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# Epigenetic Mechanisms Affecting the Development of Atherosclerosis: A Narrative Review

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**ABSTRACT**

**Introduction:** Atherosclerosis is a chronic inflammatory arterial disease that underlies several cardiovascular conditions, including coronary artery disease, heart failure, and stroke. A hallmark of atherosclerosis is the accumulation of fatty plaques, involving various cell types and molecular pathways. The development of this disease involves complex interactions between genetic and environmental factors .

**Materials and Methods:** A review was conducted using bibliographic databases such as PubMed and Scopus to examine articles on epigenetic alterations and their impact on atherosclerosis and cardiovascular disease. Furthermore, in silico data analyses related to epigenetics and atherosclerosis were also performed.

**Results:** Epigenetic processes, including DNA methylation, histone modifications, and non-coding RNA, are dynamic modifications that play a crucial role in various stages of atherosclerotic plaque progression. Transgenerational transmission of epigenetic modifications increases cardiovascular disease risk, even in offspring. Inflammation within blood vessel walls is linked to lipid metabolism and is crucial for atherosclerosis development. Macrophages and monocytes, when exposed to inflammatory stimuli, undergo alterations in their genetic and epigenetic profiles, thereby contributing to atherosclerosis progression. Our in silico analysis identified HDAC1, HDAC9, HDAC3, SIRT1, and HDAC6 as key hub genes within a protein-protein interaction network of atherosclerosis-related genes.

**Conclusion:** Understanding epigenetic regulation can provide insights into gene activation related to functions such as inflammation, lipid metabolism, and vascular remodeling, thus influencing atherosclerosis progression. Considering both genetic and epigenetic mechanisms can provide insight into the molecular processes driving atherosclerosis and inform the development of new therapeutic strategies.

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## Introduction

Atherosclerosis is a chronic inflammatory condition characterized by the accumulation of cholesterol in arterial walls, leading to plaque development, rupture, heart attacks, and strokes. The disease begins with damage to the endothelial layer, followed by lipid accumulation, chronic inflammation, plaque rupture, and thrombosis. Atherosclerosis can result in blood vessel obstruction, myocardial tissue death, peripheral vascular disease, organ failure, and stroke. The process involves persistent inflammation, characterized by the presence of macrophages, T cells, and clonal expansion of smooth muscle cells. Endothelial cells (ECs),

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which make up the innermost layer of an artery, are the first to experience metabolic imbalances such as hyperglycemia, blood lipids, and blood flow stress (1).

Risk factors such as hypertension, smoking, diabetes, obesity, and a sedentary lifestyle also contribute to atherosclerosis development. Lifestyle modifications such as a heart-healthy diet, regular exercise, smoking cessation, and weight management are important for reducing atherosclerosis risk (2).

The pathogenesis and progression of atherosclerosis are mechanistically linked to various pathophysiological factors, including angiogenesis, inflammatory reactions, cholesterol metabolism, high blood pressure, cellular proliferation, and apoptosis (Figure 1). Cardiovascular diseases (CVDs) are multifactorial and result from a complex interaction between genetic predisposition and external influences (3).

In recent years, our understanding of the involvement of genes and their expression in various biological processes and diseases has improved significantly. Given the intricate nature of atherosclerosis and its multifaceted origins, it is essential to investigate the influence of epigenetic factors on its onset. This narrative review synthesizes prior research to provide a comprehensive overview of the epigenetic effects on atherosclerosis, identifying, categorizing, and analyzing the evidence. Furthermore, this review includes bioinformatic analyses of related genes.

### *Process of Atherosclerosis Formation*

Healthy endothelium maintains vascular homeostasis through various mechanisms. Pathological factors induce endothelial cell death, disrupting ECs and triggering apoptosis. Over time, hemodynamic stresses, including hyperglycemia, elevated blood lipids, and hypertension, compromise EC function. The adventitia, the outer layer of the vessel's wall, consists of fibroblasts, collagen, elastic fibers, adrenal nerves, and lymphatic vessels. In response to vascular injury, fibroblasts multiply and migrate to the vessel interior, secreting factors that regulate the growth of ECs and vascular smooth muscle cells (VSMCs), and attract inflammatory cells. VSMCs can transform into macrophage-like cells, thereby influencing atherosclerosis progression (Figure 2). Atherosclerotic lesions, known as atheromas, often develop in low-shear stress regions of arteries, such as bends and branches, due to the accumulation of lipids and inflammatory cells within the arterial walls. If the plaque ruptures or becomes severely thickened, it can block blood flow and cause a heart attack (4).

