI27, IFI44L, GBP5, BST2, OAS3, MX1, MX2, and EPSTI1 signature genes. In addition, the topological analysis based on degree metric recognized ten core proteins as IL6, IL1A, IL1B, CXCL1, CXCL2, CXCL5, CXCL6, CSF2, CSF3, and MX1, which are presented in Fig. 5D. These hub proteins might be considered as prospective therapeutic targets to treat COVID-19.

### 3.5. Gene regulatory network analysis discovered highly active regulatory biomolecules

The regulatory interactome between the TF (Transcription Factor) proteins and their regulated genes was obtained from NetworkAnalyst

software [49], which contained a total of 93 nodes with 288 interactions. Among these, 27 nodes belong to the shared DEGs as shown in Fig. 6. We found the top 5 genes, i.e. TYPM, ZC3H12 A, CFB, CXCL2, and SOCS3 to be regulated by a maximum number of TFs, which was 31, 27, 26, 22, and 22 TFs, respectively. On the contrary, we identified the top 5 TFs considering the number of interactions with DEGs, which are IRF1, MAZ, TRIM22, ZBTB11, and NR2C2. IRF1 and MAZ regulated 8 and 7 genes, respectively whereas each of TRIM22, ZBTB11, and NR2C2 has interactions with 6 genes. Similarly, we obtained a gene–miRNA interaction network with a total of 81 nodes and 547 interactions, as shown in Fig. 7. We identified 43 regulatory bio-molecules (miRNAs) that may influence the regulation of 38 DEGs at post-transcriptional levels. Notably, IFI44L was involved with a maximum of 26 miRNAs, whereas IL6, BST2, CXCL2, and MX1 had connections with 26, 25, 24, and 24 miRNAs, respectively. Among 43 identified miRNAs, hsa-mir-21-3p, hsa-mir-27a-5p, hsa-mir-129-2-3p, hsa-mir-16-5p, and hsa-mir-34a-5p had the most predominant role in gene regulation at post-transcriptional level from the perspective of number of interactions.

### 3.6. Identification of candidate drug/compounds

Using all the identified common DEGs, we have obtained 93 most significantly enriched candidate drugs/compounds from the EnrichR platform [38] based on the adjusted  $p$ -value < 0.001 threshold. Among these 93 drug molecules, the top 30 significantly enriched drugs/compounds are shown in Fig. 8 based on their enrichment adjusted  $p$ -value. Notably, we found some drug molecules to be associated with a higher number of DEGs, such as *tetradioxin*, *estradiol*, *arsenous acid*, and *zinc* were linked with 33, 27, 22, and 17 DEGs, respectively.

