Am J Transl Res 2025;17(3):2339-2351
www.ajtr.org /ISSN:1943-8141/AJTR0164080

Original Article

Mechanistic insights into COVID-19 mRNA vaccine-associated myocarditis: a bioinformatics analysis

Yanjie Jiang[1,2*], Yaxue Xie[1*], Yingnan You[1,3*], Bo Han[1,3]

[1]*Department of Pediatrics, Shandong Provincial Hospital, Shandong University, Jinan, Shandong, China;* [2]*Department of Pediatrics, The Affiliated Taian City Central Hospital of Qingdao University, Tai'an, Shandong, China;* [3]*Department of Pediatrics, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, China.* [*]*Equal contributors.*

Received February 21, 2025; Accepted March 8, 2025; Epub March 15, 2025; Published March 30, 2025

Abstract: Purpose: While COVID-19 vaccination offers significant public health benefits, it also has potential risks, such as myocarditis. The mechanisms underlying myocarditis after COVID-19 vaccination remain poorly understood. The purpose of this study was to identify potential pathogenic genes and molecular pathways related with COVID-19 mRNA vaccine-associated myocarditis. Methods: Differentially expressed genes (DEGs) were analyzed from a fulminant myocarditis (FM) cohort and a COVID-19 mRNA vaccination dataset. Shared DEGs were intersected, followed by functional enrichment, protein-protein interaction (PPI) network construction, and hub gene identification. Transcriptional and miRNA regulatory networks, as well as therapeutic drug predictions were also performed. Results: Eighty shared DEGs were identified by interacting DEGs from the FM cohort and the vaccination cohort, we identified. Functional enrichment analysis revealed that DEGs are significantly involved in immune cell-mediated responses, highlighting the critical role of immune dysregulation. PPI network analysis revealed three hub genes (CXCR3, NKG7, and GZMH), which may be involved in the pathogenesis of vaccine-associated myocarditis. Furthermore, transcriptional networks highlighted TBX21 and STAT4 as key regulators of all hub genes, while hsa-mir-146a-5p targeted CXCR3 and NKG7. PhIP, a compound targeting CXCR3 and NKG7, emerged as a potential therapeutic candidate. Conclusion: This study implicates immune dysregulation driven by CXCR3, NKG7, and GZMH in post-vaccination myocarditis, supported by regulatory networks and therapeutic insights using a bioinformatics analysis. These findings advance mechanistic understanding of this rare adverse event and propose potential treatment strategy for further investigation.

Keywords: COVID-19 mRNA vaccination, myocarditis, bioinformatics analysis, protein-protein interaction network, BNT162b2

Introduction

International efforts to promote vaccination are crucial for restoring global health and facilitating social recovery as the COVID-19 pandemic persists. Vaccination remains one of the most effective strategies to combat COVID-19 [1]. As of December 2, 2024, more than 13.64 billion doses of COVID-19 vaccines have been administered worldwide (https://covid19.who.int/). While several vaccines have successfully passed phase III clinical trials, rare adverse events often emerge only during post-marketing surveillance in real-world settings. Among these, myocarditis has been identified as a rare but significant adverse reaction associated with the COVID-19 vaccination. In individuals aged 5-39 years, the incidence of myocarditis was approximately 1 per 200,000 doses after the first dose, 1 per 30,000 doses after the second dose of the primary series, and 1 per 50,000 doses following the first booster [2]. Notably, compared to the reference population (data from the previous three years), the mortality rate ratios for myocarditis were significantly higher among those who received the COVID-19 vaccine [3]. As reports of COVID-19 vaccine-associated myocarditis have gradually increased, concerns over potential adverse events have contributed to vaccine hesitancy [4].

Given the rarity of myocarditis, sample collection poses a significant challenge. However,

https://doi.org/10.62347/CCVM6311

owing to the severity of this adverse effect, investigating myocarditis following COVID-19 vaccination is of paramount importance. Currently, the available RNA-seq data on myocarditis after COVID-19 vaccination are limited to a single patient [5], which lacks statistical power for robust conclusions. In the present study, we aimed to determine potential genes related with myocarditis by analyzing transcriptomic changes induced by vaccination. Specifically, we investigated the shared transcriptomic alterations and potential mechanisms underlying COVID-19 vaccine-associated myocarditis by interacting differentially expressed genes (DEGs) from a fulminant myocarditis (FM) cohort and a COVID-19 mRNA vaccination dataset.

Materials and methods

Collection of samples

mRNA sequencing data for myocarditis were obtained from a previous study by our research team [6], which included fulminant myocarditis (FM) patients (N=3) and healthy controls (HCs, N=3). Detailed clinical information of this cohort was provided in Supplementary Table 1.

To obtain mRNA sequencing data related to COVID-19 mRNA vaccination, Gene Expression Omnibus (GEO) database was searched using the term "COVID-19 mRNA vaccination/vaccine". The selection of the dataset was guided by the following criteria: 1) the dataset should include samples collected before and after COVID-19 mRNA vaccination; and 2) all samples must be from individuals without pre-existing medical conditions prior to vaccination. Based on these inclusion criteria, only GSE169159 [7], generated using GPL15520 Illumina MiSeq met these criteria. Based on previous reports, myocarditis has been observed more frequently in younger individuals (median age 21 years) and males, after a second dose [8, 9]. Therefore, we selected expression data from this dataset on the following conditions: 1) samples from males closest to the median age of myocarditis cases; and 2) samples collected before and after the second dose. Finally, three samples were selected, and their specific information is provided in Supplementary Table 1.

Detection of DEGs and functional enrichment analysis

The gene expression data for FM and HC were normalized via the quantile method based on the "limma" R package [10], and the differences between FM and HC were calculated using a t-test. To identify DEGs before and after the second BNT162b2 vaccination (SBV), the "DEseq2" R package was used [11]. DEGs were determined based on the following thresholds: 1) for FM, a p-value less than 0.05 and $|\log2$ fold change (FC)$|$ no less than 1; 2) for SBV, a p-value less than 0.05 and $|\log2\ FC|$ no less than 0.5. Volcano plots were plotted to present these DEGs. The "venneuler" R package was used to identify the shared genes between FM and SBV. Gene Ontology (GO) enrichment analysis was performed to explore the biological processes associated with common genes [12]. Additionally, pathway enrichment analysis was performed using four pathway databases (KEGG Pathway, Reactome, BioCyc, and PANTHER) from KOBAS 3.0 [13]. The findings were sorted by p-values and gene counts, with a p-value less than 0.05 considered statistically significant. Top ten biological processes and pathways were presented as bar charts using Graphpad Prism 8.0.

Protein-protein interaction (PPI) analysis and hub gene screening

To analyze protein interactions and identify hub genes, a PPI network of shared DEGs was built using the STRING database (https://cn.string-db.org/) and visualized with Cytoscape 3.7.2 [14]. Two Cytoscape plugins, MCODE and CytoHubba, were used. MCODE was employed to identify significant gene clusters, with parameters configured as follows: Degree Cutoff =2, Node Score Cutoff =0.2, K-Core =2, and Max. Depth =100. CytoHubba was employed to detect core genes using five topological algorithms (DMNC, MCC, MNC, EPC and Degree) [15]. The top five genes from each algorithm were selected, and hub genes were determined by intersecting the results. An UpSet plot was generated using the "upSetR" R package to visualize the overlap [16]. Additionally, the GeneMANIA platform was used to explore the co-expression network of these hub genes (http://www.genemania.org/), which provides

insights into gene functions and inner connections [17].

Establishment of transcription factor (TF)/miRNA-mRNA regulatory network

To further investigate the regulatory mechanisms of these hub genes at both transcriptional and post-transcriptional levels, TFs and miRNAs associated with the hub genes were predicted [18]. TFs, which regulate gene expression by binding to specific DNA sequences upstream of 5'-end of genes, were identified using the ChIP-X Enrichment Analysis 3 (ChEA3) database [19]. miRNAs, which regulate gene expression post-transcriptionally via binding to the 3'-untranslated region (UTR) of target mRNAs, were predicted by the integrating data of Tarbase (v8.0), miRTarBase (v8.0) and miRecords databases through the OmincsNet2.0 platform [20] (http://www.omicsnet.ca). Then, the TF-mRNA and miRNA-mRNA networks were established and presented using Cytoscape 3.7.2.

