

## The Co-pathogenic Target Gene CNTN1 Involved in Coronary Artery Disease and Pulmonary Arterial Hypertension Has Potential for Diagnosis of Coronary Artery Disease

### ABSTRACT

**Background:** We aimed to find a gene for coronary artery disease (CAD) early diagnosis by detecting co-pathogenic target gene involved in CAD and pulmonary arterial hypertension (PAH).

**Methods:** Datasets were obtained from the Gene Expression Omnibus (GEO) database, including GSE113079, GSE113439, and GSE12288, to investigate gene expression patterns in cardiovascular diseases. Weighted Gene Co-expression Network Analysis (WGCNA) was performed to identify gene modules associated with clinical traits. Differential gene expression analysis and functional enrichment analysis were carried out. Protein–protein interaction (PPI) networks were constructed. JASPAR database and FIMO tool were utilized to predict transcription factor (TF) binding sites.

**Results:** Fifteen key genes were identified in CAD and PAH, with CNTN1 being prioritized for further investigation due to its high connectivity degree. Upstream regulation analysis identified potential TFs (DRGX, HOXD3, and RAX) and 7 miRNAs targeting CNTN1. The expression profile of CNTN1 was significantly upregulated in CAD samples, and ROC analysis indicated potential diagnostic value for CAD. CMap database analysis predicted potential targeted drugs for CAD.

**Conclusion:** CNTN1 was detected as a co-pathogenetic gene for CAD and PAH. It is highly expressed in CAD patients and has potential value for CAD diagnosis. CNTN1 is potentially regulated by 3 TFs and 7 miRNAs.





**Keywords:** Coronary artery disease, pulmonary arterial hypertension, CNTN1, diagnosis

### INTRODUCTION

Coronary artery disease (CAD) is a pervasive and life-altering cardiovascular condition, representing the third leading cause of morbidity and mortality globally.<sup>1</sup> This disease is characterized by the gradual buildup of atherosclerotic plaques within the coronary arteries, impeding the crucial blood supply to the heart muscle.<sup>2</sup> The interplay of genetic predispositions, environmental factors, and lifestyle choices converges to initiate and perpetuate this pathological process.<sup>3</sup> Coronary artery disease manifests in a spectrum of clinical presentations from asymptomatic atherosclerosis to debilitating angina and potentially fatal myocardial infarctions.<sup>4</sup> Coronary artery disease commands attention due to its profound impact on public health. Diagnostic techniques, therapeutic interventions, and preventive strategies continually evolve to address the multifaceted challenges posed by CAD. The confirmation of CAD diagnosis currently relies on invasive methods like coronary angiography. There is a crucial need to establish noninvasive biomarkers for the early diagnosis of CAD.<sup>5</sup>

Pulmonary arterial hypertension (PAH) is a progressive and debilitating disorder characterized by elevated pulmonary artery pressure, leading to right ventricular failure and a decline in functional capacity.<sup>6</sup> Pulmonary arterial hypertension is primarily driven by pathological changes in the pulmonary vasculature, including vascular remodeling, vasoconstriction, and endothelial dysfunction.<sup>7</sup> The intricate

### ORIGINAL INVESTIGATION

Kun Cheng<sup>1</sup>   
Qixuan Zhai<sup>1</sup>   
Jieqiong Song<sup>2</sup>   
Bing Liu<sup>1</sup> 

<sup>1</sup>Department of Cardiovascular Surgery, Zibo Central Hospital, Shandong Province, China

<sup>2</sup>Department of Blood Transfusion, Zibo Central Hospital, Shandong Province, China

#### Corresponding author:

Bing Liu  
✉ lb19840404@126.com

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interplay of these mechanisms contributes to elevated pulmonary vascular resistance, impairing blood flow and leading to the clinical manifestations of PAH, such as dyspnea, fatigue, and exercise intolerance, even ultimately death.<sup>8</sup> Patients diagnosed with PAH face a 4-fold higher risk of CAD when compared to the general population.<sup>9</sup> Pulmonary hypertension is commonly observed in CAD-associated heart failure.<sup>10</sup> A significant occurrence of CAD has been found in individuals with pulmonary hypertension secondary to chronic obstructive pulmonary disease.<sup>11</sup> In systemic sclerosis patients, CAD and PAH were also shown considerable overlap.<sup>12</sup>

Due to the close relationship of CAD and PAH, we aimed to enhance CAD early diagnosis by exploring co-pathogenic target gene in CAD and PAH, aiming to uncover common genetic factors that may explain the observed overlap between these cardiovascular disorders and provide valuable insights into the underlying biological pathways and potential therapeutic targets especially for CAD.

## METHODS

### Research Object

Datasets with accession numbers GSE113079, GSE113439, and GSE12288 were retrieved from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). GSE113079 comprised 93 CAD samples and 48 samples from normal control subjects. GSE113439 included 15 PAH samples and 11 control samples. GSE12288 consisted of 110 CAD samples and 112 normal samples.

### Weighted Gene Co-expression Network Analysis

The weighted gene co-expression network analysis ("WGCNA") package<sup>13</sup> (version 1.72-1) in R language was employed to conduct co-expression network analysis based on gene expression values. The top 25% of genes were filtered based on variance analysis for subsequent WGCNA analysis. Pearson correlation coefficients were calculated between each pair of genes, and an appropriate soft threshold ( $\beta$ ) was selected to ensure that the constructed network adhered to the scale-free network criteria. A one-step approach was used to construct the gene network, and the adjacency matrix was transformed into the Topological Overlap Matrix (TOM). A hierarchical clustering tree for genes was generated. The significance of the association between genes and

clinical information was measured. Module-trait associations were analyzed to further obtain target gene modules.

### Differential Gene Expression Analysis

The R package "limma"<sup>14</sup> (version 3.52.4) was utilized for differential gene expression analysis. Differentially expressed genes (DEGs) were screened based on the criteria of a log-transformed fold change  $|\text{Log}_2\text{FC}| > 0.3$  (equivalent to a fold change greater than 1.23) and an adjusted *P*-value (*p.adjust*) less than .05.

### Functional Enrichment Analysis Based on Differentially Expressed Genes

The R package "clusterProfile"<sup>15</sup> (version 4.71.2) was employed for Gene Ontology (GO) enrichment analysis, including Biological Process (BP), Molecular Function (MF), and Cellular Component (CC), as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway enrichment analysis. A significance threshold of *P*-value  $< .05$  was used to filter for significantly enriched GO terms and KEGG pathways.

### Protein-Protein Interaction Networks

The STRING database was utilized for the analysis and prediction of protein functional associations and protein-protein interactions. We employed STRING<sup>16</sup> (<https://string-db.org/>, version 11.0) to analyze protein functional connections and interactions. Subsequently, Cytoscape<sup>17</sup> (version 3.7.2) was used to visualize the PPI network. To gain insights into the complex interactions between proteins and evaluate potential pathways, we further utilized the online database GeneMANIA (<http://www.genemania.org>).

### Functional Enrichment Analysis Based on Groups with Different Expression Levels of Target Gene

Based on the median expression levels of the target gene, samples with expression levels higher than the median were classified as the high-expression group, while those with expression levels lower than the median were classified as the low-expression group. Gene Set Enrichment Analysis (GSEA) was then performed between these groups, and pathways with a *P*-value  $< .05$  were selected as significantly enriched pathways.

Simultaneously, differential gene expression analysis was conducted using the R package "limma." Following the identification of the DEGs, the "DOSE" package<sup>18</sup> (version 3.26.1) in R was employed for Disease Ontology (DO) enrichment analysis on these DEGs.

### Transcription Factor Binding Site Prediction

Firstly, the sequence file for the upstream 3000 bp from the gene start site was downloaded from UCSC (<http://genome.ucsc.edu/>). Subsequently, the motif files corresponding to TFs were obtained from the JASPAR database (<https://jaspar.genereg.net/>). Next, the online tool FIMO (<https://meme-suite.org/meme/tools/fimo>) was used to predict whether transcription factor binding motifs were present in the upstream region of the gene promoter.

