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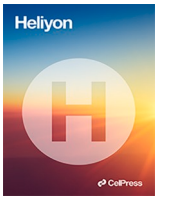

Research article

Stimulation of PSTPIP1 to trigger proinflammatory responses in asymptomatic SARS-CoV-2 infections

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ABSTRACT

Background: A hyperinflammatory response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection gravely worsens the clinical progression of coronavirus disease 2019 (COVID-19). Although the undesirable effects of inflammasome activation have been correlated to the severity of COVID-19, the mechanisms of this process in the asymptomatic infection and disease progression have not yet been clearly elucidated.

Methods: We performed strand-specific RNA sequencing in 39 peripheral blood mononuclear cell (PBMC) samples from asymptomatic individuals (n = 10) , symptomatic patients (n = 16) and healthy donors (n = 13) .

Results: Dysregulation of pyrin inflammasomes along with the proline-serine-threonine phosphatase-interacting protein 1 (*PSTPIP1*) gene was identified in SARS-COV-2 infection. Notably, the *PSTPIP1* expression level showed a significant negative correlation with an adjacent long-noncoding RNA (lncRNA) *RP11-797A18.6* in the asymptomatic individuals compared with the healthy controls. In addition, a decline in the nuclear factor kappa B subunit 1 (*NFKB1*) gene expression was observed in asymptomatic infection, followed by a rise in the mild and moderate disease stages, suggesting that altered *NFKB1* expression and associated proinflammatory signals may trigger a disease progression.

Conclusions: Overall, our results indicate that PSTPIP1-dependent pyrin inflammasomes-mediated pyroptosis and NF-κB activation might be potential preventive targets for COVID-19 disease development and progression.

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1. Introduction Background

The severity of coronavirus disease 2019 (COVID-19) ranges widely, from asymptomatic to lethal. The majority of research on gene expression profiling to date has focused on the identification of risk factors for severe diseases in COVID-19 patients [1–4]. Numerous asymptomatic COVID-19 person-to-person transmission cases have been reported [5–11]. However, little is known regarding the molecular characteristics related to asymptomatic COVID-19 individuals and the risk factors associated with the progression from asymptomatic to mild and moderate disease stages. Investigations of asymptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections might lead to the identification of key regulators protecting the infected individuals from more severe symptoms.

A rising amount of clinical evidence indicates that an excessive immune response known as a cytokine storm is associated with COVID-19 severity and is perhaps one of the critical hallmarks of mortality from COVID-19 [12,13]. However, the immunological mechanism for asymptomatic individuals remains incompletely understood. Previous studies indicated that inadequate immune activation from asymptomatic infections may have delayed viral clearance [14,15]. On the contrary, some reports also provided evidence that asymptomatic SARS-CoV-2 infected individuals do not exhibit weakened antiviral immunity, but develop effective virus-specific immune responses instead [16].

Innate immunity, as a front-line guard of alerting the host defensive mechanism, sensing of SARS-CoV-2 by a variety of host pattern recognition receptors (PRRs) that include membrane receptors such as the Toll-like receptors (TLRs), and cytosolic receptors such as the NOD-like receptors (NLRs), which trigger the production of proinflammatory cytokines such as interferons (IFNs), and inflammatory cell death such as pyroptosis [17]. Pyroptosis is characterized by the loss of plasma membrane integrity, which results in lytic cell death and prevents microbial spread, and is caused by the activation of inflammasome sensors. Inflammasomes are cytoplasmic multiprotein complexes that are assembled by a sensor protein, in some situations, an adapter protein, and inflammatory caspases. Inflammasome complexes are classified by their protein domain structures into the NLR family such as (NLR family pyrin domain containing) NLRP subfamily, and non-NLR inflammasomes such as pyrin, absent in melanoma 2 (AIM2) and interferon gamma-inducible protein 16 (IFI16) inflammasomes [18,19]. Recently, there have been numerous reports of the NLRP3 inflammasome as a critical mediator in the activation of inflammatory macrophages, which releases mature forms of the pro-inflammatory cytokines interleukin-1 beta (IL-1β) and IL-18 and triggers pyroptosis through the activation of caspase-1 (CASP1) in severe COVID-19 [20–22]. In general, early innate immune-mediated inflammatory responses play a critical role in protecting hosts from viral infections, while late inflammatory responses can cause tissue damage and organ dysfunction if not controlled [17]. Besides, many models have been proposed to explain how immune cell and molecule dynamics affect SARS-CoV-2 infiltration and disease progression [23–25].

Apart from proteins, long-noncoding RNAs (lncRNAs) are now known to regulate immune responses through a variety of mechanisms to eradicate viral infections [26,27]. For instance, metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) and nuclear paraspeckle assembly transcript 1 (*NEAT1*) have been reported dysregulated in the lung and peripheral blood mononuclear cell (PBMC) samples of severe COVID-19 patients and SARS-CoV-2 infected cells could be associated with innate immune responses and inflammation development [28–31]. However, the functions and roles of lncRNAs in the asymptomatic SARS-CoV-2 infection remain uncharacterized. In this study, to explore the immunological features associated with the asymptomatic and mild to moderate SARS-CoV-2 infection, we performed strand-specific RNA-seq using PBMCs from 26 SARS-CoV-2 infected individuals and 13 healthy donors. Our results for the first time reveal that pyroptosis is mediated by proline-serine-threonine phosphatase-interacting protein 1 (PSTPIP1)-dependent pyrin inflammasomes in asymptomatic COVID-19. In addition, lncRNA *RP11-797A18.6* was found negatively correlated with the expression of *PSTPIP1*. Functional prediction revealed that *RP11-797A18.*6 might regulate *PSTPIP1* through a direct binding on its genomic sequences, indicating *RP11-797A18.*6 might be considered as a regulator controlling PSTPIP1 transcription and pyroptosis.

2. Methods

2.1. Patients

Twenty-six individuals diagnosed with SARS-CoV-2 infection and 13 healthy donors were enrolled from the three hospitals including Guangzhou Eighth People's Hospital, Shunde Hospital of Guangzhou University of Chinese Medicine, and Fourth People's Hospital of Foshan from April to May in 2020. All participants or their surrogates provided written informed consent.

According to the Guidelines for the Diagnosis and Treatment of COVID-19 (7th edition) of National Health Commission of China, patients who had positive SARS-CoV-2 RT-PCR tests or specific blood IgM antibodies but without clinically recognizable symptoms, were diagnosed with asymptomatic COVID-19, and patients with mild cases displayed mild clinical symptoms with no radiological findings of pneumonia. Moderate cases are characterized by fever (temperature above 37.3 °C) and respiratory symptoms with radiological findings of pneumonia. In our study, ten cases were diagnosed with asymptomatic COVID-19, and 5 cases were defined as mild symptoms, 11 cases were defined as moderate symptoms. The demographics and clinical features of all the groups are summarized in Additional file 1: Table S1. Among the 26 cases, ten asymptomatic individuals at a median age of 36.5, and 16 symptomatic patients at a median age of 33. No significant differences in age or gender were found between the healthy control group and any of the infection groups. The PBMC samples were collected from all the infected individuals and healthy donors within 4 days following the exact diagnosis.

