Received: 2 August 2023 | Revised: 9 October 2023 | Accepted: 23 October 2023

DOI: 10.1002/hsr2.1686

ORIGINAL RESEARCH

Health Science Reports Open Access WILEY

Exploration of the common pathogenic link between COVID-19 and diabetic foot ulcers: An in silico approach

Xueyao Cai[1] | Ruijin Yang[1] | Wenjun Shi[2] | Yuchen Cai[2] | Zhengzheng Ma[1]

[1]Department of Burn and Plastic Surgery, Dongguan Tungwah Hospital, Dongguan, China

[2]Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Correspondence

Yuchen Cai, Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhi-Zao-Ju Rd., Huangpu District, Shanghai 200011, China.
Email: cyc0419@126.com

Zhengzheng Ma, Department of Burn and Plastic Surgery, Dongguan Tungwah Hospital, No.1 Dongcheng East Rd., Dongguan 523000, China.
Email: zzhengma@126.com

Abstract

Background and Aims: The Coronavirus Disease-19 (COVID-19) is posing an ongoing threat to human health. Patients of diabetic foot ulcer (DFU) are susceptible to COVID-19-induced adverse outcomes. Nevertheless, investigations into their mutual molecular mechanisms have been limited to date. In the present work, we tried to uncover the shared pathogenesis and regulatory gene targets of COVID-19 and DFU.

Methods: In this study, we chose GSE161281 as the COVID-19 data set, which contained severe acute respiratory syndrome coronavirus 2 infected human induced embryonic stem cell-derived peripheral neurons ($n = 2$) with uninfected controls ($n = 2$). The GSE134431 designated as the DFU data set, comprising full-thickness DFU ($n = 13$) and diabetic foot skin ($n = 8$) samples from diabetic patients. The differential expressed genes (DEGs) were identified from GSE161281 and GSE134431, and the common DEGs between COVID-19 and DFU were extracted. Multifactor regulatory network and co-expression network of the common DEGs were analyzed, along with candidate drug prediction.

Results: Altogether, six common DEGs (*dickkopf-related protein 1 [DKK1], serine proteinase inhibitor A3 [SERPINA3], ras homolog family member D [RHOD], myelin protein zero like 3 [MPZL3], Claudin-11 [CLDN11], and epidermal growth factor receptor pathway substrate 8-like 1 [EPS8L1]*) were found between COVID-19 and DFU. Functional analyses indicated that pathways of apoptotic and Wnt signaling may contribute to progression of COVID-19. Gene co-expression network implied the shared pathways of immune regulation and cytokine response participated collectively in the development of DFU and COVID-19. A multifactor regulatory network was constructed integrating the corresponding microRNAs (miRNAs) and transcription factors. Additionally, we proposed potential drug objects for the combined therapy.

Conclusion: Our study revealed the shared molecular mechanisms underlying COVID-19 and DFU. The identified pivotal targets and common pathways can

Xueyao Cai and Ruijin Yang contributed equally to this study.

provide new perspectives for further research and assist the development of management strategies in patients of DFU complicated with COVID-19.

KEYWORDS
COVID-19, diabetic foot ulcer, differentially expressed genes, regulatory network, therapy

1 | INTRODUCTION

The Coronavirus Disease-19 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is posing an ongoing threat to human health.[1] According to the World Health Organization Coronavirus dashboard, a total of 770,875,433 COVID-19 cases have been confirmed by the end of September 27, 2023, including 6,959,316 deaths.[2] Despite an ever-increasing advance in understanding its pathophysiology and manufacture of vaccines, COVID-19 is still a severe burden on global healthcare systems.[3] According to recent statistics, symptoms of COVID-19, especially the Omicron variation, usually resulted in asymptomatic infection or mild upper respiratory tract illness.[4] Nevertheless, severe viral pneumonia or acute respiratory distress syndrome (ARDS) may occur upon SARS-CoV-2 infection in immunosuppressed patients with chronic disorders, including coronary heart disease, chronic kidney disease, and diabetes.[5–7]

As one of the most important healthcare problems, diabetes is estimated to affect 536.6 million people in 2021 and reach 783.2 million by 2045.[8] Diabetic foot ulcer (DFU) is a multifactorial clinical problem defined by the International Working Group on the Diabetic Foot as a set of symptoms secondary to current or previous diabetes, including skin ulceration, neuropathy, infection, or destruction of the lower extremities.[9,10] It is one of the most commonly observed and severe chronic complications of diabetes, with a global prevalence of 6.3%.[11] Recent advances in molecular and genetic studies have greatly expanded the scope of research in the prevention, diagnosis, and treatment of DFU. From a molecular perspective, studies have indicated that prolonged exposure to elevated glucose levels is associated with a significant alteration in the expression of numerous key mediators, including vascular endothelial growth factor, hypoxia-inducible factor 1, and proinflammatory cytokines such as tumor necrosis factor-α and interleukin (IL)-1, ultimately leading to impaired angiogenesis and chronic inflammation in diabetic wound healing.[12,13] Based on DNA sequencing technology, researchers have identify pivotal molecular factors essential for the wound healing process in DFU, such as IL-10, MMP-9, and NRF2.[14–16] The application of these molecules has been employed in synthesized nanoparticles and gene therapy, showing promising results for diabetes and diabetic wounds.[12] Although substantial progress has been made in genetic research, the current state of knowledge of DFU remains limited. Exploration into the specific molecular mechanisms underlying this debilitating condition has the potential to yield valuable insights that could aid in the development of novel therapeutics for effective clinical management.

Emerging evidence has suggested a high prevalence (33%–58%) of diabetes among critically ill COVID-19 patients, indicating a link between severe COVID-19 and diabetes.[17,18] At the same time, numerous studies have suggested that patients with diabetes are highly susceptible to COVID-19-induced adverse outcomes and complications.[19] Diabetic patients have a higher risk of developing more serious neuropathic complications from COVID-19 infection.[20] Due to exasperated inflammation and microcirculation damage, the amputation and mortality rate in DFU patients is seemingly higher during the COVID-19 pandemic.[21,22] On a molecular level, in vitro studies reveal that the SARS-CoV-2 viral particles gain primary entry in human cells by leveraging the angiotensin-converting enzyme 2 (ACE2) receptor, which is also a major player involved in the pathogenesis of diabetes.[23] Diabetes-associated ACE2 dysfunctions might have a role in viral activities of SARS-CoV-2.[24] As a result, diabetes may induce abnormal viral invasion leading to profound inflammatory response and aggravation of diabetic ulcerations. Nevertheless, the impact of COVID-19 on DFU patients is mostly studied in terms of their adverse reactions and mortality, with limited investigations into their molecular mechanisms to date. Explaining the intricate interplay between these two diseases is crucial in facilitating appropriate treatment approaches.

2 | MATERIALS AND METHODS

2.1 | Study design

The objective of this original research is to utilize bioinformatics approaches to investigate the shared molecular mechanism underlying COVID-19 and DFU, and to propose potential pharmaceuticals for therapy. The workflow diagram for our study is illustrated in Figure 1. In brief, using online transcriptome data sets, we performed functional assessment of COVID-19. The shared differential expressed genes (DEGs) between COVID-19 and DFU were analyzed for gene co-expression and transcription factors (TFs)- microRNAs (miRNAs)–mRNAs networks. In addition, we identified drug candidates for the combined therapy of COVID-19 and DFU.

