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RESEARCH ARTICLE

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Upregulation of hsa_circ_0004812 promotes COVID-19 cytokine storm via hsa-miR-1287-5p/IL6R, RIG-I axis

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Abstract

Background: SARS-CoV-2 is one of the most contagious viruses in the Coronaviridae (CoV) family, which has become a pandemic. The aim of this study is to understand more about the role of hsa_circ_0004812 in the SARS-CoV-2 related cytokine storm and its associated molecular mechanisms.

Materials and Methods: cDNA synthesis was performed after total RNA was extracted from the peripheral blood mononuclear cells (PBMC) of 46 patients with symptomatic COVID-19, 46 patients with asymptomatic COVID-19, and 46 healthy controls. The expression levels of hsa_circ_0004812, hsa-miR-1287-5p, IL6R, and RIG-I were determined using qRT-PCR, and the potential interaction between these molecules was confirmed by bioinformatics tools and correlation analysis.

Results: hsa_circ_0004812, IL6R, and RIG-I are expressed higher in the severe symptom group compared with the negative control group. Also, the relative expression of these genes in the asymptomatic group is lower than in the severe symptom group. The expression level of hsa-miR-1287-5p was positively correlated with symptoms in patients. The results of the bioinformatics analysis predicted the sponging effect of hsa_circ_0004812 as a competing endogenous RNA on hsa-miR-1287-5p. Moreover, there was a significant positive correlation between hsa_circ_0004812, RIG-I, and IL-6R expressions, and also a negative expression correlation between hsa_circ_0004812 and hsa-miR-1287-5p and between hsa-miR-1287-5p, RIG-I, and IL-6R.

Conclusion: The results of this in-vitro and in silico study show that hsa_circ_0004812/hsa-miR-1287-5p/IL6R, RIG-I can play an important role in the outcome of COVID-19.

KEYWORDS
cytokine storm, hsa_circ_0004812, hsa-miR-1287-5p, SARS-CoV-2, sponging effect

1 | BACKGROUND

The COVID-19 pandemic has become a very challenging and controversial problem. The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus is a newly emerging single-stranded RNA virus that belongs to the beta-coronavirus family and causes COVID-19 (coronavirus disease 2019). COVID-19, with a dramatic fatality rate,[1–3] triggers the immune system to respond and simultaneously represses the immune response to allow the virus replication. The interplay among the infected cells, viruses, and immune system induces a high level of cytokine and chemokine secretion known as the "cytokine storm".[3,4]

CircRNAs are created through the back-splicing process, which results in a closed-loop structure without poly(A) tails and 5′ caps.[5] These RNA molecules are highly stable and resistant to exonuclease-mediated degradation. It has been found that circRNAs, as the key regulators, could modulate gene expression through various molecular mechanisms, including a sponging effect on miRNA or acting as protein scaffolds.[6]

It has been reported that noncoding RNAs could have an effect on the inflammatory cytokine storm.[7] CircRNAs, which are regarded as endogenous short RNAs that may be classified into coding and noncoding circRNAs,[8] play a proven role in COVID-19-related pathogenesis.[9] For example, Wu et al.[9] found that differentially expressed circRNAs in COVID-19 patients with recurrent disease are mainly involved in the regulation of host cell immunity and inflammation, substance and energy metabolism, cell cycle, and cell apoptosis .

The most well-known function of circRNAs is their activity as competing endogenous RNAs (ceRNAs) through a circRNA/miRNA/mRNA regulatory network.[10,11] Recent evidence suggested the role of circRNAs in various viral infections such as human papilloma virus infection, herpes simplex virus, Epstein–Barr virus, human immunodeficiency virus, Middle East respiratory syndrome coronavirus, and hepatitis B virus infection and confirmed it as a potential biomarker between viral and non-viral states.[12]

One of the circRNAs that has been recognized for its contribution to human viral-associated infection is hsa_circ_0004812. Hsa_circ_0004812 originated from the NINL gene and is located on chromosome 20.[13] In 2020, Zhang and colleagues revealed that hsa_circ_0004812 was upregulated in chronic hepatitis B (CHB) and HBV infected hepatoma cells. Additionally, this circRNA was associated with immune suppression by HBV infection through the hsa_circ_0004812/hsa-miR-1287-5p/FSTL1 pathway.[14] According to some studies, CircRNAs play an important role in gene expression as microRNA sponges via their microRNA response elements (MREs).[5]

MicroRNAs have also been indicated as potential biomarkers for COVID-19 diagnosis.[15] High-throughput sequencing was used during the study in 2020 to evaluate the expression levels of different miRNAs. Then, they revealed that in human patients with COVID-19, 35 miRNAs were upregulated, and 38 miRNAs were downregulated.[16]

MicroRNAs are small noncoding RNAs that are about 20 to 25 nucleotides in length and act as regulators of gene expression by affecting the stability and translation of their target mRNAs.[17] In this study, hsa-miR-1287-5p located on chromosome 10 was selected, which was previously predicted to have a significant binding site in the SARS-CoV-2 genome.[18]