T cells and macrophages are particularly involved in this process. Monocytes migrate to vessel walls and differentiate into dendritic cells or macrophages. Activated macrophages locate and engulf oxidized lipoproteins, becoming foam cells. These macrophages generate collagen fibers and form a fibrous cap that stabilizes the plaque by clearing apoptotic cells. Insufficient macrophage proliferation and increased apoptosis can weaken the fibrous cap, potentially leading to plaque rupture and thrombosis. Activated ECs and macrophages can trigger VSMC dedifferentiation through paracrine growth factors (5).

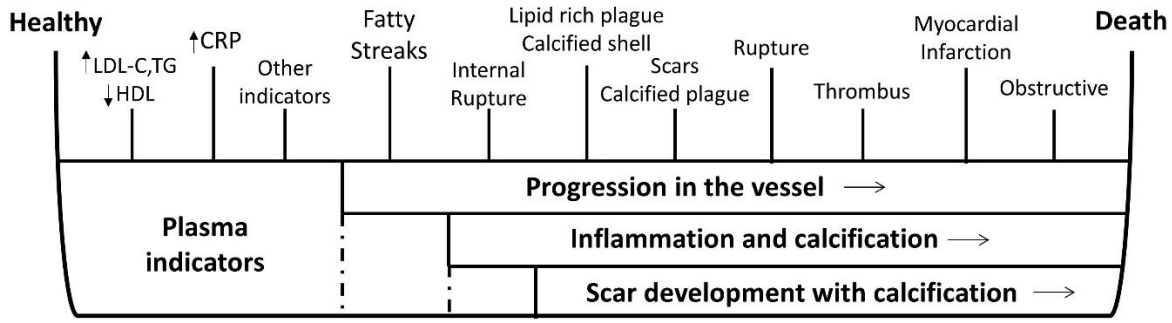


**Figure 1.** Atherosclerosis progression in the human body.

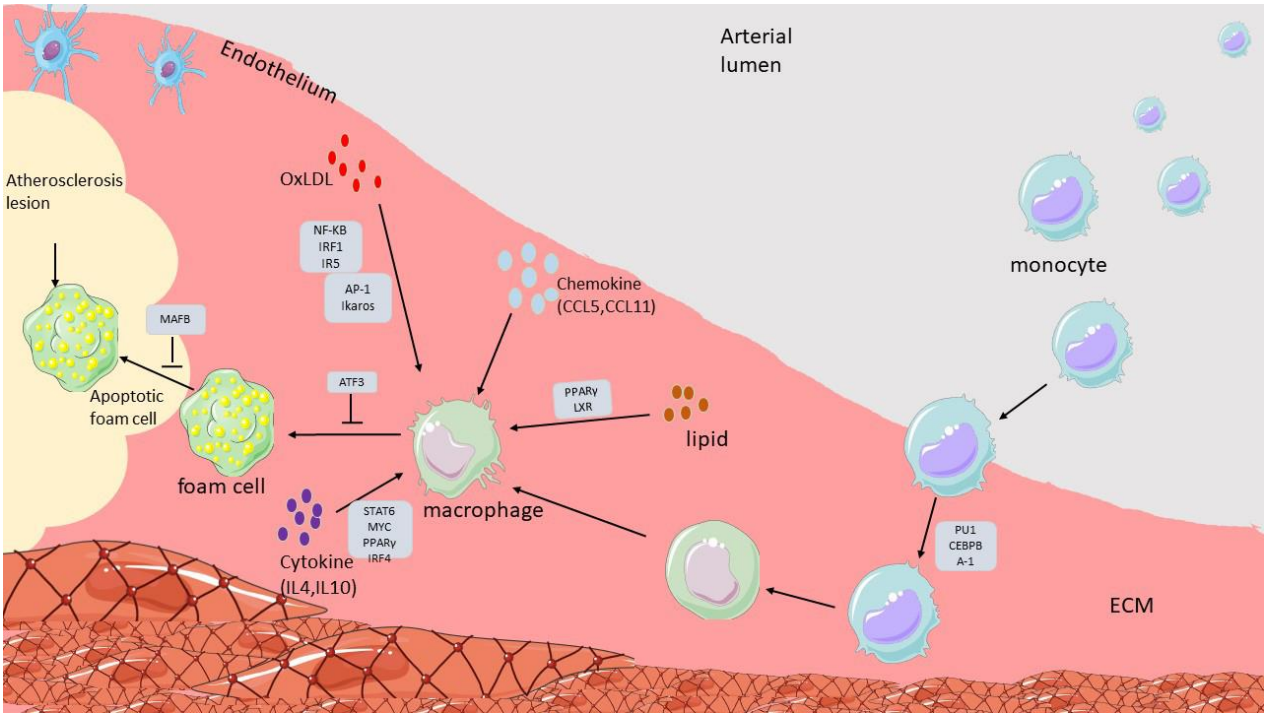


**Figure 2.** Immunological factors contribute to the formation of atherosclerosis. Endothelial activation triggers the expression of cytokines and chemokines, increases endothelial cell layer permeability, and elevates the expression of adhesion molecules. These chemokines activate immune and inflammatory cells, including monocytes and T lymphocytes, leading to transendothelial migration. Subsequently, these cells infiltrate the subendothelial space of the vessel wall, playing a crucial role in the development of fatty streaks, which constitute the earliest discernible lesions.

CD4 positive T cells play a crucial role in the formation of atherosclerosis, with the T helper cell type 1 (TH1) subset being particularly predominant in plaques, while T helper cell type 2 (TH2) cells may contribute to aneurysm formation. Regulatory T cells (Tregs) protect against atherosclerosis by maintaining the balance between TH1 and TH2 responses (6).

Lipid mediators play a role in regulating inflammation within vascular walls. Dyslipidemia and cholesterol imbalances contribute to the accumulation of low-density lipoprotein (LDL), plaque formation, and the release of pro-inflammatory molecules. Elevated LDL levels can promote the formation of atherosclerotic plaques, potentially leading to arterial blockage and serious cardiovascular