2.2 | Data set collection

All transcriptome data were obtained from the National Center for Biotechnology Information database Gene Expression Omnibus.[25] We chose GSE161281 as the COVID-19 data set, which contained

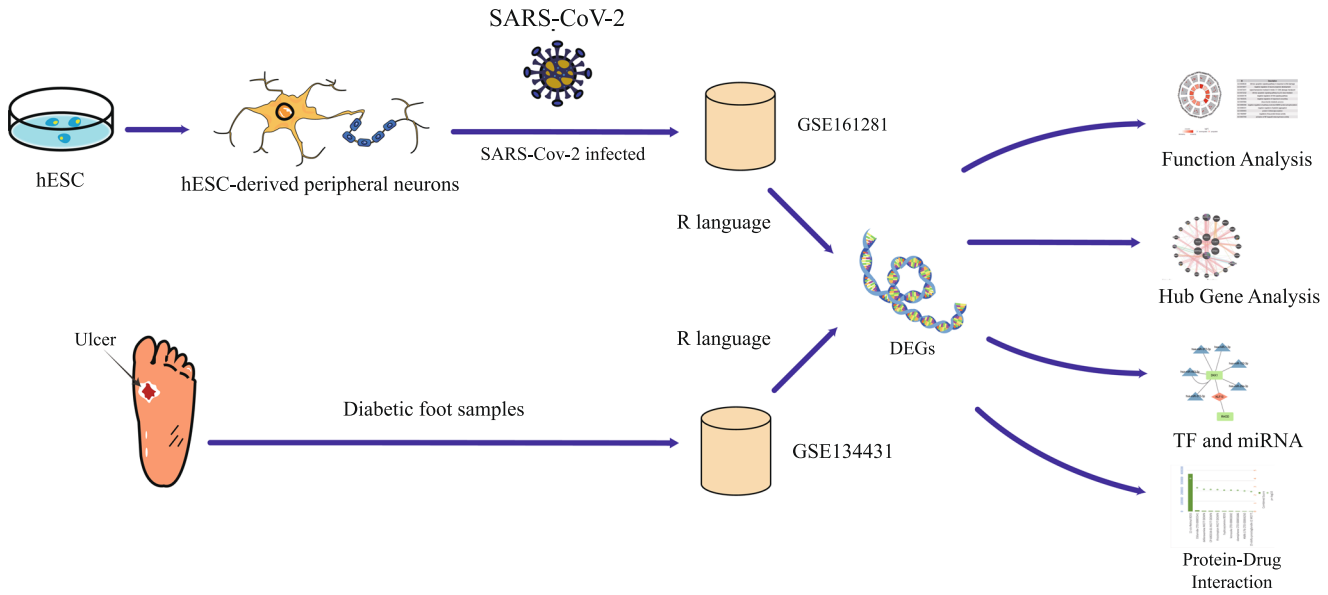


FIGURE 1 Workflow diagram of the present study.

SARS-CoV-2 infected human induced embryonic stem cell-derived peripheral neurons ($n = 2$) with uninfected controls ($n = 2$).[26] The GSE134431 was comprised of full-thickness DFU ($n = 13$) and diabetic foot skin ($n = 8$) samples from diabetic patients.[27]

2.3 | Functional enrichment analysis

We performed the gene ontology (GO) using the "clusterProfiler R" package including biological processes (BP), cellular components (CC), and molecular functions (MF). Kyoto encyclopedia of genes and genomes (KEGG) enrichment was performed using the KEGG Orthology-Based Annotation System.[28] The significant enrichment set at adjusted $p < 0.05$ and count ≥2.

2.4 | Identification of common DEGs between COVID-19 and DFU

We extracted the common DEGs between the COVID-19 data set GSE161281 and DFU data set GSE134431 using the limma package in R software, based on adjusted $p < 0.05$ and |log2 fold change (FC)| >1.0. Jvenn, a plug-in for the jQuery Javascript library, was used to plot the Venn diagram for DEGs visualization.

2.5 | Construction of TFs-miRNAs–mRNAs and gene co-expression network

In this study, the six common DEGs (mRNAs) were used to screen for their corresponding miRNAs and TFs in databases including starBase, TargetScan, miRTarBase, and Enrichr.[29–32] The predicted multifactor TFs-miRNAs–mRNAs regulatory network was constructed and visualized by Cytoscape.[33] To illustrate the gene co-expression network of our identified common DEGs, we performed the gene-gene interaction analysis in GeneMANIA, a database to search for gene lists with predict gene functions.[34]

2.6 | Analysis of target drugs

Using the Drug Signatures database (DSigDB) in online Enrichr server, we screened for candidate pharmaceuticals associated with hub genes. The searching criteria was set at $p < 0.05$ and combined score >100, and the top 10 candidate drugs were listed.

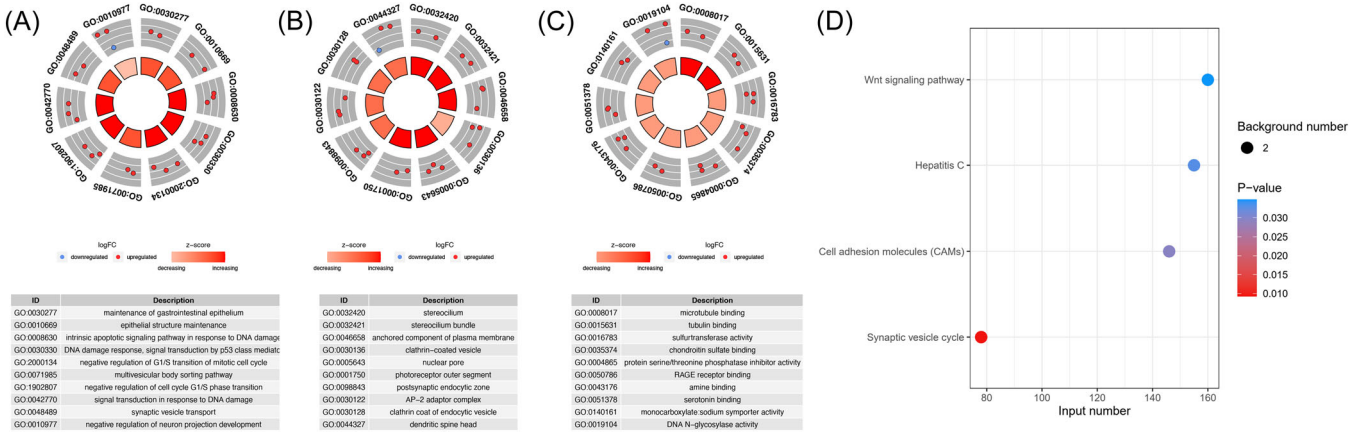
2.7 | Statistical analysis

R software (version 4.1.2) and GraphPad Prism (version 7.0) were applied for data processing. We used the Benjamini-Hochberg method was to calculate the adjusted p value. $p < 0.05$ was regarded as statistically significant.