JAK/STAT is one of the pathways involved in inflammatory responses of COVID-19, such as cytokine storm.[14] Interleukin 6 (IL-6) and other inflammatory components such as IL-12 and TNF are important components of cytokine storm and play a role in the pathogenesis of COVID-19 associated pneumonia.[19] IL-6 can activate STAT3 during inflammatory processes, and both of them play a regulatory role in the cytokine storm in COVID-19 infection.[20] Some studies indicate that IL-6R antagonists can improve the hyperinflammation state in hospitalized COVID-19 patients.[21] Retinoid-inducible gene 1 (RIG-I) is one of the retinoic acid-inducible genes I (RIG-I)-like receptor (RLR) family, playing an important role in sensing viral nucleic acids and production of pro-inflammatory and antiviral proteins.[22] Interestingly, we find that hsa_circ_0004812/hsa-mir-1287-5p/IL6R, RIG-I have an impact on cytokine storm through JAK/STAT and STAT3 signaling pathways.

The aim of this study was to develop a better understanding of the molecular mechanisms involved in COVID-19 inflammatory responses. According to the primary *in-silico* analysis and literature studies, we had a specific focus on the hsa_circ_0004812/hsa-miR-1287-5p/IL6R, RIG-I axis to evaluate its potential role in COVID-19 inflammatory responses. In this regard, we assessed the expression of the members of our candidate axis, and its correlation with JAK/STAT and STAT3 signaling pathways was measured.

2 | MATERIALS AND METHODS

2.1 | Patient characteristics and sample collection

To investigate the role of hsa_circ_0004812/hsa-miR-1287-5p/IL6R, RIG-I axis in COVID-19 viral infection, patients with SARS-CoV-2 severe symptoms ($n = 46$), without symptoms ($n = 46$), and a negative control group ($n = 46$) were selected for the study. The first group of patients was hospitalized at Valiasr and Shariati (Fasa, Fars, Iran). All samples were confirmed by reverse transcription-polymerase chain reaction (RT-PCR). Individuals presenting as negative controls had no respiratory insufficiency or hyperinflammation, such as bacterial and fungal infections, inflammatory bowel disease, autoimmune disorders, or cancer. We work on peripheral blood mononuclear cells (PBMC) whole blood from samples after collecting 5 μl blood samples in tubes containing EDTA in the shortest possible time. All of the participants signed a consent form, and this study was approved by the ethics committee of FUMS (IR.FUMS.REC.1399.182).

2.2 | Isolation of total RNA and cDNA synthesis

After exposing the blood cells to the reagent, PBMC were isolated using the density-gradient method. Trizol isolation reagent (Invitrogen, Thermo Fisher) was used to extract total RNA. Then,

RNA was extracted from PBMC according to the manufacturer's instructions. The RNA purity was assessed with a NanoDrop spectrophotometer (BioTek, HTX multi-mode reader) and gel electrophoresis. The first-strand cDNA was synthesized using the PrimeScript™ RT Reagent Kit (BioFact™, Cat. No: BR441-096) following the manufacturer's recommendations.

2.3 | Real time PCR

The relative expression of genes was determined using Power SYBR® Green PCR Master Mix (ABI, USA) on the 7500 real-time PCR system (ABI, life technology). A 15 μl reaction contained 1 μl of cDNA, 7.5 μl BioFACT™ master mix including SYBR Green (Ampliqon, Cat. No: A325402-25), 0.75 μl of each primer, and 5 μl DNase-free deionized H2O. Thermal cycling was performed at 45 cycles of 95°C for 20 s and then 60°C for 30 s. We used two internal controls, including ACTB and U48. The sequences of each primer are stated in Table 1. The Livak method ($2^{-\Delta\Delta C_t}$) was utilized to calculate the relative expression.

2.4 | Statistical analysis

Data analysis was performed in SPSS software v.26, and graphs were drawn with GRAPHPAD PRISM v.8. Comparison of expression between the three groups of samples, including symptomatic COVID-19, asymptomatic COVID-19, and negative control, was evaluated using the Kruskal–Wallis H non-parametric test. The Chi-square test was applied to compare sex and blood type between groups, and the one-way ANOVA test was used to compare age. The correlation of the elements in the hsa_circ_0004812/hsa-miR-1287-5p/IL6R, RIG-I pathway was measured by the Spearman correlation coefficient test. A *p*-value less than .05 was considered a significant level.

