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Research Article

Identification of Key Genes and Pathways in First Acute Myocardial Infarction Based on Gene Expression Profiling by Bioinformatics Analysis

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ABSTRACT

Patients with acute myocardial infarction (AMI) have high morbidity and mortality. This study explored the molecular mechanisms of first acute myocardial infarction (FAMI) with integrated bioinformatic analyses of the feature genes and the correlative gene functions. The expression profile of GSE24519 was downloaded from the Gene Expression Omnibus database. Differentially expressed genes (DEGs) between FAMI and normal specimens were identified. Gene ontology (GO) enrichment analysis on gene functions and Kyoto Encyclopedia of Gene and Genome (KEGG) pathway analysis were performed by using the Database for Annotation, Visualization and Integrated Discovery (DAVID). Cytoscape was used to visualize the protein-protein interaction (PPI) of these DEGs. GO analysis revealed that the "transcription", "DNA binding" and "cilium" were the enriched GO terms belonging to BP, MF and CC, respectively. The KEGG pathway analysis showed that the aberrant expression of long-term potentiation, amphetamine addiction and calcium signaling pathway were closely associated with FAMI occurrence. Importantly, JUN, PRKACB, GNB4, MTOR, HDAC2, PPP3CB, PPP3R1 and, FGFR3 were predicted to be significantly related to FAMI. Our discovery provides a registry of genes and pathways that are disrupted in FAMI, which has the potential to be used in the clinic for diagnosis and targeted therapy of FAMI in future.

Introduction

Myocardial infarction is the single most critical event in coronary disease, and acute myocardial infarction (AMI) is one of the top ten leading causes of death worldwide [1, 2]. Although the incidence of myocardial infarction decreased between 1997 and 2008 in the United States, approximately 155,000 new asymptomatic silent cases occur annually [3, 4]. Especially in developing countries, the morbidity of AMI is still high, and it is becoming a significant health burden [5]. Epidemiological studies showed that AMI is associated with many risk factors including older age, smoking, high blood pressure, diabetes mellitus, and total cholesterol and high-density lipoprotein levels et al [6]. However, these environmental factors cannot fully explain the progression and etiology of AMI. Several molecular markers, such as ANP, BNP, CRP, and some other serum biochemical markers have gained increasing attention but only provided modest increases in predictive capacity [7]. Therefore, the molecular mechanisms contributing to the pathogenesis of AMI should be further demonstrated to identify potential target genes for the prevention and therapy of AMI.

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There is growing evidence showed that genetic predisposition plays an essential role in the development of AMI, and that family history of ischemic heart disease may affect the incidence of AMI [8]. Previous studies have been conducted to identify key genes that participate in the occurrence and development of AMI. Suresh et al. established a gene expression profiling by microarray and found that modulation of cholesterol transport genes, including ABCA1, CETP, APOA1, and LDLR, were associated with clinical outcome [9]. Gao et al. predicted several crucial genes in AMI progression, such as CCL5, BCL3, and NCOA7 [10]. However, the present knowledge is inadequate to explain how these candidate genes are involved in AMI progression. Therefore, there is a great need to identify novel genes and to develop novel methods to elucidate the mechanism controlling AMI.

Bioinformatics analysis of gene expression profiles has shown remarkable promise in uncovering potential key genes and pathways in complex diseases [11]. It might also be useful to identify novel genes as well as biological processes implicated in AMI pathogenesis. Therefore, in this study, we downloaded the original data (GSE24519), provided by Bellin M, from the publicly available Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo/) to identify DEGs and the associated biological processes in AMI using comprehensive bioinformatics analyses. Function enrichment and pathway analysis of DEGs were carried out. Moreover, the protein-protein interaction (PPI) network was constructed to screen for key gene nodes. Analysis of the biological functions and pathways in these disorders may provide further insights regarding AMI development at the molecular level and pave the way toward understanding potential disease pathogenesis mechanisms to facilitate diagnosis, prognosis, and the identification of drug targets.

Materials and methods

I Microarray data

The gene expression profile GSE24519 was downloaded from GEO database, including 38 samples obtained from 17 FAMI patients and 4 from normal people. Gene expression profiles were obtained by using the GPL2895 platform (GE Healthcare/Amersham Biosciences CodeLink Human Whole Genome Bioarray).

II Data preprocessing and analysis of DEGs

GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r /) is a useful web tool for comparing GEO datasets data from two groups (NCBI 2012) [12]. GEO2R was used to analyze the published GSE24519 microarray dataset to compare the FAMI group and the control group. Genes with $P < 0.05$ and $|\log2FC \text{ (fold change)}| > 2$ were regarded as DEGs. A heatmap of the top 60 DEGs was generated using the online tool ClustVis (a web tool for visualizing clustering of multivariate data), which was also used to carry out a principal component analysis (PCA).

III GO function and pathway enrichment analysis of DEGs

GO analysis is a commonly used method for the functional annotation of large-scale genomic data [13]. The KEGG pathways database is a comprehensive and well recognized database with a wide range of biochemical pathways [14]. Pathway analysis was used to assess significant pathways that DEGs participated according to the KEGG database [15]. The Database for Annotation, Visualization, and Integrated Discovery (DAVID, https ://david .ncifc rf.gov/) is a series of functional annotation tools which was used to enrich GO functions and KEGG pathways for the systematic and integrative analysis of large gene lists. A value of $P < 0.05$ was considered statistically significant.

IV PPI network construction

Search Tool for the Retrieval of Interacting Genes (STRING; http://string.embl.de/) database is an online tool containing known and predicted protein interactions to conduct functional PPI analysis. In this study, we utilized the identified DEGs to construct a PPI network with STRING. The interactions between proteins with a score of ≥ 0.4 were visualized using Cytoscape. The top ten DEGs with high degrees of connectivity were selected as the hub genes for FAMI.

V Module selection from the PPI network

Molecular complex detection (MCODE) is a cytoscape plugin that was used to detect strongly connected regions of the PPI network. Subsequently, based on modules selected from the PPI network, GO functional and KEGG pathway enrichment analyses of the most significant modules were performed using EXCEL at a significance of $P < 0.05$.

VI Pathway-net analysis of the significant pathways

Pathway-net analysis, the interaction network of the significant pathways of DEGs, was built according to the interaction among pathways of the KEGG database to identify the relationship among the significant pathways directly and systemically. Each pathway in the network was measured by counting its number of upstream and downstream pathways, which were displayed as 'in-degree' and 'out-degree'. A higher degree of a pathway indicated that it regulated or was regulated by other pathways, implying a more important role in the signaling network. Pathway-net analysis could summarize the pathways interaction of DEGs and discover why some kinds of pathway were activated [16].

