

Identification of Key Genes and Biological Pathways Related to Myocardial Infarction through Integrated Bioinformatics Analysis

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What's Known

- As high-throughput technologies such as microarrays are developed, genome-wide expression analysis profiles are now more feasible. To date, various studies on myocardial infarction patients were conducted using this technology.
- Integrating huge data from numerous research found in Gene Expression Omnibus (GEO) was suggested as one of the innovative methods to increase the statistical power of such studies. Accordingly, this strategy may offer a new method for precisely identifying crucial genes and pathways that underlie the pathophysiology of both coronary artery disease (CAD) and myocardial infarction (MI).

What's New

- In the present study, to identify the key deregulated genes and pathways in coronary heart disease (CHD), three gene expression datasets were combined into a single matrix using an integrative technique.
- As a result, we identified five signaling pathways utilizing the systems biology approach, including IL-17 signaling pathway, TNF signaling pathway, toll-like receptor signaling pathway, C-type lectin receptor signaling pathway, and rheumatoid arthritis signaling pathway as the potent underlying pathogenesis of both CAD and MI.

Abstract

Background: Coronary heart disease is the leading cause of death worldwide. Myocardial infarction (MI) is a fatal manifestation of coronary heart disease, which can present as sudden death. Although the molecular mechanisms of coronary heart disease are still unknown, global gene expression profiling is regarded as a useful approach for deciphering the pathophysiology of this disease and subsequent diseases. This study used a bioinformatics analysis approach to better understand the molecular mechanisms underlying coronary heart disease.

Methods: This experimental study was conducted in the department of cardiology, Aja University of Medical Sciences (2021-2022), Tehran, Iran. To identify the key deregulated genes and pathways in coronary heart disease, an integrative approach was used by merging three gene expression datasets, including GSE19339, GSE66360, and GSE29111, into a single matrix. The *t* test was used for the statistical analysis, with a significance level of $P < 0.05$.

Results: The limma package in R was used to identify a total of 133 DEGs, consisting of 124 upregulated and nine downregulated genes. KDM5D, EIF1AY, and CCL20 are among the top upregulated genes.

Moreover, the interleukin 17 (IL-17) signaling pathway and four other signaling pathways were identified as the potent underlying pathogenesis of both coronary artery disease (CAD) and MI using a systems biology approach. Accordingly, these findings can provide expression signatures and potential biomarkers in CAD and MI pathophysiology, which can contribute to both diagnosis and therapeutic purposes.

Conclusion: Five signaling pathways were introduced in MI and CAD that were primarily involved in inflammation, including the IL-17 signaling pathway, TNF signaling pathway, toll-like receptor signaling pathway, C-type lectin receptor signaling pathway, and rheumatoid arthritis signaling pathway.

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Keywords • Myocardial infarction • Coronary disease • Bioinformatics

Introduction

Coronary heart disease (CHD) is the leading cause of global mortality, accounting for an estimated 17 million deaths, or

nearly one-third of all deaths worldwide.¹ In addition, the underlying cause of myocardial infarction (MI), also known as heart attack, is coronary artery disease. MI occurs when an *atherosclerotic* plaque recaptures and causes a blood clot in an artery, completely blocking the blood flow through a coronary artery.²

A significant number of people affected by CAD were studied, and it was found that it occurs in apparently normal individuals with a few or no traditional risk factors, such as high LDL cholesterol level, low HDL cholesterol level, high blood pressure level, family history, diabetes, and smoking.³

Traditional family pedigrees analysis in twins revealed the heritability trait of coronary artery disease, with the rate of genetic variance in CAD being around 50%. Consequently, the findings emphasize the genetic contribution to this disease.⁴

Despite the tendency in the aggregation of CAD in families, it is still a common disorder. A strong genetic contribution was identified in genome-wide association studies (GWAS) and by comparing the disease cases and disease-free controls. The GWAS identified several physiological pathways related to the genetic loci that were linked to coronary artery disease. However, since the causal genes and variants for most loci were not identified definitively, most of these loci were selected based on their proximity to the nearest genes.⁵

Genome-wide gene expression analysis is a powerful tool for GWAS validation. Nowadays, genome-wide expression analysis profiles are becoming more feasible with the development of high-throughput technologies such as microarray and Ribonucleic acid (RNA) sequencing.⁶ One of the innovative approaches proposed for increasing the statistical power of such studies is the integration of big data from existing studies in the Gene Expression Omnibus (GEO). Accordingly, this strategy may provide a new method of accurately recognizing crucial genes and pathways as underlying pathophysiology in both CAD and MI.

