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THE NOVEL LINCRNA JUNI FIND BY COMPUTATIONAL INVESTIGATION OF THE EXPRESSION PATTERNS OF LNCRNAS IN CORONARY ARTERY DISEASE

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ABSTRACT

Background: Long non-coding RNAs (lncRNAs) have emerged as important regulators of cardiovascular pathology and may serve as promising biomarkers for coronary artery disease (CAD). However, the contribution of many lncRNAs to CAD remains unclear. We aimed to identify novel CAD-associated lncRNAs by integrating publicly available transcriptomic datasets.

Methods: Three Gene Expression Omnibus (GEO) datasets were analyzed to identify differentially expressed lncRNAs between CAD patients and healthy controls. To explore the potential functions of the identified lncRNAs, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using lncRNA-associated genes. The expression of JUNI was further validated by quantitative real-time PCR (qRT-PCR).

Results: We identified 107 differentially expressed genes across the three datasets, including 14 dysregulated lncRNAs (11 upregulated and 3 downregulated) in CAD patients relative to controls. Functional enrichment analysis suggested that these lncRNAs are associated with transcriptional regulation, apoptotic signaling, and pathways potentially involved in CAD pathogenesis. qRT-PCR analysis confirmed significant upregulation of JUNI in CAD samples.

Conclusions: JUNI may represent a novel CAD-associated lncRNA. Our findings provide several of candidate lncRNAs for further investigation as potential biomarkers and regulatory molecules in CAD, although independent validation and mechanistic studies are required before clinical application.

KEYWORDS: LncRNAs, CAD, Differentially expressed lncRNAs, lincRNA JUNI (LINC01135).

1. INTRODUCTION

Coronary artery disease (CAD) is a major contributor to mortality and disability worldwide (Ahari 2025). The most common pathological basis of CAD is atherosclerosis, a chronic inflammatory process of atherosclerosis and inflammation. This condition arises when the coronary arteries, responsible for delivering oxygenated blood to the myocardium, become constricted or obstructed owing to the accumulation of cholesterol and lipid deposits (Kong 2022) . Atherosclerotic plaques are composed of smooth muscle cells, inflammatory cells, lipids, necrotic cells, calcium deposits, and proteins from the extracellular matrix. Furthermore, matrix metalloproteinases are released by endothelial cells as a response to various stimuli such as oxidation and inflammatory factors, contributing to tissue remodeling and the degradation of the extracellular matrix. A key stage in the development of human atherosclerosis involves smooth muscle cells migrating from the media to the intima, where they then proliferate (Pandit 2025) . CAD may be classified into three distinct categories: stable angina, unstable angina, and acute myocardial infarction (AMI) (Nakahara 2017). Various risk factors for CAD are known, which include hypertension, dyslipidemia, diabetes mellitus, obesity, tobacco use, unhealthy diet, and gender, among others. Despite advances in the clinical interventions of CAD, early detection and treatment remain formidable tasks due to a lack of understanding of its pathological mechanisms and limitations in the current diagnostic biomarkers and therapeutic targets, which often have low sensitivity and Malakar (Malakar 2019).

Long non-coding RNAs (lncRNAs) are a class of transcripts longer than 200 nucleotides with limited or no protein-coding potential. They are essential in various biological functions, including regulating genes, facilitating cellular development, and responding to environmental changes (Gong 2021, Jiang 2024). Recent studies have emphasized the significance of lncRNAs in diverse cellular functions, such as chromatin remodeling, transcriptional control, and post-transcriptional regulation (He, R.-Z. 2019). Studies have demonstrated that lncRNAs are located in both the

cytoplasm and the nucleus and are also present in detectable concentrations in human body fluids such as plasma, serum, and exosomes or extracellular vesicles. Besides, lncRNAs have remarkable stability and can effectively reflect the in vivo health or disease, suggesting their possible application as non-invasive biomarkers for diagnosis and prognosis (Mattick 2023, Bridges 2021, Gu 2024). CAD typically arises from irregular functions of biological processes, including cell motility, immune response, metabolism, and inflammatory response which lncRNAs play a direct or indirect role in these processes (Devaux Y 2015). These advancements provide opportunities to improve our comprehension of the complex pathological processes associated with CAD.

In the present study, we performed an integrated analysis of three GEO datasets to identify differentially expressed lncRNAs between CAD patients and healthy controls. We further explored the potential biological roles of these candidate lncRNAs using functional enrichment analysis of associated genes and performed preliminary qRT-PCR validation of JUNI expression. This study aimed to identify novel CAD-associated lncRNAs and provide a basis for future investigations into their diagnostic and biological significance.

2. METHODS AND MATERIALS

2.1. Data source

Three publicly available transcriptomic datasets related to CAD were retrieved from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The datasets included GSE159657, comprising plasma exosomal long RNA profiles from 18 patients with CAD and 10 controls; GSE166780, including peripheral blood monocyte RNA-seq data from 16 CAD patients and 8 non-CAD individuals; and GSE205255, containing RNA-seq data from peripheral blood mononuclear cells (PBMCs) obtained from 5 CAD patients and 5 healthy controls.

2.2. Bioinformatics Pipeline

All bioinformatic analyses were conducted in R (version 4.4.1). Data import, formatting, and harmonization of gene identifiers across datasets were performed using standard R workflows (including the dplyr package for data handling). Where applicable, inter-dataset technical variation was accounted for using ComBat-seq implemented in the sva package. Differential expression analysis was performed using the DESeq2 package. Genes/lncRNAs with an absolute log2 fold change $|\log2\ FC| > 1$ and adjusted p-value < 0.05 were considered differentially expressed. A volcano plot was utilized to illustrate the highly and lowly expressed genes in the datasets.

2.3 Analysis of Functional Enrichment

To explore the potential functional relevance of the identified lncRNAs, enrichment analyses were performed using multiple resources, including NcPath, RNAenrich, and LncSEA 2.0. Gene Ontology (GO) terms (biological process, cellular component, and molecular function) and pathway-level annotations were examined. Enrichment results with adjusted p-value < 0.05 were considered statistically significant. Enrichment findings were interpreted as hypothesis-generating and were used to prioritize candidate biological processes/pathways potentially related to CAD.