Table 1: The top60 significant up-regulated and down-regulated DEGs involved in FAMI according to fold change.

Group	Gene Symbol
Upregulated genes	LALBA, NPIPB5, SCAMP3, SRI, P3H2, MARS2, F10, LOC102467216, LOC105378604, GPRC5C, PDE1C, ATP2B1, STS, PTH, SSX3, PDE4DIP, GJB6, NRAV, LOC105377480, PPP1R1A, CCDC153, DIAPH3-AS2, LDOC1L, RERE, RAB11FIP4, TMEM184A, LDB2, MACC1, TMPRSS6, ZNF786
Downregulated genes	ATXN7L1, PURA, KRI1, C2orf48, HNRNPA2B1, IL1RL1, LINC01410, ICOSLG, ZNF721, TIFAB, DKK1, ZNF720, BTBD7, STAM2, GNL3, WDR26, PLPPR5, LOC101929288, LPA, RAP2B, LRRC31, CLEC4G, BAGE2, RGS2, CLK4, ZNF681, LOC101928569, SMG8, ARHGEF7-IT1, FYB

Results

I Identification of DEGs

A total of 28,321 genes from 34 FAMI platelets samples and 4 control samples were obtained. Therein, a total of 712 DEGs were identified as significant in FAMI patients compared to normal controls, including 46 (6.5%) upregulated and 666 (93.5%) downregulated genes. The top 30 up- and down-regulated genes for FAMI and control are listed in (Table 1). We chose the 60 genes with the most significant differences to make the heatmap as showed in (Figure 1), in which these DEGs could well distinguish the two kinds of sample, indicating the DEGs could be used for further analysis. The PCA plot is shown in (Figure 2), and it is clear that the samples from FAMI and control were grouped in different PCA plots, indicating an obvious distinction between the control and FAMI patients.

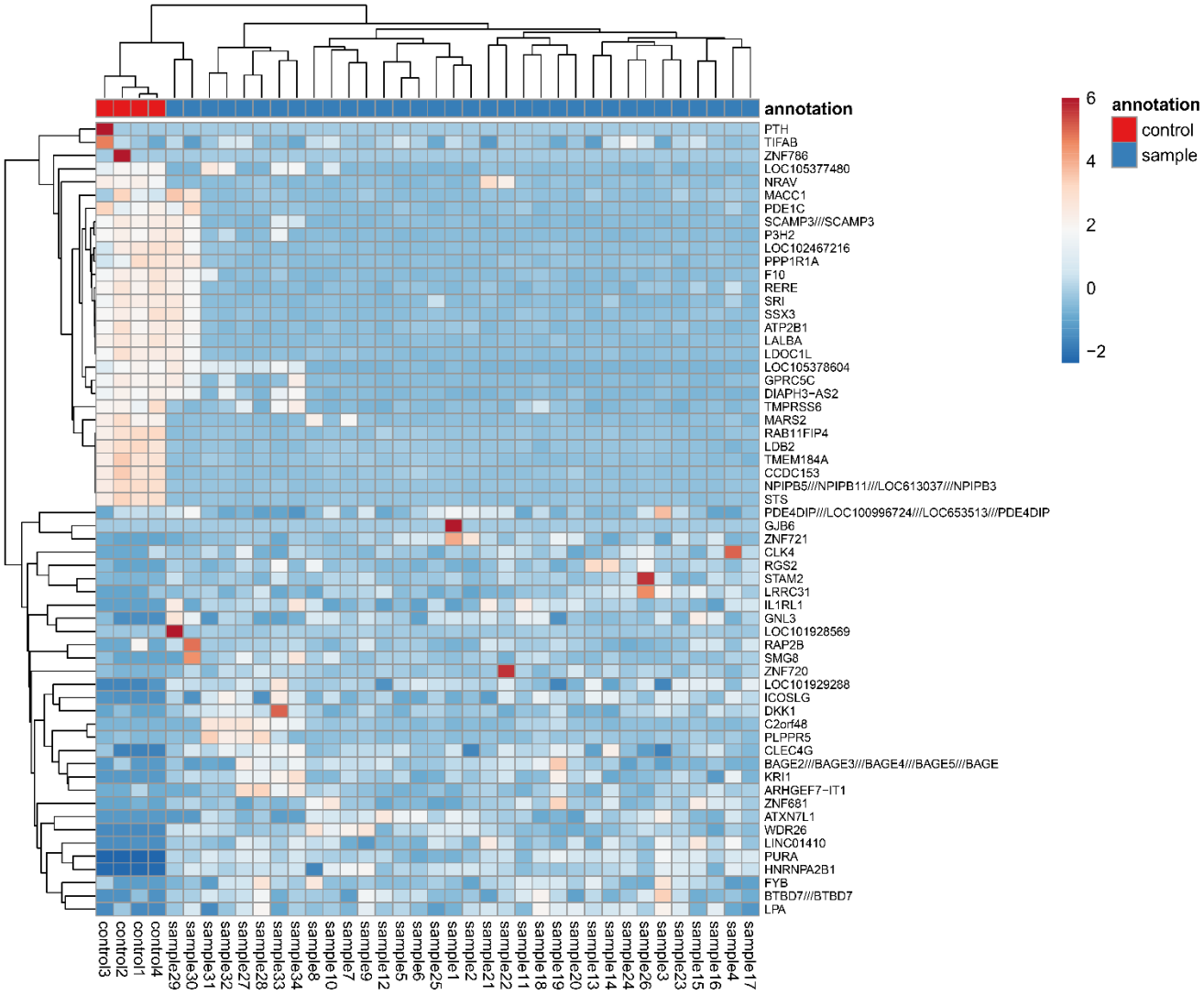

Figure 1: Heatmap plot of differentially expressed genes (DEGs) across all samples. X-axis represents the names of each sample in dataset GSE24519, and Y-axis represents the clusters of genes. The color toward red represents high expression values, and color toward blue represents lower expression values.