### Drug Prediction

The CMap database (<https://clue.io>)<sup>19</sup> enables the exploration of networks involving drugs/small molecules, genes, and disease states based on cell expression profile data treated

## HIGHLIGHTS

- CNTN1 is relatively higher expressed in coronary artery disease than in normal samples.
- CNTN1 has been identified as a co-pathogenic target gene in both coronary artery disease and pulmonary arterial hypertension.
- Diagnostic value has been detected in CNTN1 for coronary artery disease.
- The study identifies transcription factors (DRGX, HOXD3, and RAX) and microRNAs (hsa-miR-6835-3p, hsa-miR-182-5p, hsa-miR-5590-3p, hsa-miR-142-5p, hsa-miR-506-3p, hsa-miR-141-3p, hsa-miR-200a-3p) that may regulate CNTN1.

with 164 drugs/small molecules and overexpression or gene knockout tools. Using the L1000 analysis platform, we categorized samples into high-expression and low-expression groups based on target gene expression levels. Differential expression analysis was then conducted between the 2 groups. Subsequently, potential effective drugs of the gene set consisting of top 10 upregulated genes and the target gene were predicted based on this analysis.

### Statistical Analysis

All boxplot comparisons in this article were conducted using the Wilcoxon rank-sum test, including gene expression level and immune cell infiltration ratio comparisons between different groups. The "cor" function was used for Pearson correlation analysis, and the "ggstatsplot" package was used to perform *t*-test based on Student's *t*-distribution and plot the correlation between expression levels of our target gene and TFs. Receiver operating characteristic (ROC) curves were generated using the "pROC" package in R<sup>20</sup> (version 1.18.4) to demonstrate whether the target gene have diagnostic value. Differences were considered statistically significant when *P* < .05. All statistical analyses were performed using R software version 4.3.1.

## RESULTS

### Identification of Potential Pathogenic Target Genes in Coronary Artery Disease

Firstly, we identified potential pathogenic target genes in CAD. Using GSE113079 for WGCNA analysis, a soft threshold ( $\beta$ ) of 14 was selected (Figure 1A), and a gene network was constructed, resulting in 8 gene modules (Figure 1B). Samples in the dataset were divided into CAD and control groups, and they were used as trait data for WGCNA. The correlation between each gene module and the 2 types of samples was calculated (Figure 1C-D). Modules with a *P*-value < .05 and |correlation| > 0.5 were selected as CAD-related gene modules, specifically the brown, yellow, and black modules, comprising a total of 959 genes.

Differential gene expression analysis was performed on CAD vs Control using GSE113079. There were 8100 DEGs in CAD samples compared to normal samples, including 4070 upregulated genes and 4030 downregulated genes (Figure 1E). After taking the intersection of WGCNA module-related genes and upregulated DEGs in CAD samples, a total of 463 intersecting genes were obtained and considered as CAD-related genes (Figure 1F, Supplementary Table 1).

Subsequently, GO and KEGG enrichment analyses were conducted for the 463 CAD-related genes. There were 12 significantly enriched KEGG pathways (*P*-value < .05) such as neuroactive ligand-receptor interaction, and 350 significantly enriched BP terms, 75 MF terms, and 31 CC terms like sensory perception (Supplementary Table 2). The top 10 enriched KEGG pathways and the top 10 enriched GO pathways were shown in Figure 1G-H.

### Identification of Potential Pathogenic Target Genes in Pulmonary Arterial Hypertension

Coronary artery disease is common among PAH patients,<sup>21</sup> and severe CAD can lead to heart failure, which may result

in increased pressure in the pulmonary arteries, contributing to PAH. To screen potential pathogenic target genes in PAH, we applied WGCNA analysis based on GSE113439 with a  $\beta$ -value of 7 was selected (Figure 2A), resulting in a total of 11 gene modules (Figure 2B). Samples in the dataset were categorized into PAH and Control groups, serving as trait data for WGCNA. The correlation between each gene module and the 2 types of samples was computed (Figure 2C-D). Modules with a *P*-value < .05 and |correlation| > 0.5 were considered correlated with PAH revealing 3 gene modules (turquoise, brown, black), comprising a total of 4453 genes.

Using GSE113439, differential gene expression analysis was conducted for PAH vs Control. A total of 7987 DEGs were identified in PAH samples compared to normal samples, including 4054 upregulated and 3933 downregulated genes (Figure 2E). Intersection of WGCNA module-associated genes and upregulated DEGs in PAH contained 2631 common genes, designated as PAH-related genes (Figure 2F, Supplementary Table 3). Gene ontology and KEGG enrichment analyses were performed on the 2631 PAH-related genes. There were 56 significantly enriched KEGG pathways (*P*-value < .05) such as NOD-like receptor signaling pathway and cell cycle, and 1186 significantly enriched BP terms, 220 MF terms, and 198 CC terms like chromosome segregation, DNA replication, etc. The top 10 enriched KEGG pathways and the top 10 enriched GO pathways were presented in Figure 2G-H, with detailed enrichment results available in Supplementary Table 4.

### Identification of Co-pathogenic Target Gene CNTN1 in the Context of Coronary Artery Disease and Pulmonary Arterial Hypertension

In this study, we aimed to pinpoint shared pathogenic mechanisms by identifying common target genes in the context of both CAD and PAH. To achieve this, we initially performed a comprehensive analysis involving 463 CAD-related genes and 2631 PAH-related genes, resulting in the identification of 15 genes common to both conditions (Figure 3A, Supplementary Table 5). These 15 genes were subsequently considered as candidate genes for further investigation.

To elucidate potential PPIs among the candidate genes, we utilized the STRING database, applying a minimum required interaction score of >.15 as a threshold for filtering interaction pairs. The PPI network visualized using Cytoscape software comprised 22 nodes and 27 edges (Figure 3B). Each node represented a gene, and edges denoted the interactions between them. Simultaneously, a complementary interaction network was constructed using GENEMANIA (<http://genemania.org/search/>) based on the identified candidate genes (Figure 3C, Supplementary Table 6), revealing 114 interaction sites, encompassing 64 co-expression interactions and 50 genetic interactions. Drawing insights from literature reviews and the results of the PPI network, we prioritized CNTN1 (contactin 1) for further investigation due to its highest connectivity degree.

