

Original Research

# Macrophage-Related Genes Biomarkers in Left Ventricular Remodeling Induced by Heart Failure

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## Abstract

**Background:** Elevated left ventricular mass index contributes to morbidity and mortality induced by heart failure and M2 macrophages play a critical role in left ventricular remodeling. Here, our aim was to investigate the roles of M2 macrophage-related genes in heart failure. **Methods:** GSE10161 was downloaded and the abundance of immune cells were estimated utilizing the CIBERSORT algorithm. Using the limma test and correlation analysis, differentially expressed plasm B cells and M2 macrophages-related genes (DEBRGs and DEMRGs) were documented. Functional pathways and the protein-protein interaction network were analyzed and the hub DEMRGs were obtained. The hub DEMRGs and their interactions were analyzed using NetworkAnalyst 3.0 and for validation, the hub DEMRGs expressions were analyzed using the GSE135055, GSE116250 and GSE74144 datasets. **Results:** 103 differentially expressed genes were correlated with the abundance of M2 Macrophages and were identified as DEMRGs (PCC >0.4), which were mainly enriched in extracellular matrix organization, cell adhesion molecule binding and postsynaptic membrane. After screening out, 5 hub DEMRGs were obtained, including FN1 (degree = 21), COL3A1 (degree = 13), COL1A2 (degree = 13), FBN1 (degree = 12), and MMP2 (degree = 11). However, no hub DEBRGs were obtained in the network. The expression patterns of the screened DEMRGs were further validated in the patients with heart failure, dilated cardiomyopathy, ischemic cardiomyopathy or hypertension. **Conclusions:** The results can improve our understanding of the macrophages-associated molecular mechanisms in heart failure induced by dilated cardiomyopathy, ischemic cardiomyopathy or hypertension and 5 hub DEMRGs may help prevent the adverse left ventricular remodeling to decrease mortality and morbidity.

**Keywords:** macrophages; plasm B cells; PPI; differentially expressed genes; left ventricular remodeling; CIBERSORT; GO/KEGG pathways analysis

## 1. Introduction

Currently, coronary artery disease (CAD) is still one of the leading causes of death in patients [1]. In addition, acute myocardial infarction (MI) mortality has increased 5.6-fold in the past 30 years [2]. Young patients with type 2 diabetes and MI have higher long-term cardiovascular and all-cause mortality and more than one-third of patients die within 10 years, which may emphasize more aggressive secondary prevention for those patients [3]. Acute heart failure (HF) contributes to the mortality above mentioned, which is characterized by an acute or subacute deterioration in cardiac function due to the underlying heart diseases and precipitating factors.

Isabelle *et al.* [4] reported that 23,291 patients with HF from 40 countries in 8 different world regions were investigated and there were 4460 (19%) deaths, 3885 (17%)

HF hospitalizations, and 6949 (30%) instances of either adverse event, suggesting that HF has become the leading problem in CAD. Previous researches have confirmed the importance of neurohormonal systems, such as the renin-angiotensin-aldosterone axis in the pathogenesis of heart failure phenotypes [5,6]. However, a large number of drug trials have failed, highlighting the limitations of animal models in replicating complex diseases and investigating critical regulators in HF [7]. The underlying critical genes and interactions that control the transformation of heart failure remain largely unknown.

Elevated left ventricular mass (LVM) contributes to morbidity and mortality induced by heart failure, which is partially regulated by hemodynamic indicators [8]. However, only a small portion of LVM variation is investigated by hemodynamic effects, and it has been suggested that genetic influences may also be important.



In this study, GSE10161 was downloaded and immune cells analysis was carried out to screen out the hub genes associated with immune cells. Then the expression levels of the hub genes in heart failure, dilated cardiomyopathy, ischemic cardiomyopathy and hypertension patients were demonstrated to validate the correlation to left ventricular remodeling, which will help to diagnose the adverse left ventricular remodeling at an early stage and reduce the patients' mortality.

## 2. Methods

### 2.1 Microarray Data

Using the keywords "left ventricular" in "Homo sapiens", GSE10161 from the Gene Expression Omnibus (GEO) database was downloaded and analyzed [9]. There were 7 left ventricular biopsies from control patients and 20 cardiac biopsies from aortic stenosis (AS) patients based on the Affymetrix Human Genome U133A Array. Firstly, the raw transcriptomic data were processed with log<sub>2</sub> transformation for normalization utilizing a robust multichip average algorithm [10].

### 2.2 Evaluation of Immune Cells in Left Ventricle

Based on their gene expression profiles CIBERSORT is a deconvolution approach to characterize the cell compositions in bulk tissues [11]. To obtain the abundance of immune cells in the left ventricle, the CIBERSORT algorithm with 100 permutations was applied in the GSE10161 dataset, utilizing the LM22 matrix as reference. CIBERSORT outputs a deconvolution *p*-value for each sample to determine the reliability of the results. In this study, we retained the samples with *p* < 0.05 to analyze the fractions of immune cells.

### 2.3 Identification of DEMRGs and DEBRGs

The differentially expressed genes (DEGs) between left ventricle samples from control patients and aortic stenosis patients were investigated utilizing the "limma" package. The genes with |fold change| > 1.5 or *p* < 0.05 were considered as differentially expressed genes (DEGs). Then, Pearson correlation analysis was applied to obtain the genes associated with the abundance of M2 Macrophages and B cells. The DEGs with Pearson correlation coefficients (PCC) > 0.4 were regarded as DEMRGs and DEBRGs, respectively.

### 2.4 Enrichment Analysis

To understand the functions of DEMRGs and DEBRGs, GO and KEGG pathway analysis, as well as Gene Set Enrichment Analysis (GSEA) analysis, were applied utilizing the "clusterProfiler" package in R [12]. A *p* < 0.05 was set as the significance threshold for enrichment analyses.