II Functional enrichment analysis of DEGs

To gain insights into the biological roles of the DEGs from FAMI and normal control samples, we performed GO categories enrichment analysis. In this study, all DEGs were uploaded to DAVID in order to identify overrepresented GO categories. GO term enrichment analysis results varied according to GO classification and expression change of DEGs. With the criterion of $P<0.05$ (top 10), transcription, regulation of transcription, negative regulation of transcription from RNA polymerase II promoter exhibited highly significant enrichment within the GO biological process category (Figure 3). For the cellular component category, DEGs were significantly enriched in cilium, intracellular and nuclear membrane (Figure 4). In addition, the molecular function category contained DEGs significantly enriched in transcription factor activity, sequence-specific DNA binding, nucleic acid binding and syntax in binding (Figure 5). KEGG pathway enrichment analysis was then used to gain a better understanding of the signaling pathway enrichment of DEGs. With the criterion of $P<0.05$, the top enriched biological pathways associated with FAMI included long-term potentiation, amphetamine addiction, calcium signaling pathway, pathways in cancer, inositol phosphate metabolism, histidine metabolism, glutamatergic synapse, Wnt signaling pathway and Glucagon signaling pathway (Figure 6).

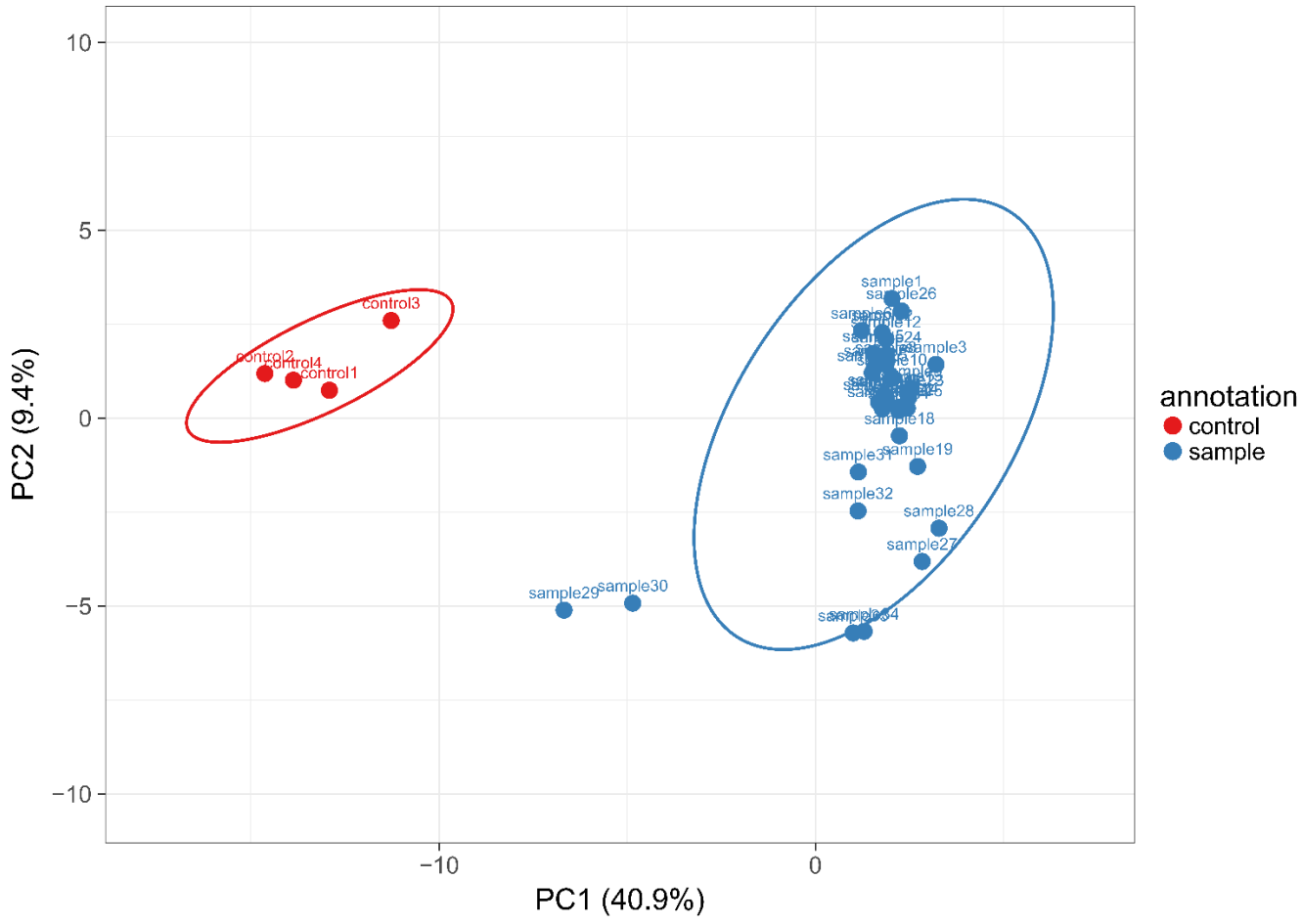

Figure 2: Principal component analysis of (PCA) of 38 samples.

