

Differential gene expression analysis and network construction of recurrent cardiovascular events

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Abstract. Recurrent cardiovascular events are vital to the prevention and treatment strategies in patients who have experienced primary cardiovascular events. However, the susceptibility of recurrent cardiovascular events varies among patients. Personalized treatment and prognosis prediction are urged. Microarray profiling of samples from patients with acute myocardial infarction (AMI), with or without recurrent cardiovascular events, were obtained from the Gene Expression Omnibus database. Bioinformatics analysis, including Gene Oncology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), were used to identify genes and pathways specifically associated with recurrent cardiovascular events. A protein-protein interaction (PPI) network was constructed and visualized. A total of 1,329 genes were differentially expressed in the two group samples. Among them, 1,023 differentially expressed genes (DEGs; 76.98%) were upregulated in the recurrent cardiovascular events group and 306 DEGs (23.02%) were downregulated. Significantly enriched GO terms for molecular functions were nucleotide binding and nucleic acid binding, for biological processes were signal transduction and regulation of transcription (DNA-dependent), and for cellular component were cytoplasm and nucleus. The most significant pathway in our KEGG analysis was Pathways in cancer ($P=0.000336681$), and regulation of actin cytoskeleton was also significantly enriched ($P=0.00165229$). In the PPI network, the significant hub nodes were GNG4, MAPK8, PIK3R2, EP300, CREB1 and PIK3CB. The present study demonstrated the underlying molecular differences between patients with AMI,

with and without recurrent cardiovascular events, including DEGs, their biological function, signaling pathways and key genes in the PPI network. With the use of bioinformatics and genomics these findings can be used to investigate the pathological mechanism, and improve the prevention and treatment of recurrent cardiovascular events.

Introduction

With the significant advances in medication, reperfusion therapy, cardiac rehabilitation and organ transplantation, cardiovascular disease remains one of the major causes of mortality worldwide (1). Evaluation of cardiovascular disease based on risk factors is important in the clinical prevention and treatment of cardiovascular disease, which may alter the risk stratification and guide the treatment and prognosis (2,3). More and more indexes are included in the risk stratification as clinical and experimental research develops, including brain natriuretic peptide, C reactive protein and blood homocysteine. However, the prediction of cardiovascular disease is not so satisfying (4), particularly in personalized prevention and treatment. Sensitivity of risk factors varies in different individuals, and clinical doctors must be aware of this and objective to the current risk factors and stratification (5). More superior and systematic algorithms for stratification remain to be elucidated (6).

The evaluation and stratification of cardiovascular diseases depend more on primary cardiovascular events, which elevate the stratification and enhance the treatment once they occur. However, recurrent cardiovascular events are also vital, which indicate that the current intervention is not marked enough to prevent disease progression. Although patients receive standard treatment based on the risk factors stratification, recurrent cardiovascular events still occur, which indicates that certain individuals are more prone to recurrent cardiovascular events. These patients may require more aggressive therapies, involving susceptibility screening and personalized treatment (7). With the development and application of clinical genomics technology and bioinformatics, novel biomarkers are used in the diagnosis and prognosis of cardiovascular disease (8,9). Previous research revealed that the expression of different genes varies in different stages of cardiovascular diseases, and these genes are involved in the pathological process, and may even predict

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the cardiovascular events (10). With the help of genomics and bioinformatics, patient susceptibility to recurrent cardiovascular events may be screened out, and personalized treatment can be made. This may reduce the recurrence of cardiovascular events and improve the prognosis. The present study used genomics and bioinformatics technology, and associated software to analyze the differentially expressed genes (DEGs) associated with recurrent cardiovascular events. The present study also aimed to identify the key genes in the pathological process and provide alternative guidance in the preventions, and personalized treatment of recurrent cardiovascular events.

Materials and methods

Microarray data and clinical characteristics. The microarray dataset, GSE48060 with GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array platform, was obtained from the Gene Expression Omnibus (GEO) database (11). The data samples were peripheral blood samples collected from patients with acute myocardial infarction (AMI) 48 h within the primary AMI. All 27 samples were divided into two groups, according to the recurrence of cardiovascular events in the 18 month follow-up. A total of five patients exhibited recurrent cardiovascular events and 22 did not. The definition of recurrent cardiovascular events is recurrent myocardial infarction, re-vascularization, evidence of restenosis, hospitalization for unstable angina or heart failure, cardiovascular mortality, stroke or transient ischemic attack, or amputation due to peripheral vascular disease.

Raw data processing. All 27 sample files were downloaded from the GEO database and were reanalyzed using R software (version 3.1.1; <http://www.r-project.org/>). The Affy package was applied to read the probe set data from the CEL files (12). Robust Multiarray Averaging was used to normalize the original data. Following standardization, a total of 54,675 probe set IDs' expression levels in different samples were obtained.

Screening and annotation of the DEGs. The limma package in the R software was used to compare the expression levels of the probe sets between the two groups (13). The threshold was set as $P < 0.05$ or a fold change > 1.5 . The annotate package was used to annotate the DEGs.

Enrichment analysis of DEGs. GeneCodis online tools (<http://genecodis2.dacya.ucm.es/>) were used to annotate and analyze the DEGs (14,15). The annotation and analysis were predominantly focussed on the molecular function, the biological process and the cellular component of Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The threshold was set as $P < 0.05$.

Protein-protein interaction (PPI) network analysis. Cytoscape (version 3.1.1; The Cytoscape Consortium, San Diego, CA, USA) and reactome plugin were applied to analyze the DEGs (16), and to construct and visualize the PPI network.

Further analysis of the key nodes in the PPI network were processed.

Results

Clinical characteristics of the two group samples. According to Suresh *et al* (11), the clinical characteristics between the recurrence and no recurrence groups, including age, sex, body mass index, cardiovascular risk factor score, lipid profile and severity of AMI, were similar with the exception of the usage of beta-blockers. The clinical characteristic details are listed in Table I.

Recurrent cardiovascular event-associated DEGs. By comparing the two group samples of with or without recurrent cardiovascular events in the 18 month follow-up following primary AMI, 1,329 genes (2.43% of total probe set) were identified to be differentially expressed and annotatable. A total of 1,023 DEGs (76.98%) were upregulated and 306 DEGs (23.02%) were downregulated in the recurrent cardiovascular events group. The top 10 markedly up or downregulated genes with a fold change > 1.5 are listed in Tables II and III, respectively.

Significant GO enrichment. To gain insights into the biological roles of the DEGs in recurrent cardiovascular events, a GO categories enrichment analysis was performed using GeneCoDis. GO categories are predominantly in three groups: Biological process, cellular component and molecular function. The significantly enriched GO terms for molecular functions were nucleotide binding (GO:0000166, $P = 8.24 \times 10^{-19}$) and nucleic acid binding (GO:0003676, $P = 1.94 \times 10^{-07}$), for biological processes were signal transduction (GO:0007165, $P = 5.26 \times 10^{-08}$) and regulation of transcription, DNA-dependent (GO:0006355, $P = 9.19 \times 10^{-06}$), and for cellular component were cytoplasm (GO:0005737, $P = 8.98 \times 10^{-25}$) and nucleus (GO:0005634, $P = 1.91 \times 10^{-23}$; Fig. 1).

Significant pathways. KEGG pathway enrichment analysis was performed to further evaluate the biological significance of the DEGs. The most significant pathway in our KEGG analysis was Pathways in cancer ($P = 0.000336681$). Furthermore, Melanoma ($P = 0.000336681$) and Regulation of actin cytoskeleton ($P = 0.00165229$) were revealed to be highly enriched. The top 15 enriched KEGG pathways of the DEGs are listed in Table IV.