events. This process involves cholesterol accumulation in vessel walls, triggering inflammation and plaque development that may rupture, resulting in blood clots. The initiation of atherosclerosis involves endothelial dysfunction resulting from oxidized low-density lipoprotein (Ox-LDL). Ox-LDL metabolism via Lectin-like oxidized LDL receptor-1 (LOX-1) impairs endothelial function and attracts monocytes to injury sites (7).

High homocysteine levels are associated with an increased risk of CVDs and atherosclerosis, potentially through mechanisms involving DNA demethylation and altered DNA replication. Elevated homocysteine levels may contribute to vascular diseases by influencing DNA methylation processes (8).

## *Epigenetics*

Epigenetic mechanisms regulate smooth muscle cell (SMC) function and play a significant role in atherosclerosis development and progression through differentiation and activation. While all cells in the body share the same genetic material, each cell type functions uniquely. This unique function is governed by gene regulation mechanisms, including epigenetic mechanisms. Epigenetics regulates gene expression patterns without altering the underlying genetic code. Altered gene expression patterns can be inherited by daughter cells after mitotic cell division. Epigenetic mechanisms modulate chromatin

accessibility primarily through DNA methylation and histone post-translational modifications (PTMs). Accessible chromatin allows gene regulatory proteins, including transcription factors, to interact with their binding sites within gene regulatory regions, such as promoters, enhancers, and silencers (9).

Epigenetic modification systems consist of three key components: writers (enzymes that add modifications), readers (proteins that recognize and bind epigenetic modifications), and erasers (enzymes that remove modifications). For example, DNA methyltransferases (DNMTs) are writer enzymes responsible for DNA methylation, while ten-eleven translocation methylcytosine dioxygenases (TET) are eraser enzymes that remove these marks (Figure 3) (10,11).

