Comparisons of the immunological landscape of COVID-19 patients based on sex and disease severity by multi-omics analysis

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ABSTRACT

Objective: To determine the differences in the immune response against SARS-CoV-2 infection of patients based on sex and disease severity.
Methods: We used an analytical framework of 382 transcriptional modules and multi-omics analyses to discriminate COVID-19 patients based on sex and disease severity.
Results: Male and female patients overexpressed modules related to the innate immune response. The expression of modules related to the adaptive immune response showed lower enrichment levels in males than females. Inflammation modules showed ascending overexpression in male and female patients, while a higher level was observed in severe female patients. Moderate female patients demonstrated significant overexpression to interferon, cytolytic lymphocyte, T & B cells, and erythrocytes modules. Moderate female patients showed a higher adaptive immune response than males matched group. Pathways involved in metabolism dysregulation and Hippo signaling were upregulated in females than in male patients. Females and moderate cases showed higher levels of metabolic dysregulation.
Conclusions: The immune landscape in COVID-19 patients was noticeably different between the sexes, and these differences may highlight disease vulnerability in males. This study suggested that certain treatments that increase or decrease the immune responses to SARS-CoV-2 might be necessary for male and female patients at certain disease stages.

1. Introduction

Since the outbreak of the COVID-19 pandemic in late November 2020, around 228 million cases and 4.6 million deaths have been reported globally as of September 20, 2021 [1]. Several reports have shown that male COVID-19 patients have a higher risk for disease severity, including death, than female patients [2,3]. In the USA, approximately 321,760 deaths every month from COVID-19 are reported in men, compared to 264,899 in women [4]. In England, a large cohort study revealed a strong association between sex and death from COVID-19 (hazard ratio 1.59, 95% confidence interval (CI): [1.53,1.65])

[5].

Sex is a biological variable that plays a vital role in immune responses to different antigens such as viruses and bacteria [6]. It is defined by the disparity in the organization of chromosomes, sex steroid levels, and reproductive tissues; it is dissimilar from gender, which includes behaviors and activities determined by the society in humans [7]. Human immunological responses may be affected by sex and gender. In sex, physiological and anatomical differences impact exposure, recognition, and transmission of microbes. On the other hand, gender may reflect behaviors that influence vulnerability to microbes [7]. Several reports suggested that sex has a substantial role in infection

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outcomes and is associated with fundamental differences in immune responses to infection. For example, most autoimmune diseases more frequently occur in females. Men show a higher risk of death from cancer than women. In addition, responses to influenza vaccines are steadily stronger in women than men [7,8].

Since the COVID-19 outbreak, several studies reported the immune responses against respiratory disease in male and female patients by assessing viral loads, plasma cytokines or chemokines, and different blood-cell phenotypes [7,8].

To determine the immune response differences to SARS-CoV-2 infection between male and female patients, we performed multi-omics analyses of immune phenotypes in male and female COVID-19 patients stratifying by sex and disease severity. This study aimed to provide new insights into the different immunological landscapes between male and female COVID-19 patients.

2. Methods

2.1. Data processing

We collected five COVID-19 datasets (Table 1), which contained a total of 287 COVID-19 patients and 324 healthy controls. The 287 COVID-19 patients included 141 (49%) females and 146 (51%) males. Patients' median age was 60 years (interquartile range, 42.50–72.50 years) and. 77 (27%), 120 (42%), and 90 (31%) of these patients were mild, moderate, and severe cases, respectively. In the five datasets, one dataset involved COVID-19 patients from Hong Kong and Atlanta, one from China, and three from the USA.

We merged the five raw datasets using the "merge" function in the R package "base" and performed data curation and normalization using the "ComBat" function in the R package "sva." These datasets were downloaded from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) and ArrayExpress (https://www.ebi.ac.uk/arrayexpress/). A description of these datasets is shown in Table 1.

2.2. mRNA data analysis

We compared predefined sample groups using the Mann–Whitney *U* test or limma [9]. The Benjamini–Hochberg method was used to calculate the false discovery rate (FDR) to adjust for multiple tests [10]. We used the R package "BloodGen3Module" to analyze and interpret blood transcriptome data and visualize the fingerprint data [11]. A total of 985 unique blood transcriptome profiles derived from different diseases were annotated to 382 distinctive modules. We compared normally distributed variables using the Student's t-test (for two comparisons) or ANOVA (for three classes of comparisons). In comparisons of non-normally-distributed variables, we used Mann–Whitney U tests (for two classes of comparisons) or Kruskal–Wallis tests (for three classes of comparisons). A threshold of P < 0.05 defined the statistical significance.

2.3. miRNA data analysis

We used the R package "edger" [12] to identify differentially expressed miRNAs between predefined groups. The validated target genes for miRNAs were searched in 14 databases using the R package "multiMiR" [13]. Furthermore, we identified miRNA sponge interactions using the function "spongeMethod" in the R package "miR-spongeR" [14], and the miRHomology method was used to identify sponges. Finally, We conducted pathway enrichment analysis using the R package "clusterProfiler" [15].

2.4. mRNA-lncRNA data analysis

We performed module detection analysis in the mRNA-lncRNA data across the predefined groups based on a robust interconnection measure using the DynamicTreeCut algorithm and the R package "WGCNA" [16]. We conducted pathway enrichment analysis using the R package "anRichment." The Cytoscape software was used to demonstrate the network of hub genes and lncRNAs [17].

2.5. Metabolomic analysis

We utilized the principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) for multivariate analysis to discriminate between predefined groups. Univariate analysis was performed using the Student's t-test. Mapping metabolites to KEGG pathways was conducted by the R package "FELLA." [18].