TABLE 1 primers sequences

Gene symbol (Gene name)	Primer sequence (5′–3′)
Hsa_circ_0004812 (F)	GAAGAAAATCAAAGCCTGGACTTC
Hsa_circ_0004812 (R)	GCTCAGCTCCTGGTGGTA
Hsa-miR-1287-5p (F)	GCGTGCTGGATCAGTGGTT
Hsa-miR-1287-5p (RT)	GTCGTATCCAGTGCAGGGTCCGAGGTA TTCGCACTGGATACGACGACTCG
hsa-miR-149-5p and U48 (R)	GTGCAGGGTCCGAGGT
U48 (F)	AGGTAACTCTTGAGTGTGTCGCT
U48 (RT)	GTCGTATCCAGTGCAGGGTCCGAGGTA TTCGCACTGGATACGACGGTCAGA
RIG-I (F)	ATGGGACGAAGCAGTATTTAG
RIG-I (R)	GCTTGGGATGTGGTCTACTC
IL6R (F)	CCACTCCTGGAACTCATCTTTC
IL6R (R)	ACTCCTCCTGGGCACGAA
ACTB (F)	TGGAACGGTGAAGGTGACAG
ACTB (R)	CTGTAACAACGCATCTCATATTTGG

Abbreviations: ACTB, actin beta; IL6R, interleukin 6 receptor; RIG-I, RNA sensor RIG-I; U48, SNORD48, small nucleolar RNA.

2.5 | Bioinformatics Predictions

Hsa_circ_0004812/hsa-miR-1287-5p/IL6R, RIG-I axis was constructed based on circRNA-miRNA pair, miRNA-mRNA pairs. CircRNA-miRNA interaction was downloaded from the database Circinteractome.[23] The MiRWalk2.0[24] and miRTargetLink[25] databases were used to predict the interaction between miRNA and mRNAs. Then, the pathway enrichment analysis of miRNA and mRNAs was performed using miRPathDB 2.0[26] and Enrichr[27] databases.

3 | RESULTS

3.1 | Basic and demographic information of patients

Participants in the current study included: group (1) 46 patients with symptomatic COVID-19, including 19 (41%) females and 27 (59%) males, with a mean age of 41.54 years, group (2) 46 patients with asymptomatic COVID-19, with 21 (46%) females and 25 (54%) males, with a mean age of 47.90 years, and group (3) the negative control which included 24 (52%) males and 22 (48%), females, with a mean age of 42.65. The patients and control groups were matched in terms of age, sex, and blood group (Table 2). The most common underlying diseases in the symptomatic and asymptomatic COVID-19 groups were cardiovascular disease with 10 (22%), 12 (26%) patients and immunodeficiency disease with 4 (9%), 0 (0%) patients, respectively (Table 2). Table 3 demonstrates the different symptoms of patients with symptomatic COVID-19 participating in this study.

3.2 | Evaluation of hsa_circ_0004812, hsa-miR-1287-5p, RIG-I, and IL6R expression in symptomatic COVID-19 patients compared with asymptomatic COVID-19 patients and negative controls

We used quantitative real-time PCR to evaluate the gene expression levels in two different subgroups of COVID-19 patients and negative controls, with a total of 46 samples in each group. The expression of hsa_circ_0004812 is significantly higher in symptomatic COVID-19 samples in comparison with other groups, and it was also upregulated in asymptomatic patients compared with negative controls (Table 4, Figure 1A). The hsa-miR-1287-5p expression is obviously lower in symptomatic COVID-19 patients compared with negative controls and asymptomatic patients (Table 4, Figure 1B). Furthermore, the

TABLE 2 Participants' baseline and demographic information (*p*-values 1: in comparison with negative control, 2: in comparison with asymptomatic COVID-19)

		Negative control		Asymptomatic covid		Symptomatic covid		
		Count	Column *N* %	Count	Column *N* %	Count	Column *N* %	*p*-value
Blood group	A+	12	26.1%	12	26.1%	11	23.9%	.355
	A−	6	13.0%	2	4.3%	3	6.5%	
	B+	10	21.7%	16	34.8%	10	21.7%	
	B−	0	0.0%	5	10.9%	3	6.5%	
	O+	11	23.9%	8	17.4%	12	26.1%	
	O−	4	8.7%	3	6.5%	4	8.7%	
	AB+	3	6.5%	0	0.0%	3	6.5%	
Sex	Male	24	52.2%	25	54.3%	27	58.7%	.815
	Female	22	47.8%	21	45.7%	19	41.3%	
Underlying disease	None	0	0.0%	34	73.9%	32	69.6%	.120
	Cardiovascular disease	0	0.0%	12	26.1%	10	21.7%	
	Immune deficiency	0	0.0%	0	0.0%	4	8.7%	

Age	Mean	SD	*p*-value 1	*p*-value 2
Negative control	42.65	15.44		
Asymptomatic covid	47.90	15.40	.234	
Symptomatic covid	41.54	14.31	.934	.121

results indicate that RIG-I and IL6R are significantly upregulated in symptomatic and asymptomatic patients compared with negative controls (Table 3, Figure 1C,D).

