

Six Hub Genes Associated with Erectile Dysfunction And Acute Myocardial Infarction Based On GEO

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Research

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Abstract

Background: Erectile dysfunction(ED) is a common male sexual dysfunction that significantly affects quality of life in men and induce a significant public health problem. Nowadays, vascular dysfunction is known as a common cause of ED. Acute myocardial infarction (AMI) became co-morbidity with ED as vascular event. The increasing number of males with both diseases is a public health problem that deserves our attention.

Results: Seven common genes of DEGs were obtained in ED and AMI. Fifteen terms mainly including I-kappaB kinase/NF-kappaB signaling et al and five KEGG pathways mainly including Toll-like receptor signaling pathway were also obtained. Further, the diagnostic value of top 6 hub genes were identified.

Conclusion: The hub genes IKBKG, RIPK1, TNF, RPS27A, TLR2, and TNFAIP3 were selected and their diagnostic values were validated. They may play an important potential role in the occurrence and offer viable targets for treating inflammation-mediated vascular dysfunction in the ED and AMI.

Introduction

Although erectile dysfunction is not a fatal disease, effective erectile function is essential for human reproduction, and males always view an erect penis as a sign of masculinity. Once erectile dysfunction occurs, it is bound to cause harm to male's physical and mental health[1]. Therefore, up to now, the studies and explorations of erectile dysfunction are still everlasting. Erectile dysfunction (ED) is defined as a persistent inability to achieve or maintain an erection that is sufficient for satisfactory sexual performance, which affects a considerable proportion of males. It is a complex neurovascular process that penile erection involves the synchronic effects of smooth muscle, vascular endothelium, and psychological and neuroendocrine systems[2]. Therefore, the normal coordination of these parts is essential for maintaining good erectile function, and any changes and disturbances may lead to ED. Various factors including smoking, hypertension and hyperlipidaemia have been identified to influence the development of ED. Among these factors, the vascular component is dominant[3]. Importantly, studies in recent years have shown that erectile dysfunction is no longer confined to sexual behavior alone, but serves as an indicator of systemic endothelial dysfunction. From a clinical perspective, erectile dysfunction usually precedes cardiovascular events and can be used as an early marker to identify men at high risk for major cardiovascular diseases[4].

Coronary artery disease (CAD) was classified into nonobstructive coronary atherosclerosis (NOCA), stable angina pectoris (SA), unstable angina pectoris (UA), and acute myocardial infarction (AMI) according to clinical symptoms, degree of arterial blockage and extent of myocardial injury[5]. Coronary artery disease (CAD) remains one of the main causes of death worldwide. According to studies, CAD was responsible for an estimated 8.14 million deaths globally that year. Several studies have reported an association between ED and coronary artery disease. Some researchers support that ED and CAD should be

considered two distinct manifestations of the same systemic disease. ED is usually a precursor to coronary artery disease[6].

Up to now, ED has not been well solved as a public health problem. With the development of technology, the differentially expressed genes (DEGs) between disease samples and normal controls in ED and AMI were found. However, the common expressed genes of these 2 diseases have not been obtained. We try to reveal the common expressed genes for further early diagnosis, prevention and treatment between ED and AMI, especially in AMI patients with ED.

Results

Differential expression analysis of DEGs in ED and AMI

In the ED dataset GSE10804, 118 DEGs were filtered when we compared the 5 ED samples with 7 normal controls. The AMI datasets (GSE66360) was enrolled in the study, the merged dataset contained 49 AMI patient samples and 50 of their control samples. Heatmaps showing the gene expression profiles of ED and AMI are presented in Figs. 1 and 2, after homogenization, there were 118 and 460 DEGs achieved with adjusted-P < 0.05, respectively. The 7 DEGs include SAMS1N1, ALDH1A1, VCAN, RNASE1, TNFAIP3, ARG1, DAPK1. Using the Xiantao online tool, 7 intersecting common genes of 2 diseases were obtained and are shown in Fig. 3. The information on the whole study process is presented in Fig. 4.

PPI network analysis and hub gene selection

To distinguish the hub genes from the common genes, a PPI network was constructed. As seen in Fig. 5, IKBKG, RIPK1, TNF, RPS27A, TLR2, TNFAIP3, ARG1, RNASE1, TNFRSF1A, TNIP1 interact with other proteins by 2, which was the central node of the protein interaction network. Figure 6 shows the concrete scores of these hub genes. Finally, we chose the top 6 hub genes for study in further research.

GO and KEGG enrichment pathway analysis of Hub genes

Functional enrichment and KEGG pathway analyses of 6 hub genes were performed in ED and AMI at the threshold of P-value < 0.05. Changes in GO biological processes (BP) mainly included I - kappaB kinase/NF - kappaB signaling, regulation of DNA - binding transcription factor activity, toll - like receptor signaling pathway, positive regulation of NF - kappaB transcription factor activity, and regulation of response to cytokine stimulus shown in Fig. 7A. In Fig. 7B, changes in cellular component (CC) were notably focused on enrichment of cytosolic part, membrane raft, membrane microdomain, membrane region and endocytic vesicle membrane. Moreover, in the molecular function (MF) section shown in Fig. 7C, changes were significant in K63 - linked polyubiquitin modification - dependent protein binding, ubiquitin protein ligase binding, tumor necrosis factor receptor superfamily binding, ubiquitin - like protein ligase binding, and polyubiquitin modification - dependent protein binding. Interestingly, changes in the KEGG pathway were mostly enriched in Epstein - Barr virus infection, NF - kappa B signaling

pathway, Toll – like receptor signaling pathway, TNF signaling pathway, and NOD – like receptor signaling pathway in Fig. 7D.