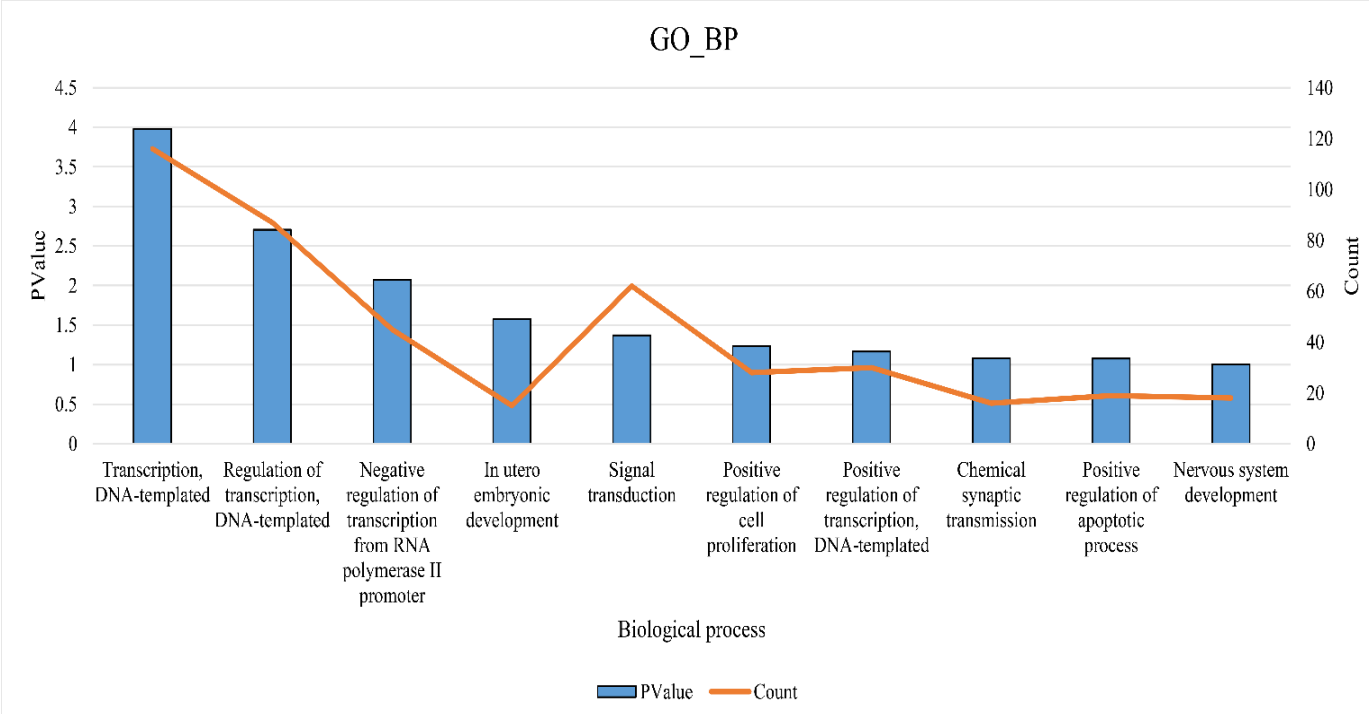

Figure 3: Top ten GO-biological process functional enrichment analyses of all DEGs.

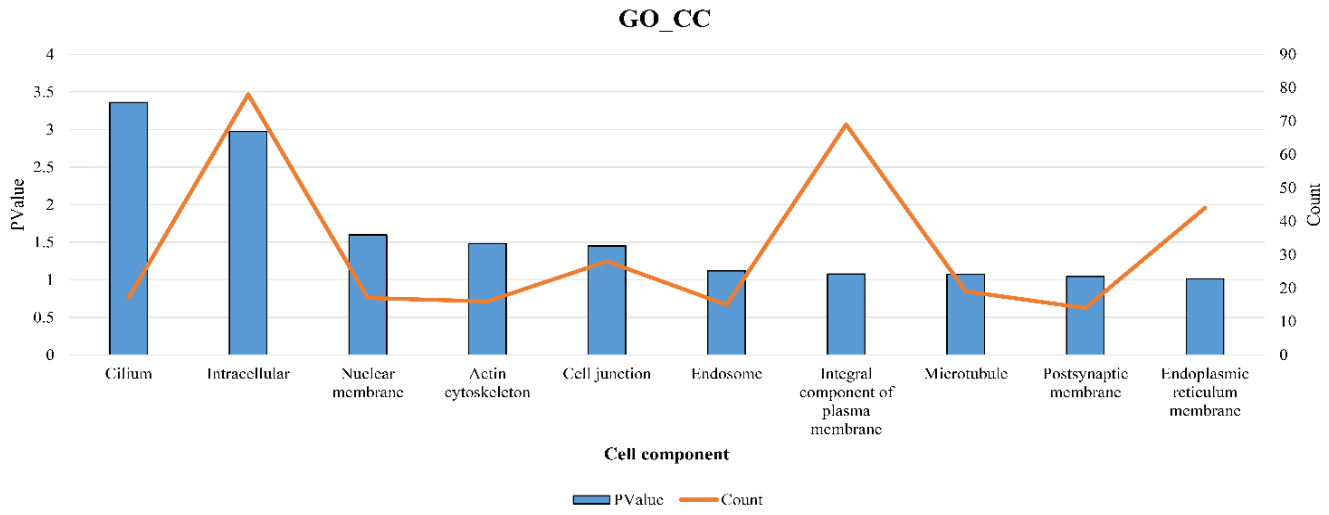

Figure 4: Top ten GO-cellular component functional enrichment analyses of all DEGs.

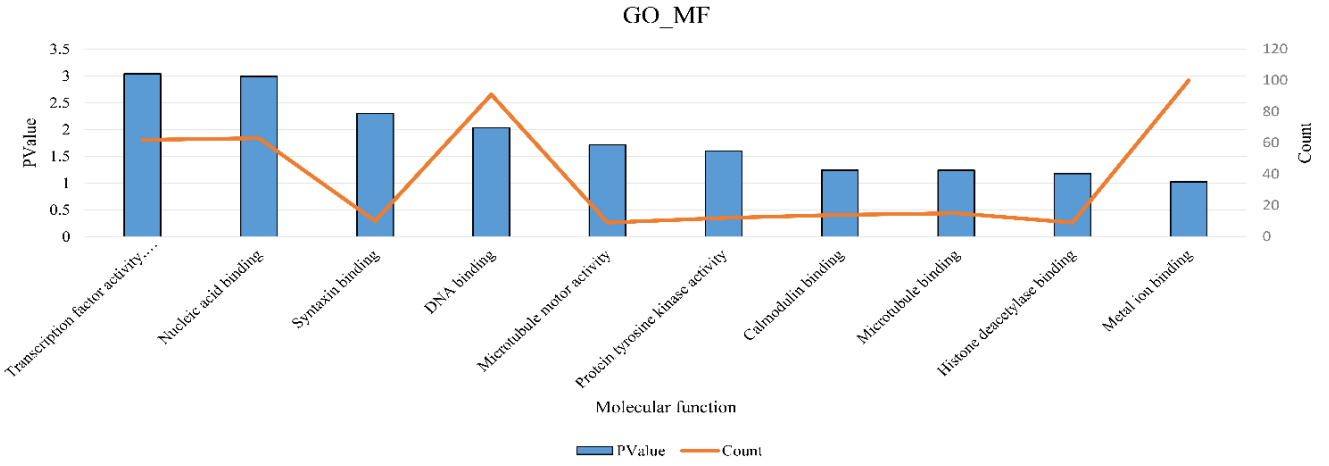

Figure 5: Top ten GO-molecular function functional enrichment analyses of all DEGs.