3. Results

3.1. Transcriptional biosignatures in COVID-19 patients

We analyzed transcriptomic data from peripheral blood samples of 287 COVID-19 patients (including 120 mild, 100 moderate, and 67 severe cases) and 324 healthy controls to define the whole blood biosignature of COVID-19. Samples were divided into two independent cohorts ("male" and "female" sets). We first identified transcriptional signatures of both cohort sets, including 4097 differentially expressed genes (DEGs) between 141 female COVID-19 patients and 23 healthy female controls and 3063 DEGs between 146 male patients and 25 healthy male controls. We found 2857 overlaps between both sets of DEGs (Fig. 1A). To compare the host immune response between male and female COVID-19 patients, we used an analytical framework of 382 transcriptional modules divided into 38 clusters that group genes with shared expression patterns and similar biologic functions. Module maps were derived for the male (Fig. 1B, D, E) and female (Fig. 1C, D, E) sets, respectively, with their respective healthy control groups as references. Overall, both male and female patients overexpressed modules related to the innate immune response (inflammation (M12.10, M13.2, and M14.19), interferon (M10.1, M13.17, M15.127, M15.64, and M15.86], monocytes (M14.65 and M15.70), and neutrophils (M15.26 and M15.35)). However, the expression of modules related to the adaptive immune response differed between male and female patients. In males, antigen-presenting cells (M15.16 and M16.77), B cells (M12.8, M13.18, and M13.27), and T cells (M12.6, M14.42, and M15.33) showed lower enrichment levels compared to females (Fig. 1E).

3.2. Distinct transcriptional profiles in mild, moderate, and severe COVID-19 patients

To identify specific transcriptional profiles in mild, moderate, and

Table 1
A summary of the five datasets included in this study.

Dataset	Data type	No. of patients	No. of healthy controls	Tissue source
GSE157859	mRNA, miRNA, lncRNA	18	9	peripheral blood mononuclear cells (PBMCs)
GSE152418	mRNA	17	17	PBMCs
E-MTAB-9357	mRNA, metabolomics	139	258	blood
GSE157103	mRNA	100	26	leukocytes from whole blood
GSE161778	mRNA	13	14	PBMCs
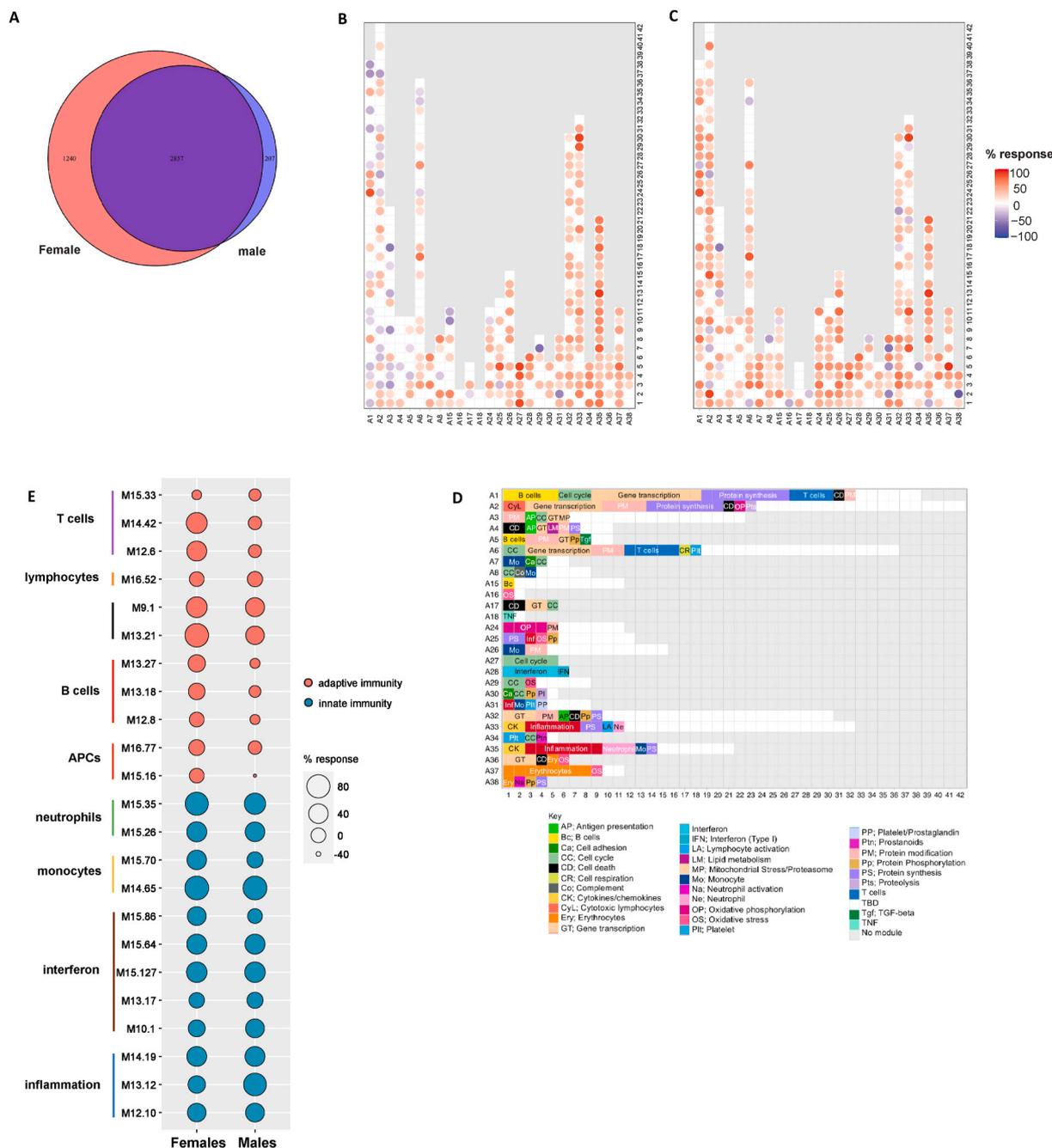
Fig. 1. Robust transcriptional biosignature in adults COVID-19 patients. **(A)** Venn diagram displaying the overlap among the significant transcripts identified in the males (3063 DEGs) and females (4097 DEGs) with common transcripts among the two groups (2857 DEGs) obtained from a nonparametric test (*P* value < 0.05), >1.10-fold change, and Benjamini–Hochberg multiple-test correction. **(B)** Transcriptional Average analytical framework of 382 modules divided into 38 clusters that group genes with shared expression patterns and similar biologic functions of male patients and **(C)** Female patients compared to healthy controls. Results (at the module level) are expressed as the percentage of increased or decreased genes. **(D)** Module functional annotations legend. **(E)** Mean modular transcriptional fingerprint for males (146 patients and 25 matched controls) and females (141 patients and 23 matched controls). Modules are ordered based on their relation to the innate and adaptive immune response. The expression of modules related to the adaptive immune response differed between male and female patients. In males, antigen-presenting cells, B cells, and T cells showed lower enrichment levels than females. Results (at the module level) are expressed as the mean value of increased or decreased genes.