In the present study, an integrative approach was initially used to identify the differentially expressed genes (DEGs) in both MI patients and healthy individuals. Thereafter, a system biology approach was used to determine the involved

signaling pathways and potential molecular interactions in order to gain insight into the underlying pathogenesis of both CAD and MI. The obtained results can provide expression signatures and potential biomarkers in CAD and MI pathophysiology, which can contribute to both diagnosis and treatment.

Overall, this study used bioinformatics analysis to gain a better understanding of the molecular mechanisms underlying coronary heart disease.

Materials and Methods

Data Collection and Preprocessing

This experimental study was conducted in the department of cardiology, Aja University of Medical Sciences (2021-2022), Tehran, Iran. The study was approved by the Ethics Committee of Aja University of Medical Sciences (IR.AJAUMS.REC.1399.135).

Exploring the GEO database resulted in the selection of three microarray expression datasets comprising 125 samples, including 89 samples from MI patients and 54 healthy individuals. The datasets were obtained using the GEO query package in R software (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria, <https://www.R-project.org/>). 125 samples were involved in the analysis (table 1). No treatment was given to any of the samples. All datasets were preprocessed with Robust multiarray average (RMA). Each probe set ID was mapped to a distinct gene symbol. For the genes that were mapped to the multiple probes, the average expression value was taken into account.

Data Integration and Batch Effect Removal

The combat method was used to eliminate the batch effect across all datasets. The Reduce function in R was used to merge the preprocessed expression datasets into a single global expression matrices dataset according to gene symbol.

Identification of Differentially Expressed Genes (DEGs)

The limma package in R (v 3.5.1) was used to determine the DEGs between patients and normal samples, with the cut-off criteria of $|\log_2FC| > 1$ and significance of $P < 0.05$.

Table 1: Gene Expression Omnibus (GSE) list

GEO dataset	Platform	MI	Normal	Total	Samples discarded
GSE29111	GPL570	36 (69%)	-	52 (100%)	16 (31%)
GSE66360	GPL570	49 (49%)	50 (51%)	99 (100%)	-
GSE19339	GPL570	4 (50%)	4 (50%)	8 (100%)	-

Construction of Protein-protein Interaction Network

The protein-protein interaction network (PPIN) among DEGs was reconstructed using the Search Tool for Retrieval of Interacting Genes/Proteins online database (STRING) (<http://string-db.org>).⁷ The degree and betweenness centrality (BC) were assessed using the Network-analyzer in Cytoscape from the US National Institute of General Medical Sciences (NIGMS). The PPIN was visualized by Cytoscape 3.7.2.

Functional and Pathway Enrichment Analysis

The up- and downregulated DEGs were enriched in DAVID (US DAVID Bioinformatics Resources) and Enrichr Package (US Ma'ayan Lab) to determine the gene ontology and pathways enriched with them.

Results

The GEOquery package in R software was used to download three datasets (GSE19339, GSE66360, GSE29111).

Screening of DEGs

The limma package in R was used to identify a total of 133 DEGs, which included 124 upregulated and 9 downregulated genes. Table 2 summarizes the top ten most significantly up- and downregulated DEGs. The heatmap of the top 100 DEGs between case and controls is shown in figure 1A. The difference in the gene expression values between the two groups was

obvious. Moreover, the volcano plot indicates all differentially expressed genes, with green and red dots representing upregulated and downregulated genes, respectively (figure 1B). The KDM5 transcript is marked as the top-ranked gene among upregulated genes. The KDM5 family is involved in a wide variety of molecular processes, including cell proliferation, migration, and angiogenesis. Among the upregulated genes, the EIF1AY transcript has the second-ranked transcript. This gene, which is found in the non-recombining region of the human Y chromosome (NRY), encodes for human male-specific minor histocompatibility antigens that contribute to immune-mediated disease. The third-ranked transcript among the upregulated genes belongs to CCL20. CCL20 is a chemokine that promotes the migration of immune cells. In this study, downregulated genes were not further investigated.