3.3 | The hsa_circ_0004812 as a potential regulator of RIG-I and IL6R through sponging hsa-miR 1287-5p

To confirm whether hsa_circ_0004812 may act as the molecular sponge for hsa-miR-1287-5p, the expression correlation between these ncRNAs was evaluated in the samples. There was a significant negative correlation between the expression of hsa_circ_0004812 and hsa-miR-1287-5p ($r = -0.283$, p value = .0.001, Figure 2B). Using bioinformatics tools, we have predicted that RIG-I and IL6R are potential targets of the hsa_circ_0004812/hsa-miR-1287-5p, and correlation analysis between scale results of RT-PCR revealed a positive expression correlation between hsa_circ_0004812 and IL6R and RIG-I ($r = 0.760$, p value = <.001/$r = .236$, p value = .005, Figure 2C,D). In addition, the negative expression correlation between hsa-miR-1287-5p and IL6R and RIG-I was studied ($r = -0.234$, p value = .006/$r = -0.514$, p value = <.001, Figure 2E,F). According to the results from the expression pattern of the hsa_circ_0004812/hsa-miR-1287-5p/RIG-I, IL6R axis, a distinct expression profile between COVID-19 samples and negative controls is shown by the heatmap analysis (Figure 2A). The final output confirms the significance of this hsa_circ_0004812/hsa-miR-1287-5p/RIG-I, IL6R pathway in the COVID-19 cytokine storm.

3.4 | Functional enrichment analysis of hsa_circ_0004812/hsa-miR-1287-5p/ RIG-I, IL6R axis

Using the circRNA-miRNA pair and the miRNA-mRNA pair, we generated the hsa_circ_0004812/hsa-miR-1287-5p/RIG-I, IL6R triple network. We used the miRPathDB and Enrichr databases to evaluate the association between miRNA and mRNA related pathways, respectively.[26,27] "Regulatory circuits of the STAT3 signaling pathway" is the significant pathway related to hsa-miR-1287-5p via WikiPathways data (p-value = .006). Additionally, the significant gene ontology (GO) associated with IL6R and RIG-I has been shown in Figure 3. GO analysis displayed that IL6R, RIG-I, and hsa-miR-1287-5p in our proposed ceRNA regulatory network were involved in the regulation of JAK–STAT and PI3K-AKT signaling pathways.

4 | DISCUSSION

Despite the quick progression in COVID-19 research, due to the manifestation of new variants in SARS-CoV-2, COVID-19 remains a global crisis. This is largely owing to our lack of understanding of the virus's molecular processes of pathogenesis. While the characteristics of COVID-19 vastly range from symptomatic to asymptomatic,

TABLE 3 Features of symptomatic COVID-19 patients

		Symptomatic covid	
		Count	Column *N* %
Underlying disease	None	31	67.4%
	Cardiovascular disease	10	21.7%
	Immune deficiency	5	10.9%
Fever-tremor	No	21	45.7%
	Yes	25	54.3%
cough	No	17	37.0%
	Yes	29	63.0%
Vomiting-nausea	No	36	78.3%
	Yes	10	21.7%
stomachache	No	26	56.5%
	Yes	20	43.5%
dizziness	No	33	71.7%
	Yes	13	28.3%
headache	No	21	45.7%
	Yes	25	54.3%
sore throat	No	23	50.0%
	Yes	23	50.0%
BMI	≤25	24	52.2%
	25–29	14	30.4%
	≥30	8	17.4%
fatigue	No	27	58.7%
	Yes	19	41.3%
diarrhea	No	34	73.9%
	Yes	12	26.1%

TABLE 4 Expression levels of hsa_circ_0004812, hsa-miR-1287-5p, RIG-I, and IL6R in asymptomatic and symptomatic COVID-19 patients, and negative controls (*p*-value computed from Kruskal–Wallis Test. *p*-value 1: In comparison with negative control. *p*-value 2: In comparison to asymptomatic covid)

Gene name	Group	*N*	Mean	SD	Median	*p*-value 1	*p*-value 2
hsa_circ_0004812	Negative control	46	3.667	6.892	1.096		<.001
	Asymptomatic covid	46	21.154	51.223	2.536	.037	
	Symptomatic covid	46	46.431	62.662	26.719	<.001	
hsa-miR-1287-5p	Negative control	46	2.811	5.895	1.011		.246
	Asymptomatic covid	46	0.221	0.315	0.115	<.001	
	Symptomatic covid	46	0.225	0.611	0.047	<.001	
RIG1	Negative control	46	4.805	7.503	1.145		.153
	Asymptomatic covid	46	50.362	68.598	23.595	<.001	
	Symptomatic covid	46	121.466	113.529	88.392	<.001	
IL6R	Negative control	46	4.028	6.615	0.854		.485
	Asymptomatic covid	46	15.514	19.057	7.105	<.001	
	Symptomatic covid	46	20.332	17.129	18.700	<.001	

the cytokine storm has been triggered in the respiratory system of some of the patients.[1,2] Thus, it is crucial to conduct some original research on the genetic factors that cause a dysfunctional innate immune system.