2.4 Specimen Collection

The MASHAD study is a cohort study that included 9,704 men and women aged 35 to 65, all of whom had no chronic illnesses (e.g., CVD, cancer, and chronic kidney disease) at the outset. Its goal was to explore the relationship between various cardiovascular disease (CVD) risk factors, such as nutrition, environment, and genetic factors, and the development of CVD. Participants were chosen through a stratified cluster random sampling technique from three areas in Mashhad, Iran, which were divided into nine regions surrounding Mashhad Healthcare Centers. Recruitment began in 2010, with follow-up extending throughout 5 to 6 years. After six years, a cardiologist confirmed CVD events. Those participants who did not complete the follow-up were excluded. The Ethics Committee of the Mashhad University of Medical Sciences approved the study, and written informed consent was obtained from all participants (Ghayour-Mobarhan 2015, Asadi 2019). In the present study, a total of fifty CAD and fifty healthy individuals' serum specimens were selected from the MASHAD study cohort, which was stored at −80°C.

2.5 Quantitative Real-time PCR (qRT-PCR) assay

The TRIZOL solution was used to extract total RNA from serum samples according to the manufacturer's instructions (Sinaclon, Tehran, Iran). After extraction, the quality and quantity of all total RNA samples were assessed using 2% Agarose Gel Electrophoresis and the Nanodrop spectrophotometer (NanoDrop® 2000; Thermo Fisher Scientific, USA), which measured the 260/280 OD ratio. Then, all specimens were stored at -80 °C until used.

The primer pairs for each gene were developed under optimal conditions by Gene Runner software (version 6.5.52). Following the selection of the suitable primers, their sequences were checked for specificity using BLAST on the NCBI website. All primers utilized for RT-PCR were produced by Genfanavaran (Tehran, Iran).

We examined the expression of lincRNA JUNI in CAD patients and the healthy control group by One-Step qRT-PCR. To conduct the One-Step RT-qPCR assay using the Ultra–MMLV Reverse Transcriptase kit (Parstous, Tehran, Iran). The qRT-PCR reactions were conducted in a final volume of 15 µl, which included 4 µl of total RNA, 1 µl RT Enzyme , 0.4 µl of forward and reverse primers, 7.5 µl of SyBr Green Mix, and 1.7 µl of ddH2O. The qRT-PCR thermal cycling protocol included an initial pre-incubation step for 15 min at 50°C and 3 min at 95°C, followed by 40 cycles of 10 seconds at 95°C, 20 seconds at 56°C, and 10 second at 72°C. The final extention was 3 min at 72°C. The sequences

of the JUNI and GAPDH primers were as follows: forward: 5′-CGGACACTCGCATAAAGTCA-3′, reverse: 5′-AGCGCTTCCTAGAGGCTACC-3′, forward:, 5′-GCCACATCGCTCAGACAC-3′, reverse: 5′-GCAACAATATCCACTTTACCAGAG-3′. Each sample was analyzed in duplicate, and GAPDH was used as an internal control to normalize all gene expression data. Differences in expression levels due to ploidy were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.6 Statistical analysis

Statistical evaluations were conducted using SPSS (version 25.0), GraphPad Prism (version 8.0), and R software (version 3.5.1). The Mann-Whitney test was utilized to compare gene expression levels between the two groups: CAD and the healthy individuals group. Data that are continuous variables are shown as mean ± standard deviation (SD). In order to evaluate the potential prognostic role of lncRNA JUNI expression on time-to-event outcomes, Cox proportional hazards regression analysis was performed.

Receiver operating characteristic (ROC) curve analysis was carried out to assess the potential of lincRNA JUNI as a diagnostic biomarker in CAD. All analyses considered a 95% confidence interval, with P-values less than 0.05 indicating statistical significance.

3. RESULTS

3.1 LncRNA expression profiles of CAD patients and the control group

A meta-analysis was performed on RNA sequencing datasets associated with CAD to identify genes that are differentially expressed. Using the DESeq2 package, our investigation identified 107 genes with differential expression in CAD patients compared to controls across three datasets. Among the genes investigated were those with significantly different lncRNAs. We found 14 novel lncRNAs that were specifically altered in CAD patients, with 11 (namely MKNK1-AS1, ST7-AS2, NFE4, ITGB1-DT, DISC1-IT1, LINC00102, ARHGAP31-AS1, ZNF341-AS1, LINC01135 (JUNI), TOB1-AS1, HOTAIRM1) exhibiting upregulation and 3 downregulation (namely LDLRAD4-AS1, FAM225A, PART1) (FC > 1, $p < 0.05$, **Table 1**). The genes with altered expression were visualized using a volcano plot (**Fig 1**).

3.2 Gene ontology analysis of over-expressed lncRNAs in CAD patients

LncRNAs are known for their role in regulating the expression of associated protein-coding genes. Therefore, we conducted Gene Ontology (GO) term analysis, encompassing BP, CC, MF, and KEGG pathway analysis to investigate the functions of the altered expression lncRNAs which found in CAD patients compared to the healthy individual group. **Fig 2A and 2B** show the top 20 most enriched GO terms according to the RNAenrich database.

Apoptotic signaling pathway (GO:2001233), gland development (GO:0048732), and reproductive structure development (GO:0048608) were the most enhanced BP terms. RNA polymerase II transcription regulator complex, transcription regulator complex, and PML body were among most enriched CC terms. The most enriched MF terms were DNA-binding transcription activator activity, RNA polymerase II-specific, DNA-binding transcription activator activity, and ubiquitin-like protein ligase binding, and all were significant after statistical adjustment ($adj\_p < 0.05$).

Moreover, these 11 overexpressed lncRNAs exhibit combinatorial histone binding patterns with H3T11P, H3K4me2-3, and H3K122ac, implying a complex regulatory network where multiple histone modifications are integrated to influence gene transcriptional regulation (**Table 2**). In heart tissue, ARHGAP31-AS1, LINC01135, and TOB1-AS1 lncRNAs regulate super enhancers. Additionally, MKNK1-AS1, ZNF341-AS1, and LINC01135 regulate super enhancers in peripheral blood primary cells (**Fig 2C**).

3.3 Pathway Enrichment Analysis

A KEGG pathway analysis was carried out to find the pathways with the most enrichment for DElncRNAs. Afterward, the KEGG pathways related to lncRNAs with increased expression are shown in **Fig 3A and B**. The enriched pathways were the platinum drug resistance, thermogenesis, axon guidance, tight junction, small cell lung cancer, FoxO signaling pathway, and cell cycle. Of note, the FoxO signaling pathway, which has been reported to be associated with the pathogenesis of CAD, was observed in our results (**Fig 3A**). In order to gain a further understanding of DE lncRNAs in various biological pathways, we conducted a Reactome pathway enrichment analysis. Through this analysis, it was determined that CAD is influenced by several major pathways, such as interleukin-4 and interleukin-13 signaling, oncogene-induced senescence, and cellular senescence (**Fig 3B**).