Construction of PPIN and Identification of Hub Genes

The identified DEGs were mapped into the STRING database to determine the interactions between the corresponding proteins. As shown in figure 2, the resulted PPIN has 99 nodes and 1280 edges interactions.

The top five genes in the PPI network based on the highest degree of expression (The number of connections a node has to other nodes in the network.), and BC (the number of times a node is on the shortest path between other nodes) are CXCL8 (degree 47, BC 0.09), TLR2 (degree 46, BC 0.09), MMP9 (degree 40, BC 0.09), IL1B (degree 40, BC 0.06), and CXCL1

Table 2: The top up- and downregulated DEGs

Gene. symbol	logFC	P value	Adjusted P value
Upregulated			
KDM5D	2.63	<0.001	0.02
EIF1AY	2.40	<0.001	0.01
CCL20	2.37	<0.001	<0.001
RPS4Y1	2.13	<0.001	0.02
NR4A2	2.05	<0.001	<0.001
ITLN1	1.82	<0.001	<0.001
USP9Y	1.79	<0.001	0.02
IL1B	1.78	<0.001	<0.001
PTX3	1.75	<0.001	<0.001
TREM1	1.73	<0.001	<0.001
Down-regulate			
XIST	-2.36	<0.001	<0.001
TSIX	-2.30	<0.001	<0.001
CCR2	-1.10	<0.001	<0.001
B3GALT2	-1.07	<0.001	<0.001
EOMES	-1.07	<0.001	<0.001
GIMAP4	-1.06	<0.001	<0.001
GIMAP7	-1.05	<0.001	<0.001
GIMAP6	-1.03	<0.001	<0.001
WDR86-AS1	-1.00	<0.001	<0.001

