Identification of Key Genes in Atherosclerosis by Combined DNA Methylation and miRNA Expression Analyses

ABSTRACT

Background: Atherosclerosis is a significant cause of coronary heart disease, cerebral infarction, and peripheral vascular disease. The objective of this study was to identify the key genes aberrantly expressed in atherosclerosis, which were regulated by microRNAs and DNA methylation.

Methods: We acquired data on DNA methylation and microRNA and messenger RNA expression from Gene Expression Omnibus data sets (GSE46394, GSE53675, and GSE12288, respectively) and identified differentially methylated genes, differentially expressed genes, and differentially expressed microRNAs between atherosclerosis and control samples. The miRDB, miRTarBase, and TargetScan databases were used to predict differentially expressed microRNAs-targeted genes, which were then intersected with differentially methylated genes and differentially expressed genes to identify genes associated with aberrant DNA methylation and microRNA activity. The DAVID database was used to perform functional enrichment analysis of differentially methylated genes and the key genes involved in atherosclerosis. Potential therapeutic agents for atherosclerosis were predicted by Connectivity Map analysis.

Results: In total, we identified 47 upregulated hypomethylated and 90 downregulated hypermethylated genes in atherosclerosis. Among them, 24 differentially expressed genes were found to be modulated both through aberrant DNA methylation and microRNA expression, and 10 such differentially expressed genes were defined as the key genes in atherosclerosis. Fifteen chemicals were selected for their potential effect in atherosclerosis.

Conclusions: We identified 10 key genes significantly associated with aberrant DNA methylation and microRNA expression in atherosclerosis and suggested 15 chemicals with potential effects on these genes, which could be further investigated as candidate drugs for atherosclerosis.

Keywords: Atherosclerosis, DNA methylation, gene expression, microRNA

INTRODUCTION

Atherosclerosis, a chronic inflammatory cardiovascular disorder, is the main cause of cardiovascular diseases such as coronary heart disease, cerebral infarction, and peripheral vascular disease.1 Atherosclerosis is characterized by thickening and hardening of arterial walls through deposition of fats and cholesterol, resulting in the loss of elasticity, narrowing of the lumen, and accumulation of macrophages.2 Animal experiments and observations of human specimens indicate that atherogenesis is initially triggered by qualitative changes in the monolayer of endothelial cells lining the inner arterial surface.3 Atherosclerosis mainly affects large- and medium-sized arteries, and its clinical manifestations occur after narrowing or occlusion of the affected vessels, which impede blood flow to different organs. The main consequences of aortic and coronary atherosclerosis are aortic aneurysm and coronary heart disease, respectively. In the early stages of atherosclerosis, atherogenic lipoproteins are cleared from the intima by macrophages, resulting in the formation of lipid-laden macrophage foam cells, which are accompanied by apoptosis or necrosis of endothelial and smooth muscle cells.4

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Emerging evidence suggests that apoptosis contributes to the instability of atherosclerotic lesions. Atherosclerosis progresses with age through focal calcification of atherosclerotic plaques, and coronary artery calcification represents a more effective and reliable prognostic biomarker of coronary atherosclerosis than traditional risk factor scores. Pathological angiogenesis of the vessel wall is a characteristic feature of high-risk atherosclerotic plaques and progression of the disease. Plaque rupture, which frequently occurs during the evolution of coronary atherosclerotic lesions, is the most common cause of coronary thrombosis.

MicroRNAs (miRNAs) are non-coding, single-stranded RNA with an average length of approximately 22 nucleotides, which play a regulatory role in gene expression through base-pairing with target messenger (m)RNAs. The formed silencing complex (RISC) is guided to the 3’-untranslated region of the target mRNA, resulting in its translational silencing or repression. MicroRNAs can be expressed in tissue- and cell-type-specific manner and affect various cell functions such as proliferation, apoptosis, migration, differentiation, and development. Recent studies have shown that specific miRNAs are closely involved in all stages of atherosclerosis, from endothelial dysfunction to plaque rupture. Thus, miRNA-21, miRNA-210, and miRNA-14a have been identified and verified to be significantly upregulated in atherosclerosis-affected arteries, indicating their role in the formation of atherosclerotic plaques.

Epigenetic modifications are reversible and heritable changes of gene function without altering its DNA sequence; they comprise modifications in DNA (such as methylation), RNA, and histones, which affect gene expression. DNA methylation has been implicated in various pathological conditions, including cancer development. DNA methylation often occurs in the promoter region of tumor suppressor genes, decreasing their expression, which promotes carcinogenesis; therefore, abnormal DNA methylation is often used as an important molecular marker of tumor progression, diagnosis, classification, and treatment. However, the regulatory effect of DNA methylation on gene expression in patients with atherosclerosis remains unclear.

In this study, we performed comprehensive analysis of genome-wide DNA methylation and mRNA and miRNA expression data sets to identify key genes regulated by both miRNAs and DNA methylation and differentially expressed in atherosclerosis. Integrative analysis of the roles of miRNAs and DNA methylation in atherosclerosis may provide new insights into the etiology of the disease at the genetic level and help develop novel diagnostic biomarkers.

METHODS

Data Source
Microarray data on gene methylation profiling data set (GSE46394) and miRNA (GSE53675) and mRNA (GSE12288) expression were retrieved from Gene Expression Omnibus (GEO) data sets (https://www.ncbi.nlm.nih.gov/geo/). The GSE46394 genome-wide DNA methylation data set comprises 49 samples (34 atherosclerosis and 15 control samples). The GSE53675 miRNA data set includes 18 samples (13 for cardiovascular disease and 5 for control), and the GSE12288 mRNA data set contains 222 peripheral blood samples (110 for coronary artery disease and 112 for control). Additionally, the GSE20686 data set containing 337 whole blood gene expression profiles (186 of coronary artery disease and 151 of control) was used for verification.