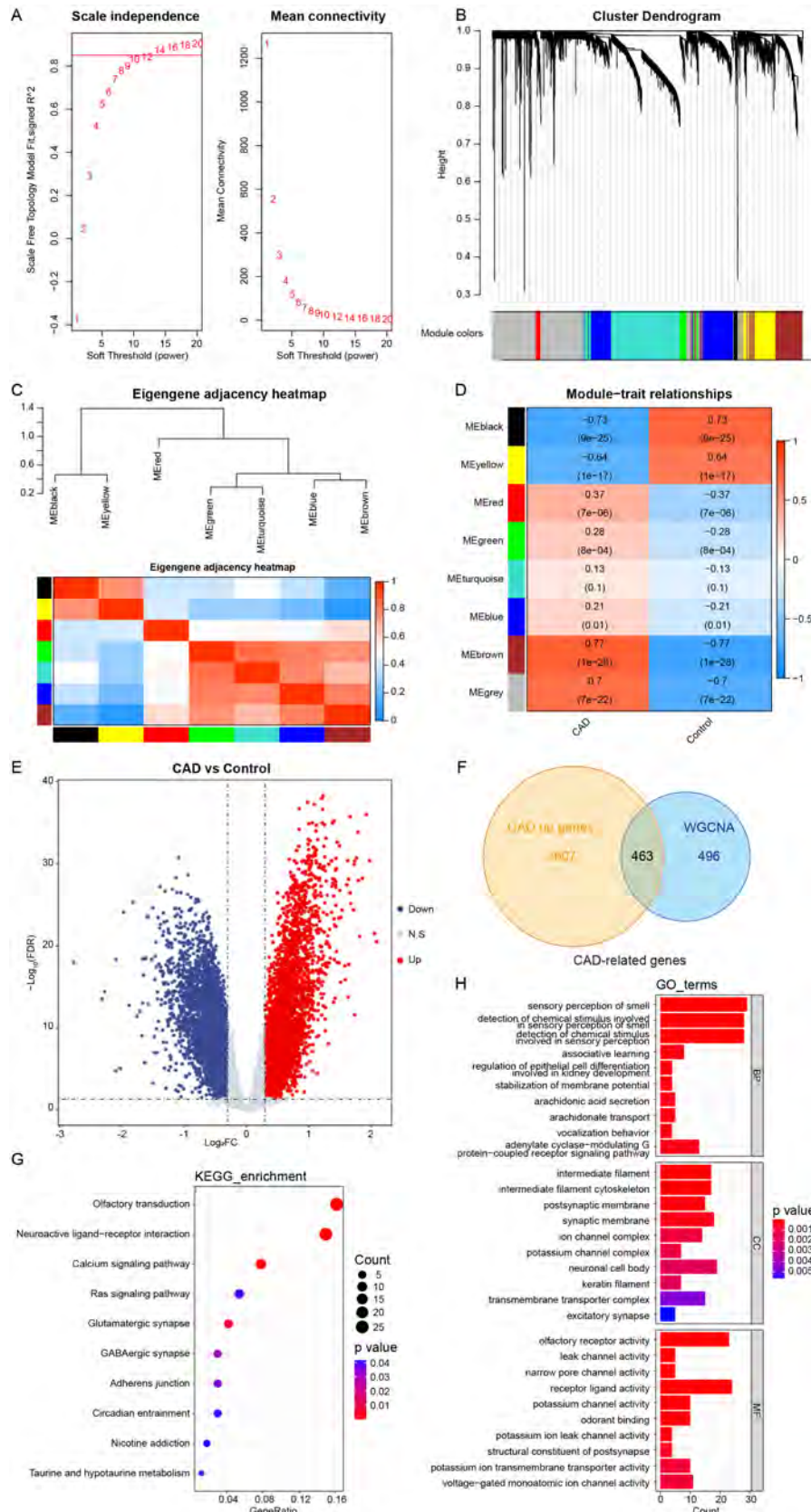


Figure 1. Identification of potential pathogenic target genes in CAD. (A) Determination of soft threshold  $\beta$ . (B) Cluster dendrogram. Each color represents a module, and the gray parts represent genes can't be clustered into other modules. (C) Heatmap of dendrogram and traits. (D) Relationship of module and traits. (E) Differential gene expression analysis of CAD samples and normal samples. (F) Venn of obtaining of CAD-related genes. (G, H) Top 10 enriched KEGG pathways and GO terms of each aspect.

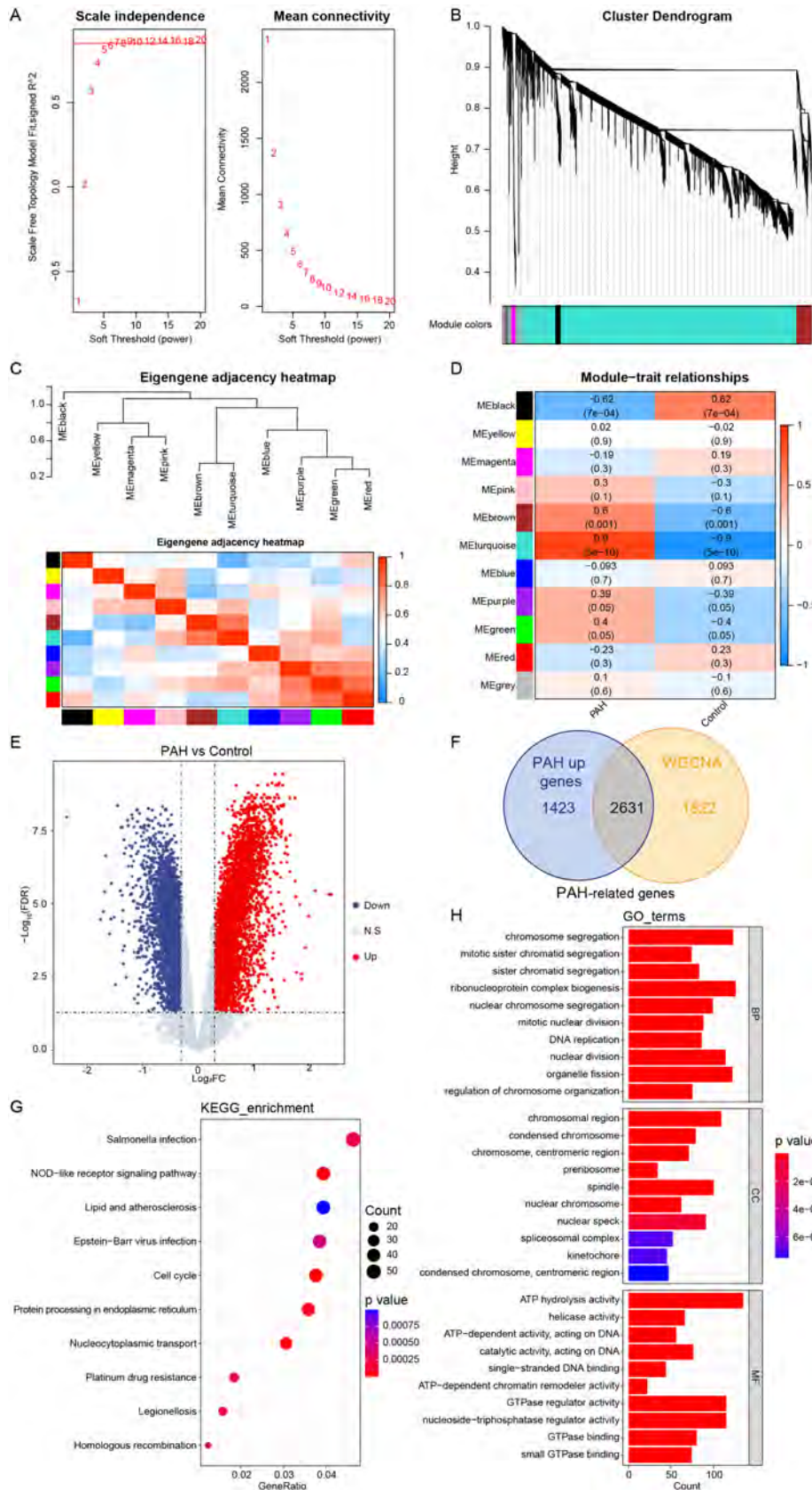
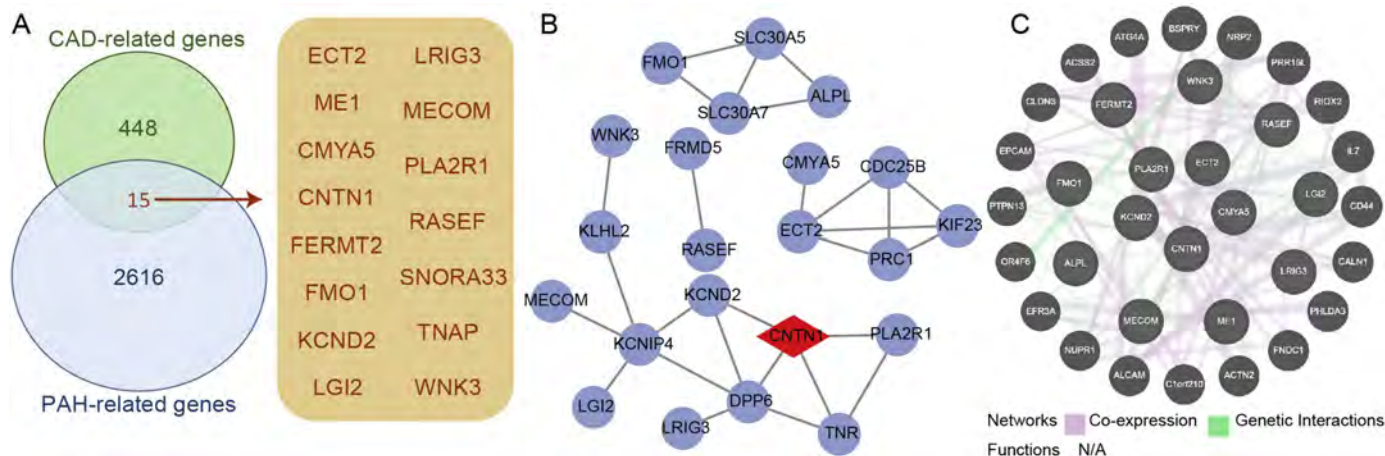


Figure 2. Identification of potential pathogenic target genes in PAH. (A) Determination of soft threshold  $\beta$ . (B) Cluster dendrogram. Each color represents a module, and the grey parts represent genes can't be clustered into other modules. (C) Heatmap of dendrogram and traits. (D). Relationship of module and traits. (E) Differential gene expression analysis of PAH samples and normal samples. (F) Venn of obtainment of PAH-related genes. (G, H) Top 10 enriched KEGG pathways and GO terms of each aspect.



