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Original Article

Identification of key gene modules and pathways of human platelet transcriptome in acute myocardial infarction patients through co-expression network

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Abstract: Acute myocardial infarction (AMI) seriously threatens human life. In this study we aimed to systemically analyze the function of key gene modules in human platelets in AMI. We used weighted gene co-expression network analysis (WGCNA) to construct a co-expression module, and analyzed the relationship between potential modules and clinical characteristics based on platelet RNA-seq RPKM count reads of 16 ST-segment elevation myocardial infarction (STEMI) patients and 16 non-STEMI (NSTEMI) patients provided by the GEO database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed with the DAVID tool. Hub genes were calculated by the Cytohubba package. A total of 3653 genes was selected to construct the co-expression modules. A significant correlation between BMI and the module with color of sky-blue in STEMI. In NSTEMI, there was a significant correlation between the sky blue module and CAD, the Salmon module and HT, and the Cyan module and HT. In STEMI, the Hub genes were mainly enriched in functions related to cell membrane signal transduction including *Aqp1, Armcx1, Gsta4, Hist3h2a* and *Il17re*. In NSTEMI, the Hub genes are related mainly to energy metabolism in the sky-blue module including *Olr1, Nap1l3, Gfer, Dohh, Crispld1* and *Ccdc8b*; they are mainly related to extracellular space and calcium binding in the Cyan module, including *Clec12b, Chd4, Asgr1, Armcx4, Chid1* and *Alkbh7*. The hub genes in the Salmon module include *Ell3, Aldh1b1, Cavin4, Cabp4, Eif1ay* and *Dus3l*. Our results provide a framework for co-expression gene modules in STEMI and NSTEMI patients, and identify key targets as biomarkers for patients with different subtypes of AMI.

Keywords: RNA-seq, RPKM count reads data, co-expression network, Hub gene, coronary atherosclerosis

Introduction

The characteristic pathological changes of acute myocardial infarction (AMI) are acute myocardial ischemia and necrosis, which are caused mainly by coronary atherosclerosis [1]. According to electrocardiogram characteristics, AMI can be divided into two types: ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI). Because of the several differences in clinical manifestations and complications between STEMI and NSTEMI, the specific treatment and prognosis for these AMI variants are also different [2].

High platelet reactivity (HPR) is associated with the occurrence of acute myocardial infarction [3, 4]. Platelet activity is the main factor of intracoronary thrombosis [5]. Platelets may play an important role in the development of atherosclerosis by regulating the inflammatory response [6]. Plaque rupture of coronary atherosclerosis and percutaneous coronary intervention (PCI) could damage vascular endothelium and promote activation and aggregation of platelets on the endothelial surface, which may lead to AMI or the formation of acute thrombosis in stents [7]. Several investigators have reported that platelet activation occurs during thrombosis and plays a role in cardiovascular diseases and events [6, 8]. Differences in platelet transcription levels may reveal causal or reactive mechanisms that lead to platelet reactions and the occurrence and/or prognosis

of various cardiovascular events. Other studies have reported that HPR is associated with adverse outcomes in STEMI and NSTEMI patients undergoing surgical treatment [9]. Platelet reactivity and antiplatelet resistance in STEMI patients are higher than those in NSTEMI patients. The differences in platelet reactivity and disease progression between STEMI and NSTEMI patients may be due to differences in gene expression. Identifying transcripts of the two AMI clinical subtypes will facilitate identification of the pathways relevant to STEMI and NSTEMI and development of specially tailored therapies for NSTEMI and STEMI patients.

Weighted correlation network analysis, also known as weighted gene co-expression network analysis (WGCNA) [10], has been widely used in genomics and in explaining expression patterns in disease transcriptomes. It has also been used in AMI research [11]. Hub genes such as *Cd81* and T-cell receptor CD3ζ were identified by WGCNA in blood transcriptional profiles of the Toll-like receptor (TLR), TCR, and B-cell receptor (BCR) signaling pathways in asymptomatic atherosclerosis, acute ischemic stroke, and myocardial infarction patients [12]. WGCNA of microarray expression profiling of peripheral blood in patients with AMI identified TBX21 and PRF1 as potential diagnostic biomarker candidates and as possible regulatory targets in AMI [13]. In recent research, WGCNA was used to identify the critical genes in development of heart failure after AMI [14]. WGCNA is designed to identify higher-order correlation between gene products, while standardization analysis with DEG aims to detect the individual genes associated with disease. Cluster analysis and classification analysis of biological networks can more accurately reflect the network characteristics of biological systems, which is obviously superior to one-dimensional differential expression analysis [15]. Gene co-expression network analysis constructs gene modules based on all human coding genes, and the data are all from published literature, with a small bias [16]. The WGCNA algorithm can greatly simplify the unavoidable multiple detection problem in microarray expression profile analysis.

In this study, we used WGCNA to analyze the different gene modules of platelet RNA-seq in NSTEMI and STEMI in AMI patients, and analyzed the effects of the main functions and pathways of genes based on different modules; Our analysis identified key genes in the modules that are significantly correlated with HPR in AMI patients.

Material and methods

Expression analysis of RNA-seq RPKM count reads

RNA sequence data and patient clinic traits were obtained from GEO (http://www.ncbi.nlm.nih.gov/geo; accession number was GSE65705). The cohort was comprised of 16 patients with STEMI and 16 with NSTEMI. 32 samples of the platelet transcriptome in arterial blood were profiled using the chip-based platform GPL11154 Illumina HiSeq 2000 (*Homo sapiens*). The number of exons, annotated transcripts, protein domains, coding sequence size, homology information, and GC percent content were retrieved using the biomaRt R package [17]. After the gene expression data were read, those genes with larger variance are selected according to the variance interquartile range. The WGCNA algorithm was used to evaluate gene expression. In addition, flashClust tools in R language were used for cluster analysis of samples with the appropriate threshold value [16].

Analysis of co-expression module construction

The power value is filtered out in module construction by the WGCNA algorithm. The independence and average connectivity of different modules with different power values (between 1 and 30) were tested by the gradient method. When the degree of independence reached 0.8, the appropriate power value was determined, and the WGCNA algorithm was then used to construct the module. The corresponding gene information of each module was extracted. In order to ensure high reliability of the results, the minimum number of genes was set to 30. The WGCNA algorithm was used to identify the co-expression module, and the Heatmap toolkit was used to analyze the intensity of interaction. Genes that could not be included in any modules were sorted into the grey module and removed in subsequent analysis.

Construction of module-trait relationships

The correlation between Eigengene module and phenotype (clinical traits) was used to es-