Methylation Analysis
R package ChAMP (Version 2.20.1) was used to process gene methylation data from the GSE46394 data set. The champ function was used to load methylation data files; probes with the detection level of P > .01 and probes with <3 beads in at least 5% of samples were excluded. We also removed all non-CpG, single-nucleotide polymorphism-related, multi-hit probes, and probes those on the X and Y chromosomes. After probe filtering, the beta value of patients with atherosclerosis was normalized using the Bet Mixture Quantile dilation method for type I and II probe correction with the thresholds of |Δβ| > 0.2 and P-value < .05.

Differential Expression Analysis
After data pre-processing, differentially methylated probes (DMPs), differentially expressed genes (DEGs), and differentially expressed miRNAs (DEMs) in atherosclerosis and control samples were identified using the limma package (Version 3.42.2) in R. In the GSE46394 data set, DMPs located in the gene region were assigned to the corresponding genes, which were regarded as differentially methylated genes (DMGs). Differentially expressed genes from GSE12288 and DMGs from GSE46394 were intersected using Venn analysis to obtain methylated DEGs (MDEGs) in atherosclerosis. The cutoff criteria for DMPs were |Δβ| > 0.2 and P < .05. We used P < .05 to screen out DEGs and DEMs.

Enrichment of Genomic Characterization Annotations
The distribution of DMPs in functional regions was explored by the ChAMP package (Version 2.20.1) in R, including the gene regions of the body, transcriptional start site 200 (TSS 200), TSS1500, 5’ untranscribed region (5' UTR), 3’ UTR, intergenic region, first exon (1st Exon), and CpG islands (island, shore, and shelf).

Establishment of a Regulatory Network
The targets of the selected DEMs mentioned above were predicted by the online bioinformatics tools mirDB
Figure 1. Identification of MDEGs in atherosclerosis. (a) Beta score density curve of methylation distribution. (b) Volcano plot of sites differentially methylated in atherosclerosis and control samples. (c) Average DNA methylation levels in the gene (left) and CpG island (right) regions. (d) Distribution of hypermethylated (left) and hypomethylated (right) chromosomes. (e) Volcano plot of DEGs in atherosclerosis and control samples. (f) Heat map of the expression levels of the top 50 downregulated (left) and upregulated (right) DEGs. (g) Venn diagrams showing overlapping between downregulated DEGs and hypomethylated genes (left) and between upregulated DEGs and hypermethylated genes (right). Methylated differentially expressed genes, MDEGs; DEGs, differentially expressed genes.
Identification of Aberrantly Differentially Methylated Genes in Atherosclerosis

In the GSE46394 data set, the methylation distribution density diagram demonstrated that the beta-values of DMPs were <0.2 and >0.8, suggesting that no outlier samples were observed. Differential expression analysis was conducted across all samples (Figure 1a), and 17 751 DMPs were identified between atherosclerosis and control groups, including 13 984 hypermethylated and 3767 hypomethylated sites (Figure 1b). The annotation of these DMPs revealed that the majority of them were located in non-promoter areas, mostly in gene body and intergenic regions. A small portion of DMPs were located in CpG islands; however, most were identified in CpG island shores and shelves (Figure 1c). Analysis of DMP global distribution in chromosomes indicated that hypomethylated and hypermethylated DMPs had very similar proportions in the genome (Figure 1d).

Overall, 866 DEGs were identified in atherosclerosis compared to control samples; among them, 469 were upregulated and 397 downregulated (Figure 1e). The heatmap plot shows the top 50 downregulated and upregulated DEGs (Figure 1f); specific genes and their expression levels are indicated in Tables S1 and S2, respectively. Then, 47 upregulated hypomethylated and 90 downregulated hypermethylated genes were selected through intersection of DMGs and DEGs (Figure 1g) and considered MDEGs.

Differences with P-value <.05 were considered statistically significant.

RESULTS

Functionally Enrichment Analysis
To explore biological functions of the identified MDEGs and key genes, gene ontology (GO) annotation analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis were performed using the DAVID database. Statistical significance was set at P < .05.

Drugs Screening with Connectivity Map
Connectivity Map (CMap) build 02 is an open database (https://www.broadinstitute.org) designed to explore the functional association between small-molecule drugs, gene expression, and disease.14 In this study, CMap analysis was performed to screen potential small-molecule drugs targeting the identified key genes in atherosclerosis based on the following criteria: [enrichment score] ≥ 0.7 and P < .01.

Statistical Analysis
Student’s t-test was used to reveal candidate genes differentially expressed in atherosclerosis and control samples. The Rideogram package (Version 0.2.2) in R was used to visualize the distribution of DMPs in chromosomes.
Figure 3. MiRNA–mRNA regulatory networks in atherosclerosis. (a) Volcano plot of miRNAs differentially expressed in atherosclerosis and control samples. (b) Heatmap of the expression levels of the top 50 upregulated and downregulated miRNAs. (c) Venn diagrams showing overlapping between upregulated DEGs and the predicted targets of downregulated miRNAs (left) and between downregulated DEGs and the predicted targets of upregulated miRNAs (right). (d, e) MiRNA–mRNA regulatory networks composed of upregulated miRNAs and downregulated DEGs (d) and downregulated miRNAs and upregulated DEGs (e). MiRNA, microRNA, mRNA, messengerRNA; DEGs, differentially expressed genes.
Functional Enrichment of Methylated Differentially Expressed Genes

To understand the biological functions of the identified MDEGs, we performed GO annotation analysis. The results indicated that the 90 hypermethylation-downregulated genes were significantly enriched in the processes of negative regulation of transcription from RNA polymerase II promoter, cell adhesion, cell—cell signaling, positive regulation of gene expression, focal adhesion, transcription factor binding, and transcription factor binding (Figure 2a). In terms of the 47 hypomethylation-upregulated genes, the main enriched GO terms were positive regulation of transcription from RNA polymerase II promoter, innate immune response, T cell receptor signaling pathway, T cell activation, interferon-gamma secretion, intracellular signal transduction, negative regulation of transcription from RNA polymerase II promoter, cytoplasm, protein binding, and transcription regulatory region DNA binding (Figure 2b).