Validation of diagnostic value of hub genes

To validate the diagnostic value of the hub genes obtained from the above analysis, we constructed ROC curves and calculated the corresponding AUC of these gene expression levels in the ED and AMI datasets. Figure 8A shows the results of ED patients. The AUC for IKBKG, RIPK1, TNF, RPS27A, TLR2, and TNFAIP3 in ED patients and normal controls were 0.686 [95% confidence interval (CI), 0.333–1.000; $P < 0.05$], 1.000 (95% CI, 1.000–1.000; $P < 0.05$), 0.600 (95% CI, 0.193–1.000; $P < 0.05$), 0.629 (95% CI, 0.237–1.000; $P < 0.05$), 0.686 (95% CI, 0.345–1.000; $P < 0.05$) and 1.000 (95% CI, 1.000–1.000; $P < 0.05$). Figure 8B shows the ROC curve in AMI patients and non-AMI controls. The AUC for 6 hub genes in AMI were 0.512 [95% CI, 0.397–0.627; $P < 0.05$], 0.526 (95% CI, 0.411–0.642; $P < 0.05$), 0.742 (95% CI, 0.643–0.842; $P < 0.05$), 0.682 (95% CI, 0.575–0.789; $P < 0.05$), 0.853 (95% CI, 0.526–0.746; $P < 0.05$) and 0.858 (95% CI, 0.770–0.946; $P < 0.05$).

Discussion

With the increase of life pace and work pressure, there are more and more diseases caused by oxidative stress, metabolic abnormalities and neurological disorders, such as hypertension, diabetes, coronary artery disease, erectile dysfunction, acute myocardial infarction and so on. According to some authors, ED and CAD should be regarded as “two different manifestations of the same systemic disorder.” [4]. As the gradual deepening of the studies, it is well established that common pathogenesis of ED and AMI include endothelial dysfunction, innate and chronic inflammatory response and et al. With longer course of disease, the risk increases gradually, so it is necessary to explore the molecular mechanisms in these 2 diseases and discover the early targets to prevent disease development.

In this study, through searching the datasets of ED and AMI from GEO, we found 7 common DEGs between these diseases. And a PPI network was constructed to identify the hub genes from among the common DEGs. We chose top 6 hub genes to verify diagnostic value in ED and AMI patients ($P < 0.05$). These genes may have an important ability to predict risk of AMI and ED.

Previous studies have indicated that AMI-triggered inflammatory responses play a critical role in determining AMI size, indicating that inflammation is a potential therapeutic target for improving clinical outcomes in AMI patients[7, 8]. In our study, inhibitor of nuclear factor kappa B kinase regulatory subunit gamma (IKBKG) is the hub gene with highest score. Gao et al[9] found new insights that Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and IKBKG are close relations. Their results also further identified that NF κ B1 and IKBKG directly combine with the the receptor-interacting protein kinase 3 (RIPK3, a multi-functional protein best known for facilitating cellular necroptosis and inflammation) promoter and prevent TNF α -induced RIPK3 transcription in cultured human primary endothelial cells. TNF α is a major inflammatory cytokine[10],which can result in cell death. Therefore, NF κ B1 and IKBKG

act in opposition to TNF α . Interestingly, the tumor necrosis factor (TNF) and RIPK1 (receptor-interacting serine/threonine-protein kinase 1) was verified in our study as the hub gene. Different with RIPK3, RIPK1 primarily drives NF- κ B-dependent inflammation in early atherogenic lesions. Karunakaran et al identified RIPK1 as a central driver of inflammation in atherosclerosis by its ability to activate the NF- κ B pathway and promote inflammatory cytokine release. RIPK1 will be a promising therapeutic target to reduce residual inflammation in patients at high risk of coronary artery disease[11].

TNF is tremendously necessary for human immunity and cellular homeostasis[12]. The role of TNF as a master regulator in balancing cell survival, apoptosis and necroptosis has been extensively studied in various cell types and tissues. TNF regulates development of the cell survival signaling pathways, immune system, proliferation and governs metabolic processes. TNF-induced NF- κ B and MAP pro-survival kinase activities constitute its major biochemical functions, while TNF can also stimulate cell death in certain pathological situations[13]. In recent years, there is growing interest in the novel role of TNF as a regulator of inflammatory response. As we all know, obesity is closely related to ED and AMI. The results of several studies have suggested that obesity progressively leads to the recruitment of inflammatory cell(monocyte/macrophage) in the visceral adipose tissue, so beginning the low-grade inflammatory response that, through the expression of inflammatory cytokines such as tumor necrosis factor (TNF)- α [14, 15]. And a low-grade inflammation of white adipose tissue in obese patients can subsequently lead to insulin resistance, impaired glucose tolerance, and even diabetes. In addition, evidence in previous researches suggested cytokines in general and tumour necrosis factor (TNF) in particular play an important role in cardiovascular disease. In the course of vascular damage, TNF could be responsible for further ventricular remodelling; down-regulation of myocardial contractility; increased rate of apoptosis of the endothelial cell and of the myocytes, alteration of the expression and function of the enzymes regulating nitric oxide production[16]. It is excited that El-Makaky et al reported a randomized clinical trial aiming to explore the relationship between periodontal therapy with patients having chronic periodontitis and erectile dysfunction[17]. Their study showed that the non-surgical periodontal therapy could significantly improve the severity of erectile dysfunction in addition to periodontal and serological parameters by detecting the level of an inflammatory mediator (TNF- α) and the clinical variables. Salivary TNF- α could be a new diagnostic tool to detect the severity of erectile dysfunction.

