

Unveiling the Hypoxia-Driven Molecular Mechanisms in Ischemic Heart Failure Based on Bioinformatics, Machine Learning and Bayesian Networks

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Abstract

Hypoxia is a critical determinant in the etiology and progression of ischemic heart failure, but its underlying molecular mechanisms and regulatory interactions remain largely enigmatic. In our study, we employed differential analysis, coupled with WGCNA and the MSigDB database, to identify nine genes associated with hypoxia in heart failure. GO and KEGG enrichment analyses indicated that these genes are predominantly involved in hypoxia, immune responses, inflammation, apoptosis, and aging processes. Employing Bayesian networks, we elucidated four regulatory relationships among the hypoxia genes: PIM1-CDKN1A, IL6-CDKN1A, PLIN2-ANGPTL4, and SERPINE1-PLAUR. We amalgamated 12 machine learning algorithms, comprising 113 distinct combinations, to refine our findings and identified four pivotal hypoxia genes: SLC2A1, PLIN2, FOSL2, and PIM1. The SHAP analysis was instrumental in interpreting the predictive outcomes of the optimal model, the Random Forest algorithm, SLC2A1 was the most influential gene in the model. Immune infiltration analysis revealed the presence of seven types of dysregulated immune cells within the failing myocardium. Colocalization analysis suggested that hypoxia genes and heart failure are not likely to be independently influenced by the same genetic factors, although the potential for intermediary factors or shared biological pathways was not excluded. Overall, our research offers significant insights into the molecular mechanisms of hypoxia genes in the pathophysiology of heart failure, paving the way for the development of targeted and immunomodulatory therapies.

INTRODUCTION

Heart failure (HF) represents a significant global health concern, affecting millions worldwide. It is estimated that over 26 million individuals suffer from HF globally, and this number continues to rise due to an aging population and the prevalence of risk factors such as hypertension, diabetes, and obesity [1]. Despite advancements in medical therapies and interventions, HF remains a leading cause of morbidity and mortality, characterized by high rates of hospital readmissions and poor prognosis [2]. Current treatment strategies, although effective in symptom management and quality of life improvement, often fail to markedly alter disease progression or reduce mortality rates [3]. Furthermore, with advancements in interventional techniques and pharmacotherapy, the survival rate of patients with ischemic heart disease and acute myocardial infarction has improved, consequently increasing the number of individuals with ischemic heart failure [4]. The immense public health burden compels us to seek effective targeted therapies and novel pathological mechanisms for ischemic heart failure.

The heart is a high-energy-consuming organ, highly sensitive to oxygen and energy supply. In heart failure, reduced cardiac pumping function leads to inadequate tissue perfusion and circulatory congestion. Consequently, hypoxia is one of the primary features of ischemic heart failure. Under hypoxic conditions, alterations in nitric oxide (NO) production, energy metabolism, and reactive oxygen species (ROS) levels contribute to cardiac dysfunction [5]. During heart failure, mitochondrial function is impaired, resulting in decreased oxidative capacity. This impairment can shift metabolism from oxidative phosphorylation back to glycolysis, which, while suitable under low oxygen conditions, is less efficient

[6]. Hypoxia-induced inflammatory responses can lead to myocardial cell degeneration and necrosis, potentially promoting myocardial fibrosis, which is a critical factor in ventricular remodeling [7]. Hypoxia-inducible factors (HIFs) are key transcription factors in hypoxic environments, playing a crucial role in cellular responses to low oxygen levels. In heart failure, HIFs help counteract hypoxic conditions by promoting glycolysis and angiogenesis in myocardial cells, thereby playing a significant role in myocardial injury and repair processes [8]. Furthermore, pathological hypoxia can disrupt tissue function and disease progression through immune cell dysregulation, with the impact of hypoxia on immunity and inflammation varying depending on the microenvironment and immune processes occurring within specific niches [9]. Given that hypoxia is now recognized as a fundamental driver of inflammatory and immune responses, the heterogeneous landscape of immune cells in failing myocardium under hypoxic conditions warrants further investigation [10].

In this study, we aim to investigate the role of hypoxia-related genes in ischemic heart failure and their impact on immune cell infiltration. We identified hypoxia-related genes and their associated biological processes in heart failure through differential gene expression analysis, weighted gene co-expression network analysis (WGCNA), and functional enrichment analysis. By constructing a Bayesian regulatory network, we elucidated the regulatory relationships of these hypoxia genes. Utilizing a combination of 113 algorithmic approaches across 12 machine learning algorithms and validating them in multiple external datasets, we identified key hypoxia genes associated with heart failure and employed SHAP values to interpret model predictions. Immune cell infiltration in failing myocardium was assessed using the CIBERSORT package, and a heatmap depicting the correlation between key hypoxia genes and immune cells was generated. Our analysis reveals significant associations between these genes and immune cell infiltration, suggesting that hypoxia may modulate immune responses in heart failure. Gene co-localization analysis further indicates that some hypoxia-related genes share genetic variations with heart failure, supporting their potential role in the disease. The flowchart of our study is illustrated in Fig. 1.

MATERIALS AND METHODS

Data Acquisition and Preprocessing

The data for this study were obtained from the public GEO database. The data set obtained the consent and ethical approval of the relevant participants, so this study did not require ethical approval from the institutional review board [11]. The heart failure data set we selected was GSE57338, which contains left ventricular myocardial samples from 95 patients with ischemic heart failure and 136 normal people [12]. We randomly selected 70% of the samples as the training set and the remaining 30% of the samples as the test set. The external data sets we selected were GSE5406, GSE16499, and GSE161472 [13–15]. We used R software (R version 4.4.0) to complete the data preprocessing. We removed probes corresponding to multiple molecules. For multiple probes corresponding to the same molecule, only the probe with the largest signal value was retained. Finally, we removed the batch effect of the data and converted the probe ID into a gene symbol according to the annotation file of the platform. The filtered

data will use the ComBat function of the sva package to remove the batch effect [16]. We used the LIMMA package for differential analysis. We selected genes with $p\text{-value} < 0.05$ and $|\log_2(\text{FC})| \geq 0.5$ as differentially expressed genes [17].