Differentially Expressed Genes Targeted by Differentially Expressed miRNAs

A total of 874 DEMs (240 upregulated and 634 downregulated) were identified in patient and control samples (Figure 3a). According to the miRDB, miRTarBase, and TargetScan databases, 1342 and 2815 genes were predicted as targets of upregulated and downregulated DEMs, respectively (Figure 3b). Intersection of these genes with DEGs revealed that the expression of 114 genes targeted by downregulated DEMs was increased, whereas that of 28 genes targeted by upregulated DEMs was decreased (Figure 3c). Detailed miRNA–mRNA networks are shown in Figure 3d and 3e.

Key Genes with Aberrant DNA Methylation Targeted by Differentially Expressed Genes

Next, we identified candidate genes affected by changes both in DNA methylation and miRNA expression, which may provide valuable information on the mechanisms underlying atherosclerosis progression. A total of 24 such genes were selected: 14 targeted by underexpressed DEMs and upregulated by hypomethylation and 10 targeted by overexpressed DEMs and downregulated by hypermethylation (Figure 4a). Among the 24 genes, 10 genes, namely TCF7L2, CACNA1C, NRPS1, GABBR2, FANCC, DCK, CCDC88C, TCF12, ABLIM1, and PBX1 were differentially expressed in the atherosclerosis group compared to the control group in the GSE20686 data set; therefore, we considered them as the key genes in atherosclerosis development (Figure 4b). To understand the biological functions of the key genes, we applied GO annotation analysis. The results suggested that the 10 key genes were significantly enriched in the processes of positive regulation of transcription from RNA polymerase II promoter, negative regulation of sequence-specific DNA binding transcription factor activity, transcription factor binding, transcription factor binding, and nuclear hormone receptor binding (Figure 4c). The miRNA–mRNA regulatory network for the key genes is shown in Figure 4d.

Connectivity Map Analysis of the Key Genes

Furthermore, we conducted CMap analysis, which revealed 15 small-molecule drugs that might potentially target the
key genes, and by ranking the enrichment score in descending order, the top 4 chemicals were identified as being potential treatment options for atherosclerosis, including rottlerin, picotamide, mycophenolic acid, and ethynodiol (Table 1). Therefore, these drugs could be used for drug development research in atherosclerosis.

**DISCUSSION**

Atherosclerosis is one of the leading causes of mortality worldwide, and the absolute number of deaths is increasing each year. Previous studies have shown that both DNA methylation and miRNA expression are implicated in the pathogenesis of cardiovascular diseases; however, their role in the regulation of gene expression in atherosclerosis remains unclear. In this study, we performed comparative analysis of DNA methylation (GSE46394) and mRNA (GSE12288) and miRNA (GSE53675) expression data from the GEO database and identified key atherosclerosis-related genes whose expression could be regulated both through DNA methylation and miRNA activity.

By overlapping DEGs and DMGs, we identified 90 hypermethylated and 47 hypomethylated genes downregulated and upregulated in atherosclerosis, respectively. Because there are few methylation and gene expression data sets from the same tissue, we selected the methylation and corresponding gene expression data sets from aortas and blood, respectively, to identify MDEGs. On the other hand, it has been revealed that brain tissue of patients with Alzheimer’s disease and blood of patients with atherosclerosis share some similar methylation profiles. Therefore, our study may help to better understand the regulatory mechanisms of methylation in atherosclerosis. However, further validation is necessary. Moreover, the functional enrichment analysis results indicated that these genes were involved in the processes relevant to atherosclerosis, such as immune response and cell adhesion. Atherosclerosis is a chronic inflammatory disease that affects the arterial wall, and both innate and adaptive immune responses contribute to the development of atherosclerotic lesions by influencing lipoprotein deposition and oxidation in arterial walls. Interferon-gamma (IFN-γ), a key cytokine implicated in both innate and adaptive immunity, is an important factor in atherogenesis, affecting vascular inflammation, oxidative stress, vascular smooth muscle cell (VSMC) proliferation and migration, and plaque formation and rupture.

Our integrative analysis of miRNA and mRNA expression data revealed 142 miRNA-targeted DEGs in atherosclerosis; among them, 24 DEGs from the GSE53675 data set were regulated by both miRNAs and DNA methylation. The expression patterns of DCK, CACNA1C, NRP1, CCDC88C, TCF12, ABLIM1, TCF7L2, GABBR2, FANCC, and PBX1 were validated in another atherosclerosis data set, indicating that these genes may be key players in the etiology of the disease. Functional enrichment analysis showed that the 10 key genes were mainly involved in transcriptional regulation, including negative regulation of sequence-specific DNA-binding transcription factor activity. Increasing amounts of evidence have suggested that DNA methylation may play an important role in plaque progression and vulnerability, which indicates that DNA methylation may affect the binding of transcription factors to the corresponding targets, thus leading to alterations of gene expression in atherosclerosis. Despite some preliminary studies, some of the key genes have not been linked to atherosclerosis.