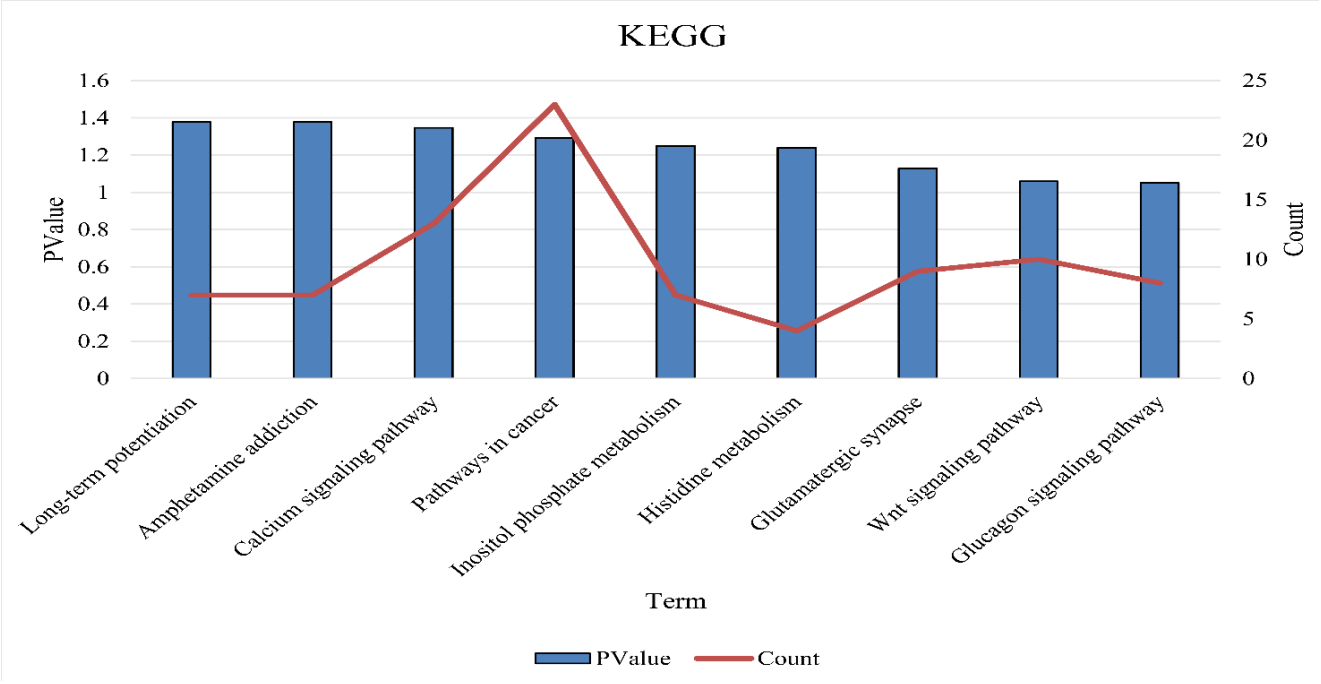

Figure 6: Top enriched KEGG pathways of all DEGs.

Table 2: Top hub genes in the PPI network according to degree.

Gene	Degree	Eccentricity	Stress	Betweenness Centrality	Clustering Coefficient
TOP2B	66	7	274962	0.18211583	0.07132867
ACACA	54	7	203934	0.09273358	0.10062893
ACACB	52	7	184984	0.07756531	0.10859729
PIKFYVE	43	7	215308	0.12596274	0.07973422
JUN	39	7	131144	0.09022732	0.09041835
PRKACB	34	6	118896	0.0705608	0.06238859
GNB4	29	7	82874	0.03860331	0.21428571
MTOR	28	7	107822	0.05075502	0.14550265
HDAC2	26	7	63424	0.04655674	0.14461538
PPP3CB	25	7	81700	0.0392962	0.14666667
ABL1	25	7	96470	0.05512343	0.15666667
NR3C1	24	7	82498	0.0454593	0.10144928
CDC20	23	6	69044	0.04353424	0.15019763
NTSR1	22	6	32376	0.02463689	0.23376623
DCN	19	7	65484	0.04207577	0.13450292
PGK2	18	7	62058	0.03546438	0.1503268
MGP	17	6	34046	0.02859951	0.14705882
PPP3R1	17	7	34272	0.01602586	0.13970588
FGFR3	17	7	57608	0.03273839	0.07352941
BTRC	17	7	18952	0.01143977	0.22794118