PPI network construction and visualization. By analyzing the identified 1,329 DEGs using Cytoscape and the reactome plugin, 330 genes (node) and 796 gene-gene interactions (edge) were identified. The result was visualized in Cytoscape and the majority of the nodes were located within one network. To modify the PPI network, the sizes of the nodes were set according to their interaction density with the other nodes. The node color of the upregulated DEGs were made red and those downregulated were made blue (Fig. 2). The more that one gene interacts with the other genes, the larger the node was and the more central this gene occurs within the network. The genes, GNG4, MAPK8 and PIK3R2 were the three predominantly upregulated genes, while EP300, CREB1 and PIK3CB were the predominantly downregulated genes in the PPI network. The details of the nodes are listed in Table V.

Table I. Baseline clinical characteristics of AMI patients with or without recurrent events following primary AMI, who underwent whole-genome blood gene expression microarray analysis.

Variable	Event (n=5)	No event (n=22)	P-value
Age, years	51 (41-53)	56.5 (48-65)	0.110
Gender, male, n (%)	3 (60)	13 (59)	0.972
Body mass index, kg/m ²	36.5 (25.0-46.5)	31.5 (22.9-48.4)	0.140
Cardiovascular risk factor score	5 (4-6)	4 (1-6)	0.266
Cardiovascular history, n (%)			
Arterial hypertension, n (%)	5 (100)	13 (59)	0.080
Smoking, n (%)	2 (40)	16 (73)	0.161
Diabetes mellitus, n (%)	0 (0)	3 (14)	0.381
Family history of coronary artery disease, n (%)	5 (100)	12 (55)	0.057
Lipid profile, mg/dl			
Total cholesterol, mg/dl	128 (110-219)	191 (126-325)	0.190
Low density lipoprotein cholesterol, mg/dl	65 (41-152)	115 (70-254)	0.169
HDL cholesterol, mg/dl	31 (25-47)	38 (25-72)	0.165
Medication			
Statin therapy, n (%)	3 (60)	7 (33)	0.271
Aspirin, n (%)	4 (80)	12 (55)	0.296
ACE inhibitor, n (%)	1 (20)	4 (19)	0.961
Beta blocker, n (%)	4 (80)	5 (22)	0.014
Severity of AMI			
Ejection fraction, %	55 (43-65)	57 (35-71)	0.240
Troponin, ng/ml	2.24 (0.11-9.51)	0.47 (0.04-16.43)	0.142
STEMI, n (%)	2 (40)	7 (32)	0.726

AMI, acute myocardial infarction.

Table II. Top 10 upregulated genes.

Gene	Fold change	Mean of intensity		P-value	Official gene name
		Recurrence	No recurrence		
LRRC18	1.58	21.38	13.50	1.14 ⁻⁰⁴	Leucine rich repeat containing 18
IRAK1BP1	1.77	90.64	51.19	6.19 ⁻⁰⁴	Interleukin-1 receptor-associated kinase 1 binding protein 1
MGAT4A	1.57	385.69	245.89	6.00 ⁻⁰³	Mannosyl (α-1,3-)-glycoprotein β-1, 4-N-acetylglucosaminyltransferase, isozyme A
BZW2	1.74	921.80	529.93	9.33 ⁻⁰³	Basic leucine zipper and W2 domains 2
LOC152586	1.64	19.38	11.84	1.25 ⁻⁰²	MGAT4 family, member D
ADTRP	1.78	272.08	153.19	1.46 ⁻⁰²	Androgen-dependent TFPI-regulating protein
SMC1B	1.78	24.76	13.94	2.02 ⁻⁰²	Structural maintenance of chr 1B
LOC283788	1.53	147.03	96.39	2.39 ⁻⁰²	Hypothetical protein LOC283788
CLDN12	1.52	37.24	24.58	2.56 ⁻⁰²	Claudin 12
LEF1-AS1	1.79	74.60	41.72	3.32 ⁻⁰²	LEF1 antisense RNA 1

Genes with a fold change >1.5 are shown. Data are sorted by P-value.

Table III. Top 10 downregulated genes.

Gene	Fold change	Mean of intensity		P-value	Official gene name
		Recurrence	No recurrence		
HIST1H2AC	1.57	2086.82	3273.74	2.50 ⁻⁰³	Histone cluster 1, H2ac
NEXN	1.65	199.36	329.40	4.46 ⁻⁰³	Nexilin (F actin binding protein)
SIRPB2	1.76	102.91	181.12	4.77 ⁻⁰³	Signal-regulatory protein β 2
TUBB1	1.56	462.45	719.52	5.44 ⁻⁰³	Tubulin β 1
HIST1H2BD	1.56	254.17	397.28	5.81 ⁻⁰³	Histone cluster 1, H2bd
TSPAN2	1.76	142.35	250.81	6.02 ⁻⁰³	Tetraspanin 2
GUCY1A3	1.55	12.14	18.80	6.57 ⁻⁰³	Guanylate cyclase 1, soluble, α 3
FAR2	1.77	129.96	230.38	7.84 ⁻⁰³	Fatty acyl CoA reductase 2
STON2	1.66	91.55	151.83	9.06 ⁻⁰³	Nexilin (F actin binding protein)
DLEU2	1.62	63.13	102.52	1.11 ⁻⁰²	Deleted in lymphocytic leukemia 2

Genes with a fold change >1.5 are shown. Data are sorted by P-value.

