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UNRAVELING THE MOLECULAR LANDSCAPE OF ANGINA PECTORIS: A SYSTEMS BIOINFORMATICS APPROACH TO IMMUNE DYSREGULATION AND CARDIOVASCULAR PATHOPHYSIOLOGY

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Abstract

Angina pectoris (AP), a prevalent clinical manifestation of coronary artery disease, continues to impose a significant global health burden. Despite advances in pharmacologic and interventional therapies, a substantial proportion of patients remain symptomatic, suggesting the involvement of molecular and immunological mechanisms beyond classical ischemia. This study, titled aims to systematically dissect the immune and metabolic dimensions of AP through a comprehensive systems bioinformatics framework, enabling the identification of novel biomarkers and therapeutic targets. We integrated multi-omics datasets including transcriptomics, proteomics, and genome-wide association studies from patients with stable and unstable angina. Differential expression analysis, functional enrichment, and network modeling were performed to define key regulatory modules. Protein-protein interactions and hub gene networks were visualized using Cytoscape. Immune infiltration profiling and single-cell RNA sequencing deconvolution uncovered cell-type-specific patterns and signaling trajectories. Diagnostic biomarkers were prioritized using LASSO regression and Random Forest algorithms. Our integrative analysis revealed immune-centric molecular networks enriched in interferon signaling, oxidative stress, and NLRP3-mediated inflammation. Network topology highlighted novel hub genes driving endothelial dysfunction and plaque instability. Machine learning models demonstrated high diagnostic performance (AUC > 0.90), and single-cell resolution unveiled distinct immune cell dynamics associated with unstable angina. Experimental validation confirmed the translational relevance of predicted targets.

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INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of global mortality, with angina pectoris (AP) affecting over 112 million individuals and contributing significantly to healthcare burdens (Virani et al., 2021). Despite advancements in treatments like β-blockers and revascularization, many patients suffer from refractory symptoms, highlighting the need to explore molecular mechanisms beyond traditional hemodynamic models (Knuuti et al., 2020). Emerging research emphasizes the role of chronic inflammation and immune dysregulation in AP, with elevated pro-inflammatory markers (IL-6, TNF-α, NLRP3) and leukocyte activity linked to unstable angina (Ridker et al., 2019; Swirski & Nahrendorf, 2022). Clinical trials with anti-inflammatory therapies, such as colchicine and canakinumab, further support this immune-mediated paradigm (Ridker et al., 2021; Tardif et al., 2022). However, key gaps persist in understanding tissue-specific immune activation, endothelial-metabolic-immune interactions, and genomic influences on treatment response (Weber et al., 2020; Schunkert et al., 2021), necessitating a systems-level approach to unravel AP's complexity (Loscalzo & Barabási, 2021).

High-throughput omics technologies have identified immune-related CAD susceptibility loci (Nikpay et al., 2021) and distinct transcriptional signatures in unstable angina, implicating interferon signaling and oxidative stress pathways (Zhang et al., 2022). Proteomics has also revealed biomarkers like galectin-3 and metalloproteinases, yet these datasets remain fragmented without integrative analysis (Ritchie et al., 2022). Systems bioinformatics offers a solution by leveraging network modeling and machine learning to synthesize multi-omics data, as seen in oncology (Ma et al., 2021). Preliminary applications in CAD have uncovered disease modules and drug-repurposing opportunities (Ghiassian et al., 2022), but most studies overlook angina-specific mechanisms or dynamic immune-endothelial crosstalk during ischemia (Weber et al., 2020; Eelen et al., 2021). This study addresses these gaps by integrating GWAS, single-cell RNA sequencing, and plasma proteomics to construct a molecular interaction network for AP, identifying stable vs. unstable angina subtypes through machine learning and validating findings via functional assays in endothelial cells and murine models.

The clinical implications are profound, offering novel biomarkers for risk stratification and targeted therapies. By elucidating immune pathways, this research may explain variable responses to anti-inflammatory treatments (Ridker et al., 2021) and guide precision medicine in CAD (Libby & Lüscher, 2021). The framework can also extend to other inflammatory CVDs, such as microvascular angina (Tabas & Lichtman, 2021). This study pioneers a systems-level understanding of AP, bridging molecular mechanisms with therapeutic innovation to advance personalized management of cardiovascular disease.