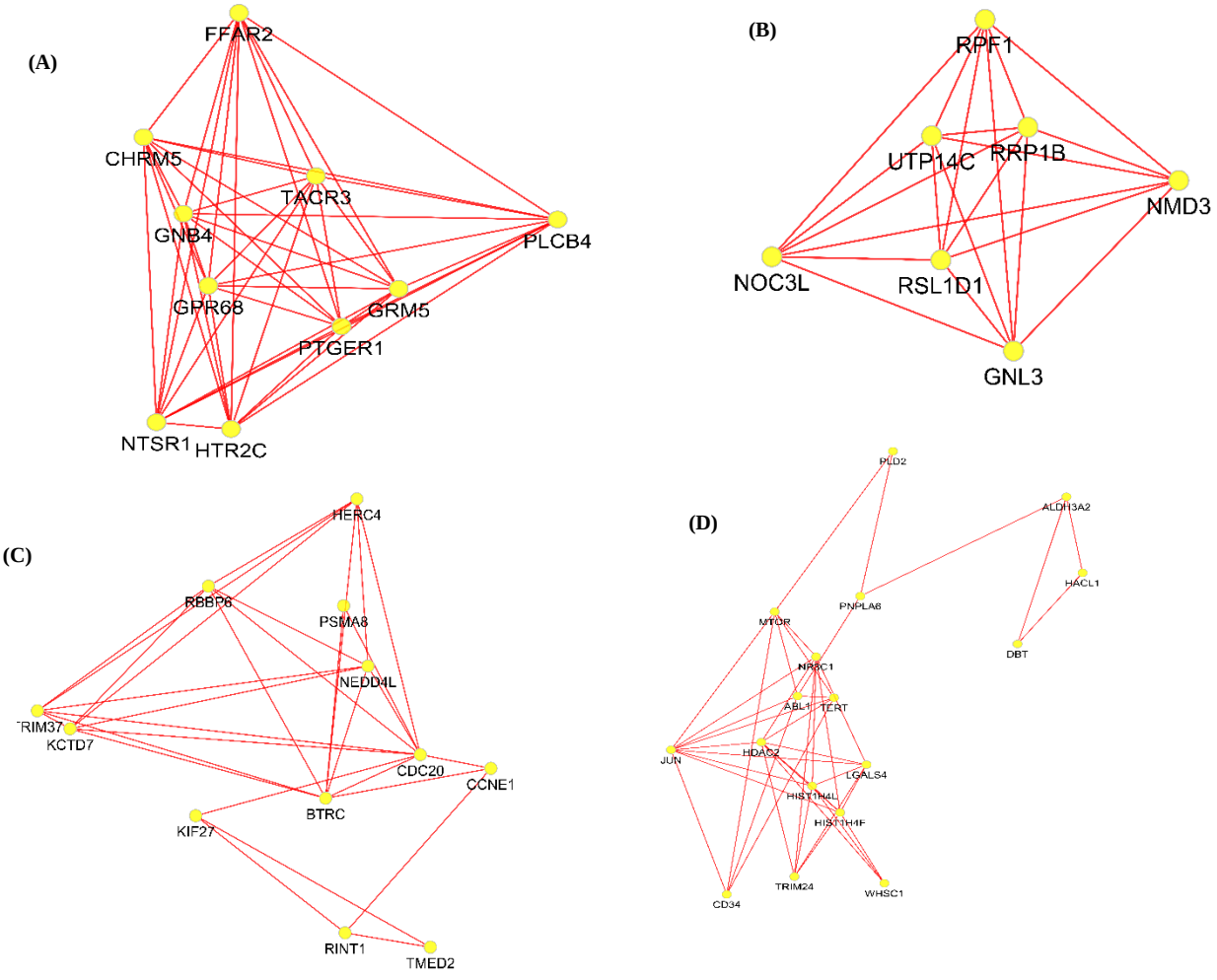

Figure 7: Top four significant modules in PPI network with MCODE score ≥ 4. Each node represents the relevant genes.

III PPI network analysis of DEGs

To further explore the relationships between DEGs at the protein level, we constructed the PPI networks based on the interactions of DEGs. With the predefined criterion of a combined score > 0.7, a total of 1507 interactions and 547 nodes were screened to establish the PPI network between FAMI and control samples. In this network, the first 20 DEGs with high connectivity were selected as the hub genes of the FAMI. The hub genes may play a significant role in AMI progression. The top 20 key genes with the highest degree scores are shown in (Table 2).

IV Module analysis

Module analysis was conducted utilizing MCODE. The four most significant sub-modules of DEGs were extracted from the PPI network, as shown in (Figure 7). The GO categories enrichment analysis showed that these genes mainly participate in regulation of the cell cycle and negative regulation of transcription from RNA polymerase II promotor. Similarly, KEGG pathway enrichment analysis showed that the calcium signaling pathway is the top enriched biological pathway associated with FAMI (Figure 8 and Figure 9). Significantly changed pathways are connected in a Path-net to show the relationship between them (Figure 10). A higher degree of pathway indicates that it regulates or is regulated by other pathways, implying a more crucial role in the signaling network.

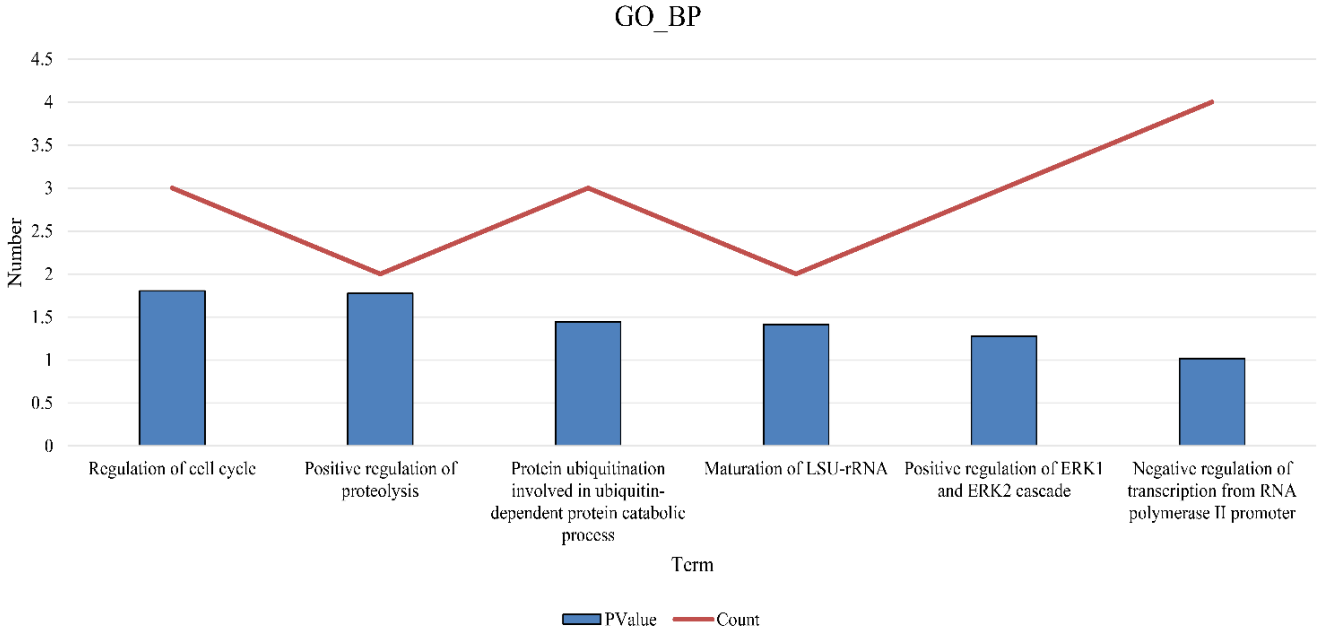

Figure 8: Top GO functional enrichment analyses in the most significant DEG modules.