Ribosomal protein S27a(RPS27a) gene encodes a fusion protein consisting of ubiquitin(UB) at the N terminus and ribosomal protein S27a at the C terminus[18]. When expressed in yeast, the protein is post-translationally processed, generating free UB monomer and ribosomal protein S27a. Therefore, RPS27a is closely related to ubiquitin. Ubiquitination will be completed after covalent conjugation of ubiquitin with lysine (K) residues of target proteins. The well-known function of ubiquitination is to target substrate proteins for degradation in the 26S proteasome. Ubiquitination is necessary for multifarious biological processes, including different aspects of immune functions. Stephanie L C Scofield et al injected vehicle or exogenous UB into target mice for the condition of myocardial ischemia-reperfusion (I/R) injury. Their study reveals that exogenous UB plays a protective role in myocardial remodeling post-I/R with effects on area at risk/infarct size, cardiac function, the inflammatory response, and levels of serum

cytokines/chemokines[19]. As a result, RPS27a closely related to UB may provide therapeutic regimens for patients with ischemic heart disease.

Inflammation is initiated by an innate immune response often mediated by Toll-like receptors (TLRs). The TLRs are evolutionarily conserved members of the pattern recognition receptor family. Abundant in immune cells, TLRs are also expressed in vascular cells, and their presence provides a mechanism for innate immune activation upon detection of molecular patterns of pathogens or endogenous molecules released during cell injury and death[20–22]. The TLR2 was also demonstrated to be involved in neo-intima formation after arterial injury in mice, mediating the effect of the virulence factor of *Porphyromonas gingivalis* (*P. gingivalis*), a common bacteria in periodontal disease[23]. The TLR1/2 heterodimer was shown to involve in a lipid raft complex that induced endothelial cell activation by *P. gingivalis*, indicating an important role for TLR1/2 in atherosclerosis in a cell culture model[24]. TLR2 and the TLR1/2 dimer can result in activation of the pro-inflammatory transcription factor $\text{NF-}\kappa\text{B}$, which contribute to vascular disease and were shown in human atherosclerotic plaque[25]. In addition, some direct impacts of the inflammatory cytokine on erectile function has been shown in mice infused with, or genetically deficient in, $\text{TNF-}\alpha$ [26]. Given TLR2 is activated in conditions characterized by vascular dysfunction and that co-exist with ED, TLR2 signaling could be a promising target.

Tumor necrosis factor alpha-induced protein 3 (TNFAIP3) is also known as A20, which is an endogenous negative regulator of $\text{NF-}\kappa\text{B}$ signaling, has been widely studied in several autoimmune and inflammatory disorders[27]. A recent study showed that TNFAIP3 was directly targeted by MiR-125b promoted the $\text{NF-}\kappa\text{B}$ -mediated inflammatory response in Non-alcoholic fatty liver disease (NAFLD)[28]. Deletions of the TNFAIP3 gene in innate immune cells of mice would develop autoinflammatory disease, which revealed the negative regulation of TNFAIP3 to innate immune cells[29]. TNFAIP3 has a variety of pathophysiologic functions, especially inflammation. Clinically, Inflammation functions holds a key role in the pathology of a host of conditions ranging from coronary artery disease, rheumatoid arthritis, inflammatory bowel disease, osteoporosis, depression and neurodegenerative diseases. Firstly, mechanistic studies revealed that A20 overexpression in mice with obesity-induced heart injuries reversed myocardial dysfunction, hypertrophy, and fibrosis through reducing cardiac inflammation and apoptosis[30]. Moreover, it was identified increased A20 expression in adipose tissue was shown to ameliorate adipose tissue inflammation[31]. The study of the relationship between ED and TNFAIP3 is unknown. However, based on the relationship between inflammation functions and ED, it's possible that TNFAIP3 plays an important role in ED.

All in all, our study showed that IKBKG, RIPK1, TNF, RPS27A, TLR2, and TNFAIP3 are closely correlated under the mediation of inflammatory pathways. They may offer viable targets for treating inflammation-mediated vascular dysfunction in the penis. There are many treatment paradigms of diseases with co-pathogenesis in medicine. One case was reported by Masanori et al that a female infant with incontinentia pigmenti complicated by severe pulmonary arterial hypertension was markedly improved by tadalafil administration[32]. As co-morbidity, ED and AMI will share some common therapeutic targets. Our study identified some of these targets that could be of real benefit to patients with ED and AMI. While

our results are consistent with previous studies, there are some limitations of our study. Firstly, because penis specimens were too difficult to obtain, our study was lacked of vivo and vitro experiments. We hope that we can make up for this in the near future to confirm these results. Secondly, our results do not necessarily apply to all populations, and research is needed in other populations. Finally, our sample sizes were relatively small, and larger-sample, multi-center research is needed.