3 | RESULTS

3.1 | Pathway enrichment analysis for COVID-19

To uncover the molecular features and enriched pathways underlying COVID-19, we performed the GO and KEGG analyses of the DEGs (Figure 2) in data set GSE161281, which contained SARS-CoV-2 infected human peripheral neurons with uninfected controls. Supporting Information S1: Table S1–S3 summarized the top 10



ID	Description
GO:0030277	maintenance of gastrointestinal epithelium
GO:0010669	epithelial structure maintenance
GO:0008630	intrinsic apoptotic signaling pathway in response to DNA damage
GO:0030330	DNA damage response, signal transduction by p53 class mediator
GO:2000134	negative regulation of G1/S transition of mitotic cell cycle
GO:0071985	multivesicular body sorting pathway
GO:1902807	negative regulation of cell cycle G1/S phase transition
GO:0042770	signal transduction in response to DNA damage
GO:0048489	synaptic vesicle transport
GO:0010977	negative regulation of neuron projection development

ID	Description
GO:0032420	stereocilium
GO:0032421	stereocilium bundle
GO:0046658	anchored component of plasma membrane
GO:0030136	clathrin-coated vesicle
GO:0005643	nuclear pore
GO:0001750	photoreceptor outer segment
GO:0098843	postsynaptic endocytic zone
GO:0030122	AP-2 adaptor complex
GO:0030128	clathrin coat of endocytic vesicle
GO:0044327	dendritic spine head

ID	Description
GO:0008017	microtubule binding
GO:0015631	tubulin binding
GO:0016783	sulfurtransferase activity
GO:0035374	chondroitin sulfate binding
GO:0004865	protein serine/threonine phosphatase inhibitor activity
GO:0050786	RAGE receptor binding
GO:0043176	amine binding
GO:0051378	serotonin binding
GO:0140161	monocarboxylate:sodium symporter activity
GO:0019104	DNA N-glycosylase activity

FIGURE 2 Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the differentially expressed genes (DEGs) in COVID-19. GO analysis including (A) biological process (BP), (B) cellular component (CC), and (C) molecular function (MF). (D) KEGG analysis.

terms in three categories including BP, CC, and MF. Among the subset of BP, these common DEGs mainly participated in epithelial structure maintenance, intrinsic apoptotic signaling pathway in response to DNA damage, signal transduction by p53 class mediator, negative regulation of G1/S transition of mitotic cell cycle, multivesicular body sorting pathway, negative regulation of cell cycle G1/S phase transition, signal transduction in response to DNA damage, synaptic vesicle transport, and negative regulation of neuron projection development (Figure 2A, Supporting Information S1: Table S1). As for CC, these DEGs were enriched in stereocilium, anchored component of plasma membrane, clathrin-coated vesicle, nuclear pore, photoreceptor outer segment, postsynaptic endocytic zone, AP-2 adaptor complex, and dendritic spine head (Figure 2B, Supporting Information S1: Table S2). Besides, MF was primarily focused on the microtubule binding, tubulin binding, sulfurtransferase activity, chondroitin sulfate binding, protein serine/threonine phosphatase inhibitor activity, RAGE receptor binding, amine binding, serotonin binding, monocarboxylate: sodium symporter activity, and DNA N-glycosylase activity (Figure 2C, Supporting Information S1: Table S3). Furthermore, KEGG revealed that theses DEGs were mostly enriched in Wnt signaling pathway, hepatitis C, cell adhesion molecules, and synaptic vesicle cycle (Figure 2D, Supporting Information S1: Table S4).

3.2 | Identification of shared DEGs between COVID-19 and DFU

A total of 135 DEGs were obtained from the COVID-19 data set GSE161281, including 123 upregulated and 12 downregulated genes. The DFU data set GSE134431 contained 3055 DEGs, of which 1150 genes were upregulated and 1905 were downregulated. The Venn diagram of DEGs between COVID-19 and DFU is shown in Figure 3, wherein six shared DEGs (*dickkopf-related protein 1 [DKK1], serine proteinase inhibitor A3 [SERPINA3], ras homolog family member D [RHOD], myelin protein zero like 3 [MPZL3], Claudin-11 [CLDN11], and epidermal growth factor receptor pathway substrate 8-like 1 [EPS8L1]*) were acquired, accounting for 0.19% of the total 3184 DEGs.

3.3 | Gene co-expression and TFs-miRNAs–mRNAs network

Based on our six common DEGs between COVID-19 and DFU, we built a gene co-expression network to decipher their correlations among co-expression, physical interactions, prediction, and genetic interactions (Figure 4). Altogether, we identified 20 predicted genes which were strongly correlated with our shared DEGs. Functionally, all these interactive genes participated in the negative regulation of Wnt signaling pathway, pattern specification process, regulation of canonical Wnt signaling pathway, positive regulation of T cell activation, positive regulation of leukocyte cell–cell adhesion, and cell–cell adhesion mediated by integrin.

TFs and miRNAs play an essential role in transcriptional and posttranscriptional level of gene expression.[35] To understand the regulation of gene expression, we analyzed the TF-miRNA network with predicted interactions with our extracted common DEGs. Figure 5 demonstrated the TFs-miRNAs–mRNAs regulatory network containing six miRNAs (has-miR-373-3p, has-miR-493-3p, has-miR-372-3p, has-miR-1-3p, has-miR-152-3p, and has-miR-29a-3p) and one TFs (KLF13) that interacted with *DKK1* and *RHOD*, which were designated as hub genes. Notably, *DKK1* was at the center among this interactive network.

3.4 | Recognition of candidate drugs

We uploaded the hub genes (*DKK1* and *RHOD*) to the DSigDB database in Enrichr server to explore the pharmaceutical compounds that could potentially affect the disease transformation of COVID-19 and DFU. We determined the top 10 therapeutic molecules according to their combined scores, including 11-cis-retinal,