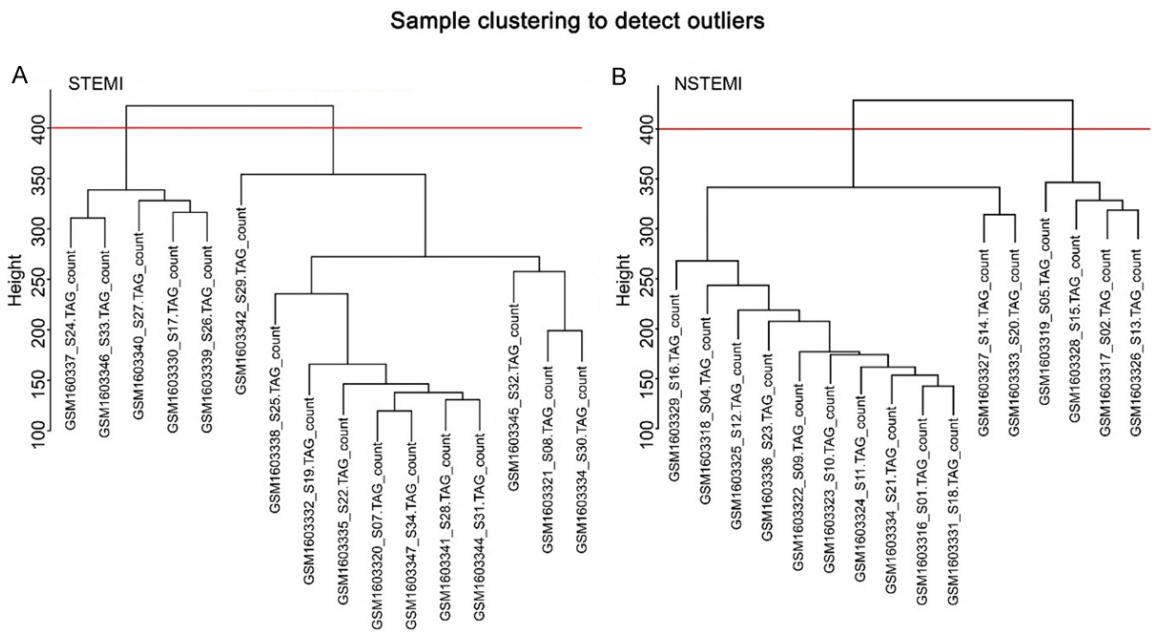

Figure 1. Sample clustering to detect outliers. The top 25% gene expression (3563 genes) out of 14610 genes from different samples were clustered. A. STEMI samples can be separated into two clusters: one includes 5 samples and the other includes 11 samples; B. NSTEMI samples can be separated into two clusters: one with 12 samples and the other with 4 samples. Red lines indicate the outlier samples. The threshold was set as 400; no outlier samples were found.

timate the module-trait association, and facilitate identification of highly phenotypically related expression sets (modules). For each expression profile, gene saliency (GS) was calculated as the absolute value of the correlation between the expression profile and each feature; module member (mm) is defined as the correlation between the expression profile and each module feature value [16].

Functional enrichment analysis of co-expression modules

To analyze the correlation between clinical traits and modules, the genes in modules with a *P* value less than 0.05 were selected for GO and KEGG analysis. The analysis tool was DAVID Functional Annotation Bioinformatics Microarray Analysis (https://david.ncifcrf.gov/). GO annotations were divided into three categories: Molecular Function (MF), Biological Process (BP), and Cellular Components (CC). According to the number of genes contained in each item, the functional modules whose *P* value is less than 0.05 after enrichment analysis were selected for tabular display and visualization. If more than ten records were recorded, the first ten records were extracted. Interested modules were calculated by Cytohubba package in Cytoscape software, and the Hub genes in the module analyzed [18].

Results

Construction of co-expression modules of STEMI and NSTEMI

Gene co-expression networks of STEMI and NSTEMI samples were constructed using WGCNA software package tools. Gene expression variance was calculated and 3653 genes (top 25% of expression value) were selected for WGCNA analysis. These samples were clustered using the flashClust toolkit; results are shown in **Figure 1A** and **1B**. In construction of co-expression modules, there were no outlier samples. All sample were divided into two clusters; we chose cluster I. Finally, there were 10 samples in STEMI and 12 samples in NSTEMI. We identified a module containing highly related genes and used sample clustering to detect abnormal samples (**Figure 2A** and **2B**).

Power value is one of the most critical parameters in construction of the WGCNA model, which mainly affects the independence and aver-

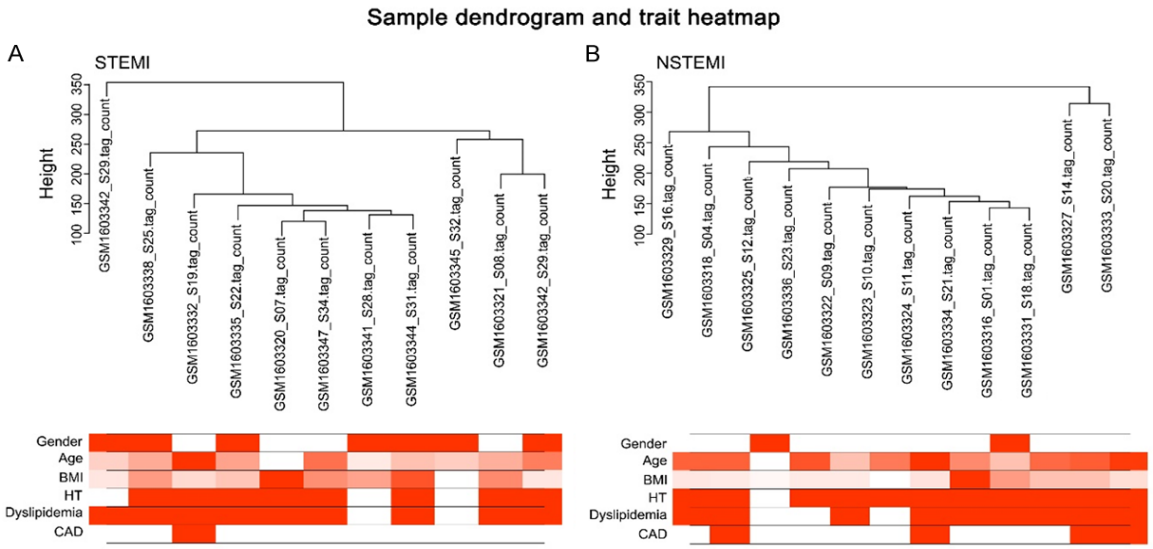

Figure 2. Sample dendrogram and trait heatmap. A. STEMI; B. NSTEMI. No abnormal samples were detected by sample clustering.

age connectivity of the co-expression module. First, the power value is filtered out to establish a scale-free distribution (**Figure 3A** and **3B**). In the STEMI samples, when the power value is equal to 20, the degree of independence can reach 0.85 with high average connectivity. In NSTEMI samples, when the power value is equal to 18, the degree of independence can reach 0.85 with high average connectivity. After determining the appropriate power value, the genes were clustered and the co-expression module was constructed. Seven gene co-expression modules were established in STEMI, and 10 gene co-expression modules were established in NSTEMI. The heatmap describes the topological overlap matrix (Tom) between all the genes in the analysis. Light colors indicate low overlap, while darker colors indicate high overlap. The dark block along the diagonal line is the module. Genotype maps and module assignments are also shown on the left and top (**Figure 4A-D**).

Construct module-trait relationships

The clinical features were provided by GSE-65705 RNA-seq in GEO database (GSE65705_RNA-seq_ClinicalVars_GEO_13Nov2014.xls). We selected data on risk factors related to myocardial infarction, including gender, age, body mass index (BMI), hypertension (HT), dyslipidemia and coronary artery disease (CAD). The co-expression modules associated with specific features were analyzed (**Figure 5A** and **5B**). The correlation between genes and associated traits in the module was validated by using an Eigengene adjacency Heatmap and Module Membership scatter plot (**Figure 6**). In STEMI, the genes in the sky-blue module were significantly correlated with clinical characteristics of BMI, with a correlation coefficient of 0.67 (P=0.02). In NSTEMI, the genes in the sky-blue module were significantly correlated with clinical characteristics of CAD, with a correlation coefficient of -0.63 (P=0.03); the genes in the cyan module were significantly correlated with HT clinical traits, with a correlation coefficient of 0.82 (P=0.001); and the genes in salmon modules were significantly correlated with HT clinical traits with a correlation coefficient of 0.64 (P=0.02).