Prediction of candidate drugs

Candidate drug-protein pairs were predicted using the Drug Signatures Database (DSigDB) via the Enrichr platform [21]. A *p*-value less than 0.05 was considered statistically significant. The detailed candidate drug-proteins information was also displayed.

Statistical analysis

DEGs were identified based on the predefined criteria. All analyses were performed using the established statistical thresholds, and results were visualized using R 4.0.5, GraphPad Prism (v.8.0.2), and Cytoscape (v.3.7.2) as described above.

Results

Identification of 80 shared DEGs between myocarditis and vaccination

The workflow of this study is illustrated in **Figure 1**. As mentioned above, the clinical information of the samples in the FM group, and the samples before and after SBV is summarized in Supplementary Table 1. DEGs in the FM and SBV groups were identified using the t-test and the "limma" R package, respectively. As illustrated in **Figure 2**, 1890 DEGs were detected in the FM group, with 1279 down-regulated genes and 611 up-regulated (**Figure 2A**). A total of 775 DEGs were observed in the SBV group, including 331 down-regulated genes and 444 up-regulated (**Figure 2B**). To identify myocarditis-related genes within the transcriptional changes induced by SBV, DEGs were intersected between these two groups (**Figure 2C, 2D**). This analysis revealed 80 shared DEGs, including 42 down-regulated genes and 38 up-regulated.

Functional and pathway enrichment of 80 shared DEGs

To further explore the biological significance of these DEGs, GO biological process analysis was performed using the Metascape database, while the KORBAS 3.0 database was used to conduct pathway enrichment analyses. GO analysis demonstrated that common DEGs were predominantly enriched in immune cell activation processes, including T cells, leukocytes and lymphocytes (**Figure 2E**). Pathway enrichment analysis revealed that the most significantly enriched pathway was related with the "immune system" (**Figure 2F**).

Identification of hub genes (CXCR3, NKG7, GZMH)

To establish a PPI network, all common DEGs were incorporated into the STRING database, which was also visualized using Cytoscape (**Figure 3A**). To identify the significant cluster (score =5) within the PPI network, module analysis was performed using the MCODE plugin in Cytoscape. This cluster consisted of 5 nodes (NKG7, CXCR3, GNLY, GZMH, and CD3D) and 10 edges (**Figure 3B**). Additionally, the hub genes were predicted using five topological algorithms (DMNC, MCC, MNC, EPC and Degree). Then, we intersected the results of the five above-mentioned algorithms, and eventually identified three hub genes (CXCR3, GZMH, and NKG7) that appeared in all five analyses (**Figure 3C**). These hub genes were centrally located within the MCODE-identified cluster (**Figure 3B**). Furthermore, the network of three hub genes and their co-expression genes were built and analyzed by GeneMANIA (Supplementary Figure 1). The three hub genes displayed a complex PPI network. The biological functions of hub genes and their associated

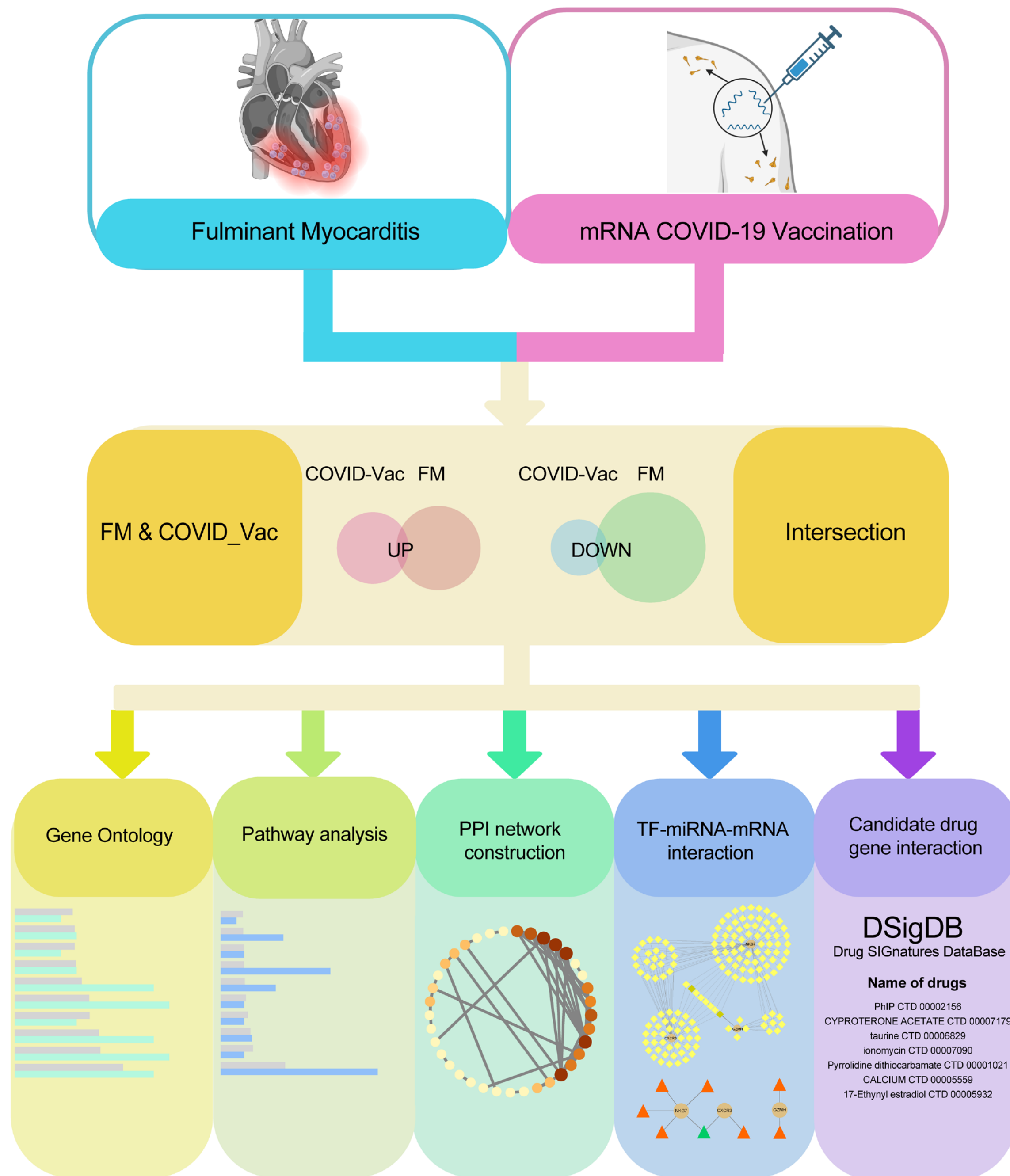


Figure 1. The flowchart of this study.

genes are consistent with the critical roles of cytokine and chemokine functions in myocarditis [22] and post-SBV responses [7].