3.4 Construction of the lncRNA-mRNA network

The RNAenrich database was utilized to construct a network of interactions between lncRNAs and mRNAs to uncover the functional connections between DEGs and lncRNAs. The results of this analysis revealed that three significant lncRNAs, including HOTAIRM1, ARHGAP31-AS1, and LINC01135, target genes and control their expression. This interaction is visualized by the lncRNA-mRNA network, which highlights the role of lncRNAs in gene expression regulation in CAD (**Fig 4**).

3.5 Overexpression of lincRNA JUNI in CAD serum and its associationwith clinicopathological features of CAD patients

Analysis of JUNI expression data indicated a significant overexpression of this LincRNA in CAD serum compared with non-CAD specimens (P-value <0.05; **Fig 5**).

The present study investigated the association between the high level of JUNI expression and clinicopathological features including age, gender, Glocuse, LDL, Chleosterol, HDL,Triglysrides, Dyslipidemia, Diabete, HsCRP, SmokingStatus, Hypertension history, Diabet history, Hyperlipidemia history, HTN2 (Hypertension >=140), BMI, CADevent in CAD patients. The obtained findings demonstrated that there was a significant association between JUNI overexpression and CHDevent (p-value< 0.001) in CAD patients.

Cox proportional hazards regression analysis was performed to evaluate the association between lncRNA JUNI expression and time to event in the study population. The results showed that JUNI expression was not significantly associated with the hazard of the outcome (HR = 1.026 95% CI: 0.987–1.026, $p > 0.05$). In contrast, group status (CAD vs. normal) demonstrated a strong and statistically significant association with time to event (HR = 0.19, 95% CI: 0.003–0.138, $p < 0.001$). These findings indicate that individuals in the CAD group had a significantly different risk of experiencing the event over time compared with the normal group. However, the expression level of lncRNA JUNI did not independently predict the hazard of the outcome in the Cox regression model (**Table 3**).

The ROC curve analysis showed a maximum area under the curve of 0.64 (95% CI 0.53–0.75, P = 0.012) with a sensitivity and specificity of 78%, and 42%, respectively (**Fig 6**).

4. DISCUSSION

Coronary artery disease (CAD) is a significant and growing global health concern, acting as a leading factor in deaths and disabilities (Coronado 2023). Although considerable research has been conducted in recent decades regarding the molecular mechanisms underlying CAD, there remains much more to learn (Kim 2023). Recent advances in genomics and transcriptomics have established that lncRNAs represent a major class of non-coding RNAs. They are implicated in a diverse array of functions in many biological processes, and their dysregulation contributes to the pathogenesis of many diseases (Statello 2021). CAD has also been identified among those diseases. Recent observations indicated that lncRNAs play a vital functional role in the development as well as the initiation of CAD (Ahadi 2021). However, lncRNA regulation and functional significance in CAD progression remain unclear. Noncoding RNAs, including lncRNAs, have been found to possess the possibility of being circulating biomarkers for CAD and other cardiovascular diseases. lncRNAs play a role in many biological functions and hold potential as novel molecular biomarkers for various diseases (Le 2023).

In this study, we analyzed the expression patterns of lncRNA in patients with CAD and in healthy individuals through RNA-seq analysis. This analysis identified 14 differentially expressed lncRNAs in CAD samples. Among them, 11 lncRNAs were upregulated and 3 lncRNAs were downregulated in patients with CAD compared with the healthy control group, with some of these lncRNAs being documented for the first time in our research. In order to define the functional importance of lncRNAs that are expressed differently in CAD, we conducted GO and KEGG analyses, which revealed several related biological processes and signaling pathways. The most highly enriched BP terms were apoptotic signaling pathway, gland development, and reproductive structure development. The CC terms that were most significantly enriched included the RNA polymerase II transcription regulator complex, the transcription regulator complex, and the PML body. The most notable MF terms were DNA-binding transcription activator activity, RNA polymerase II-specific activity, and DNA-binding transcription activator activity along with ubiquitin-like protein ligase binding. KEGG analyses identified the signaling pathways, specifically the FoxO signaling pathway and the signaling pathways related to interleukin-4 and interleukin-13. The dysregulation of the Apoptosis, FoxO, and interleukin-4 and interleukin-13 signaling pathways has been associated with various factors of CVD, particularly CAD (Kim 2010, Ronnebaum 2010, Bakhshian 2022).

In the current study, up-regulated lncRNA-JUNI, which is linked to the MCM2 gene, has revealed associations with CAD events in CAD patients. This result indicates that lncRNA-JUNI might be involved in signaling pathways associated with atherosclerosis progression and cardiovascular events via regulatory interactions with minichromosome maintenance complex component 2 (MCM2). The MCM2 protein is one of the components of MCM2-7 proteins which is responsible for functioning as a DNA helicase important for DNA replication. Elevated expression levels of MCM2 usually indicate proliferative cellular function as MCM2 has been reported to be highly expressed in actively dividing cells (Snyder 2009). In the setting of CVD, uncontrolled proliferation of vascular smooth muscle cells (VSMCs) and endothelial cells leads to the formation and progression of atherosclerotic lesions. Up-regulation of JUNI expression associated with CAD events may indicate proliferative activity of vascular cells. By means of its regulatory interaction with MCM2, JUNI can affect cellular proliferation via influencing DNA replication in vascular cells which results in smooth muscle cells proliferation. These events have an important influence on plaque expansion and can even result in luminal narrowing and instability of the plaque itself, making it more prone to cause coronary disorders like myocardial infarction and unstable angina. Furthermore, high levels of

JUNI may indicate some important connection with inflammatory signaling pathways in the process of atherosclerotic disease development. It is well-known that chronic inflammation in blood vessels causes endothelial activation, immune cell infiltration, and the development of lipid-rich plaques. There is a possibility of the interaction between proliferative events and inflammation. The lincRNA JUNI (JUN-DT, LINC01135) is responsible for modulating cell processes under stress. It does this by influencing the levels of c-Jun, a protein responsible for cell growth and apoptosis, and modulating cell migration and survival. It modulates these processes via the DUSP14-JNK pathway, a signaling pathway. JUNI function suggests possible significance in a range of biological processes, including cellular stress response, migration, and survival pathways. Research into this pathway could offer clues to potential therapeutic targets concerning stress responses (Kumar 2024). To examine the potential of lncRNA JUNI expression in predicting the hazard ratio in time-to-event outcomes, Cox proportional hazards regression analysis was conducted. According to the obtained results, there were no statistically significant differences between lncRNA JUNI expression and the hazard ratio of the outcome in the study population. In contrast, disease status showed a strong and statistically significant association with event risk. Patients in the CAD group demonstrated a significantly higher hazard of experiencing the event compared with the normal group. These findings suggest that although the presence of CAD is a major determinant of time-to-event risk, the expression level of lncRNA JUNI does not independently predict the occurrence of the event in this cohort. Therefore, JUNI expression may not serve as a reliable prognostic biomarker for event timing in CAD based on the current Cox regression model.