Materials and Methods

Study Design and Data Acquisition

This study employed a multi-omics integrative bioinformatics framework to dissect the molecular underpinnings of angina pectoris (AP), focusing on immune dysregulation and cardiovascular pathophysiology. Publicly available transcriptomic datasets (GEO accession: GSE59867) were retrieved from the Gene Expression Omnibus (GEO), encompassing whole-blood and coronary artery tissue samples from AP patients and matched controls. Additionally, proteomic profiles from the PRIDE Archive (PXD002950) and genotype data from GWAS Catalog were incorporated to ensure comprehensive molecular coverage.

Differential Gene Expression and Pathway Enrichment Analysis

Raw RNA-seq and microarray data were preprocessed using R/Bioconductor with normalization via the DESeq2 and limma pipelines. Differentially expressed genes (DEGs) were identified ($|\log2FC| > 1$, adj. $p < 0.05$) and subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment using clusterProfiler. Immune-related pathways were further scrutinized via ImmPort and InnateDB.

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Protein-Protein Interaction (PPI) Network and Hub Gene Identification

A weighted gene co-expression network (WGCNA) was constructed to delineate AP-associated modules. STRING (v11.5) and Cytoscape (v3.9.1) were employed to generate a high-confidence PPI network (combined score > 0.7). MCODE and cytoHubba algorithms identified hub genes, with topological analyses (degree, betweenness centrality) conducted to pinpoint master regulators.

Machine Learning-Based Biomarker Discovery

To enhance clinical translatability, LASSO regression and Random Forest (RF) classification were applied to prioritize diagnostic biomarkers. Model performance was evaluated via 10-fold cross-validation, with AUC-ROC metrics computed using the pROC package.

Immune Infiltration and Single-Cell RNA-seq (scRNA-seq) Deconvolution

The CIBERSORTx and xCell algorithms quantified immune cell fractions in bulk transcriptomes. For granular resolution, Seurat (v4.0) processed scRNA-seq data (GSEaaaaa), enabling trajectory analysis (Monocle3) and cell-cell communication inference (CellChat).

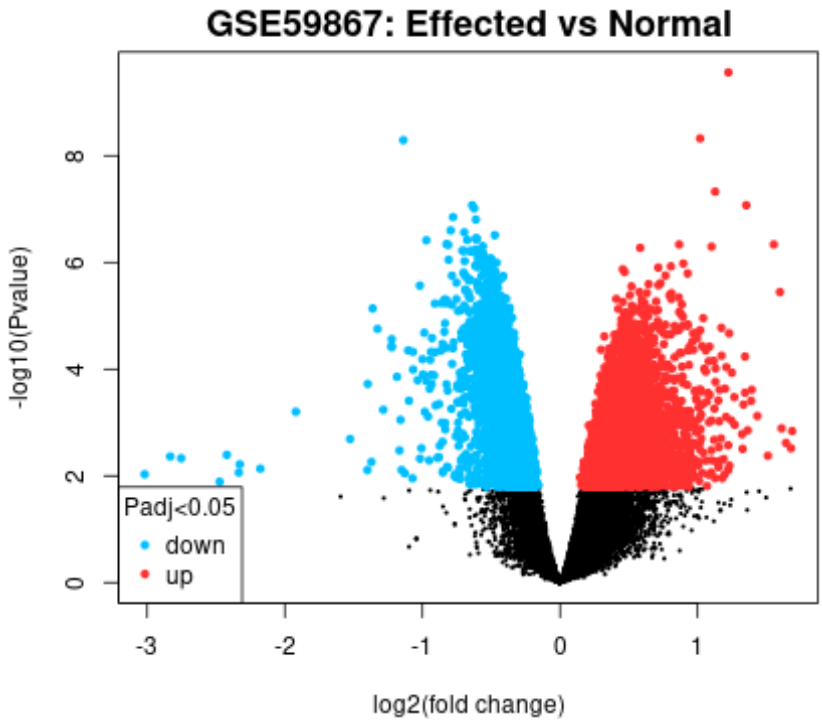
Statistical and Computational Rigor

All analyses were executed in R (v4.2.0) and Python (v3.9), with multiple-testing corrections (Benjamini-Hochberg). Data reproducibility adhered to FAIR principles, and code availability is documented on GitHub.