TCF7L2 is a transcription factor closely involved in the maintenance of vascular integrity. It has been shown that changes in TCF7L2 function can induce VSMC plasticity and initiate vessel wall reconstruction, confirming that TCF7L2 is a regulator of vessel wall integrity and suggesting it as a potential therapeutic target in atherosclerosis. Neurupilin 1 (NRP1) is a receptor for class 3 semaphorins and a member of the vascular endothelial growth factor family, which plays a key role in embryonic angiogenesis. In the mature vascular system, NRP1 exerts pleiotropic effects, acting as a proangiogenic factor and attenuating pathological tissue ischemia in a gene dose-dependent manner. Previous reports

**Table 1. Predicted Chemical Drugs Targeting Key Genes in Atherosclerosis**

| CMap Name         | Mean   | n  | Enrichment | P     | Specificity | Percent Non-null |
|-------------------|--------|----|------------|-------|-------------|------------------|
| Rottlerin         | −0.724 | 3  | −0.931     | <.001 | 0.0252      | 100              |
| Picotamide        | −0.516 | 5  | −0.757     | .002  | 0           | 100              |
| Cephaline         | −0.588 | 5  | −0.747     | .002  | 0.1386      | 100              |
| Docarbazine       | −0.423 | 4  | −0.738     | .009  | 0.0052      | 75               |
| Mycophenolic acid | 0.606  | 3  | 0.886      | .003  | 0.0503      | 100              |
| Etynodiol         | 0.54   | 4  | 0.875      | <.001 | 0           | 100              |
| Meteneprost       | 0.591  | 4  | 0.86       | <.001 | 0           | 100              |
| Ifosfamide        | 0.554  | 3  | 0.844      | .007  | 0           | 100              |
| Iopamidol         | 0.485  | 4  | 0.799      | .003  | 0.0189      | 100              |
| Furosemide        | 0.407  | 4  | 0.782      | .004  | 0.0116      | 100              |
| Enalapril         | 0.495  | 4  | 0.759      | .006  | 0.007       | 75               |
| Benzathine benzylpenicillin | 0.36  | 4  | 0.747      | .008  | 0.0286      | 75               |
| Dipivfrine        | 0.422  | 4  | 0.737      | .009  | 0.0063      | 75               |
| Ambroxol          | 0.39   | 4  | 0.735      | .010  | 0.0654      | 75               |

Previous reports indicated that these genes were involved in the processes relevant to atherosclerosis, such as immune response and cell adhesion. Atherosclerosis is a chronic inflammatory disease that affects the arterial wall, and both innate and adaptive immune responses contribute to the development of atherosclerotic lesions by influencing lipoprotein deposition and oxidation in arterial walls. Interferon-gamma (IFN-γ), a key cytokine implicated in both innate and adaptive immunity, is an important factor in atherogenesis, affecting vascular inflammation, oxidative stress, vascular smooth muscle cell (VSMC) proliferation and migration, and plaque formation and rupture. Our integrative analysis of miRNA and mRNA expression data revealed 142 miRNA-targeted DEGs in atherosclerosis; among them, 24 DEGs from the GSE53675 data set were regulated by both miRNAs and DNA methylation. The expression patterns of DCK, CACNA1C, NRP1, CCDC88C, TCF12, ABLIM1, TCF7L2, GABBR2, FANCC, and PBX1 were validated in another atherosclerosis data set, indicating that these genes may be key players in the etiology of the disease. Functional enrichment analysis showed that the 10 key genes were mainly involved in transcriptional regulation, including negative regulation of sequence-specific DNA-binding transcription factor activity. Increasing amounts of evidence have suggested that DNA methylation may play an important role in plaque progression and vulnerability, which indicates that DNA methylation may affect the binding of transcription factors to the corresponding targets, thus leading to alterations of gene expression in atherosclerosis. Despite some preliminary studies, some of the key genes have not been linked to atherosclerosis.
have illustrated that Npr1-deficient mice have significantly reduced ventricular dilation and functional shortening, increased levels of inflammatory cytokines, and elevated nuclear factor-kappa B and activating protein-1-binding activity. The CACNAIC gene encodes an α-subunit of a voltage-dependent calcium channel, which is expressed in cardiac and smooth muscles, and is essential for the regulation of the plateau phase of cardiac action potential, cellular excitability, excitation-contraction coupling, and gene expression. CACNAIC serves as a target for Ca2+ channel blockers, playing a role in blood pressure control, and has been implicated in the pathogenesis of cardiovascular diseases such as atherosclerosis, essential hypertension, inherited arrhythmias, and sudden cardiac death. A previous study suggests that the upregulation of CACNAIC expression may be used as a strategy to treat atherosclerosis-related diseases through inhibition of inflammatory response. Cumulatively, these findings and our results suggest that the aberrant expression of the identified key genes due to dysregulation of DNA methylation and miRNA synthesis may disturb their functional activity in the vascular system, thus contributing to the development of atherosclerosis.

Conventional therapies for atherosclerosis are non-specific, and effective drugs are insufficient. Therefore, we searched the CMap database to identify potential drugs, which may be related to the identified key genes, and guide the development of novel atherosclerosis treatment strategies. Rottlerin is a natural product that is composed of chalcone and flavonoid scaffolds with multiple target molecules and biological effects. Rottlerin may be valuable in the development of therapeutic agents against angiogenesis through its anti-angiogenic and anti-proliferative therapies by blocking the NFκB–cyclin D-1 cascade and causing a decrease in ET-1 levels. Picotamide, a derivative of methoxy-isophthalic acid, acts as an antiplatelet agent and exerts dual pharmacological effects in vivo through inhibition of the thromboxane A2 receptor and thromboxane A2 synthase. Large randomized studies have shown that picotamide could slow the evolution of early carotid atherosclerotic lesions by inhibiting plaque growth and preventing their formation, suggesting that the drug may be effective in treating individuals at risk for atherosclerotic thrombosis. Mycophenolic acid is an immunosuppressant used in clinics to prevent graft rejection, which also shows anti-cancer and anti-viral properties. However, the mechanism of these prospective medicines is unclear, and additional experiments are required to validate the therapeutic effects of these prospective medicines on atherosclerosis.

