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.1 This disease is characterized by the gradual buildup of atherosclerotic plaques within the coronary arteries, impeding the crucial blood supply to the heart muscle.2 The interplay of genetic predispositions, environmental factors, and lifestyle choices converges to initiate and perpetuate this pathological process.3 Coronary artery disease manifests in a spectrum of clinical presentations from asymptomatic atherosclerosis to debilitating angina and potentially fatal myocardial infarctions.4 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.

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.6 Pulmonary arterial hypertension is primarily driven by pathological changes in the pulmonary vasculature, including vascular remodeling, vasoconstriction, and endothelial dysfunction.7 The intricate
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.\textsuperscript{8} Patients diagnosed with PAH face a 4-fold higher risk of CAD when compared to the general population.\textsuperscript{9} Pulmonary hypertension is commonly observed in CAD-associated heart failure.\textsuperscript{10} A significant occurrence of CAD has been found in individuals with pulmonary hypertension secondary to chronic obstructive pulmonary disease.\textsuperscript{11} In systemic sclerosis patients, CAD and PAH were also shown considerable overlap.\textsuperscript{12}

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, aiding 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\textsuperscript{13} (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 (\(k\)) 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"\textsuperscript{14} (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 \(|\log_{2}FC| > 0.3\) (equivalent to a fold change greater than 1.23) and an adjusted \(P\)-value (\(p\text{-adj}\)) less than .05.

Functional Enrichment Analysis Based on Differentially Expressed Genes

The R package "clusterProfile"\textsuperscript{n15} (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\textsuperscript{16} (https://string-db.org/, version 11.0) to analyze protein functional connections and interactions. Subsequently, Cytoscape\textsuperscript{17} (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\textsuperscript{18} (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)\textsuperscript{19} 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 between expression levels of our target gene and immune cell infiltration ratio comparisons between different groups. 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 R20 (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 \(|\text{correlation}| > 0.5 \) 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-associated genes and upregulated DEGs in CAD samples, a total of 463 intersecting genes were obtained and considered as CAD-related genes. 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) Relationship of module and traits. (F) Differential gene expression analysis of CAD samples and normal samples. (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.
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 and DEGs between CAD and normal...
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 $P < 0.05$ 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 945bp 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/), 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 ≥ 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).
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 neuron-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. Contactin 1 is a glycosylphosphatidylinositol (GPI)-anchored protein that is primarily expressed in the nervous system, particularly during early embryonic development. It is involved in cell adhesion and is known to participate in axon guidance, neuronal migration, and the formation of neural circuits. Overexpression of CNTN1 has been proven to promote cell proliferation in a breast cancer cell line. 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. Its expression level is also significantly related to incident heart failure. In menopausal women, CNTN1 expression level has already been found to be a marker for detecting and diagnosing CAD, consistent with our research. Being identified as a co-pathogenetic gene in CAD and PAH, CNTN1 has been rarely detected in relationship with PAH, but CNTN1 is associated with lung cancer and contributes to drug resistance to lung adenocarcinoma. 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, as CNTN1. HOXD3 is involved in the regulation of anterior-posterior
identity during embryogenesis and is essential for proper limb development,34 playing a vital role in neural system. HOX TFs function in cardiovascular development,35 and the administration of retinoic acid leads to an upregulation in the expression of HOXD3 in cardiac explants of chick embryos.36 RAX, also known as retinal antioxidant X, is a transcription factor that plays a crucial role in eye development and photoreceptor differentiation.37 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.38 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.39 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.40 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.41 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.42 Hsa-miR-6835-3p not only regulates CNTN1, but also influences pancreatic islet cell function by regulating AdipoR1.43 Hsa-miR-5590-3p is able to inhibit renal cell metastasis.44 Levels of hsa-miR-142-5p may serve as an independent indicator for forecasting cardiovascular incidents in individuals with peripheral artery disease.45 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.46 MiR-141-3p inhibition is able to reduce apoptosis induced by hypoxia,47 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.48

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 Figure 1. Correlation between CNTN1 expression and TFs.