Weighted Gene Co-expression Network Analysis

We employed the WGCNA package to investigate gene modules associated with heart failure [18]. Initially, a filter criterion of 0.5 was applied to exclude genes with minimal variation in expression levels. Subsequently, sample identification and clustering were performed using the gsub function in conjunction with hclust and the dist function to differentiate samples and detect outliers, with the abline function employed to add a threshold line. The selection of the soft threshold was achieved through the pickSoftThreshold function, aimed at identifying the optimal power parameter that satisfies the scale-free topology model fit. A subsequent step involved the construction of an adjacency matrix and a TOM similarity matrix, laying the groundwork for gene clustering. Dynamic cutting of modules was executed using the cutreeDynamic function, with a minimum module size set at 60 to form gene modules. The correlation analysis between modules and phenotypes was completed by calculating the correlation between MEs and phenotype data, followed by a significance test. We selected the module with the highest correlation as the characteristic gene module for heart failure.

Screening Candidate Hypoxia Genes in HF

The Molecular Signatures Database (MSigDB) serves as a repository for tens of thousands of annotated gene sets for the GSEA software, where hallmark gene sets encapsulate and represent specific well-defined biological states or processes, exhibiting consistent expression patterns [19]. We selected the "Human Gene Set: HALLMARK_HYPOXIA," from which we derived 200 hypoxia-related genes [20].

Functional Enrichment Analysis and Bayesian Network

To explore the biological processes and functions executed by hypoxia genes in the progression of heart failure, we utilized the clusterProfiler package to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses and visualizations [21–23]. To ascertain the regulatory relationships among genes under hypoxic conditions, we employed the CBNplot package to conduct Reactome pathway enrichment analysis and to plot the Bayesian regulatory network of hypoxia genes [24].

Screening Key Hypoxia Genes by Machine Learning

We integrated a suite of 12 machine learning algorithms, including Elastic Net, Lasso Regression, Ridge Regression, Stepwise Logistic Regression, Support Vector Machine, Linear Discriminant Analysis, Generalized Linear Model Boosting, Partial Least Squares Regression with Generalized Linear Model, Random Forest, Gradient Boosting Machine, Extreme Gradient Boosting, and Naive Bayes, to identify key hypoxia genes in heart failure [25]. Initially, we utilized the intersect function to identify genes common to both the training and testing sets, based on which we constructed the data matrix. Subsequently,

through parameter tuning, custom parameter combinations, and cross-validation, we derived 113 distinct machine learning algorithm configurations. We identified the optimal model by comparing the average AUC of different models. Ultimately, we ascertained the key hypoxia-related genes in heart failure through the optimal model.

Interpreting Machine Learning Models Using SHAP

SHAP (SHapley Additive exPlanations) is a powerful tool for interpreting machine learning model predictions, based on the Shapley values from game theory, which quantify the contribution of each feature to the model's prediction outcome [26]. We employed the SHAP method to elucidate the predictions of the optimal model. Utilizing the kernelshap function, we calculated the SHAP values for the test data, indicating the contribution of each gene to the model's results. With the shapviz library, we generated a beehive plot and a SHAP value waterfall chart to represent the importance of the genes.

Immune Infiltration Analysis

Hypoxia can lead to immune cell dysregulation. We utilized the CIBERSORT package to assess the content of immune and stromal cells in myocardial samples, thereby delineating the cellular heterogeneity landscape of the myocardial expression profile and completing the analysis of immune cell infiltration [27]. Bar charts were employed to visualize the proportion of each type of immune cell across different samples. The Wilcoxon rank-sum test was applied to compare the distribution differences of cells between the heart failure (HF) and normal groups, with a cutoff value set at $p < 0.05$. Using Spearman's correlation analysis, we evaluated the interrelationships among immune cells and recorded the correlation coefficients. Finally, we employed the linkET package to plot the correlation heatmap between key hypoxia genes and immune cells.

Co-localization Analysis

We conducted Bayesian colocalization analysis using the coloc package, employing a Bayesian probability model to estimate whether hypoxia genes and heart failure are independently influenced by the same genetic factors [28]. Within the coloc.abf algorithm, we calculated the posterior probabilities of five mutually exclusive hypotheses (PPH0–PPH4). When the PPH4 exceeds 0.8, this indicates a connection between the protein and heart failure through shared genetic variants in that region. If the PPH4 is less than 0.8, it suggests that the gene and heart failure may not be interrelated through the same genetic mechanisms. Of course, heart failure is a complex multifactorial disease that likely involves the interplay of multiple genes and environmental factors. Even if certain genetic loci are significantly associated with the disease, it is not straightforward to infer a direct causal relationship.

RESULTS

Screening Hypoxia Genes of HF by DEGs and WGCNA

After completing data preprocessing, we utilized the LIMMA package for differential analysis, identifying a total of 566 differentially expressed genes. Through WGCNA, we obtained six gene co-expression modules, among which the turquoise module showed the highest correlation with heart failure (correlation coefficient = 0.5, $p = 2e-11$), encompassing 522 genes. Consequently, we selected the turquoise module as the key module for subsequent analysis. By intersecting the differentially expressed genes, genes from the turquoise module, and Hypoxia-related Genes, we identified nine hypoxia genes closely associated with heart failure. The results are depicted in Fig. 2.

Functional Enrichment Analysis and Bayesian Network

We conducted GO and KEGG enrichment analyses on the nine hypoxia genes to explore the biological processes and functions affected by hypoxia in heart failure, using a p -adjust threshold of less than 0.05 for selection. The GO analysis yielded 196 biological processes (BP) and one molecular function (MF), with no statistically significant cellular components (CC) identified. Among the BPs, the most prominent were regulation of epithelial cell apoptotic process, epithelial cell apoptotic process, positive regulation of homotypic cell-cell adhesion, replicative senescence, and wound healing. The MF was related to enzyme inhibitor activity. KEGG enrichment analysis indicated that these nine hypoxia genes are primarily involved in pathways such as HIF-1 signaling pathway, AGE-RAGE signaling pathway in diabetic complications, Cellular senescence, JAK-STAT signaling pathway, and Human T-cell leukemia virus 1 infection, totaling 11 pathways. Reactome pathway enrichment analysis suggested that STAT5 activation downstream of FLT3 ITD mutants, Dissolution of Fibrin Clot, Interleukin-4 and Interleukin-13 signaling, Signaling by FLT3 ITD and TKD mutants, and Signaling by FLT3 fusion proteins may be key pathways involved with these nine hypoxia genes. The Bayesian regulatory network indicated that PIM1 and IL6 can regulate CDKN1A, PLIN2 can regulate ANGPTL4, and SERPINE1 can regulate PLAUR. The results are depicted in Fig. 3.