### *Post-translational modifications of histones in atherosclerosis*

Histone PTMs are intricate chemical alterations occurring at the N-termini of histones H3 and H4, and are essential for gene regulation. These PTMs vary across different tissues and include acetylation, methylation, phosphorylation, ubiquitination, and SUMOylation. Ubiquitination, another type of PTM, has also been shown to play a role in transcription activation and elongation. Histone methyltransferase enzymes (HMEs) act as writers to modify specific histone residues involved in interactions with transcription factors. Transcription factors typically bind to specific DNA motifs within gene promoters or enhancers to regulate transcription. Efficient binding to DNA requires accessible chromatin. Only a limited number of transcription factors can bind to closed chromatin motifs within compacted chromatin (12).

Chromatin remodeling complexes facilitate changes in local chromatin structure. Euchromatin exhibits a more accessible structure, thereby allowing other factors to bind to DNA. Chromatin can undergo remodeling, transitioning from a compacted heterochromatin state to an open euchromatin state. The euchromatin state is characterized by promoter DNA hypomethylation and specific histone PTMs, including H3K4me3, H3K27ac, and H3K36me3. In contrast, heterochromatin is characterized by promoter DNA hypermethylation, along with the presence of H3K27me3 and H3K9me3.

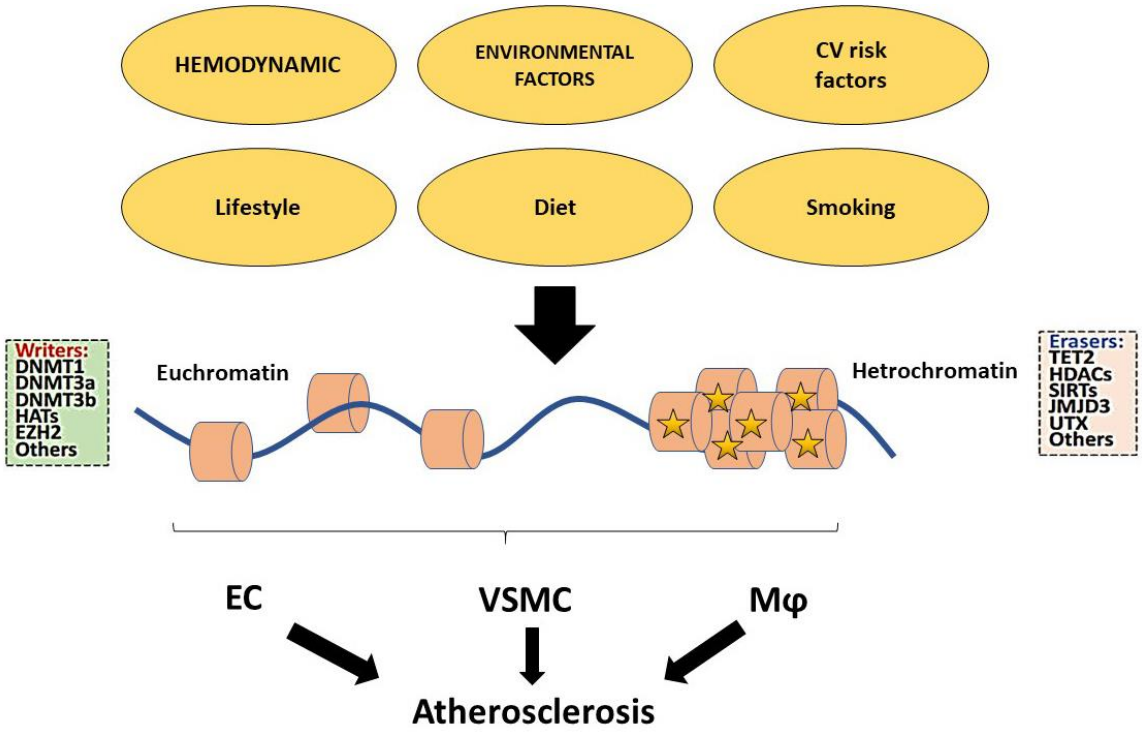


**Figure 3.** Epigenetic targeting in atherosclerosis involves writers and readers, such as DNMTs, HATs, and EZH2, that regulate the epigenetic process of atherosclerosis formation.