severe COVID-19 cases in males and females, we compared gene expression profiles between the three groups of patients with different disease severities for males and females. There were 20 mild, 61 moderate, and 79 severe cases in males and 57 mild, 59 moderate, and 11 severe cases in females. Statistical group comparisons between the mild and moderate controls in males identified 60 up-regulated DEGs and 266 down-regulated DEGs. In comparison, the matched group in females identified 23 up-regulated DEGs and 1158 down-regulated DEGs. A

similar approach revealed 81 up-regulated DEGs and 235 down-regulated DEGs in males between severe and moderate controls. In comparison, the female matched group identified 233 up-regulated DEGs and 297 down-regulated DEGs.

We performed the modular analysis to characterize better the biological significance of the differences in gene expression profiles identified in the three groups per set. Moderate male patients showed slight overexpression in modules associated with B cells and platelets (gene
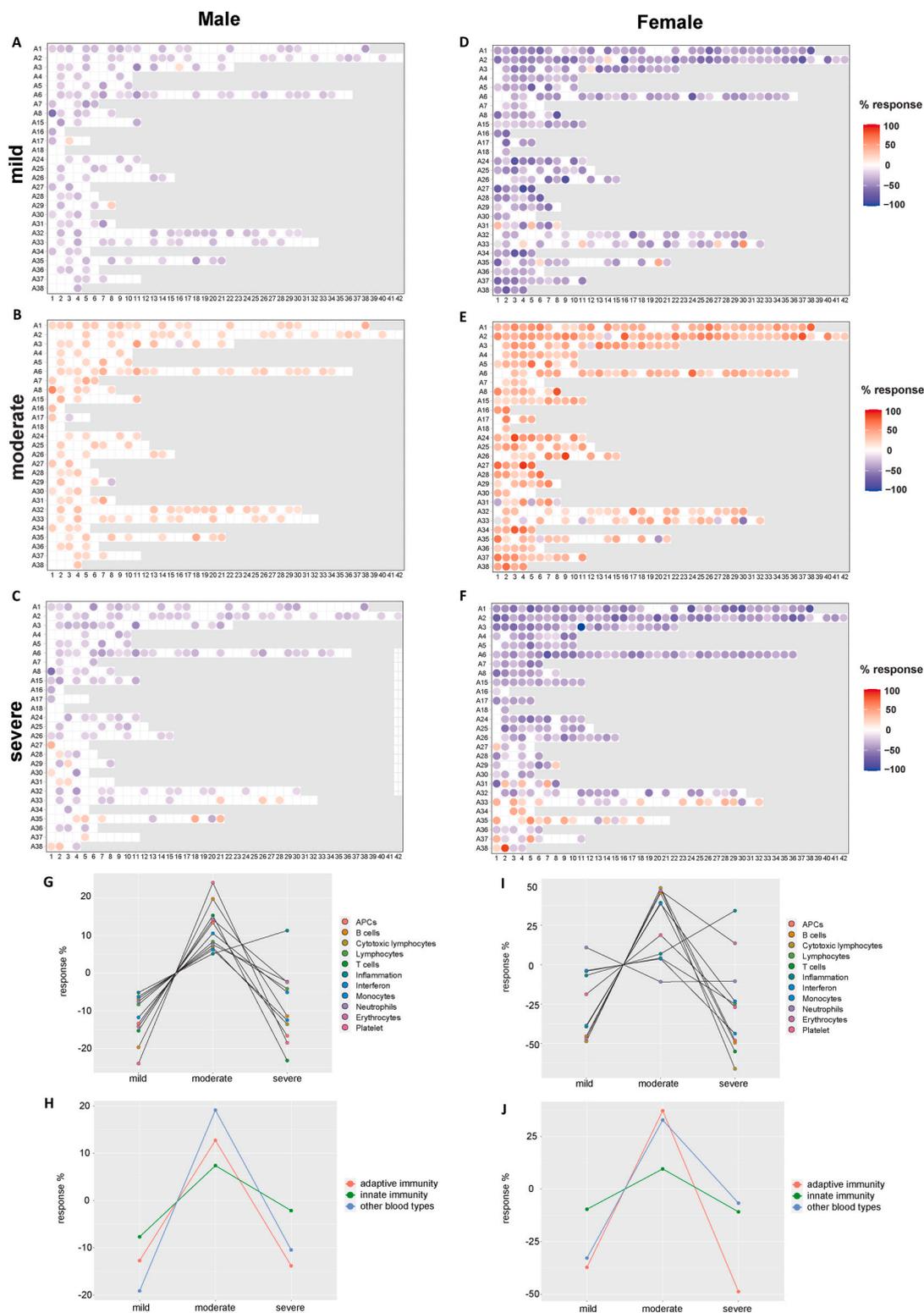
Fig. 2. Distinct transcriptional profiles in patients with mild, moderate, and severe COVID-19. Transcriptional Average analytical framework of 382 modules divided into 38 clusters that group genes with shared expression patterns and similar biologic functions of mild **(A)**, moderate **(B)**, severe **(C)** male patients. Transcriptional Average analytical framework of 382 modules divided into 38 clusters of mild **(D)**, moderate **(E)**, severe **(F)** female patients. Results (at the module level) are expressed as the percentage of increased or decreased genes. **(G)** Mean immune response modular transcriptional fingerprint for mild, moderate, and severe male patients, based on 11 different immune cells representing innate and adaptive immune response beside erythrocytes and platelets. **(H)** Mean immune response modular fingerprint for mild, moderate, and severe male patients. Modules are ordered based on their relation to the innate and adaptive immune response or other blood types. **(I)** Mean immune response modular transcriptional fingerprint for mild, moderate, and severe female patients **(J)** Mean immune response fingerprint for mild, moderate, and severe female patients. Modules are ordered based on their relation to the innate and adaptive immune response or other blood types. Results (at the module level) are expressed as the percentage of increased or decreased genes.