Identification of Key Genes via Machine Learning

We subjected the nine hypoxia genes to computation using 12 machine learning algorithms and 113 combinations on the GSE57338 test set, followed by validation on an internal validation set and three external validation sets to derive the optimal predictive model. The results indicated that the Random Forest (RF) algorithm demonstrated the highest accuracy, with an AUC greater than 0.8 for each gene set and an average AUC of 0.873. Utilizing the RF model, we identified four key hypoxia-related genes associated with heart failure, namely SLC2A1, PLIN2, FOSL2, and PIM1. The results are illustrated in Fig. 4.

Interpreting Random Forest Model

We quantified the contribution of each gene to the RF model's predictive outcomes using SHAP. The results revealed that SLC2A1 had the highest contribution to the RF model, with a SHAP value of 0.144. Among the five genes excluded from the model, PLAUR had a SHAP value of 0.0269, and the combined SHAP values of the remaining four genes totaled 0.0854. Overall, the SHAP values indicated that

SLC2A1, PLIN2, FOSL2, and PIM1 are the most critical genes in the RF model's predictions. The findings are presented in Fig. 5.

Immune Cell Infiltration Analysis

We performed an immune infiltration analysis on the training set of myocardial samples using the CIBERSORT algorithm. Bar charts clearly displayed the content of different subpopulations in each sample. We assessed the heterogeneity of cellular composition between heart failure samples and healthy samples. The results indicated significant differences in the infiltration of seven types of immune cells. Specifically, Plasma cells, T cells CD4 naive, Macrophages M0, and Mast cells resting were expressed at higher levels in heart failure samples compared to healthy samples. Conversely, T cells regulatory, Macrophages M2, and Neutrophils were expressed at lower levels in heart failure samples. The correlation heatmap of immune cells revealed that Tregs had a high negative correlation with T cells CD4 memory resting, and Macrophages M0 had a high negative correlation with Macrophages M2, suggesting potential interplay and coordination among these immune cells. The correlation heatmap between key hypoxia genes and immune cells showed that SLC2A1 was positively correlated with Monocytes. PLIN2 was positively correlated with Macrophages M2. FOSL2 was negatively correlated with Mast cells resting and Tregs. PIM1 was negatively correlated with Mast cells resting and positively correlated with B cells naive. This suggests that the immune cell dysregulation in failing myocardium may be closely related to hypoxia. The results are depicted in Fig. 6.

Co-localization Analysis

Colocalization analysis indicates that although the rs4665420 locus is significantly associated with both FOSL2 expression and heart failure, the H4 value is less than 0.8, suggesting that the expression of FOSL2 and heart failure are unlikely to be independently influenced by the same genetic factors. Similarly, the rs12197850 locus is significantly associated with PIM1 and heart failure, but the H4 value is also less than 0.8. PLIN2 and SLC2A1 are also significantly associated with SNPs that are significantly related to heart failure, but the H4 values are both less than 0.8. Overall, these four hypoxia genes and heart failure are unlikely to be independently influenced by the same genetic factors. However, it should be noted that these SNP loci may affect the risk of heart failure by influencing the expression of hypoxia genes, but this effect may not be direct and may involve other intermediary factors or biological pathways. Therefore, further research is needed to fully elucidate their interactions and potential biological mechanisms. The colocalization analysis results are shown in Fig. 7.

DISCUSSION

With the increasing survival rates from ischemic heart disease and the growing prevalence of an aging population, heart failure has become a significant public health burden [29]. Heart failure (HF) is a severe clinical syndrome characterized by the heart's inability to pump sufficient blood to meet the body's demands. Consequently, patients with heart failure are perpetually in a state of oxygen deficiency and impaired exercise tolerance. The vicious cycle of heart failure and hypoxia can lead to the continuous

deterioration of the patient's condition, making it crucial to uncover the underlying molecular mechanisms and therapeutic targets.

In this study, we explored the biological processes and functions involved in ischemic heart failure by hypoxia genes through three enrichment analyses. The results indicated that these hypoxia genes are primarily associated with processes such as hypoxia, immunity, inflammation, apoptosis, and senescence. The HIF-1 signaling pathway is a key regulator of the cellular response to hypoxia, crucial for cellular adaptation to low-oxygen conditions. Hypoxia-inducible factor 1 (HIF-1) is a transcription factor that regulates the expression of various genes involved in angiogenesis, erythropoiesis, and metabolism under hypoxic conditions. Studies have shown that HIF-1 α is significantly upregulated in heart failure, highlighting its role in promoting glycolysis and angiogenesis under hypoxic conditions [30]. This upregulation is part of a broader metabolic shift observed in the failing heart, where energy metabolism shifts from fatty acid oxidation to glycolysis [31]. Activation of HIF-1 α has been shown to improve ventricular function in rats and remodel the state post-myocardial infarction, protecting the failing heart [32]. However, persistent activation of the HIF-1 α signaling pathway can increase oxidative stress, inflammation, and fibrosis, impairing cardiac function. This damage can be mitigated by HIF-2 α , indicating that the balance between these subtypes is essential for cardiac health [33].

In this study, we identified key hypoxia genes in heart failure by integrating 113 algorithmic combinations of 12 machine learning algorithms and fitting them across multiple external datasets. The optimal predictive model was the Random Forest (RF) model, which yielded four critical genes: SLC2A1, PLIN2, FOSL2, and PIM1. The average AUC value of the RF model was 0.873, indicating that these four genes can serve as characteristic genes for ischemic heart failure. While the Random Forest algorithm effectively prevents overfitting, its drawback is the difficulty in interpreting the results. We utilized SHAP to quantify the contribution of each gene to the RF model's predictive outcomes, assisting us in gaining a better understanding of the model's predictions.