Conclusions

The hub genes IKBKG, RIPK1, TNF, RPS27A, TLR2, and TNFAIP3 were selected and their diagnostic values were validated. They may offer viable targets for ED and AMI patients to treat inflammation-mediated vascular dysfunction in the penis.

Material And Methods

Microarray data collection

The microarray datasets of ED, AMI, and normal controls were downloaded from the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo>). The probes were transformed into the corresponding gene symbols on the basis of annotation information on the platform. The GSE10804 datasets downloaded from GEO (Affymetrix GPL571platform, Affymetrix Human Genome U133A 2.0 Array) had 3 groups: 5 samples are cultured human corpus cavernosum endothelial cells (HCCEC) from a donor with erectile dysfunction; 3 samples are cultured human umbilical vein endothelial cells (HUVEC) from a donor without erectile dysfunction[33]. Then, same methods were employed for AMI datesets. 49 AMI patients and 50 non-AMI controls were also selected from GSE66360 datesets downloaded from GEO (Affymetrix GPL571platform, Affymetrix Human Genome U133A 2.0 Array)[34]. Because these gene expression profiles all originated from a free open-access database on the internet, our research did not require Ethics Committee approval.

Identification of differentially expressed genes (DEGs) in ED and AMI

DEGs between ED and normal controls, AMI patients, and corresponding controls were identified using the limma R package, which is an efficient analysis method in bioinformatics[35]. The selected criteria in ED datasets were set as adjusted- $P < 0.05$ and $|\log_2FC| > 1$. In AMI section, the cut-off values were adjusted- $P < 0.05$ and $|\log_2FC| > 1$. After we utilized these screening conditions, 2 sets of DEGs were identified, then we put these DEGs that came from the 2 diseases into an online analysis tool Xiantao (<http://www.xiantao.love>) to obtain their intersection genes. These intersecting common genes were used for subsequent analysis.

Construction of protein–protein interaction (PPI) network and identification of hub genes

To further explore the interaction among the common genes obtained above, we used the Search Tool for the Retrieval of Interacting Genes (STRING) 11.0 (<http://string-db.org/>) to construct a PPI network. The minimum required interaction score was considered high confidence (0.700) as the criteria of statistical significance[36]. Cytoscape 3.8.2 (<https://cytoscape.org>) was utilized to present the results. In the network outcome, the nodes represented the proteins, while the lines represented the interactions between proteins[37].

Functional enrichment analyses for hub genes

The Gene Ontology (GO) classification[38], which contains molecular functions, biological processes, and cellular component, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed on the hub genes using the R package[39]. P-values < 0.05 were defined as statistically significant.

Statistical analysis

Using Proc package (version 3.2–10) of R software (version 3.6.3), we constructed receiver operating characteristic (ROC) curves and calculated the area under the curve (AUC) of the hub genes to compare the AUC as the index of the model. These results showed the diagnostic efficiency of genes. P-values < 0.05 were regarded as statistically significant.

Abbreviations

ED

Erectile dysfunction; AMI:Acute myocardial infarction; CAD:Coronary artery disease; NOCA:nonobstructive coronary atherosclerosis; SA:stable angina pectoris; UA:unstable angina pectoris; GEO:Gene Expression Omnibus; HCCEC:human corpus cavernosum endothelial cells; HUVEC:human umbilical vein endothelial cells; GO:Gene Ontology; KEGG:Kyoto Encyclopedia of Genes and Genomes; STRING:Search Tool for the Retrieval of Interacting Genes; ROC:receiver operating characteristic; AUC:area under the curve; SAMS1N1:SH3 domain and nuclear localization signals 1; ALDH1A1:aldehyde dehydrogenase1 family member A1; VCAN:versican; RNASE1:ribonuclease A family member 1; TNFAIP3:Tumor necrosis factor alpha-induced protein 3; ARG1:arginase 1, DAPK1:death associated protein kinase 1; NAFLD:Non-alcoholic fatty liver disease; IKBKG:inhibitor of nuclear factor kappa B kinase regulatory subunit gamma; NF-κB:Nuclear Factor kappa-light-chain-enhancer of activated B cells; RIPK1:receptor-interacting serine/threonine-protein kinase 1; RIPK3:receptor-interacting protein kinase 3; TNF:tumour necrosis factor; RPS27a:Ribosomal protein S27a; UB:ubiquitin; TLRs:Toll-like receptors.

Declarations

Acknowledgment

None.

Authors' contributions

ZKX performed the data analyses and wrote the manuscript; the XJA and JNX contributed to analysis and manuscript preparation; YXW and NXG were contributed to the provision of study materials; CSP were contributed to the conception and helped perform the analysis with constructive discussions. All authors read and approved the final manuscript.

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Availability of data and materials

The raw data of this study are derived from the GEO data portal ([https:// www. ncbi. nlm. nih. gov/ geo/](https://www.ncbi.nlm.nih.gov/geo/)), which is publicly available database. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflicts of interest

The authors declare no conflict of interest.