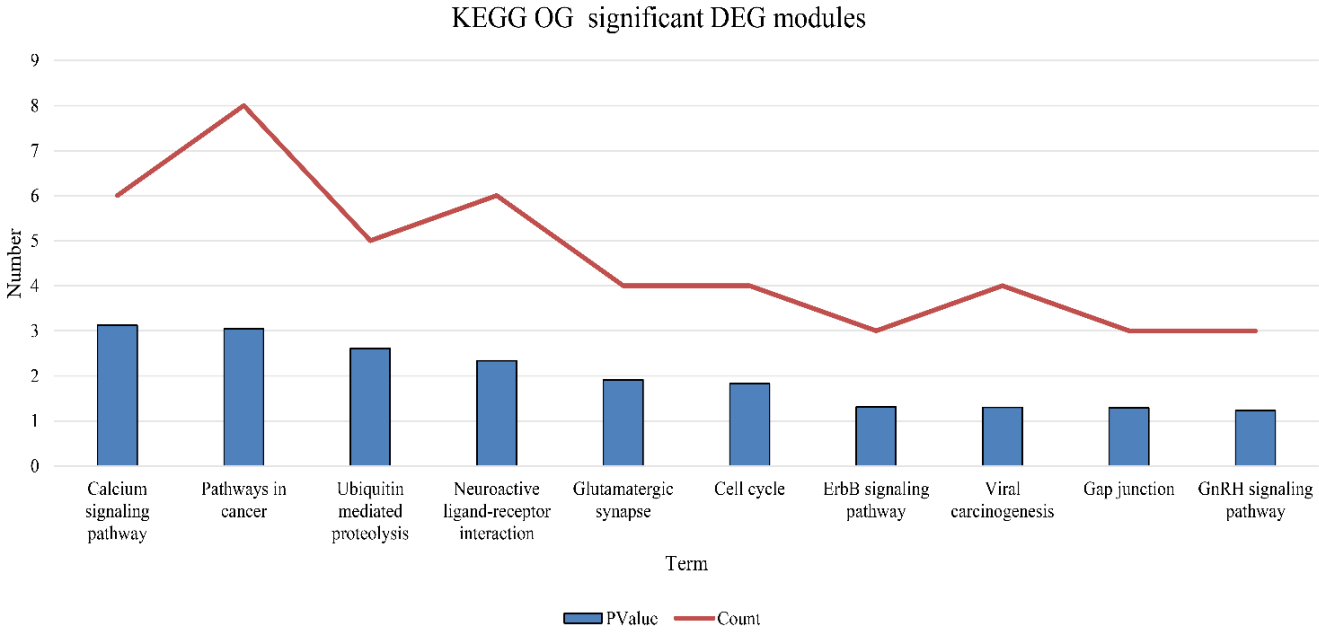

Figure 9: Top KEGG pathway enrichment analyses in the most significant DEG modules.

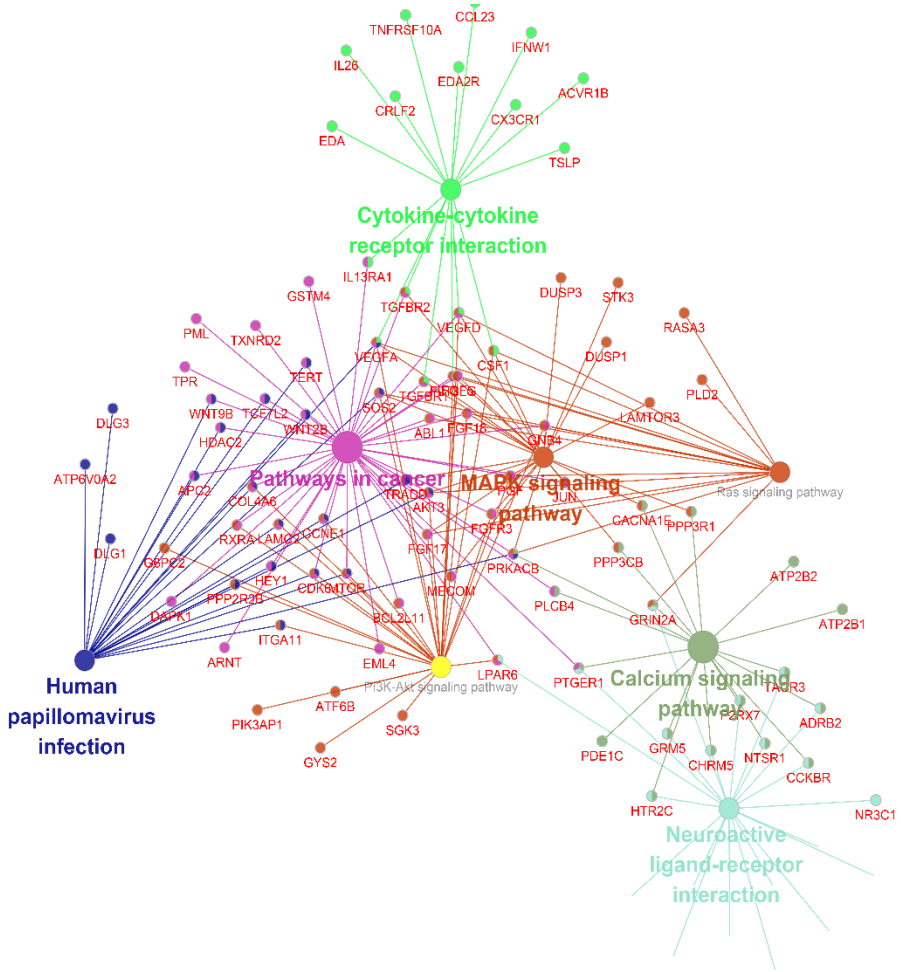

Figure 10: Pathway network (Path-net) analysis with ClueGO and CluePedia. Significantly changed pathways are connected in a Path-net to show the relationship between these pathways. A higher degree of crosstalk genes indicates that it associates with more than two pathways and serves as bridges implying a more important role in the signaling network.

Discussion

As a leading cause of morbidity and mortality worldwide, AMI represents a major public health issue in most developing countries [17]. It is characterized by a necrosis of myocardium caused by platelet-rich thrombi and reduced blood supply to the heart and is caused by multiple environmental and genetic factors and the interaction between them. An early and correct diagnosis may help to reduce the mortality rate [18]. Lots of efforts and advances have been made for the diagnosis and therapy of AMI, though the development of clinically validated useful markers represents huge challenges. Current advances in gene microarray technology and bioinformatics analysis can offer new chances to detect the potential key genes for certain diseases. In this study, 34 FAMI samples and 4 normal control samples were obtained from the GEO GSE24519 dataset. A total of 712 DEGs were identified, including 46 significantly upregulated and 666 significantly downregulated genes. To obtain further understanding of these DEGs, GO function and KEGG pathway analyses of the DEGs were performed. The results showed that the DEGs were mainly enriched in the following pathways: long-term potentiation, amphetamine addiction, MAPK signaling pathway, Pi3k-Akt signaling pathway, calcium signaling pathway, pathways in cancer, Wnt signaling pathway, and cytokine-cytokine receptor interaction. In addition, genes with high degrees of connectivity were obtained using a PPI network and modules analysis. TOP2B, ACACA, ACACB, PIKFYVE, JUN, PRKACB, GNB4, MTOR, HDAC2, PPP3CB, ABL1, NR3C1, CDC20, NTSR1, DCN, PGK2, MGP, PPP3R1 FGFR3 and BTRC were recognized as hub nodes. The four most significant submodules of DEGs were extracted from the PPI network, and we also carried out gene function and pathway analysis (Figure 8 and 9).