SLC2A1, also known as GLUT1, is a glucose transporter that plays a crucial role in cellular glucose uptake. Studies have shown that the expression of SLC2A1 significantly increases under hypoxic conditions to enhance glucose uptake, thereby meeting the energy demands of cardiomyocytes [34]. Additionally, SLC2A1 is involved in regulating intracellular oxidative stress responses, mitigating the damage to cardiomyocytes under hypoxia by increasing the expression of antioxidant enzymes [35]. Under hypoxic conditions, HIF-1 α binds with its co-activator HIF-1 β to form an active complex, which in turn promotes the transcription of the SLC2A1 gene [36]. Oxidative stress responses under hypoxia can also enhance the expression of SLC2A1 through the activation of specific signaling pathways, such as the PI3K/Akt pathway [37]. Cardiomyocytes primarily rely on anaerobic glycolysis to obtain energy under hypoxic conditions, and the high expression of SLC2A1 ensures an adequate supply of glucose [38]. Moreover, the increased expression of SLC2A1 can promote the breakdown and utilization of lipids, thereby reducing the accumulation of lipids in cardiomyocytes and preventing lipid toxicity-induced damage [39]. Therefore, SLC2A1 not only plays a vital role in glucose metabolism but also protects cardiomyocytes from lipid toxicity by regulating lipid metabolism.

PLIN2 (Perilipin 2) is a key protein on the surface of lipid droplets, primarily involved in the regulation of lipid metabolism. Lipid droplets are the main structures for intracellular lipid storage, and PLIN2 plays an essential role in the formation and stabilization of these droplets. PLIN2 is also closely related to the metabolism of fatty acids, as it can regulate the release and utilization of fatty acids by interacting with lipases [40]. Studies have found that the expression level of PLIN2 in cardiomyocytes of patients with heart failure is significantly increased, which may be associated with an increase in lipid accumulation within the cardiomyocytes [41]. This lipid accumulation not only affects the normal function of cardiomyocytes but can also lead to apoptosis and myocardial fibrosis, thereby exacerbating the condition of heart failure [42]. Research suggests that the overexpression of PLIN2 in cardiomyocytes of patients with heart failure may lead to lipid metabolic disorders, which in turn affect the energy supply and function of cardiomyocytes [43]. Therefore, PLIN2 is not only a crucial regulator of lipid metabolism, but changes in its expression levels may also serve as an important indicator for assessing cardiac function and the severity of heart failure.

FOSL2 (Fos-like antigen 2) is a member of the AP-1 family of transcription factors, participating in a variety of biological processes, including cell proliferation, differentiation, and apoptosis [44]. In heart failure, the transcriptional regulatory role of FOSL2 is particularly significant. Studies have shown that FOSL2 can promote angiogenesis and the survival of cardiomyocytes by regulating the expression of Wnt5a [45]. Hypoxia can activate the expression of FOSL2 through the HIF-1 α -FOSL2 axis, thereby promoting anti-atherosclerotic and endothelial protective effects [46]. Additionally, FOSL2 can regulate autophagy in cardiac fibroblasts during the process of myocardial fibrosis [47]. Overall, the expression changes of FOSL2 in heart failure may represent a protective mechanism for cardiomyocytes to cope with hypoxic and inflammatory environments.

PIM1 is a serine/threonine kinase that promotes cell survival and proliferation by phosphorylating its substrate proteins, thereby regulating the cell cycle and anti-apoptotic signaling pathways [48]. Studies have shown that PIM1 can stabilize HIF-1 α and HIF-2 α , and stimulate angiogenesis in a HIF-1 dependent manner [49]. PIM1 can also inhibit pathological damage by promoting cardioprotective signaling [50]. Recent research indicates that PIM1 can improve the senescent characteristics of human cardiac progenitor cells, thereby restoring their phenotypic and functional properties [51]. Therefore, incorporating PIM1 modification into cell-based therapeutic approaches holds promising prospects for the treatment of heart failure patients with advanced age and concomitant myocardial injury [52].

Immune infiltration analysis indicates that there is a dysregulation of seven types of immune cells in the failing myocardium. Immune cells, including T lymphocytes, monocytes, macrophages, and Tregs, play a dual role in heart failure. On one hand, they can mediate tissue repair and anti-inflammatory effects, thus providing protection. On the other hand, they may also lead to chronic inflammation, tissue damage, and adverse cardiac remodeling [53]. T lymphocytes and monocytes/macrophages infiltrate the cardiovascular tissue, releasing cytokines such as interleukin-6 (IL-6), interleukin-17 (IL-17), tumor necrosis factor-alpha (TNF- α), and interferon-gamma (IFN- γ), thereby promoting vascular senescence and cardiac fibrosis [54]. Macrophages, particularly the M0 subtype, are associated with the onset and

progression of heart failure due to their roles in phagocytosis, antigen presentation, and cytokine production [55]. We also found that compared to healthy controls, HF samples have higher levels of plasma cells and lower levels of Tregs. This imbalance indicates that the immune regulatory environment in HF is disrupted, which may lead to chronic inflammation and impaired tissue repair. Plasma cells are typically involved in antibody production, which may suggest ongoing immune activation and autoimmunity in HF patients [56]. Conversely, reduced levels of Tregs may lead to uncontrolled inflammation and exacerbate cardiac damage. Our study also found a strong positive correlation between the key hypoxia-related gene SLC2A1 and monocytes. This is particularly interesting because monocytes are known to differentiate into macrophages upon tissue infiltration, and they can mitigate or exacerbate inflammation based on microenvironmental cues [57]. The upregulation of SLC2A1 in monocytes may indicate a metabolic adaptation to the common hypoxic conditions in HF, potentially affecting their inflammatory response and contributing to disease progression [58]. In summary, our findings enhance our understanding of the immune state in HF under hypoxic conditions and provide insights for the development of targeted immunotherapies to improve the clinical symptoms of patients with heart failure.

Colocalization analysis suggests that although some SNP loci are significantly associated with key hypoxia genes and heart failure, the lower H4 values indicate that these hypoxia genes and heart failure are unlikely to be independently influenced by the same genetic factors. However, this does not imply a lack of biological connection between them. While the influence may not be direct, there could still be other intermediary factors or biological pathways at play. Therefore, further research may be necessary to explore how these SNP loci affect heart failure through hypoxia genes or other pathways.

Despite the integration of various bioinformatics and machine learning methods in our study, there are some limitations. Firstly, the study did not include laboratory experiments, which are an important step in validating bioinformatics results and understanding underlying biological mechanisms. Secondly, our findings lack clinical studies for validation, meaning that these discoveries should be interpreted with caution before they are confirmed in a clinical setting. Lastly, our study results are based on transcriptomics, whether changes in mRNA will lead to parallel changes in proteins still requires further investigation. In future research, if conditions permit, we will add experimental designs to assess protein level changes and immune cell infiltration to further validate our conclusions.