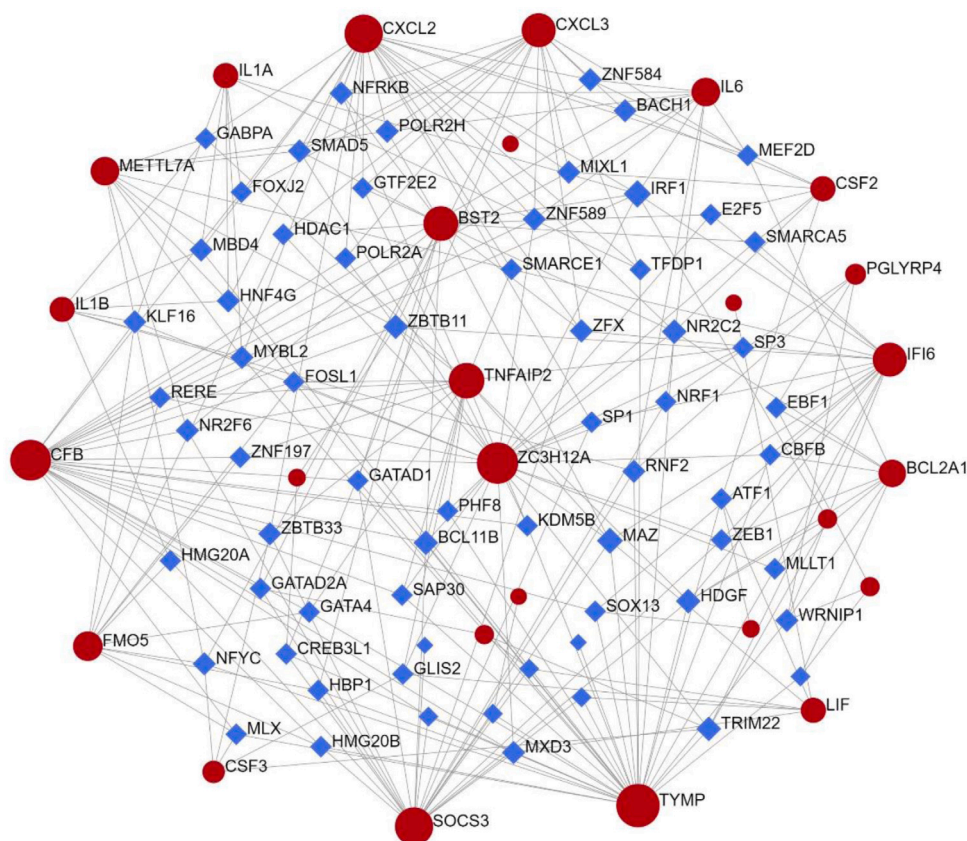


Fig. 6. The TF-gene interaction network obtained in regulatory analysis for the shared DEGs among SARS-CoV-2 infection and each of the asthma and vitamin D deficiency. The network was organized via the web-based platform NetworkAnalyst [49] using the ENCODE database [50]. The dimension of a node indicates the number of interactions the node regulates. The circular nodes correspond to DEGs and square nodes represent Transcription factors (TFs). The network was filtered with degree centrality  $\geq 3$ .

#### 4. Discussion

SARS-CoV-2 infection can cause acute respiratory infection with substantial morbidities and mortalities. Being a chronic inflammatory disease of the airways of lungs, asthma could be a lethal health complication for COVID-19 patients. Besides, due to the immunomodulatory activities, vitamin D status could have significant association with the consequences of COVID-19 [53]. Therefore, this study primarily focused to explore the genetic relationships of SARS-CoV-2 with asthma and vitamin D treatment through a series of network-based bioinformatics methodologies. We compared the genome-wide RNA-sequencing of SARS-CoV-2 infection in lung epithelial cells, asthma infection in bronchial epithelial cells and vitamin D treated individuals' samples from ASM cells with corresponding healthy individuals. Our analysis revealed 41 DEGs being common between SARS-CoV-2 and asthma, while 14 DEGs were common to SARS-CoV-2 and vitamin D deficiency, and a total of 50 distinct DEGs were common between these two sets. The subsequent analyses were conducted engaging these 50 common DEGs through the gene set enrichment analyses for GO terms and cell signalling pathways, PPI analysis, gene-regulatory interactome, and drug-candidate identification.

The gene-set enrichment analysis for the total shared DEGs between SARS-CoV-2 and each of asthma and vitamin D deficiency identified significant GO terms and cell signalling pathways. Markedly, we observed that among the most significantly enriched GO terms were the inflammatory-related GO terms, including cytokine and chemokine activities, cytokine- and chemokine-mediated signalling pathways, positive regulation of granulocyte chemotaxis and leukocyte chemotaxis, inflammatory response, type I interferon signalling pathway, and cellular response to type I interferon (Fig. 4A). The comprehensive activity of these pathways may exacerbate inflammatory responses that results

in release of large amount of pro-inflammatory cytokines, this condition is termed as cytokine storm. Studies reported that severe COVID-19 patients might develop cytokine storm characterized by acute respiratory distress syndrome (ARDS) and subsequent haemophagocytic lymphohistiocytosis [54]. The phenomenon cytokine storm has also been considered as an important contributor to multiple organ dysfunction syndrome [55]. We also found inflammatory pathways that were enriched significantly, as shown in Fig. 4B. Amongst those, IL-17 signalling pathway, cytokines and inflammatory response, JAK-STAT signalling pathway, TNF signalling pathway, cytokine signalling in immune system, interferon (IFN) alpha/beta signalling, interferon signalling, NOD-like receptor signalling pathway, signalling by interleukins, interleukin-6 family signalling, IL-10 anti-inflammatory signalling pathway, and T-cell antigen receptor (TCR) signalling pathway are noteworthy. Most of these inflammatory pathways represent the pathogenesis of COVID-19 [56]. They also play a significant role in immune system related different issues and modulate other physiological and pathological activities. For instance, cytokines and chemokines are the key modulators of the inherent and adaptive immune system of the host [57,58].