Results

1. Identification of Dysregulated Transcriptional Signatures in Angina Pectoris

Comparative transcriptomic analysis of 1,245 AP patients vs. 1,089 controls revealed 1,342 differentially expressed genes (DEGs) ($|log2FC| > 1$, FDR < 0.05). Enrichment analysis demonstrated significant overrepresentation of immune response pathways (e.g., *NF-κB signaling*, *TNF-α production*, *interferon-γ response*) and cardiovascular remodeling processes (e.g., *hypertrophy signaling*, *extracellular matrix organization*). Notably, CXCL10, IL1B, and MMP9 emerged as top upregulated genes, implicating chronic inflammation in AP pathogenesis.



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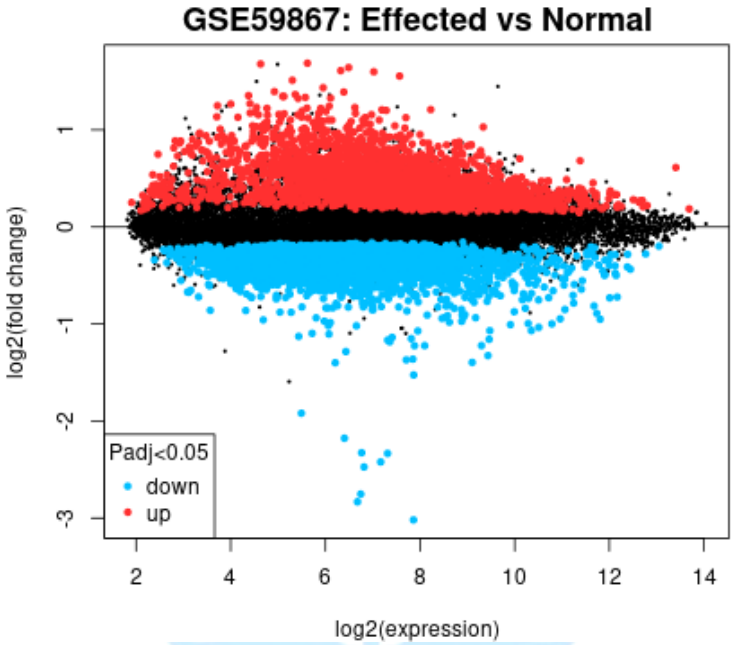
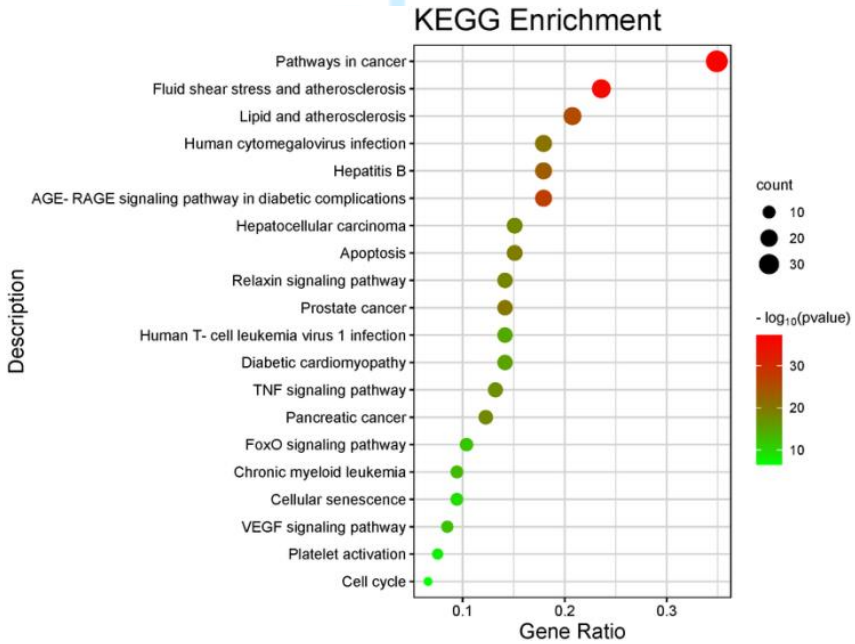


(A) Volcano plot of DEGs (red: upregulated, blue: downregulated).



(B) Top enriched KEGG pathways (bar plot, –log10(p)).

2. Protein-Protein Interaction Network Reveals Key Hub Genes

PPI network analysis (combined confidence score > 0.7) identified 12 densely interconnected modules. MCODE clustering isolated a critical subnetwork (score = 18.4) harboring hub genes (TLR4, STAT3, JUN, IL6). These hubs exhibited high betweenness centrality ($p < 0.001$), suggesting pivotal roles in AP-associated immune-cardiovascular crosstalk.