CONCLUSION

We explored the role of hypoxia-related genes in ischemic heart failure and their impact on immune cell infiltration using bioinformatics and machine learning methods. Through differential analysis, WGCNA, and a combination of 12 machine learning algorithms, we identified four key hypoxia-related genes in heart failure: SLC2A1, PLIN2, FOSL2, and PIM1. Using the CIBERSORT algorithm, we found that seven types of immune cells were dysregulated in the failing myocardium, with the dysregulation of five immune cells being related to hypoxia genes. Overall, our study results contribute to the further

exploration of key hypoxia-related mechanisms in the process of heart failure and provide new ideas for the development of targeted and immunotherapies.

Declarations

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CRediT authorship contribution statement

Quancheng Han: Supervision, Software, Data curation, Visualization, Writing – original draft, Writing – review & editing.

Jingjing Ma: Software, Data curation, Writing – review & editing.

Huajing Yuan: Methodology, Validation, Writing – review & editing.

Yonghong Guo: Writing – review & editing.

Yitao Xue: Project administration, Writing – review & editing.

Yan Li: Project administration, Writing – review & editing, Funding acquisition.

Yiding Yu: Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing.

Consent for publication

Not applicable.

Competing interests

The authors have no conflict of interest to disclose.

Ethics approval and consent to participate

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Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: GSE57338, GSE5406, GSE16499, and GSE161472.

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Figures

Workflow

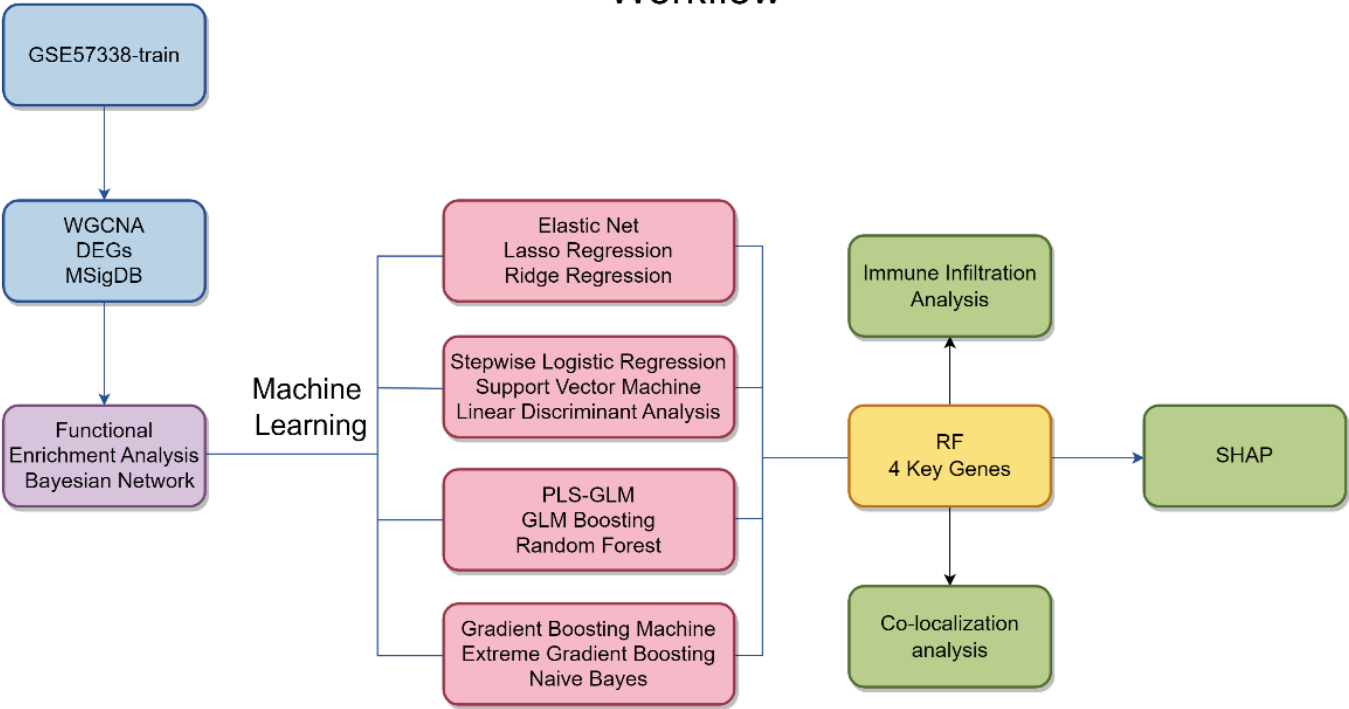
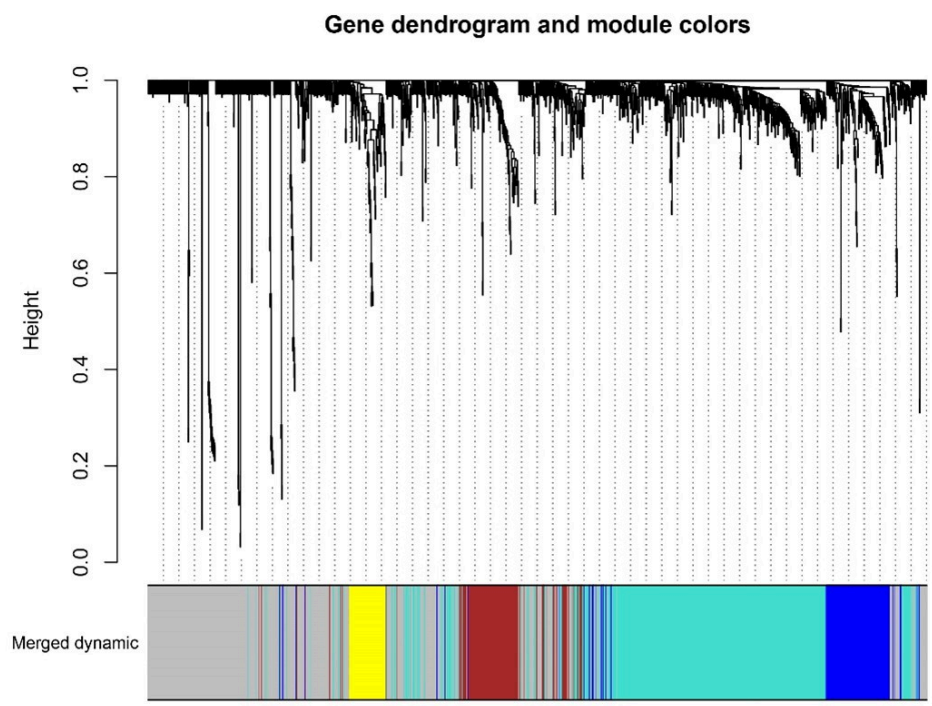