response above 15%, Fig. 2B, G) than mild and severe patients (Fig. 2A, C, G). In contrast, inflammation modules showed ascending over-expression, although the response was lower in severe male patients than severe female patients (Fig. 2G). On the other hand, female patients with moderate status demonstrated significant overexpression to modules related to the interferon, cytolytic lymphocyte, T & B cells, and erythrocytes (gene response above 25%, Fig. 2E and I) compared to mild and severe female patients (Fig. 2D, F, I). In contrast, inflammation modules showed ascending overexpression, especially in severe female cases than the males matched group (Fig. 2I).

Moderate female patients generally showed a higher adaptive immune response (Fig. 2J) than moderate male patients (Fig. 2H). However, this response declined assertively in severe female patients than in severe male patients. Meanwhile, innate immune response in both male and female patients showed almost the same pattern of ascending in moderate cases and descending in severe cases (Fig. 2H and J). However, this innate immune response was related to different modules over-expression in males due to inflammation and in females due to interferon.

3.3. Identification and validation of COVID-19 miRNA sponge interactions

We compared miRNA expression profiles between COVID-19 patients and healthy controls. We found 61 miRNAs differentially expressed between patients and controls. Furthermore, we identified target genes of these miRNAs by searching for databases. Using the miRHomology [14]. We identified miRNA sponge interactions based on these miRNAs and their target genes. We found two significant sponges of sharing miRNAs by each RNA-RNA pair (adjusted P value < 0.01).

Furthermore, we identified five modules from the miRNA sponge interaction (Table 2), GO and KEGG pathways are significantly associated with these modules. These pathways were mainly involved in metabolism regulation, extracellular matrix, MAPK signaling, Hippo signaling, Wnt signaling, TGFβ pathway, and immune response (Fig. 3A and B). Similarly, we identified 25 differentially expressed miRNAs between male and female patients and their target genes. The pathways associated with the target genes mainly involved regulating mRNA stability, metabolism dysregulation, Hippo signaling, and stem cell signaling that were upregulated in females than in male patients (Fig. 3C and D).

3.4. Identification of gene modules based on mRNA-lncRNA expression profiles in COVID-19 patients

We identified gene modules based on mRNA-lncRNA expression profiles in COVID-19 patients across two groups (males versus females). This analysis was carried out by the dynamic branch-cutting algorithm [19]. A total of 23 gene modules were identified, each indicated by a unique color label. Four modules (indicated in brown, gray, thistle, and pale violet red) were the most significant (Fig. 4A). GO analysis revealed that protein demethylation, macrophage activation involved in immune response, cellular response to radiation, and translational initiation module were significantly associated with these modules, respectively.

Table 2
Modules identified from the miRNA sponge interaction network.

Module	Gene set
1	ZBTB34, TMEM170B
2	TNRC6B, STXBP5
3	ARFGEF1, MBTD1
4	ONECUT2, GXYLT1, SZRD1, ZFHX3, AFF4, TRPV3, PLK2, GAB1, TNPO1, DCUN1D4, KDM7A, RPS6KA5, AKIRIN1, NRK, GPAM, CDS1, RO60, SOS1, KCNK2, GRIN2D, IPMK, EHF, PDIA5, ZHX1
5	RALGAPA2, SEMA6A, FBXW7

Notably, three modules were more enriched in the female than in the male group, including protein demethylation (R = −0.62, P = 0.01), macrophage activation involved in immune response (r = −0.60, p = 0.01), and cellular response to radiation (r = −0.53, p = 0.04), (Fig. 4A). In contrast, one module was more enriched in males (translational initiation module (r = 0.96, p = 3E-07, Fig. 4A). Furthermore, we showed robust intramodular connectivity of the top 100 hub genes and lncRNAs in the four modules with other genes and lncRNAs (Fig. 4B–E).