### 2.5 Protein-Protein Interaction (PPI) Network Analysis

STRING (<https://string-db.org>) is a biological resource that provides systematic screens of human protein interactions [13]. To investigate the hub genes, DEMRGs and DEBRGs were uploaded to STRING to investigate the protein network interaction diagram and significant PPIs were identified with a combined score > 0.4. Then the result was imported into Cytoscape v.3.7.1 (<https://cytoscape.org/>) and the hub genes were investigated by applying the Cytoscape plug-in (MCODE and Cytohubba). Nodes with a connectivity degree  $\geq 10$  and ranked by MCC in the network were selected as hub DEMRGs or DEBRGs.

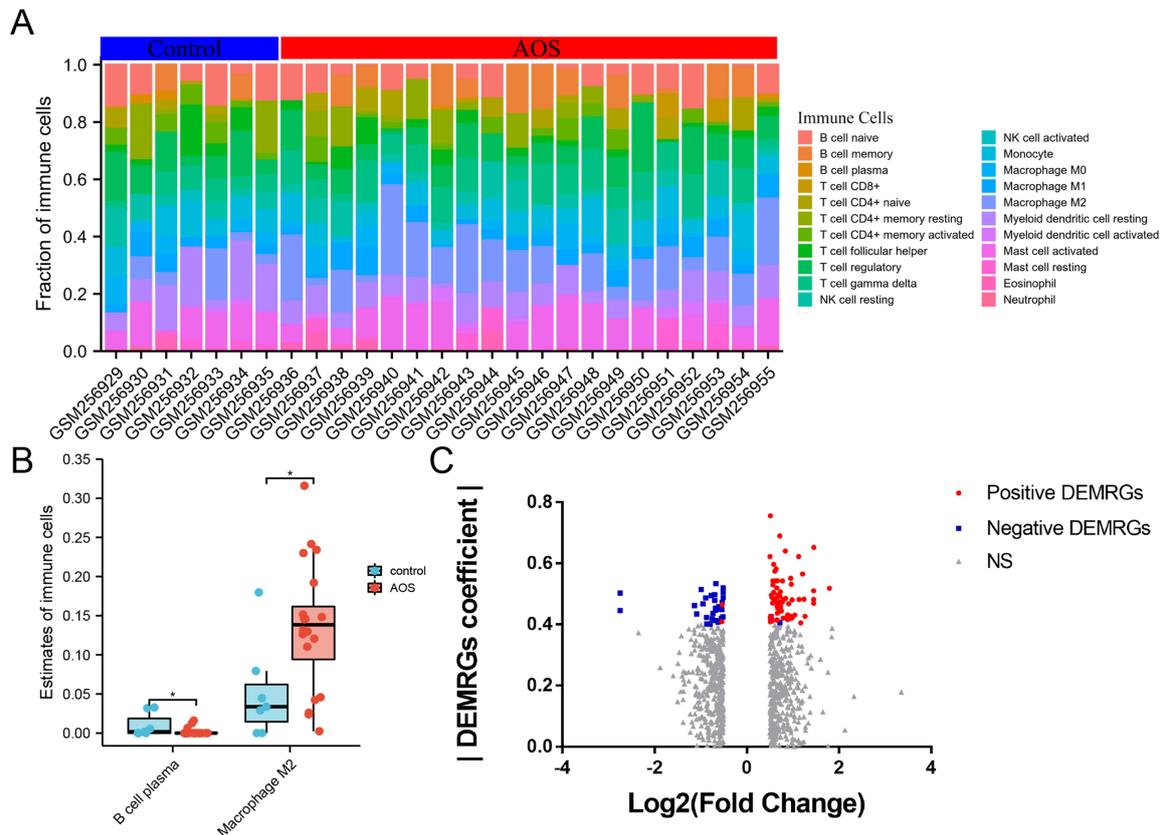
### 2.6 The Hub DEMRGs and Their Interactions

NetworkAnalyst 3.0 (<https://www.networkanalyst.ca/>) is a comprehensive network visual analyzed platform for gene expression analysis [14]. The hub DEMRGs and their interactions were analyzed using NetworkAnalyst 3.0. Specifically, transcription factors (TFs)-miRNA coregulatory interactions with 5 screened hub DEMRGs were shown using the RegNetwork repository where the literature curated regulatory interaction information was collected (Applicable for human and mouse data only). Left ventricle tissue-specific PPI were shown using the DifferentialNet database (Filter is 15), which shows the differential protein-protein interactions across human tissues. The hub DEMRGs-chemical interactions were shown using the Comparative Toxicogenomics Database (CTD). Left ventricle tissue-specific co-expression with 5 screened hub DEMRGs were shown using the TCSBN database. The hub DEMRGs-drugs interactions were shown using the DrugBank database (Version 5.0) (<https://go.drugbank.com/releases/5-0-11>). MMP2 and its edges were validated in THP-1 cells from the Immuno-Navigator database.

### 2.7 Validation of Hub Genes in Left Ventricular Remodeling Induced by Heart Failure

To view the correlation between the hub DEMRGs and left ventricular remodeling, we performed unsupervised hierarchical clustering in the GSE135055 dataset utilizing the "Pheatmap" package [15]. Then, the expression patterns of the screened DEMRGs in left ventricular remodeling process induced by heart failure were validated in three independent datasets, including GSE135055 (*n* = 30) [15], GSE116250 (*n* = 64) [16,17] and GSE74144 (*n* = 28). The detailed information for the datasets was shown in **Supplementary Table 1**.

Receiver operation characteristic (ROC) curve and joint ROC curve analysis were conducted to investigate the diagnostic performance of the DEMRGs and the area under the curve (AUC) was determined using the "pROC" package.



**Fig. 1. Identification of DEMRGs in left ventricular biopsies from control patients and aortic stenosis patients.** (A) Abundance of 22 immune cells across 27 samples estimated by the CIBERSORT algorithm. (B) Fraction of plasm B cells and M2 Macrophages between left ventricular biopsies from control patients and aortic stenosis patients. (C) Volcano plot for DEMRGs between left ventricular biopsies from control patients and aortic stenosis patients. DEMRGs, differentially expressed M2 Macrophages-related genes.