Transcriptional regulatory network analysis of hub genes

A TF-gene interaction network was further established based on the ChEA3 database, which included 153 nodes and 196 edges (**Figure 4**). Among the hub genes, NKG7 was regulated by 107 TFs, CXCR3 by 69 TFs, and GZMH by 20 TFs, respectively. The top ten TFs ranked by the ChEA3 database are listed in **Table 1**, with TBX21 and STAT4 identified as the top two TFs capable of interacting with all three hub genes.

miRNA regulatory networks of hub genes

miRNA-mRNA regulatory networks were predicted using experimentally validated data from three databases (Tarbase, miRTarBase, and

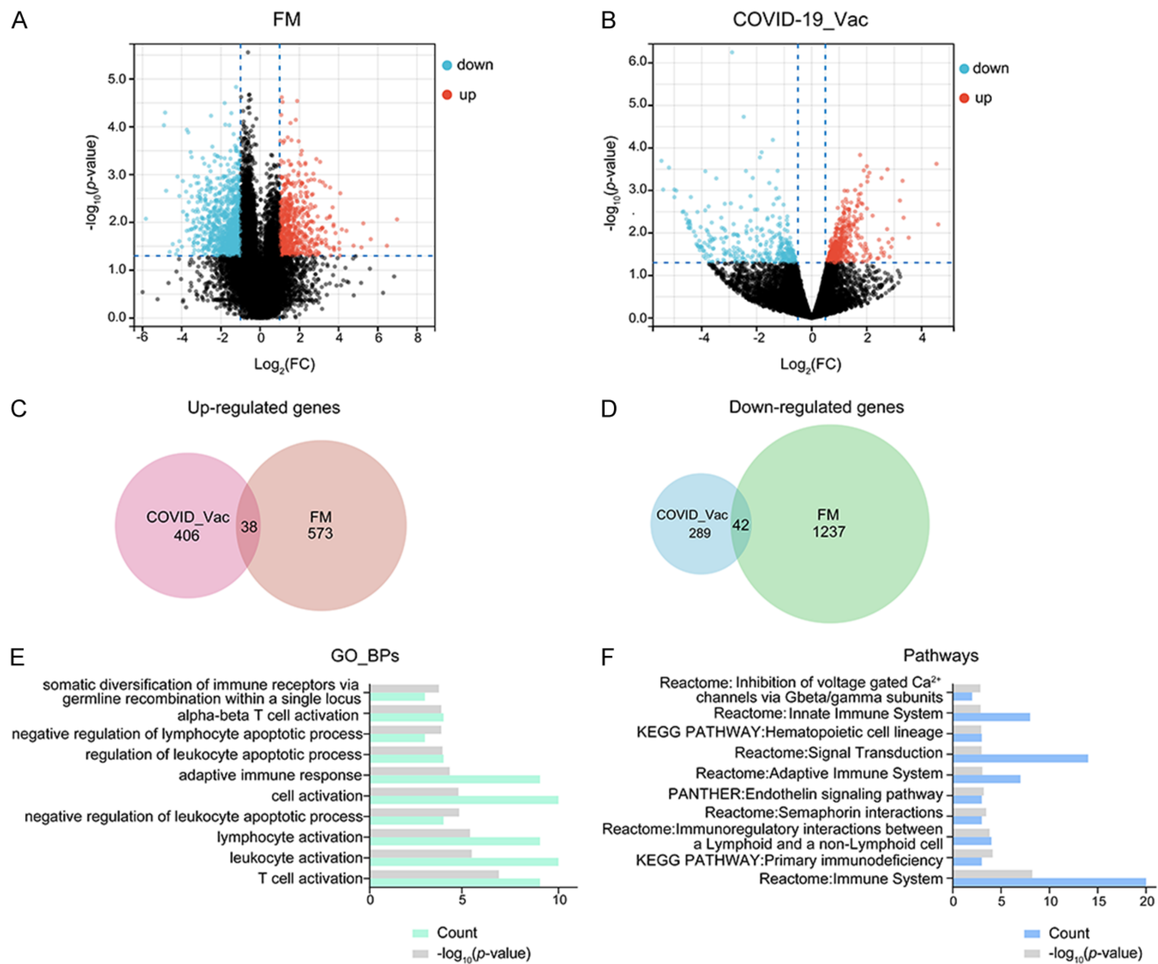


Figure 2. Identification and functional enrichment analysis of common DEGs between FM and SBV. A. Volcano plot of FM vs HC. B. Volcano plot of before and after SBV. C. The 38 up-regulated overlapped DEGs between FM and SBV. D. The 42 down-regulated overlapped DEGs between FM and SBV. E. Biological process enrichment analysis of the common DEGs. F. Pathway enrichment analysis of the common DEGs. FM: fulminant myocarditis, SBV: the second BNT162b2 vaccination, HC: healthy control, DEGs: differentially expressed genes.

miRecords) through OmincsNet2.0 platform. The analytical miRNA-mRNA network, comprising 11 nodes and nine edges, is shown in **Figure 5** and **Table 2**. Notably, hsa-mir-146a-5p was found to interact with two of the three hub genes (CXCR3 and NKG7).

Candidate therapeutic drug (PhIP) targeting hub genes

Potential therapeutic drugs targeting the hub genes were identified using the DSigDB database on the Enrichr platform. The most significant drug interactions are showed in **Table 3**. PhIP (CTD 00002156) was identified as a potential drug that interacted both CXCR3 and NKG7, with the highest statistical significance. No statistically significant drug interactions were predicted for GZMH.

Discussion

The link between COVID-19 mRNA vaccines and acute myocarditis has attracted worldwide concern, with emerging evidence highlighting its potential pathophysiological link [4, 23-27]. Although cardiac recovery rates are generally high in vaccine-related myocarditis, the psychosocial impact of myocarditis remains substantial and frequently overlooked [28]. To address the limited understanding of transcriptomic alterations in vaccine-associated myocarditis, we conducted a systematic bioinformatic analysis integrating datasets from ful-

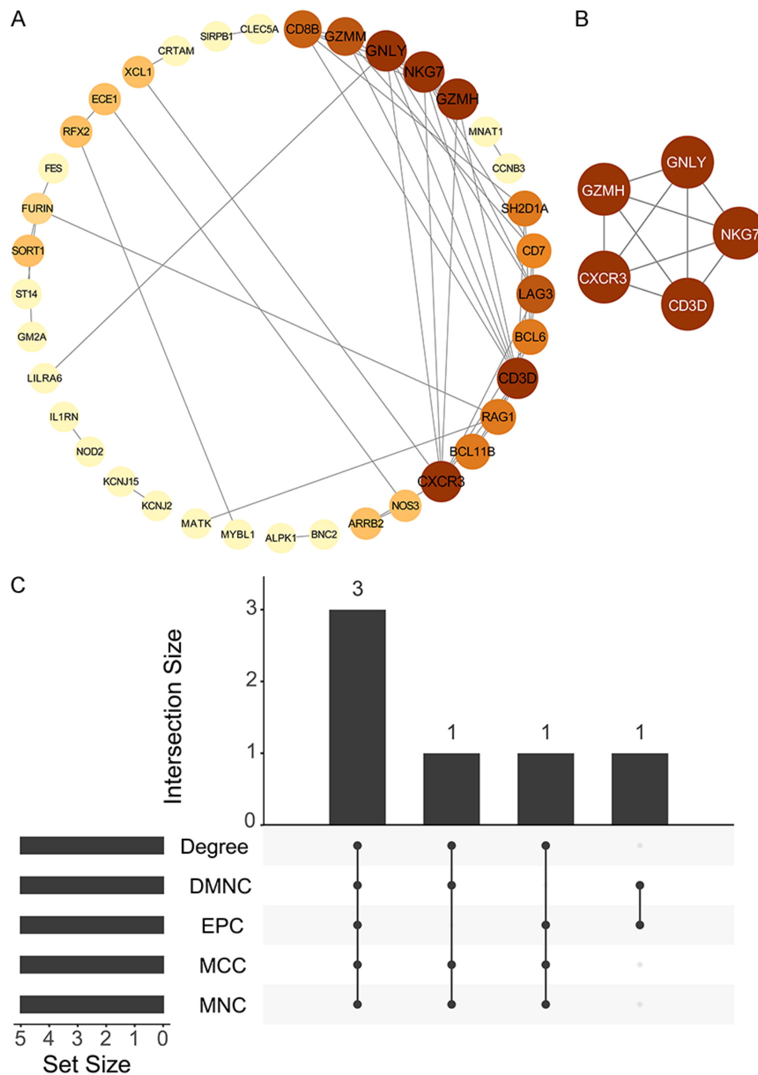


Figure 3. Integrated Network Analysis Reveals Hub Genes in COVID-19 mRNA Vaccine-Associated Myocarditis. A. Protein-protein interaction (PPI) network of common DEGs between FM and SBV cohorts (37 nodes, 51 edges). B. Key functional cluster identified by MCODE analysis (5 nodes, 10 edges). Different colors and sizes of nodes represent different scores calculated by MCODE. Larger and darker node represent greater degree. C. UpSet plot demonstrating the common and specific hub genes in the five algorithms of CytoHubba (DMNC, MCC, MNC, EPC and Degree). FM: fulminant myocarditis, SBV: the second BNT162b2 vaccination, DEGs: differentially expressed genes, PPI: Protein-protein interaction.

minant myocarditis and post-vaccination. These findings reveal novel molecular insights and propose potential therapeutic targets, advancing the mechanistic understanding of this rare adverse event associated with COVID-19 mRNA vaccination.