2.2. The strand-specific RNA sequencing and data processing

PBMCs were isolated by density gradient centrifugation, followed by total RNA isolation and purification according to the manufacturer's procedure. The quality and yield of the isolated RNA were evaluated using an Agilent 2100 Bioanalyzer. After ribosomal RNA removal, strand-specific RNA sequencing libraries were generated using VAHTS® Universal V6 RNA-seq Library Prep Kit for Illumina (Vazyme, cat.NR604-02, Nanjing, China) and sequenced in 150 bp paired-end mode on Illumina NovaSeq 6000 at Guangzhou Huayin Medical Laboratory Center Ltd. (Guangzhou, China).

The summary of sample quality control and RNA-seq outcomes were presented in Additional file 2: Table S2. Cutadapt (v1.16) and SOAPnuke were used to trim the adaptor reads, repeat reads and low-quality reads [32,33], followed by rRNA removal using bowtie 2 (v2.2.9) with default settings [34]. Trimmed reads were mapped to human genome annotation hg19 using Hisat (v2-2.0.5) [35]. The expression levels of transcripts were calculated using StringTie-eB (v1.3.3b) and normalized to Fragments Per Kilobase Per Million reads (FPKM) [36], and the low-abundance transcripts with FPKM value < 0.5 were discarded. LncRNA identification was based on the criteria that more than two exons, bases greater than 200 bp, and locations do not overlap with mRNAs. The known lncRNAs were annotated using the Cuffcompare (v2.1.1). Further, CPC (cpc-0.9-r2) and Pfam-scan (v1.3) were used to predict the unknown transcripts [37]; the transcripts identified by one tool as having coding potential were classified as the transcripts of uncertain coding potential (TUCPs), while those identified by both tools as having no coding potential were predicted as novel lncRNAs. The Ballgown package in R was used to filter the differentially expressed transcripts with a fold change of >1.5 ($|\log 2$ fold change$| > 0.5849625$) and a p-value of <0.05 [38].

2.3. Construction of Co-expression modules and networks analysis

For constructing co-expression networks, we have used the weighted gene co-expression network analysis (WGCNA) method [39], which integrates modules with similar eigengene expression profiles. The option for the soft threshold parameter was set at 14 to achieve a scale-free topology fit threshold >0.85. Then, we obtained 43 modules of genes, followed by module merging with a dissimilarity threshold of 0.25.

A heatmap was used to find modules of interest that were significantly related to clinical traits, Modules correlated with Asy traits with $|\text{cor}| > 0.35$ and p-value <0.05 were considered as the hub modules of As. After the identification of the hub modules, transcripts derived from the collected hub modules with gene significance (GS) > 0.2 and module membership (MM) > 0.8 were marked as hub transcripts including hub protein coding genes (PCGs) and hub lncRNAs. The hub transcripts that related to the five key pathways of

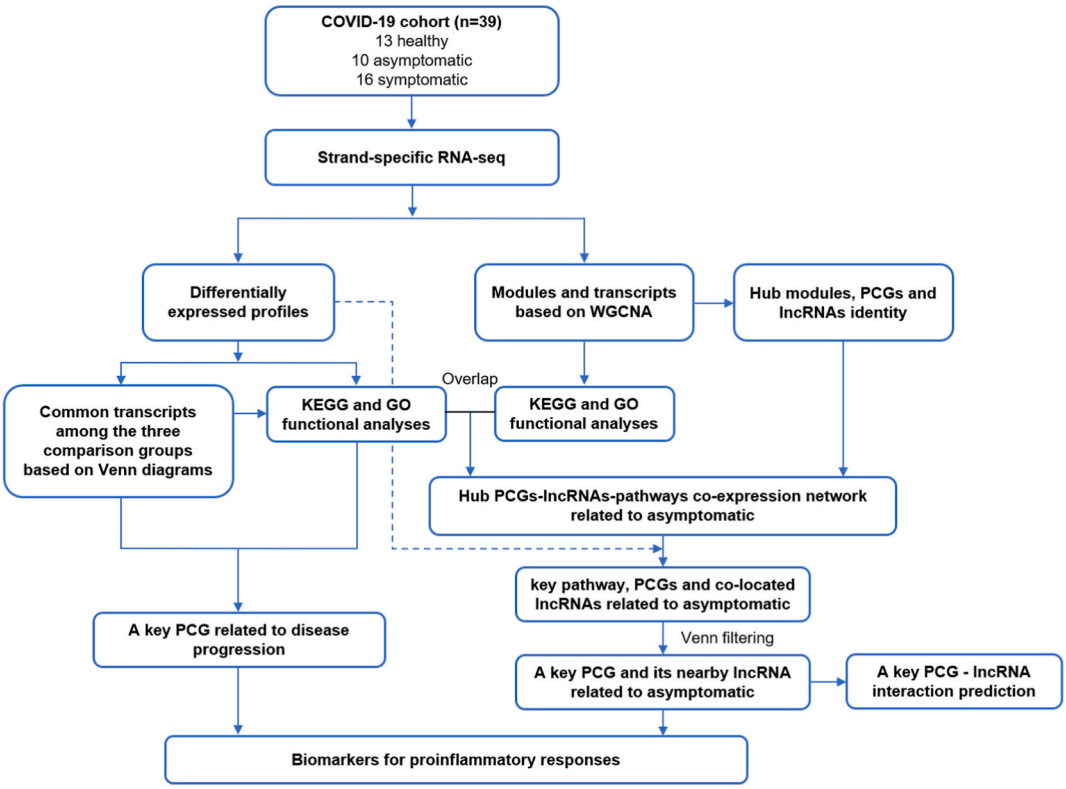

Fig. 1. Research workflow. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCG, protein coding genes; WGCNA, weighted gene co-expression network analysis.

each six hub modules were used to visualize the PCGs-lncRNAs-pathways networks using the Cytoscape (v3.9.1).

2.4. Functional enrichment of protein coding genes

Gene functional enrichment analysis was performed using clusterProfiler R package to map DE PCGs to pathways and terms against the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) database, respectively [40]. A cut-off of p-value <0.05 was applied to select the significant KEGG pathways and GO terms, of which top enriched pathways and terms were visualized using the ggplot 2 R package.

2.5. Prediction of the interplay between protein coding genes and lncRNAs

The co-located target genes of *cis*-acting lncRNAs were predicted by searching for genomic regions at 100,000 bp upstream and downstream of the lncRNAs. Silencers with the nearest PCGs were predicted using the SilencerDB tool. The lncRNA-DNA interaction prediction of lncRNA *RP11-797A18.6* and its co-located gene *PSTPIP1* was based on RNAplex.

2.6. Statistical analysis

Statistical analysis was performed using R version 4.1.2. Demographic data on age and gender were compared using Fisher's exact test. Pearson's correlation coefficient was used to calculate the relationship between the expression of lncRNAs and that of PCGs. A p-value <0.05 was considered significant.