### 3. Results

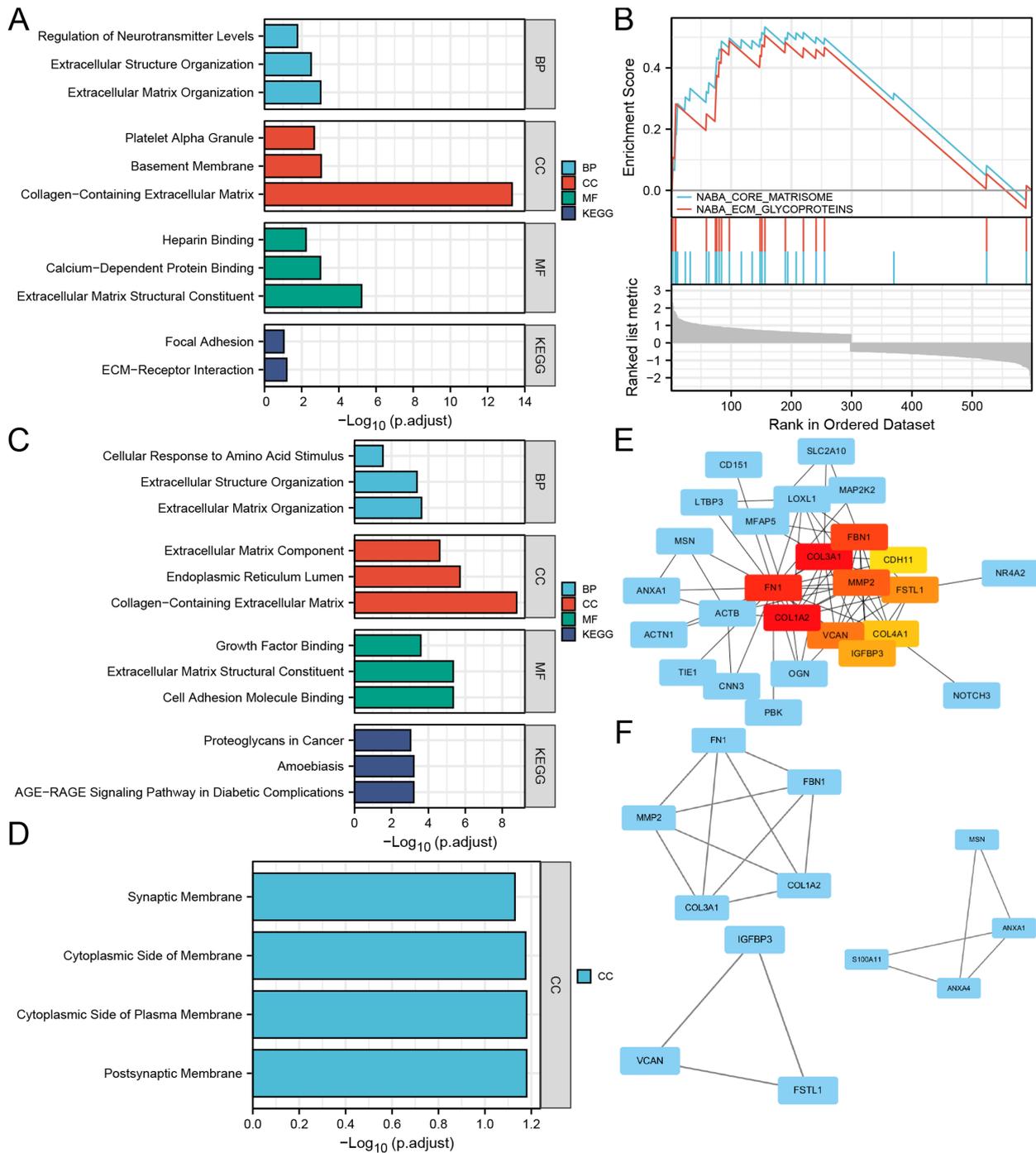
#### 3.1 DEMRGs and DEBRGs in Afterload Overload

The CIBERSORT algorithm was conducted to obtain the immune cell compositions in the GSE10161 dataset and Fig. 1A summarized the results investigated from the 7 left ventricular biopsies from control patients and 20 cardiac biopsies from AS patients. Compared with control biopsies, the biopsies from AS patients exhibited a higher infiltration of M2 Macrophages and a lower infiltration of plasm B cells (Fig. 1B; **Supplementary Fig. 1**). Utilizing the “limma” package, a total of 860 DEGs were obtained and of these DEGs, 103 were correlated ( $PCC > 0.4$ ) with the abundance of M2 Macrophages and were identified as DEMRGs (Fig. 1C), while 43 were correlated with the abundance of plasm B cells and were considered as DEBRGs (**Supplementary Fig. 2**). Compare to control biopsies, 69 DEMRGs were demonstrated to be high-expressed and 34 DEMRGs were demonstrated to be low-expressed in AS biopsies. Compare to control biopsies, 31 DEBRGs were demonstrated to be high-expressed and only 12 DEBRGs were demonstrated to be low-expressed in AS biopsies.

#### 3.2 Functional Enrichment for DEGs, DEMRGs and DEBRGs

GO and KEGG pathway analysis, as well as GSEA analysis, were performed to analyze the functions of DEGs, DEMRGs and DEBRGs. These DEGs were mainly involved in extracellular matrix organization, extracellular structure organization, collagen-containing extracellular matrix, calcium-dependent protein binding and ECM-receptor interaction. Moreover, GSEA analysis showed that NABA\_CORE\_MATRISOME and NABA\_ECM\_GLYCOPROTEINS were top2 gene sets associated with DEGs (Fig. 2A,B; Table 1).