Functional enrichment analysis of co-expression modules

GO enrichment analysis and KEGG analysis were performed on genes in STEMI (**Table 1** and **Figure 7**) and NSTEMI (**Table 2** and **Figure 7**) modules that were significantly correlated with clinical traits [19]. In sky-blue modules in STEMI, CC was enriched mainly in the GO: 0005886~plasma membrane, GO: 0070062~extracellular exosome, and GO: 0005887~integral component of plasma membrane, BP was enriched mainly in the GO: 0045766~positive component, regulation of angiogenesis, GO:

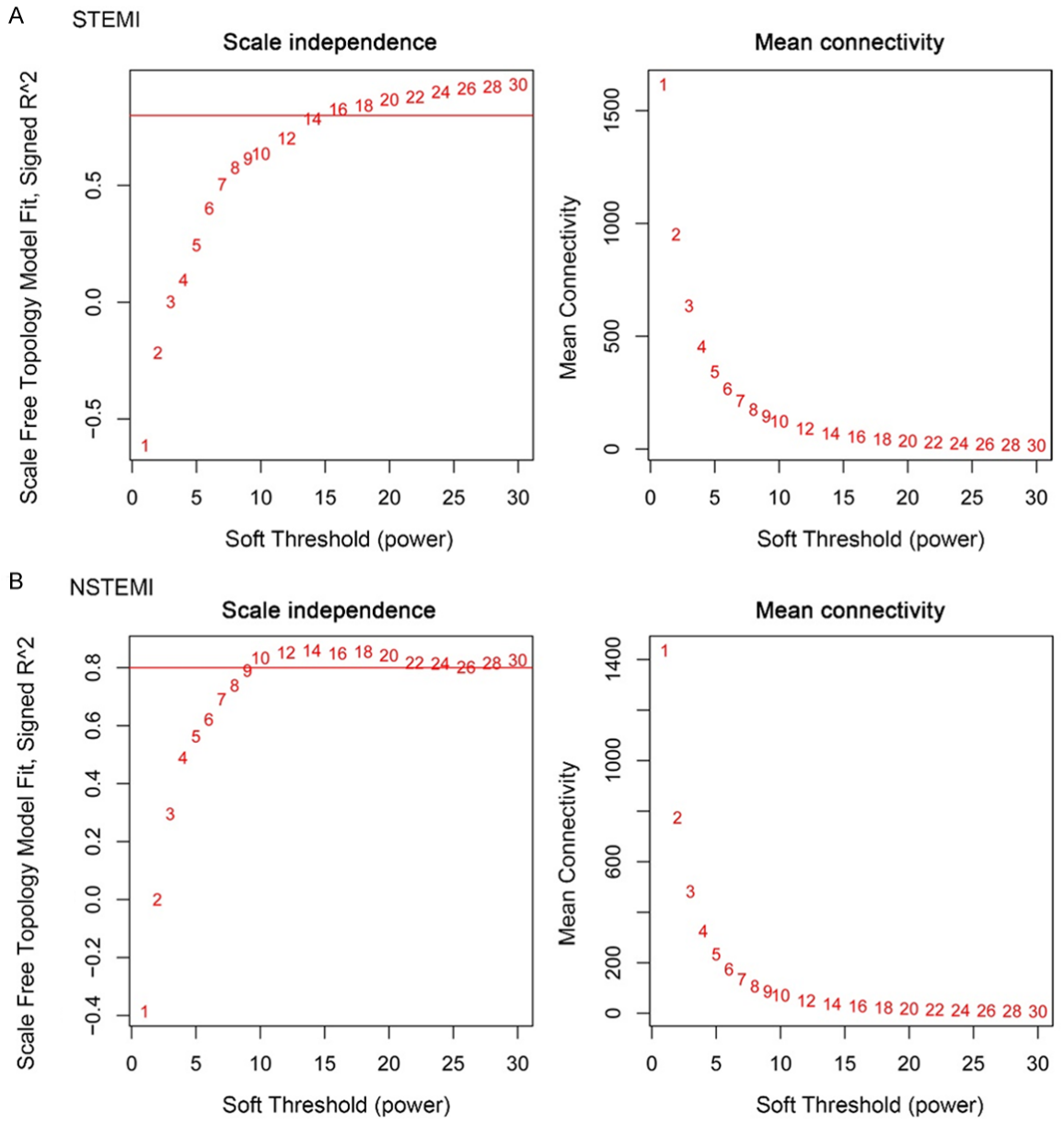

Figure 3. Analysis of network topology for various soft-thresholding powers. A. STEMI; B. NSTEMI. Both scale independence and mean connectivity are shown.

0071805~potassium transmembrane transport, and GO: 0007155~cell adhesions. In sky-blue modules in NSTEMI, genes were concentrated mainly in GO: 0006351~transcription and DNA-templated, GO: 0005739. In the mitochondrion, the main pathways were enriched in hsa00520: Amino sugar and nucleotide sugar metabolism. In cyan modules in NSTEMI, genes were mainly enriched in GO: 0005615~extracellular space and GO: 0005509~calcium binding. In salmon modules in NSTEMI, the gene was mainly enriched in BP: GO: 00098-87~organ morphogenesis, GO: 0006457~protein folding, GO: 0044770~cell cycle phase transition, and MF: GO: 0050253~retinyl-palmitate esterase activity.

The connectivity of the module and hub gene was analyzed by Cytoscape software using the Cytohubba package. The hub genes obtained by the 12 algorithms are shown in **Tables 3-6**. The hub genes in the sky-blue modules in STEMI were *Aqp1*, *Armcx1*, *Gsta4*, *Hist3h2a*, and *Il17re* (**Table 3**). Hub genes in the sky-blue