The current literature on transcriptomic alterations in COVID-19 mRNA vaccination-associated myocarditis is limited. A PubMed search (up to November 8th, 2024) identified only two relevant studies: a longitudinal analysis of peripheral blood mononuclear cells (PBMCs) in a single myocarditis patient post-BNT162b2 vaccination [5], and a system-level study demonstrating cytokine-driven lymphocyte activation and profibrotic monocyte responses in 23 patients [29]. Notably, the latter study reported elevated levels of CD16+ NK cells and CXCR3+ cytotoxic T cells, consistent with our identification of NKG7 and CXCR3 as central hub genes. Based on these findings, our study uniquely combined FM transcriptomics with post-vaccination datasets to identify shared pathogenic pathways, bridging critical gaps in current knowledge. Regarding the pathogenic genes of myocarditis, the mRNA-seq of FM published by our own research team was used. The overlapping DEGs in these two groups were defined as myocarditis-related genes after SBV.

A previous descriptive study reported that the median age of myocarditis patients was 21 years, with males comprising 82% of cases [8]. To minimize confounding factors, males in GSE169159 closest to the median age were selected for subsequent analyses. In addition, it has been reported that most COVID-19 vaccine associated myocarditis occurs after the second BNT162b2 vaccination [2]. Therefore, in our case, we focused on transcriptional alterations before and after the second dose of the vaccine. By intersecting DEGs from the FM and post-vaccination groups, we identified 80 common genes enriched in immune activation process including T cells activation, leukocyte and lymphocyte activation process, negative regulation of leukocyte

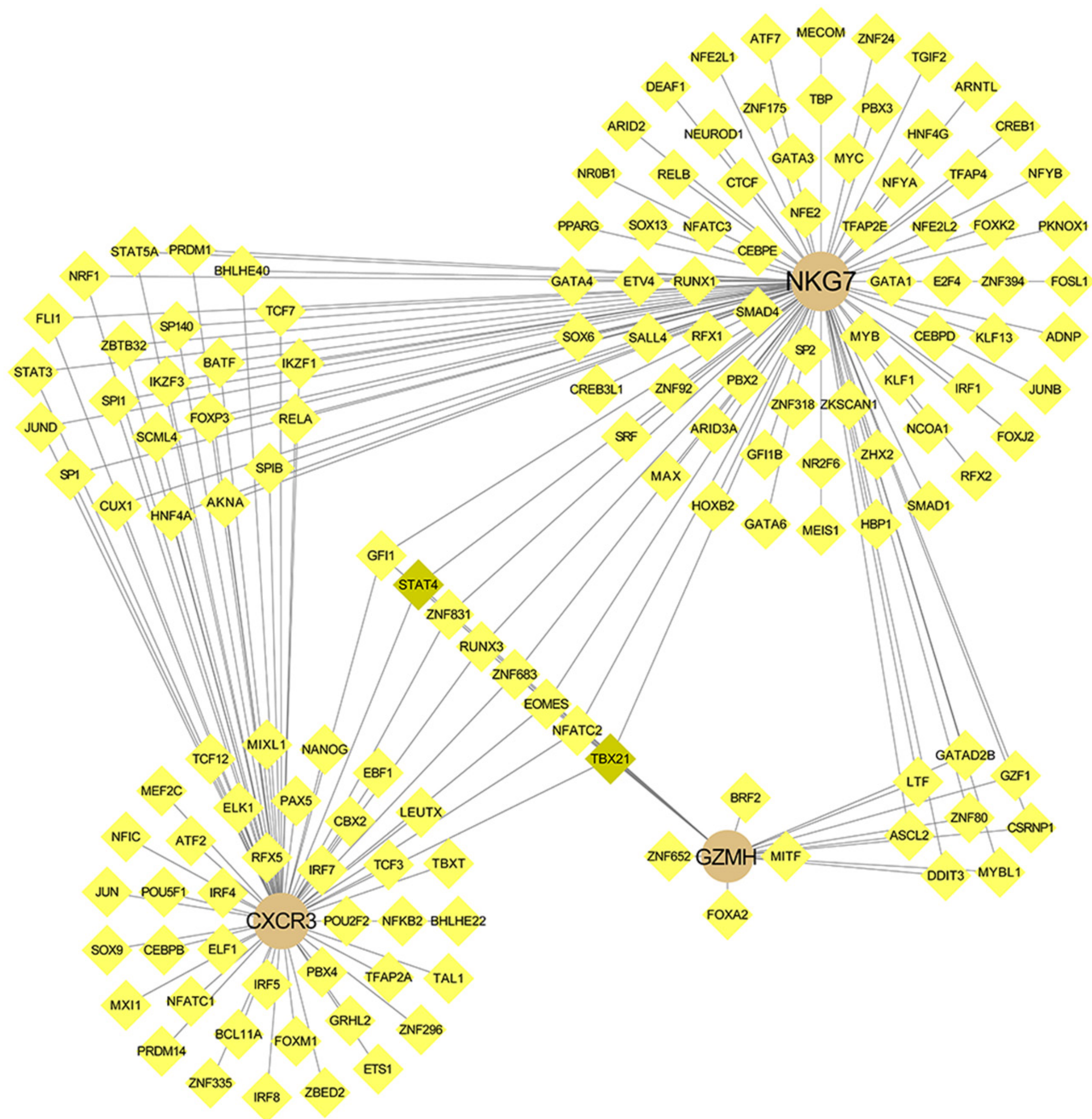


Figure 4. The network of hub gene-TF interaction. The network consists of 153 nodes and 196 edges. Brown round nodes represent hub genes; yellow diamond nodes represent TFs, and brown green diamond nodes represent the top two TFs with the highest ranks. TF: transcription factor.

Table 1. Top ten TFs according to the Rank in ChEA3

Rank	TF	Overlapping_Genes	Score
1	TBX21	CXCR3, NKG7, GZMH	5.5
2	STAT4	CXCR3, NKG7, GZMH	8.5
3	ZNF80	NKG7, GZMH	12.33
4	IKZF3	CXCR3, NKG7	13
5	FOXP3	CXCR3, NKG7	32
6	BATF	CXCR3, NKG7	34.6
7	CSRNP1	NKG7, GZMH	38
8	SP140	CXCR3, NKG7	39.67
9	EOMES	CXCR3, NKG7, GZMH	59
10	GFI1	CXCR3, NKG7, GZMH	61.67

and lymphocyte apoptotic process, adaptive immune response, αβ T cells activation process and somatic diversification of immune receptors via germline process. Among them, infiltration of T cells [30, 31] and eosinophils [32] has been detected in the endomyocardial biopsy (EMB) in mRNA COVID-19 vaccination-associated myocarditis. Furthermore, emerging evidence indicates that monocytes may be critical to the development of myocarditis associated with BNT162b2 vaccination [5, 33].

Among the top ten enriched pathways, immune system pathways had the highest confidence level. Among them, it could be noted that

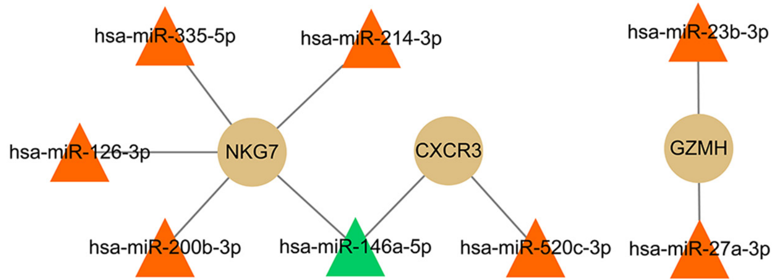


Figure 5. The hub genes-miRNA co-regulatory network. The network consists of 11 nodes and 9 edges. Brown round nodes represent hub genes; triangle nodes represent miRNAs, of which green triangle can bind with most mRNAs.