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Figures

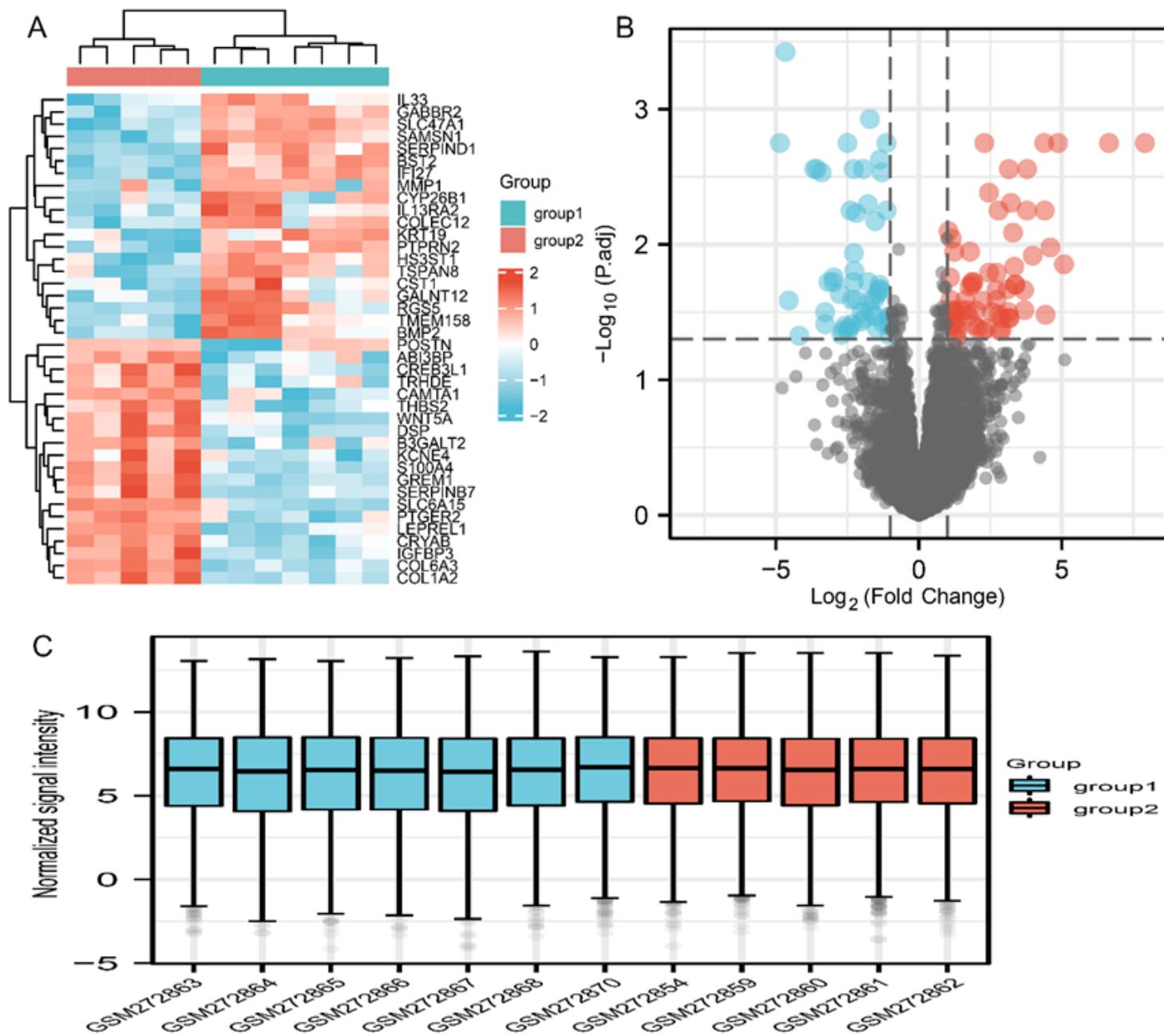


Figure 1

A and B: Heatmap showing the expression changes in Erectile dysfunction (ED). Group 1 – control; Group 2 – ED (DEGs were selected with a fold change >1 and P-value < 0.05 among the mRNA expression profiling set GSE10804). C: the median of each sample is basically on a horizontal line.