A research found that lncRNA NEAT1 enhances cell survival and inhibits apoptosis in CAD cells by potentially activating the miR-140-3p/MAPK1 pathway. In addition, lncRNA NEAT1 could act as a possible treatment target for CAD (Zhang 2020). A study of prostate cancer assessed the state of a core autophagy-related lncRNA by a competing endogenous RNA network. It indicated that the variation of MKNK1-AS1 expression was an independent predictor, and the C-index was 0.882 (Li 2021). Further, there is currently no study on MKNK1-AS1 in other diseases, specifically CAD. Research has shown that autophagy is both protective and detrimental in CAD. It can help remove dysfunctional cellular content and reduce inflammation, and defend cells. Excessive or defective autophagy, however, will induce cell death and disease progression (Jiang 2023). The LDLRAD4 gene, also referred to as C18orf1, is situated on chromosome 18p11.2 and contains eight possible antisense lncRNAs, with LDLRAD4-AS1 being the longest (Nakano 2014). Research has shown that lncRNA LDLRAD4-AS1 resides in the nucleus of colorectal cancer (CRC) cells, where it is expressed at higher levels in the majority of CRC samples and cell lines. The heightened expression of lncRNA LDLRAD4-AS1 is correlated with poorer prognoses for patients with CRC. Additionally, the overexpression of lncRNA LDLRAD4-AS1 has been found to promote the migration and invasion capabilities of CRC cells in vitro and to aid in CRC metastasis in animal models (Mo 2020). Du Q et al. performed a functional enrichment analysis on the genes within the identified ceRNA networks related to EC, revealing a significant correlation between ST7-AS2 and survival rates of EC patients (Du 2022). NFE4 functional analysis identified a gene associated with m6A and cuproptosis-related lncRNAs in ccRCC. Caki-1 /OS-RC-2 cell proliferation and migration were also found to be inhibited in the NFE4 knockdown group. Also, NFE4 is highly expressed in patients with brain metastasis and has a good diagnostic effect (Feng 2024, Sun 2024). ITGB1, known as integrin β1, is upregulated and has been demonstrated to have oncogenic functions in various cancer types, such as lung adenocarcinoma, stomach adenocarcinoma, and breast cancer (Chang 2021, Jiang 2022, Esfandiari 2023). In a study focused on lung adenocarcinoma, the integrated analysis of lncRNA revealed that DISC1-IT1 lncRNA was upregulated and can function as a possible prognostic biomarker for individuals with LUAD (Wu 2020). Another study has reported that ARHGAP31-AS1 expression is altered aberrantly in Dilated cardiomyopathy (Zhang 2023). TOB1-AS1 is a TOB1 gene cluster lncRNA and has been reported as a tumor suppressor; it has been found to play a regulatory function in cell cycle and cancer processes with negative correlations with disease markers (e.g., EDSS for multiple sclerosis) and its inhibition of proliferation, migration, and invasion across various cancer contexts via pathways such as Wnt/beta-catenin and miRNA axes (e.g., miR-23a/NEU1), indicating a multi-facet role in disease control (Dehghanzad 2020, Jiang 2019). HOTAIRM1 levels increase significantly in human umbilical vein endothelial cells (HUVECs) when stimulated by oxidized LDL. When HOTAIRM1 is knocked down, HUVEC growth slows down and cell death increases, both with and without oxidized LDL. HOTAIRM1 is mainly found in the nuclei of these cells. HOXA4 interacts with the HOTAIRM1 promoter, boosting its expression and forming a positive feedback loop. HSPA5 also interacts with HOTAIRM1, affecting HUVEC growth (Zhou 2024).

Our research has some limitations. Our information mainly comes from publicly accessible databases, and applying our findings in clinical settings requires additional exploration. Also, the RNA-seq sample size was small. A larger multicenter trial will be needed to provide reproducible and reliable validation of lncRNA biomarkers. Lastly, since these lncRNAs are novel, the molecular mechanism of their dysregulation in CAD patients remains unclear and the signature needs validation from external sources.

5. CONCLUSION

In current research, we analyzed the expression profiles of lncRNA in patients with CAD compared to a control group using RNA sequencing and from multiple GEO datasets. We identified a novel lncRNA landscape in CAD , highlighting 14 differentially expressed transcripts implicated in critical pathogenic processes like FoxO signaling and cellular senescence. Through integrated network and epigenetic analyses, we identified key regulatory hubs, including HOTAIRM1, ARHGAP31 AS1, and JUNI, that coordinate complex gene expression networks in cardiac tissue. Furthermore, clinical validation reveals that JUNI is significantly overexpressed in CAD serum and correlates with disease events. Collectively, these findings establish these lncRNAs as potential diagnostic biomarkers and provide a mechanistic foundation for future therapeutic targeting in CAD.

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Availability of data and material

All utilized and reported data in this project can be available with a reasonable request from the corresponding author.

Author Contributions

All authors contributed to the study conception and design. Conceptualization, R.A..; writing—original draft preparation, R.A.; review and editing, R.A., S.R., S.S.S, and G.M.M.; supervision, S.R., S.S.S, and G.M.M. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate and patient consent for publication

The Ethics Committee of the Mashhad University of Medical Sciences approved the study, and written informed consent was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

AI tools was employed for improving the language of the article, and not for generating scientific content or drawing scientific conclusions, neither for analysing or interpreting scientific data.

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Fig1. Volcano plot of differentially expressed genes in CAD patients compared with healthy individuals.