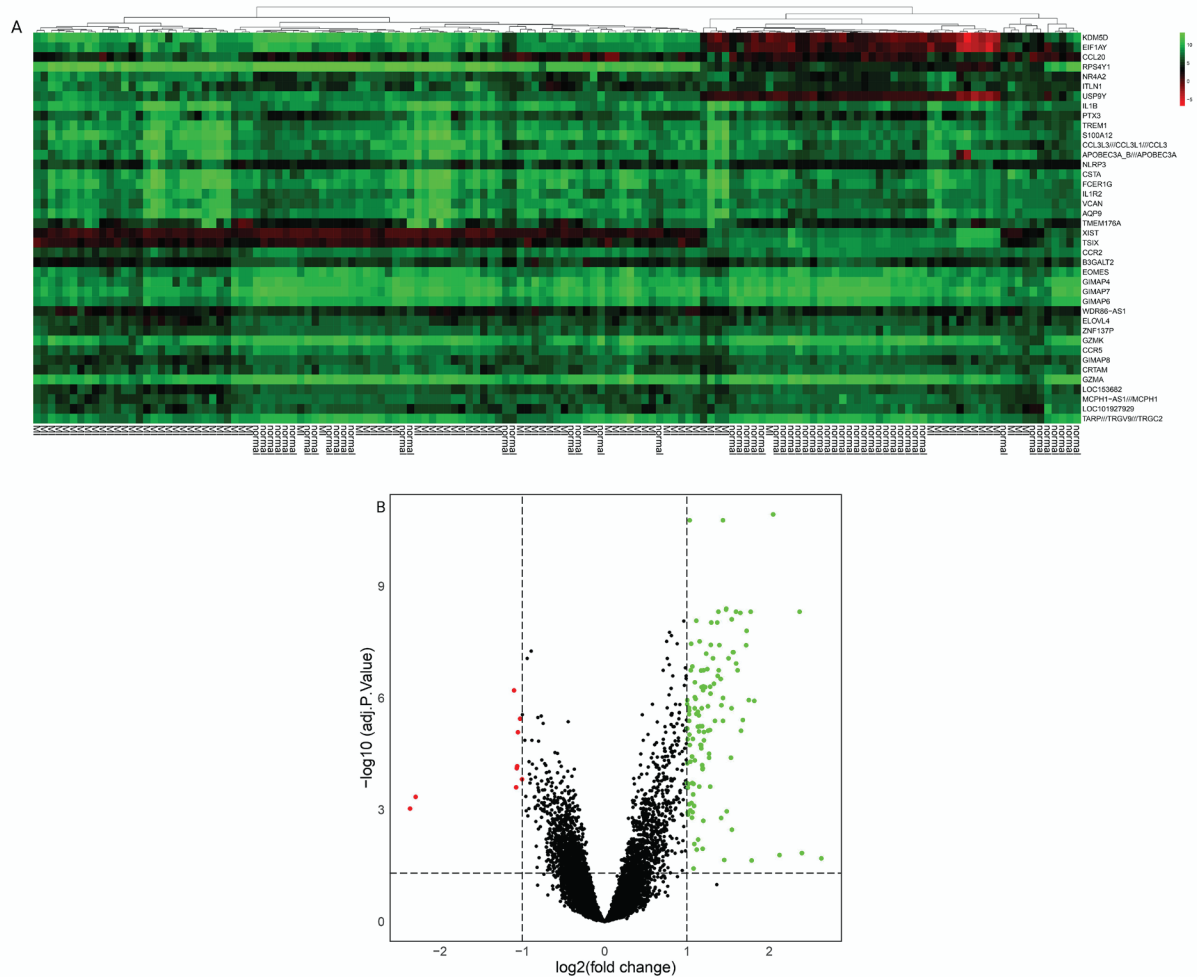


Figure 1: Different visualization of differentially expressed genes (DEGs) between myocardial infarction (MI) and normal individuals. A: The heatmap of top 40 DEGs. The row and column represent gene symbol and patient type, respectively. B: Volcano plot visualized the DEGs. Green and red points represent upregulated and downregulated DEGs, respectively

(degree 37, BC 0.04).

Functional Annotation Analysis

To determine the biological functions of the identified DEGs, the upregulated and downregulated genes were separately enriched. However, the focus of this study was on the genes that were upregulated for molecular function categories, biological processes, and cellular components. The upregulated DEGs in the molecular function class were primarily enriched in the IL-17 signaling pathway, tumor

necrosis factor (TNF) signaling pathway, and toll-like receptor signaling pathway, all of which are involved in the immune and inflammation systems (table 3). Moreover, according to DAVID Gene Ontology (GO) data, the upregulated DEGs in the biological process were primarily enriched in the inflammatory response and neutrophil chemotaxis. The upregulated DEGs for cellular components were primarily enriched in extracellular space, and for KEGG Pathway, they were primarily enriched for TNF signaling pathway and receptor interaction (figure 3).

ID	Pathways	Gene No	P value	Genes
hsa04657	IL-17 signaling pathway	14	<0.001	JUN, CXCL8, CCL20, CXCL1, FOS, CXCL3, PTGS2, CXCL2, MMP9, NFKBIA, IL1B, FOSB, S100A9, S100A8
hsa04668	TNF signaling pathway	11	<0.001	NFKBIA, JUN, EDN1, CCL20, IL1B, CXCL1, FOS, CXCL3, PTGS2, CXCL2, MMP9
hsa04620	Toll-like receptor signaling pathway	9	<0.001	NFKBIA, JUN, CXCL8, IL1B, CCL4, CCL3, CD14, FOS, TLR2
hsa04625	C-type lectin receptor signaling pathway	9	<0.001	NFKBIA, JUN, CLEC4D, EGR3, FCER1G, IL1B, NLRP3, CLEC4E, PTGS2
hsa05323	Rheumatoid arthritis	5	<0.001	JUN, CXCL8, CCL20, IL1B, CCL3, CXCL1, FOS, TLR2