The PPI analysis with combined shared DEGs identified two significant functional sub-networks and ten hub proteins. The first sub-network consisted of ten proteins in which three (IL1 A, IL1B, and IL6) belong to the interleukin family, five proteins (CXCL1, CXCL2, CXCL3, CXCL5, and CXCL6) to the CXC chemokine family, and two proteins (CSF2 and CSF3) to growth factors. Likewise, the second sub-network was comprised of ten interferon-inducible proteins (XAF1, IFI6, IFI27, IFI44L, GBP5, BST2, OAS3, MX1, MX2, and EPSTI1). These proteins enhance inflammatory responses by inducing pro-inflammatory cytokines and cause COVID-19 more severe. CSF2 was evidenced to be linked with the pervasiveness of asthma and other atopic conditions [59,60].



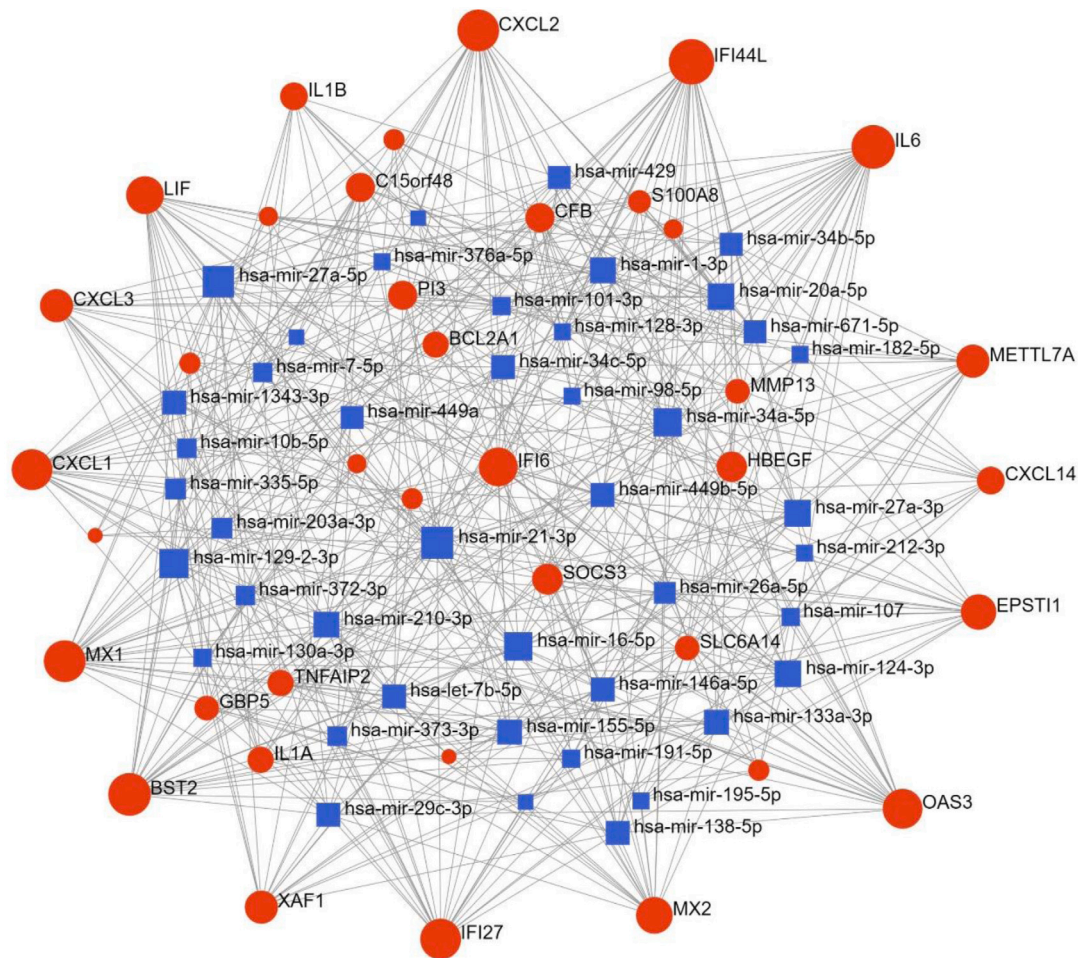


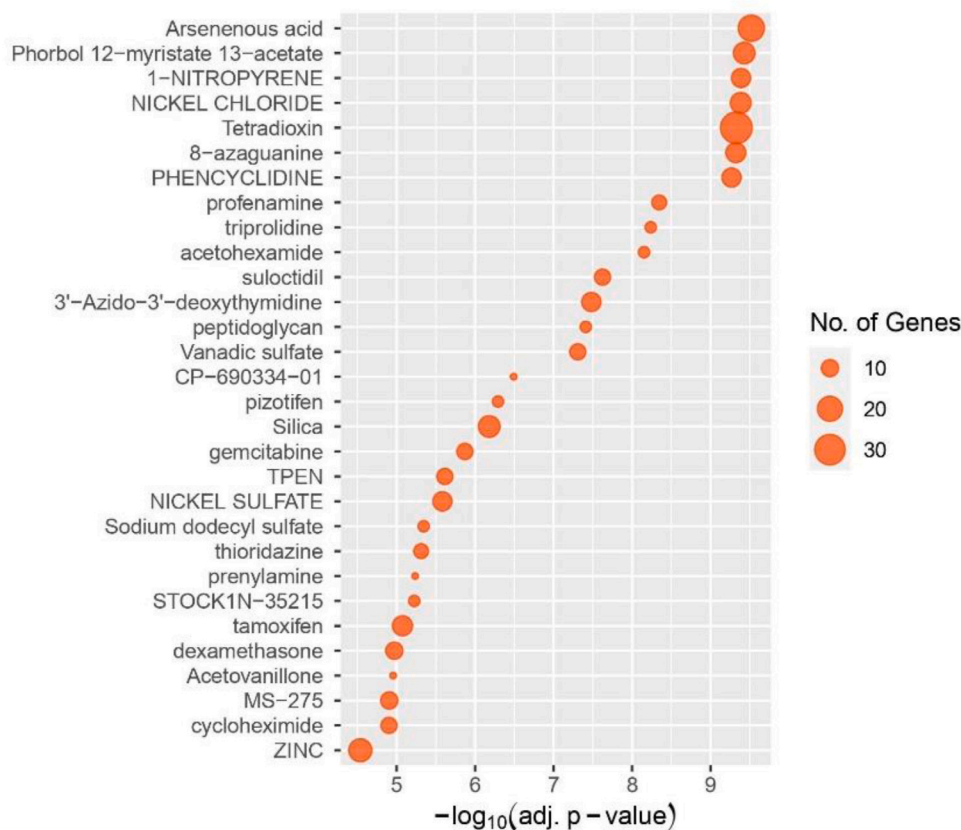
Fig. 7. The Gene-miRNA interaction network obtained in regulatory analysis with all the identified common DEGs. The network was built via the web-based tool NetworkAnalyst [49] by employing the TarBase 8.0 database [51] and filtered with degree centrality  $\geq 7$ .

Moreover, a recent study found CSF3 to have increased regulation in bronchial epithelial cells infected with SARS-CoV-2, which triggers the recruitment process of innate immunity by activating neutrophils and alveolar macrophages [61]. Furthermore, the expression of CSF3 in the lung correlates to the acuteness of pulmonary neutrophilia in ARDS [62]. Consequently, CSF3 might be suggested as a potential drug