IL1RN (IL-1RA) and NOD2 are involved. In accordance with our results, a study showed that elevated IL-1 receptor antagonist (IL-1RA) levels were observed in patients with myocarditis following COVID-19 mRNA vaccination [34]. Additionally, IL-1RA and IL-1RA antibodies have been reported to be associated with the course of myocarditis after COVID-19 mRNA vaccination [35]. In addition, NOD2 plays a key role in mediating the inflammatory response during myocarditis pathogenesis [36]. These findings align with prior studies, further underscoring the reliability of our bioinformatics analysis results.

Three hub genes, CXCR3, NKG7, and GZMH, emerged from integrative network analyses, and were all downregulated in both the FM and SBV groups. CXCR3 is primarily expressed on CD4+, CD8+ T cells and NK cells, and has three ligands: CXCL9, CXCL10 and CXCL11. We found that CXCL9 was upregulated after SBV, whereas CXCL10 and CXCL11 remained unchanged (Supplementary Figure 2). CXCL9 has been demonstrated to promote inflammation by driving the polarization of Th1 and Th17 cells [37-39]. In contrast, CXCL11, which binds to CXCR3 [40], exhibits anti-inflammatory properties through inducing the polarization of Treg cells [38, 39]. An elevated Th17/Treg ratio by promoting the maturation of Th17 cells, is a critical factor in the initiation of experimental autoimmune myocarditis (EAM) in mice [22]. NKG7, downregulated in both the FM and SBV groups, is a member of the NK cell granule protein family that facilitates the cytotoxic activity of both T and NK cells [41]. Dynamic changes in NKG7 during T cell activation and differentiation have been well characterized. In less differentiated or stem-like T cells, downregulation of NKG7 during early activation may prolong immune synapse formation with antigen-presenting cells (APCs), boosting T cell activation while preventing APC lysis. Conversely, terminally differentiated CD8+ T cells upregulate NKG7 to enhance cytotoxicity and limit widespread inflammation [42-44]. Additionally, reduced NKG7 expression is associated with increased cytokine secretion, further implicating its role in modulating immune responses [42]. A few studies have shown that another hub gene, GZMH, can induce cell death and inactivate viral proteins, further underscoring dysregulated immune effector mechanisms [45].

From the TF-gene interaction network, TBX21, STAT4, EOMES, GFI1, RUNX3, NFATC2, ZNF831 and ZNF683 could regulate all three hub genes. Among them, TBX21 (T-bet) and STAT4 had the lowest scores, suggesting a higher confidence in regulating hub genes. TBX21, a Th1-specific T box TF, participates in inflammation by regulating CXCR3 [46] and NKG7 [47]. TBX21 (T-bet) deficiency exacerbates myocarditis in mouse models [48], along with elevated IL-17 production in heart-infiltrating T cells, which is associated with myocarditis pathogenesis [49]. Additionally, studies reported that the reduced TBX21 level decreases NK cell proportion and impairs CD8+ T cell exhaustion, which could in turn reprogram a sustained inflammatory environment, inducing multisystem inflammatory syndrome in children (MIS-C) [50]. However, whether TBX21 functions in the development of COVID-19 mRNA vaccine-associated myocarditis warrants further investigation.

STAT4 is involved in signal transduction and activation of transcription proteins. Research has demonstrated that impairment of the IL-12/STAT4/IFN-γ pathway can exacerbate chronic Coxsackievirus B3 myocarditis [51]. Conversely, another study found that STAT4 silencing mitigates experimental autoimmune myocarditis (EAM) via modulating the Th1/Th2 cell immune response [52]. However, the specific role of STAT4 in vaccine-associated myo-

Table 2. miRNA-gene interaction

Hub genes	miRNAs
CXCR3	hsa-miR-146a-5p, hsa-miR-520c-3p
NKG7	hsa-miR-126-3p, hsa-miR-146a-5p, hsa-miR-200b-3p, hsa-miR-214-3p, hsa-miR-335-5p
GZMH	hsa-miR-23b-3p, hsa-miR-27a-3p

Table 3. Prediction of candidate drugs for hub genes

Name of drugs	*p*-value	Genes
PhIP CTD 00002156	2.40E-04	CXCR3, NKG7
CYPROTERONE ACETATE CTD 00007179	0.004642964	CXCR3
taurine CTD 00006829	0.008675133	CXCR3
ionomycin CTD 00007090	0.016855113	NKG7
Pyrrolidine dithiocarbamate CTD 00001021	0.023809528	NKG7
CALCIUM CTD 00005559	0.026611554	CXCR3
17-Ethynyl estradiol CTD 00005932	0.045057855	CXCR3

carditis remains to be elucidated. Additionally, miRNAs regulate target gene expression post-transcriptionally. Identifying miRNAs targeting hub genes could improve our understanding of myocarditis pathogenesis. In the present study, we analyzed mRNA-associated miRNAs. Among these, miR-146a-5p regulates the expression of most hub genes and has been extensively reported to play a role in the inflammatory process [53, 54].

Except for the role of COVID-19 mRNA vaccination in myocarditis [55], treatment options remain unclear [56]. In this analysis, we also predicted several chemical compounds that target the hub genes. Drug prediction using DSigDB identified PhIP (CTD 00002156) as a candidate inhibitor of CXCR3/NKG7, with known dose-dependent immunosuppressive effects [57, 58]. PhIP, also called 2-Amino-1-methyl-6-phenylimidazo[4,5-b] pyridine, a heterocyclic amine generated during high-temperature cooking, is a known carcinogen but also exhibits dose-dependent immunomodulatory effects. Although preliminary, these findings provide a rationale for experimental validation in preclinical models. Of course, its genotoxic risks necessitate further validation of therapeutic potential versus toxicity in myocarditis models.

Certainly, this study has several limitations that need to be noted. First, the small sample size (n=3 per group) and demographic disparities between the FM and post-vaccination cohorts necessitate cautious interpretation. The ages and ethnicity of patients in FM group and SBV group was not comparable. However, intra-group paired analyses (pre-/post-vaccination) diminished age-related confounding factors. Second, the reported RNA-seq data on myocarditis after COVID-19 vaccination was obtained from only one patient. The lack of pre-vaccination controls in the existing datasets underscores the need for longitudinal studies. Nonetheless, this is the first study to explore the underlying mechanism of myocarditis after COVID-19 vaccination via bioinformatic analysis. Future research should prioritize multi-omics profiling in larger cohorts to verify these results.

Our integrative bioinformatics analysis proposed a potential molecular framework for COVID-19 mRNA vaccine-associated myocarditis, centering on CXCR3, NKG7, and GZMH as key mediators of immune dysregulation during disease development. The identified TF/miRNA networks and candidate drugs offer valuable insights for mechanistic validation and therapeutic development. As global vaccination efforts continue, these findings underscore the urgency of vaccine safety monitoring with robust translational research to address this rare adverse event.

Acknowledgements

The study was funded by Special Expert of Taishan Scholars (no. 201511099); Jinan Sci-

ence and Technology Development Plan (no. 202134015); and Shandong Provincial Natural Science Foundation for Youths (ZR2023-QH310).

Disclosure of conflict of interest

None.