Dysregulation in circRNA expression has been linked to various diseases, including cancer, heart disease, neurological problems, and some cases of viral infection, while the underlying mechanisms remain unknown. For example, Wu et al.[9] found that 114 differentially

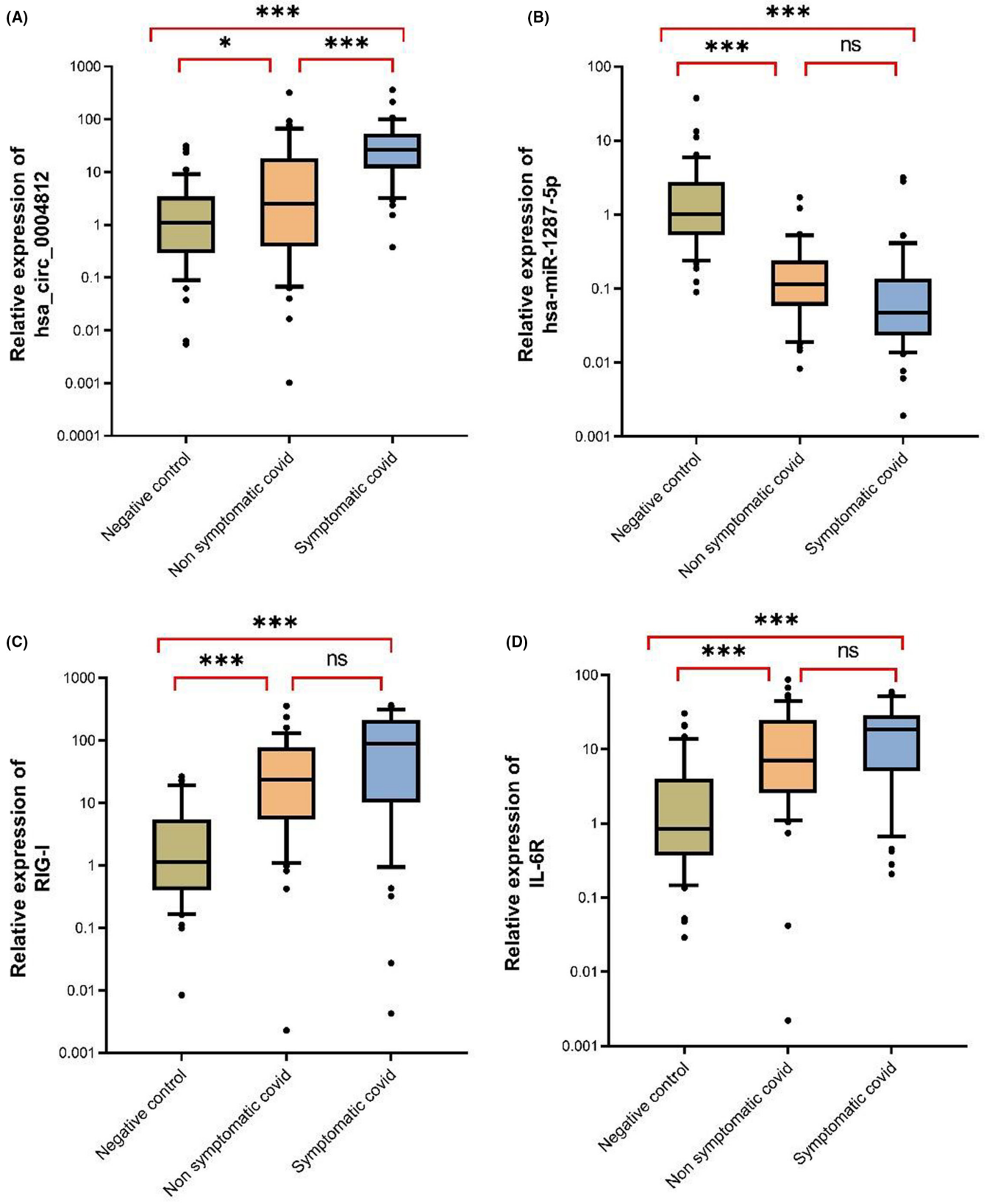

FIGURE 1 Relative expression levels of hsa_circ_0004812, hsa-miR-1287-5p, IL6R, and RIG-I were shown in a box and whisker plot (10–90 percentile). (ns: $p > .05$; * $p \leq .05$; *** $p < .001$). (A) Upregulation of hsa_circ_0004812 in COVID-19 patients compared with healthy. (B) Down expression of hsa-miR-1287-5p in COVID-19 patients compared with healthy controls. (C) Upregulation of RIG-I in COVID-19 patients compared with healthy controls. (D) Upregulation of IL6R in COVID-19 patients compared with healthy controls.