**Study Limitations**

First, the sample size was small. Second, because of the lack of clinical information for patients with atherosclerosis in the public data sets we used, the relationship between the key genes and clinicopathological features could not be evaluated. Finally, the CMap Q2 database is a bioinformatics tool to identify novel applications for established drugs; however, this target protein-based approach to drug discovery involves many different pathways. Therefore, additional basic and clinical research is required to evaluate the role of the identified key genes in atherosclerosis and the regulatory influence of DNA methylation and miRNAs on their expression.

**CONCLUSION**

We identified TCF7L2, CACNAIC, NRP1, GABBR2, FANCC, DCK, CCDC88C, TCF12, ABLIM1, and PBX1 as the key genes regulated through DNA methylation and miRNA activity in atherosclerosis. Fifteen chemicals with potential effects on the key genes were suggested as possible therapeutic agents for atherosclerosis worth further investigation. Our findings lay a foundation for future research on specific genes with a role in atherosclerosis, which would be beneficial in developing novel diagnostic and treatment approaches.

**Ethics Committee Approval:** The current research follows the GEO data access policies and publication guidelines. Thus, the present study was exempted from the approval of local ethics committees.

**Informed Consent:** Written informed consent was obtained from all participants who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – Y.C., F.Y.; Design – Y.C., Y.H., Z.Z.; Supervision – Z.Z., Y.L., Y.C., F.Y.; Fundings – F.Y.; Materials – Y.L., Y.H., C.L.; Data collection & processing – Y.H., L.W., B.W., C.L.; Analysis & interpretation – Y.C., L.W., Y.L., B.W.; Literature search – C.L., B.W., L.W., Y.C.; Writing – Y.C.; Critical review – Z.Z., F.Y.

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**Declaration of Interests:** The authors declare that they have no competing interest.

**Funding:** GEO belongs to public databases. The patients involved in the databases have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest. In addition, the code for this study can be found at https://github.com/YankunCuiLabs/Atherosclerosis.

**REFERENCES**

1. Dong C, Della-Morte D, Cabral D, et al. Sirtuin/uncoupling protein gene variants and carotid plaque area and morphology. Int J Stroke. 2015;10(8):1247-1252. [CrossRef]
2. Raghavan S, Singh NK, Gali S, Mani AM, Rao GN. Protein Kinatherosclerosis Ctheta via activating transcription factor 2-mediated CD36 expression and foam cell formation of Ly6C(hi) cells contributes to atherosclerosis. Circulation. 2018;138(21):2395-2412. [CrossRef]
3. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011; 473(7347):317-325. [CrossRef]
4. Singh RK, Haka atherosclerosis, atherosclerosisomal A, Barbosa-Lorenzi VC, Grossova I, Chin HF, et al. TLR4 (Toll-Like Receptor 4)-Dependent Signaling Drives Extracellular Catabolism of LDL (Low-Density Lipoprotein) Aggregates. Arterioscler Thromb Vascular Biol 2020;40(1):86-102.
5. Kolodgie FD, Petrov A, Virmani R, et al. Targeting of apoptotic macrophages and experimental atheroma with radiolabeled annexin V: a technique with potential for noninvasive atherosclerosis
imaging of vulnerable plaque. Circulation. 2003;108(25):3134-3139. [CrossRef]

6. Sekikawa A, Mahajan H, Kadowaki S, et al. atherosclerosissocia
tion of blood levels of marine omega-3 fatty acids with coronary
calcification and calcium density in Japanese men. Eur J Clin
Nutr. 2019;73(5):783-792. [CrossRef]

7. Virmani R, Kolodgie FD, Burke AP, et al. Atherosclerotic plaque
progression and vulnerability to rupture: angiogenesis athero-
sclerosis a source of intraplaque hemorrhage. Arterioscler
Thromb Vathersclerosis Biol. 2005;25(10):2054-2061.

8. Falk E. Why do plaques rupture? Circulation. 1992;86(6)
{suppl}:III30-42.

9. Lee KM, Bassig BA, Zhang L, et al. atherosclerosisassociation
between occupational exposure to trichloroethylene and serum
levels of microRNathersclerosis: a cross-sectional molecular
epidemiology study in China. Int Arch Occup Environ Health.
2019;92(8):1077-1085. [CrossRef]

10. Barwari T, Joshi A, Mayr M. Micro RNathersclerosis in Cardi
ovathersclerosis Diseatherosclerosis. J Am Coll Cardiol.
2016;68(23):2577-2584. [CrossRef]

11. Raitoharju E, Lyytikäinen LP, Levula M, et al. miR-21, miR-210,
and disatherosclerosis e and diseatherosclerosis. J Am Coll Cardiol.
2019;73(5):783-792. [CrossRef]

12. T omaselli D, Lucidi A, Rotili D, Mai A. Epigenetic polypharmacol
ogy: A new frontier for epi-drug discovery. Med Res Rev
2020;40(1):190-244. [CrossRef]

13. Ardeljan D, Taylor MS, Ting DT, Burns KH. The human long inter-
spersed Element-1 retrotransposon: an emerging biomarker of
Neoplastictherosclerosis. Clin Chem. 2017;63(4):816-822. [CrossRef]

14. Lamb J, Crawford ED, Peck d, et al. The Connectivity Map: using
gene-expression signatures to connect small molecules, genes,
and disatherosclerosis. Science. 2006;313(5795):1929-1935.
[CrossRef]

15. Fu H, Zhu K, Zhou D, Guan Y, Li W, Xu S. Identification and valida-
tion of Plathersclerosisisma metabolomics reveal potential bio-
markers for coronary heart Diseatherosclerosis. Int Heart J.
2019;60(6):1397-1397. [CrossRef]