3.5. Metabolomic analysis of COVID-19 patients

The metabolomic analysis is the measurement of minute molecules named metabolites [20]. We performed a metabolomic analysis of COVID-19 patients by comparing COVID-19 patients and healthy controls, male and female patients, moderate with mild and severe. PCA of metabolites could discriminate between healthy controls and COVID-19 cases to some extent (Fig. 5A), while it could not separate male from female COVID-19 patients (Fig. 5B). However, PLS-DA slightly discriminated between healthy controls from moderate and severe cases but did not present significant differences between themselves (Fig. 5C). This discrimination capability was confirmed by analyzing the model accuracy, which revealed that healthy versus COVID-19 patients, mild versus moderate and severe, moderate versus mild and severe, and severe cases versus mild and moderate in the PLS-DA model could significantly discriminate between those groups (Fig. 5D, ANOVA, P value < 0.05). PLS-DA loadings plot showed that the most significant metabolites contributing to these separations were mainly from carbohydrates and lipids classes.

We found eight metabolite classes that were more highly enriched in COVID-19 cases than in healthy controls, including bioactive peptides, carbohydrates, lipids, nucleic acids, organic acids, peptides, phytochemical compounds, and vitamins & cofactors (Fig. 5E). Five metabolite classes had significantly higher enrichment levels in females than in male patients, including carbohydrates, lipids, organic acids, peptides, and phytochemical compounds (Fig. 5F). On the other hand, moderate and severe cases compared to mild cases presented mostly the same differential metabolites (Fig. 5G). Between mild, moderate, and severe cases, we found that mild and moderate cases showed higher levels of carbohydrates, lipids, and organic acids than severe cases. In comparison, moderate cases showed higher levels of organic compounds and nucleic acids compared to others.

Mapping of these metabolites to KEGG pathways identified several metabolic pathways differentially enriched between COVID-19 and healthy individuals (Fig. 5H), between male and female COVID-19 patients (Fig. 5I), and between mild versus moderate (Fig. 5J), and severe versus moderate (Fig. 5K). Several pathways related to lipid metabolism, immune system activation, and inflammation were included in these pathways. The pathways related to inflammation had significantly different enrichment levels between male and female patients, suggesting different immune responses between them. Moderate cases showed higher activation of various metabolic and immune response pathways than mild cases. In contrast, severe cases demonstrated higher levels in the pathways related to immunosuppression and metabolic dysregulation than moderate cases.

4. Discussion and conclusion

Since increasing immune responses are necessary for identifying and responding to different pathogens, it requires metabolic properties that might be used for other biological processes, such as growth and sex characteristics. Among males, several theories suggest that increased pathogen loads and reduced immune function are considered opposing side effects of positive selection for other features that increase reproductive success and survival [21].

Among humans, males and females differ in immune responses, proposing that some sex differences may be germline encoded. For
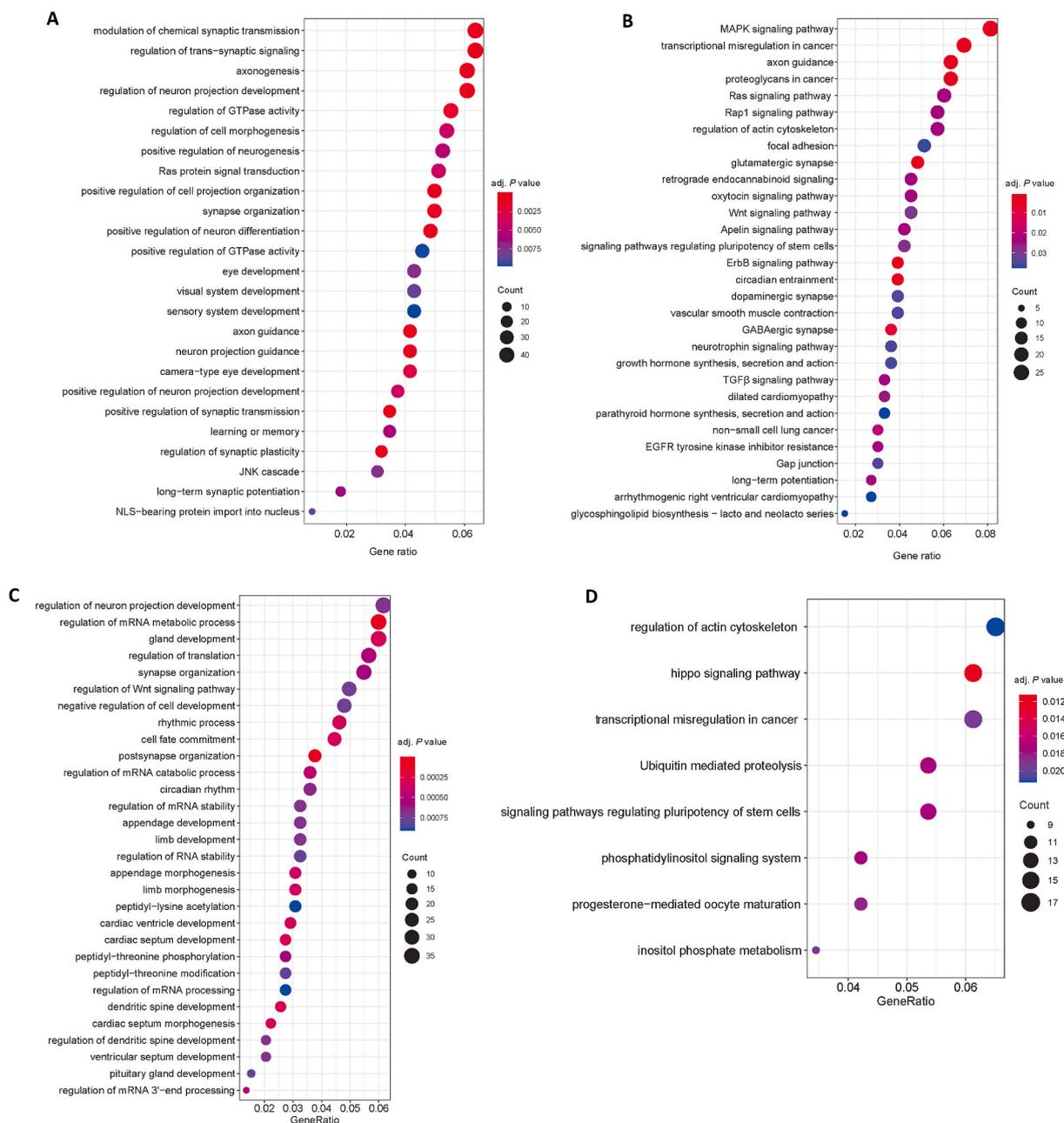
Fig. 3. Identification and validation of COVID-19 miRNA targeted genes pathways. **(A)** GO and **(B)** KEGG enrichment analysis of differentially miRNAs targeted genes in COVID-19 patients versus healthy. **(C)** GO and **(D)** KEGG enrichment analysis of differentially miRNAs targeted genes in COVID-19 female patients versus males. The pathways associated with the targeted genes mainly involved in regulating mRNA stability, metabolism dysregulation, Hippo signaling, and stem cell signaling were upregulated in females than in male patients.