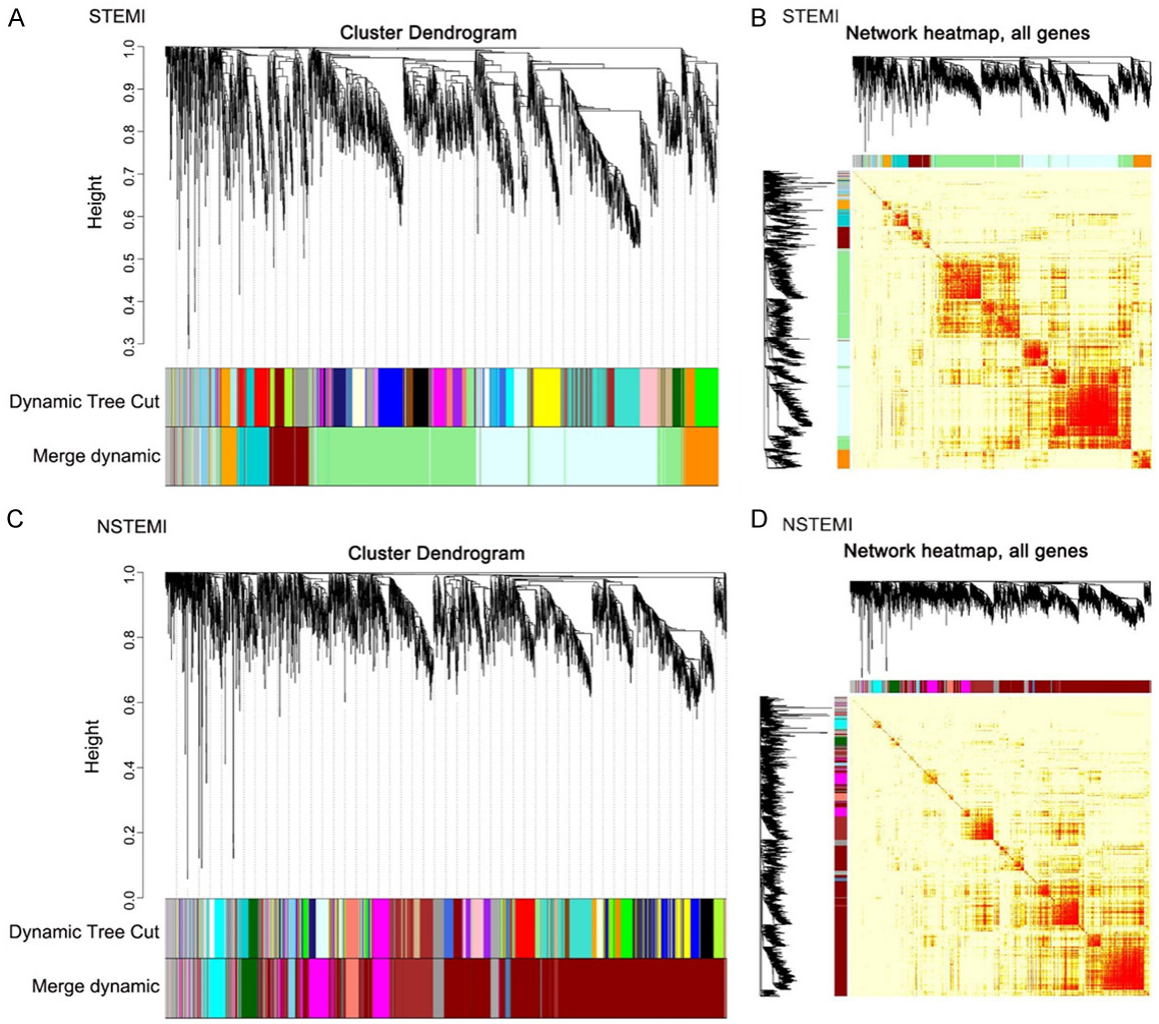

Figure 4. Cluster dendrogram and gene network heatmap plot. A. Co-expression module in STEMI; B. Topological overlap matrix (Tom) among all the genes in the STEMI analysis; C. Co-expression module in NSTEMI; D. Topological overlap matrix among all the genes in the NSTEMI analysis.

module of NSTEMI were *Olr1, Nap1l3, Gfer, Dohh,* and *Crispld1* (**Table 4**); hub genes in the cyan module were *Clec12b, Chchchd4, Asgr1, Armcx4, Chid1,* and *Alkbh7* (**Table 5**); hub genes in the salmon module were *Ell3, Aldh1b1, Cavin4, Cabp4, Eif1ay,* and *Dus3l* (**Table 6**).

Discussion

Gene co-expression analysis and genome module network detection enable deep exploration of inter-gene relationships [20]. WGCNA aims to distinguish high-order relationships among gene products. By focusing on the correlation between co-expression modules and clinical characteristics, the results of WGCNA analysis have higher reliability and biological significance [21]. The identified biologically related modules and hub genes may eventually serve as biomarkers for detection or treatment of AMI. In this study, the WGCNA method was used to analyze 3653 genes from RNA-seq data of 16 NSTEMI and 16 STEMI samples. Correlation between genes and clinical traits was analyzed by establishing a co-expression module combined with clinical traits. We established seven gene co-expression modules in STEMI and 10 gene co-expression modules in NSTEMI.

The hub genes in the sky-blue module in STEMI are closely associated with cardiovascular disease. The sky-blue module in STEMI is significantly correlated with BMI. A large number of recent studies have shown that a high percentage of obese people are associated with some

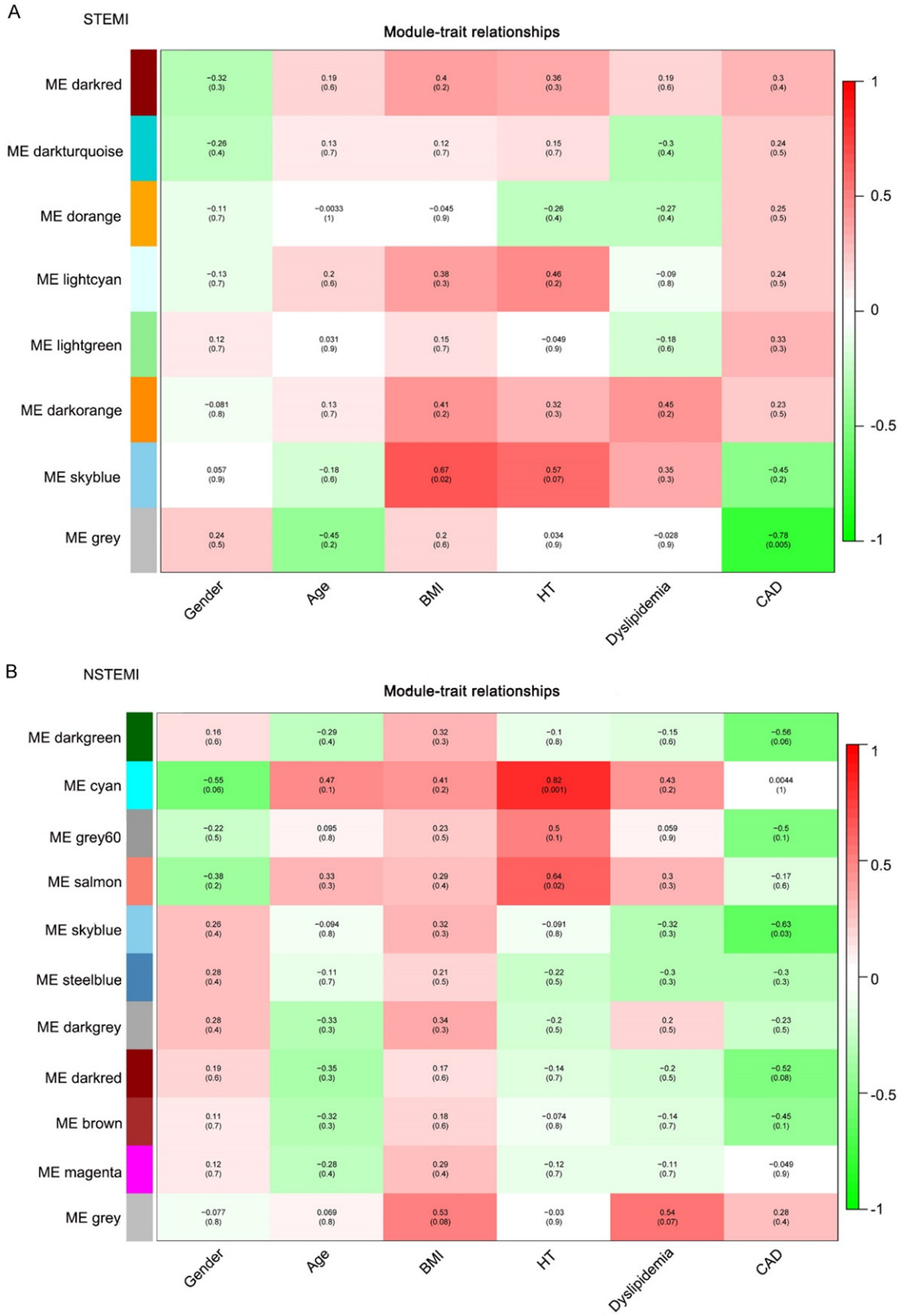

Figure 5. Module-trait relationship. Each row corresponds to a module eigengene, each column corresponds to a clinical trait. Each cell contains the corresponding correlation and *p*-value. The table is color-coded by correlation according to the color legend.