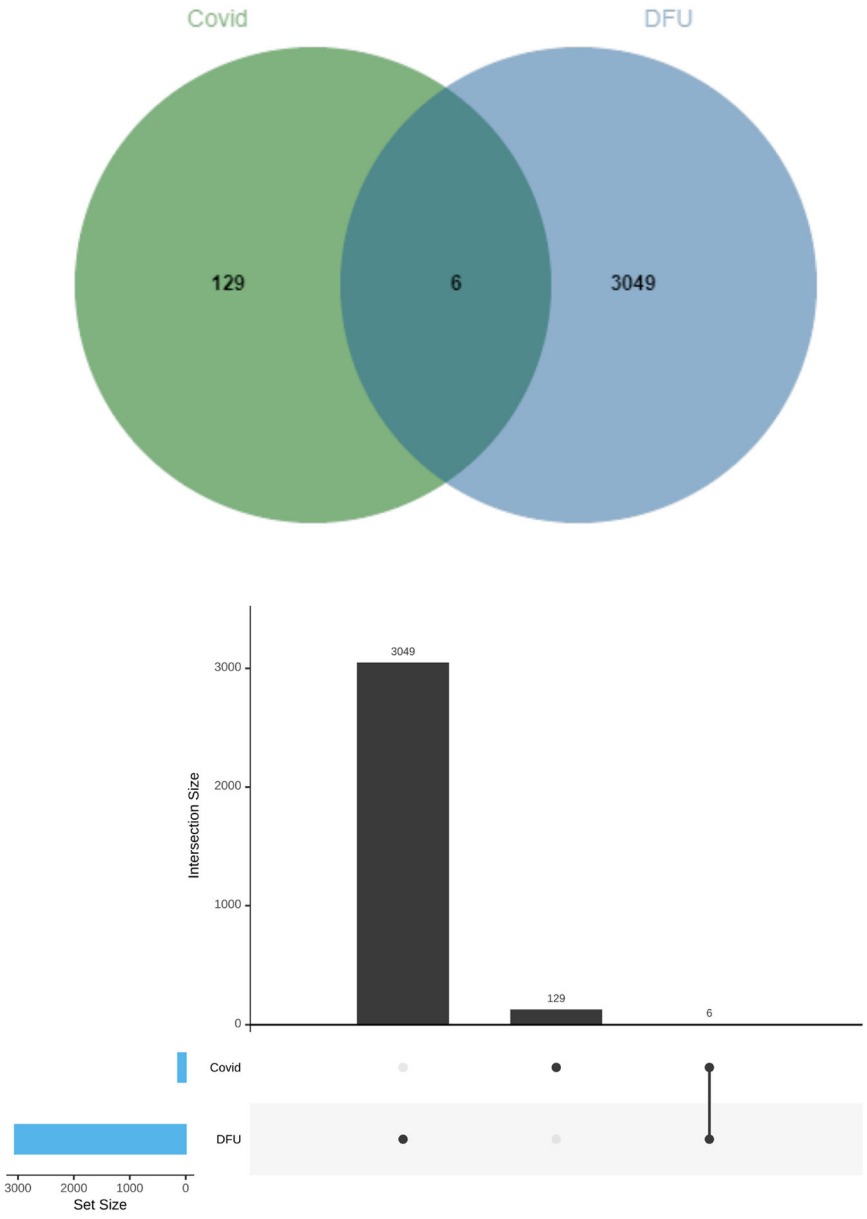


FIGURE 3 Venn diagram identified an overlap of six common differentially expressed genes (DEGs) between COVID-19 and diabetic foot ulcer (DFU).

etilamide, deferoxamine, CP-690334-01, thiostrepton, hydroxylamine, letrozole, clomiphene, HMN-176, and 15-delta prostaglandin (Figure 6, Supporting Information S1: Table S5).

4 | DISCUSSION

SARS-CoV-2 infected patients could develop or show worsening of diabetic complications such as DFU, which is characterized by neuropathic dysfunction in the lower extremities.[20,23] Although many research have proposed the mechanistic hypothesis for DFU in association with COVID-19, a more precise nature of their intrinsic relationship is yet to be elucidated.[36,37] Understanding how COVID-19 affects DFU is of great help to develop new strategies to delay the disease progression. In the present work, we tried to uncover the shared pathogenesis and regulatory gene targets of COVID-19 and DFU. We identified their common DEGs and revealed collective pathways participated in the development of COVID-19 and DFU. Based on the selected hub genes, we established the gene co-expression network and TFs-miRNAs–mRNAs regulatory network and performed the candidate drug analysis. As far as we are concerned, the present work is the first to demonstrated the shared molecular mechanism and gene targets in COVID-19 and DFU.

Our work identified six common DEGs including *DKK1*, *RHOD*, *SERPINA3*, *CLDN11*, *MPZL3*, and *EPS8L1*. Among them, *DKK1* and *RHOD* were determined as hub genes. DKK1 is a Wnt signaling inhibitor. Growing evidence has shown its regulatory role in the metabolic process of diabetes, representing a novel therapeutic target for diabetes.[38] Intriguingly, a recent study reported that serum levels of DKK1 can predict disease outcomes in patients with COVID-19. It is revealed that the metabolic signature associated with SARS-CoV-2 infection was mirrored by circulating DKK1

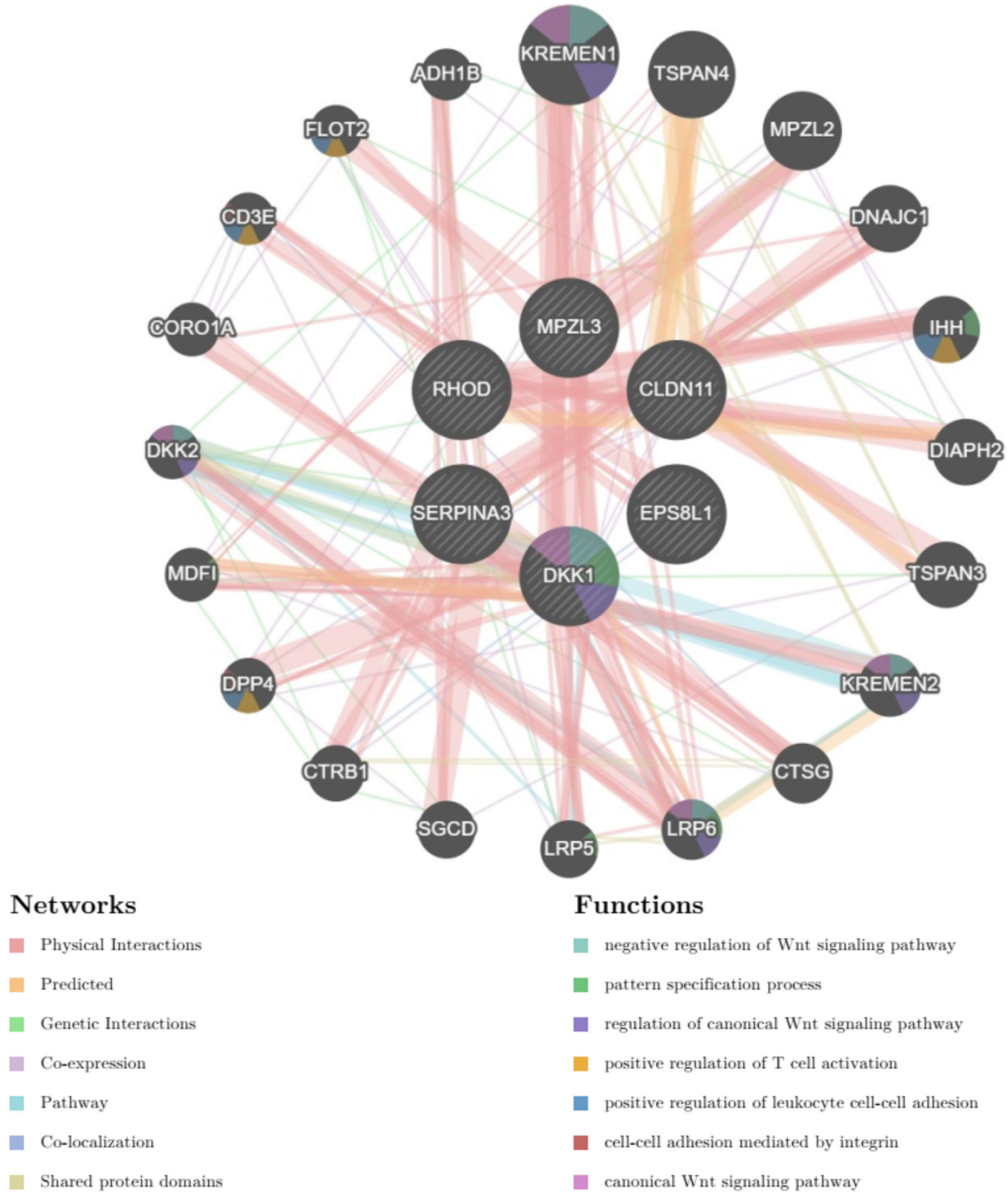


FIGURE 4 Common differentially expressed genes (DEGs) and their co-expression genes analyzed via GeneMANIA.