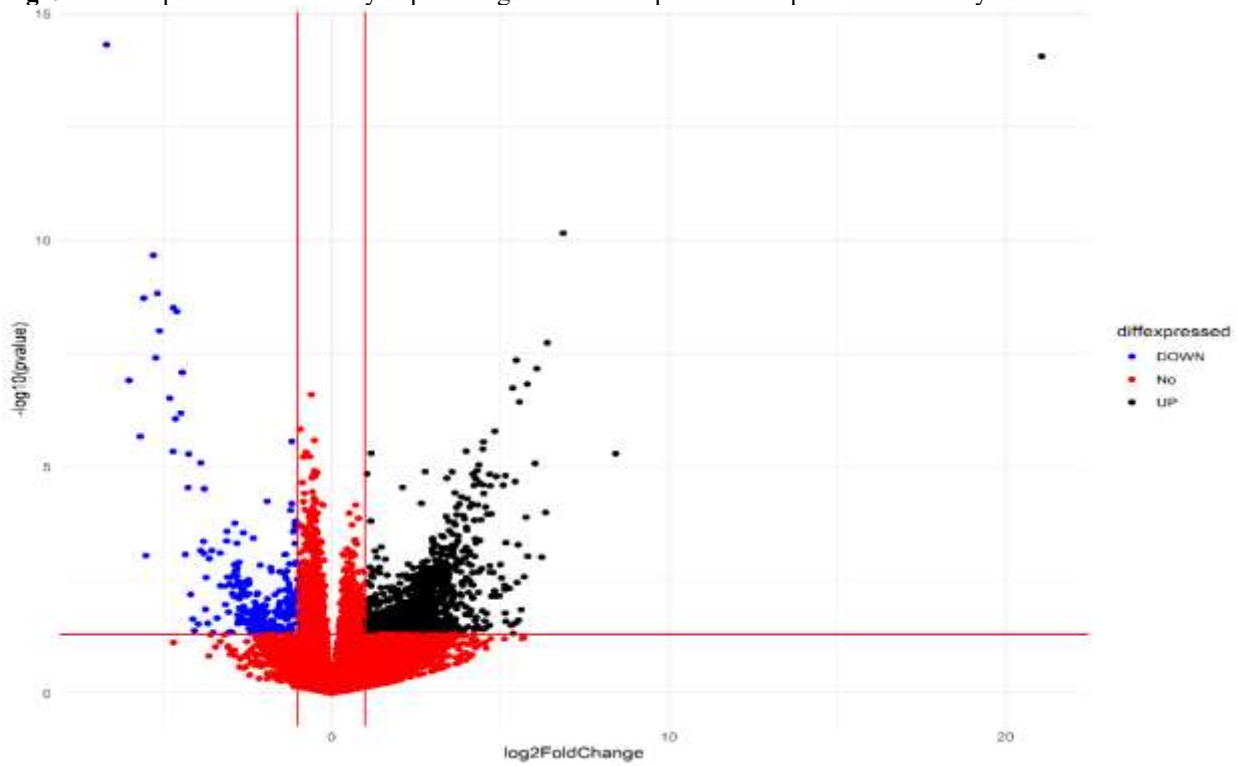
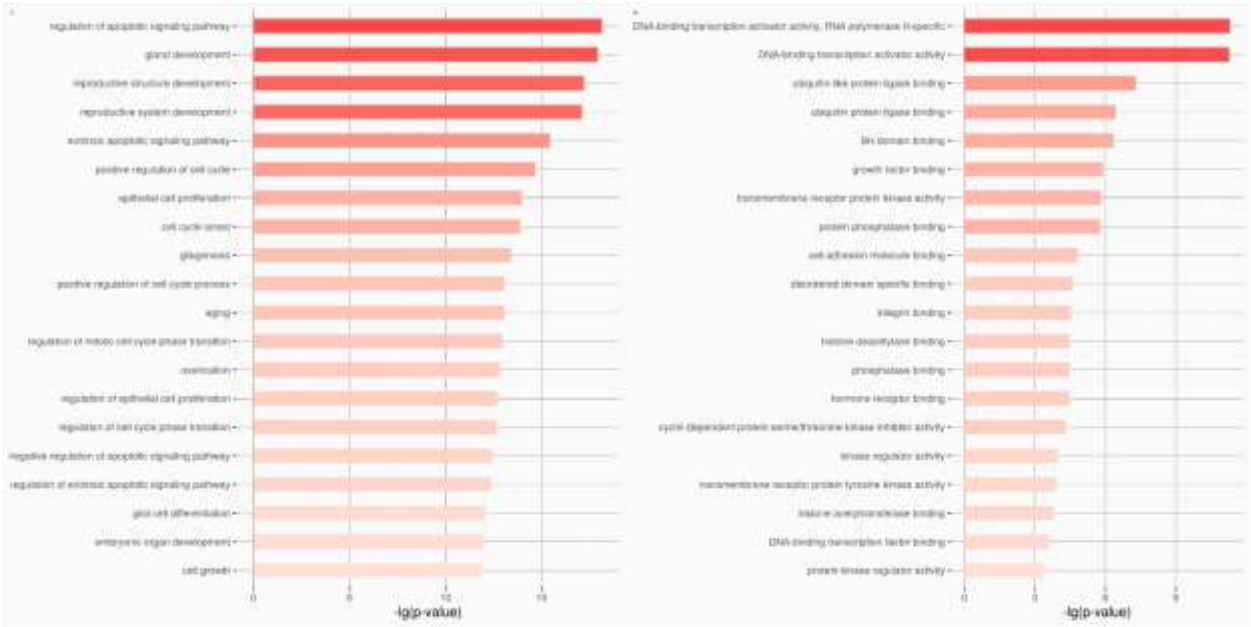


Fig 2. GO classification of overexpression lncRNAs in CAD patients compared with the healthy individual group. **(2A).** Top 20 biological processes enriched for over-expressed lncRNAs. **(2B).** Top 20 Molecular functions enriched for lncRNAs with increased expression. **(2C).** Overexpressed lncRNAs in CAD patients serve as super enhancers in CAD.


-lg(p-value)
-lg(p-value)