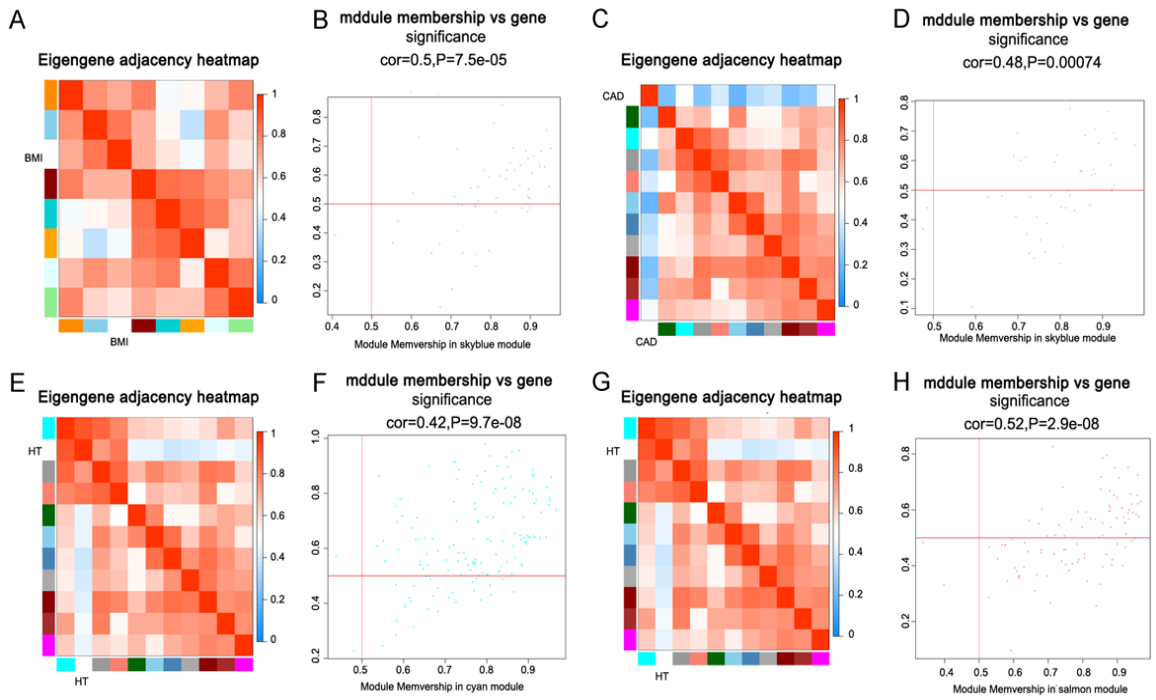

Figure 6. The eigengene dendrogram and heatmap identify groups of correlated eigengenes termed meta-modules. As a result, (A, B) the sky-blue module is highly correlated with BMI in STEMI patients, (C, D) the sky-blue module is highly correlated with CAD in NSTEMI patients, (E, F) the cyan module is highly correlated with HT in NSTEMI patients, and (G, H) the salmon module is also highly correlated with HT in NSTEMI patients. The heatmap in the panel indicates eigengene adjacency.

Table 1. GO enrichment analysis of genes in co-expression modules of STEMI

Module	Category	Term	Count	P	Genes
Sky-blue	CC	GO: 0005886~plasma membrane	20	0.015	*Ret, Kcnc3, Ptprf, C3, Gpr63, Gnrhr, Itga3, Il17re, Ptpru, Npr3, Aqp1, Xpnpep2, Kcnk10, Ddr1, Kcnq4, Arhgap33, Cd34, Kcnh2, Traf4, Lct*
Sky-blue	CC	GO: 0070062~extracellular exosome	14	0.049	*Ddr1, Bhlhb9, Ptprf, Nit2, C3, Sh3d21, Ephx2, Cd276, Hist3h2a, Itga3, Npr3, Aqp1, Ecm1, Xpnpep2*
Sky-blue	CC	GO: 0005887~integral component of plasma membrane	13	<0.001	*Ddr1, Ret, Ptprf, Cd34, Gnrhr, Il17re, Npr3, Ptpru, Aqp1, Gpr1, Lct, Dcstamp, Kcnk10*
Sky-blue	BP	GO: 0045766~positive regulation of angiogenesis	5	<0.001	*Cd34, C3, Aqp1, Ecm1, Angpt4*
Sky-blue	BP	GO: 0071805~potassium ion transmembrane transport	5	<0.001	*Kcnq4, Kcnc3, Kcnh2, Aqp1, Kcnk10*
Sky-blue	BP	GO: 0007155~cell adhesion	5	0.047	*Ddr1, Ptprf, Cd34, Itga3, Ptpru*
Sky-blue	BP	GO: 0050900~leukocyte migration	4	0.006	*Cd34, Itga3, Mmp1, Angpt4*
Sky-blue	CC	GO: 0043235~receptor complex	4	0.006	*Ddr1, Ret, Gpr63, Itga3*
Sky-blue	BP	GO: 0008217~regulation of blood pressure	3	0.016	*Cd34, Ephx2, Npr3*
Sky-blue	BP	GO: 0034765~regulation of ion transmembrane transport	3	0.043	*Kcnq4, Kcnc3, Kcnk10*

Abbreviations: CC, cellular components; BP, biological process.

types of cardiovascular disease, especially with coronary heart disease [22, 23]. Our analysis suggests that high BMI is a high-risk factor for cardiovascular events. In this study, the results of GO enrichment analysis of genes significantly correlated with BMI suggest that the genes in this module are mainly related to Cellular Component. The exosomes are closely associated with endothelial cell function [24]. The functions of these genes are mainly involved in cell membrane transport. Earlier studies [25] found that regulatory proteins on endothelial