3. Results

3.1. Differential transcription profiles of healthy controls, asymptomatic and symptomatic COVID-19 individuals

To define the transcriptome responses to the severity of COVID-19, we conducted strand-specific RNA-seq on PBMCs collected from asymptomatic and symptomatic individuals with SARS-CoV-2 infection and healthy donors. An overview of the study design,

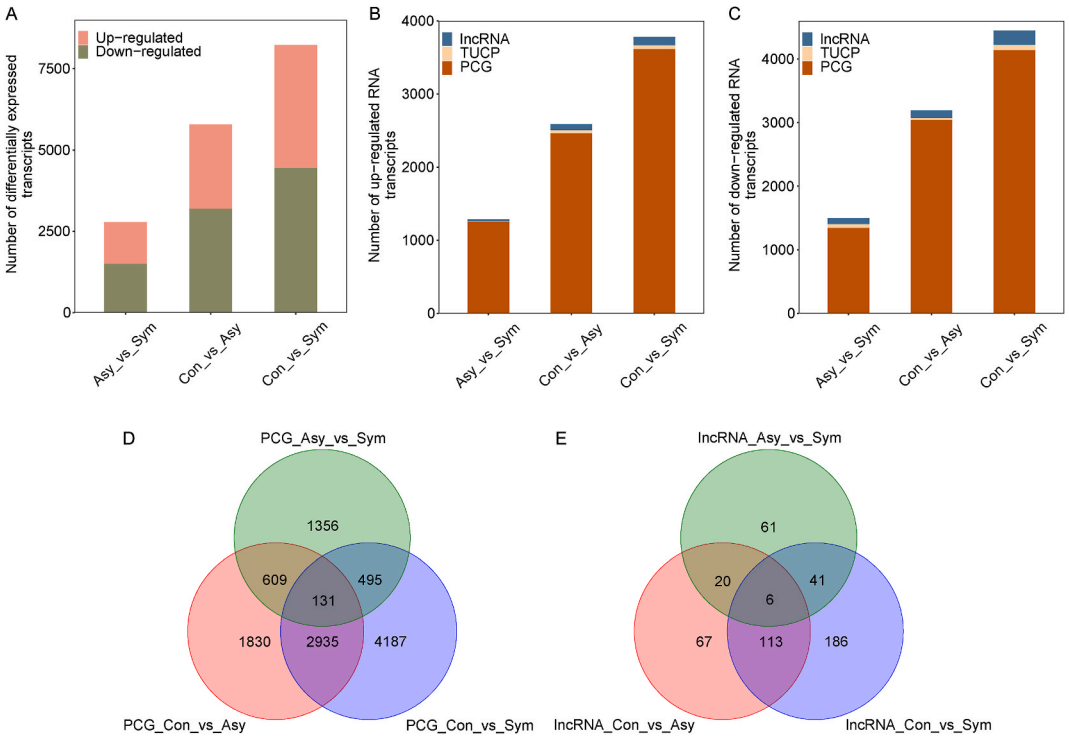

Fig. 2. Differential expression patterns of protein coding genes and lncRNAs in COVID-19 infections. **A**, number of differentially expressed transcripts observed in Asy and Sym individuals in comparison with the Con group, and that in Sym comparing to Asy individuals. Number of the different types of up-regulated (**B**) and down-regulated (**C**) transcripts in Asy and Sym individuals in comparison with the Con group, and that in Sym comparing to Asy individuals. Venn diagrams considering DE PCGs (**D**) and lncRNAs (**E**) comparing the groups "Con vs Asy", "Con vs Sym" and "Asy vs Sym". Asy, asymptomatic; Con, healthy controls; DE PCGs, differentially expressed protein coding genes; Sym, symptomatic; TUCPs, transcripts of uncertain coding potential.

consisting of the sample distribution and transcriptomic analysis, is shown in Fig. 1. The differentially expressed transcripts among different groups were identified based on the Ballgown R package under the conditions of foldchange >1.5 and *p*-value <0.05. The landscape of transcriptional dysregulation in individuals with different disease severity associated with infections by SARS-CoV-2 was presented in Fig. 2. Globally, severer disease resulted in an increase in the number of both up-regulated and down-regulated transcripts (Fig. 2A), displaying a preferential up-regulation pattern where the PCGs were more abundant, according to an analysis of the distinct types of transcripts (Fig. 2B–C). Additionally, both the asymptomatic and symptomatic groups exhibited a larger fraction of down-regulated transcripts represented by lncRNAs in comparison with the healthy controls (Additional file 3: Fig. S1). A Venn diagram of the transcripts of PCGs and lncRNA detected for each of the three comparison groups was created (Fig. 2D–E). The results showed that common differentially expressed transcripts including 131 PCGs and six lncRNAs were defined to be associated with asymptomatic SARS-CoV-2 infections at high risk for progression to symptomatic infections.

We performed KEGG pathway enrichment analysis of the differentially expressed protein coding genes (DE PCGs) in the three comparison groups to explore the specific changes in different disease severity after SARS-CoV-2 infections (Fig. 3A–C). The most represented enrichments that DE PCGs involved in among the top 20 pathways according to the *p*-value influenced by the asymptomatic and symptomatic infections were associated with pathway clusters such as Folding, sorting and degradation, Development and regeneration, and Immune system. In particular, pathways of the Immune system including the C-type lectin receptor signaling pathway and NOD-like receptor signaling pathway were more robust in both the asymptomatic and symptomatic infections.