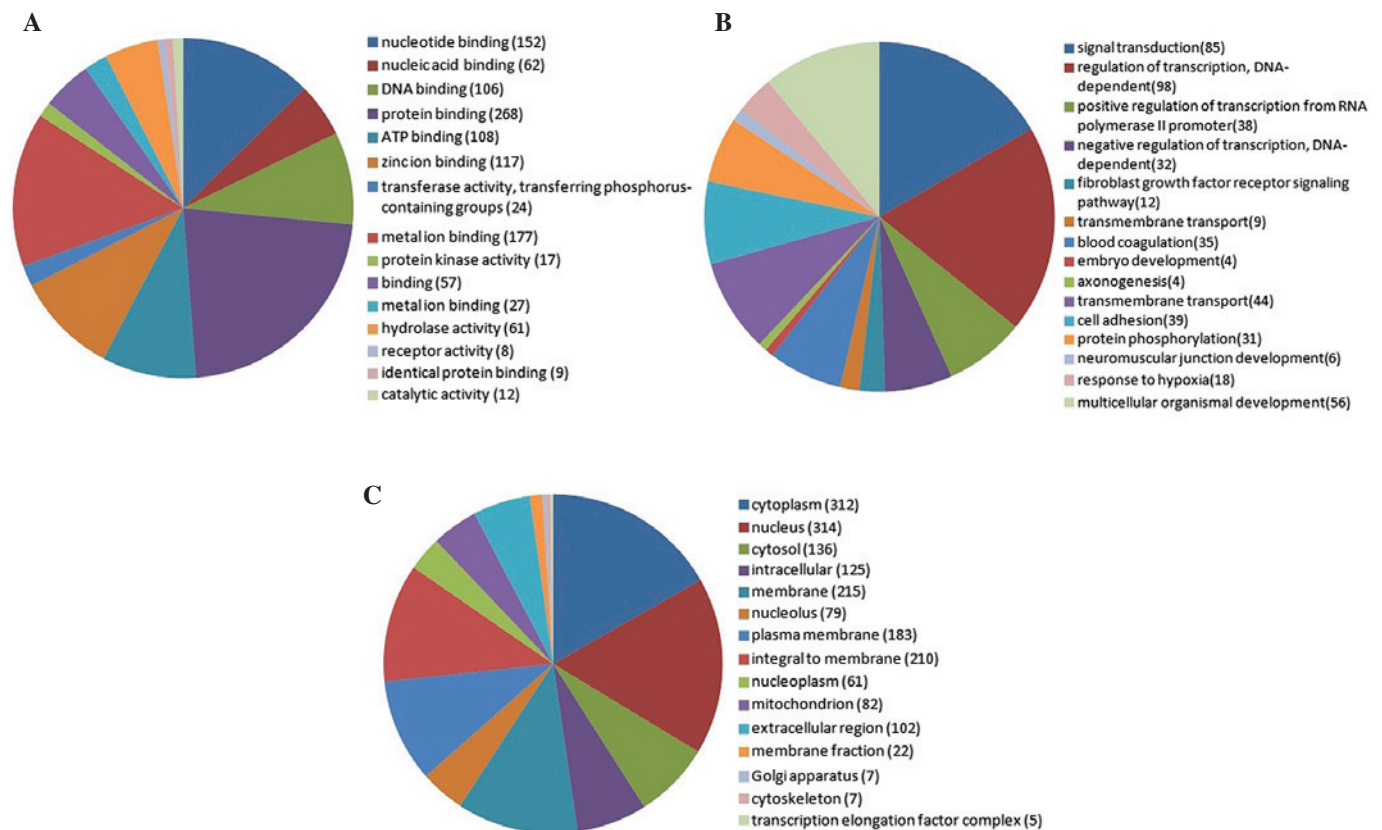


Figure 1. GO enrichment. (A) Molecular functions, (B) biological processes and (C) cellular components for the differentially expressed genes. The order of GO enrichment terms were sorted by P-value. GO, gene ontology.

Discussion

Cardiovascular events are important in the prevention and treatment of cardiovascular diseases. When it occurs in patients with risk factors, the heart function must be re-evaluated, and the prevention and treatment strategy must be adjusted. For patients who had experienced cardiovascular events, the prevention and treatment strategies are not uniform between

different regions and hospitals. There are divergences between different area and different grades of hospitals (17), conservative and aggressive strategies are being used, not to mention the circumstances vary among individuals, efficient and effective personalized evaluation and treatment are urged (18). Previous research has revealed that whole-genome sequencing can be used in cardiovascular disease risk-prediction algorithms, to more accurately forecast whether patients will develop

Table IV. Top 15 enriched KEGG pathways of differentially expressed genes.

KEGG ID	KEGG pathway	Gene no.	P-value
hsa05200	Pathways in cancer	12	3.37 ⁻⁰⁴
hsa05218	Melanoma	12	3.37 ⁻⁰⁴
hsa04810	Regulation of actin cytoskeleton	9	1.65 ⁻⁰³
hsa05215	Prostate cancer	11	1.77 ⁻⁰³
hsa04010	MAPK signaling pathway	23	2.04 ⁻⁰³
hsa04120	Ubiquitin mediated proteolysis	14	6.34 ⁻⁰³
hsa04660	T cell receptor signaling pathway	4	7.80 ⁻⁰³
hsa05340	Primary immunodeficiency	4	7.80 ⁻⁰³
hsa05214	Glioma	7	8.34 ⁻⁰³
hsa05222	Small cell lung cancer	10	9.31 ⁻⁰³
hsa04510	Focal adhesion	6	1.68 ⁻⁰²
hsa04144	Endocytosis	16	1.98 ⁻⁰²
hsa03030	DNA replication	4	2.07 ⁻⁰²
hsa03430	Mismatch repair	4	2.07 ⁻⁰²
hsa04115	p53 signaling pathway	8	2.28 ⁻⁰²

KEGG, Kyoto Encyclopedia of Genes and Genomes.

disease (19). However, there remains a lack of research about microarray profiling in recurrent cardiovascular events. The present study performed a microarray profiling of peripheral blood samples from patients with AMI, downloaded from the GEO database, to focus on the DEGs of those with or without recurrent cardiovascular events 18 months following AMI.

R is an integrated suite of software facilities for data manipulation, calculation and graphical display. Using R software and certain packages, the present study identified the DEGs between patients with AMI, with or without recurrent cardiovascular disease. A total of 1,329 genes were identified and 1,023 were upregulated in recurrent group compared with the no recurrent group, while 306 of them were downregulated. The genes with the most significant P-value and fold change >1.5 in the up and downregulated DEGs are listed in Tables II and III. Among them, TUBB1 (tubulin β 1, class VI; P=0.00544; fold change=1.56) encodes a member of the β tubulin protein family, and this protein is specifically expressed in platelets and megakaryocytes, and may be involved in proplatelet production and platelet release. Previous research revealed that the prevalence of TUBB1 was higher among healthy individuals compared with patients with cardiovascular disease (20). This may be associated with the TUBB1 function of suppressing microtubule dynamics, fragmenting microtubules and inhibiting cell division (21). Although there is little previous research about other significant genes involved in cardiovascular diseases, the method in the present study may be the initial and alternative way to explore the pathological mechanism of recurrent cardiovascular events.

To further investigate the roles of the DEGs identified in the pathological mechanism of recurrent cardiovascular events, GO enrichment analysis and KEGG pathway analysis was used. GO is widely used as the tool for the organization and functional annotation of molecular aspect (22). It was revealed that the significantly enriched GO terms for molecular

functions were nucleotide binding and nucleic acid binding, for biological processes were signal transduction and regulation of transcription (DNA-dependent), and for cellular component were cytoplasm and nucleus. The GO terms mentioned above are basic and vital to the biological and pathological process. Fibroblast growth factor receptor signaling pathway (GO:0008543; P=0.00151494), blood coagulation (GO:0007596; P=0.00166723) and cell adhesion (GO:0007155; P=0.00170222) were also significantly enriched in GO biological process. Ronca *et al* (23) reported that fibroblast growth factor receptor-1 gene knockout impairs cardiac and haematopoietic development in murine embryonic stem cells, and the fibroblast growth factor receptor is required for cardiomyocyte differentiation. Yukawa *et al* (24) demonstrated that impaired fibroblast growth factor receptor gene would suppress the growth of vascular smooth muscle. As for blood coagulation and cell adhesion, which are associated with the formation and breaking off of thrombosis, they are important in both primary and recurrent cardiovascular events.

In KEGG pathway analysis, regulation of actin cytoskeleton is significantly enriched. Actin cytoskeleton is involved in the inward remodeling process associated with cytoskeletal modifications. It is also involved in reducing the passive diameter of resistance vessels, which are the vascular components of the circulatory system, and exert a preponderant role in the regulation of blood flow and the modulation of blood pressure (25). Therefore, the regulation of actin cytoskeleton may have profound consequences on the incidence of cardiovascular events.