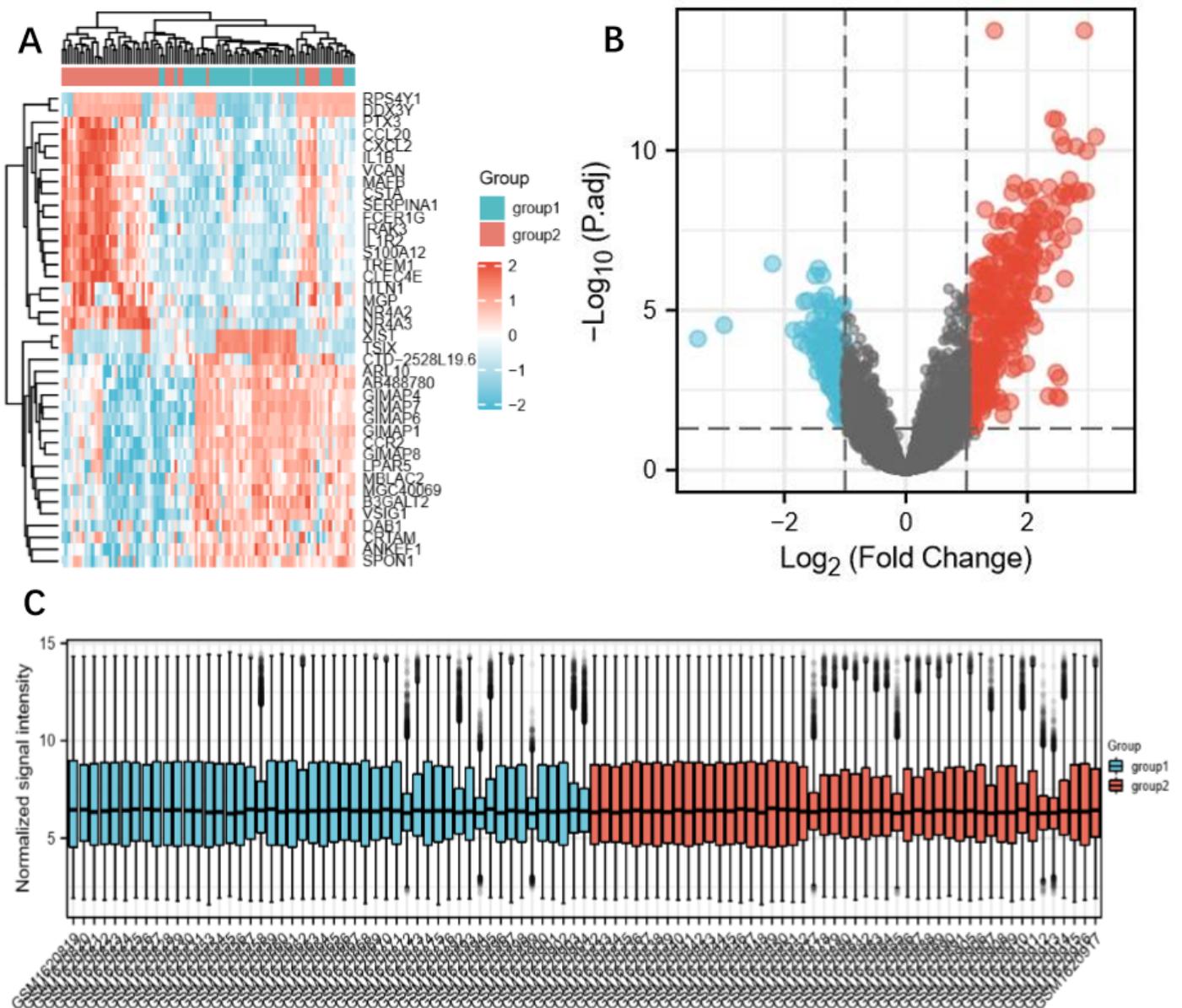


Figure 2

A and B: Heatmap showing the expression changes in acute myocardial infarction (AMI). Group 1 – control; Group 2 – AMI (DEGs were selected with a fold change >1 and P-value < 0.05 among the mRNA expression profiling set GSE66360). C: the median of each sample is basically on a horizontal line.

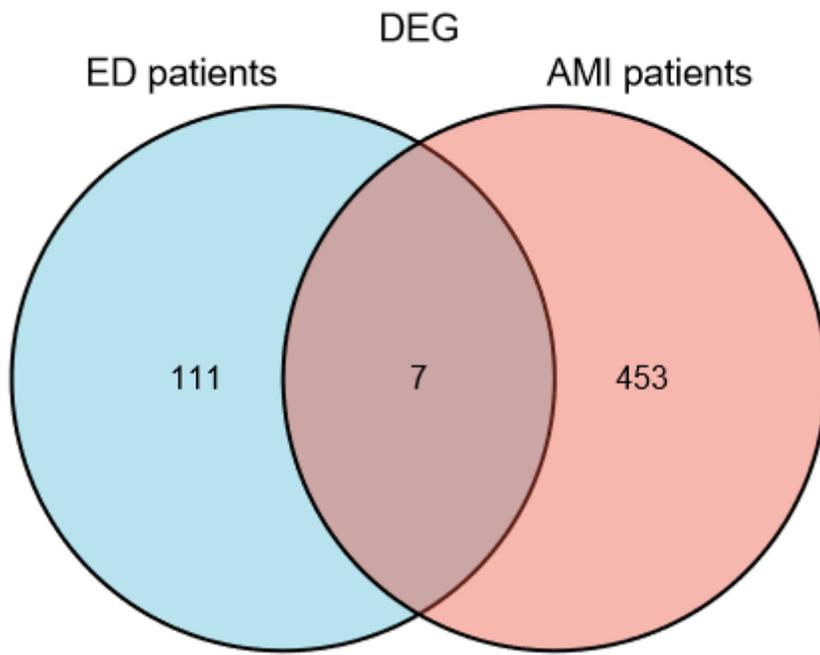


Figure 3

Venn diagram of intersecting common genes identified by differential genes (DEGs) from ED and AMI.