H3K27me3 levels are elevated in ECs isolated from early and advanced human atherosclerotic plaques, highlighting a crucial role for H3K27me3 in the development of atherosclerotic lesions. The relationship between changes in H3K27me3 levels and atherosclerotic plaque formation in human vascular tissue has been studied using immunohistochemistry.

Immunohistochemical analysis revealed decreased H3K27me3 levels in atherosclerotic blood vessels, associated with VSMC development and proliferation. Human monocytes exposed to Ox-LDL exhibited elevated H3K4me3 levels in inflammatory mediators such as IL-18, MMP, MCP-1, TNF-α, and IL-1β, which play a significant proatherogenic role under in vitro conditions (13).

H3K9me2 and H3K9me3 are associated with chromatin compaction and transcriptional repression (Figure 4, Table 1).

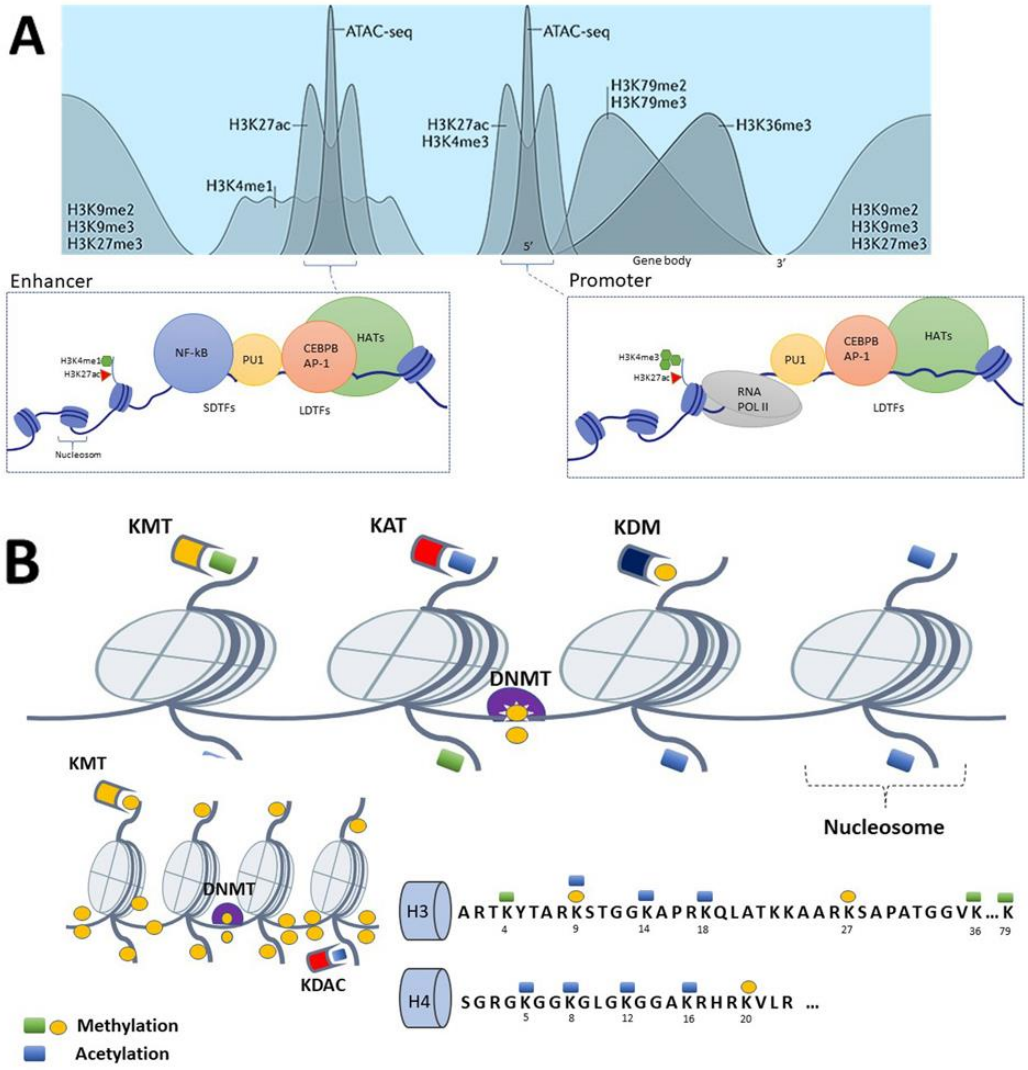


**Figure 4.** A) Active enhancers are marked by the presence of H3K4me1, H3K27ac, and signals of open chromatin on an assay for transposase-accessible chromatin using sequencing (ATAC-seq). In macrophages, regulatory elements bind lineage-determining transcription factors (LDTFs) such as PU.1, CCAAT/enhancer-binding protein β (C/EBPβ), and activator protein 1 (AP-1). These elements may be activated by signal-dependent transcription factors (SDTFs) like nuclear factor-κB or peroxisome proliferator-activated receptor γ (PPARγ), which are induced by lipids. Transcription factors that activate gene expression can recruit histone-modifying enzymes, such as HATs, to create a chromatin landscape permissive for transcription. B) DNA is wrapped around histone octamers to form nucleosome structures. Lysine acetyltransferases (KATs) are responsible for histone acetylation, whereas lysine methyltransferases (KMTs) catalyze methylation. It is postulated that KDACs may remove histone methylation marks, although this process has not been conclusively proven. KDACs are known to eradicate acetylation marks, which are typically associated with activation.

**Table 1**. Examples of histone marks and histone-modifying enzymes are linked to transcription activation and gene silencing. These include Lysine Methyltransferase (KMT), Lysine Demethylase (KDM), methylation (me), and acetylation (ac).

| Histone mark | Function | Location | Writers (methyltransferases) | | Erasers (demethylases) | |