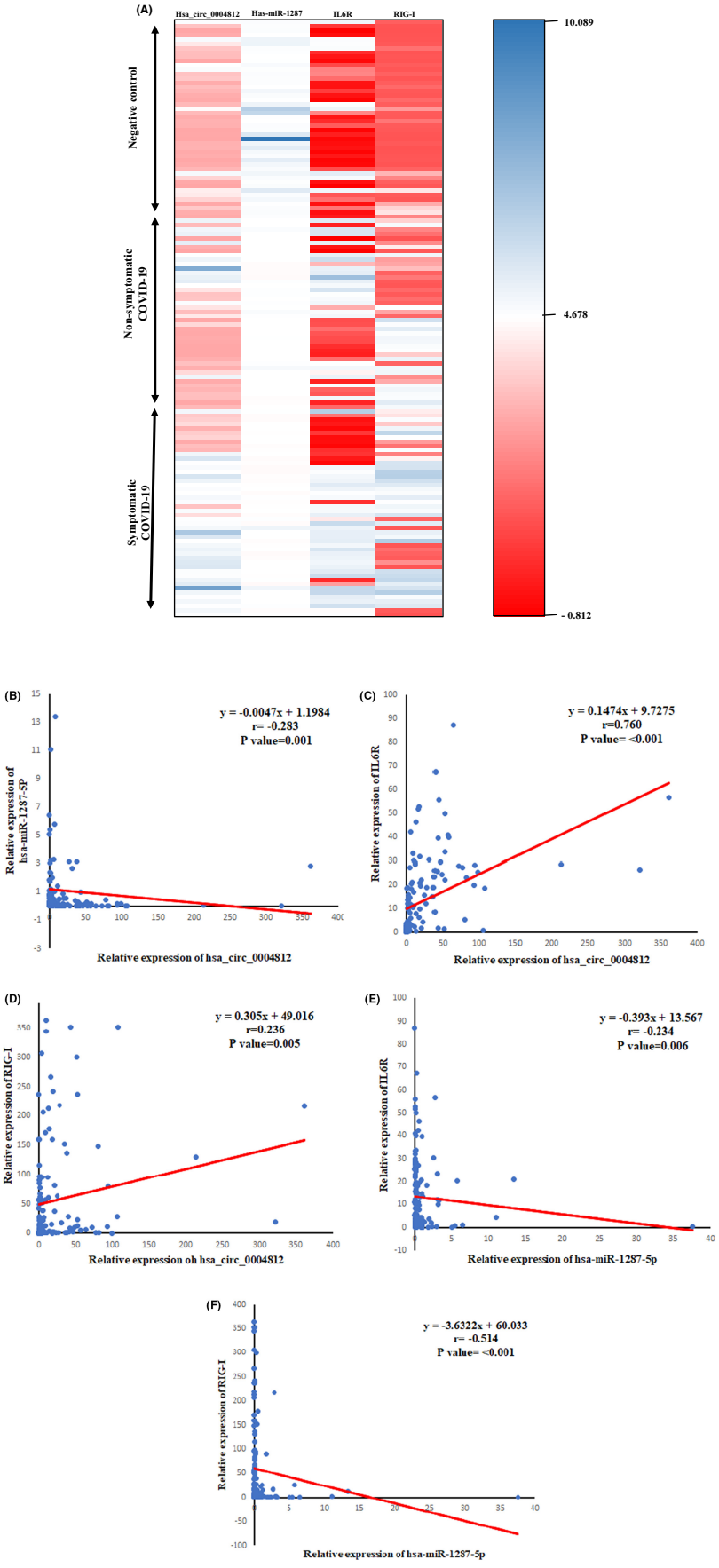

FIGURE 2 (A) LogFC heatmap of gene expression. (B) The negative correlation between hsa_circ_0004812 and hsa-miR-1287-5p expression levels in COVID-19 ($r = -0.283$, p value = .001). (C) The positive correlation between hsa_circ_0004812 and IL6R expression levels in COVID-19 ($r = 0.760$, p value = <.001). (D) The positive correlation between hsa_circ_0004812 and RIG-I expression levels in COVID-19 ($r = 0.236$, p value = .005). (E) The negative correlation between hsa-miR-1287-5p and IL6R expression levels in COVID-19 ($r = -0.234$, p value = .006). (F) The negative correlation between hsa-miR-1287-5p and RIG-I expression levels in COVID-19 ($r = -0.514$, p value = <.001).