The results from PPI network analysis of the top 10 up and downregulated DEGs revealed the significant nodes, including GNG4, MAPK8, PIK3R2, EP300, CREB1 and PIK3CB. MAPK8 is one member of the MAPK family, which has vast implications in signaling and crosstalk with other signaling networks. The MAPK signal pathway is highly associated

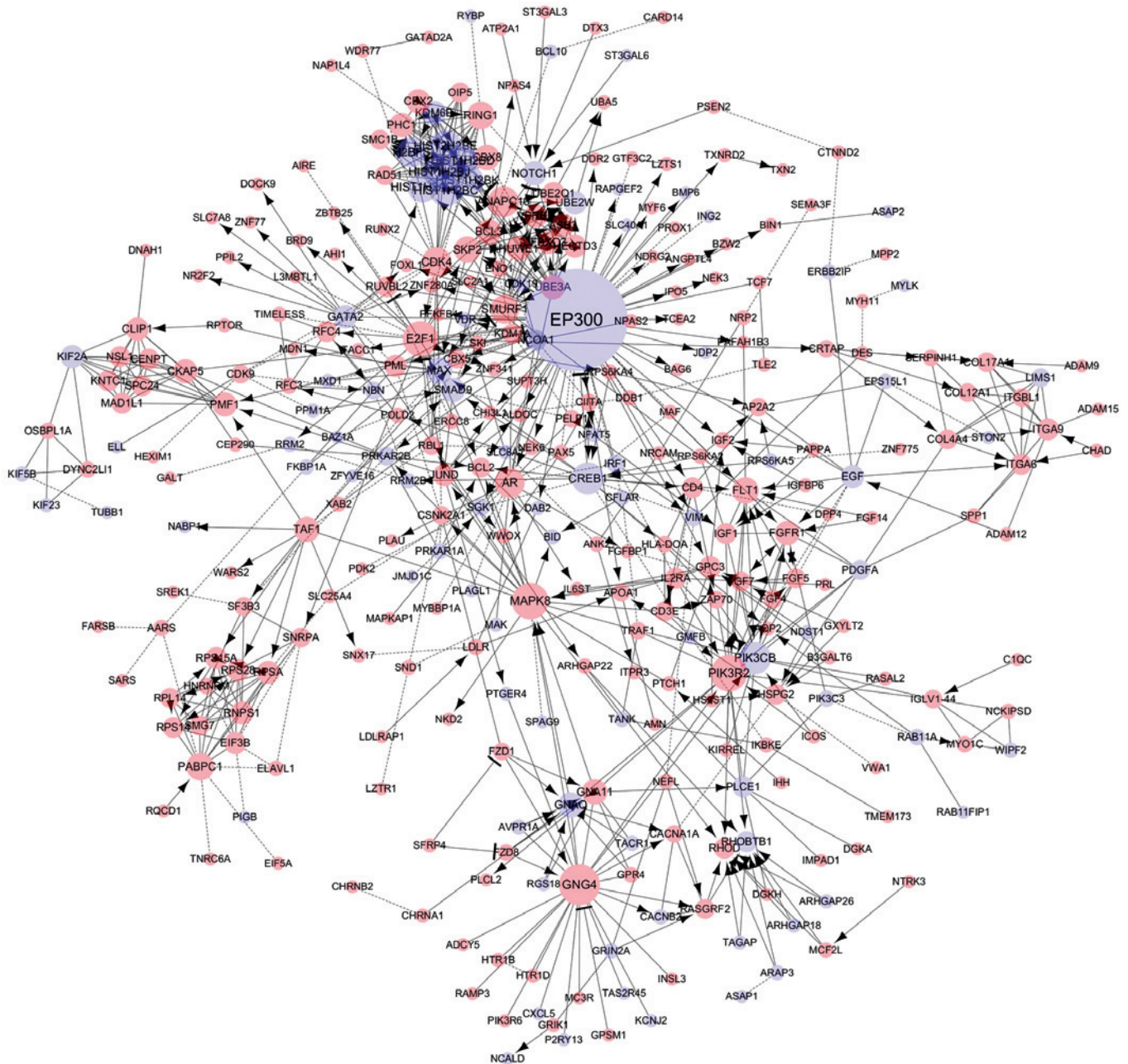


Figure 2. Protein-protein interaction network visualization of differentially expressed genes. The nodes in red represent the upregulated genes and those in blue represent the downregulated genes. The larger size nodes interact more with other nodes. The type of edges represents the interaction between the nodes, → to indicate activating/catalyzing, - to indicate inhibition, - for functional interactions extracted from complexes or inputs and --- for predicted functional interactions.

with mitochondria, the power houses of the cell, which provide >80% of ATP for normal cardiomyocyte function and have a crucial role in cell death (26). EP300 is the node with the most interactions with other nodes in the PPI network, and previous research revealed that it is associated with arterial stiffness prior to hypertension, increased pulse pressure, and structural vessel wall changes (27). CREB1, also termed CREB, phosphorylation induced by the prostacyclin/IP pathway may suppress cardiac fibrosis, which is a consequence of numerous cardiovascular diseases, and contributes to impaired ventricular function (28). The PPI results suggested that MAPK8, EP300 and CREB1 may be important in the development of recurrent cardiovascular events.

The results from the present study suggested that DEGs exist between patients with AMI, with and without recurrent cardiovascular events. These genes are involved in different GO enrichment terms and signaling pathways, from which insights into the pathological processes of recurrent events can be obtained. Several genes, including TUBB1, GNG4, MAPK8, PIK3R2, EP300 and CREB1, with or without previous research, may provide potential candidates for distinguishing the susceptibility to recurrent cardiovascular events in the future. Therefore, the present research may provide important references for the prevention and treatment strategies in patients with primary cardiovascular events. Nevertheless, the genes and the associated GO enrichment

Table V. Network features of differentially expressed genes included in protein-protein interaction network sorted by degree.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
EP300	E1A binding protein p300	0.03886829	81	25	56	Down
GNB4	Guanine nucleotide binding protein (G protein), γ 4	0.00246082	26	9	17	Up
MAPK8	Mitogen-activated protein kinase 8	0.00373044	22	12	10	Up
PIK3R2	Phosphoinositide-3-kinase, regulatory subunit 2 (p85 β)	0.00275468	22	17	5	Up
E2F1	E2F transcription factor 1	0.00881333	21	5	16	Up
CREB1	CAMP responsive element binding protein 1	0.0020355	18	4	14	Down
PIK3CB	Phosphoinositide-3-kinase, catalytic, β polypeptide	0.00123254	18	13	5	Down
HIST1H2BC	Histone cluster 1, H2bc	0.00045679	18	8	10	Down
HIST1H2BK	Histone cluster 1, H2bk	0.00044752	18	11	7	Down
HIST1H2BJ	Histone cluster 1, H2bj	0.00044752	18	10	8	Down
HIST2H2BE	Histone cluster 2, H2be	0.00084908	18	11	7	Down
HIST1H2BD	Histone cluster 1, H2bd	0.00039501	17	8	9	Down
ANAPC10	Anaphase promoting complex subunit 10	0	17	0	17	Up
AR	Androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)	0	16	0	16	Up
CDK4	Cyclin-dependent kinase 4	0.00042396	16	1	15	Up
HIST1H2AC	Histone cluster 1, H2ac	0.000341	16	5	11	Down
H2BFS	H2B histone family, member S	0.00003574	16	4	12	Down
SMURF1	SMAD specific E3 ubiquitin protein ligase 1	0.00112437	15	11	4	Up
PABPC1	Poly (A) binding protein, cytoplasmic 1	0.00046983	14	4	10	Up
RING1	Ring finger protein 1	0.00051894	14	13	1	Up
MAX	MYC associated factor X	0.00190278	13	5	8	Down
FLT1	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	0.00358728	13	7	6	Up
SKP2	S-phase kinase-associated protein 2 (p45)	0.0016332	13	8	5	Up
CBX8	Chromobox homolog 8 (Pc class homolog, Drosophila)	0.00101008	12	1	11	Up
TAF1	TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 250 kDa	0.00086181	12	11	1	Up
BCL3	B-cell CLL/lymphoma 3	0.00123249	12	1	11	Up
GNAI1	Guanine nucleotide binding protein (G protein), α 11 (Gq class)	0.00031838	12	3	9	Up
GNAQ	Guanine nucleotide binding protein (G protein), q polypeptide	0.00064179	12	6	6	Down
PMF1	Polyamine-modulated factor 1	0.00076915	11	9	2	Up
CBX2	Chromobox homolog 2 (Pc class homolog, <i>drosophila</i>)	0	11	0	11	Up
HUWE1	HECT, UBA and WWE domain containing 1	0.00050967	11	4	7	Up
KDM6B	Lysine (K)-specific demethylase 6B	0	11	9	2	Down
UBE3A	Ubiquitin protein ligase E3A (human papilloma virus E6-associated	0.00000927	11	10	1	Up