The up-regulated DEMRGs were mainly involved in extracellular matrix organization, collagen-containing extracellular matrix, cell adhesion molecule binding and AGE-RAGE signaling pathway in diabetic complications, while the down-regulated DEMRGs were mainly involved in GO-cellular components, such as postsynaptic membrane and postsynaptic membrane (Fig. 2C,D; Tables 2,3). The up-regulated DEBRGs were mainly involved in regulation of cell growth, cell growth, external side of plasma membrane and Glycine, serine and threonine metabolism, while the down-regulated DEBRGs were mainly involved



**Fig. 2. Enrichment pathway analysis and protein-protein interaction network for the DEMRGs.** (A) The significant GO and KEGG pathways enriched by DEGs. (B) The top2 gene sets associated with DEGs using GSEA analysis. (C) The significant GO and KEGG pathways enriched by up-regulated DEMRGs compared left ventricular biopsies from aortic stenosis patients to control patients. (D) The significant GO-Cellular Component enriched by down-regulated DEMRGs. (E) Protein-protein interaction network for the DEMRGs using Cytohubba. The nodes were ranked by MCC, which were labeled successively as red, yellow and blue. (F) 3 modules were screened out using MCODE in Cytoscape.

in Retrograde endocannabinoid signaling and Chemokine signaling pathway (**Supplementary Fig. 3; Supplementary Tables 2,3**).

### 3.3 PPI Network Analysis

The PPI network of DEMRGs and DEBRGs constructed utilizing the STRING database was a scale-free

**Table 1. The significant GO and KEGG pathways enriched by DEGs.**

Gene ONTOLOGY	ID	Description	GeneRatio	BgRatio	p value	p.adjust	q value
BP	GO:0030198	extracellular matrix organization	31/555	368/18670	2.07e-07	9.52e-04	8.77e-04
BP	GO:0043062	extracellular structure organization	32/555	422/18670	1.36e-06	0.003	0.003
BP	GO:0001505	regulation of neurotransmitter levels	26/555	354/18670	2.31e-05	0.017	0.015
BP	GO:0007596	blood coagulation	25/555	336/18670	2.66e-05	0.017	0.015
BP	GO:0050849	negative regulation of calcium-mediated signaling	7/555	31/18670	2.79e-05	0.017	0.015
CC	GO:0062023	collagen-containing extracellular matrix	48/568	406/19717	8.82e-17	4.82e-14	4.19e-14
CC	GO:0005604	basement membrane	13/568	95/19717	3.30e-06	9.02e-04	7.85e-04
CC	GO:0031091	platelet alpha granule	12/568	91/19717	1.16e-05	0.002	0.002
CC	GO:0005925	focal adhesion	27/568	405/19717	5.13e-05	0.006	0.005
CC	GO:0005924	cell-substrate adherens junction	27/568	408/19717	5.82e-05	0.006	0.005
MF	GO:0005201	extracellular matrix structural constituent	22/552	163/17697	7.63e-09	5.99e-06	5.43e-06
MF	GO:0048306	calcium-dependent protein binding	11/552	61/17697	2.50e-06	9.82e-04	8.90e-04
MF	GO:0008201	heparin binding	17/552	169/17697	2.23e-05	0.006	0.005
MF	GO:0005539	glycosaminoglycan binding	20/552	229/17697	3.48e-05	0.007	0.006
MF	GO:0005178	integrin binding	14/552	132/17697	6.55e-05	0.010	0.009
KEGG	hsa04512	ECM-receptor interaction	11/283	88/8076	2.34e-04	0.064	0.059
KEGG	hsa04510	Focal adhesion	17/283	201/8076	6.68e-04	0.091	0.084

DEGs, Different Expressed Genes; GO, Gene ONTOLOGY; BP, Biological Process; CC, cellular component; MF, Molecular Function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

network and the genes with connective degrees  $\geq 10$  and ranked by MCC were regarded as hub DEMRGs or DEBRGs in the network. After screening out, 5 hub DEMRGs were obtained, including FN1 (degree = 21), COL3A1 (degree = 13), COL1A2 (degree = 13), FBN1 (degree = 12), and MMP2 (degree = 11) (Fig. 2E,F; **Supplementary Fig. 4**). All the hub genes were up-regulated in the AS biopsies. However, no hub DEBRGs were obtained in the network (**Supplementary Fig. 5**).

### 3.4 The Hub DEMRGs and Their Interactions

The hub DEMRGs and their interactions were demonstrated (Fig. 3). TFs-miRNA- hub DEMRGs coregulatory network, left ventricle tissue-specific co-expression and left ventricle tissue-specific PPI were constructed. Besides, the hub DEMRGs-chemical interactions were shown and 7 chemicals, including 4-(5-benzo(1,3)dioxol-5-yl-4-pyridin-2-yl-1H-imidazol-2-yl)benzamide, (6-(4-(2-piperidin-1-ylethoxy)phenyl))-3-pyridin-4-ylpyrazolo(1,5-a)pyrimidine, Copper, (+)-JQ1 compound, Paclitaxel, Phenylmercuric Acetate, Tretinoin and Valproic Acid, can all be interacted with 5 screened hub DEMRGs. The hub DEMRGs-drugs interactions were also shown utilizing the DrugBank database and 6 drugs seemed to have antagonistic effects on adverse left ventricular remodeling. Besides, MMP2 and its edges were validated in THP-1 cells, which can provide further evidence that MMP2 was high-expressed in M2 Macrophages for left ventricular remodeling.