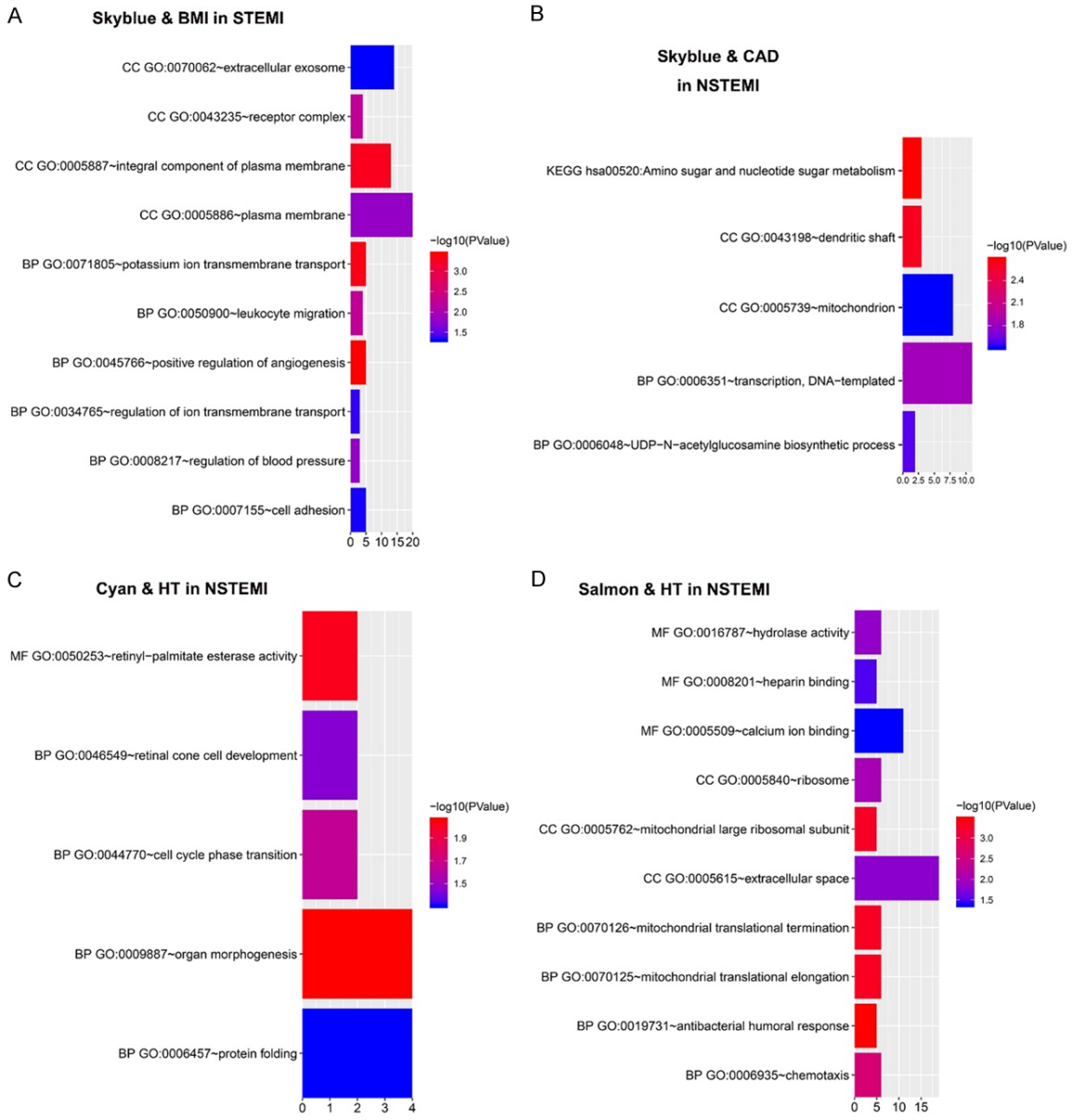

Figure 7. GO enrichment analysis of genes in modules associated with clinical traits. A. Sky-blue modules in STEMI. B. Sky-blue modules in NSTEMI. C. Cyan modules in NSTEMI. D. Salmon modules in NSTEMI.

cell membranes play an important role in the occurrence of thromboembolic disease. For example, thrombomodulin acts as a cofactor of thrombin-mediated protein C activation, and impaired function of thrombomodulin cofactors may also lead to abnormal thrombosis in thromboembolic diseases. The Hub genes of this module were *Aqp1, Armcx1, Gsta4, Hist3h2a*, and *Il17re*. AQP1 is a key member of the aquaporins (AQPs) family, which plays an important role in promoting water transport by regulating osmotic pressure and enhancing the membrane permeability to water. AQP protein plays a potential pathophysiological role in myocardial edema [26, 27]. GSTA4 plays an important role in regulating oxidative stress in human atherosclerosis [28]. IL-17 is widely involved in the regulation of myocardial inflammation, and has been reported in acute coronary syndrome (CAD) and atherosclerosis [29, 30]. HIST3H2A is also involved in atherosclerosis [31, 32]. The roles of the remaining HUB genes in cardiovascular disease have not been further studied. We carried out transcriptome analy-

Table 2. GO enrichment analysis of genes in co-expression modules of NSTEMI

Module	Category	Term	Count	P	Genes
Sky-blue	BP	GO: 0006351~transcription, DNA-templated	11	0.014	*Sbno2, Znf593, Rasl11a, Ing2, Zscan22, Znf697, Znf649, Znf511, Mad2l2, Znf219, Kank2*
Sky-blue	CC	GO: 0005739~mitochondrion	8	0.031	*Maff, Atpaf2, Myom2, Gfer, Romo1, Ntsr1, Abcb6, Kank2*
Sky-blue	CC	GO: 0043198~dendritic shaft	3	0.002	*Jph4, Gper1, Ntsr1*
Sky-blue	BP	GO: 0006048~UDP-N-acetylglucosamine biosynthetic process	2	0.026	*Renbp, Uap1l1*
cyan	CC	GO: 0005615~extracellular space	19	0.016	*Cmtm2, Ltbp2, Rnase3, Enpp2, Podxl, Il1rn, Plbd1, Igf2, Pomc, Tcn2, C1qc, Cxcl10, Chid1, Defa1b, Adm, Lrg1, Vegfa, Ltf, Defa1*
cyan	MF	GO: 0005509~calcium ion binding	11	0.042	*Fkbp9, Prrg4, Lrp1, Ltbp2, Enpp2, Mex3b, Rph3al, Ryr1, Dsc2, Celsr3, Cabyr*
cyan	BP	GO: 0070125~mitochondrial translational elongation	6	<0.001	*Mrpl53, Mrpl2, Mrpl23, Mrpl14, Mrps22, Mrpl54*
cyan	BP	GO: 0070126~mitochondrial translational termination	6	<0.001	*Mrpl53, Mrpl2, Mrpl23, Mrpl14, Mrps22, Mrpl54*
cyan	BP	GO: 0006935~chemotaxis	6	0.002	*Defa1b, Cmtm2, Enpp2, Znf580, Defa1, Cxcl10*
cyan	CC	GO: 0005840~ribosome	6	0.009	*Mrpl53, Mrpl2, Mrpl23, Mrpl14, Mrps22, Mrpl54*
cyan	MF	GO: 0016787~hydrolase activity	6	0.014	*Psmc3, Enpp2, Nudt7, Abhd17c, Dhx58, Afmid*
cyan	BP	GO: 0019731~antibacterial humoral response	5	<0.001	*Defa1b, Adm, Rnase3, Ltf, Defa1*
cyan	CC	GO: 0005762~mitochondrial large ribosomal subunit	5	<0.001	*Mrpl53, Mrpl2, Mrpl23, Mrpl14, Mrpl54*
cyan	MF	GO: 0008201~heparin binding	5	0.032	*Ltbp2, Vegfa, Ltf, Mdk, Cxcl10*
salmon	BP	GO: 0009887~organ morphogenesis	4	0.009	*Hras, Gmnn, Gamt, Bhlhe41*
salmon	BP	GO: 0044770~cell cycle phase transition	2	0.023	*Timeless, Tipin*
salmon	BP	GO: 0046549~retinal cone cell development	2	0.036	*Cabp4, Gnat2*
salmon	BP	GO: 0006457~protein folding	4	0.049	*Tbcc, Ppil6, Hscb, Gnat2*
salmon	MF	GO: 0050253~retinyl-palmitate esterase activity	2	0.01	*Plb1, Pnpla4*

Abbreviations: CC, cellular components; BP, biological process; MF, molecular function.