Table V. Continued.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
FGFR1	protein, Angelman syndrome) Fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	0.00350998	11	6	5	Up
PHC1	Polyhomeotic homolog 1 (<i>Drosophila</i>)	0	11	10	1	Up
SMAD9	SMAD family member 9	0.0020102	11	8	3	Down
CKAP5	Cytoskeleton associated protein 5	0.000139	11	2	9	Up
NOTCH1	Notch homolog 1, translocation-associated (<i>Drosophila</i>)	0.00167253	11	6	5	Down
FBXO3	F-box protein 3	0	10	2	8	Up
UBE2Q1	Ubiquitin-conjugating enzyme E2Q (putative) 1	0.00000927	10	8	2	Up
KIF2A	Kinesin heavy chain member 2A	0.00010193	10	4	6	Down
UBE2W	Ubiquitin-conjugating enzyme E2W (putative)	0.00000927	10	8	2	Down
HECTD3	HECT domain containing 3	0	10	3	7	Up
ASB3	Ankyrin repeat and SOCS box-containing 3	0	10	1	9	Up
EGF	Epidermal growth factor (β -urogastrone)	0.00189305	10	2	8	Down
GATA2	GATA binding protein 2	0.00200163	10	4	6	Down
RPSA	Ribosomal protein SA	0.00015167	10	7	3	Up
VPRBP	Vpr (HIV-1) binding protein	0	10	10	0	Up
CLIP1	CAP-GLY domain containing linker protein 1	0.0000556	10	2	8	Up
RNPS1	RNA binding protein S1, serine-rich domain	0.00017607	10	2	8	Up
GPC3	Glypican 3	0.00296725	9	3	6	Up
EIF3B	Eukaryotic translation initiation factor 3, subunit B	0	9	0	9	Up
RPS28	Ribosomal protein S28	0.00004973	9	6	3	Up
RPS15A	Ribosomal protein S15a	0.00004973	9	5	4	Up
PML	Promyelocytic leukemia	0.00137496	9	6	3	Up
ITGA9	Integrin, α 9	0.00008649	9	6	3	Up
JUND	Jun D proto-oncogene	0.00140147	9	4	5	Up
NSL1	NSL1, MIND kinetochore complex component, homolog (<i>S. cerevisiae</i>)	0	8	6	2	Up
RHOD	Ras homolog gene family, member D	0.00038457	8	7	1	Up
HSPG2	Heparan sulfate proteoglycan 2	0.00096904	8	5	3	Up
SPC24	SPC24, NDC80 kinetochore complex component, homolog (<i>S. cerevisiae</i>)	0	8	8	0	Up
CD4	CD4 molecule	0.00166231	8	2	6	Up
NCOA1	Nuclear receptor coactivator 1	0.0000556	8	6	2	Down
IGF1	Insulin-like growth factor 1 (somatomedin C)	0.00081239	8	2	6	Up
ITGA6	Integrin, α 6	0.00008649	8	5	3	Up

Table V. Continued.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
RPS14	Ribosomal protein S14	0.00000185	8	4	4	Up
RHOBTB1	Rho-related BTB domain containing 1	0.00038457	8	7	1	Down
RFC4	Replication factor C (activator 1) 4, 37 kDa	0.00023013	8	6	2	Up
KNTC1	Kinetochore associated 1	0	8	4	4	Up
CENPT	Centromere protein T	0	8	0	8	Up
RUVBL2	RuvB-like 2 (<i>E. coli</i>)	0	8	8	0	Up
IL2RA	Interleukin 2 receptor, α	0.0002709	8	2	6	Up
MAD1L1	MAD1 mitotic arrest deficient-like 1 (yeast)	0	8	5	3	Up
PLCE1	Phospholipase C, epsilon 1	0	8	8	0	Down
RASGRF2	Ras protein-specific guanine nucleotide-releasing factor 2	0.0002085	7	5	2	Up
FGF7	Fibroblast growth factor 7 (keratinocyte growth factor)	0.00004633	7	2	5	Up
SIX5	SIX homeobox 5	1	7			Up
SMC1B	Structural maintenance of chromosomes 1B	0	7	7	0	Up
KDM1A	Lysine (K)-specific demethylase 1	0.0001058	7	5	2	Up
CD3E	CD3e molecule, epsilon (CD3-TCR complex)	0	7	0	7	Up
PDGFA	Platelet-derived growth factor α polypeptide	0.0009675	7	4	3	Down
COL4A4	Collagen, type IV, α 4	0	7	0	7	Up
RAD51	RAD51 homolog (RecA homolog, <i>E. coli</i>) (<i>S. cerevisiae</i>)	0	7	7	0	Up
BCL2	B-cell CLL/lymphoma 2	0	7	0	7	Up
SMG7	Smg-7 homolog, nonsense mediated mRNA decay factor (<i>C. elegans</i>)	0	7	7	0	Up
FGF5	Fibroblast growth factor 5	0.00007058	7	2	5	Up
OIP5	Opa interacting protein 5	0	7	7	0	Up
RPL14	Ribosomal protein L14	0	7	2	5	Up
SNRPA	Small nuclear ribonucleoprotein polypeptide A	0	7	7	0	Up
COL17A1	Collagen, type XVII, α 1	0.00126029	7	1	6	Up
FGF4	Fibroblast growth factor 4 (heparin secretory transforming protein 1, Kaposi sarcoma oncogene)	0	6	0	6	Up
LIMS1	LIM and senescent cell antigen-like domains 1	0	6	6	0	Down
ITGBL1	Integrin, β -like 1 (with EGF-like repeat domains)	0	6	5	1	Up
COL12A1	Collagen, type XII, α 1	0	6	0	6	Up
RBL1	Retinoblastoma-like 1 (p107)	0.00010657	6	5	1	Up
CBX5	Chromobox homolog 5 (HPI α homolog, <i>Drosophila</i>)	0.00021578	6	1	5	Up
PRKAR2B	Protein kinase, cAMP-dependent, regulatory, type II, β	0	6	6	0	Down
SF3B3	Splicing factor 3b, subunit 3, 130 kDa	0.0001529	6	2	4	Up
AP2A2	Adaptor-related protein complex 2, α 2 subunit	0	6	0	6	Up
IGLV1-44	Immunoglobulin lambda variable 1-44	0.00012974	6	1	5	Up