### 3.5 Validation of Hub Genes in Left Ventricular Remodeling Induced by Heart Failure

Unsupervised hierarchical clustering was shown based on the expressions of hub DEMRGs in the GSE135055 dataset (Fig. 4A). Next, the DEMRGs expression patterns were validated in three independent datasets (GSE135055 (n = 30), GSE116250 (n = 64) and GSE74144 (n = 28)). In the GSE135055 dataset, MMP2, FN1, COL1A2 and COL3A1 were highly expressed and the AUC of 4 DEMRGs were 0.905 (Fig. 4B–D). Interestingly, although the AUC of FBN1 expression was 0.556, the AUC of all 5 DEMRGs was 1 (**Supplementary Fig. 6A**). In the GSE116250 dataset, MMP2, COL1A2 and COL3A1 were highly expressed in patients with dilated cardiomyopathy and MMP2, FN1, COL1A2 and COL3A1 were highly expressed in patients with ischemic cardiomyopathy. The ROC curves and joint ROC curves demonstrated the 5 hub DEMRGs can be diagnostic biomarkers for heart failure patients with dilated and ischemic cardiomyopathy (Fig. 4E–I; **Supplementary Fig. 6B,C**). In the GSE74144 dataset, FN1, FBN1, COL1A2 and COL3A1 were highly expressed in patients with hypertension and left ventricular remodeling and the AUC of the above 4 hub DEMRGs was 0.964 (Fig. 4J–L; **Supplementary Fig. 6D**). Taken together, MMP2, FN1, FBN1, COL1A2 and COL3A1 can be the prognostic biomarkers for the patients with left ventricular remodeling induced by heart failure and the kit of the 5 hub DEMRGs may test all the conditions of left ventricular remodeling events, which can help prevent the adverse left ventricular remodeling to decrease the mortality and morbidity.

**Table 2. The significant GO and KEGG pathways enriched by up-regulated DEMRGs.**

Gene ONTOLOGY	ID	Description	GeneRatio	BgRatio	p value	p.adjust	q value
BP	GO:0030198	extracellular matrix organization	10/57	368/18670	1.48e-07	2.36e-04	2.01e-04
BP	GO:0043062	extracellular structure organization	10/57	422/18670	5.23e-07	4.16e-04	3.54e-04
BP	GO:0071230	cellular response to amino acid stimulus	4/57	68/18670	5.50e-05	0.029	0.025
BP	GO:0001101	response to acid chemical	7/57	343/18670	7.98e-05	0.032	0.027
BP	GO:0046651	lymphocyte proliferation	6/57	272/18670	1.76e-04	0.046	0.039
CC	GO:0062023	collagen-containing extracellular matrix	14/58	406/19717	8.78e-12	1.65e-09	1.28e-09
CC	GO:0005788	endoplasmic reticulum lumen	10/58	309/19717	2.07e-08	1.95e-06	1.51e-06
CC	GO:0044420	extracellular matrix component	5/58	51/19717	3.91e-07	2.45e-05	1.89e-05
CC	GO:0005604	basement membrane	5/58	95/19717	8.74e-06	4.11e-04	3.18e-04
CC	GO:0098644	complex of collagen trimers	3/58	19/19717	2.26e-05	5.45e-04	4.21e-04
MF	GO:0050839	cell adhesion molecule binding	12/56	499/17697	4.00e-08	4.53e-06	3.56e-06
MF	GO:0005201	extracellular matrix structural constituent	8/56	163/17697	4.25e-08	4.53e-06	3.56e-06
MF	GO:0019838	growth factor binding	6/56	137/17697	4.56e-06	2.59e-04	2.04e-04
MF	GO:0048407	platelet-derived growth factor binding	3/56	11/17697	4.86e-06	2.59e-04	2.04e-04
MF	GO:0098641	cadherin binding involved in cell-cell adhesion	3/56	19/17697	2.81e-05	0.001	9.39e-04
KEGG	hsa04933	AGE-RAGE signaling pathway in diabetic complications	5/24	100/8076	9.28e-06	6.19e-04	5.22e-04
KEGG	hsa05146	Amoebiasis	5/24	102/8076	1.02e-05	6.19e-04	5.22e-04
KEGG	hsa05205	Proteoglycans in cancer	6/24	205/8076	2.28e-05	9.21e-04	7.77e-04
KEGG	hsa04926	Relaxin signaling pathway	5/24	129/8076	3.20e-05	9.69e-04	8.18e-04
KEGG	hsa04510	Focal adhesion	5/24	201/8076	2.63e-04	0.006	0.005

DEGs, Different Expressed Macrophage(M2)-Related Genes; GO, Gene ONTOLOGY; BP, Biological Process; CC, cellular component; MF, Molecular Function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

**Table 3. The significant GO and KEGG pathways enriched by down-regulated DEMRGs.**

Gene ONTOLOGY	ID	Description	GeneRatio	BgRatio	p value	p.adjust	q value
CC	GO:0045211	postsynaptic membrane	4/27	323/19717	9.21e-04	0.066	0.055
CC	GO:0009898	cytoplasmic side of plasma membrane	3/27	154/19717	0.001	0.066	0.055
CC	GO:0098562	cytoplasmic side of membrane	3/27	178/19717	0.002	0.067	0.056
CC	GO:0097060	synaptic membrane	4/27	432/19717	0.003	0.074	0.062