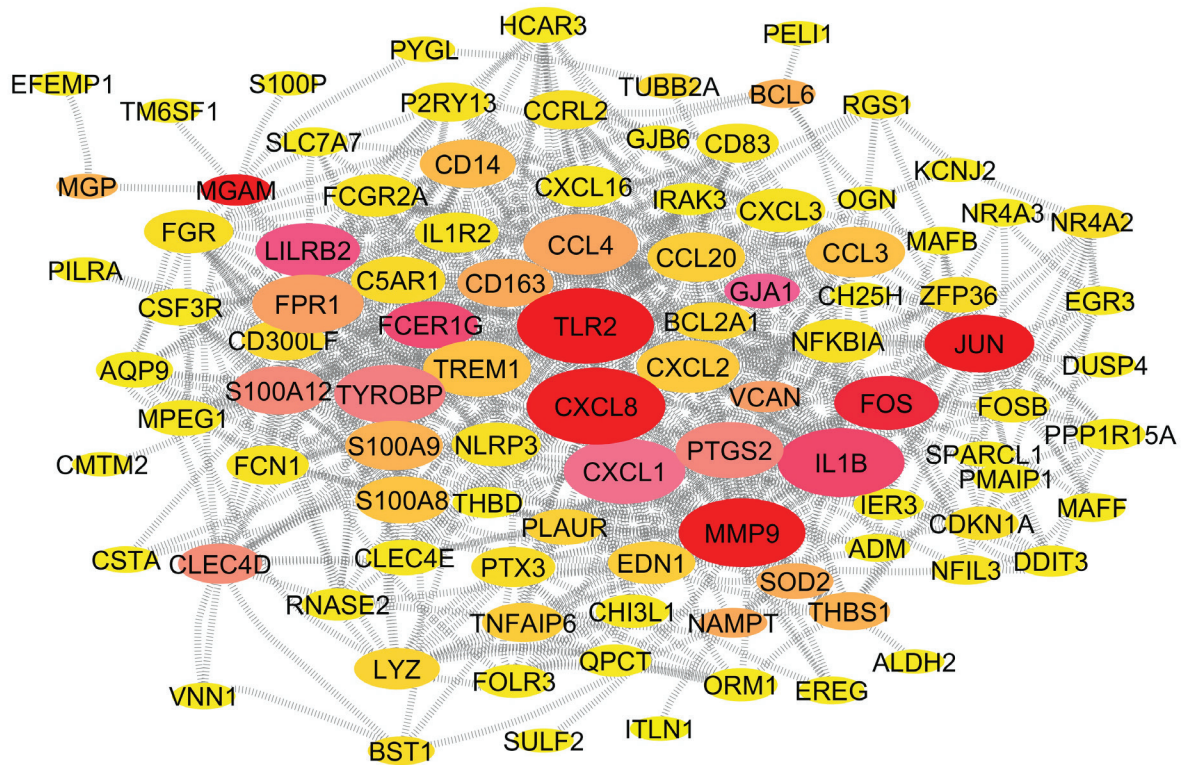


Figure 2: Protein-protein interaction network including differentially expressed genes (DEGs). The circular nodes represent the proteins, and the grey lines represent interactions. Only upregulated genes are included in the protein-protein interaction network (PPIN). The thicker edge represents the higher STRING combined score, and the bigger node size is indicative of the higher degree.