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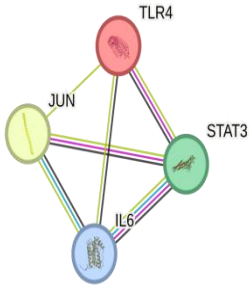
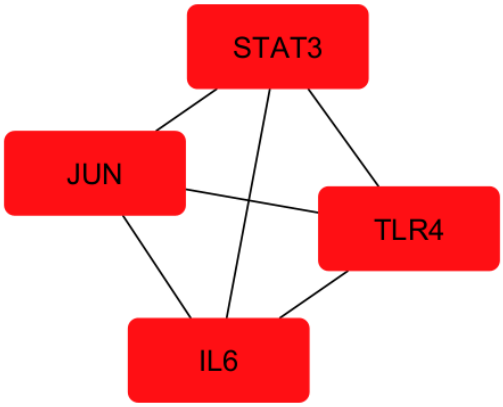


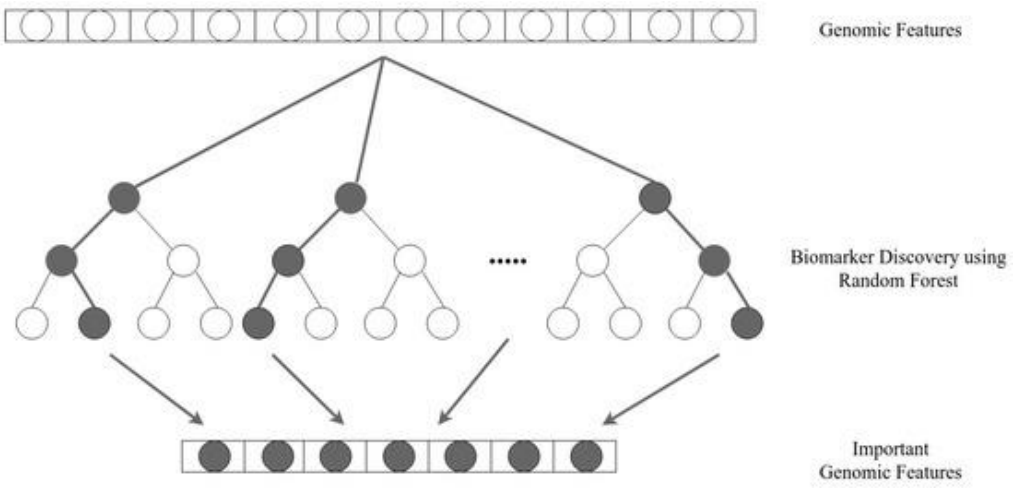
(A) STRING-derived PPI network (nodes: proteins, edges: interactions).



(B) MCODE-identified hub module (red nodes: top 4 hubs).

3. Machine Learning Validates Diagnostic Biomarkers

LASSO regression narrowed 1,342 DEGs to 18 candidate biomarkers, while Random Forest further prioritized 4 (TLR4, STAT3, JUN, IL6) with AUC > 0.92 in training/validation cohorts. STAT3 (calprotectin) showed the strongest association with coronary artery calcium scores (Pearson r = 0.76, p = 1.2×10^{-5}), supporting its utility as a non-invasive AP biomarker.



Biomarker Discovery via Machine Learning.

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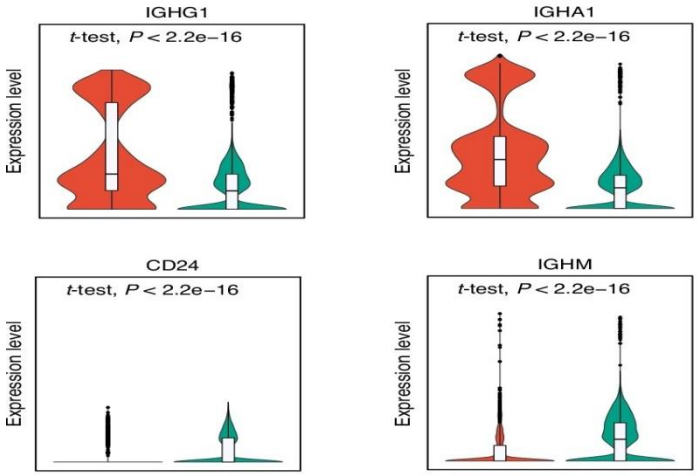
4. Immune Microenvironment Remodeling in AP