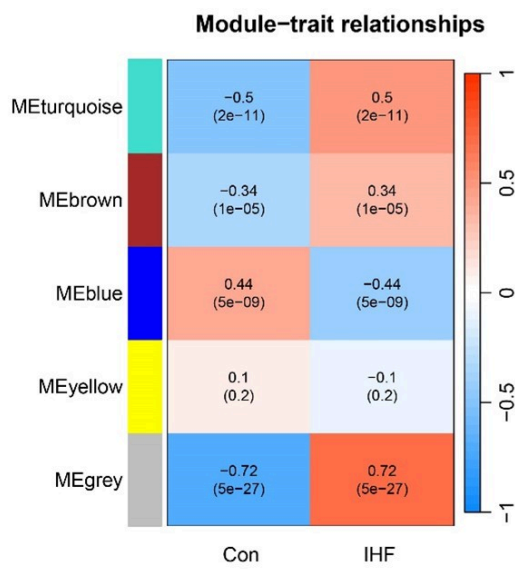
Figure 1

The workflow.

A



B



C

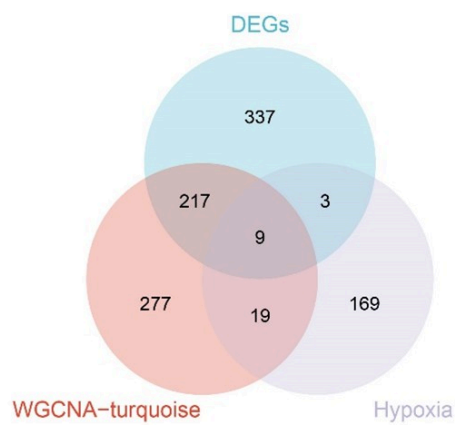


Figure 2

Results of Hypoxia Genes. (A) Gene co-expression modules represented by different colors under the gene tree. (B) 5 gene co-expression modules. (C) Intersection of genes in Hypoxia, DEGs, and WGCNA.

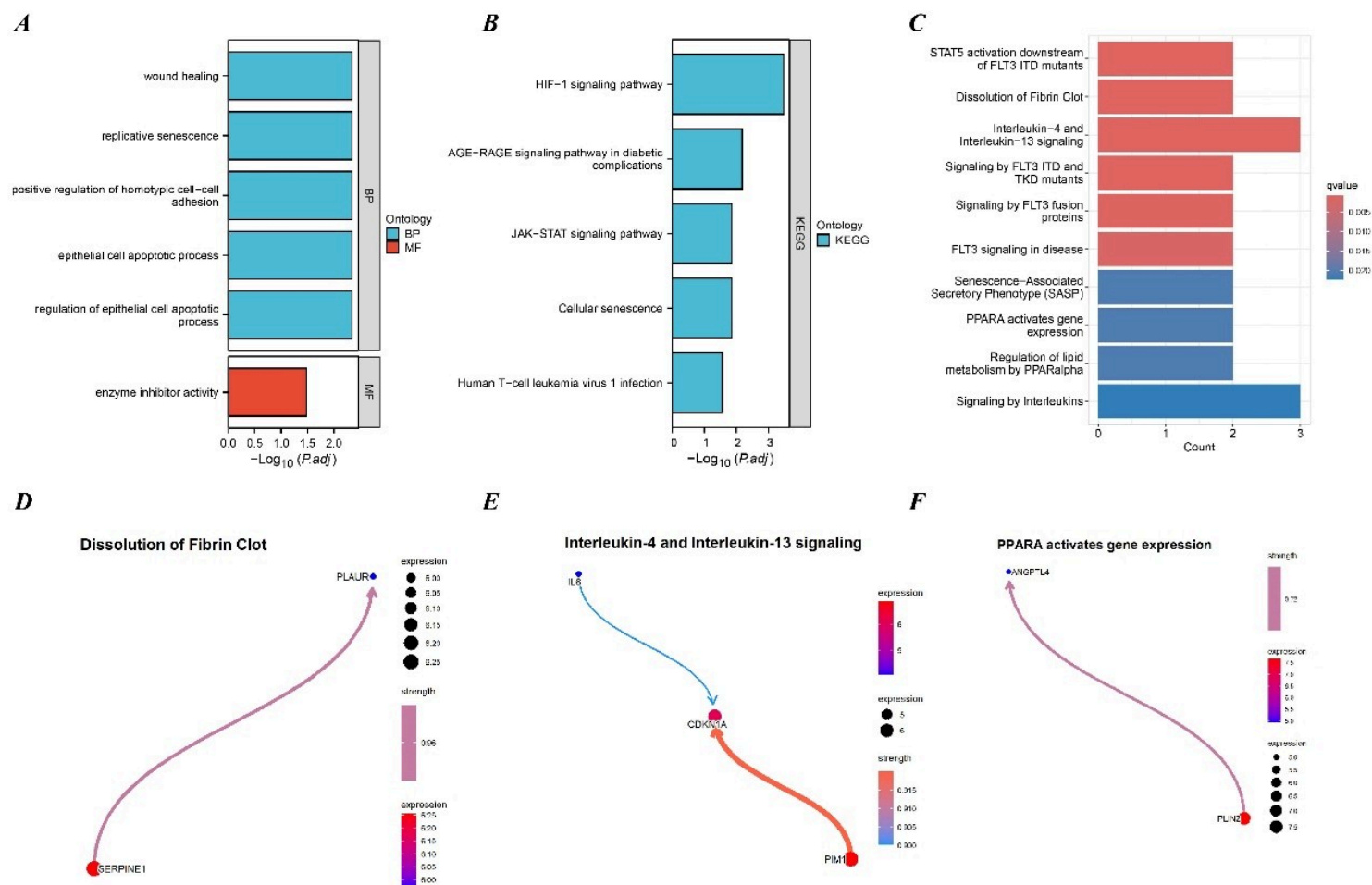


Figure 3

Functional Enrichment Analysis and Bayesian Network. (A) *GO enrichment analysis results*. (B) *KEGG enrichment analysis results*. (C) *Reactome pathway enrichment analysis results*. (D-F) *Bayesian Network of Reactome pathway*.