**Figure 3. Identification of co-pathogenic target gene CNTN1 in the context of CAD and PAH. (A) Intersection of CAD-related genes and PAH-related genes. (B) PPI network based on 15 candidate genes. (C) PPI network constructed in GeneMANIA.**

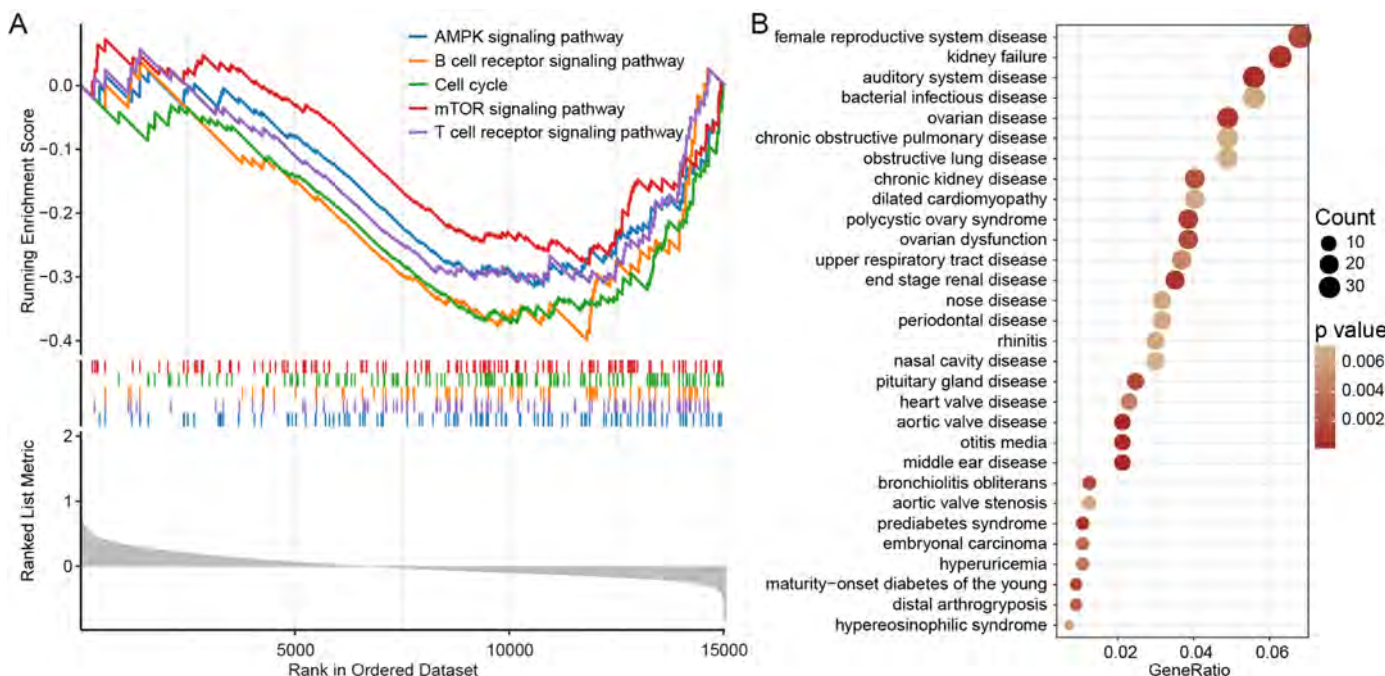
**Potential Functional Pathways of CNTN1 in Coronary Artery Disease**

To explore the functional implications of CNTN1 in CAD, we conducted GSEA using CAD samples from the GSE113079 dataset. The CAD samples were stratified based on CNTN1 expression levels, with those above the median considered as the high-expression group and those below the median as the low-expression group. GSEA revealed significant enrichment of 113 pathways in the high-expression group compared to the low-expression group ( $P < .05$ ). Noteworthy pathways enriched included the AMPK signaling pathway, B-cell receptor signaling pathway, cell cycle, mTOR signaling pathway, and T-cell receptor signaling pathway, as illustrated in Figure 4A.

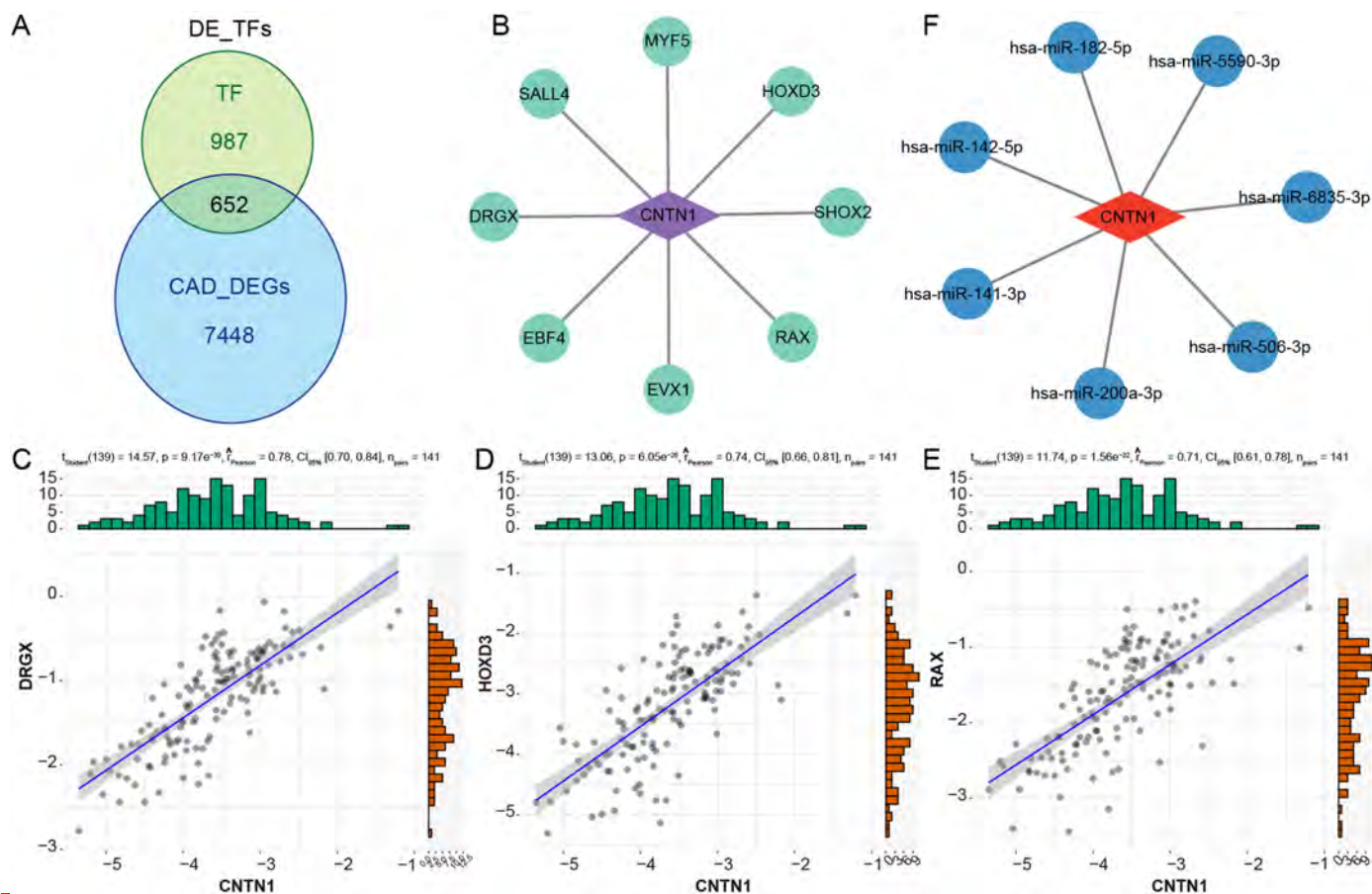
Furthermore, DEGs of high and low expression groups mentioned above were subjected to DO enrichment analysis, identifying 121 significantly enriched pathways ( $P < .05$ ). The top 30 significantly enriched pathways were depicted in Figure 4B. Detailed results of the enrichment analyses were provided in Supplementary Table 7.