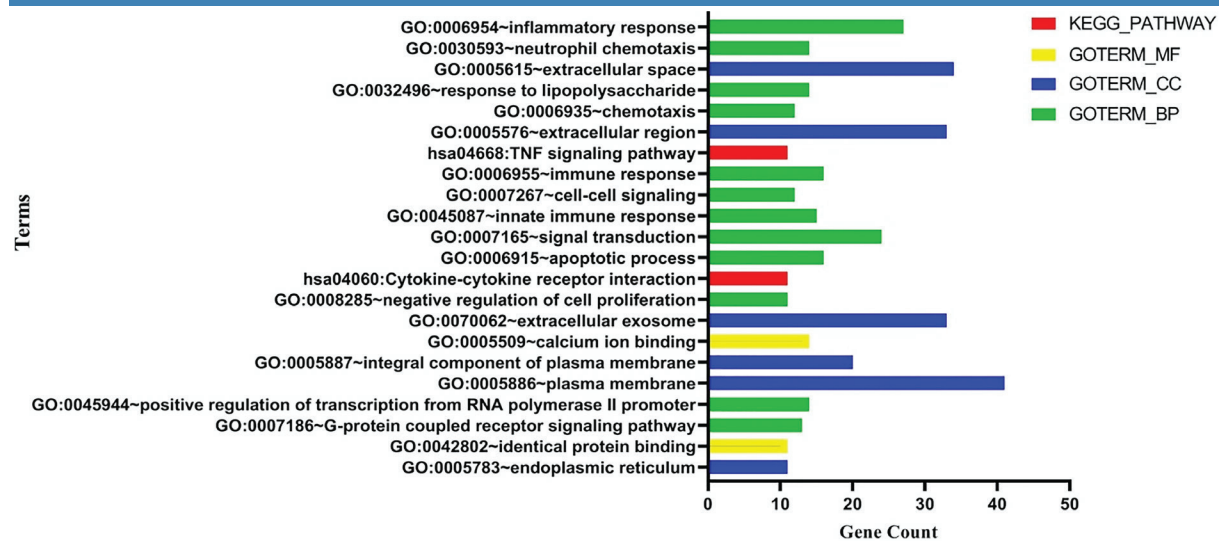


Figure 3: Gene ontology (GO) enrichment analysis of upregulated genes. The significant GO terms were selected based on the gene count and the chart was displayed according to the data obtained from DAVID GO term enrichment analysis. Moreover, the lines were sorted from top to bottom based on how significant that term is ($P < 0.05$). BP: Biological process; CC: Cellular component; MF: Molecular function

Discussion

In the present study, in order to provide a meta-data as well as to obtain a deeper understanding of the functional importance of critical genes and pathways in coronary heart disease and MI, three expression array datasets were integrated with MI patients and normal individuals.

KDM5 is the first ranked upregulated gene. Since the KDM5 family is involved in all mechanisms, including cell proliferation, migration, and angiogenesis, they play a significant role in the development of atherosclerosis.⁸ In an *in vitro* study by Marika Mokou and others, KDM5 was introduced as a novel pharmacological candidate with increased

expression in cardiovascular diseases.⁹

In the current study, EIF1AY was the second-ranked transcript among the upregulated genes. Yingfang Tian and others previously reported this gene's up-regulation in ischemic stroke. This could imply that these genes play a sex-specific role in stroke.¹⁰ Moreover, in another study, Wei Zhao and colleagues reported the Trans effects association of this gene with the Chr9p21 region, which is one of the main loci associated with CAD and MI.¹¹

The third-ranked transcript among upregulated genes is CCL20. Accordingly, it is a chemokine that selectively attracts immature dendritic cells, effector/memory T lymphocytes, and naive B cells.¹²

Olivier Calvayrac and others for the first time in their study reported CCL20 up-regulation in cardiovascular diseases. They discovered a link between this gene and the inflammatory process of atherosclerosis.¹³ Furthermore, Safa and others reported CCL20 overexpression in patients with ischemic heart disease.¹⁴ Furthermore, bioinformatics analysis revealed five upregulated DEGs that were found to be enriched in the immune system, specifically the inflammation pathway (table 3).