target to treat COVID-19. Again, GBP5 acts as an important mediator of the inflammatory immune response [63]. The preventive characteristic of GBP5 against viral infections was also evidenced against HIV-1 and respiratory syncytial virus [64,65]. The host defense protein BST2 acts as a molecular tether between the host cell membrane and the budding viral membrane to restrict virus entry that suggests BST2 as a crucial regulator of inherent antiviral response in infected host cells [66]. Among the identified hub proteins, 9 (IL1 A, IL1B, IL6, CXCL1, CXCL2, CXCL5, CXCL6, CSF2, and CSF3) were from the first sub-network and MX1 alone came from the second sub-network. A recent study reported that SARS-CoV-2 stimulates IL-1 (IL1 A and IL1B) in macrophages and mast cells activating other pro-inflammatory cytokines such as IL6 and TNF [67]. Elevated expression of IL6 provokes high expression of ACE2, which in turn lead to inflammatory factor storms [68]. Moreover, Reza et al. recently reported notable rise in expression level of IL-6 in COVID-19 patients [69]. More promisingly, drugs targeting IL6 showed efficacy in reducing severity and mortality of medium to severe stages of COVID-19 patients [70]. The association of cytokine storm resulted by SARS-CoV-2 infection with the disease fatality is well established [71]. Yong et al. evidenced the regulation of a group of pro-inflammatory cytokines including CXCL1 and CXCL2 induced cytokine storm in SARS-CoV-2 infection [72]. Tamizhini et al.