**Exploration of Upstream Regulation of CNTN1 in Coronary Artery Disease**

To further investigate the potential regulatory network involving CNTN1 in the pathogenesis of CAD, an analysis of potential upstream regulation was conducted. We curated a total of 652 differentially expressed TFs in CAD by intersecting TFs<sup>22</sup> and DEGs between CAD and normal



**Figure 4. Potential functional pathways of CNTN1 in CAD. (A) GSEA analysis of CNTN1 high-expression and low-expression group. (B) DO analysis of DEGs between CNTN1 high and low expression groups.**



**Figure 5.** Exploration of upstream regulation of CNTN1 in CAD. (A) Intersection of TFs list and CAD-related genes. (B) Network of CNTN1 and TFs. (C–E) Correlation of CNTN1 and DRGX, HOXD3, RAX expression levels. The degrees of freedom of  $t$ -test were 139. CI 95% represented 95% confidence interval. (F) Network of CNTN1 and its corresponding miRNAs.

samples (Figure 5A) and calculated the expression correlation between each of these TFs and CNTN1 using mRNA expression data from the GSE113079 dataset. Based on the criteria of  $P < .05$  and  $|\text{correlation}| > 0.7$ , we identified 8 TFs with significantly correlated expression levels out of the initial 652 (Supplementary Figure 1). Using Cytoscape, a network diagram depicting the relationships between these TFs and CNTN1 was generated (Figure 5B).

To explore potential TF binding sites in the upstream 3000bp region of the CNTN1 promoter, we conducted a sequence analysis. Based on a significance threshold of  $P$ -value  $< 10^{-4}$ , we identified putative binding sequences for TFs DRGX (MA1481.1.meme) around 620bp upstream, HOXD3 (MA0912.2.meme) around 620bp upstream, and RAX (MA0718.1.meme) around 945 bp upstream of the CNTN1 promoter (Supplementary Table 8). These findings suggested that DRGX, HOXD3, and RAX might regulate CNTN1 gene expression by binding to its upstream region. TFs DRGX, HOXD3, and RAX expression levels all displayed significantly positive correlation with CNTN1 expression level with  $r$  value of 0.78, 0.74, 0.71, respectively (Figure 5C-E).

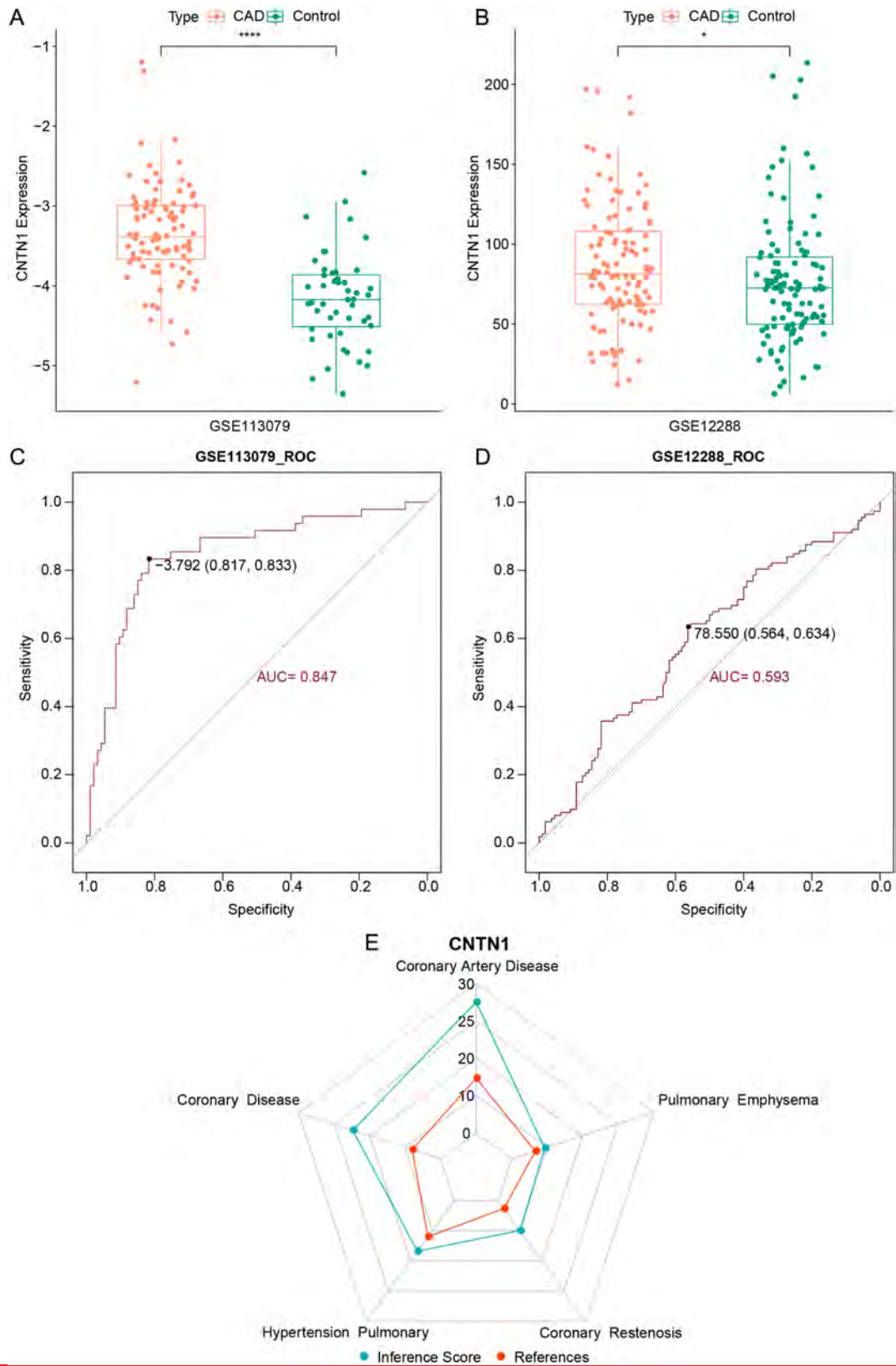
Furthermore, using the TargetScan database ([https://www.targetscan.org/vert\\_80/](https://www.targetscan.org/vert_80/)), we predicted 10 miRNAs targeting

the CNTN1 gene. Simultaneously, utilizing the miRDB database (<https://mirdb.org/>), we predicted 132 miRNAs targeting the CNTN1 gene (score  $\geq 80$ ). The intersection of miRNAs from both databases yielded a total of 7 miRNAs (hsa-miR-6835-3p, hsa-miR-182-5p, hsa-miR-5590-3p, hsa-miR-142-5p, hsa-miR-506-3p, hsa-miR-141-3p, hsa-miR-200a-3p). A network diagram illustrating the interactions between the CNTN1 gene and these miRNAs was generated using Cytoscape (Figure 5F). Predicted miRNAs from both databases were detailed in Supplementary Table 9.

#### Analysis of the Expression and Clinical Value of CNTN1 in Coronary Artery Disease

The expression profile of the gene CNTN1 in CAD samples compared to normal samples was analyzed using GSE113079 and GSE12288 datasets, revealing a significant upregulation in CAD samples in both datasets (Figure 6A-B). Additionally, ROC curves were generated using both datasets to assess the diagnostic value of CNTN1 in CAD. The AUC values for the ROC curves were 0.847 and 0.593 in the 2 datasets, indicating that CNTN1 held potential diagnostic value for CAD (Figure 6C-D).