Table V. Continued.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
CDK9	Cyclin-dependent kinase 9	0	6	0	6	Up
CSNK2A1	Casein kinase 2, α 1 polypeptide	0.00045515	6	1	5	Up
PELP1	Proline, glutamic acid and leucine rich protein 1	0.00034009	5	4	1	Up
TRAF1	TNF receptor-associated factor 1	0	5	5	0	Up
VIM	Vimentin	0	5	5	0	Down
CRTAP	Cartilage associated protein	0.00468901	5	3	2	Up
NBN	Nibrin	0.00107495	5	2	3	Down
CACNA1A	Calcium channel, voltage-dependent, P/Q type, α 1A subunit	0	5	0	5	Up
CIITA	Class II, major histocompatibility complex, transactivator	0	5	0	5	Up
IRF1	Interferon regulatory factor 1	0.00002008	5	4	1	Down
FZD1	Frizzled homolog 1 (Drosophila)	0.00061315	5	1	4	Up
SGK1	Serum/glucocorticoid regulated kinase 1	0	5	5	0	Down
APOA1	Apolipoprotein A-I	0.00117688	5	1	4	Up
ZAP70	Zeta-chain (TCR) associated protein kinase 70kDa	0	5	5	0	Up
DYNC2LI1	Dynein, cytoplasmic 2, light intermediate chain 1	0	4	0	4	Up
OSBPL1A	Oxysterol binding protein-like 1A	0	4	4	0	Up
RFC3	Replication factor C (activator 1) 3, 38kDa	0.00014364	4	2	2	Up
SERPINH1	Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	0	4	4	0	Up
HNRNPM	Recombinant Heterogeneous nuclear ribonucleoprotein M	0.00003243	4	1	3	Up
PIK3C3	Phosphoinositide-3-kinase, class 3	0.00103422	4	1	3	Down
PRKAR1A	Protein kinase, cAMP-dependent, regulatory, type I, α (tissue specific extinguisher 1)	0.00000463	4	3	1	Down
AARS	Alanyl-tRNA synthetase	0	4	0	4	Up
FZD8	Frizzled homolog 8 (Drosophila)	0	4	0	4	Up
MYO1C	Myosin IC	0.00003707	4	1	3	Up
RAB11A	RAB11A, member RAS oncogene family	0.00047261	4	3	1	Down
HLA-DOA	Major histocompatibility complex, class II, DO α	0.00003707	4	3	1	Up
IGF2	Insulin-like growth factor 2 (somatomedin A)	0.00076297	4	2	2	Up
LDLR	Low density lipoprotein receptor (familial hypercholesterolemia)	0.00007105	4	2	2	Up
ENO1	Enolase 1, (α)	0.00003398	4	2	2	Up
ZNF280A	Zinc finger protein 280A	0	3	3	0	Up
ARHGAP22	Rho GTPase activating protein 22	0	3	0	3	Up
DDB1	Damage-specific DNA binding protein 1, 127kDa	0	3	0	3	Up
ERCC8	Excision repair cross-complementing rodent repair deficiency, complementation group 8	0.0000139	3	1	2	Up

Table V. Continued.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
VDR	Vitamin D (1,25- dihydroxyvitamin D3) receptor	0	3	3	0	Down
POLD2	Polymerase (DNA directed), delta 2, regulatory subunit 50kDa	0.00012047	3	1	2	Up
CACNB2	Calcium channel, voltage-dependent, β 2 subunit	0	3	1	2	Down
PTCH1	Patched homolog 1 (Drosophila)	0	3	3	0	Up
SPP1	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	0	3	3	0	Up
KIF5B	Kinesin family member 5B	0.00000927	3	1	2	Down
ARAP3	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3	0	3	0	3	Down
SKI	V-ski sarcoma viral oncogene homolog (avian)	0.00040774	3	2	1	Up
EPS15L1	Epidermal growth factor receptor pathway substrate 15-like 1	0.0000278	3	2	1	Down
FKBP1A	FK506 binding protein 1A, 12kDa	0.00009267	3	1	2	Down
RPS6KA4	Ribosomal protein S6 kinase, 90kDa, polypeptide 4	0	3	3	0	Up
BID	BH3 interacting domain death agonist	0.00000927	3	1	2	Down
GRIN2A	Glutamate receptor, ionotropic, N-methyl D-aspartate 2A	0.0000556	3	1	2	Down
NEFL	Neurofilament, light polypeptide 68kDa	0.00001853	3	1	2	Up
ANK2	Ankyrin 2, neuronal	0	3	0	3	Up
TIMELESS	Timeless homolog (Drosophila)	0	3	3	0	Up
SND1	Staphylococcal nuclease and tudor domain containing 1	0	3	3	0	Up
RPS6KA5	Ribosomal protein S6 kinase, 90kDa, polypeptide 5	0.00006487	3	2	1	Down
RPS6KA2	Ribosomal protein S6 kinase, 90kDa, polypeptide 2	0	3	3	0	Up
RRM2	Ribonucleotide reductase M2 polypeptide	0.00009267	3	2	1	Down
RRM2B	Ribonucleotide reductase M2 B (TP53 inducible)	0	3	3	0	Down
GPR4	G protein-coupled receptor 4	0	3	3	0	Up
WIPF2	WAS/WASL interacting protein family, member 2	0	3	3	0	Down
CFLAR	CASP8 and FADD-like apoptosis regulator	0.00104252	3	1	2	Down
ELAVL1	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R)	0	3	1	2	Up
IKBKE	Inhibitor of α light polypeptide gene enhancer in B-cells, kinase epsilon	0.00011429	3	1	2	Up
ERBB2IP	ErbB2 interacting protein	0.00003707	3	2	1	Down
PAX5	Paired box 5	0.00012371	3	2	1	Up
TACR1	Tachykinin receptor 1	0	3	3	0	Down
MCF2L	MCF2 cell line derived transforming sequence-like	0	3	0	3	Up
NRP2	Neuropilin 2	0.00045407	3	2	1	Up
XAB2	XPA binding protein 2	0	3	3	0	Up
WWOX	WW domain containing oxidoreductase	0	3	3	0	Up

Table V. Continued.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
TANK	TRAF family member-associated NFKB activator	0.00001853	3	2	1	Down
AVPR1A	Arginine vasopressin receptor 1A	0	3	0	3	Down
NCKIPSD	NCK interacting protein with SH3 domain	0	3	2	1	Up
NEK6	NIMA (never in mitosis gene a)-related kinase 6	0.00010116	3	1	2	Up
CDK19	cyclin-dependent kinase 19	0	3	0	3	Down
B3GALT6	UDP-Gal:βGal β 1,3-galactosyltransferase polypeptide 6	0	2	0	2	Up
NFAT5	Nuclear factor of activated T-cells 5, tonicity-responsive	0	2	2	0	Down
HS2ST1	Heparan sulfate 2-O-sulfotransferase 1	0.00042627	2	1	1	Up
ARHGAP18	Rho GTPase activating protein 18	0	2	0	2	Down
TACC1	Transforming, acidic coiled-coil containing protein 1	0	2	2	0	Up
SNX17	Sorting nexin 17	0.00003398	2	1	1	Up
TPD52L1	Tumor protein D52-like 1	1	2			Up
ALDOC	Aldolase C, fructose-bisphosphate	0	2	0	2	Up
FGFBP1	Fibroblast growth factor binding protein 1	0	2	2	0	Up
GRIK1	Glutamate receptor, ionotropic, kainate 1	0	2	0	2	Up
PTGER4	Prostaglandin E receptor 4 (subtype EP4)	0	2	2	0	Down
TXNRD2	Thioredoxin reductase 2	0	2	2	0	Up
FOXLI	Forkhead box L1	0	2	1	1	Up
MXD1	MAX dimerization protein 1	0	2	1	1	Down
TLE2	Transducin-like enhancer of split 2 (E(sp1) homolog, Drosophila)	0	2	2	0	Up
NRCAM	Neuronal cell adhesion molecule	0.00001853	2	1	1	Up
KCNAB1	Potassium voltage-gated channel, shaker-related subfamily, β member 1	1	2			Up
UBA5	Ubiquitin-activating enzyme E1-domain containing 1	0	2	0	2	Up
TCF7	Transcription factor 7 (T-cell specific, HMG-box)	0.00037994	2	1	1	Up
WDR77	WD repeat domain 77	0	2	2	0	Up
KIF23	Kinesin family member 23	0	2	1	1	Down
CHRNA1	Cholinergic receptor, nicotinic, α 1 (muscle)	0	2	0	2	Up
AQP2	Aquaporin 2 (collecting duct)	0	2	0	2	Up
MAK	Male germ cell-associated kinase	0.000000927	2	1	1	Down
SFRP4	Secreted frizzled-related protein 4	0	2	2	0	Up
TAGAP	T-cell activation GTPase activating protein	0	2	2	0	Down
DPYSL5	Dihydropyrimidinase-like 5	1	2			Up
CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)	0	2	0	2	Up
CEP290	Centrosomal protein 290kDa	0	2	0	2	Up
MYH11	Myosin, heavy chain 11, smooth muscle	0.000000927	2	1	1	Up