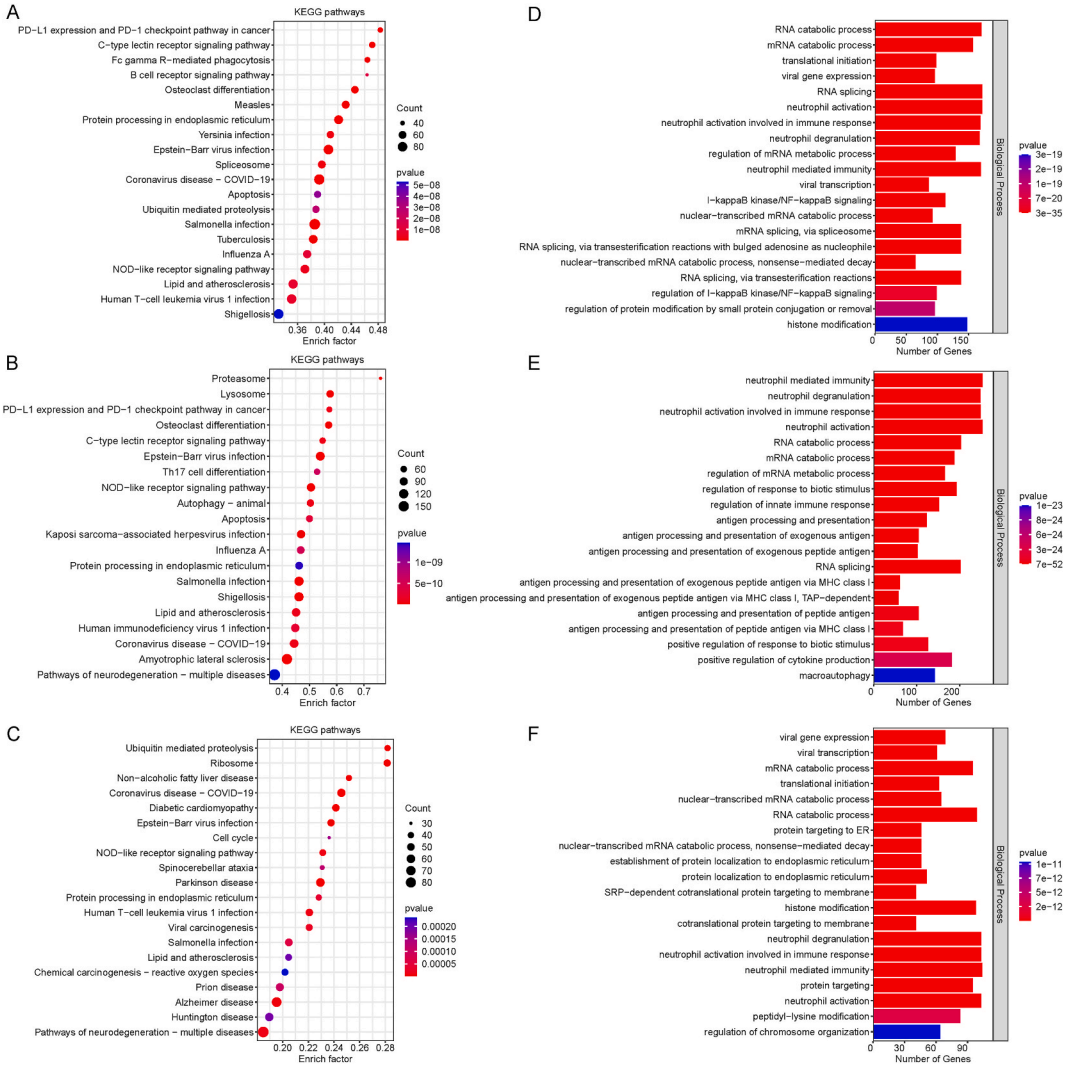

Fig. 3. KEGG and GO enrichment analysis of differentially expressed protein coding genes (DE PCGs) in COVID-19 infections. **A-C**, the top 20 significantly enriched KEGG pathways of DE PCGs in the "Con vs Asy", "Con vs Sym" and "Asy vs Sym" groups, respectively. **D-F**, the top 20 significantly enriched GO terms of DE PCGs in the "Con vs Asy", "Con vs Sym" and "Asy vs Sym" groups, respectively. Asy, asymptomatic; Con, healthy controls; DE PCGs, differentially expressed protein coding genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; Sym, symptomatic.

Interestingly, the NOD-like receptor signaling pathway is the only pathway for the Immune system among the top 20 enrichments in the symptomatic compared with the asymptomatic COVID-19 individuals (Fig. 3C). Furthermore, GO biological processes enrichment analysis of the DE PCGs in the three comparison groups was also conducted. We found that both the asymptomatic and symptomatic groups shared the same terms of biological processes when compared to the healthy controls, such as neutrophil activation, neutrophil degranulation, and neutrophil mediated immunity (Fig. 3D–F). Besides, biological processes of viral gene expression/transcription and I-kappaB kinase/NF-kappaB signaling were indeed changed in the asymptomatic infections in comparison to the healthy controls. Instead, when we compared the asymptomatic and symptomatic infections, we found that enrichment in terms of translational initiation, protein targeting to endoplasmic reticulum, and SRP-dependent cotranslational protein targeting to membrane were more robust (Fig. 3F). Overall, these functional analysis results above suggested that immune dysregulation is prevalent not only in symptomatic COVID-19 patients but also in asymptomatic individuals.

3.2. *Weighted co-expression network and gene function associated with asymptomatic SARS-CoV-2 infections*

In addition, to obtain the asymptomatic associated characteristics depending on multi-methods, we further used the WGCNA package to identify the patterns of gene enrichment and connectivity. The optimal soft threshold parameter was set as 14 due to it met an approximate scale-free topology. A cluster dendrogram depicts the original clustered modules that each represented a collection of eigengenes, as well as the 43 merged modules, which are detailed in the Additional file 4: Fig. S2. The correlation coefficients between the modules and the traits under the degree of COVID-19 severity are shown in the form of a heatmap, in which red denoted a positive relationship and green denoted a negative one (Fig. 4A). Six modules including MEpaleturquoise, MEpalevioletred3, MEdarkslateblue, MEblack, MEwhite, and MEsalmon2 were selected as hub modules correlated with the asymptomatic trait following the criteria: $|cor| > 0.35$ and p-value < 0.05. The MEblack module was composed of more highly correlated mRNAs and lncRNAs with the asymptomatic trait (Fig. 4B). Then, we extracted the transcripts from the collected six hub modules and performed KEGG pathways enrichment

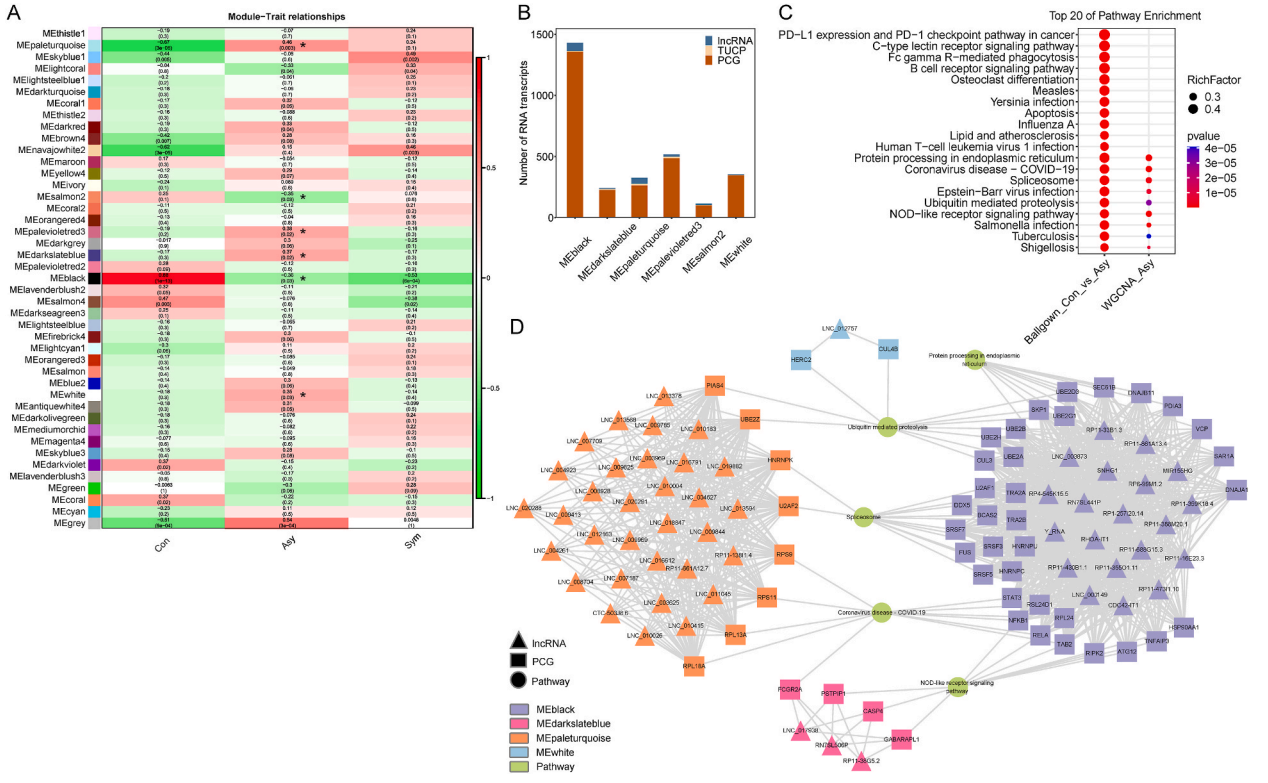

Fig. 4. Weighted specific gene-modules, co-expression network, and gene function associated with asymptomatic SARS-CoV-2 infections. **A**, heatmap of the correlation between module eigengenes and traits including Con, Asy, and Sym; Modules significantly associated with the Asy traits with identified with $|cor| > 0.35$ and p-value < 0.05, and are indicated by asterisks. **B**, number of the different types of transcripts observed in six hub modules. **C**, As-associated enrichment in the top 20 significant KEGG pathways identified by the Ballgown and WGCNA methods. **D**, a PCGs-lncRNAs-pathway network was constructed with 56 hub lncRNAs and 48 hub PCGs that were involved in five KEGG pathways; triangle nodes represent hub lncRNAs, square nodes represent hub PCGs and circle nodes represent pathways; the purple, pink, orange, blue and green nodes represent MEblack, MEdarkslateblue, MEpaleturquoise and MEwhite modules, and all pathways, respectively. Asy, asymptomatic; Con, healthy controls; PCGs, protein coding genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; Sym, symptomatic; TUCPs, transcripts of uncertain coding potential; WGCNA, weighted gene co-expression network analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