Address correspondence to: Bo Han, Department of Pediatrics, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 251000, Shandong, China. E-mail: bhan@email.sdfmu.edu.cn

References

[1] Thompson MG, Burgess JL, Naleway AL, Tyner H, Yoon SK, Meece J, Olsho LEW, Caban-Martinez AJ, Fowlkes AL, Lutrick K, Groom HC, Dunnigan K, Odean MJ, Hegmann K, Stefanski E, Edwards LJ, Schaefer-Solle N, Grant L, Ellingson K, Kuntz JL, Zunie T, Thiese MS, Ivacic L, Wesley MG, Mayo Lamberte J, Sun X, Smith ME, Phillips AL, Groover KD, Yoo YM, Gerald J, Brown RT, Herring MK, Joseph G, Beitel S, Morrill TC, Mak J, Rivers P, Poe BP, Lynch B, Zhou Y, Zhang J, Kelleher A, Li Y, Dickerson M, Hanson E, Guenther K, Tong S, Bateman A, Reisdorf E, Barnes J, Azziz-Baumgartner E, Hunt DR, Arvay ML, Kutty P, Fry AM and Gaglani M. Prevention and attenuation of Covid-19 with the BNT162b2 and mRNA-1273 vaccines. N Engl J Med 2021; 385: 320-329.

[2] Goddard K, Hanson KE, Lewis N, Weintraub E, Fireman B and Klein NP. Incidence of myocarditis/pericarditis following mRNA COVID-19 vaccination among children and younger adults in the United States. Ann Intern Med 2022; 175: 1169-1771.

[3] Watanabe S and Hama R. SARS-CoV-2 vaccine and increased myocarditis mortality risk: a population based comparative study in Japan. medRxiv 2022.

[4] Kim HW, Jenista ER, Wendell DC, Azevedo CF, Campbell MJ, Darty SN, Parker MA and Kim RJ. Patients with acute myocarditis following mRNA COVID-19 vaccination. JAMA Cardiol 2021; 6: 1196-1201.

[5] Hwang N, Huh Y, Bu S, Seo KJ, Kwon SH, Kim JW, Yoon BK, Ahn HS and Fang S. Single-cell sequencing of PBMC characterizes the altered transcriptomic landscape of classical monocytes in BNT162b2-induced myocarditis. Front Immunol 2022; 13: 979188.

[6] Liu Q, Kong Y, Han B, Jiang D, Jia H and Zhang L. Long non-coding RNA expression profile and functional analysis in children with acute fulminant myocarditis. Front Pediatr 2019; 7: 283.

[7] Arunachalam PS, Scott MKD, Hagan T, Li C, Feng Y, Wimmers F, Grigoryan L, Trisal M, Edara VV, Lai L, Chang SE, Feng A, Dhingra S, Shah M, Lee AS, Chinthrajah S, Sindher SB, Mallajosyula V, Gao F, Sigal N, Kowli S, Gupta S, Pellegrini K, Tharp G, Maysel-Auslender S, Hamilton S, Aoued H, Hrusovsky K, Roskey M, Bosinger SE, Maecker HT, Boyd SD, Davis MM, Utz PJ, Suthar MS, Khatri P, Nadeau KC and Pulendran B. Systems vaccinology of the BNT162b2 mRNA vaccine in humans. Nature 2021; 596: 410-416.

[8] Oster ME, Shay DK, Su JR, Gee J, Creech CB, Broder KR, Edwards K, Soslow JH, Dendy JM, Schlaudecker E, Lang SM, Barnett ED, Ruberg FL, Smith MJ, Campbell MJ, Lopes RD, Sperling LS, Baumblatt JA, Thompson DL, Marquez PL, Strid P, Woo J, Pugsley R, Reagan-Steiner S, DeStefano F and Shimabukuro TT. Myocarditis cases reported after mRNA-based COVID-19 vaccination in the US from December 2020 to August 2021. JAMA 2022; 327: 331-340.

[9] Knudsen B and Prasad V. COVID-19 vaccine induced myocarditis in young males: a systematic review [J]. Eur J Clin Invest 2023; 53: e13947.

[10] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W and Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015; 43: e47.

[11] Love MI, Huber W and Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014; 15: 550.

[12] Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C and Chanda SK. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun 2019; 10: 1523.

[13] Wu J, Mao X, Cai T, Luo J and Wei L. KOBAS server: a web-based platform for automated annotation and pathway identification. Nucleic Acids Res 2006; 34: W720-724.

[14] Smoot ME, Ono K, Ruscheinski J, Wang PL and Ideker T. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics 2011; 27: 431-432.

[15] Chin CH, Chen SH, Wu HH, Ho CW, Ko MT and Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 2014; 8 Suppl 4: S11.

[16] Conway JR, Lex A and Gehlenborg N. UpSetR: an R package for the visualization of intersecting sets and their properties. Bioinformatics 2017; 33: 2938-2940.

[17] Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, Maitland A, Mostafavi S, Montojo J, Shao Q, Wright G, Bader GD and Morris

Q. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 2010; 38: W214-220.

[18] Zhou Y, Ferguson J, Chang JT and Kluger Y. Inter- and intra-combinatorial regulation by transcription factors and microRNAs. BMC Genomics 2007; 8: 396.

[19] Keenan AB, Torre D, Lachmann A, Leong AK, Wojciechowicz ML, Utti V, Jagodnik KM, Kropiwnicki E, Wang Z and Ma'ayan A. ChEA3: transcription factor enrichment analysis by orthogonal omics integration. Nucleic Acids Res 2019; 47: W212-W224.

[20] Zhou G, Pang Z, Lu Y, Ewald J and Xia J. OmicsNet 2.0: a web-based platform for multi-omics integration and network visual analytics. Nucleic Acids Res 2022; 50: W527-533.

[21] Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A, McDermott MG, Monteiro CD, Gundersen GW and Ma'ayan A. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 2016; 44: W90-97.

[22] Tschöpe C, Ammirati E, Bozkurt B, Caforio ALP, Cooper LT, Felix SB, Hare JM, Heidecker B, Heymans S, Hübner N, Kelle S, Klingel K, Maatz H, Parwani AS, Spillmann F, Starling RC, Tsutsui H, Seferovic P and Van Linthout S. Myocarditis and inflammatory cardiomyopathy: current evidence and future directions. Nat Rev Cardiol 2021; 18: 169-193.

[23] Marshall M, Ferguson ID, Lewis P, Jaggi P, Gagliardo C, Collins JS, Shaughnessy R, Caron R, Fuss C, Corbin KJE, Emuren L, Faherty E, Hall EK, Di Pentima C, Oster ME, Paintsil E, Siddiqui S, Timchak DM and Guzman-Cottrill JA. Symptomatic acute myocarditis in 7 adolescents after Pfizer-BioNTech COVID-19 vaccination. Pediatrics 2021; 148: e2021052478.

[24] Montgomery J, Ryan M, Engler R, Hoffman D, McClenathan B, Collins L, Loran D, Hrncir D, Herring K, Platzer M, Adams N, Sanou A and Cooper LT Jr. Myocarditis following immunization with mRNA COVID-19 vaccines in members of the US military. JAMA Cardiol 2021; 6: 1202-1206.

[25] Diaz GA, Parsons GT, Gering SK, Meier AR, Hutchinson IV and Robicsek A. Myocarditis and pericarditis after vaccination for COVID-19. JAMA 2021; 326: 1210-1212.

[26] Mevorach D, Anis E, Cedar N, Bromberg M, Haas EJ, Nadir E, Olsha-Castell S, Arad D, Hasin T, Levi N, Asleh R, Amir O, Meir K, Cohen D, Dichtiar R, Novick D, Hershkovitz Y, Dagan R, Leitersdorf I, Ben-Ami R, Miskin I, Saliba W, Muhsen K, Levi Y, Green MS, Keinan-Boker L and Alroy-Preis S. Myocarditis after BNT162b2 mRNA vaccine against Covid-19 in Israel. N Engl J Med 2021; 385: 2140-2149.

[27] Witberg G, Barda N, Hoss S, Richter I, Wiessman M, Aviv Y, Grinberg T, Auster O, Dagan N, Balicer RD and Kornowski R. Myocarditis after Covid-19 vaccination in a large health care organization. N Engl J Med 2021; 385: 2132-2139.

[28] Ammirati E and Cooper LT Jr. Recovery from mRNA COVID-19 vaccine-related myocarditis. Lancet Child Adolesc Health 2022; 6: 749-751.