Table V. Continued.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
ZNF341	Zinc finger protein 341	0	2	2	0	Up
DES	Desmin	0	2	0	2	Up
NDST1	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1	0	2	2	0	Down
SLC8A1	Solute carrier family 8 (sodium/calcium exchanger), member 1	0.00000927	2	1	1	Down
PAPPA	Pregnancy-associated plasma protein A, pappalysin 1	0	2	2	0	Up
ITPR3	Inositol 1,4,5-triphosphate receptor, type 3	0.00000927	2	1	1	Up
GMFB	Glia maturation factor, β	0.00015136	2	1	1	Down
SLC25A4	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	0	2	2	0	Up
CTNND2	Catenin (cadherin-associated protein), delta 2 (neural plakophilin-related arm-repeat protein)	0	2	0	2	Up
ADAM12	ADAM metalloproteinase domain 12 (meltrin α)	0	2	0	2	Up
IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)	0	2	2	0	Up
IGFBP6	Insulin-like growth factor binding protein 6	0	2	2	0	Up
GTF3C2	General transcription factor IIIC, polypeptide 2, β 110kDa	0	2	1	1	Up
LZTS1	Leucine zipper, putative tumor suppressor 1	0	2	2	0	Up
GSTO2	Glutathione S-transferase omega 2	0.66666667	2			Up
NPAS2	Neuronal PAS domain protein 2	0	2	2	0	Up
ARHGAP26	Rho GTPase activating protein 26	0	2	0	2	Down
DAB2	Disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)	0	2	0	2	Down
EIF4E2	Eukaryotic translation initiation factor 4E family member 2	1	2			Up
RPTOR	Regulatory associated protein of MTOR, complex 1	0	2	2	0	Up
RASAL2	RAS protein activator like 2	0	2	2	0	Up
FGF14	Fibroblast growth factor 14	0	2	0	2	Up
GXYLT2	Glucoside xylosyltransferase 2	0.00042627	2	1	1	Up
BIN1	Bridging integrator 1	0.00114908	2	1	1	Up
PSEN2	Presenilin 2 (Alzheimer disease 4)	0	2	2	0	Up
RGS18	Regulator of G-protein signaling 18	0	2	2	0	Down
PPM1A	Protein phosphatase 1A (formerly 2C), magnesium-dependent α	0.00007413	2	1	1	Down
KIRREL	Kin of IRRE like (Drosophila)	0	2	0	2	Up
CYP2E1	Cytochrome P450, family 2, subfamily E, polypeptide 1	0.66666667	2			Up
BCL10	B-cell CLL/lymphoma 10	0	2	0	2	Down
ICOS	Inducible T-cell co-stimulator	0	2	0	2	Up
HTR1B	5-hydroxytryptamine (serotonin) receptor 1B	0	2	1	1	Up
HTR1D	5-hydroxytryptamine (serotonin) receptor 1D	0	2	2	0	Up
MAF	V-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	0	2	2	0	Up

Table V. Continued.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
SUPT3H	SUPpressor of Ty 3 homolog (<i>S. cerevisiae</i>)	0	2	2	0	Up
CHAD	Chondroadherin	0	2	0	2	Up
CAD	Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase	0	1			Up
CTPS2	CTP synthase II	0	1			Up
PDCD11	Programmed cell death 11	0	1			Up
RBM28	RNA binding motif protein 28	0	1			Up
EIF5A	Eukaryotic translation initiation factor 5A	0	1	0	1	Up
CPD	Carboxypeptidase D	0	1			Up
DPP4	Dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)	0	1	1	0	Up
NCALD	Neurocalcin delta	0	1	1	0	Down
ZNF169	Zinc finger protein 169	0	1			Up
GABARAPL1	GABA(A) receptor-associated protein like 1	0	1			Down
TECPR2	Tectonin β -propeller repeat containing 2	0	1			Down
PPRC1	Peroxisome proliferator-activated receptor γ , coactivator-related 1	0	1			Up
GPSM1	G-protein signaling modulator 1 (AGS3-like, <i>C. elegans</i>)	0	1	1	0	Up
MRPL14	Mitochondrial ribosomal protein L14	0	1			Up
MRPL4	Mitochondrial ribosomal protein L4	0	1			Up
TNRC6A	Trinucleotide repeat containing 6A	0	1	1	0	Up
RAPGEF2	Rap guanine nucleotide exchange factor (GEF) 2	0	1	1	0	Down
KCNA1	Potassium voltage-gated channel, shaker-related subfamily, member 1 (episodic ataxia with myokymia)	0	1			Up
PAFAH1B3	Platelet-activating factor acetylhydrolase, isoform Ib, γ subunit 29kDa	0	1	1	0	Up
AIRE	Autoimmune regulator	0	1	0	1	Up
P2RY13	Purinergic receptor P2Y, G-protein coupled, 13	0	1	1	0	Down
GATAD2A	GATA zinc finger domain containing 2A	0	1	0	1	Up
BAG6	BCL2-associated athanogene 6	0	1	0	1	Up
PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	0	1	1	0	Up
BZW2	Basic leucine zipper and W2 domains 2	0	1	0	1	Up
CHRNB2	Cholinergic receptor, nicotinic, β 2 (neuronal)	0	1	1	0	Up
SARS	Seryl-tRNA synthetase	0	1	1	0	Up
RYBP	RING1 and YY1 binding protein	0	1	1	0	Down
ADCY5	Adenylate cyclase 5	0	1	0	1	Up