analysis. To investigate the key pathways associated with asymptomatic infections, the KEGG pathway enrichment patterns of Ballgown and WGCNA methods screened asymptomatic-related gene sets were then merged in a combined bubble plot (Fig. 4C). Both methods revealed a relationship between asymptomatic and enrichment in pathways of Coronavirus disease-COVID-19, Protein processing in endoplasmic reticulum, Spliceosome, NOD-like receptor signaling pathway, and Ubiquitin mediated proteolysis, excluding other disease-related pathways.

Subsequently, we identified 56 hub lncRNAs and 48 hub PCGs that were involved in these five KEGG pathways with the criteria of gene significance (GS) > 0.2 and module membership (MM) > 0.8. A co-expression network was used to assess the presence of hub PCGs-hub lncRNAs-pathways relationships in the six hub modules, of which MEpalevioletred3 and MEsalmon2 were filtered out since the hub PCGs and hub lncRNAs belong to them are not related to any of the key pathways (Fig. 4D). We found that the several hub PCGs, for instance, nuclear factor kappa B subunit 1 (*NFKB1*), NF-κB subunit *RELA* and TGF-beta-activated kinase 1 binding protein 2 (*TAB2*) were associated with both the Coronavirus disease-COVID-19 and NOD-like receptor signaling pathway, which indicated that NOD-like receptor signaling was strongly correlated with COVID-19 disease. In addition, three hub lncRNAs *RP11-38G5.2*, *RN7SL506P*, and *LNC_017938* were all closely related to four hub PCGs *CASP4*, Fc gamma receptor IIa (*FCGR2A*), GABA type A receptor associated protein like 1 (*GABARAPL1*) and *PSTPIP1* that involved in the NOD-like receptor signaling pathway, suggesting that these lncRNAs together with PCGs may play a contributing role in the proinflammatory responses to COVID-19 asymptomatic infections.

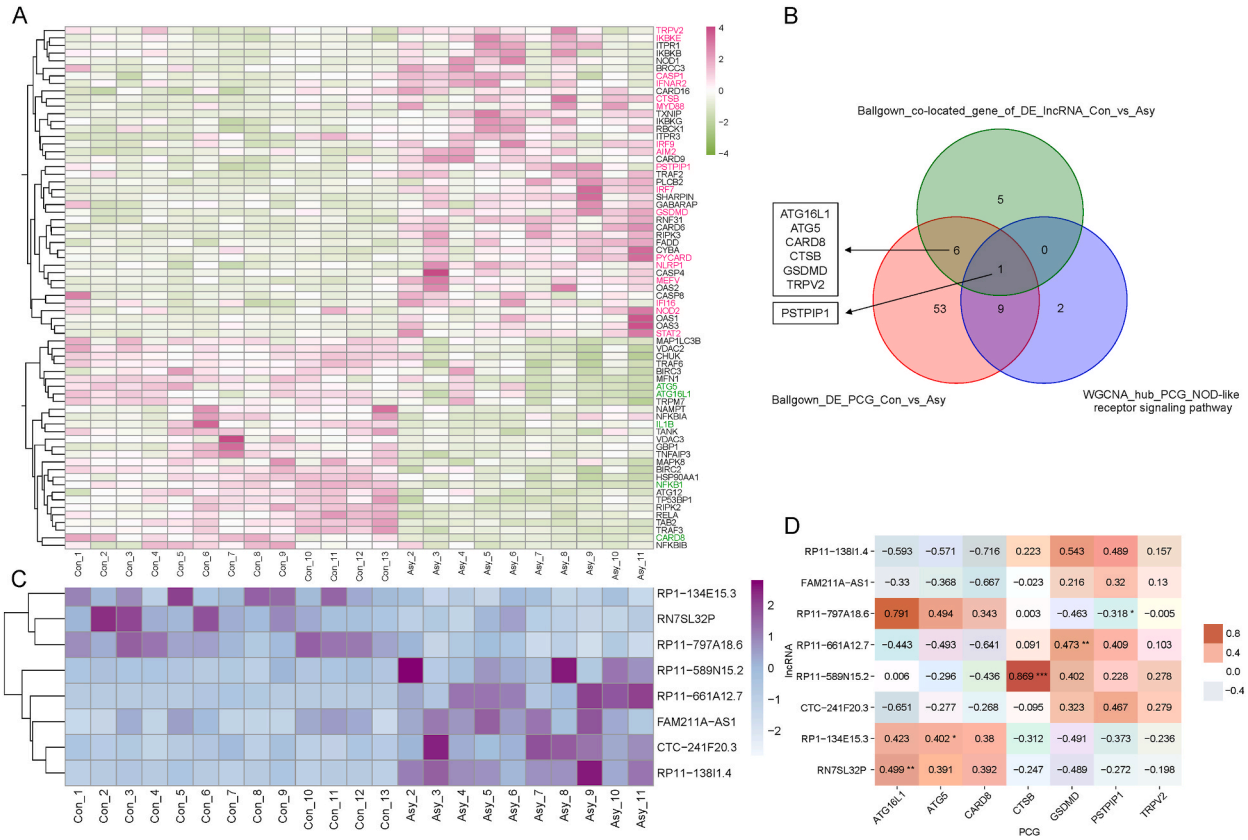

Fig. 5. Differential transcription patterns in the NOD-like receptor signaling pathway between healthy and asymptomatic COVID-19 individuals. **A**, heatmap and hierarchical clustering of the PCGs related to this pathway showing differential expression between the Con and Asy groups; up-regulated and down-regulated PCGs that are closely associated with responses of type I IFN and inflammasomes are indicated by pink and green color, respectively. **B**, Venn diagram of DE PCGs, co-located target genes of DE lncRNA, and the "hub PCGs in WGCNA" related to the NOD-like receptor signaling pathway. **C**, heatmap and hierarchical clustering of differential expression patterns of eight lncRNAs, of which co-located genes are associated with this pathway. **D**, Pearson's correlation analysis between the eight candidate PCGs and co-located lncRNA pairs that are shown in the diagonal line, of which TRPV2 is co-located with the two lncRNAs (*FAM211A-AS1* and *RP11-138I1.4*); *p*-value <0.05, 0.01 and 0.001 are indicated by *, ** and ***, respectively. Asy, asymptomatic; Con, healthy controls; DE PCGs, differentially expressed protein coding genes; IFN, interferon; WGCNA, weighted gene co-expression network analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. PSTPIP1-mediated hyperinflammatory responses in asymptomatic individuals