[29] Barmada A, Klein J, Ramaswamy A, Brodsky NN, Jaycox JR, Sheikha H, Jones KM, Habet V, Campbell M, Sumida TS, Kontorovich A, Bogunovic D, Oliveira CR, Steele J, Hall EK, Pena-Hernandez M, Monteiro V, Lucas C, Ring AM, Omer SB, Iwasaki A, Yildirim I and Lucas CL. Cytokinopathy with aberrant cytotoxic lymphocytes and profibrotic myeloid response in SARS-CoV-2 mRNA vaccine-associated myocarditis. Sci Immunol 2023; 8: eadh3455.

[30] Nguyen TD, Mall G, Westphal JG, Weingärtner O, Möbius-Winkler S and Schulze PC. Acute myocarditis after COVID-19 vaccination with mRNA-1273 in a patient with former SARS-CoV-2 infection. ESC Heart Fail 2021; 8: 4710-4714.

[31] Won T, Gilotra NA, Wood MK, Hughes DM, Talor MV, Lovell J, Milstone AM, Steenbergen C and Čiháková D. Increased interleukin 18-dependent immune responses are associated with myopericarditis after COVID-19 mRNA vaccination. Front Immunol 2022; 13: 851620.

[32] Parmar K, Mekraksakit P, Del Rio-Pertuz G, Sethi P, Motes A, Hughes M, Wischmeyer J, Carbajal L and Sosa EA. Myocarditis following COVID-19 mRNA vaccination. Proc (Bayl Univ Med Cent) 2022; 35: 209-213.

[33] Yoon BK, Oh TG, Bu S, Seo KJ, Kwon SH, Lee JY, Kim Y, Kim JW, Ahn HS and Fang S. The peripheral immune landscape in a patient with myocarditis after the administration of BNT162b2 mRNA vaccine. Mol Cells 2022; 45: 738-748.

[34] Muthukumar A, Narasimhan M, Li QZ, Mahimainathan L, Hitto I, Fuda F, Batra K, Jiang X, Zhu C, Schoggins J, Cutrell JB, Croft CL, Khera A, Drazner MH, Grodin JL, Greenberg BM, Mammen PPA, Morrison SJ and de Lemos JA. In-depth evaluation of a case of presumed myocarditis after the second dose of COVID-19 mRNA vaccine. Circulation 2021; 144: 487-498.

[35] Thurner L, Kessel C, Fadle N, Regitz E, Seidel F, Kindermann I, Lohse S, Kos I, Tschöpe C, Kheiroddin P, Kiblboeck D, Hoffmann MC, Bette B, Carbon G, Cetin O, Preuss KD, Christofyllakis K, Bittenbring JT, Pickardt T, Fischer Y, Thiele H,

Baldus S, Stangl K, Steiner S, Gietzen F, Kerber S, Deneke T, Jellinghaus S, Linke A, Ibrahim K, Grabmaier U, Massberg S, Thilo C, Greulich S, Gawaz M, Mayatepek E, Meyer-Dobkowitz L, Kindermann M, Birk E, Birk M, Lainscak M, Foell D, Lepper PM, Bals R, Krawczyk M, Mevorach D, Hasin T, Keren A, Kabesch M, Abdul-Khaliq H, Smola S, Bewarder M, Thurner B, Böhm M, Pfeifer J and Klingel K. IL-1RA antibodies in myocarditis after SARS-CoV-2 vaccination. N Engl J Med 2022; 387: 1524-1527.

[36] Tschöpe C, Müller I, Xia Y, Savvatis K, Pappritz K, Pinkert S, Lassner D, Heimesaat MM, Spillmann F, Miteva K, Bereswill S, Schultheiss HP, Fechner H, Pieske B, Kühl U and Van Linthout S. NOD2 (nucleotide-binding oligomerization domain 2) is a major pathogenic mediator of coxsackievirus B3-induced myocarditis. Circ Heart Fail 2017; 10: e003870.

[37] Karin N and Wildbaum G. The role of chemokines in adjusting the balance between CD4+ effector T cell subsets and FOXp3-negative regulatory T cells. Int Immunopharmacol 2015; 28: 829-835.

[38] Karin N, Wildbaum G and Thelen M. Biased signaling pathways via CXCR3 control the development and function of CD4+ T cell subsets. J Leukoc Biol 2016; 99: 857-862.

[39] Zohar Y, Wildbaum G, Novak R, Salzman AL, Thelen M, Alon R, Barsheshet Y, Karp CL and Karin N. CXCL11-dependent induction of FOXP3-negative regulatory T cells suppresses autoimmune encephalomyelitis. J Clin Invest 2014; 124: 2009-2022.

[40] Cole KE, Strick CA, Paradis TJ, Ogborne KT, Loetscher M, Gladue RP, Lin W, Boyd JG, Moser B, Wood DE, Sahagan BG and Neote K. Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3. J Exp Med 1998; 187: 2009-2021.

[41] Turman MA, Yabe T, McSherry C, Bach FH and Houchins JP. Characterization of a novel gene (NKG7) on human chromosome 19 that is expressed in natural killer cells and T cells. Hum Immunol 1993; 36: 34-40.

[42] Lelliott EJ, Ramsbottom KM, Dowling MR, Shembrey C, Noori T, Kearney CJ, Michie J, Parish IA, Jordan MA, Baxter AG, Young ND, Brennan AJ and Oliaro J. NKG7 enhances CD8+ T cell synapse efficiency to limit inflammation. Front Immunol 2022; 13: 931630.

[43] Ng SS, De Labastida Rivera F, Yan J, Corvino D, Das I, Zhang P, Kuns R, Chauhan SB, Hou J, Li XY, Frame TCM, McEnroe BA, Moore E, Na J, Engel JA, Soon MSF, Singh B, Kueh AJ, Herold MJ, Montes de Oca M, Singh SS, Bunn PT, Aguilera AR, Casey M, Braun M, Ghazanfari N, Wani S, Wang Y, Amante FH, Edwards CL, Haque A, Dougall WC, Singh OP, Baxter AG, Teng MWL, Loukas A, Daly NL, Cloonan N, Degli-Esposti MA, Uzonna J, Heath WR, Bald T, Tey SK, Nakamura K, Hill GR, Kumar R, Sundar S, Smyth MJ and Engwerda CR. The NK cell granule protein NKG7 regulates cytotoxic granule exocytosis and inflammation. Nat Immunol 2020; 21: 1205-1218.

[44] Luckey CJ, Bhattacharya D, Goldrath AW, Weissman IL, Benoist C and Mathis D. Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells. Proc Natl Acad Sci U S A 2006; 103: 3304-3309.

[45] Voskoboinik I, Whisstock JC and Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. Nat Rev Immunol 2015; 15: 388-400.

[46] Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB and Campbell DJ. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. Nat Immunol 2009; 10: 595-602.

[47] Hahtola S, Tuomela S, Elo L, Häkkinen T, Karenko L, Nedoszytko B, Heikkilä H, Saarialho-Kere U, Roszkiewicz J, Aittokallio T, Lahesmaa R and Ranki A. Th1 response and cytotoxicity genes are down-regulated in cutaneous T-cell lymphoma. Clin Cancer Res 2006; 12: 4812-4821.

[48] Rangachari M, Mauermann N, Marty RR, Dirnhofer S, Kurrer MO, Komnenovic V, Penninger JM and Eriksson U. T-bet negatively regulates autoimmune myocarditis by suppressing local production of interleukin 17. J Exp Med 2006; 203: 2009-2019.

[49] Hua X, Hu G, Hu Q, Chang Y, Hu Y, Gao L, Chen X, Yang PC, Zhang Y, Li M and Song J. Single-cell RNA sequencing to dissect the immunological network of autoimmune myocarditis. Circulation 2020; 142: 384-400.