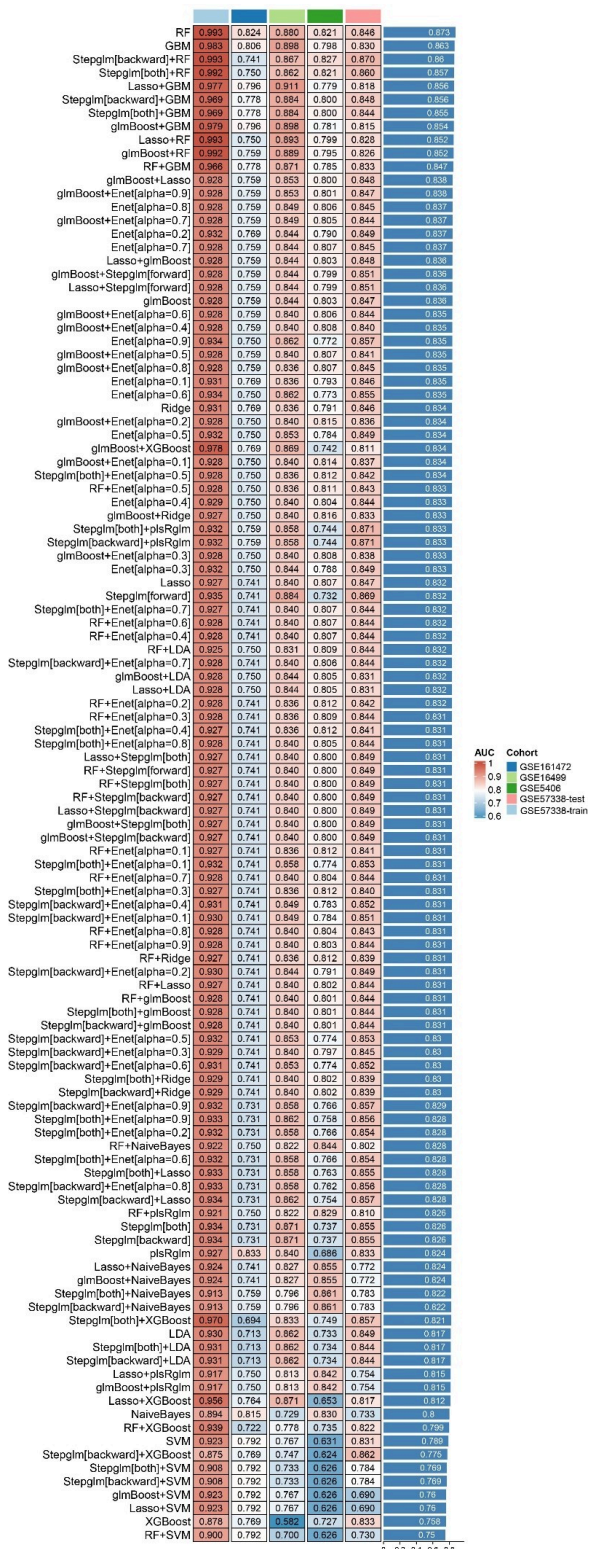
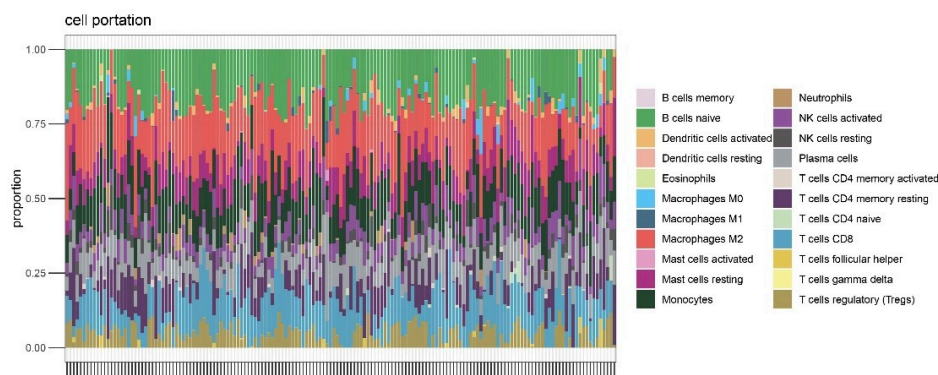


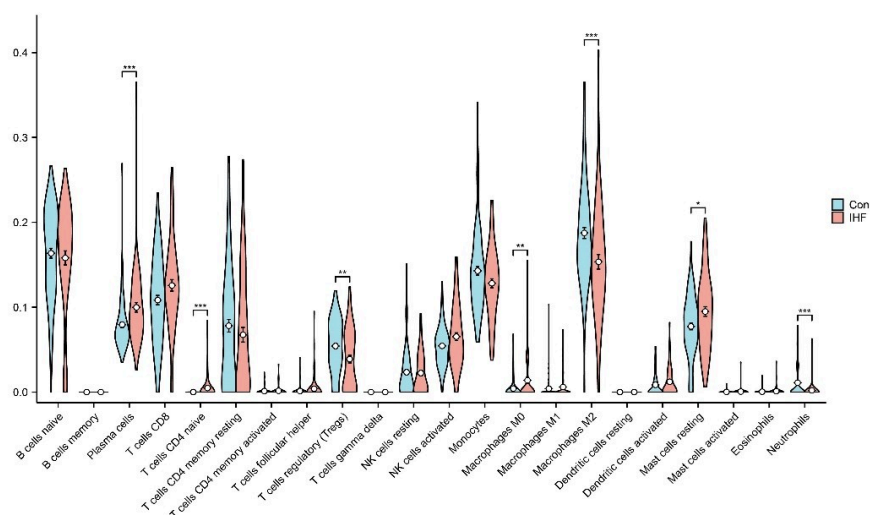
Figure 4

Machine learning in screening key hypoxia genes. RF algorithm demonstrated the highest accuracy.