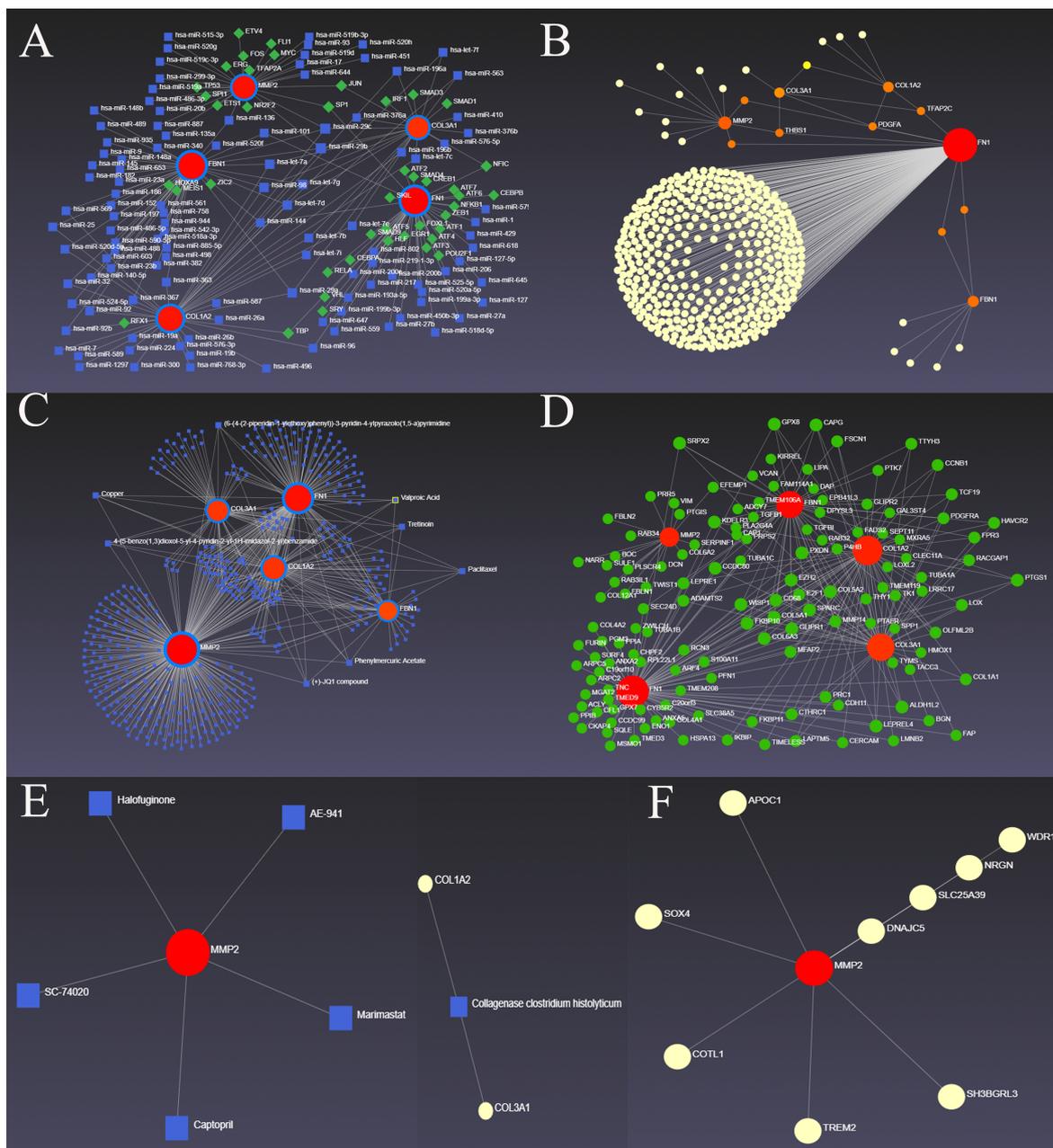
DEGs, Different Expressed Macrophage(M2)-Related Genes; GO, Gene ONTOLOGY; CC, cellular component.

#### 4. Discussion

Heart failure contributes to increased morbidity and mortality and affects >24 million patients worldwide [18]. Near half of the patients with heart failure die of cardiac sudden death due to adverse left ventricular remodeling and ventricular arrhythmia. So, prevention of adverse left ventricular remodeling and cardiac dysfunction can improve the prognosis of patients with heart failure [19,20]. Previous studies have reported that MI promotes the release of progenitor cells and hematopoietic stem cells from the bone marrow niche. These progenitor cells subsequently colonize the spleen, thereby increasing the number of monocytes in the blood and macrophage in the myocardium and arteries, which further promotes atherosclerosis and thus myocardial infarction progression [21,22].

In the event of acute inflammation, a large number of immune cells, especially monocytes, are recruited to the site

of inflammation to repair the tissue to eventually restore the tissue to homeostasis [23,24]. The monocytes differentiated into M1-type macrophages and combined with resident M1 macrophages to remove foreign bodies or necrotic tissues. In the fiber repair stage of the disease course, M1-type macrophages have been gradually replaced by M2-type macrophages, which play a role in promoting tissue repair [25,26]. Besides, a network of macrophages can actively take up material, including mitochondria, derived from cardiomyocytes and support mitochondrial homeostasis in the heart [27]. Macrophages can also facilitate electrical conduction in the physiological and pathological heart through gap junction [28–30]. However, the specific mechanism of the differentiation of M1-type macrophages to M2-type macrophages and the role of M2-type macrophages has not been fully clarified and needs further studies. In this study, GSE10161 was downloaded and immune cells analysis was