Using the CTD database (<http://ctdbase.org/>), an inference score analysis was conducted for the CNTN1 gene. The results indicated that CNTN1 is implicated in CAD, highlighting its



**Figure 6.** Analysis of the expression and clinical value of CNTN1 in CAD. (A, B) CNTN1 expression in CAD and control samples of GSE113079 and GSE12288, respectively. (C, D) ROC curve based on GSE113079 and GSE12288, respectively. (E) Analysis of CNTN1 inference score according to CMap database (\* $P < .05$ , \*\*\*\* $P < .0001$ ).



**Table 1. Drug Prediction Results in CMap Database**

Rank	Score	ID	Name	Description
8486	-99.54	BRD-K71103788	Duloxetine	Serotonin reuptake inhibitor
8490	-99.58	BRD-A37052580	Physostigmine	Acetylcholinesterase inhibitor
8491	-99.58	BRD-K40782193	QX-222	Sodium channel blocker
8501	-99.65	BRD-K73824630	Skatole	Thrombin inhibitor
8503	-99.66	BRD-K20313525	Rosmarinic acid	GABA transaminase inhibitor
8508	-99.68	BRD-K37130656	Rivaroxaban	Coagulation inhibitor
8512	-99.72	BRD-K77998258	Ganglioside	SRC activator
8514	-99.72	BRD-K93176058	AC-55649	Retinoid receptor agonist
8515	-99.75	BRD-K65786282	CGP-7930	GABA receptor modulator
8519	-99.79	BRD-K17823458	Danoprevir	HCV inhibitor
8521	-99.82	BRD-K93258693	GW-9662	PPAR receptor antagonist
8532	-99.86	BRD-A01787639	Naftopidil	Adrenergic receptor antagonist
8533	-99.86	BRD-K14550461	Doxercalciferol	Vitamin D receptor agonist
8534	-99.86	BRD-A71009679	KUC103420N	-666
8535	-99.86	BRD-K32107296	Temozolomide	DNA alkylating agent
8539	-99.89	BRD-K68332390	Ponalrestat	Aldose reductase inhibitor
8540	-99.89	BRD-K64514229	Toltrazuril	Antiprotozoal
8554	-99.93	BRD-K81783531	VX-222	HCV inhibitor
8555	-99.93	BRD-K84639753	Safinamid	Dopamine uptake inhibitor
8556	-99.93	BRD-A07395371	Esmolol	Adrenergic receptor antagonist

strong relevance to the development and occurrence of CAD (Figure 6E).

We selected top 10 upregulated DEGs between high and low CNTN1 expression groups in CAD and the key gene CNTN1, totaling 11 genes, to consist a gene set for drug prediction. The CMap database was then utilized to predict targeted drugs for this gene set, aiming to identify potential treatments for CAD. Based on a |score| > 99 criterion, 46 drugs were identified, with the top 15 predicted drugs listed in Table 1. Additional results could be found in Supplementary Table 10. Negative CMap scores suggested that specific perturbations induced a gene expression pattern opposite to that of the high CNTN1 expression group, indicating potential therapeutic effects on the high CNTN1 expression group following such disturbances.

## DISCUSSION

The study identified 15 CAD and PAH-related genes by intersecting genes obtained from WGCNA analysis. CNTN1 was selected as a co-pathogenic target gene for CAD and PAH due to its high connectivity in PPI network. Transcription factors and miRNAs regulating CNTN1 were also investigated. CNTN1 was potentially diagnostically valuable in CAD.

By functional enrichment analysis of CAD and PAH-related genes, CAD-related genes were enriched mostly on neuro-related terms, and PAH-related genes were mostly enriched on terms about cell cycle or DNA replication. CNTN1 is a gene closely associated with these functions. CNTN1, also known as Contactin 1, is located on human chromosome 12q11 and encodes a neural cell adhesion molecule.<sup>23</sup> This gene plays a crucial role in the development and function of the nervous

system.<sup>24</sup> Contactin 1 is a glycosylphosphatidylinositol (GPI)-anchored protein that is primarily expressed in the nervous system, particularly during early embryonic development.<sup>25</sup> It is involved in cell adhesion and is known to participate in axon guidance, neuronal migration, and the formation of neural circuits.<sup>26</sup> Overexpression of CNTN1 has been proven to promote cell proliferation in a breast cancer cell line.<sup>27</sup> In a research of protein biomarkers in cardiovascular diseases, CNTN1 has been identified as a biomarker of myocardial infarction by single marker analyses from iTRAQ mass spectrometry.<sup>28</sup> Its expression level is also significantly related to incident heart failure.<sup>29</sup> In menopausal women, CNTN1 expression level has already been found to be a marker for detecting and diagnosing CAD,<sup>30</sup> consistent with our research. Being identified as a co-pathogenic gene in CAD and PAH, CNTN1 has been rarely detected in relationship with PAH, but CNTN1 is associated with lung cancer<sup>31</sup> and contributes to drug resistance to lung adenocarcinoma.<sup>32</sup> Differentially expressed genes between CNTN1 high and low expression groups in CAD indicated to be enriched on chronic obstructive pulmonary disease and obstructive lung disease.

To dig into the potential regulatory network of CNTN1 in CAD, we explored TFs expressed differentially between CAD and normal samples, and found TFs DRGX, HOXD3, and RAX might regulate CNTN1 gene expression by binding to its upstream region. DRGX, also known as Dorsal Root Ganglia Homeobox transcription factor, is a critical regulator involved in the development and maintenance of dorsal root ganglia in vertebrates. It plays a key role in the specification of sensory neurons during embryogenesis,<sup>33</sup> as CNTN1. HOXD3 is involved in the regulation of anterior-posterior

identity during embryogenesis and is essential for proper limb development,<sup>34</sup> playing a vital role in neural system. HOX TFs function in cardiovascular development,<sup>35</sup> and the administration of retinoic acid leads to an upregulation in the expression of HOXD3 in cardiac explants of chick embryos.<sup>36</sup> RAX, also known as retinal antioxidant X, is a transcription factor that plays a crucial role in eye development and photoreceptor differentiation.<sup>37</sup> Our research reveals the potential regulatory relationships between the TFs and CNTN1, which have been rarely researched, suggesting their potential application value on CAD treatment.

Besides TFs, we detected 7 miRNAs, namely hsa-miR-6835-3p, hsa-miR-182-5p, hsa-miR-5590-3p, hsa-miR-142-5p, hsa-miR-506-3p, hsa-miR-141-3p, hsa-miR-200a-3p, potentially regulating CNTN1. In previous study, miRNAs has been identified to have potential to predict CAD prognosis.<sup>38</sup> The role of miRNAs in the context of CAD has been investigated across various cellular populations. Among these, endothelial cells represent the primary focus of such studies.<sup>39</sup> For example, miR-206 has been observed to reduce the survival and invasiveness of endothelial progenitor cells in CAD patients and to promote their apoptosis, while also decreasing VEGF expression.<sup>40</sup> Another study suggests that the levels of hsa-miR-182-5p in the bloodstream may serve as a biomarker for detecting unprotected left main CAD.<sup>41</sup> Exosomal miR-182-5p has been shown to reduce cell pyroptosis and inflammation triggered by hypoxia/reoxygenation injury, enhancing cardiac function and decreasing myocardial infarct size in vivo by mitigating these effects.<sup>42</sup> Hsa-miR-6835-3p not only regulates CNTN1, but also influences pancreatic islet cell function by regulating AdipoR1.<sup>43</sup> Hsa-miR-5590-3p is able to inhibit renal cell metastasis.<sup>44</sup> Levels of hsa-miR-142-5p may serve as an independent indicator for forecasting cardiovascular incidents in individuals with peripheral artery disease.<sup>45</sup> MiR-506-3p exacerbates damage in vascular endothelial cells by reducing BECN1 expression, which leads to decreased proliferation and migration in HUVECs and increased apoptosis.<sup>46</sup> MiR-141-3p inhibition is able to reduce apoptosis induced by hypoxia,<sup>47</sup> which might be one of the mechanism influencing CAD. In rats, miR-200a-3p has been proven to reduce myocardial damage caused by coronary microembolization.<sup>48</sup>

This study still has some limitations including the reliance on existing datasets, which may not fully represent the diversity of CAD and PAH cases. The sample size, while sufficient for bioinformatics analysis, may not be large enough to capture all relevant genetic variations and interactions. Future research should incorporate larger, more diverse cohorts and include experimental validation to confirm the findings and explore the potential clinical applications of the identified genes and pathways.