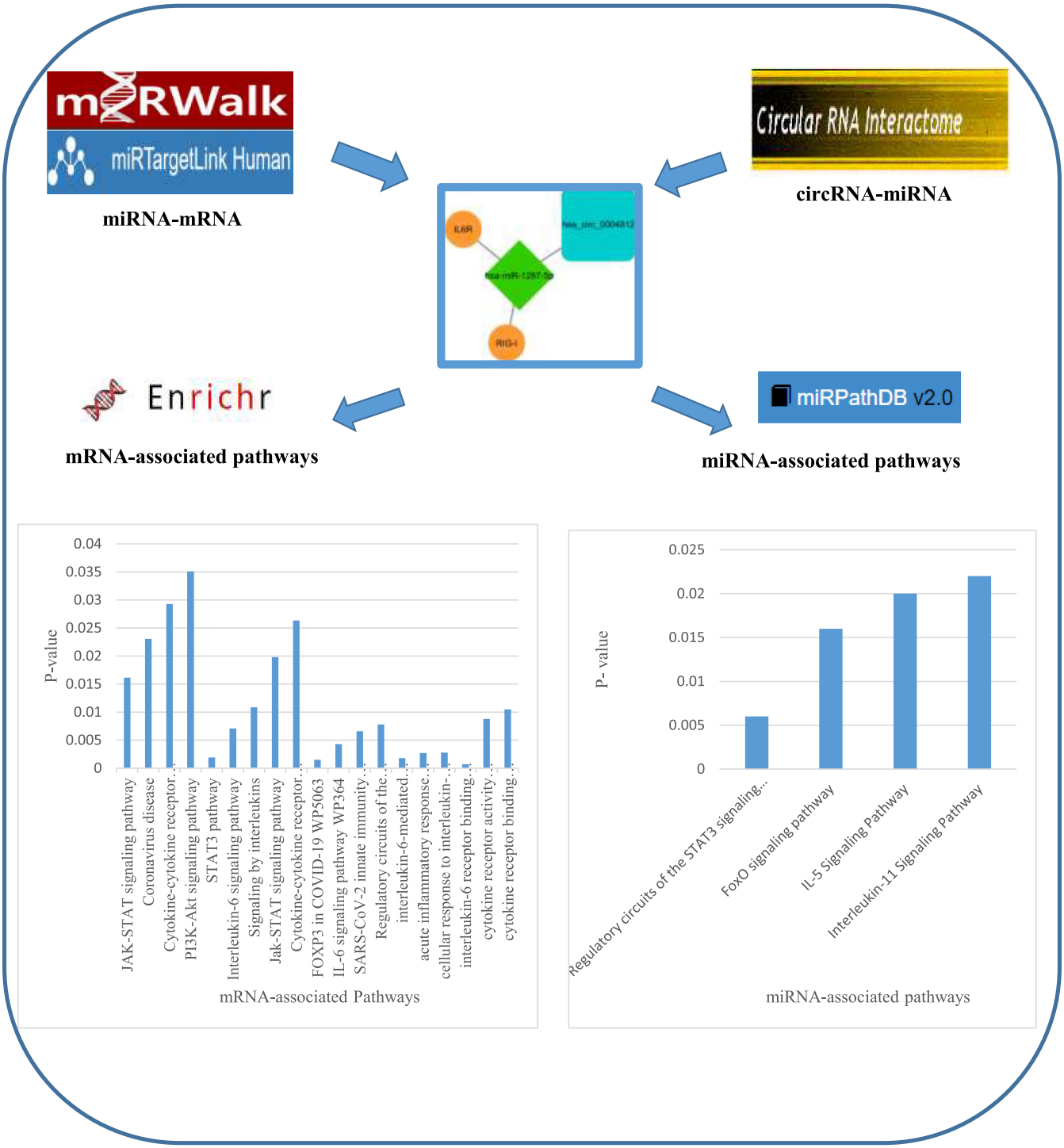

FIGURE 3 Overall workflow of bioinformatics analyses

expressed circRNAs were related to exosomes that were extracted from COVID-19 patients and healthy people. In this study, using qRT-PCR, we assessed the hsa_circ_0004812, hsa-miR-1287-5p, RIG-I, and IL6R expression among the three groups of participants. Further analysis was performed to discover circRNA-miRNA, miRNA-mRNAs, and circRNA-mRNAs interactions. For confirmation of the qPCR results, *in silico* validation was also conducted using several databases.

Circular RNAs (circRNAs) can bind to miRNAs and act as sponges or decoys, enhancing the expression of miRNA target genes.[28] Recently, circRNAs have been proposed as biomarkers to differentiate viral from non-viral pneumonia. Circular RNAs are involved in various activities, including immune tolerance and escape, and thus could be helpful in the emerging COVID-19 infection.[9] The cellular mechanisms underlying circRNA dysregulation are still poorly understood in SARS-CoV-2 infection. The *in silico* representation

identified a ceRNA network in SARS-CoV-infected cells by Arora et al.[29] RNA sequencing was carried out by Zhou et al. and they discovered a total number of 99 dysregulated circRNAs that were linked to chronic hepatitis B (CHB). The study of the CHB-related circRNA-miRNA-mRNA pathway suggested that TGFb2 was controlled by hsa _circ _0000650 through miR-6873-3p sponging.[30] We chose hsa_circ_0004812, which is generated from the ninein-like (NINL) gene, for further investigation among the circRNAs that have been shown to play a function in infectious illnesses.[13]

The experimental data showed that hsa_circ_0004812 is significantly overexpressed in symptomatic COVID-19 patients as compared with asymptomatic COVID-19 patients and negative controls. In line with our analysis, previous research has indicated upregulated hsa_circ_0004812 could modulate the expression of follistatin-like 1 (FSTL1) through sponging of miR-1287-5p in CHB patients and HBV-infected hepatoma cells.[14]