abundance.[39] DKK1 may also contributed to deficits in β-cell function and increased glucose toxicity leading to the development of diabetic inflammatory state and complications.[40] RhoD, encoded by *RHOD*, belongs to the Rho GTPase family, which are essential regulators of basic cellular processes, including actin dynamics and membrane trafficking.[41] Using high throughput microarray techniques, researchers have identified *RHOD* as one of the distinct gene signatures for diabetes and diabetic nephropathy.[42] Interestingly, RhoD may also

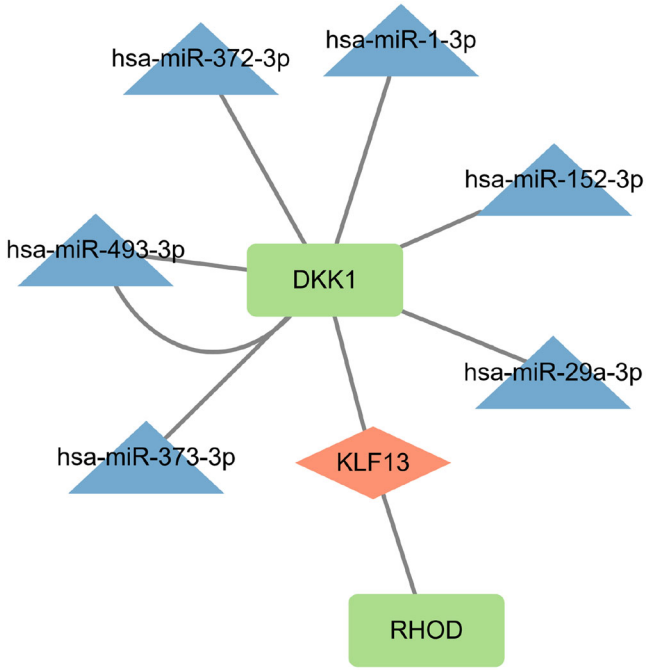


FIGURE 5 Gene regulatory network integrating the hub genes (mRNAs), microRNAs (miRNAs), and transcription factors (TFs). The green rectangles represent for hub genes (mRNAs), blue triangles for miRNAs, and red diamonds for TFs.

participate in virus infection by regulating RhoC-ROCK-dependent cell contraction.[43] SERPINA3 has been found to be involved in various physiological functions including apoptosis, extracellular matrix remodeling, and wound healing.[44] Through plasma proteome analysis, a recent proteomics and machine learning study revealed SERPINA3 as a putative protein biomarker for predicting the COVID-19 severity progression.[45] Similarly, by comparing host and SARS-CoV-2 RNA sequencing data, researchers highlighted that *SERPINA3* was specifically altered in SARS-CoV-2-infected respiratory cells, showing its potential as a therapeutic target to reduce the severity of COVID-19.[46] Various studies also indicated that *SERPINA3* participated in the progression of diabetes and different complications.[47,48] CLDN11 is a junctional protein associated with epithelial-mesenchymal transition and cell–cell adhesion.[49] Members of the claudin family have been found to act in the entry process after virus infection and binding.[50,51] Reportedly, SARS-CoV-2 infection can induce the disruption of CLDN11 proteins on the male reproductive system through the release of proinflammatory cytokines.[52] As for *MPZL3* and *EPS8L1*, they have been studied in several chronic inflammatory diseases, such as obesity, dermatitis, and chronic obstructive pulmonary disease.[53–55] Taken together, our results demonstrated the promising role of these genetic biomarkers, especially *DKK1* and *RHOD*, for the targeting of COVID-19 infection complicated with DFU.

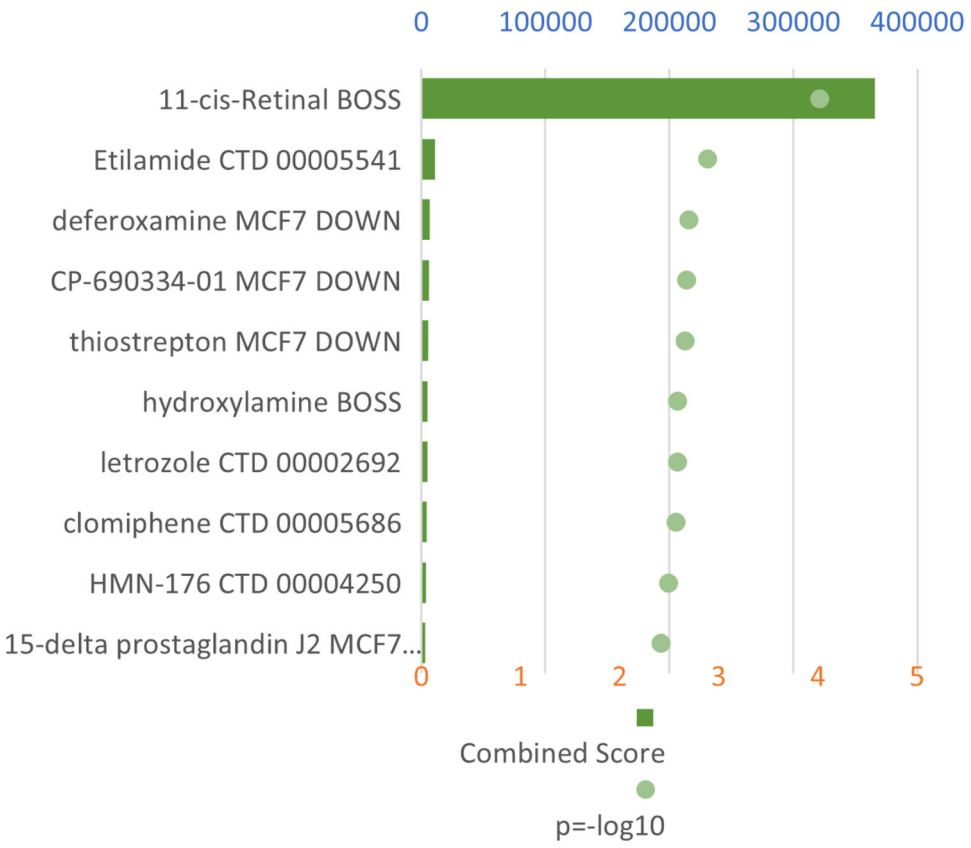


FIGURE 6 Analysis of top 10 candidate drugs associated with hub genes between COVID-19 and diabetic foot ulcer (DFU).