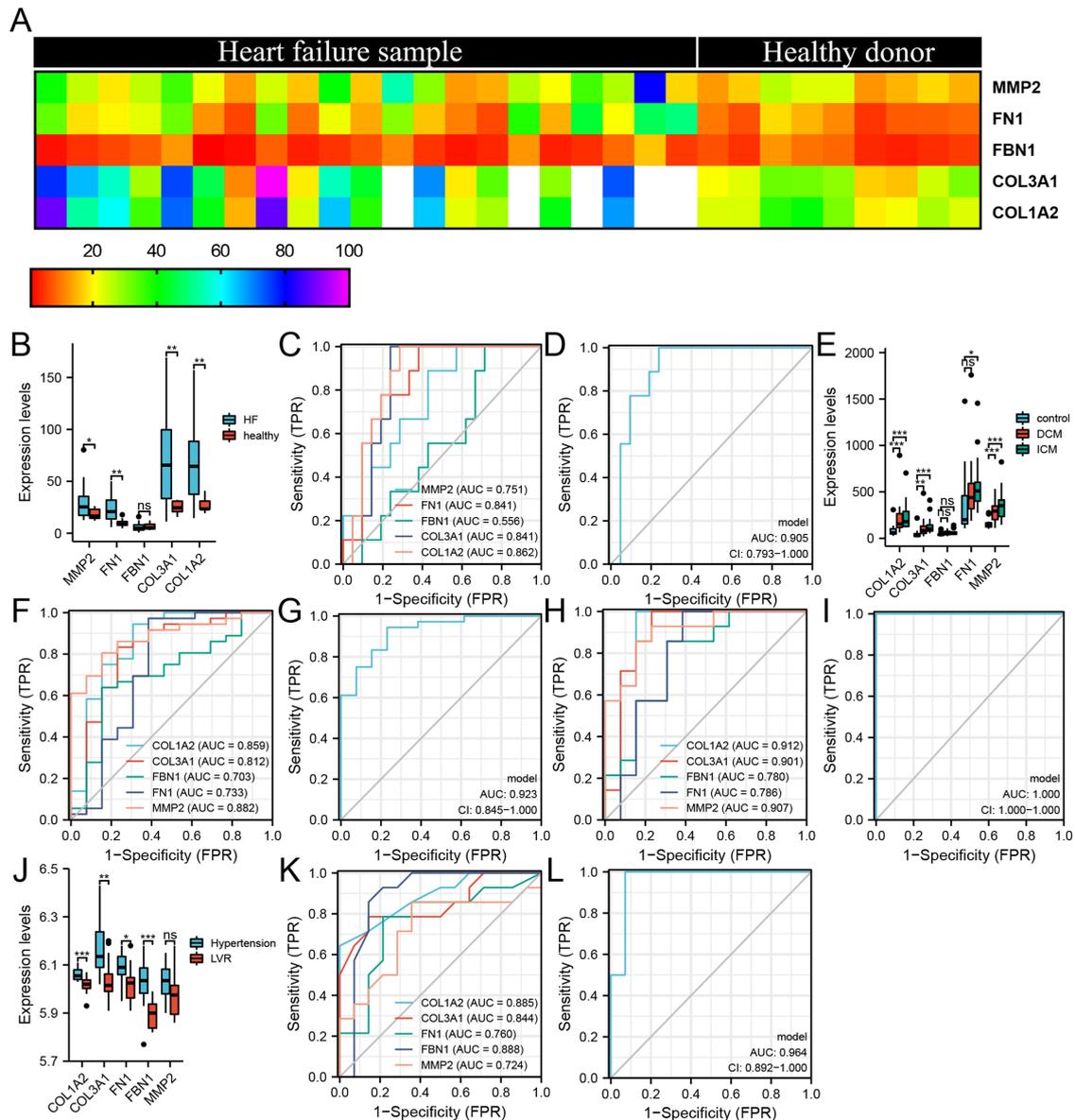


**Fig. 3. The hub DEMRGs and their interactions.** (A) TF-miRNA coregulatory interactions with 5 screened hub DEMRGs. (B) Left ventricle tissue-specific PPI, including 5 screened hub DEMRGs and their edges. (C) The hub DEMRGs-chemical interactions. (D) Left ventricle tissue-specific co-expression with 5 screened hub DEMRGs. (E) The hub DEMRGs-drugs interactions. (F) MMP2 and its edges validated in THP-1 cells.

carried out to screen out the hub genes associated with immune cells. DEMRGs and DEBRGs were screened out and after further GO/KEGG enrichment analysis and PPI analysis, 5 hub DEMRGs was investigated, which may be the critical hub genes to promote left ventricular remodeling induced by heart failure. Then the expression levels of the hub genes in heart failure, dilated cardiomyopathy, ischemic cardiomyopathy and hypertension patients were demonstrated to validate the correlation to left ventricular remodeling, which will help to diagnose the adverse left

ventricular remodeling at an early stage and reduce the patients' mortality.

5 hub DEMRGs, including MMP2, FN1, FBN1, COL3A1 and COL1A2, were investigated to be associated with M2 Macrophages phenotype. Matrix metalloproteinase 2 (MMP2), as a fibrosis-related gene, affected extracellular matrix remodeling after an ischemic myocardial injury and can be the detection of gelatinase expression after MI [31,32]. Treatment with verapamil can decline calpain-1 and MMP-2 activities and ameliorate cardiac hypertro-



**Fig. 4. Expression patterns of hub DEMRGs in left ventricular remodeling induced by heart failure.** (A) The heatmap for unsupervised hierarchical clustering of the five hub DEMRGs in GSE135055 dataset. (B) Expression levels of the hub DEMRGs in GSE135055 dataset. (C–D) ROC curve of 5 hub DEMRGs (C) and joint ROC curve of MMP2, FN1, COL1A2 and COL3A1 (D) in GSE135055 dataset. (E) Expression levels of the hub DEMRGs in GSE116250 dataset. (F–G) ROC curve of 5 hub DEMRGs (F) and joint ROC curve of MMP2, COL1A2 and COL3A1 (G) in DCM. (H–I) ROC curve of 5 hub DEMRGs (H) and joint ROC curve of MMP2, FN1, COL1A2 and COL3A1 (I) in ICM. (J) Expression levels of the hub DEMRGs in GSE74144 dataset. (K–L) ROC curve of 5 hub DEMRGs (K) and joint ROC curve of FN1, FBN1, COL1A2 and COL3A1 (L) in GSE74144 dataset. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; ns, not significant.

phy [33]. MMP-2/JNK apoptotic pathway was activated in the border zone after MI in adult sheep [34]. Transverse aortic constriction in mice model induced cardiac hypertrophy, collagen deposition, and the expression of transforming growth factor (TGF)- $\beta$  and MMP-2 in the left ventricle [35]. Besides, MMP2 was highly expressed in the left and right ventricles of the patients with hypoplastic left heart through single-cell RNA sequencing [36]. Fibronectin 1