We specifically investigated the NOD-like receptor signaling pathway as it relates to proinflammatory responses in the differential transcription profiles. Heatmap visualizing DE PCGs in NOD-like receptor signaling indicated the activated responses of type I IFN and inflammasomes (Fig. 5A, Additional file 5: Table S3), the former including the upstream signals of myeloid differentiation primary

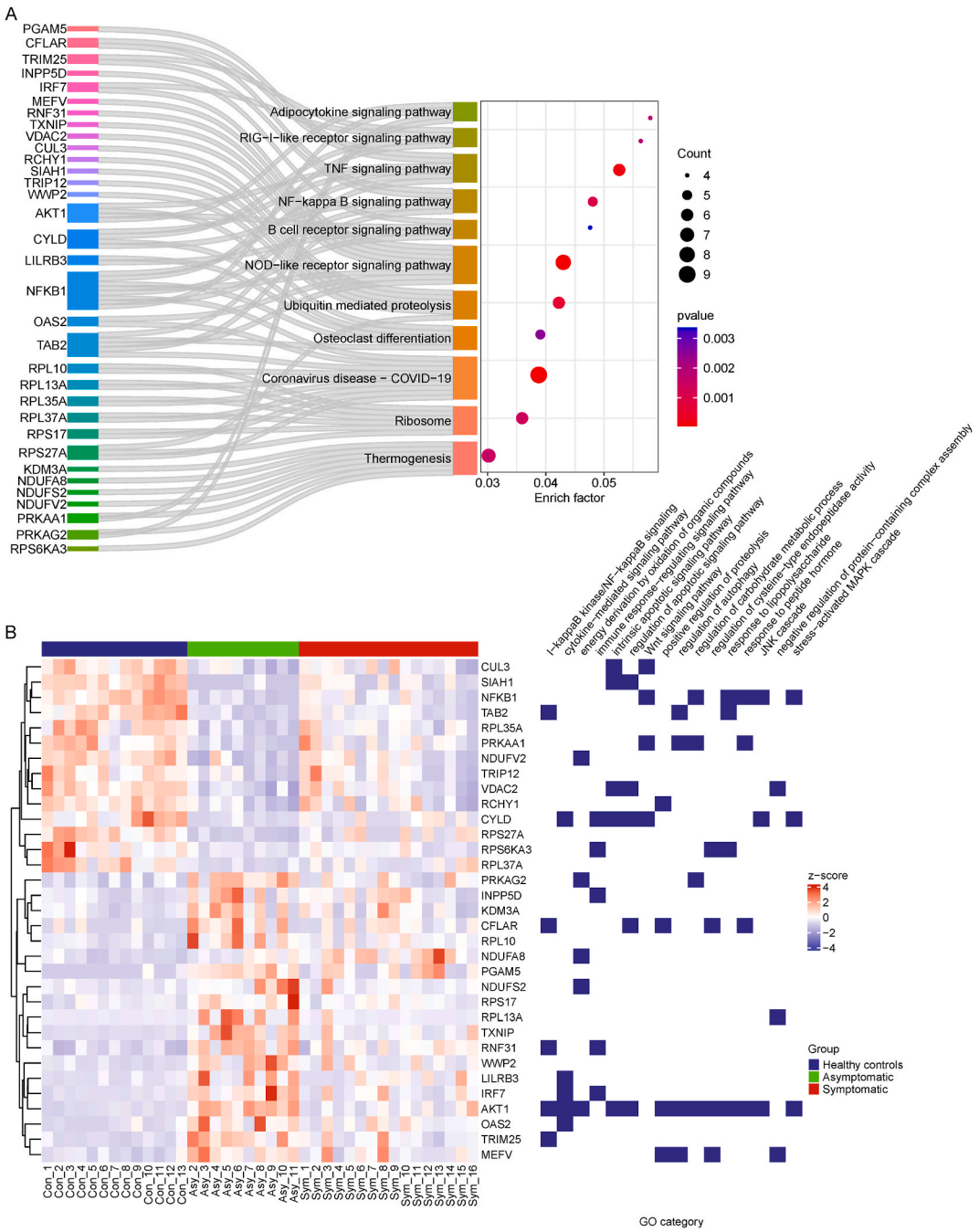

Fig. 6. Differential expression profiles of COVID-19 disease progression-related PCGs and functional enrichment analysis by KEGG and GO. **A**, Sankey diagram based on analysis of significantly enriched KEGG pathways of the overlapping PCGs in the comparisons "Con vs Asy", "Con vs Sym" and "Asy vs Sym"; 11 out of the 20 most enriched KEGG pathways following filtering out other disease-related pathways are visualized. **B**, gene expression heatmap and GO ontology of 33 DE PCGs mentioned in the Sankey diagram; the GO categories which have a greater number of genes are displayed. Asy, asymptomatic; Con, healthy controls; DE PCGs, differentially expressed protein coding genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; Sym, symptomatic.

response protein 88 (*MYD88*), nucleotide binding oligomerization domain containing 2 (*NOD2*), inhibitor of nuclear factor kappa B kinase subunit epsilon (*IKBKE*) and interferon regulatory factor 7 (*IRF7*), and the downstream signals of IFN-α receptor 2 (*IFNAR2*), signal transducer and activator of transcription 2 (*STAT2*) and *IRF9*. Besides, genes encoding inflammasomes components pyrin/*MEFV*, NLRP1, AIM2, and IFI16 showed a similar rising trend in the asymptomatic individuals compared with the healthy controls as the apoptosis-associated speck like protein containing a caspase recruitment domain (ASC/*PYCARD*), *CASP1* and gasdermin-D (*GSDMD*) did, while other NLRs such as NLRP3, NLRP6, and NLRP12 did not. The activation of the inflammasome causes CASP1 maturation, which catalyzes the cleavage of GSDMD and pro-inflammatory cytokines such as IL-1β, leading to pyroptosis and IL-1β release [41]. On the contrary, our findings showed a downregulation of genes encoding IL-1β and NF-κB, which implied that NF-κB-dependent IL-1β production and release were inhibited after asymptomatic infection.

Additionally, when focusing on the DE lncRNAs related to the NOD-like receptor signaling pathway comparing the asymptomatic and healthy control groups, we identified the co-located target genes of *cis*-acting DE lncRNAs based on searching for the genomic regions 100,000 bp upstream and downstream of the DE lncRNAs. For functional analysis, the co-located target gene function was derived from KEGG pathway enrichment. We obtained 12 DE lncRNA cis target genes that were also enriched in the NOD-like receptor signaling pathway (Additional file 6: Table S4). A Venn diagram showed the overlapped areas of circles between DE PCGs and DE lncRNA cis target gene sets as the candidate key PCGs associated with proinflammatory response to asymptomatic infection, namely autophagy related 16 like 1 (*ATG16L1*), autophagy related 5 (*ATG5*), caspase recruitment domain-containing protein 8 (*CARD8*), cathepsin B (*CTSB*), *GSDMD*, transient receptor potential vanilloid 2 (*TRPV2*) and *PSTPIP1* (Fig. 5B). We attempted to determine the key PCGs using multiple methods; the "hub PCGs in WGCNA" related to the NOD-like receptor signaling pathway were intersected with the seven candidate key PCGs. In particular, we found *PSTPIP1* was the only common gene between method-dependent key gene sets. Furthermore, we calculated Pearson's correlation coefficients to reveal the potential expression correlation between the seven candidate key PCGs and eight co-located lncRNA. In general, the results showed that *PSTPIP1*, considered as the key PCG, was up-regulated and significantly negatively correlated with its adjacent lncRNA *RP11-797A18.6* in PBMCs after asymptomatic infection (Fig. 5A, C-D, Additional file 5: Table S3).