Table V. Continued.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
GALT	Galactose-1-phosphate uridylyltransferase	0	1	0	1	Up
STON2	Stonin 2	0	1	1	0	Down
DGKA	Diacylglycerol kinase, α 80kDa	0	1	0	1	Up
PLXNA4	Plexin A4	0	1			Up
MYLK	Myosin, light chain kinase	0	1	1	0	Down
MYF6	Myogenic factor 6 (herculin)	0	1	1	0	Up
MDN1	MDN1, midasin homolog (yeast)	0	1	1	0	Up
AMN	Amnionless homolog (mouse)	0	1	0	1	Up
DOCK9	Dedicator of cytokinesis 9	0	1	0	1	Up
PDK2	Pyruvate dehydrogenase kinase, isozyme 2	0	1	0	1	Up
MOB1B	MOB kinase activator 1B	0	1			Down
SAV1	Salvador homolog 1 (Drosophila)	0	1			Up
PIK3R6	phosphoinositide-3-kinase, regulatory subunit 6	0	1	1	0	Up
IMPAD1	Inositol monophosphatase domain containing 1	0	1	0	1	Up
NR2F2	Nuclear receptor subfamily 2, group F, member 2	0	1	1	0	Up
JMJD1C	Jumonji domain containing 1C	0	1	1	0	Down
IPO5	Importin 5	0	1	1	0	Up
PPIL2	Peptidylprolyl isomerase (cyclophilin)-like 2	0	1	1	0	Up
CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1	0	1			Up
ELP2	Elongation protein 2 homolog (<i>S. cerevisiae</i>)	0	1			Up
IKBKAP	Inhibitor of α light polypeptide gene enhancer in B-cells, kinase complex-associated protein	0	1			Down
BRD9	Bromodomain containing 9	0	1	0	1	Up
RAMP3	Receptor (G protein-coupled) activity modifying protein 3	0	1	1	0	Up
NAP1L4	Nucleosome assembly protein 1-like 4	0	1	1	0	Up
NTRK3	Neurotrophic tyrosine kinase, receptor, type 3	0	1	1	0	Up
CXCL5	Chemokine (C-X-C motif) ligand 5	0	1	0	1	Down
NDRG2	NDRG family member 2	0	1	1	0	Up
SEMA3F	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F	0	1	1	0	Up
RUNX2	Runt-related transcription factor 2	0	1	0	1	Up
MPP2	Membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2)	0	1	1	0	Up
SLC40A1	Solute carrier family 40 (iron-regulated transporter), member 1	0	1	1	0	Down
SREK1	Splicing regulatory glutamine/lysine-rich protein 1	0	1	1	0	Up
ING2	Inhibitor of growth family, member 2	0	1	1	0	Down

Table V. Continued.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
AKIRIN2	Akirin 2	0	1			Down
CDH6	Cadherin 6, type 2, K-cadherin (fetal kidney)	0	1			Up
CDH7	Cadherin 7, type 2	0	1			Up
KCNK1	Potassium voltage-gated channel, Shaw-related subfamily, member 1	0	1			Up
TMEM173	Transmembrane protein 173	0	1	1	0	Up
ZNF775	Zinc finger protein 775	0	1	1	0	Up
DTX3	Deltex 3 homolog (Drosophila)	0	1	0	1	Up
EIF4G2	Eukaryotic translation initiation factor 4 γ , 2	0	1			Up
ASAP1	ArfGAP with SH3 domain, ankyrin repeat and PH domain 1	0	1	1	0	Down
ZNF133	Zinc finger protein 133	0	1			Up
SPAG9	Sperm associated antigen 9	0	1	1	0	Down
SLC4A4	Solute carrier family 4, sodium bicarbonate cotransporter, member 4	0	1			Up
SLC4A7	Solute carrier family 4, sodium bicarbonate cotransporter, member 7	0	1			Up
MYBBP1A	MYB binding protein (P160) 1a	0	1	1	0	Up
WARS2	Tryptophanyl tRNA synthetase 2, mitochondrial	0	1	1	0	Up
KCNJ2	Potassium inwardly-rectifying channel, subfamily J, member 2	0	1	1	0	Down
DGKH	Diacylglycerol kinase, eta	0	1	0	1	Up
ANGPTL4	Angiopoietin-like 4	0	1	0	1	Up
MAPKAP1	Mitogen-activated protein kinase associated protein 1	0	1	0	1	Up
RAB11FIP1	RAB11 family interacting protein 1 (class I)	0	1	1	0	Down
IIH	Indian hedgehog homolog (Drosophila)	0	1	0	1	Up
SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1	0	1	1	0	Up
ADAM9	ADAM metalloproteinase domain 9 (meltrin γ)	0	1	0	1	Up
ST3GAL6	ST3 β -galactoside α -2,3-sialyltransferase 6	0	1	1	0	Down
ST3GAL3	ST3 β -galactoside α -2,3-sialyltransferase 3	0	1	1	0	Up
PIGB	Phosphatidylinositol glycan anchor biosynthesis, class B	0	1	1	0	Down
SLC7A8	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	0	1	1	0	Up
AHI1	Abelson helper integration site 1	0	1	0	1	Up
DDR2	Discoidin domain receptor family, member 2	0	1	0	1	Up
MC3R	Melanocortin 3 receptor	0	1	1	0	Up
TXN2	Thioredoxin 2	0	1	0	1	Up
PROX1	Prospero homeobox 1	0	1	1	0	Up
NABP1	Nucleic acid binding protein 1	0	1	0	1	Down
TUBB1	Tubulin, β 1	0	1	1	0	Down
PLCL2	Phospholipase C-like 2	0	1	1	0	Up

Table V. Continued.

Gene	Description	Betweenness centrality	Degree	Indegree	Outdegree	Regulation
CARD14	Caspase recruitment domain family, member 14	0	1	1	0	Up
JDP2	Jun dimerization protein 2	0	1	1	0	Down
ZNF77	Zinc finger protein 77	0	1	1	0	Up
ZBTB25	Zinc finger and BTB domain containing 25	0	1	1	0	Up
AP4E1	Adaptor-related protein complex 4, epsilon 1 subunit	0	1	1	0	Up
ADAM15	ADAM metalloproteinase domain 15	0	1	0	1	Up
DNAH1	Dynein, axonemal, heavy chain 1	0	1	1	0	Up
FARSB	Phenylalanyl-tRNA synthetase, β subunit	0	1	1	0	Up
INSL3	Insulin-like 3 (Leydig cell)	0	1	1	0	Up
TAS2R45	Taste receptor, type 2, member 45	0	1	1	0	Down
PRL	Prolactin	0	1	1	0	Up
ATP2A1	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	0	1	0	1	Up
EYA4	Eyes absent homolog 4 (Drosophila)	0	1			Up
BMP6	Bone morphogenetic protein 6	0	1	0	1	Down
MKNK1	MAP kinase interacting serine/threonine kinase 1	0	1			Down
TCEA2	Transcription elongation factor A (SII), 2	0	1	1	0	Up
L3MBTL1	1(3) mbt-like 1	0	1	1	0	Up
ASAP2	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2	0	1	0	1	Down
ZFYVE16	Zinc finger, FYVE domain containing 16	0	1	1	0	Down
HEXIM1	Hexamethylene bis-acetamide inducible 1	0	1	1	0	Up
NEK3	NIMA (never in mitosis gene a)-related kinase 3	0	1	1	0	Up
DDX56	DEAD (Asp-Glu-Ala-Asp) box polypeptide 56	0	1			Up
RPF1	Ribosome production factor 1 homolog	0	1			Up
STX3	Syntaxin 3	0	1			Down
STX7	Syntaxin 7	0	1			Down
GATM	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	0	1			Up
ELL	Elongation factor RNA polymerase II	0	1	1	0	Down
EZH1	Enhancer of zeste homolog 1 (Drosophila)	0	1			Up
JARID2	Jumonji, AT rich interactive domain 2	0	1	1	0	Down
NKD2	Naked cuticle homolog 2 (Drosophila)	0	1			Up
ZNF569	Zinc finger protein 569	0	1			Up
C1QC	Complement component 1, q subcomponent, C chain	0	1	0	1	Up
DPYS	Dihydropyrimidinase	0	1			Up
LZTR1	Leucine-zipper-like transcription regulator 1	0	1	0	1	Up
VWA1	Von Willebrand factor A domain containing 1	0	1	1	0	Up
PEX14	Peroxisomal biogenesis factor 14	0	1			Up

terms and pathways identified here require further investigation and confirmation.

In conclusion, the present study revealed the underlying molecular differences between patients with AMI, with and without recurrent cardiovascular events, including DEGs, their biological function, signaling pathways and key genes in the PPI network, which may contribute to the prevention of recurrent events and personalized treatment for primary cardiovascular events. Further functional studies may provide additional insights into the role of the DEGs in the pathological process of recurrent cardiovascular events.

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