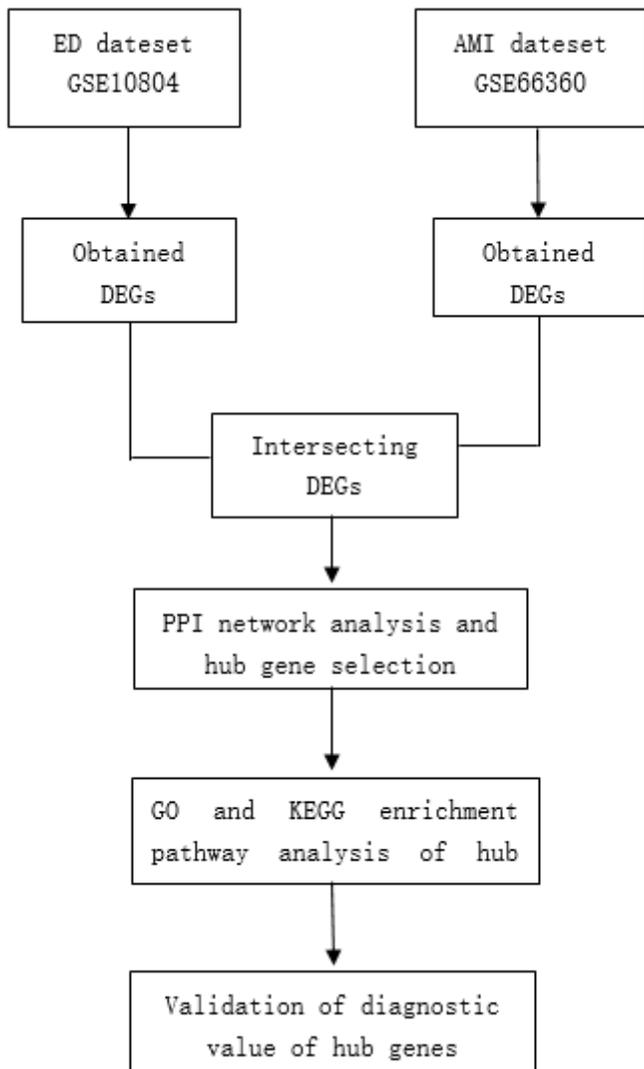


Figure 4

Flow diagram of study.

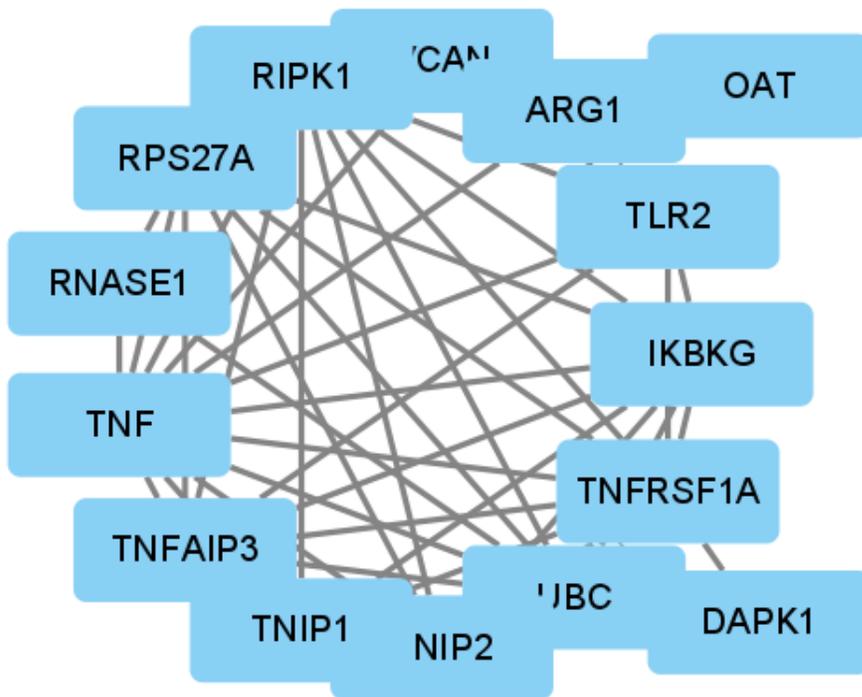


Figure 5

PPI network construction and the hub genes.