instance, contact of PBMCs to *TLR7* gene ligands in vitro cell lines causes higher production of interferon-α in cells from women than from men [22]. Furthermore, The *TLR7* gene encoded on the X chromosome may leak X deactivation, resulting in higher expression levels in females than males [23].

Our results revealed significant differences in immune responses during COVID-19 infection in male and female patients. In general, males and females with COVID-19 demonstrated overexpression of the innate immune response, especially in inflammation, monocytes, interferons, and neutrophiles but with different expression levels, while adaptive immune response considered lower in males than females. Moderate male patients showed slight overexpression in B cells and platelets modules than mild and severe patients. In contrast, inflammation modules showed ascending overexpression, especially in

moderate male patients than moderate female patients. Meanwhile, female patients with moderate status demonstrated significant overexpression in interferon, cytolytic lymphocyte, T & B cells, and erythrocytes compared to mild and severe female patients. However, inflammation modules showed higher ascending overexpression in females than males, especially in severe female cases. These immune response differences between males and females indicated that female patients might benefit from therapies that reduce innate immune activation during critical stages of COVID-19. Since the inflammation response is higher in moderate males than female patients, moderate male patients could benefit more from immunosuppressive agents than females but not in critical stages.

We also identified and validated miRNA sponge interactions in COVID-19 patients. In COVID-19 patients, we found pathways involved
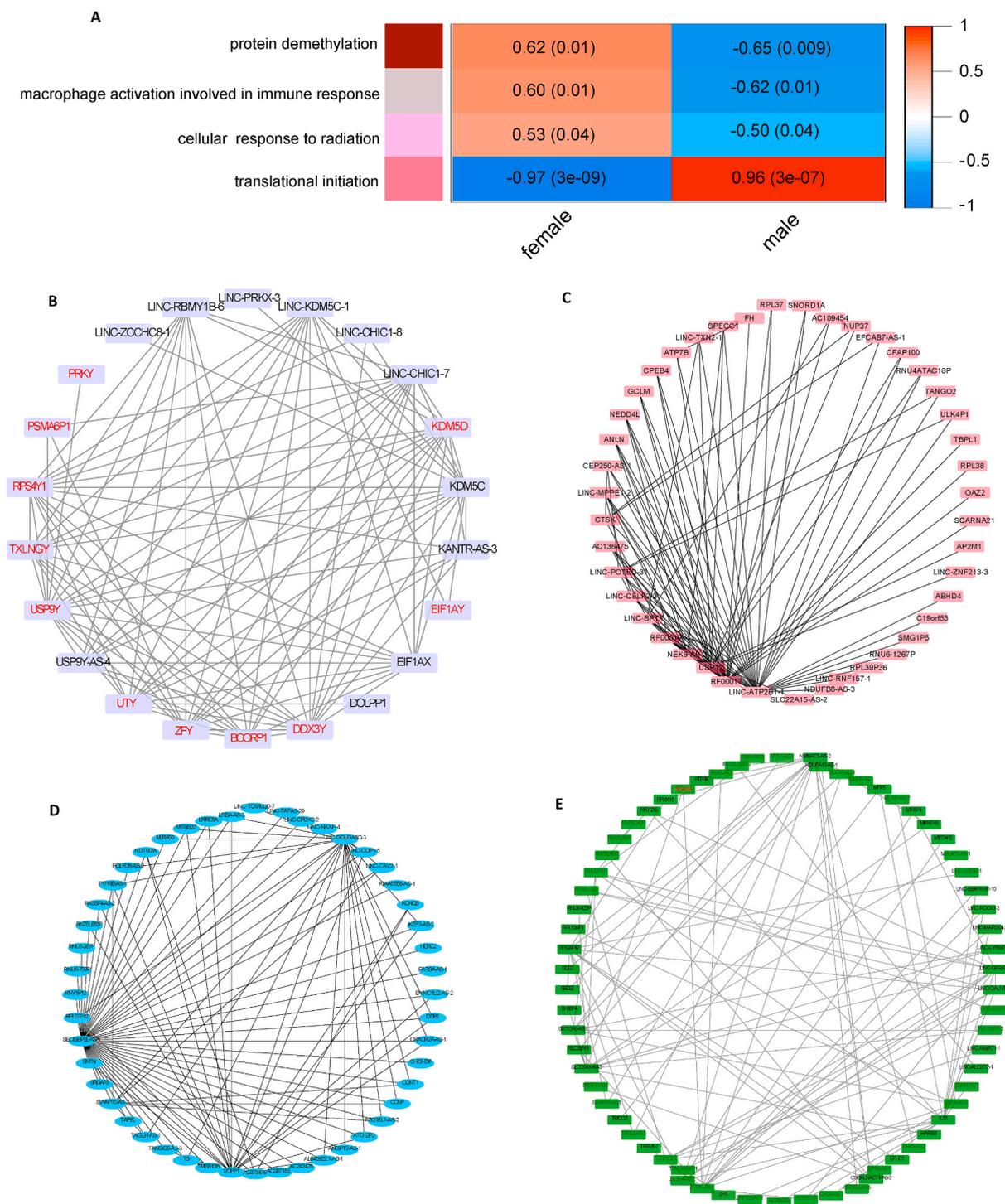
Fig. 4. WGCNA analysis of mRNA-lncRNA expression data in COVID-19 patients. **(A)** WGCNA analysis of mRNA-lncRNA expression data using sexes as analysis traits. Pearson's correlation, *P* value < 0.05. Four modules (indicated in brown, gray, thistle, and pale violet red) were the most significant. **(B)** Module network representation of top 100 hub genes in the translational initiation module, **(C)** Protein demethylation module, **(D)** Cellular response to radiation module, and **(E)** Macrophage activation involved in immune response module using Cytoscape software.