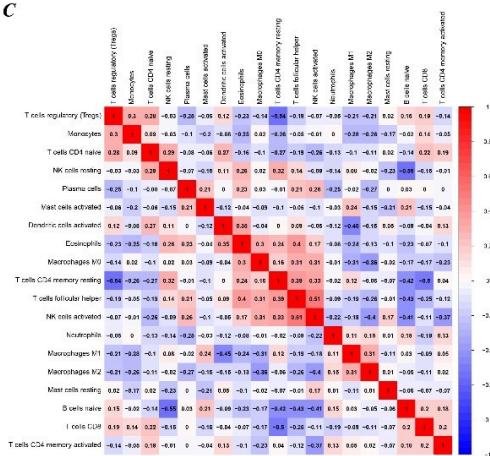
A



B



C



D

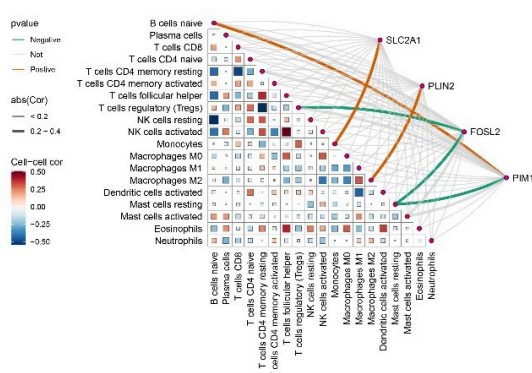


Figure 6

The Analysis of Immune Cell Infiltration in the GSE57338-train Dataset. (A) The distribution of 22 types of immune cells across different samples, depicted in a bar plot. (B) Seven dysregulated immune cells between IHF patients and controls, visualized using violin plots. (C) Heat map of correlations between immune cells. (D) Heat map of the correlation between hypoxia genes and immune cells.

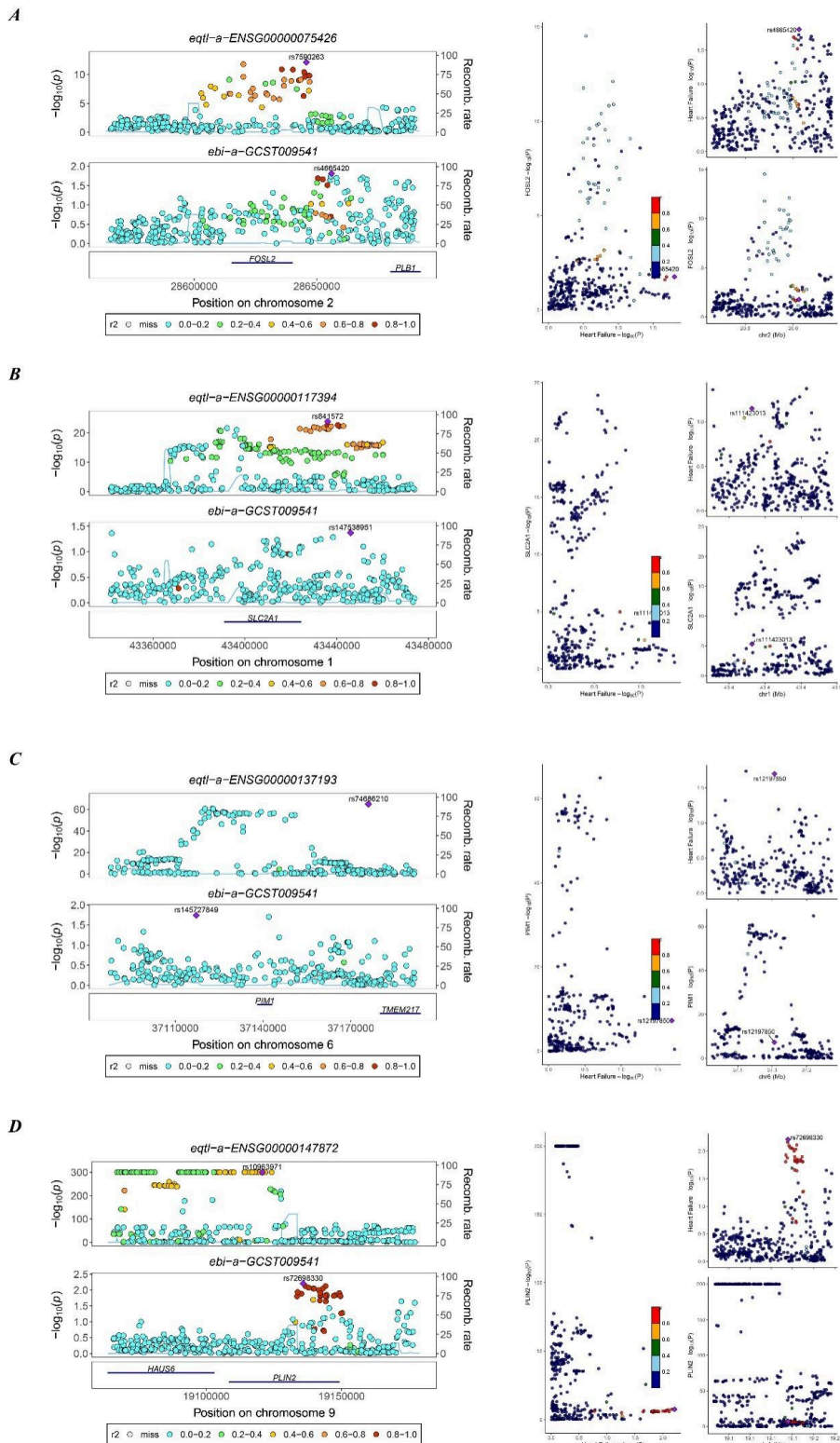


Figure 7

The Results of Co-localization Analysis. (A) *R*s4665420 locus is significantly associated with both *FOSL2* expression and heart failure. (B) *R*s111423013 locus is significantly associated with *SLC2A1* and heart failure (C) *R*s12197850 locus is significantly associated with *PIM1* and heart failure. (D) *R*s72698330 locus is significantly associated with *PLIN2* and heart failure.