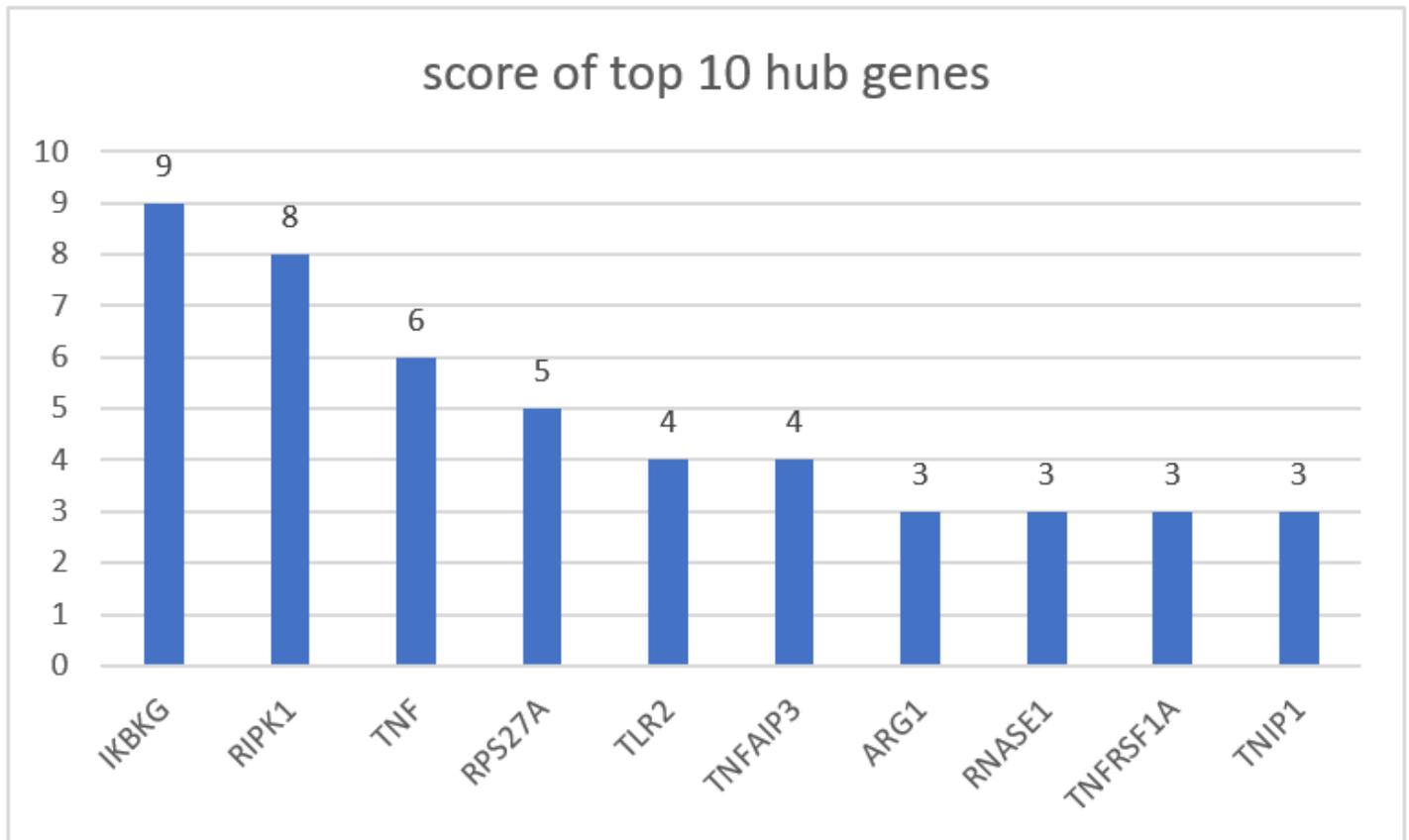


Figure 6

The accordance percentage of hub genes.

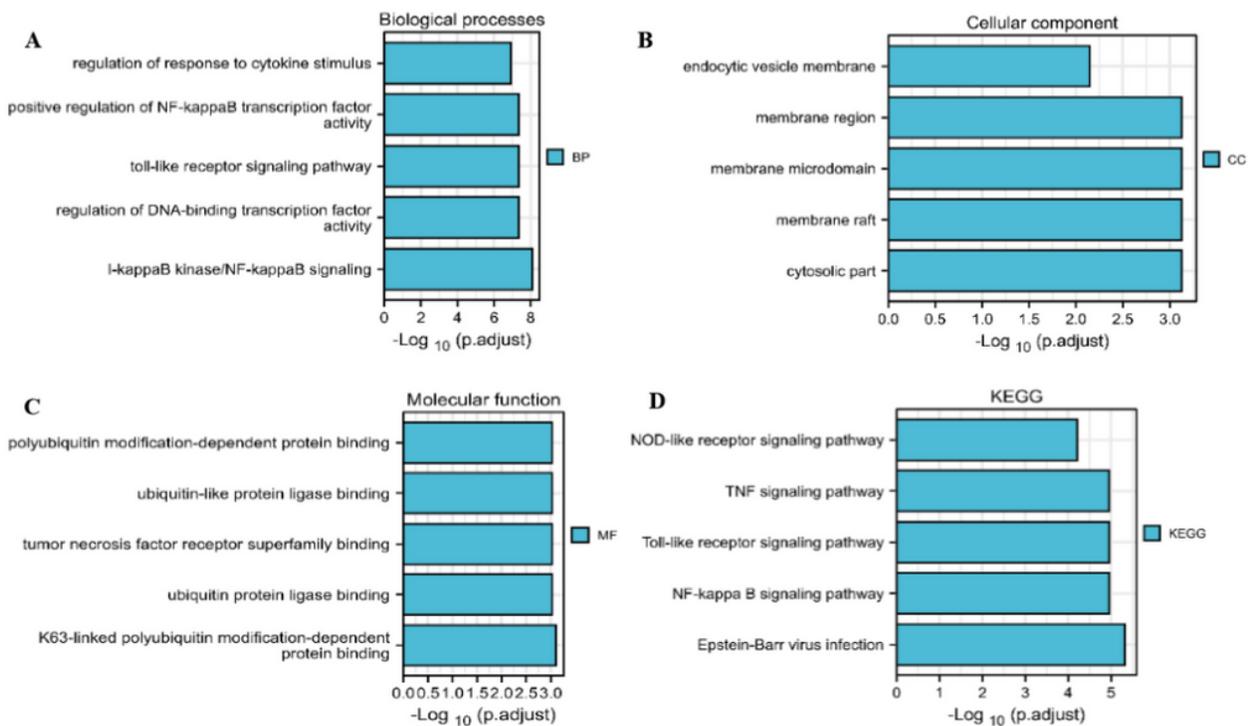


Figure 7

The BP, CC, MF, and KEGG analysis of hub genes.