3.4. Stage-specific NFKB1 expression in disease progression

Among the three comparison groups, similarly, we performed KEGG and GO enrichment analysis of 137 common transcripts, including 131 DE PCGs and six DE lncRNAs, which had significantly varying expression patterns in the different disease stages and suggested associations with disease progression. Interestingly, the NOD-like receptor signaling pathway remained the most enriched immune system in which both the DE PCGs and DE lncRNA cis target genes were involved. As shown in Fig. 6A, 11 out of the 20 most enriched KEGG pathways, excluding other disease-related pathways, are highlighted in a Sankey plot. The network elucidated *NFKB1* was the key gene that mapped to multiple pathways that related to Coronavirus disease-COVID-19, NOD-like receptor signaling pathway, TNF signaling pathway, NF-kappa B signaling pathway, Adipocytokine signaling pathway, RIG-I-like receptor signaling pathway, Osteoclast differentiation, and B cell receptor signaling pathway in PBMCs during the disease progression.

We further evaluated the expression patterns of COVID-19 disease progression-associated genes that related to a variety of GO functional categories (Fig. 6B). The expression level of *MEFV* that encodes protein pyrin in symptomatic patients was significantly lower than in asymptomatic individuals, but still higher than in healthy controls. Besides, the pyrin inflammasome components genes *PSTPIP1*, *PYCARD*, and *CASP1*, and pyroptosis-related gene *GSDMD* do not exhibit significant differences between asymptomatic and symptomatic individuals (Additional file 5: Table S3), which indicated the pyrin inflammasome maintains a high transcript level after SARS-CoV-2 infection, regardless of disease severity. Furthermore, significant upregulation of *NFKB1* was identified in symptomatic patients relative to asymptomatic individuals, suggesting that the processing of NF-κB may lead to pleiotropic effects on mediating some other signaling such as Wnt signaling pathway, regulation of carbohydrate metabolic process, response to lipopolysaccharide, response to peptide hormone, JNK cascade and stress-activated MAPK cascade, and transcription of various target genes involved in inflammation development and progression, which might be a reason that initiated COVID-19 disease progression from asymptomatic to symptomatic infections.

4. Discussion

Our study aims to identify the innate immune responses that differ between healthy and asymptomatic individuals, as well as the alterations that occur in the disease course of COVID-19. Previous reports have shown the investigation of transcriptomic differences and dynamics of immune signatures on nasopharyngeal swabs or serum samples from COVID-19 patients with various severity based on RNA-seq [42,43]. However, the early proinflammatory responses and variations remain unclear, particularly at the lncRNA layer. Here, we examined the strand-specific RNA-seq of PBMCs from healthy donors, asymptomatic and mild to moderate COVID-19 samples and revealed interesting immune response patterns of the asymptomatic infections and disease progression.

Although numerous previous studies have characterized the activation of the NLRP3 inflammasome sensor, the most widely investigated member of NLRs, in response to SARS-CoV-2 infection and its role in the formation of cytokine storm [44]. Other sensor proteins may also possibly be involved in the excessive cytokine release and inflammation-driven in severe COVID-19 cases [45–48]. Similar to the latter studies, we did not detect robust *NLRP3* expression in PBMCs, while the pyrin, NLRP1, AIM2 and IFI16 inflammasomes and pyroptosis-related gene *GSDMD* showed consistent activation at both stages of COVID-19 disease compared with the healthy controls, which indicated that inflammasomes activation and pyroptosis occur not only in symptomatic infection but also in the phase of asymptomatic infection with specific components. Another interesting observation is that we were unable to find a strong

IL1B expression in either asymptomatic conditions or mild to moderate infection, which is consistent with the proposal that the IL-1 pathway is a better indicator of severe respiratory disease [49]. Although previous studies have shown NF-κB serves as a key mediator of the priming signal of NLRP3 inflammasome activation, inducing the transcriptional expression of NLRP3 and pro-IL-1β in response to inflammatory diseases [50], our current results reveal that NF-κB-dependent induction of NLRP3 and pro-IL-1β is suppressed, the former of which is replaced by other inflammasome sensors such as pyrin in both asymptomatic and mild to moderate COVID-19. It is assumed that there may be other mechanisms that enhance the downstream inflammatory responses in non-severe COVID-19 that are unexplored here. Furthermore, we discover the stage-specific changes in the expression of *NFKB1* and its target downstream signals that may signify a turning point in the course of the disease by switching from an asymptomatic incubation period to mild or moderate COVID-19.

Particularly, the pyrin inflammasome assembly in the process that pyrin recruits ASC via a pyrin domain (PYD)-PYD homotypic interaction called the ASC specks, followed by the recruitment of pro-caspase-1 to the specks via a CARD-CARD interaction to initiate CASP1 activation, if dysregulated, which is quite well known to drive autoinflammatory diseases, such as Cryopyrinopathy and familial Mediterranean Fever [51]. The pyrin protein encoded by *MEFV* consists of five different domains, of which the B-box and the coiled-coil domains have been shown to interact with PSTPIP1 which is a crucial factor for actin cytoskeleton organization [52]. Recently, Lee et al. [53] reported that *PSTPIP1* was up-regulated in platelets in severe COVID-19 patients compared with the healthy controls. Similarly, our present study has uncovered an upregulated pattern of both *MEFV* and *PSTPIP1* in PBMCs from asymptomatic COVID-19 individuals, thereby may cause the activation of PSTPIP1-dependent pyrin inflammasomes. It is also noteworthy that a neighboring antisense lncRNA *RP11-797A18.6* within 35,894 bp downstream of PSTPIP1 was identified and their expression exhibits a significant negative correlation comparing the asymptomatic and healthy control groups.

The lncRNAs have been reported to play a critical regulatory role in various biological regulatory processes to SARS-CoV-2 infection [54]. Aznaourova et al. [55] identified a down-regulated lncRNA *PIRAT* (PU.1-induced regulator of alarmin transcription) as a regulator of alarmins that drives the pathogenesis of severe COVID-19. The lncRNA *CHROMR* was found to be highly induced in SARS-CoV-2-infected cells and controls interferon-stimulated gene expression and innate immune responses [56]. Similarly, our results showed *PSTPIP1* was up- and its adjacent antisense lncRNA *RP11-797A18.6* was down-regulated in PBMCs during asymptomatic infections, indicating that they were likely jointly contributing to pyroptosis through pyrin inflammasome activation. To investigate the interplay between *RP11-797A18.6* and the corresponding sense gene PSTPIP1, we used RNAplex to conduct a lncRNA-DNA interaction prediction. We collected eight silencer regions of PSTPIP1 from the SilencerDB database prior to the prediction of lncRNA-DNA base pairing interactions based on the Minimum free energy (MFE). Interestingly, it was observed that two out of eight silencer regions base-paired with *RP11-797A18.6* show the lowest MFE, which may imply stable interactions between PSTPIP1 and *RP11-797A18.6* (Additional file 7: Fig. S3). Silencer sequences are known to be bound by repressor complexes such as histone deacetylase complexes and polycomb group proteins to inhibit transcription in immune regulation [57,58]. Investigation into lncRNA-DNA-protein interactions of these targets might provide better understandings towards the epigenetic regulation of COVID-19 infection. Taken together, these findings suggest that *RP11-797A18.6* may serve as a biomarker to suppress PSTPIP1 transcription via interaction at its silencers.