in regulating metabolism, extracellular matrix, MAPK signaling pathway, Hippo signaling, Wnt signaling pathway, TGFβ pathway, and immune response were up-regulated. In females, we found that pathways involved in regulating mRNA stability, metabolism dysregulation, Hippo signaling, and stem cell signaling pathways were more up-regulated than males suggested by that different immune response based on sexes and disease severity.

Since the MAPK pathway plays a vital role in releasing pro-inflammatory cytokines (e.g., *IL6*) associated with myocardial dysfunction and acute lung injury, targeted inhibition of this pathway could attenuate SARS-CoV-2 infection [24]. In addition, therapeutic inhibition of the Hippo pathway component may provide a potential value for antiviral treatment [25]. Furthermore, it found that in COVID-19 patients, TGFβ stimulates the WNT/β-catenin pathway, leading to an increased risk of pulmonary diseases. The inhibition of this pathway could result in attenuation of the SARS-CoV-2 infection [26].

In the metabolomic analysis of COVID-19 patients, we found that COVID-19 patients presented higher bioactive peptides, carbohydrates, lipids, nucleic and organic acids, peptides, phytochemical compounds, and vitamins & cofactors levels than healthy controls. We also identified higher levels of five metabolites carbohydrates, lipids, organic acids, peptides, and phytochemical compounds in females than males.