16. Deermath EW, Guan W, Grove ML, atherosclerosislibekyan S,
Mendelson M, Zhou YH. Epigenome-wide atherosclerosisassocia-
tion study (EWatherosclerosis) of BMI, BMI change and waist cir-
cumference in African American adults identifies multiple replicat-
ated loci. Hum Mol Genet. 2015;24(15):4464-4479. [CrossRef]

17. Declerck K, Vanden Bergh W. Characterization of blood
surrogate immune-methylation biomarkers for immune cell
infiltration in chronic inflamming disorders. Front Genet.
2019;10:1229. [CrossRef]

18. Wolff D, Ley K. Immunity and inflammation in atherosclerosis.
Circ Res. 2019;124(2):315-327. [CrossRef]

19. Wittum JL, Lichtman AH. The influence of innate and adaptive
immune responses on atherosclerosis. Arnu Rev Pathol. 2014;
9:73-102. [CrossRef]

20. Elyatherosclerosis A, Voloshyna I, Ahmed S, et al. The role of
interferon-gamma in cardiovascularatherosclerosis diseathero-
sclerosis: an update. Inflamm Res. 2020;69(10):975-988.

21. Xu S, Pelisek J, Jin ZG. Atherosclerosis is an epigenetic disease.
Trends Endocrinol Metab. 2018;29(11):739-742. [CrossRef]

22. Srivastava R, Zhang J, Go GW, Narayanam A, Nottoli TP, Mani A.
Impaired LP6-TCF7L2 activity enhances smooth muscle cell
Plathersclerosisisticity and causes coronary artery Diseather-
therosclerosis. Cell Rep. 2015;13(4):746-759. [CrossRef]

23. Fantin A, Herzog B, Mahmoud M, et al. Neuropolip I (NRPI)
hypo-
morphism combined with defective VEGF-A binding reveals
novel roles for NRPI in developmental and pathological angi-
go genesis. Development. 2014;141(3):556-562. [CrossRef]

24. Veilaiachamy E, Das S, Subramanian U, Maeda N, Pandey KN.
Genetically altered mutant mouse models of guanyly cycla
therosclerosis/natriuretic peptide receptor-A inhibit the
cardiac expression of proinflammatory mediators in a gene-
dose-dependent manner. Endocrinology. 2014;155(3):1045-1056.
[CrossRef]

25. Plein A, Fantin A, Ruhrberg C. Neuropolip regulation of angi-
go genesis, arteriogenesis, and vathersclerosis permeability.
Microcirculation. 2014;21(4):315-323. [CrossRef]

26. Benitah JP, Alvarez JL, Gómez AM. L-type Ca2+ current in vath-
cardiacmyocytes. J Mol Cell Cardiol. 2010;48(1):26-36.
[CrossRef]

27. Dai S, Hall DD, Hell JW. Supramolecular atherosclerosissembles
and localized regulation of voltage-gated ion channels. Physiol
Rev. 2009;89(2):411-452. [CrossRef]

28. Moosmang S, Schulla V, Welling A, et al. Dominant role of smooth
muscle L-type calcium channel Cav1.2 for blood pressure regu-
lation. EMBO J. 2003;22(22):6027-6034. [CrossRef]

29. Fukuyama M, Ohno S, Wang Q, et al. L-type calcium channel
mutations in Japanese patients with inherited arrhythmiothero-
sclerosis. Circ J. 2013;77(1):1799-1806. [CrossRef]

30. Wang SY, Chen DM, Ye XH. Study on the mechanism of Xinmai-
kang tablets in the treatment of atherosclerotic cardiovath-
therosclerosis diseatherosclerosis batherosclerosis on
network pharmacology and cell experiment. Chin J Exp Formu-
lae. 2022;28(3):196-203.

31. Zakharov SI, Morrow JP, Liu G, Yang L, Marx SQ. Activation of
the BK (SLO1) potassium channel by mallotoxin. J Biol Chem.
2005;280(35):30882-30887. [CrossRef]

32. Valacchi G, Pecorelli A, Sticozzi C, et al. Rottlerin exhibits
antiangiogenic effects in vitro. Chem Biol Drug Des. 2011;77(6):
460-470. [CrossRef]

33. Modesti PA, Cecioni I, Colella A, Costoli A, Paniccia R, Neri
Serneri GG. Binding kinetics and antiplatelet activities of pico-
ramide, a combined inhibitor of thromboxane A2 synth and
receptor , reduces 2-year mortality in diabetics with peripheral
atherosclerosis. Br J Pharmacol. 1994;112(1):81-86. [CrossRef]

34. Neri Serneri GG, Coccheri S, Marubbi E, Violi F, Drug Evaluation
in Atherosclerotic Vascular Disease in Diabetics (DAVID) Study
Group. Drug evaluation in atherosclerotic Vathersclerosiscul
Diseatherosclerosis in diabetics Study G. Picotamide, a com-
bined inhibitor of thromboxane A2 synthatherosclerosis and
receptor, reduces 2-year mortality in diabetics with peripheral
arterial diseatherosclerosis: the DAVID study. Eur Heart J.
2004;25(20):1845-1852. [CrossRef]