Growing studies have demonstrated the essential role of TFs and miRNAs in the development of diabetes and its related complications.[56,57] In our study, a TFs-miRNAs–mRNAs regulatory network was constructed which contained six miRNAs (has-miR-373-3p, has-miR-493-3p, has-miR-372-3p, has-miR-152-3p, has-miR-1-3p, and has-miR-29a-3p) and 1 TFs (Kruppel-like factor 13 [KLF13]). KLF13 is an essential player in cell proliferation and differentiation, and has a critical role in the virus life cycle.[58,59] It is critical for the activation of the HPV, leading to the amplification of viral genome and expression of late viral genes.[58] Notably, KLF13 is also involved in the neuropathology of diet-induced obesity and has a possible link with diabetes.[60] As for miRNAs, expression of has-miR-1-3p and has-miR-29a-3p were found in immune-related pathways in cardiomyopathy and cancer.[61,62] Nevertheless, their regulatory role in DFU and COVID-19 has been limited to date. Further studies are warranted to elucidate the role of these potential TFs and miRNAs targets to generate better therapies for DFU patients complicated with COVID-19.

SARS-CoV-2 infection can greatly affect the clinical course of DFU, leading to delayed wound healing and even increased risk of amputation and mortality. From a clinical perspective, it is important to discover novel therapeutic agents to initiate more effective strategies for the combined treatment of COVID-19 and DFU. In our work, we identified potential pharmaceuticals based on the hub genes, including 11-cis-retinal, etilamide, deferoxamine, CP-690334-01, thiostrepton, hydroxylamine, letrozole, clomiphene, HMN-176, and 15-delta prostaglandin. The use of vitamin A and its derivative 11-cis-retinal in COVID-19 therapy has been extensively researched in recent literature.[63] Clinically, their protective immunomodulatory and antiviral effects can be of great help in adverse events related to COVID-19, such as ARDS.[64,65] Much evidence has also accumulated that 11-cis-retinal plays a vital role in diabetic metabolism.[66] This antioxidant can improve oxidative stress in the onset and progression of diabetes, demonstrating its ability as a viable treatment option.[67] Deferoxamine, an iron chelator, is beneficial in alleviating COVID-19 by reducing macrophage-derived cytotoxicity and supplementing antioxidant capacity.[68] Deferoxamine is also reported to facilitate diabetic wound healing by mitigating inflammatory response and enhancing angiogenesis and re-epithelialization.[69] The therapeutic potential of these agents, particularly 11-cis-retinal and deferoxamine, warrants further chemical and clinical assessments in COVID-19 patients with DFU.

The high incidence and intractability of DFU bears significant economic burdens in terms of reduced productivity and increased healthcare expenses. Appropriate and prompt treatment of DFU is critical in reducing infection, hospitalization, and amputation, all of which should be based on the knowledge of DFU pathogenesis. Our bioinformatics results represented a good starting point for exploring key biomarkers for the molecular monitoring in the development of DFU. Given that diabetic patients are at higher risk of mortality from COVID-19, our results provided a reliable foundation for future clinical translational research and served as a resourceful guide for healthcare professionals.

5 | LIMITATIONS

It is important to acknowledge certain limitations in the present work. Specifically, the transcriptome data were derived from a single COVID-19 and DFU data set, with limited sample sizes. Future larger-scale RNA-seq studies are needed to verify the results. In addition, the identified common genes and drug candidates require clinical validation as part of the drug discovery process, which will be the focus of our future investigations.

6 | CONCLUSIONS

In this study, bioinformatics approaches were performed to uncover the common DEGs and mutual pathogenesis of COVID-19 and DFU based on two data sets. A total of six common DEGs (*DKK1*, *SERPINA3*, *RHOD*, *MPZL3*, *CLDN11*, and *EPS8L1*) were found. Specifically, *DKK1* and *RHOD* were found to be significant hub genes. The gene co-expression network indicated the shared pathways contributed to the development of DFU and progression of COVID-19. The TFs-miRNAs–mRNAs regulatory network was constructed, followed by a detailed analysis of potential drugs. Our findings provide a profound understanding of the molecular interactions between DFU and COVID-19. Further investigations are necessary to elucidate the underlying molecular mechanisms that may contribute to the development of more effective treatments.

AUTHOR CONTRIBUTIONS

Xueyao Cai: Data curation; investigation; methodology; resources; writing—original draft. **Ruijin Yang**: Data curation; investigation; methodology; writing—review and editing. **Wenjun Shi**: Writing—review and editing. **Yuchen Cai**: Conceptualization; supervision; validation; writing—review and editing. **Zhengzheng Ma**: Project administration; supervision.

ACKNOWLEDGMENTS

We thank the GEO database for the transcriptome data.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All authors have read and endorsed the definitive version of the manuscript. The corresponding author possessed comprehensive access to all data within this study and bears full responsibility for both the data's integrity and the accuracy of the data analysis. The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

TRANSPARENCY STATEMENT

The lead authors Yuchen Cai and Zhengzheng Ma affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have

been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

ORCID

Yuchen Cai http://orcid.org/0000-0002-7536-906X
Zhengzheng Ma http://orcid.org/0009-0004-2274-8746