Regarding disease severity status, we found that mild and moderate cases showed higher levels in carbohydrates, lipids, and organic acids than severe cases, and moderate cases showed higher levels in organic compounds and nucleic acids than others. These results indicate the potential pathological mechanisms of COVID-19 progression based on sex and disease severity.
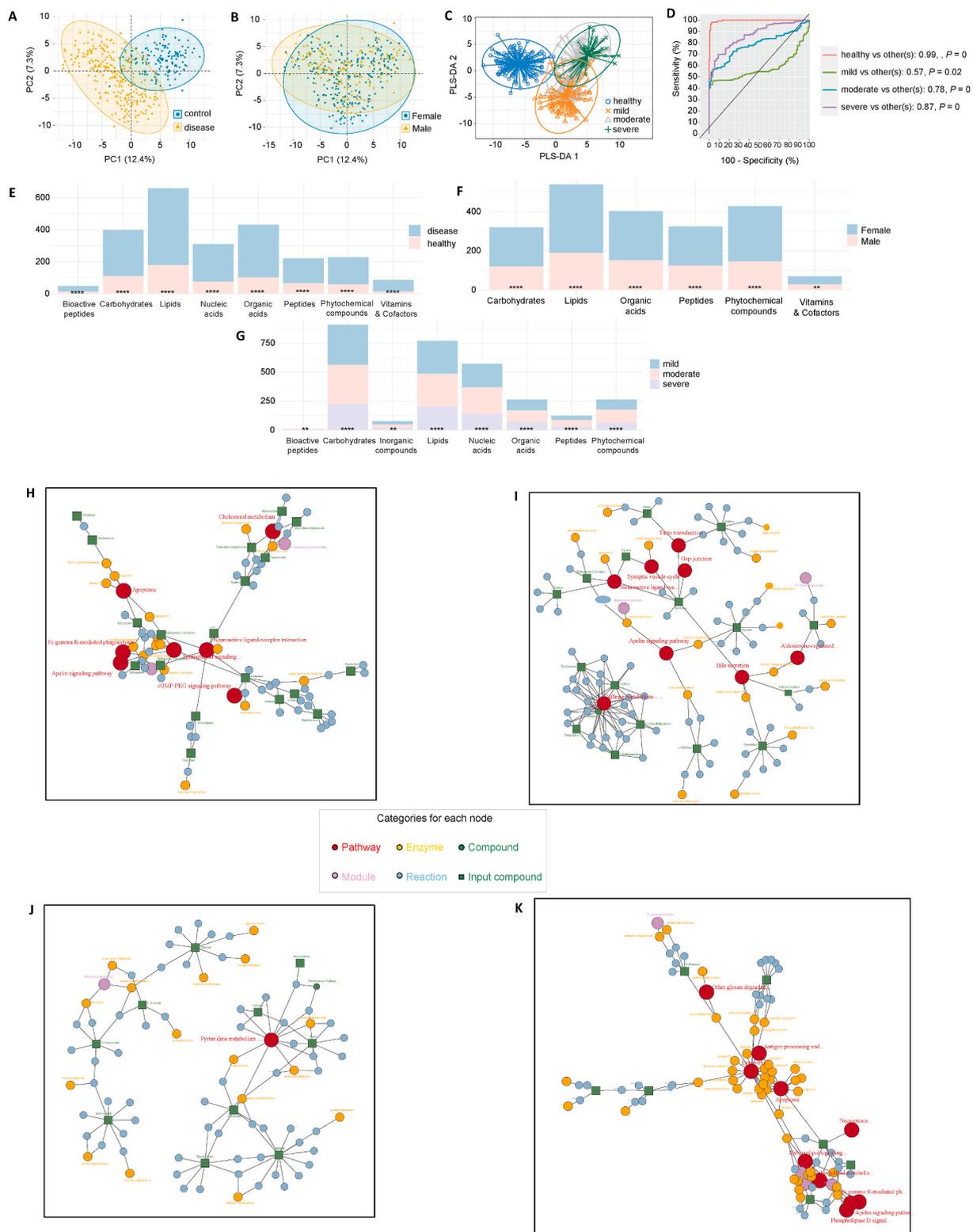
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Fig. 5. Metabolomic analysis of COVID-19 patients. **(A)** Principle component analysis (PCA) of COVID-19 patients versus healthy, and **(B)** Males versus females COVID-19 patients. PCA of metabolites could discriminate between healthy controls and COVID-19 cases, while it could not separate male from female COVID-19 patients. **(C)** Partial Least Squares discriminant analysis model (PLS-DA) of healthy controls versus mild, moderate, and severe COVID-19 patients. PLS-DA slightly discriminated between healthy controls from moderate and severe cases but did not present significant differences between themselves. **(D)** An accuracy model of the Partial Least Squares discriminant model of healthy, mild, moderate, and severe. (ANOVA, P value < 0.05). **(E)** Differentially metabolites between COVID-19 patients and healthy controls. Eight metabolite classes were highly enriched in COVID-19 cases than in healthy controls, including bioactive peptides, carbohydrates, lipids, nucleic acids, organic acids, peptides, phytochemical compounds, and vitamins & cofactors. **(F)** Differentially metabolites between male and female patients. Five metabolite classes had significantly higher enrichment levels in females than in male patients, including carbohydrates, lipids, organic acids, peptides, and phytochemical compounds. **(G)** Differentially metabolites between mild, moderate, and severe COVID-19 patients. Mild and moderate cases showed higher levels of carbohydrates, lipids, and organic acids than severe cases. Moderate cases showed higher levels of organic compounds and nucleic acids compared to others. **(H)** KEGG network pathways of altered metabolites between COVID-19 versus healthy, **(I)** Males and females COVID-19 patients, **(J)** Moderate versus mild, and **(K)** Severe versus moderate. The pathways related to inflammation had significantly different enrichment levels between male and female patients. Moderate cases showed higher activation of various metabolic and immune response pathways than mild cases. Severe cases showed higher levels in the pathways related to immunosuppression and metabolic dysregulation than moderate cases. Red nodes represent the top significant pathways.

This study suggested a potential immunological foundation of the diverse pathological mechanisms of disease evolution between sexes. These analyses also provide a possible basis for taking sex, and disease severity status dependent approaches to prognosis, care, and prevention, for COVID-19 patients.

Although our study provides a strong base for further research into how COVID-19 disease may differ between male and female patients, especially from a pathophysiological perspective, it is essential to note that some limitations to the analyses presented in this study. This study was conducted regardless of the patients underlying disease history; due to limited detailed data, we cannot rule out residual confounding due to underlying risk. Furthermore, this study's small sample size could affect the reliability of the results because it leads to a higher variability, leading to bias. However, since the COVID-19 outbreak is still emerging, and the availability of COVID-19 public data is limited, this study still provides a potential immunological foundation emphasizing the difference in response to SARS-CoV-2 infection between sexes and different disease stages, which is considered essential in disease treatment and prevention.

In conclusion, this study suggested that certain treatments that increase the immune responses to SARS-CoV-2 infection might be necessary for male and female patients at certain disease stages. Female patients might benefit from therapies that reduce innate immune activation during critical disease stages. Since the inflammation response is higher in severe female patients than severe male patients, female patients could benefit more from immunosuppressive agents such as corticosteroids than males, especially in critical cases. In contrast, moderate male cases benefit more from immunosuppressive agents than the female matched group. The immune landscape of COVID-19 patients was noticeably different between the sexes, and these differences may highlight the disease vulnerability in males.

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CRediT authorship contribution statement

Tianfang Zhang: Software, Validation, Writing – original draft, Formal analysis, Investigation, Data curation, Visualization, Writing – review & editing. **Zeinab Abdelrahman:** Software, Validation, Writing – original draft, Formal analysis, Investigation, Data curation, Visualization, Writing – review & editing. **Qian Liu:** Software, Formal analysis. **Xiaosheng Wang:** Conceptualization, Methodology, Resources, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Zuobing Chen:** Conceptualization, Methodology, Resources, Investigation, Supervision, Project administration, Funding acquisition.

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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