reported the effectiveness of CXCL2 and CXCL3 as therapeutic target against viral infections [73]. In an *in vivo* experiment on SARS-CoV-2 infected mouse models, chemokine CXCL5 demonstrated the regulation of neutrophil infiltration that indicated its prospect as a therapeutic target against pneumonia complications [74]. CXCL6 was found to be elevated in COVID-19 patient, suggesting its involvement in airway inflammation [11]. MX1 is an inhibitor and plays initial role in genome replication at the preliminary post-entry stage in the life cycle of a virus [75]. Intriguingly, a recent investigation has exhibited the protective effect of MX1 against SARS-CoV-2 infection [76]. As a whole, the identified hub proteins play important role in cytokine storm and unregulated inflammation in SARS-CoV-2 infection suggesting their prospect as target against the infection.

The gene regulatory network analysis revealed significantly high incidence of TF-gene and gene-miRNA association. We observed that interferon regulatory factor 1 (IRF1) employed the maximum gene regulation, including IL1 A, IL6, IFN, CXCL2, BST2, CFB, OAS3, and ZC3H12 A. A previous study reported that IRF1 influences antiviral, antibacterial, and tumour inhibition functions [77]. In gene-miRNA network analysis, we identified several important miRNAs, namely, hsa-mir-21-3p, hsa-mir-27a-5p, hsa-mir-129-2-3p, hsa-mir-16-5p, and hsa-mir-34a-5p. These miRNAs might be considered as key players in virus replication and pathogenesis. Recently, mir-21-3p and mir-16-5p were outlined as potential regulators of all human coronaviruses through viral RNA bindings [78].

Moreover, based on the drug-target enrichment analysis, we observed some significantly enriched drug molecules including *tetradioxin*, *estradiol*, *arsenenous acid*, and *zinc* with the shared DEGs in account.



**Fig. 8.** The top 30 most significantly enriched drugs/compounds with the shared DEGs based on their enrichment adjusted  $p$ -value. Here, we have used the DSigDB database [52] as the annotation source via Enrichr web-based tool [38]. In this plot, the size of each of the circle proportionate with the number of DEGs associated with the corresponding drug/compound.

Among the top enriched drug molecules, *estradiol* showed involvement to suppress the production of proinflammatory cytokines and thus may reduce the severity of COVID-19 [79]. Recently, *estradiol* is reported to have a preventive role for COVID-19 [80,81]. Moreover, *zinc* has the potential to have a defensive nature against SARS-CoV-2 infection and other respiratory tract infections due to its immunomodulatory activity and antiviral effect [82]. Besides, another study suggests that *zinc* supplements are fruitful to prevent and treat COVID-19 and other respiratory infections because of its therapeutic efficacy [83,84]. Therefore, the gained drug molecules could be further examined through extensive studies for their prospect to fight against COVID-19.

## 5. Conclusions

In this work, we investigated the molecular and cellular coherence of COVID-19 infection with asthma and vitamin D deficiency with an objective to investigate the putative repositioning of old drug molecules to combat the coronavirus pandemic. In this process, we explored the gene expression profiles of the health conditions and found some key biomarkers influencing their interaction. We also identified several gene signatures highlighting their therapeutic possibilities against SARS-CoV-2 infection using drug-target enrichment analysis. Furthermore, we identified some regulatory checkpoints such as TFs and miRNAs and probable drug molecules that demand further experimental validation. The key findings of this work might contribute to mitigate the knowledge gap regarding the disease mechanism and open up new research scopes for developing therapeutic intervention to alleviate the pandemic.

## CRediT authorship contribution statement

**M. Babul Islam:** Conceived and designed the study, Analysed the data, Wrote the R programming code for the development of the pipeline, Conducted all other bioinformatics analyses, Wrote the manuscript. **Utpala Nanda Chowdhury:** Conducted all other bioinformatics analyses, Wrote the manuscript. **Md. Asif Nashiry:** Conducted all other bioinformatics analyses, Wrote the manuscript. **Mohammad Ali Moni:** Conceived and designed the study, Conducted all other bioinformatics analyses, Wrote the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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