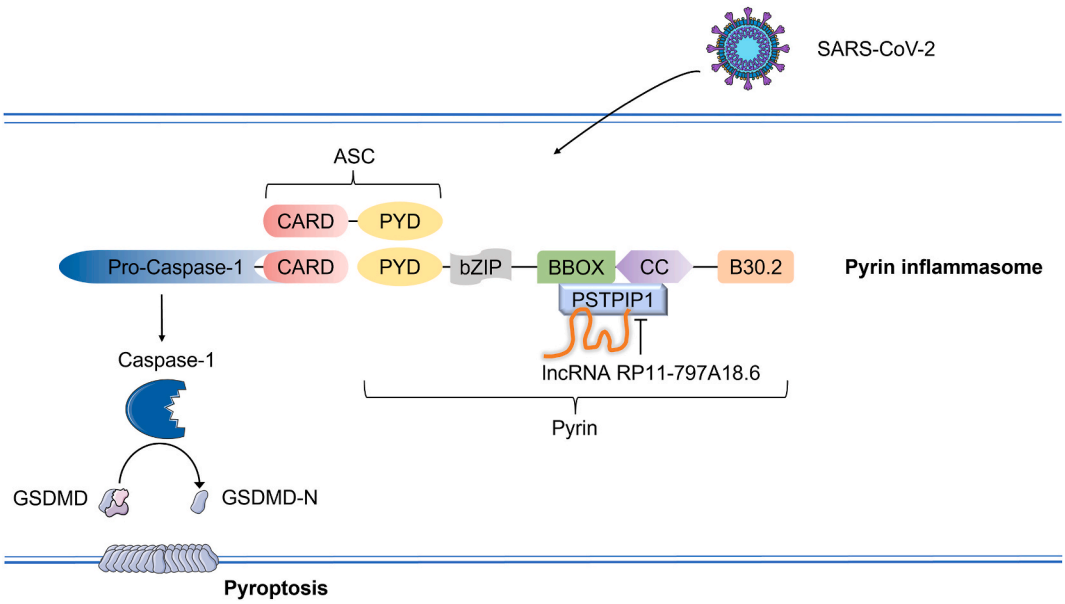

Fig. 7. A model of *RP11-797A18.6*-PSTPIP1-dependent pyrin inflammasome-mediated pyroptosis in asymptomatic SARS-CoV-2 infection. ASC, apoptosis-associated speck like protein containing a caspase recruitment domain; bZIP, basic leucine zipper domain; CARD, caspase recruitment domain; CC, coiled-coil domain; GSDMD, gasdermin-D; PYD, pyrin domain.

Despite these promising findings, our study has limitations, including that gene expression analyses were carried out in PBMCs but not in diseased respiratory tract tissues, which may have different results. On the other hand, the study lacks validation in independent cohorts, and the lack of functional experiments to substantiate the role of *RP11-797A18.6* and PSTPIP1 in pyroptosis and inflammation. Therefore, further research is needed on the interaction of PSTPIP1 and *RP11-797A18.6* in vitro and in vivo to determine their functional roles and expand their exploration in severe COVID-19 and inflammatory diseases.

5. Conclusions

Collectively, this work unveils an update on the infection of PBMCs by SARS-CoV-2 activates PSTPIP1-dependent pyrin inflammasomes and drives pyroptosis (Fig. 7). We discovered that the expression level of *PSTPIP1* exhibited a significant negative correlation with the nearby lncRNA *RP11-797A18.6* in the asymptomatic individuals. Our findings also expand the previously identified role of *NFKB1* as a multifunctional transcription factor that promotes inflammation and serves as an indicator of disease progression. Further studies of *PSTPIP1* and *RP11-797A18.6* function and interaction in vitro and in vivo in which the pyrin inflammasomes and pyroptosis are activated may shed new insight into preventive targets for COVID-19.

Ethics approval and consent to participate

The local Ethics Committee of each participating hospital approved the study. All participants enrolled in this study provided written informed consent.

Consent for publication

Not applicable.

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Data availability

Data associated with the study has been deposited into a publicly available repository. The raw data are available online on the Gene Expression Omnibus database under accession number GSE236651 and are available at the following URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE236651.

CRediT authorship contribution statement

Ruili Ji: Writing – original draft, Formal analysis. **Yue Wu:** Writing – original draft. **Yuhua Ye:** Writing – original draft. **Yanling Li:** Writing – original draft. **Yizhe Li:** Data curation. **Guojiu Zhong:** Data curation. **Wentao Fan:** Data curation. **Chengjuan Feng:** Data curation. **Hui Chen:** Formal analysis. **Xiangyun Teng:** Writing – review & editing, Supervision. **Yunli Wu:** Visualization, Supervision. **Jianhua Xu:** Writing – review & editing, Visualization, Supervision.

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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List of abbreviations

COVID-19 Coronavirus disease 2019
SARS-CoV-2 Severe acute respiratory syndrome coronavirus 2
Con Healthy controls
Asy Asymptomatic
Sym Symptomatic
PRRs Pattern recognition receptors

TLRs Toll-like receptors
NLRs NOD-like receptors
IFNs Interferons
NLRP NLR family pyrin domain containing
AIM2 Absent in melanoma 2
IFI16 Interferon gamma-inducible protein 16
IL-1β Interleukin-1 beta
CASP1 Caspase-1
lncRNAs Long-noncoding RNAs
MALAT1 Metastasis associated lung adenocarcinoma transcript 1
NEAT1 Nuclear paraspeckle assembly transcript 1
PBMCs Peripheral blood mononuclear cells
PSTPIP1 Proline-serine-threonine phosphatase-interacting protein 1
FPKM Fragments Per Kilobase Per Million reads
TUCPs Transcripts of uncertain coding potential
WGCNA Weighted gene co-expression network analysis
GS Gene significance
MM Module membership
DE Differentially expressed
PCGs Protein coding genes
KEGG Kyoto Encyclopedia of Genes and Genomes
GO Gene Ontology
NFKB1 Nuclear factor kappa B subunit 1
TAB2 TGF-beta-activated kinase 1 binding protein 2
FCGR2A Fc gamma receptor IIa
GABARAPL1 GABA type A receptor associated protein like 1
MYD88 Myeloid differentiation primary response protein 88
NOD2 Nucleotide binding oligomerization domain containing 2
IKBKE Inhibitor of nuclear factor kappa B kinase subunit epsilon
IRF7 Interferon regulatory factor 7
IFNAR2 IFN-α receptor 2
STAT2 Signal transducer and activator of transcription 2
MEFV MEFV innate immunity regulator, pyrin
ASC/*PYCARD* Apoptosis-associated speck like protein containing a caspase recruitment domain
GSDMD Gasdermin-D
ATG16L1 Autophagy related 16 like 1
ATG5 Autophagy related 5
CARD8 Caspase recruitment domain-containing protein 8
CTSB Cathepsin B
TRPV2 Transient receptor potential vanilloid 2
PYD Pyrin domain
bZIP Basic leucine zipper domain
CC Coiled-coil domain
PIRAT PU.1-induced regulator of alarmin transcription
MFE Minimum free energy

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26886.

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