(FN1) is a left ventricular extracellular matrix remodeling-related gene, and FN1, COL1A1 (Collagen Type I Alpha 1 Chain) and COL3A1 (Collagen Type III Alpha 1 Chain) can be inhibited by transient angiotensin-converting enzyme (ACE) utilizing single-cell RNA sequencing to determine the hypothesis that transient ACE inhibitor treatment can induce a persistent shift in cardiac fibroblast subpopulations [37]. Interestingly, FN1 and COL3A1 expressions differed

from sex but were not regulated by angiotensin II receptor blocker [38]. Besides, Hydrogen-containing saline treatment can decrease the phosphorylation of p38 MAPK and Smad2/3, and Col I and FN1 mRNA levels, which can alleviate pressure overload-induced cardiac dysfunction [39]. Fibrillin-1 (FBN1) is the critical gene in the pathogenesis of Marfan syndrome (MFS) and the [Fbn1C1039G/+] mouse model replicates a large number of the anomalies of MFS patients, for instance, central aortic stiffness, systolic and diastolic dysfunction [40–42]. Besides, Fbn1, Itga8, Itga11, Itgb5 and Thbs2 were the hub genes applying weighted gene co-expression network analysis (WGCNA) and PPI analysis in the GSE77798 dataset, a dataset of patients with hypoplastic left heart syndrome (HLHS) [43]. COL1A2 (Collagen Type I Alpha 2 Chain) and COL3A1 are the expressions of fibrosis indicators and the expressions increased in overexpressing a constitutively active form of the calcium-dependent phosphatase calcineurin A mice model while connexin43 expression decreased at the same time, which led to abnormal conduction and arrhythmias, similar to the results in cardiac remodeling in the patients with heart failure [44,45]. The crosstalk between macrophage and the fibroblast-like cell was investigated in both left and right atriums through the ligand-receptor interactions of COL1A1/COL1A2-CD36 [46]. Besides, COL1A1 and COL1A2 were the diagnostic biomarkers of Osteogenesis imperfecta, which may also be involved in the crosstalk between bone marrow niche and left ventricle after MI [47]. The expression of COL3A1, an extracellular matrix protein (ECM) gene, increased during the postpartum period [48]. COL3A1 as well as COL1A1, MMP9, elastin and TIMP1 expressions also increased after treatment with TGF- $\beta$ 1 to fetal cardiac fibroblasts [49]. Besides, COL3A1 expression can be elevated through the angiotensin II-ROS-HuR-TGF $\beta$  pathway in hypertensive heart disease and be inhibited by miR-29a and miR-101a after MI [50,51]. In this study, MMP2, FN1, COL1A2 and COL3A1 were highly expressed in patients with heart failure in the GSE135055 dataset. MMP2, COL1A2 and COL3A1 were highly expressed in patients with dilated cardiomyopathy and MMP2, FN1, COL1A2 and COL3A1 were highly expressed in patients with ischemic cardiomyopathy in the GSE116250 dataset. Besides, FN1, FBN1, COL1A2 and COL3A1 were highly expressed in patients with hypertension and left ventricular remodeling in the GSE74144 dataset. Taken together, MMP2, FN1, FBN1, COL1A2 and COL3A1 can be the prognostic biomarkers for the patients with left ventricular remodeling induced by heart failure and the kit of the 5 hub DEMRGs may test left ventricular remodeling events and help prevent the adverse left ventricular remodeling to decrease the mortality and morbidity.

There are some limitations which should be mentioned. Firstly, only hub DEMRGs were validated in patients with heart failure, dilated cardiomyopathy, ischemic

cardiomyopathy or hypertension and left ventricular remodeling. There may be some false negatives because of the enrichment methods and validation methods. More researches are still needed to proceed with other DEGs, such as DEBRGs and neighbor DEMRGs. Secondly, the sample sizes of included datasets were not too large, however, after validation, the results are highly reliable. Lastly, further researches are still needed to confirm the functional effects of the screened hub genes in human being to improve the prognosis and decrease the adverse left ventricular remodeling mortality.

## 5. Conclusions

Based on our current study, our research provided a bioinformatics analysis for the patients with AS. The screened hub DEMRGs, MMP2, FN1, FBN1, COL1A2 and COL3A1, may be therapeutic targets for treatment in patients with heart failure and prevention the heart failure induced dilated cardiomyopathy, ischemic cardiomyopathy or hypertension. MMP2, FN1, FBN1, COL1A2 and COL3A1 expressions increased due to the adverse left ventricular remodeling and the kit of the 5 hub DEMRGs may test left ventricular remodeling events and help prevent the adverse left ventricular remodeling to decrease the mortality and morbidity.

## Abbreviations

CAD, Coronary artery disease; MI, Myocardial infarction; HF, Heart failure; LVM, Left ventricular mass index; GEO, Gene Expression Omnibus; AS, aortic stenosis; PCC, Pearson correlation coefficients; DEGs, Differentially expressed genes; DEMRGs, Differentially expressed M2 Macrophages-related genes; DEBRGs, differentially expressed B cells-related genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene Set Enrichment Analysis; PPI, Protein-protein internet; TF, Transcription factor; CTD, Comparative Toxicogenomics Database; ROC, Receiver operation characteristic; AUC, Area under curve; WGCNA, Weighted gene co-expression network analysis; ECM, Extracellular matrix protein; MMP2, Matrix metalloproteinase 2; TGF- $\beta$ , Transforming growth factor- $\beta$ ; FN1, Fibronectin 1; FBN1, Fibrillin 1; COL1A1, Collagen type III alpha 1 chain; COL3A1, Collagen type III alpha 1 chain; COL1A2, Collagen type I alpha 2 chain; ACE, Angiotensin-converting enzyme; MFS, Marfan syndrome.

## Author Contributions

Conceptualization, YZ; Funding acquisition, WQG, YHL and TL; Investigation, YZ, WQG, BCQ, ZCQ and XMH; Visualization, YZ and YHL; Writing – original draft, YZ; Writing – review & editing, YZ, XMH and TL.

## Ethics Approval and Consent to Participate

Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://www.imrpress.com/journal/RCM/23/3/10.31083/j.rcm2303109>.

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