In a study, N Wang et al.[31] introduced the lncRNA LPAL2 in thyroid eye disease (TED) by sponging the hsa-miR-1287-5p. In contrast to our study, W. Hao argues that increased miR-1287-5p levels may inhibit LPS-induced human nasal epithelial cells (HNECs) from releasing pro-inflammatory cytokines.[32] Yajie Hu et al.[33] reported 47 novel miRNAs with differential expression in Enterovirus 71 (EV71) and coxsackievirus A16 (CA16) infections using high-throughput sequencing. This result of qRT-PCR of hsa-miR-1287-5p was in accordance with the high-throughput dataset. In a study, Caixia Li et al. (2020) identified 70 miRNAs dysregulated in human patients with COVID-19 by high-throughput sequencing.[16] In the same study, by next-generation sequencing and bioinformatics tools, 55 miRNAs were altered in 10 COVID-19 patients were identified.[34] Yajie Hu et al.[33] reported 47 novel miRNAs with differential expression in Enterovirus 71 (EV71) and coxsackievirus A16 (CA16) infections using high-throughput sequencing. This result of qRT-PCR of hsa-miR-1287-5p was in accordance with the high-throughput dataset.

We know circRNA acts as a microRNA sponge and modulates gene expression indirectly.[35] The present study explored the relationship between circRNA and microRNA using a bioinformatics database and then picked out hsa-miR-1287-5p. The circRNA-interactome indicates the 7mer-1a site type between hsa_circ_0004812 and hsa-miR-1287-5p.[23] Our results demonstrate that the hsa-miR-1287-5p is downregulated in severe symptoms COVID-19 patients compared with asymptomatic COVID-19 patients and negative controls.

Two mRNAs that we propose are related to the ceRNA are IL6R and RIG-I. Potential roles of these genes have been determined in the SARS-CoV-2 infection.[36,37] The innate immune system is also activated by SARS-CoV-2 and macrophage activation causes an overproduction of pro-inflammatory cytokines, such as IL-6.[38] In previous studies, it was found that interleukin 6 (IL-6) is a pleiotropic cytokine that controls cell proliferation and differentiation along with the immune response. Many other cytokines share this receptor component with the IL6 receptor (IL6R) and interleukin 6 signal transducer (IL6ST/GP130/IL6-beta). Innate immune responses are generated by specific families of pattern recognition receptors. A class of cytosolic RNA helicases known as RIG-I-like receptors (RLRs) recognizes non-self RNA that enters a cell as a result of intracellular virus replication. RIG-I, MDA5, and LGP2 are RLR proteins. These are expressed in immune and non-immune cells.[39,40] Retinoic acid-inducible gene I (RIG-I) can activate type I IFN in response to SARS-CoV2 in fibroblasts and dendritic cells by activating interferon regulatory factor 3 (IRF3) via kinases.[41]

We investigated the interaction between miRNA and mRNAs using three databases. We utilized bioinformatics to predict that hsa-miR-1287-5p could interact with IL6R and RIG-I. Then, in the present study, we observed that the expression of IL6 and RIG-I was increased in the SARS-CoV-2-infected patients with severe symptoms as compared with the asymptomatic and negative control groups. The correlation between gene expression is weak, according to the study that Ratner conducted. Additional tests would need to be performed in the future to ensure and continue the work's results.[42]

We evaluated the correlation between the expression levels of genes in the ceRNA regulatory network. Furthermore, we performed a correlation study between the expression of hsa_circ_0004812 and hsa-miR-1287-5p and then between hsa_circ_0004812 and IL6 and RIG-I. There was a significant negative correlation between hsa_circ_0004812 expression and hsa-miR-1287-5p expression. Additionally, our findings showed a significant positive correlation between the expression of hsa_circ_0004812 and IL6 and RIG-I. Interestingly, Zhang and colleague reported the relationships between hsa_circ_0004812 and miR-1287-5p by luciferase assays in cells transfected with pHBV.[14] Similarly, our results demonstrate that hsa-miR-1287-5p negatively regulates IL6R and RIG-I levels in SARS-CoV-2 infected patients.

5 | CONCLUSION

In conclusion, the interaction of hsa_circ_0004812/hsa-miR-1287-5p/IL6R, RIG-I axis has been verified by a bioinformatics study. Gene ontology analysis indicated that the ceRNA axis is involved in the regulation of JAK–STAT and PI3K-AKT signaling pathways. COVID-19 patients related circRNA-miRNA-mRNA pathway analysis hinted that hsa_circ_0004812 regulated RIG1 and IL6R by sponging hsa-miR-1287-5p. Moreover, on the basis of the correlation analysis, it was revealed that this candidate axis is more significant in the patients with higher COVID-19 symptoms and could be considered as the potential target to be used in understanding, identifying, and treating COVID-19.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. " cd_value_code="text

PATIENT CONSENT STATEMENT

Informed consent was obtained from all individual participants included in the study.

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