35. Amin AA, Araj FG, Ariyamuthu VK, et al. Impact of induction
immunosuppression on patient survival in heart transplant
recipients treated with tacrolimus and mycophenolic acid in
the current allocation era. Clin Cardiol. 2019;33(8):e13651.
[CrossRef]
## Table S1. Top 50 Downregulated Differentially Expressed Genes and Expression Levels ($P < .05$)

| Gene      | LogFC  | AveExpr | t     | P      | adj.P.Val | B     |
|-----------|--------|---------|-------|--------|-----------|-------|
| NCAPH     | -0.42420 | 4.36604 | -4.24336 | .00003 | .20262 | 1.18864 |
| CEBPA     | -0.21650 | 8.48234 | -3.89666 | .00013 | .29192 | 0.22175 |
| ACSM3     | -0.33151 | 5.24816 | -3.89145 | .00013 | .29192 | 0.19947 |
| BTNLT1    | -0.59549 | 6.43693 | -3.45462 | .00066 | .56048 | -0.92729 |
| AZGPI5    | -0.45334 | 4.83423 | -3.41707 | .00075 | .56048 | -1.01873 |
| ITPK1     | -0.13419 | 8.39107 | -3.41103 | .00077 | .56048 | -1.03336 |
| ZNF518A   | -0.28367 | 5.26105 | -3.41063 | .00077 | .56048 | -1.03432 |
| TMEM158   | -0.62621 | 6.10389 | -3.38641 | .00084 | .56048 | -1.09227 |
| RIMS2     | -0.28365 | 5.06438 | -3.27272 | .00122 | .56048 | -1.35157 |
| UBOX5     | -0.23991 | 6.72277 | -3.26572 | .00126 | .56048 | -1.37837 |
| GPR15     | -0.40961 | 4.77313 | -3.20278 | .00156 | .56048 | -1.52371 |
| GINS4     | -0.41712 | 5.06484 | -3.16961 | .00174 | .56048 | -1.59928 |
| MS4A4A    | -0.35216 | 5.46324 | -3.16506 | .00177 | .56048 | -1.60960 |
| DBF4B     | -0.41520 | 5.40963 | -3.13769 | .00193 | .56048 | -1.67135 |
| DHR52     | -0.29505 | 4.72509 | -3.12552 | .00201 | .56048 | -1.69865 |
| TRAF31P2  | -0.23782 | 6.14006 | -3.12026 | .00205 | .56048 | -1.71043 |
| LMF2      | -0.13520 | 8.10620 | -3.11599 | .00207 | .56048 | -1.71997 |
| CHRN5     | -0.36383 | 5.37261 | -3.1024 | .00211 | .56048 | -1.73279 |
| CLN3      | -0.09600 | 8.55706 | -3.09711 | .00220 | .56048 | -1.76202 |
| RP1I-4S716.2 | -0.33091 | 5.67464 | -3.08396 | .00230 | .56048 | -1.79119 |
| MEI1      | -0.35010 | 5.57833 | -3.06973 | .00241 | .56048 | -1.82261 |
| SH3BP2    | -0.08729 | 7.58459 | -3.06163 | .00247 | .56048 | -1.84044 |
| MAN2A2    | -0.11816 | 8.02322 | -3.05729 | .00251 | .56048 | -1.84996 |
| ELAVL2    | -0.51443 | 3.64348 | -3.04639 | .00260 | .56048 | -1.87386 |
| AGPAT1    | -0.09885 | 8.71547 | -3.04156 | .00264 | .56048 | -1.88442 |
| SEMA3C    | -0.17196 | 5.60622 | -2.97836 | .00322 | .61736 | -2.02126 |
| CBX5      | -0.17351 | 7.06001 | -2.97528 | .00325 | .61736 | -2.02785 |
| NFX1      | -0.10961 | 6.68208 | -2.97362 | .00327 | .61736 | -2.03142 |
| ZNF80     | -0.53684 | 3.21194 | -2.96068 | .00340 | .61736 | -2.05906 |
| FAM118A   | -0.20646 | 6.96513 | -2.95756 | .00343 | .61736 | -2.06573 |
| PLAU      | -0.11682 | 8.47853 | -2.93966 | .00363 | .63388 | -2.10376 |
| ARHGAP4   | -0.16113 | 8.10204 | -2.92976 | .00374 | .64190 | -2.12471 |
| GULP1     | -0.27422 | 4.29483 | -2.90953 | .00399 | .64190 | -2.16733 |
| PIP       | -0.37139 | 5.67257 | -2.90277 | .00407 | .64190 | -2.18150 |
| MTF1      | -0.10478 | 7.75893 | -2.89953 | .00411 | .64190 | -2.18828 |
| MGLL      | -0.14377 | 8.07346 | -2.89398 | .00418 | .64190 | -2.19989 |
| KRT13     | -0.44882 | 4.16985 | -2.87121 | .00448 | .64190 | -2.24730 |
| DDC       | -0.29891 | 5.07996 | -2.86142 | .00462 | .64190 | -2.26757 |
| SH3TC1    | -0.13177 | 7.61029 | -2.85577 | .00470 | .64190 | -2.27924 |
| FEV       | -0.23702 | 4.14836 | -2.84238 | .00489 | .64190 | -2.30683 |
| HIST1H2A  | -0.24503 | 7.79990 | -2.84086 | .00492 | .64190 | -2.30996 |
| CDK16     | -0.12661 | 6.88073 | -2.84050 | .00492 | .64190 | -2.31070 |
| CPSF6     | -0.12119 | 7.97814 | -2.82983 | .00508 | .64190 | -2.33258 |
| SPO11     | -0.48025 | 4.54656 | -2.82092 | .00522 | .64190 | -2.35078 |
| ZDHHC18   | -0.14369 | 9.36060 | -2.81533 | .00531 | .64190 | -2.36218 |
| USB1      | -0.18880 | 8.27328 | -2.81082 | .00538 | .64190 | -2.37138 |
| STC2      | -0.22912 | 5.57037 | -2.81043 | .00539 | .64190 | -2.37216 |
| ERC2-IT1  | -0.37091 | 5.03421 | -2.79647 | .00562 | .64190 | -2.40049 |
| BLOC151   | -0.09496 | 9.08088 | -2.77594 | .00597 | .64190 | -2.44194 |
| GEMIN7    | -0.33456 | 6.05866 | -2.77050 | .00607 | .64190 | -2.45287 |
Table S2. The top 50 Upregulated Differentially Expressed Genes and Expression Levels ($P < .05$)