[50] Beckmann ND, Comella PH, Cheng E, Lepow L, Beckmann AG, Tyler SR, Mouskas K, Simons NW, Hoffman GE, Francoeur NJ, Del Valle DM, Kang G, Do A, Moya E, Wilkins L, Le Berichel J, Chang C, Marvin R, Calorossi S, Lansky A, Walker L, Yi N, Yu A, Chung J, Hartnett M, Eaton M, Hatem S, Jamal H, Akyatan A, Tabachnikova A, Liharska LE, Cotter L, Fennessy B, Vaid A, Barturen G, Shah H, Wang YC, Sridhar SH, Soto J, Bose S, Madrid K, Ellis E, Merzier E, Vlachos K, Fishman N, Tin M, Smith M, Xie H, Patel M, Nie K, Argueta K, Harris J, Karekar N, Batchelor C, Lacunza J, Yishak M, Tuballes K, Scott I, Kumar A, Jaladanki S, Agashe C, Thompson R, Clark E, Losic B, Peters L, Roussos P, Zhu J, Wang W, Kasarskis A, Glicksberg BS, Nadkarni G, Bogunovic D, Elaiho C, Gangadharan S, Ofo-

ri-Amanfo G, Alesso-Carra K, Onel K, Wilson KM, Argmann C, Bunyavanich S, Alarcón-Riquelme ME, Marron TU, Rahman A, Kim-Schulze S, Gnjatic S, Gelb BD, Merad M, Sebra R, Schadt EE and Charney AW. Downregulation of exhausted cytotoxic T cells in gene expression networks of multisystem inflammatory syndrome in children. Nat Commun 2021; 12: 4854.

[51] Fairweather D, Frisancho-Kiss S, Yusung SA, Barrett MA, Davis SE, Steele RA, Gatewood SJ and Rose NR. IL-12 protects against coxsackievirus B3-induced myocarditis by increasing IFN-gamma and macrophage and neutrophil populations in the heart. J Immunol 2005; 174: 261-269.

[52] Xue YL, Zhang SX, Zheng CF, Li YF, Zhang LH, Hao YF, Wang S and Li XW. Silencing of STAT4 protects against autoimmune myocarditis by regulating Th1/Th2 immune response via inactivation of the NF-κB pathway in rats. Inflammation 2019; 42: 1179-1189.

[53] Yan F, Wufuer D, Ding J and Wang J. MicroRNA miR-146a-5p inhibits the inflammatory response and injury of airway epithelial cells via targeting TNF receptor-associated factor 6. Bioengineered 2021; 12: 1916-1926.

[54] Chen X, Li W, Chen T, Ren X, Zhu J, Hu F, Luo J, Xing L, Zhou H, Sun J, Jiang Q, Zhang Y and Xi Q. miR-146a-5p promotes epithelium regeneration against LPS-induced inflammatory injury via targeting TAB1/TAK1/NF-κB signaling pathway. Int J Biol Macromol 2022; 221: 1031-1040.

[55] Bozkurt B, Kamat I and Hotez PJ. Myocarditis with COVID-19 mRNA vaccines. Circulation 2021; 144: 471-484.

[56] Lazaros G, Anastassopoulou C, Hatziantoniou S, Kalos T, Soulaidopoulos S, Lazarou E, Vlachopoulos C, Vassilopoulos D, Tsakris A and Tsioufis C. A case series of acute pericarditis following COVID-19 vaccination in the context of recent reports from Europe and the United States. Vaccine 2021; 39: 6585-6590.

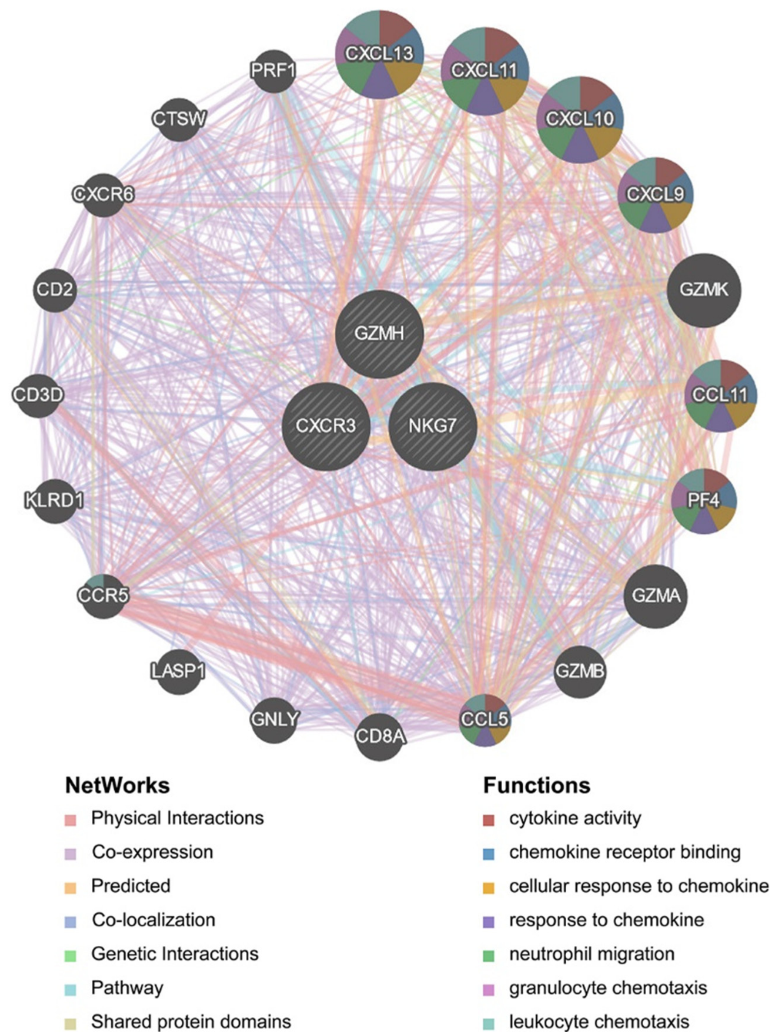
[57] Im J, Choi HS, Kim SK, Woo SS, Ryu YH, Kang SS, Yun CH and Han SH. A food-born heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), suppresses tumor necrosis factor-alpha expression in lipoteichoic acid-stimulated RAW 264.7 cells. Cancer Lett 2009; 274: 109-117.

[58] Davis DA, Archuleta MM, Born JL, Knize MG, Felton JS and Burchiel SW. Inhibition of humoral immunity and mitogen responsiveness of lymphoid cells following oral administration of the heterocyclic food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) to B6C3F1 mice. Fundam Appl Toxicol 1994; 23: 81-86.

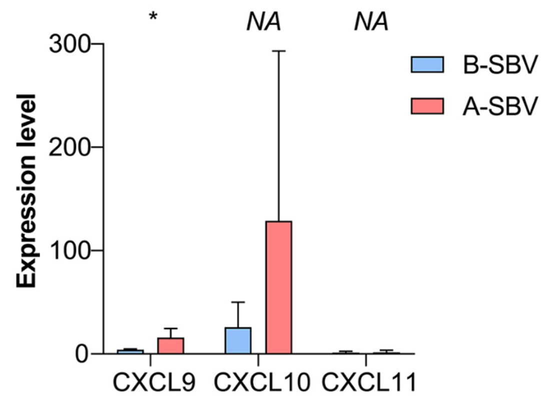
Supplementary Table 1. The basic clinical information of samples with FM vs HC and before vs after SBV in GSE169159

	FM Group		SBV Group	
	FM	HC	Day 22	Day 21
Dataset ID	Published by our team		GSE169159	
Tissue	PBMC		Blood	
GPL ID	Agilent eArray		GPL15520	
References	Liu et al.		Prabhu et al.	
No. of samples	3	3	3	3
Age, mean±SD	8.67±1.53	8.33±1.53	24.67±5.51	24.67±5.51
Male sex, No. (%)	2 (66.7)	2 (66.7)	3 (100)	3 (100)
Analysis of DEGs	t test		DEseq2	

FM: fulminant myocarditis, SBV: the second BNT162b2 vaccination.



Supplementary Figure 1. The network of three hub genes and their co-expression genes were built and analyzed by GeneMANIA. The hub genes are in the inner circle, and predicted genes are in the outer circle.



Supplementary Figure 2. Genes expression level of CXCR3 ligand in SBV-group mentioned in the discussion. B-SBV: before secondary BNT162b2 vaccination; A-SBV: after secondary BNT162b2 vaccination. Blue represents before SBV, and pink represents after SBV. **p*-value < 0.05, NA: not applicable.