## CONCLUSION

In conclusion, this study has identified CNTN1 as a potential co-pathogenic target gene in both CAD and PAH, providing insights into the shared pathogenic mechanisms between these cardiovascular disorders. The findings highlight the potential of CNTN1 as a diagnostic and therapeutic target

for CAD. The functional enrichment analysis and upstream regulation investigation suggest a complex regulatory network involving CNTN1, which warrants further research to elucidate its role in the pathogenesis of these diseases. The identification of CNTN1 and its associated pathways may pave the way for the development of novel diagnostic tools and targeted therapies, ultimately improving patient outcomes in the management of CAD.

**Availability of Data and Materials:** The data that support the findings of this study are available in the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>), STRING (<https://string-db.org/>, version 11.0), the JASPAR database (<https://jaspar.genereg.net/>), and the CMap database (<https://clue.io>).

**Ethics Committee Approval:** As this research was based on publicly available data and did not entail any experimental procedures, ethical committee approval was not applicable.

**Informed Consent:** Not applicable.

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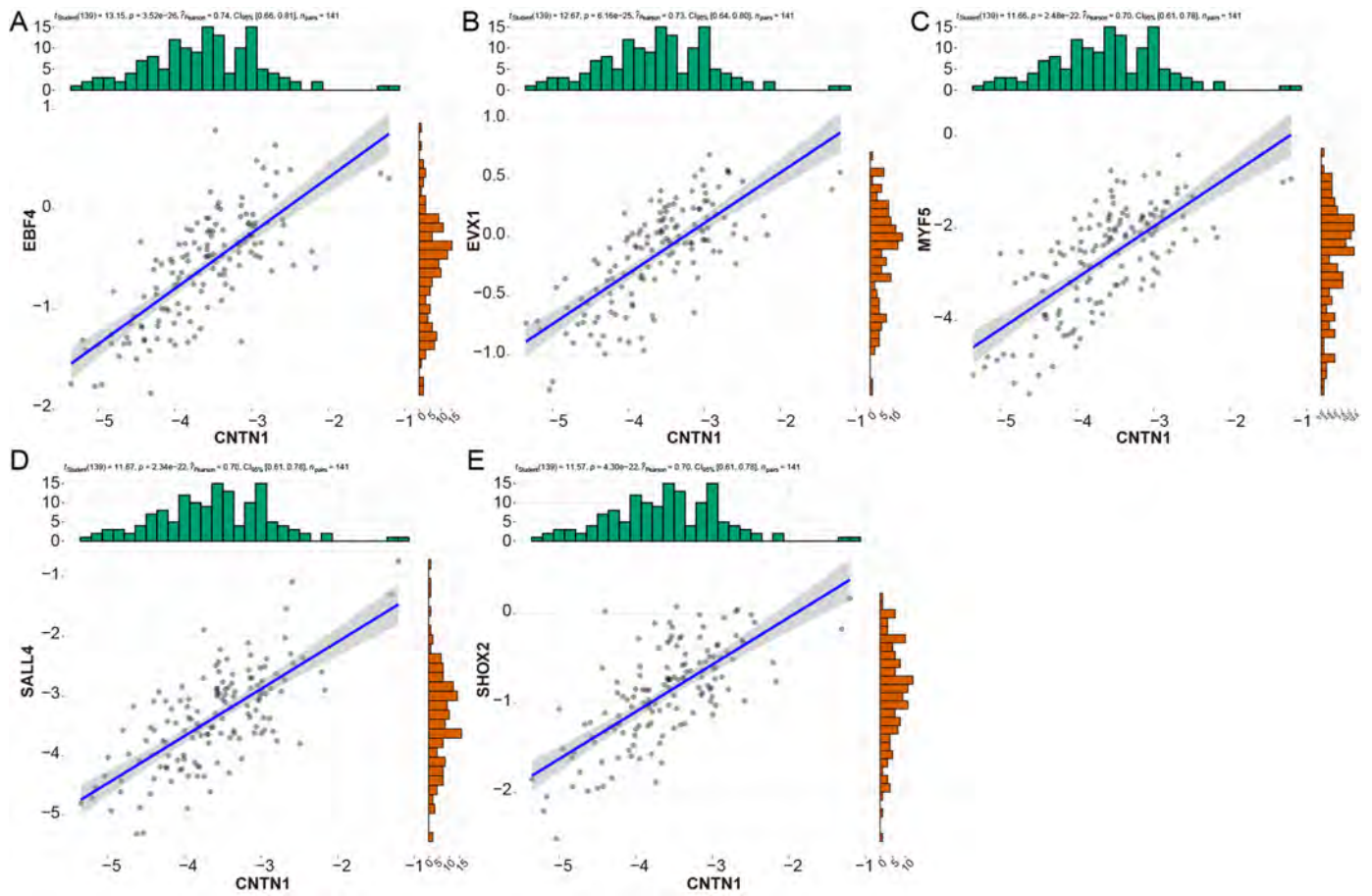
Supplementary tables can be downloaded via <https://anatoljcardiol.com/year/2024/volume/28/issue/8>.

## REFERENCES

1. Brown JC, Gerhardt TE, Kwon E. Risk Factors for Coronary Artery Disease. Disclosure: Thomas Gerhardt declares no relevant financial relationships with ineligible companies. Disclosure: Edward Kwon declares no relevant financial relationships with ineligible companies. In: *StatPearls*. Treasure Island, FL: StatPearls Publishing; 2023
2. Coronary artery disease (CAD) Centers for Disease Control and Prevention 2021. Available at: [https://www.cdc.gov/heartdisease/coronary\\_ad.htm](https://www.cdc.gov/heartdisease/coronary_ad.htm).
3. Findley AS, Richards AL, Petrini C, et al. Interpreting coronary artery disease risk through gene-environment interactions in gene regulation. *Genetics*. 2019;213(2):651-663. [CrossRef]
4. Dai X, Wiernek S, Evans JP, Runge MS. Genetics of coronary artery disease and myocardial infarction. *World J Cardiol*. 2016;8(1):1-23. [CrossRef]
5. Gilani STA, Khan DA, Rauf A, Haroon ZH, Khan KA, Hassan FU. Early diagnosis of coronary artery disease by inflammatory biomarkers of atherosclerosis in patients with angina. *J Interferon Cytokine Res*. 2022;42(9):493-500. [CrossRef]
6. Verma S, Sahni S, Vijayan VK, Talwar A. Depression in pulmonary arterial hypertension: an undertreated comorbidity. *Lung India*. 2016;33(1):58-63. [CrossRef]
7. Ranchoux B, Harvey LD, Ayon RJ, et al. Endothelial dysfunction in pulmonary arterial hypertension: an evolving land-