REFERENCES

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5
2. WHO Coronavirus (COVID-19) Dashboard | WHO Coronavirus (COVID-19) Dashboard With Vaccination Data. [cited 2023 Oct 2]. Available from: https://covid19.who.int/
3. Huang C, Huang L, Wang Y, et al. RETRACTED: 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet*. 2021;397(10270):220-232. doi:10.1016/S0140-6736(20)32656-8
4. Iacobucci G. Covid-19: runny nose, headache, and fatigue are commonest symptoms of omicron, early data show. *BMJ*. 2021;375:n3103. doi:10.1136/bmj.n3103
5. Liang C, Zhang W, Li S, Qin G. Coronary heart disease and COVID-19: a meta-analysis. *Med Clin (Barc)*. 2021;156(11):547-554. doi:10.1016/j.medcli.2020.12.017
6. Bruchfeld A. The COVID-19 pandemic: consequences for nephrology. *Nat Rev Nephrol*. 2021;17(2):81-82. doi:10.1038/s41581-020-00381-4
7. Wu J, Zhang J, Sun X, et al. Influence of diabetes mellitus on the severity and fatality of SARS-CoV-2 (COVID-19) infection. *Diabetes, Obes Metab*. 2020;22(10):1907-1914. doi:10.1111/dom.14105
8. Ogurtsova K, Guariguata L, Barengo NC, et al. IDF diabetes Atlas: global estimates of undiagnosed diabetes in adults for 2021. *Diabetes Res Clin Pract*. 2022;183:109118. doi:10.1016/j.diabres.2021.109118
9. Schaper NC, Van Netten JJ, Apelqvist J, Bus SA, Hinchliffe RJ, Lipsky BA. Practical guidelines on the prevention and management of diabetic foot disease (IWGDF 2019 update). *Diabetes Metab Res Rev*. 2020;36(S1):e3266. doi:10.1002/dmrr.3266
10. Schaper NC, Van Netten JJ, Apelqvist J, et al. Practical guidelines on the prevention and management of diabetes-related foot disease (IWGDF 2023 update). *Diabetes Metab Res Rev*. 2023:e3657. doi:10.1002/dmrr.3657
11. Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. *Ann Med*. 2017;49(2):106-116. doi:10.1080/07853890.2016.1231932
12. Mariadoss AVA, Sivakumar AS, Lee CH, Kim SJ. Diabetes mellitus and diabetic foot ulcer: etiology, biochemical and molecular based treatment strategies via gene and nanotherapy. *Biomed Pharmacother*. 2022;151:113134. doi:10.1016/j.biopha.2022.113134
13. Patel M, Patel V, Shah U, Patel A. Molecular pathology and therapeutics of the diabetic foot ulcer; comprehensive reviews. *Arch Physiol Biochem*. 2023:1-8. doi:10.1080/13813455.2023.2219863
14. Gan MS, Yang B, Fang DL, Wu BL. IL-1B can serve as a healing process and is a critical regulator of diabetic foot ulcer. *Ann Transl Med*. 2022;10(4):179. doi:10.21037/atm-22-75
15. Singh K, Agrawal NK, Gupta SK, Mohan G, Chaturvedi S, Singh K. Differential expression of matrix Metalloproteinase-9 gene in wounds of type 2 diabetes mellitus cases with susceptible -1562C>T genotypes and wound severity. *Int J Low Extrem Wounds*. 2014;13(2):94-102. doi:10.1177/1534734614534980
16. Teena R, Dhamodharan U, Jayasuriya R, Ali D, Kesavan R, Ramkumar KM. Analysis of the exonic single nucleotide polymorphism rs182428269 of the NRF2 gene in patients with diabetic foot ulcer. *Arch Med Res*. 2021;52(2):224-232. doi:10.1016/j.arcmed.2020.10.011
17. Arentz M, Yim E, Klaff L, et al. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington state. *JAMA*. 2020;323(16):1612-1614. doi:10.1001/jama.2020.4326
18. Bhatraju PK, Ghassemieh BJ, Nichols M, et al. Covid-19 in critically ill patients in the Seattle region—case series. *N Engl J Med*. 2020;382(21):2012-2022. doi:10.1056/NEJMoa2004500
19. Feldman EL, Savelieff MG, Hayek SS, Pennathur S, Kretzler M, Pop-Busui R. COVID-19 and diabetes: a collision and collusion of two diseases. *Diabetes*. 2020;69(12):2549-2565. doi:10.2337/dbi20-0032
20. Pitocco D, Viti L, Santoliquido A, et al. Diabetic neuropathy: a risk factor for severe COVID-19? *Acta Diabetol*. 2021;58(5):669-670. doi:10.1007/s00592-020-01658-7
21. Du C, Li Y, Xie P, et al. The amputation and mortality of inpatients with diabetic foot ulceration in the COVID-19 pandemic and postpandemic era: a machine learning study. *Int Wound J*. 2021;19(6):1289-1297. doi:10.1111/iwj.13723
22. Chen D, Zhou H, Yang Y, Zhang Y, Xie C. The adverse effects of novel coronavirus on diabetic foot patients. *Medicine*. 2020;99(43):e22758. doi:10.1097/MD.0000000000022758
23. Lim S, Bae JH, Kwon HS, Nauck MA. COVID-19 and diabetes mellitus: from pathophysiology to clinical management. *Nat Rev Endocrinol*. 2021;17(1):11-30. doi:10.1038/s41574-020-00435-4
24. Nassar M, Daoud A, Nso N, et al. Diabetes mellitus and COVID-19: review article. *Diabetes Metab Syndr*. 2021;15(6):102268. doi:10.1016/j.dsx.2021.102268
25. Barrett T, Troup DB, Wilhite SE, et al. NCBI GEO: mining tens of millions of expression profiles--database and tools update. *Nucleic Acids Res*. 2007;35(Database):D760-D765. doi:10.1093/nar/gkl887
26. Lyoo KS, Kim HM, Lee B, et al. Direct neuronal infection of SARS-CoV-2 reveals cellular and molecular pathology of chemosensory impairment of COVID-19 patients. *Emerg Microbes Infect*. 2022;11(1):407-412. doi:10.1080/22221751.2021.2024095
27. Sawaya AP, Stone RC, Brooks SR, et al. Deregulated immune cell recruitment orchestrated by FOXM1 impairs human diabetic wound healing. *Nat Commun*. 2020;11(1):4678. doi:10.1038/s41467-020-18276-0
28. Bu D, Luo H, Huo P, et al. KOBAS-I: intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. *Nucleic Acids Res*. 2021;49(W1):W317-W325. doi:10.1093/nar/gkab447
29. Xie Z, Bailey A, Kuleshov MV, et al. Gene Set Knowledge Discovery with Enrichr. 51.
30. Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein–RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res*. 2014;42(D1):D92-D97. doi:10.1093/nar/gkt1248
31. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *eLife*. 2015;4:e05005. doi:10.7554/eLife.05005
32. Huang HY, Lin YCD, Cui S, et al. miRTarBase update 2022: an informative resource for experimentally validated miRNA–target interactions. *Nucleic Acids Res*. 2022;50(D1):D222-D230. doi:10.1093/nar/gkab1079
33. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498-2504. doi:10.1101/gr.1239303