Atherosclerosis is a chronic inflammatory disease that involves acute coronary disorders such as AMI and is associated with cardiovascular diseases. In the present study, the IL-17 signaling pathway was the leading upregulated pathway, which primarily acts as an inflammation mediator.¹⁵ Several studies previously showed that IL-17 is a cytokine that plays a critical role in AMI. Additionally, IL-17 plays a potential role in the immune response by triggering the release of different inflammatory mediators from several types of cells involved either in the damage or the injury process in myocardial tissue.¹⁶

Ischemic damage to myocardial tissue induces a strong inflammatory response, which is followed by increased levels of some inflammatory cytokines, such as IL-17, which is primarily derived from $\gamma\delta$ T cells in cardiac tissue. Overall, some research and evidence demonstrated that IL-17 contributed to endothelial cell activation and leukocyte recruitment, which consequently stimulated the inflammatory response in ischemic tissue.¹⁶

Undoubtedly, TNF- α is a crucial proinflammatory cytokine that plays a significant role in the early inflammatory phase. Several previous *in vivo* and *in vitro* animal model studies confirmed that TNF- α promotes the emergence of several cardiovascular pathologies.¹⁷

As TNF- α is one of the most prominent proinflammatory cytokines, it is introduced as

one of the first options in the cytokine-targeted approach. TNF- α inhibitors have recently been used in clinical trials as anti-inflammatory drugs in the treatments of patients with systemic inflammatory diseases.¹⁸

One of the well-documented proinflammatory mechanisms is endothelial cell activation.¹⁹ In this regard, endothelial cells increase the expression of adhesion molecules in response to TNF- α , which regulates both the rolling and adhesion of inflammatory immune cells into the tissue. Moreover, TNF- α is known to increase reactive oxygen species (ROS) levels as well as decrease nitric oxide production in blood vessels, which consequently can cause endothelial dysfunction, as an initial step in atherogenesis.²⁰

Toll-like receptors (TLRs) are pattern recognition receptors (PRRs) that are responsible for releasing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), which induce innate immune responses. Increasing evidence in this regard suggested that TLR signaling pathway molecules were involved in the progression of heart failure.²¹

The TLR signaling pathway regulates a wide range of inflammatory mediators and serves as a prominent upstream mechanism for inflammatory signaling activation. Consequently, TLR signaling is a potential target in heart failure therapy and may provide a reliable therapeutic approach. As a result, various therapeutic agents for TLR signaling inhibition were developed to control excessive inflammation.²²

However, a few issues remain unclear. First, it is yet unknown how TLRs are activated. More ligands need to be identified in heart failure. Second, further investigation is required regarding the regulation of TLRs in heart failure. Acquiring a better understanding of these questions may lead to the development of a novel therapy for preventing or delaying the onset and progression of heart failure.^{21, 23}

A growing body of information and evidence suggests that the C-type lectin-like receptor family plays a critical role in atherothrombosis. Furthermore, since its discovery, new research revealed the structure, function, and disease relevance of C-type lectin-like receptor-2 (CLEC-2).²⁴ In an investigation, Fei and others found a strong correlation between high plasma levels and Soluble C-Type Lectin-Like Receptor 2 (sCLEC-2), which is an independent CAD risk factor.²⁵

Overall, prior integrated bioinformatics studies on MI were primarily enriched in cytokine-cytokine receptor interaction, chemokine signaling pathway, and TNF signaling pathway, which revealed the significant role of

inflammation in the pathogenesis of MI.²⁶

The present study had some potential limitations. The first limitation was the inability to find better matched GSE data for increasing the sample size. The second limitation was the lack of clinical follow-up of patients in the mentioned GSE.

Conclusion

In conclusion, five signaling pathways were identified through integrated bioinformatics analysis in MI and CAD, which were primarily associated with inflammation: the IL-17 signaling pathway, the TNF signaling pathway, the toll-like receptor signaling pathway, the C-type lectin receptor signaling pathway, and rheumatoid arthritis signaling pathway. These findings can provide reliable molecular biomarkers for the screening, diagnosis, and prognosis of novel therapeutic targets for MI and CAD.

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Authors' Contribution

N.E: Study design, data analysis, and drafting; R.A: Study design, critical revision; M.N.S: Data acquisition, drafting the manuscript, and critically revision of the manuscript; N.G: Data acquisition, data analysis and drafting the manuscript; All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions re-lated to the accuracy or integrity of any part of the work are appropriately investigated and re-solved.

Conflict of Interest: None declared.

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