- scape (2017 Grover Conference Series). *Pulm Circ*. 2018;8(1):2045893217752912. [CrossRef]
8. Lai YC, Potoka KC, Champion HC, Mora AL, Gladwin MT. Pulmonary arterial hypertension: the clinical syndrome. *Circ Res*. 2014;115(1):115-130. [CrossRef]
  9. Nickel NP, Yuan K, Dorfmueller P, et al. Beyond the lungs: systemic manifestations of pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2020;201(2):148-157. [CrossRef]
  10. Huang L, Pang L, Gu Q, et al. Prevalence, risk factors, and survival associated with pulmonary hypertension and heart failure among patients with underlying coronary artery disease: a national prospective, multicenter registry study in China. *Chin Med J (Engl)*. 2022;135(15):1837-1845. [CrossRef]
  11. Asker M, Asker S, Kucuk U, Kucuk HO, Ozbay B. Relationship between coronary artery disease and pulmonary arterial pressure in patients with chronic obstructive pulmonary disease. *Int J Clin Exp Med*. 2014;7(12):5837-5841.
  12. Komócsi A, Pintér T, Faludi R, et al. Overlap of coronary disease and pulmonary arterial hypertension in systemic sclerosis. *Ann Rheum Dis*. 2010;69(1):202-205. [CrossRef]
  13. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*. 2008;9:559. [CrossRef]
  14. Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47. [CrossRef]
  15. Yu G, Wang LG, Han Y, He QY. ClusterProfiler: an R package for comparing biological themes among gene clusters. *Omic*s. 2012;16(5):284-287. [CrossRef]
  16. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47(D1):D607-D613. [CrossRef]
  17. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498-2504. [CrossRef]
  18. Yu G, Wang LG, Yan GR, He QY. DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis. *Bioinformatics*. 2015;31(4):608-609. [CrossRef]
  19. Yu N, Liu X, Shi D, Bai L, Niu T, Liu Y. CD63 and C3AR1: the potential molecular targets in the progression of septic shock. *Int J Gen Med*. 2022;15:711-728. [CrossRef]
  20. Obuchowski NA, Bullen JA. Receiver operating characteristic (ROC) curves: review of methods with applications in diagnostic medicine. *Phys Med Biol*. 2018;63(7):07TR01. [CrossRef]
  21. Shimony A, Eisenberg MJ, Rudski LG, et al. Prevalence and impact of coronary artery disease in patients with pulmonary arterial hypertension. *Am J Cardiol*. 2011;108(3):460-464. [CrossRef]
  22. Lambert SA, Jolma A, Campitelli LF, et al. The human transcription factors. *Cell*. 2018;175(2):598-599. [CrossRef]
  23. Compton AG, Albrecht DE, Seto JT, et al. Mutations in contactin-1, a neural adhesion and neuromuscular junction protein, cause a familial form of lethal congenital myopathy. *Am J Hum Genet*. 2008;83(6):714-724. [CrossRef]
  24. Chatterjee M, Schild D, Teunissen CE. Contactins in the central nervous system: role in health and disease. *Neural Regen Res*. 2019;14(2):206-216. [CrossRef]
  25. Gu Y, Li T, Kapoor A, Major P, Tang D. Contactin 1: An important and emerging oncogenic protein promoting cancer progression and metastasis. *Genes (Basel)*. 2020;11(8). [CrossRef]
  26. Moreland T, Poulain FE. To stick or not to stick: the multiple roles of cell adhesion molecules in neural circuit assembly. *Front Neurosci*. 2022;16:889155. [CrossRef]
  27. Chen N, He S, Geng J, et al. Overexpression of Contactin 1 promotes growth, migration and invasion in Hs578T breast cancer cells. *BMC Cell Biol*. 2018;19(1):5. [CrossRef]
  28. Yin X, Subramanian S, Hwang SJ, et al. Protein biomarkers of new-onset cardiovascular disease: prospective study from the systems approach to biomarker research in cardiovascular disease initiative. *Arterioscler Thromb Vasc Biol*. 2014;34(4):939-945. [CrossRef]
  29. Lind L, Zanetti D, Ingelsson M, Gustafsson S, Ärnlöv J, Assimes TL. Large-scale plasma protein profiling of incident myocardial infarction, ischemic stroke, and heart failure. *J Am Heart Assoc*. 2021;10(23):e023330. [CrossRef]
  30. Al-Kraity WRH. Evaluation of CONTACTIN-1 level in women with coronary heart disease after menopause. *Life Sci Arch*. 2019;5(3):1636-1642.
  31. Su JL, Yang PC, Shih JY, et al. The VEGF-C/Flt-4 axis promotes invasion and metastasis of cancer cells. *Cancer Cell*. 2006;9(3):209-223. [CrossRef]
  32. Zhang R, Yao W, Qian P, et al. Increased sensitivity of human lung adenocarcinoma cells to cisplatin associated with down-regulated contactin-1. *Biomed Pharmacother*. 2015;71:172-184. [CrossRef]
  33. Ito T, Sakai A, Maruyama M, et al. Dorsal Root Ganglia Homeobox downregulation in primary sensory neurons contributes to neuropathic pain in rats. *Mol Pain*. 2020;16:1744806920904462. [CrossRef]
  34. Pineault KM, Wellik DM. Hox genes and limb musculoskeletal development. *Curr Osteoporos Rep*. 2014;12(4):420-427. [CrossRef]
  35. Roux M, Zaffran S. Hox genes in cardiovascular development and diseases. *J Dev Biol*. 2016;4(2). [CrossRef]
  36. Searcy RD, Yutzey KE. Analysis of Hox gene expression during early avian heart development. *Dev Dyn*. 1998;213(1):82-91. [CrossRef]
  37. Rodgers HM, Huffman VJ, Voronina VA, Lewandoski M, Mathers PH. The role of the Rx homeobox gene in retinal progenitor proliferation and cell fate specification. *Mech Dev*. 2018;151:18-29. [CrossRef]
  38. Onuoha CP, Ipe J, Simpson E, Liu Y, Skaar TC, Kreutz RP. MicroRNA sequencing in patients with coronary artery disease - considerations for use as biomarker for thrombotic risk. *Clin Transl Sci*. 2022;15(8):1946-1958. [CrossRef]
  39. Ghafouri-Fard S, Gholipour M, Taheri M. Role of microRNAs in the pathogenesis of coronary artery disease. *Front Cardiovasc Med*. 2021;8:632392. [CrossRef]
  40. Wang M, Ji Y, Cai S, Ding W. MiR-206 suppresses the progression of coronary artery disease by modulating vascular endothelial growth factor (VEGF) expression. *Med Sci Monit*. 2016;22:5011-5020. [CrossRef]
  41. Zhu L, Chen T, Ye W, et al. Circulating miR-182-5p and miR-5187-5p as biomarkers for the diagnosis of unprotected left main coronary artery disease. *J Thorac Dis*. 2019;11(5):1799-1808. [CrossRef]
  42. Yue R, Lu S, Luo Y, et al. Mesenchymal stem cell-derived exosomal microRNA-182-5p alleviates myocardial ischemia/reperfusion injury by targeting GSDMD in mice. *Cell Death Discov*. 2022;8(1):202. [CrossRef]
  43. Wang H, Jiang L, Li Z, Wang W, Hao C. miR-6835-3p regulates the function of pancreatic islet cells by modulating the expression of AdipoR1. *Int J Mol Med*. 2018;42(3):1317-1326. [CrossRef]
  44. Liu Q, Zhu A, Gao W, et al. miR-5590-3p inhibits the proliferation and metastasis of renal cancer cells by targeting ROCK2 to inhibit proliferation, migration and invasion. *Oncol Lett*. 2022;24(4):377. [CrossRef]

45. Barbalata T, Moraru OE, Stancu CS, et al. Increased miR-142 levels in plasma and atherosclerotic plaques from peripheral artery disease patients with post-surgery cardiovascular events. *Int J Mol Sci.* 2020;21(24). [\[CrossRef\]](#)
46. Yi F, Hao Y, Chong X, Zhong W. Overexpression of microRNA-506-3p aggravates the injury of vascular endothelial cells in patients with hypertension by downregulating Beclin1 expression. *Exp Ther Med.* 2018;15(3):2844-2850. [\[CrossRef\]](#)
47. Qin Q, Cui L, Zhou Z, Zhang Z, Wang Y, Zhou C. Inhibition of microRNA-141-3p reduces hypoxia-induced apoptosis in H9c2 rat cardiomyocytes by activating the RP105-dependent PI3K/AKT signaling pathway. *Med Sci Monit.* 2019;25:7016-7025. [\[CrossRef\]](#)
48. Chen ZQ, Zhou Y, Chen F, et al. miR-200a-3p attenuates coronary microembolization-induced myocardial injury in rats by inhibiting TXNIP/NLRP3-mediated cardiomyocyte pyroptosis. *Front Cardiovasc Med.* 2021;8:693257. [\[CrossRef\]](#)



Supplementary Figure 1. Correlation between CNTN1 expression and TFs.