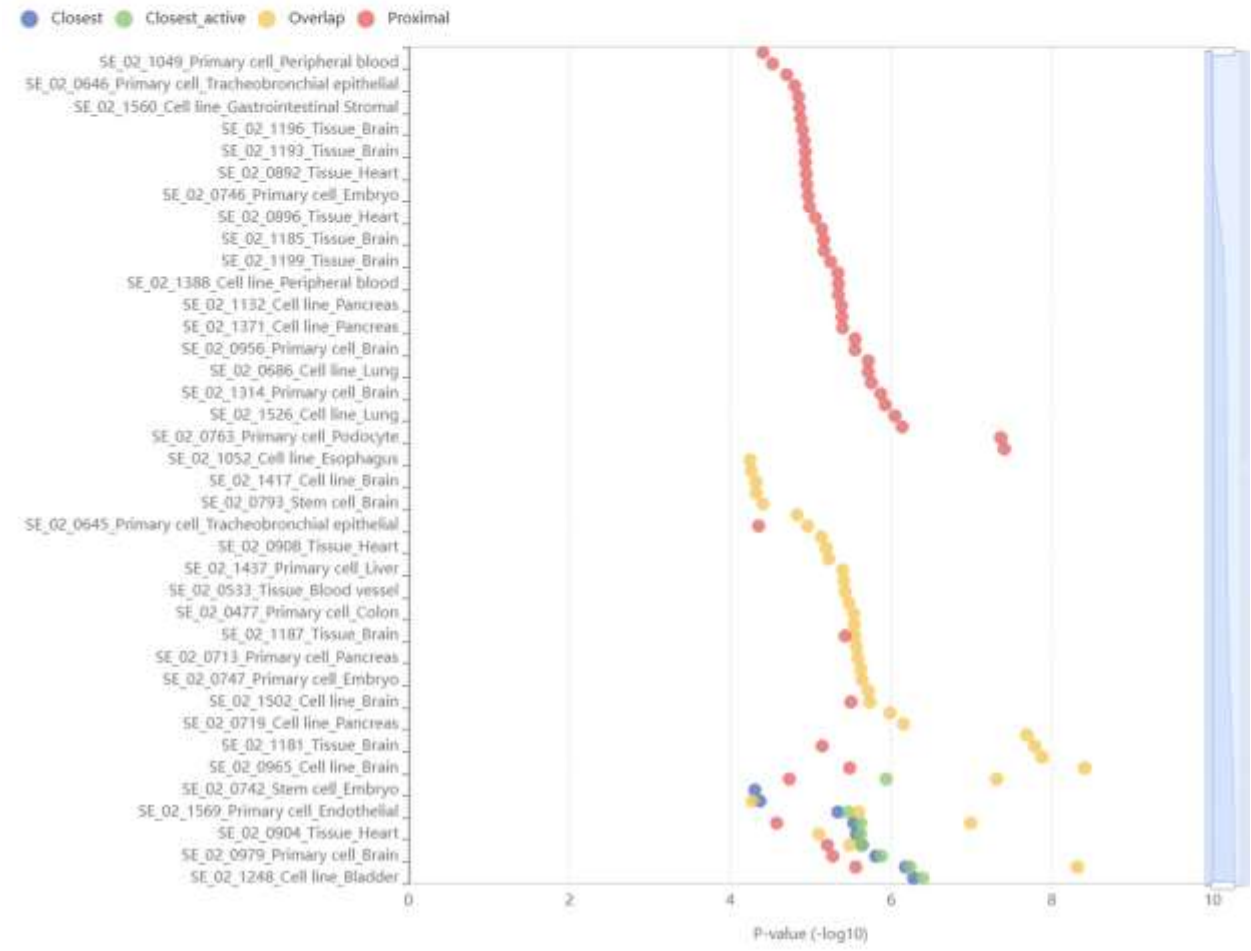

Closest
Closest_active
Overlap
Proximal
SE_02_1049_Primary cell_Peripheral blood
SE_02_0646_Primary cell_Tracheobronchial epithelial
SE_02_1560_Cell line_Gastrointestinal Stromal
SE_02_1196_Tissue_Brain
SE_02_1193_Tissue_Brain
SE_02_0892_Tissue_Heart
SE_02_0746_Primary cell_Embryo
SE_02_0896_Tissue_Heart
SE_02_1185_Tissue_Brain
SE_02_1199_Tissue_Brain
SE_02_1388_Cell line_Peripheral blood
SE_02_1132_Cell line_Pancreas
SE_02_1371_Cell line_Pancreas
SE_02_0956_Primary cell_Brain
SE_02_0686_Cell line_Lung
SE_02_1314_Primary cell_Brain
SE_02_1526_Cell line_Lung
SE_02_0763_Primary cell_Podocyte
SE_02_1052_Cell line_Esophagus
SE_02_1417_Cell line_Brain
SE_02_0793_Stem cell_Brain
SE_02_0645_Primary cell_Tracheobronchial epithelial
SE_02_0908_Tissue_Heart
SE_02_1437_Primary cell_Liver
SE_02_0533_Tissue_Blood vessel
SE_02_0477_Primary cell_Colon
SE_02_1187_Tissue_Brain
SE_02_0713_Primary cell_Pancreas
SE_02_0747_Primary cell_Embryo
SE_02_1502_Cell line_Brain
SE_02_0719_Cell line_Pancreas
SE_02_1181_Tissue_Brain
SE_02_0965_Cell line_Brain
SE_02_0742_Stem cell_Embryo
SE_02_1569_Primary cell_Endothelial
SE_02_0904_Tissue_Heart
SE_02_0979_Primary cell_Brain
SE_02_1248_Cell line_Bladder
0
2
4
6
8
10
P-value (-log10)

Fig (3A). KEGG pathway analysis by NcPth for differentially expressed lncRNAs. **(3B).** The top 20 Reactome pathways enriched of differentially expressed lncRNAs.

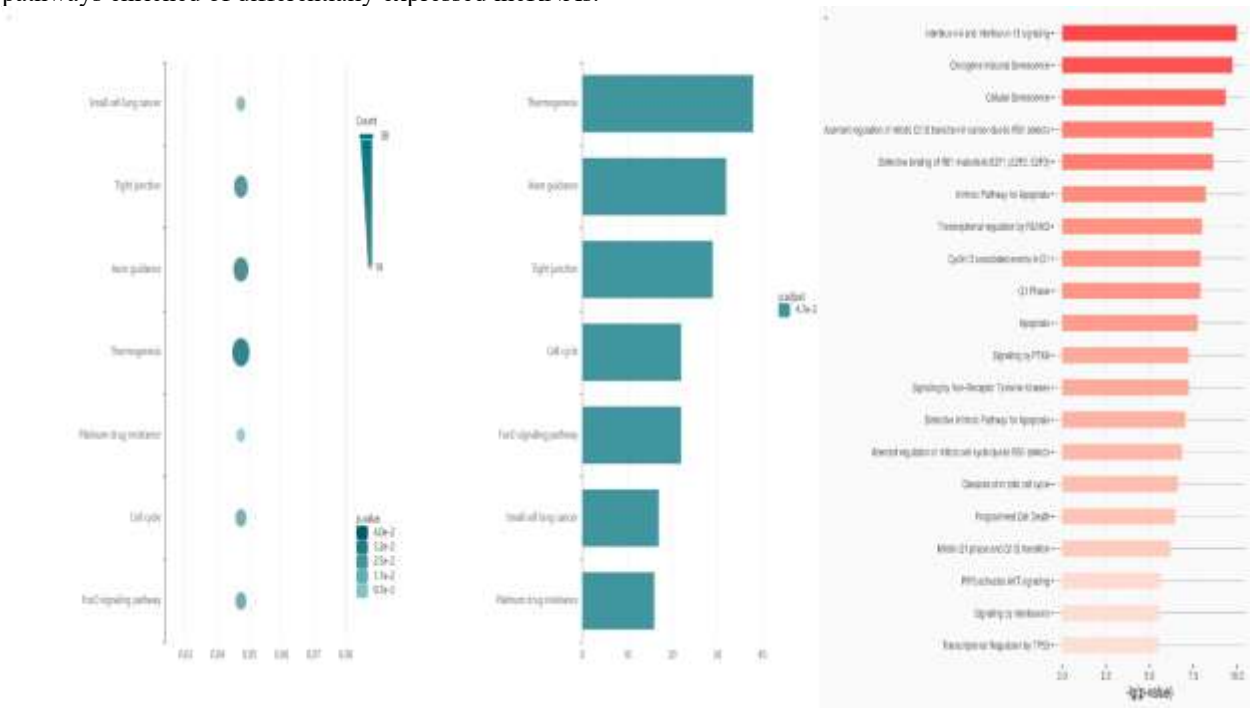


Fig 4. RNA-RNA network based on the upregulated lncRNAs. The shape represents how HOTAIRM1, ARHGAP31AS1, and LNC015 lncRNAs join in gene regulation network.