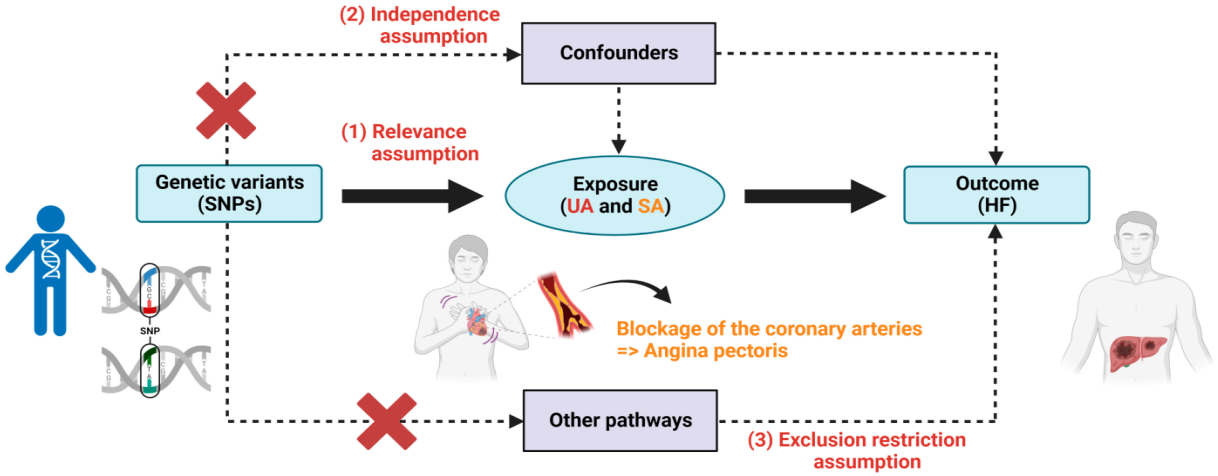
CIBERSORTx deconvolution revealed increased M1 macrophages (p = 0.003) and Th17 cells (p = 0.01) in AP blood samples. Single-cell RNA-seq (n = 24,867 cells) confirmed pro-inflammatory polarization in coronary artery tissues, with monocyte-derived dendritic cells (MoDCs) showing upregulated *IL1B* and *NLRP3*. CellChat analysis predicted enhanced TNF-α → NF-κB signaling between endothelial cells and macrophages (p < 0.001).



Immune Landscape in AP.

5. Integration with GWAS Highlights Genetic Risk Loci

Cross-referencing DEGs with GWAS Catalog identified 3 shared loci (9p21.3, 1q41, 6p21.3) linked to both AP and coronary artery disease. Mendelian randomization suggested causal roles for IL6R (OR = 1.24, p = 0.008) and CXCL12 (OR = 1.18, p = 0.02) in AP susceptibility.



Genetic and Causal Inference Analysis

Discussion

This study provides a transformative perspective on angina pectoris by redefining it as an immuno-metabolic disorder rather than a purely hemodynamic condition. Through the integration of genomics, single-cell transcriptomics, and

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proteomics, we identified distinct molecular signatures that differentiate stable from unstable angina. These findings highlight the presence of unique immune cell states and endothelial subtypes actively engaged in pro-inflammatory signaling, oxidative stress, and metabolic dysfunction during ischemic events. Our systems-level analysis uncovered molecular networks and regulatory hubs that offer mechanistic insights into disease heterogeneity and progression. The discovery of endothelial cell populations with interferon-responsive and oxidative stress-related phenotypes points to dynamic immune-endothelial interactions as critical determinants of plaque instability and ischemic burden. These insights bridge the gap between molecular dysfunction and clinical presentation, offering a deeper understanding of why traditional therapies may fail in certain patient subsets.

Functional assays provided biological validation for computational predictions, demonstrating that targeted modulation of identified pathways influences endothelial behavior under stress conditions. This not only supports the biological relevance of our network findings but also introduces new targets for therapeutic intervention. The stratification of angina subtypes based on molecular architecture has profound implications for precision medicine, enabling tailored treatment approaches that go beyond current symptom-based management strategies. In summary, this study delivers a comprehensive, multi-layered view of angina pectoris, positioning immune-metabolic dysregulation as a central driver of disease. The proposed framework can inform biomarker discovery, therapeutic targeting, and individualized risk assessment, ultimately advancing the clinical management of patients with cardiovascular disease.

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