Table 3. Hub genes in the modules of Sky-blue in STEMI

Gene	MCC	DMNC	MNC	Degree	EPC	BN	EcCentricity	Closeness	Radiality	Betweenness	Stress	CC
C3	2	0.308	2	2	5.358	1	0.127	7.95	4.293	0	0	1
Hist3h2a	3	0.308	2	3	5.714	2	0.127	8.317	4.257	34	38	0.333
Gsta4	5	0.308	2	5	7.264	9	0.158	10.5	4.68	108	190	0.2
Armcx1	4	0.308	2	4	6.511	1	0.127	8.817	4.293	17	34	0.167
Aqp1	5	0.308	2	5	6.738	5	0.127	9.7	4.469	102	220	0.1
Il17re	7	0	1	7	7.479	19	0.127	11.45	4.714	173	330	0
Ccdc28b	3	0	1	3	4.95	4	0.106	8.7	4.328	94	154	0
Ecm1	2	0	1	2	4.274	3	0.106	7.317	4.011	64	136	0
Ddr1	2	0	1	2	2.942	2	0.09	6.06	3.483	34	70	0
Cd276	2	0	1	2	5.805	1	0.158	8.417	4.433	20	64	0
Ephx2	2	0	1	2	5.566	1	0.158	8.417	4.433	20	64	0
Ccdc103	2	0	1	2	3.337	2	0.090	6.71	3.8	34	54	0
Gpr1	2	0	1	2	5.674	1	0.106	8.033	4.257	5	16	0
Bhlhb9	2	0	1	2	5.558	1	0.106	8.033	4.257	5	16	0
Cd34	3	0	1	3	2.581	6	0.067	3.833	0.88	14	14	0
Dcstamp	2	0	1	2	2.447	6	0.1	3.5	0.88	12	12	0
Gtf2h4	2	0	1	2	2.239	2	0.067	3.167	0.8	8	8	0
Gnrhr	1	0	1	1	3.991	1	0.106	7.117	4.117	0	0	0
Irak1bp1	1	0	1	1	2.92	1	0.09	6.043	3.73	0	0	0
Guca1b	1	0	1	1	3.626	1	0.106	5.85	3.659	0	0	0
Angpt4	1	0	1	1	2.325	1	0.079	5.068	3.202	0	0	0
Fam86c1	1	0	1	1	2.218	1	0.079	4.677	2.885	0	0	0

Arhgap33	1	0	1	1	1.951	1	0.05	2.583	0.72	0	0	0
Bspry	1	0	1	1	1.931	1	0.05	2.583	0.72	0	0	0
Casp12	1	0	1	1	1.797	1	0.05	2.333	0.64	0	0	0
Atp6v1g2	0	0	0	0	1	0	0	0	0	0	0	0
Fam187a	0	0	0	0	1	0	0	0	0	0	0	0
Gpr63	0	0	0	0	1	0	0	0	0	0	0	0
Eppk1	0	0	0	0	1	0	0	0	0	0	0	0
Ac020613.1	0	0	0	0	1	0	0	0	0	0	0	0

Abbreviations: MCC, maximal clique centrality; DMNC, density of maximum neighborhood; MNC, maximum neighborhood component; EPC, edge percolated component; BN, bottleneck; CC, clustering coefficient.

Table 4. The hub genes in the modules of Sky-blue in NSTEMI

Gene	MCC	DMNC	MNC	Degree	EPC	BN	EcCentricity	Closeness	Radiality	Betweenness	Stress	CC
Olr1	4	0.308	2	4	3.794	4	0.08	6.617	2.582	34	44	0.167
Nap1l3	3	0	1	3	3.361	4	0.1	6.167	2.582	28	38	0
Gfer	3	0	1	3	3.492	4	0.1	6.25	2.618	50	64	0
Dohh	3	0.308	2	3	3.58	4	0.1	6.25	2.618	32	40	0.333
Crispld1	3	0.308	2	3	3.387	3	0.08	6.117	2.545	36	36	0.333
Jph4	2	0	1	2	2.226	2	0.067	4.483	2.109	20	24	0
Atpaf2	2	0	1	2	2.732	3	0.08	5.2	2.4	36	44	0
Abcb6	2	0	1	2	2.564	2	0.067	4.867	2.255	20	20	0
Ntsr1	1	0	1	1	2.082	1	0.08	4.233	2.218	0	0	0
Mad2l2	1	0	1	1	1.68	1	0.057	3.41	1.745	0	0	0
Catip	1	0	1	1	1.875	1	0.057	3.626	1.891	0	0	0
C2orf66	1	0	1	1	2.293	1	0.067	4.367	2.218	0	0	0
Nanp	0	0	0	0	1	0	0	0	0	0	0	0
Kank2	0	0	0	0	1	0	0	0	0	0	0	0
Mln	0	0	0	0	1	0	0	0	0	0	0	0
Fzd5	0	0	0	0	1	0	0	0	0	0	0	0
Plpp7	0	0	0	0	1	0	0	0	0	0	0	0
Myo7a	0	0	0	0	1	0	0	0	0	0	0	0
Insl3	0	0	0	0	1	0	0	0	0	0	0	0
Gper1	0	0	0	0	1	0	0	0	0	0	0	0
Arl10	0	0	0	0	1	0	0	0	0	0	0	0
Pfkfb1	0	0	0	0	1	0	0	0	0	0	0	0
Myot	0	0	0	0	1	0	0	0	0	0	0	0
Myom2	0	0	0	0	1	0	0	0	0	0	0	0
Maff	0	0	0	0	1	0	0	0	0	0	0	0
Ing2	0	0	0	0	1	0	0	0	0	0	0	0
Glb1l	0	0	0	0	1	0	0	0	0	0	0	0
Fam206a	0	0	0	0	1	0	0	0	0	0	0	0
Depp1	0	0	0	0	1	0	0	0	0	0	0	0
Cenpm	0	0	0	0	1	0	0	0	0	0	0	0

Abbreviations: MCC, maximal clique centrality; DMNC, density of maximum neighborhood; MNC, maximum neighborhood component; EPC, edge percolated component; BN, BottleNeck; CC, clustering coefficient.

sis in AMI patients compared to CAD patients with no history of MI, and showed that hub genes might contribute to the recovery from AMI [33]. Our results showed the hub genes might involve in both CAD and AMI.

The sky-blue module in NSTEMI is significantly correlated with CAD. Functional enrichment analysis shows that this module mainly enriched in genes associated with energy metabolism. The heart is a high energy-consuming organ,

Table 5. The hub genes in the modules of Cyan in NSTEMI

Gene	MCC	DMNC	MNC	Degree	EPC	BN	EcCentricity	Closeness	Radiality	Betweenness	Stress	CC
Armcx4	7	0.309	3	6	12.248	1	0.173	12.867	4.125	75.476	148	0.133
Chid1	4	0.309	3	3	10.882	1	0.144	10.617	3.744	4	12	0.667
Asgr1	7	0.308	2	7	12.599	20	0.289	14.833	4.576	269.881	412	0.048
Clec12b	5	0.308	2	5	12.011	7	0.217	13.5	4.403	145	204	0.1
Cabyr	4	0.308	2	4	10.377	3	0.173	11.483	3.952	62.5	100	0.167
C1qc	4	0.308	2	4	11.01	2	0.144	11.15	3.779	31.952	60	0.167
Arhgap22	4	0.308	2	4	10.327	2	0.217	11.75	4.056	55.024	94	0.167
Adm	4	0.308	2	4	10.842	3	0.173	12.15	4.091	78.333	124	0.167
Aldh1a1	3	0.308	2	3	9.281	3	0.217	11.167	3.987	92	128	0.333
Afmid	2	0.308	2	2	9.65	1	0.144	9.817	3.605	0	0	1
Chchd4	8	0.284	4	6	12.323	5	0.173	13.283	4.229	88.524	164	0.2
Bcl2l12	3	0	1	3	9.445	3	0.217	11.083	3.987	47.809	92	0
Alkbh7	3	0	1	3	10.089	3	0.217	11.917	4.195	107.238	160	0
Abhd17c	3	0	1	3	10.732	1	0.173	11.617	4.091	27.833	62	0
Clp1	2	0	1	2	5.825	2	0.173	8.533	3.224	48	66	0
Clec4a	2	0	1	2	9.652	3	0.217	11.25	4.125	23.238	52	0
Cfap157	2	0	1	2	7.902	1	0.173	9.183	3.467	4.5	10	0
Cdc42ep4	2	0	1	2	9.629	2	0.217	11	4.056	15.238	44	0
Cchcr1	2	0	1	2	6.944	2	0.144	8.933	3.328	6.5	16	0
Catsper1	2	0	1	2	5.854	2	0.173	8.933	3.432	48	60	0
C1orf116	2	0	1	2	7.079	1	0.144	8.6	3.189	3.167	8	0
Art3	2	0	1	2	8.286	1	0.173	9.6	3.605	15.786	32	0
Arhgef40	2	0	1	2	8.291	1	0.173	9.95	3.744	18	32	0
Cinp	1	0	1	1	3.38	1	0.144	6.517	2.392	0	0	0
C20orf96	1	0	1	1	1.485	1	0.067	1	0.2	0	0	0
Borcs8	1	0	1	1	3.838	1	0.144	6.767	2.6	0	0	0
Bcl7c	1	0	1	1	1.485	1	0.067	1	0.2	0	0	0
Arhgef10l	1	0	1	1	6.506	1	0.173	8.9	3.571	0	0	0
Celsr3	0	0	0	0	1	0	0	0	0	0	0	0
Apoa2	0	0	0	0	1	0	0	0	0	0	0	0