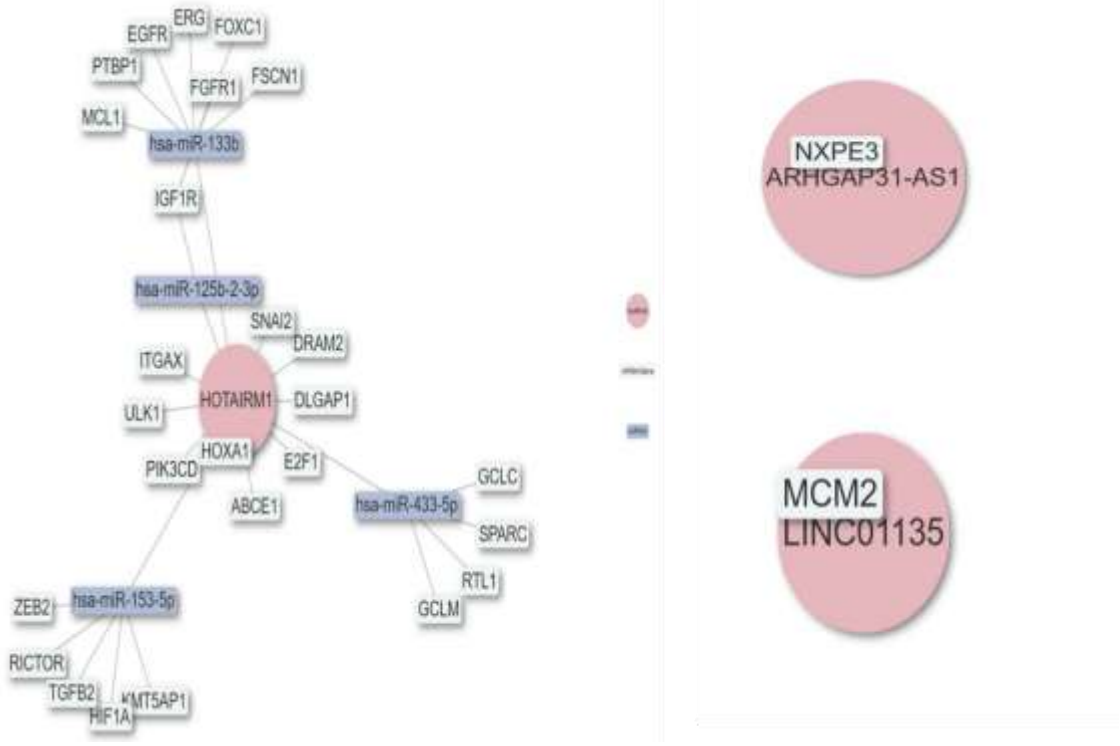


Fig 5. Overexpression of JUNI in CAD serum (mean in CAD ± SD = 5.03 ± 13.05) as compared with non-CAD (Mean of non-CAD ± SD = 1.18 ± 1.6) of CAD patients.

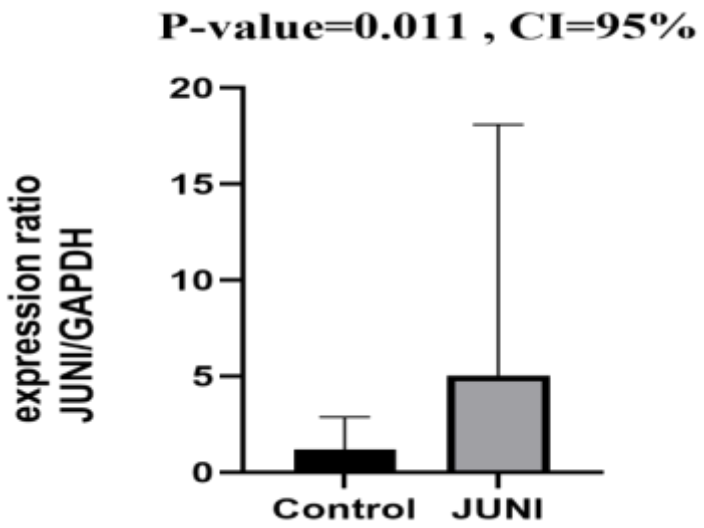


Fig 6. Receiver operating characteristic curve related to LncRNA JUNI gene expression levels. The area under the ROC curve (AUC) was 0.64 (95% CI = 0.53 - 0.75, *p*-value = 0.012)

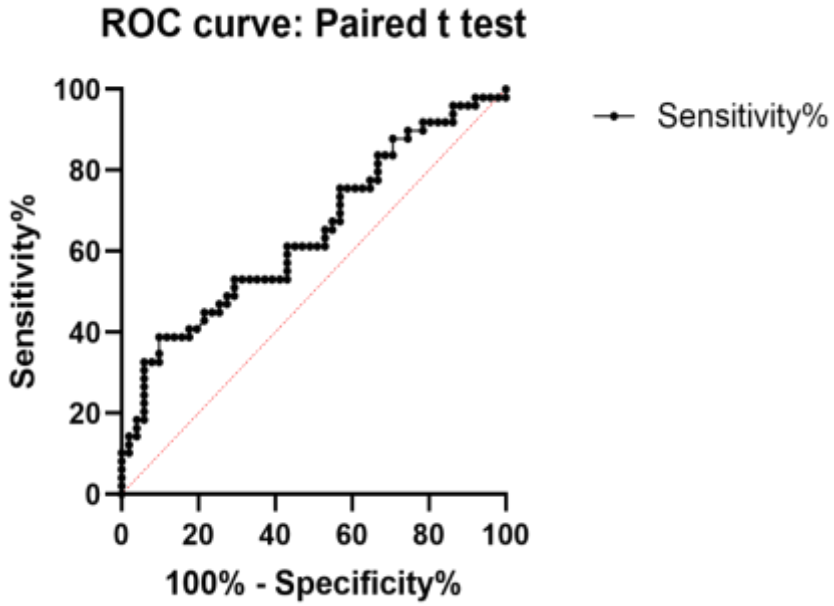


Table 1. List of differentially expressed genes in three integrated datasets. The baseMean, log2FoldChange, p-value, and p-adjust are included.