34. Franz M, Rodriguez H, Lopes C, et al. GeneMANIA update 2018. *Nucleic Acids Res*. 2018;46(W1):W60-W64. doi:10.1093/nar/gky311
35. Le TD, Liu L, Zhang J, Liu B, Li J. From miRNA regulation to miRNA-TF co-regulation: computational approaches and challenges. *Brief Bioinform*. 2015;16(3):475-496. doi:10.1093/bib/bbu023
36. Papanas N, Papachristou S. COVID-19 and diabetic foot: will the lamp burn bright? *Int J Low Extrem Wounds*. 2020;19(2):111. doi:10.1177/1534734620921382
37. Odriozola A, Ortega L, Martinez L, et al. Widespread sensory neuropathy in diabetic patients hospitalized with severe COVID-19 infection. *Diabetes Res Clin Pract*. 2021;172:108631. doi:10.1016/j.diabres.2020.108631
38. Ali H, Zmuda JM, Cvejkus RK, et al. Wnt pathway inhibitor DKK1: a potential novel biomarker for adiposity. *J Endocr Soc*. 2019;3(2):488-495. doi:10.1210/js.2018-00325
39. Jaschke NP, Funk AM, Jonas S, et al. Circulating Dickkopf1 parallels metabolic adaptations and predicts disease trajectories in patients with Covid-19. *J Clin Endocrinol Metab*. 2022;107(12):3370-3377. doi:10.1210/clinem/dgac514
40. Al-Hakeim HK, Al-Kaabi QJ, Maes M. High mobility group box 1 and Dickkopf-related protein 1 as biomarkers of glucose toxicity, atherogenicity, and lower β cell function in patients with type 2 diabetes mellitus. *Growth Factors*. 2022;40(5-6):240-253. doi:10.1080/08977194.2022.2126317
41. Gad AKB, Aspenström P. Rif proteins take to the RhoD: Rho GTPases at the crossroads of actin dynamics and membrane trafficking. *Cell Signal*. 2010;22(2):183-189. doi:10.1016/j.cellsig.2009.10.001
42. Guttula S, Rao A, Sridhar G, Chakravarthy M, Nageshwararo K, Rao P. Cluster analysis and phylogenetic relationship in biomarker identification of type 2 diabetes and nephropathy. *Int J Diabetes Dev Ctries*. 2010;30(1):52-56. doi:10.4103/0973-3930.60003
43. Durkin CH, Leite F, Cordeiro JV, et al. RhoD inhibits RhoC-ROCK-dependent cell contraction via PAK6. *Dev Cell*. 2017;41(3):315-329. doi:10.1016/j.devcel.2017.04.010
44. Aslam MS, Yuan L. Serpina3n: potential drug and challenges, mini review. *J Drug Target*. 2020;28(4):368-378. doi:10.1080/1061186X.2019.1693576
45. Suvarna K, Biswas D, Pai MGJ, et al. Proteomics and machine learning approaches reveal a set of prognostic markers for COVID-19 severity with drug repurposing potential. *Front Physiol*. 2021;12:652799. doi:10.3389/fphys.2021.652799
46. Ferrarini MG, Lal A, Rebollo R, et al. Genome-wide bioinformatic analyses predict key host and viral factors in SARS-CoV-2 pathogenesis. *Commun Biol*. 2021;4(1):590. doi:10.1038/s42003-021-02095-0
47. Takahashi E, Okumura A, Unoki-Kubota H, Hirano H, Kasuga M, Kaburagi Y. Differential proteome analysis of serum proteins associated with the development of type 2 diabetes mellitus in the KK-A(y) mouse model using the iTRAQ technique. *J Proteomics*. 2013;84:40-51. doi:10.1016/j.jprot.2013.03.014
48. Zhou W, Liu Y, Hu Q, Zhou J, Lin H. The landscape of immune cell infiltration in the glomerulus of diabetic nephropathy: evidence based on bioinformatics. *BMC Nephrol*. 2022;23(1):303. doi:10.1186/s12882-022-02906-4
49. Hashimoto I, Oshima T. Claudins and gastric cancer: an overview. *Cancers*. 2022;14(2):290. doi:10.3390/cancers14020290
50. Evans MJ, von Hahn T, Tscherne DM, et al. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature*. 2007;446(7137):801-805. doi:10.1038/nature05654
51. Zheng A, Yuan F, Li Y, et al. Claudin-6 and claudin-9 function as additional coreceptors for hepatitis C virus. *J Virol*. 2007;81(22):12465-12471. doi:10.1128/JVI.01457-07
52. Peirouvi T, Aliaghaei A, Eslami Farsani B, et al. COVID-19 disrupts the blood–testis barrier through the induction of inflammatory cytokines and disruption of junctional proteins. *Inflamm Res*. 2021;70(10-12):1165-1175. doi:10.1007/s00011-021-01497-4
53. Wikramanayake TC, Borda LJ, Kirsner RS, et al. Loss of MPZL3 function causes seborrhoeic dermatitis-like phenotype in mice. *Exp Dermatol*. 2017;26(8):736-738. doi:10.1111/exd.13150
54. Worley BL, Auen T, Arnold AC, Monia BP, Hempel N, Czyzyk TA. Antisense oligonucleotide-mediated knockdown of Mpzl3 attenuates the negative metabolic effects of diet-induced obesity in mice. *Physiol Rep*. 2021;9(9):e14853. doi:10.14814/phy2.14853
55. de Vries M, van der Plaat DA, Vonk JM, Boezen HM. No association between DNA methylation and COPD in never and current smokers. *BMJ Open Respir Res*. 2018;5(1):e000282. doi:10.1136/bmjresp-2018-000282
56. Saxena A, Mathur N, Tiwari P, Mathur SK. Whole transcriptome RNA-seq reveals key regulatory factors involved in type 2 diabetes pathology in peripheral fat of Asian Indians. *Sci Rep*. 2021;11(1):10632. doi:10.1038/s41598-021-90148-z
57. Kiyanpour F, Abedi M, Gheisari Y. A systematic integrative approach reveals novel microRNAs in diabetic nephropathy. *J Res Med Sci*. 2020;25:1. doi:10.4103/jrms.JRMS_289_19
58. Zhang W, Hong S, Maniar KP, et al. KLF13 regulates the differentiation-dependent human papillomavirus life cycle in keratinocytes through STAT5 and IL-8. *Oncogene*. 2016;35(42):5565-5575. doi:10.1038/onc.2016.97
59. Bao S, Zhang C, Aweya JJ, et al. KLF13 induces apoptotic cell clearance in *Penaeus vannamei* as an essential part of shrimp innate immune response to pathogens. *Dev Comp Immunol*. 2022;126:104242. doi:10.1016/j.dci.2021.104242
60. Wiemerslage L, Islam R, van der Kamp C, et al. A DNA methylation site within the KLF13 gene is associated with orexigenic processes based on neural responses and ghrelin levels. *Int J Obes*. 2017;41(6):990-994. doi:10.1038/ijo.2017.43
61. Qin X, Huang L, Chen S, et al. Multi-factor regulatory network and different clusters in hypertrophic obstructive cardiomyopathy. *BMC Med Genomics*. 2021;14:199. doi:10.1186/s12920-021-01036-4
62. Lv Q, Zou H, Xu Y, et al. Expression levels of chemokine (C-X-C motif) ligands CXCL1 and CXCL3 as prognostic biomarkers in rectal adenocarcinoma: evidence from gene expression omnibus (GEO) analyses. *Bioengineered*. 2021;12(1):3711-3725. doi:10.1080/21655979.2021.1952772
63. Stephensen CB, Lietz G. Vitamin A in resistance to and recovery from infection: relevance to SARS-CoV2. *Br J Nutr*. 2021;126(11):1663-1672. doi:10.1017/S0007114521000246
64. Tepasse PR, Vollenberg R, Fobker M, et al. Vitamin A plasma levels in COVID-19 patients: a prospective multicenter study and hypothesis. *Nutrients*. 2021;13(7):2173. doi:10.3390/nu13072173
65. Midha IK, Kumar N, Kumar A, Madan T. Mega doses of retinol: a possible immunomodulation in Covid-19 illness in resource-limited settings. *Rev Med Virol*. 2021;31(5):e2204. doi:10.1002/rmv.2204
66. Blaner WS. Vitamin A signaling and homeostasis in obesity, diabetes, and metabolic disorders. *Pharmacol Ther*. 2019;197:153-178. doi:10.1016/j.pharmthera.2019.01.006
67. Dakshinamurti K. Vitamins and their derivatives in the prevention and treatment of metabolic syndrome diseases (diabetes). *Can J Physiol Pharmacol*. 2015;93(5):355-362. doi:10.1139/cjpp-2014-0479
68. Poonkuzhi Naseef P, Elayadeth-Meethal M, Mohammed Salim KT, et al. Therapeutic potential of induced iron depletion using iron chelators in Covid-19. *Saudi J Biol Sci*. 2022;29(4):1947-1956. doi:10.1016/j.sjbs.2021.11.061
69. Gao SQ, Chang C, Li JJ, et al. Co-delivery of deferoxamine and hydroxysafflor yellow A to accelerate diabetic wound healing via

enhanced angiogenesis. *Drug Delivery*. 2018;25(1):1779-1789. doi:10.1080/10717544.2018.1513608

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Cai X, Yang R, Shi W, Cai Y, Ma Z. Exploration of the common pathogenic link between COVID-19 and diabetic foot ulcers: an in silico approach. *Health Sci Rep*. 2023;6:e1686. doi:10.1002/hsr2.1686