Abbreviations: MCC, maximal clique centrality; DMNC, density of maximum neighborhood; MNC, maximum neighborhood component; EPC, edge percolated component; BN, BottleNeck; CC, clustering coefficient.

which needs sufficient blood oxygen to provide energy to maintain its normal function [34]. Hub genes in this module include *Olr1, Nap1l3, Gfer, Dohh,* and *Crispld1*. OLR1 is induced by atherosclerotic stimulation and inflammatory cytokines [35], which are up-regulated in rats with ischemia-reperfusion injury [36]. The *Olr1* gene encodes the endothelial lectin-like oxidized low density lipoprotein (oxLDL) receptor, which is involved in oxLDL binding, internalization, and protein hydrolysis degradation, suggesting that this receptor may play an important role in atherosclerosis [37]. *Crispld1* rs-12115090 polymorphism plays an important regulatory role in the antiplatelet effect of clopidogrel in Han Chinese patients with coronary heart disease [38]. The remaining HUB genes have received little study with relation to in cardiovascular disease.

Genes in the cyan module and salmon module were significantly correlated with HT in NSTEMI. The functions of the cyan module group, such as GO: 0005615~extracellular space and GO: 0005509~calcium binding, play important roles in the regulation of HT [39, 40]. The Hub genes in this module were *Clec12b, Chchd4, Asgr1, Armcx4, Chid1*, and *Alkbh7*. The main function enrichment in the salmon module includes GO: 0009887~organ morphogenesis.

Numerous studies have suggested that elastin is related to arterial hypertension [41, 42], suggesting that those genes affecting arterial elasticity affect the occurrence of hypertension and lead to the occurrence of adverse cardiovascular events. The Hub genes in this module were *Ell3, Aldh1b1, Cavin4, Cabp4, Eif1ay*, and *Dus3l*. It has been reported that *Asgr1* varia-

Table 6. The hub genes in the modules of Salmon in NSTEMI

Gene	MCC	DMNC	MNC	Degree	EPC	BN	EcCentricity	Closeness	Radiality	Betweenness	Stress	CC
Aldh1b1	7	0	1	7	9.385	23	0.193	14.65	7.561	408.857	736	0
Ell3	4	0	1	4	8.52	18	0.193	12.983	7.423	256.586	528	0
Cavin4	4	0	1	4	8.366	15	0.193	11.983	7.181	182.362	470	0
Cabp4	5	0.308	2	5	7.964	3	0.138	11.342	6.49	124.167	306	0.1
Dus3l	3	0	1	3	7.499	2	0.161	11.6	7.077	89.776	210	0
Bhlhe41	3	0	1	3	7.043	2	0.161	11.3	6.974	103.333	210	0
C8orf58	3	0	1	3	7.029	6	0.161	10.883	6.836	162.143	328	0
Eif1ay	2	0	1	2	6.986	7	0.161	11.283	7.181	173.638	278	0
Fam107a	3	0	1	3	6.961	7	0.138	10.96	6.87	161.971	260	0
Gamt	2	0	1	2	6.93	5	0.161	10.3	6.801	57.181	178	0
C7orf43	3	0.308	2	3	6.782	1	0.121	9.751	6.076	24.5	44	0.333
Camkk2	2	0	1	2	6.682	1	0.161	10.3	6.801	57.181	178	0
Fbxl6	3	0	1	3	6.498	2	0.121	10.301	6.387	71.805	152	0
Cltb	3	0	1	3	6.244	3	0.121	9.885	6.283	78.5	104	0
Best3	3	0	1	3	6.2	3	0.161	10.833	6.767	106	170	0
Fdxacb1	2	0.308	2	2	6.082	1	0.121	8.58	5.662	0	0	1
Bola1	1	0	1	1	5.414	1	0.161	9.5	6.629	0	0	0
Akr1e2	2	0	1	2	5.237	2	0.161	9.583	6.56	54	76	0
Cenps	1	0	1	1	5.225	1	0.161	9.5	6.629	0	0	0
Cope	2	0	1	2	4.569	3	0.138	8.786	6.042	104	196	0
Dync2li1	1	0	1	1	4.182	1	0.138	8.26	6.145	0	0	0
Emilin1	1	0	1	1	4.162	1	0.138	8.093	6.042	0	0	0
Atp23	1	0	1	1	4.01	1	0.107	7.255	5.351	0	0	0
Gmnn	1	0	1	1	3.788	1	0.138	7.819	5.835	0	0	0
Gnat2	1	0	1	1	3.706	1	0.138	7.819	5.835	0	0	0
C16orf95	1	0	1	1	3.673	1	0.107	7.472	5.455	0	0	0
Ddx39a	2	0	1	2	3.369	2	0.121	7.421	5.179	54	100	0
C17orf51	1	0	1	1	3.264	1	0.138	7.269	5.627	0	0	0
C8orf37	1	0	1	1	2.492	1	0.107	5.903	4.246	0	0	0
Alpl	0	0	0	0	1	0	0	0	0	0	0	0

Abbreviations: MCC, maximal clique centrality; DMNC, density of maximum neighborhood; MNC, maximum neighborhood component; EPC, edge percolated component; BN, BottleNeck; CC, clustering coefficient.

tion is associated with a reduced risk of coronary heart disease [43]. Decrease in ASGR1 expression has been observed in the peripheral blood mononuclear cells of diabetic atherosclerosis patients [44]. It has been reported that MURC/Cavin-4 can be a therapeutic target for cardiac I/R injury [45]. In the mouse model of ischemia-reperfusion injury, decrease in MURC/Cavin-4 can reduce infarct size and preserve cardiac function [46]. EIFAY was found by variational Bayesian Gaussian mixture model analysis in screening target genes of microRNAs in coronary heart disease [47].

The results or our study provide a framework for co-expression gene modules in STEMI and NSTEMI patients, and identify key regulatory targets. Although only a few studies on human platelet RNA-seq have been conducted thus far, our study suggests the value of further investigation of the functions of these potential hub genes. Thus far, we found only one RNA-seq dataset of platelets from MI patients that included 16 NSTEMI and 16 STEMI samples. WGCNA algorithm (which requires a sample size greater than 15), we plan watch for to new RNA-seq data to verify the candidate target genes identified here, although that dataset met the minimum sample size requirement for use of the WGCNA.

Data availability statement

This study is a re-analysis of existing data, which are openly available at the locations cited in the reference section. Further docu-

mentation about data processing during the current study are available from the corresponding author upon request.

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Disclosure of conflict of interest

None.

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