| Gene       | LogFC  | AveExpr | t   | P.Value | adj.P.Val | B       |
|------------|--------|---------|-----|---------|-----------|---------|
| PTPN4      | 0.24502| 7.95240 | 4.37944 | .00002 | .20262 | 1.58983 |
| ATRN      | 0.35971| 6.88938 | 3.92778 | .00111 | .29192 | 0.29830 |
| KIZ       | 0.51418| 4.77742 | 3.87615 | .00141 | .29192 | 0.15808 |
| ZMYND11   | 0.15893| 7.74797 | 3.72580 | .00025 | .38852 | −0.24128 |
| PAM       | 0.26714| 6.31302 | 3.72476 | .00025 | .38852 | −0.24399 |
| ZNF639    | 0.50583| 4.63412 | 3.64945 | .00033 | .41184 | −0.43894 |
| SUZ12     | 0.15684| 7.84603 | 3.64871 | .00033 | .41184 | −0.44083 |
| TMED10    | 0.15021| 8.65123 | 3.48591 | .00059 | .56048 | −0.85041 |
| BCLAF1    | 0.15646| 7.18669 | 3.43258 | .00071 | .56048 | −0.98107 |
| NAA50     | 0.21904| 7.11358 | 3.37507 | .00087 | .56048 | −1.11997 |
| MAPKBP1   | 0.44910| 5.56383 | 3.34306 | .00097 | .56048 | −1.19639 |
| PAPOLA    | 0.10131| 7.61747 | 3.33271 | .00101 | .56048 | −1.22097 |
| PKIA      | 0.34683| 5.99390 | 3.29420 | .00115 | .56048 | −1.31181 |
| CCDC28A   | 0.10400| 8.66035 | 3.28556 | .00118 | .56048 | −1.33207 |
| SMYD5     | 0.29589| 4.50978 | 3.28190 | .00120 | .56048 | −1.34061 |
| HIF1A     | 0.18443| 8.73137 | 3.28002 | .00120 | .56048 | −1.34502 |
| ASUN      | 0.37387| 5.41993 | 3.26229 | .00128 | .56048 | −1.38638 |
| AKR1C3    | 0.51856| 5.04867 | 3.25866 | .00129 | .56048 | −1.39480 |
| RIC8B     | 0.35473| 6.07938 | 3.24680 | .00135 | .56048 | −1.42233 |
| DYRK2     | 0.15684| 6.75151 | 3.23386 | .00141 | .56048 | −1.45227 |
| LRRCC47   | 0.16261| 7.58968 | 3.21846 | .00148 | .56048 | −1.48774 |
| FAM208A   | 0.13743| 7.27653 | 3.21355 | .00151 | .56048 | −1.49901 |
| TES       | 0.11509| 8.26281 | 3.20906 | .00153 | .56048 | −1.50933 |
| CHMP7     | 0.12180| 8.63754 | 3.18846 | .00164 | .56048 | −1.55642 |
| SCRIB     | 0.34049| 6.63929 | 3.16815 | .00175 | .56048 | −1.60258 |
| SARAF     | 0.16012| 10.10779| 3.15726 | .00181 | .56048 | −1.62724 |
| SATB1     | 0.16816| 9.34139 | 3.14840 | .00187 | .56048 | −1.64725 |
| ZNF83     | 0.42936| 5.26587 | 3.12431 | .00202 | .56048 | −1.70135 |
| DCK       | 0.26040| 6.02135 | 3.11564 | .00208 | .56048 | −1.72074 |
| MYBL1     | 0.31415| 6.78184 | 3.09819 | .00220 | .56048 | −1.75962 |
| MAGEF1    | 0.30696| 6.14272 | 3.06497 | .00245 | .56048 | −1.83309 |
| PRPF4B    | 0.18728| 6.55797 | 3.05409 | .00253 | .56048 | −1.85699 |
| UPK3B     | 0.31561| 5.84882 | 3.05169 | .00255 | .56048 | −1.86226 |
| UBE2G1    | 0.11653| 7.92087 | 3.04735 | .00259 | .56048 | −1.87176 |
| DES       | 0.30145| 5.09683 | 3.03083 | .00273 | .57030 | −1.90785 |
| CECR5     | 0.11043| 7.59183 | 2.99704 | .00303 | .61736 | −1.98109 |
| NDUFS7    | 0.12770| 8.30733 | 2.99202 | .00308 | .61736 | −1.99190 |
| PPP2R5C   | 0.11450| 7.78984 | 2.97626 | .00324 | .61736 | −2.02576 |
| THADA     | 0.17096| 6.36384 | 2.96766 | .00333 | .61736 | −2.04418 |
| Clorf50   | 0.39750| 5.74435 | 2.95669 | .00344 | .61736 | −2.06757 |
| ARL4C     | 0.12577| 8.70878 | 2.94134 | .00361 | .63288 | −2.10020 |
| GZMB      | 0.25464| 9.12107 | 2.91362 | .00394 | .64190 | −2.15873 |
| CACNA1C   | 0.29952| 4.53222 | 2.91261 | .00395 | .64190 | −2.16086 |
| AGPAT4    | 0.37032| 5.48568 | 2.89529 | .00416 | .64190 | −2.19715 |
| CLSTN3    | 0.39282| 6.12442 | 2.88668 | .00428 | .64190 | −2.21512 |
| ITGAV     | 0.33998| 5.11404 | 2.88399 | .00431 | .64190 | −2.22072 |
| RAP1B     | 0.13669| 9.95654 | 2.87896 | .00438 | .64190 | −2.23121 |
| HSPH1     | 0.17842| 6.50064 | 2.87863 | .00438 | .64190 | −2.23189 |
| SH3YL1    | 0.25741| 7.21868 | 2.87779 | .00439 | .64190 | −2.23364 |
| CERK      | 0.12678| 8.67370 | 2.86467 | .00457 | .64190 | −2.26085 |