	Symbol	Log2Fold Change	P value	p-adjust	Type
lncRNAs	**MKNK1-AS1**	**6.394777822**	**1.83106E-08**	**3.4499E-05**	**Up**
	ARHGAP31-AS1	**4.702509359**	**2.61762E-05**	**0.007828354**	**Up**
	HOTAIRM1	**3.753493018**	**0.000335477**	**0.040004511**	**Up**
	ST7-AS2	**4.499481464**	**2.82508E-06**	**0.001900976**	**Up**
	NFE4	**3.577598697**	**1.29208E-05**	**0.005532762**	**Up**
	LINC00102	**4.40325518**	**2.37024E-05**	**0.007569104**	**Up**
	ITGB1-DT	**4.686599214**	**1.45559E-05**	**0.005604763**	**Up**
	DISC1-IT1	**4.269268274**	**1.80902E-05**	**0.006197048**	**Up**
	LINC01135	**3.903615392**	**0.000118564**	**0.021274941**	**Up**

ZNF341-AS1	**3.712109886**	**0.000115196**	**0.020869353**	**Up**
TOB1-AS1	**3.399816756**	**0.000123841**	**0.021482233**	**Up**
LDLRAD4-AS1	**-5.21572**	**3.91E-08**	**6.69E-05**	**Down**
FAM225A	**-4.48098**	**6.57E-07**	**0.000589**	**Down**
PART1	**-3.78269**	**3.04E-05**	**0.008688**	**Down**

Table 2. Overexpressed lncRNAs in CAD patients that could bind histones.

Set	Class	Sub_Class	LncRNA	P-value	FDR	Bonferroni	Jaccard
H3T11P	RNA_Histone_Modification	RNAInter 4.0	MKNK1-AS1;ITGB1-DT;LINC00102;ARHGAP31-AS1;LINC01135;TOB1-AS1	4.18E-07	2.09E-06	2.09E-06	0.00321
H3K4me2-3	RNA_Histone_Modification	RNAInter 4.0	ITGB1-DT;ZNF341-AS1;LINC01135;TOB1-AS1;HOTAIRM1	1.09E-05	2.72E-05	5.45E-05	0.00277
H3K122ac	RNA_Histone_Modification	RNAInter 4.0	ITGB1-DT;ARHGAP31-AS1;LINC01135;TOB1-AS1	0.000365	0.000608	0.00182	0.002

Table 3) Association between JUNI gene expression levels and clinicopathological features in CAD patients.

Variable	Number of cases (Case/Control)	Expression Mean ± SD in CAD	Expression Mean ± SD in Non-CAD	*P*-value
Age (years)				0.777
≤ 40	11/16	10.01 ± 24.84	0.7 ± 0.9	
40- 60	34/25	4.08 ± 6.97	1.5 ± 2.0	
≥ 60	8/7	1.3 ± 1.3	0.84 ± 0.81	
Gender				0.715
Male	20/19	7.5 ± 18.9	1.1 ± 1.7	
Female	29/32	3.1 ± 5.3	1.2 ± 1.6	
Glocuse				0.075
Normal (<100)	30/36	3.4 ± 7.0	1.9 ± 1.8	
Prediabetes (100–125)	10/8	12.2 ± 24.3	0.9 ± 0.6	
Diabetes (≥126)	9/7	2.4 ± 3.1	5.6 ± 14.1	
LDL				0.647
<130	30/ 32	5.6 ± 15.4	1.2 ± 1.9	
≥130	19/19	4.1 ± 7.0	1.1 ± 1.1	
Chleosterol				0.285
<200 mg/dL	17/	7.1 ± 19.4	1.1 ± 1.6	
200-239 mg/dL	23/	2.9 ± 6.0		
≥240 mg/dL	9/0	6.4 ± 8.9	0	
HDL				0.288
Low HDL <40	15/17	4.04 ± 7.46	5.46 ± 14.6	
Normal/High HDL ≥40	34/34	1.10 ± 1.3	1.22 ± 1.81	
Triglysrides				0.637
Normal: <150	28/34	6.33 ± 16.14	1.18 ± 1.73	
High: ≥150	21/17	3.29 6.06	1.18 1.53	
Dyslipidemia				0.826
Yes	43/44	3.54 ± 6.31	1.28 ± 1.76	
No	6/7	15.69 ± 30.80	0.57 ± 0.48	
Diabete				0.553
≥126	12/11	2.75 ± 2.87	1.21 ± 1.07	
<126	37/40	5.76 ± 14.70	1.17 ± 1.80	
HsCRP				0.539
Normal: <3	22/25	2.56 ± 3.01	1.08 ± 1.46	
Elevated: ≥3	28/26	7.04 ± 16.93	1.27 ± 1.84	

SmokingStatus				0.264
Non-smoker	38/32	6.00 ± 14.49	1.12 ± 1.67	
Ex-smoker	3/6	3.04 ± 1.90	0.54 ± 0.52	
Current smoker	8.13	1.15 ± 1.40	1.62 ± 1.7	
Hypertension history				0.389
Yes	11/8	12.43 ± 24.08	1.38 ± 1.05	
No	39/43	2.81 ± 5.14	1.14 ± 1.76	
Diabet history				0.864
Yes	7/8	3.52 ± 3.16	1.36 ± 1.05	
No	42/43	5.37 ± 14.03	1.15 ± 1.78	
Hyperlipidemia history				0.055
Yes	18/11	7.49 ± 19.70	0.85 ± 0.94	
No	31/40	3.70 ± 5.80	1.27 ± 1.81	
HTN2 (Hypertension >=140)				0.718
No	30/33	3.09 ± 5.71	0.89 ± 1.07	
Yes	19/18	8.08 ± 19.07	1.71 ± 2.31	
BMI				0.117
Normal	12/18	2.61 ± 2.27	1.51 ± 2.21	
Overweight	20/19	1.12 ± 1.34	0.98 ± 1.49	
Obese	18/14	10.84 ± 19.87	1.02 ± 0.78	
CHDevent				**<0.001***
Yes	46/1	5.17 ± 13.31	0.03 ± 0	
No	3/50	2.78 ± 1.92	1.20 ± 1.68	

***** The bold numbers in the table are significant.