The MAPK signaling pathway is an important pathway which is enriched by JUN, a hub gene with the highest degree in the PPI network. The MAPK pathway (also called the Ras-Raf-MEK-ERK pathway) is a chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell [19]. It governs the activities of several transcription factors, and cell cycle entry and proliferation. Previous study, using a rat model of MI, reported that the MAPK pathway is activated in the cardiomyocytes of non-infarcted myocardium after acute MI and may play an essential role in ventricular hypertrophy and the post-MI remodeling process [20]. The Wnt signaling pathways are a set of signal transduction pathways which begin with proteins that pass signals into a cell through cell surface receptors [21]. Wnt signaling pathways play an important role in developmental processes of tissue patterning, cell differentiation and cell division [22]. Wnt signaling in the adult heart is quiescent under normal

conditions, while reactivated after myocardial infarction [23]. Wnt-5a, a subtype of Wnt protein, is exclusively expressed in cardiomyocytes. It can promote the release of IL-1, IL-6 and IL-8 from mononuclear cells, suggesting a pro-inflammatory effect of Wnt signaling [24, 25]. Previous study reported that the activity of Wnt signaling pathway plays an important role in cardiac tissue repair process after MI [26]. The inhibition of Wnt signaling pathways has turned out to be beneficial in MI via improving cardiac remodeling.

Pi3k/akt pathway is an intracellular signaling pathway necessary to regulate the cell cycle. PI3K activation phosphorylates and activates AKT, localizing it in the plasma membrane [27]. CD151 can promote neovascularization and improve ventricular function after MI in rats by activating the PI3K pathway [28]. Moreover, previous studies reported that rosuvastatin may protect myocardium from injury through the PI3K/Akt signaling pathway [29]. The activation of the PI3K/Akt signaling pathway mobilizes cardiac stem cells in myocardium, and may improve prognosis [30, 31]. Calcium ions play an important role in cellular signaling, as once they enter the cytosol of the cytoplasm, they exert allosteric regulatory effects on various enzymes and proteins. Calcium can act in signal transduction arising from activation of ion channels or as a second messenger caused by indirect signal transduction pathways such as G protein-coupled receptors. Increased expression of calcium-sensing receptors in atherosclerosis confers hypersensitivity to acute myocardial infarction in rats, which may be related to an increase of myocardial apoptosis [32]. Calcium is still believed to play a central role in initiating all cell death pathways in AMI.

KEGG analysis also contained the pathways like long-term potentiation, amphetamine addiction and pathways in cancer, which appears to have little relationship with MI. Therefore, additional research is needed to explain the cause and mechanism. The cell-division cycle protein 20 is a key regulator of cell division that is encoded by the CDC20 gene in humans. Its most important function is to activate the post-promotion complex (APC/C), which is a large 11-13 subunit complexes that can start the staining monomer separation and into the later stage. The APC/CCdc20 protein complex has two main downstream targets [33]. CDC20 participates in the response to a hormone and endogenous stimulus to regulate the development of congestive heart failure after MI [34]. The mammalian target of rapamycin (mTOR) is a kinase that is encoded by the MTOR gene in humans [35]. MTOR is a fellow of the phosphatidylinositol 3-kinase-related kinase family of protein kinases [36]. The mTOR complex is a key regulator of cardioprotection against cell stressors. Reperfusion therapy with mTOR inhibitor reduces infarct size in adult mouse hearts [37]. Histone deacetylase 2 (HDAC2) is an enzyme that is encoded by the HDAC2 gene in humans [38]. This gene product belongs to the histone deacetylase family. Histone deacetylases act via the formation of large multiprotein complexes and are responsible for the deacetylation of lysine residues on the N-terminal region of the core histones (H2A, H2B, H3 and H4). The protein also forms a transcription inhibitor complex by binding with many different proteins, containing the mammalian zinc-finger transcription factor YY1. Thus, it plays an important role in transcriptional regulation, cell cycle progression and developmental events [39]. HDAC2 is up-regulated during myocardial infarction-induced cardiac hypertrophy [40].

Matrix GLA protein (MGP) is a member of the vitamin K2 dependent GLA protein family, which has a high affinity binding to calcium ions, similar to other Gla-containing proteins. The protein serves as an inhibitor of vascular mineralization [41]. MGP is an important inhibitor of blood vessels and cartilage calcification, which is strongly expressed in human calcification, atherosclerotic plaques, and can regulate plaque calcification and coronary heart disease risk. The alleles of the MGP gene may confer an increased risk of plaque calcification and MI [42]. Glucocorticoid receptors (GR or GCR) also known as NR3C1 (nuclear receptor sub-family 3, Group C, member 1) are receptors for cortisol and other glucocorticoid binding. The GR is expressed in almost every cell in the body and regulates genes controlling development, metabolism, and immune response. The activated GR complex up-regulates the expression of anti-inflammatory proteins in the nucleus or represses the expression of pro-inflammatory proteins in the cytosol [43]. GR is a potential key mediator in post-MI remodeling. GR contributes to the increase in left ventricular mass after MI [44]. GR signaling in macrophages, playing a crucial role in tissue-repairing mechanisms, could be a potential therapeutic target during wound healing after ischemic myocardial injury [45].

Additionally, some pathways and genes with high degrees of differential expression may contribute to FAMI progress and merit further discussion. JUN, PRKACB, GNB4, MTOR, HDAC2, NR3C1, PPP3CB, PPP3R1, CDC20, MGP and FGFR3 were differentially expressed in AMI samples and might play vital roles in the development and progression of AMI. They may be regarded as potential biomarkers for the diagnosis and treatment of AMI. Additionally, the MAPK signaling pathway and the Pi3k-Akt signaling pathway might also play critical roles in myocardial remodeling in the development of AMI. However, further studies and more experiments with tissue or cells to validate the expression level of these DEG are required in order to confirm our present results. The present findings have significant implications for future research and could help in the design of specific treatments for patients with AMI. We expect more high-quality research to be conducted in the future.

In summary, these microarray data and bioinformatics analyses provide a useful method for the identification of key genes and pathways associated with AMI. Moreover, some crucial DEGs, such as JUN, PRKACB, GNB4, MTOR, HDAC2, PPP3CB, PPP3R1 and, FGFR3, potentially play a critical role in the development and progression of AMI.

Disclosure statement

No competing financial interests exist.

Acknowledgments

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