|---|---|---|---|---|---|---|
| | | | Family | Members | Family | Members |
| **Transcription activation** | | | | | | |
| **H3K4me1** | Facultative euchromatin | Enhancer | KMT2 | KMT2A, KMT2B, KMT2C, KMT2D, SETD1A | KDM1 | KDM1A, KDM1B |
| | | | KMT3 | SMYD2 | KDM5 | KDM5B, RIOX1 |
| | | | KMT7 | SETD7, SETD9 | | |
| **H3K4me3** | Facultative euchromatin | promoters | KMT2 | KMT2A, KMT2D, SETD1A, SETD1B | KDM5 | KDM5A, KDM5B, KDM5C, KDM5D, RIOX1 |
| | | | KMT8 | PRDM9 | | |
| **H3K27ac** | Active transcription | Promoters and enhancers | KAT3 (HAT) | CREBBP, EP300 | HDACs | HDAC3 |
| **H3K36me3** | Active transcription | Gene body | KMT3 | SETD2 | KDM4 | KDM4A–KDM4E |
| **H3K79me2** | Active transcription | Gene body | KMT4 | DOT1L | Unknown | Unknown |
| **Gene silencing** | | | | | | |
| **H3K9me2** | Facultative heterochromatin | Gene deserts | KMT1 | EHMT1, EHMT2 | KDM3 | KDM3A |
| | | | | | KDM4 | KDM4D–KDM4E |
| **H3K9me3** | Constitutive heterochromatin | Intergenic domains, promoters, and enhancers | KMT1 | SETDB1, SUV39H1, SUV39H2 | KDM3 | KDM3B |
| | | | | | KDM4 | KDM4A–KDM4E |
| **H3K27me3** | Facultative heterochromatin | Intergenic domains (bivalent), promoters and enhancers | KMT6 | EZH1, EZH2 | KDM6 | KDM6B, UTX, UTY |

Chromatin modification profiling, utilizing chromatin immunoprecipitation followed by sequencing, can help identify regulatory factors and transcriptional activity. Several epigenetic enzymes participate in regulating classical macrophage phenotypes including histone methyltransferases and demethylases like enhancer of zeste homolog 2 (EZH2) and Jumanji domain-containing protein-3 (JMJD3), as well as histone acetyltransferases (HATs) and deacetylases such as P300, Sirtuin 6 (SIRT6), and histone deacetylases (HDACs) like HDAC3 and HDAC9. EZH2 promotes foam cell formation and atherosclerosis development by regulating ABCA1 expression, hindering levels impede cholesterol efflux from macrophages (14,15).

Histone-modifying enzymes also regulate macrophage polarization toward M2 phenotypes. M1 and M2 macrophages are differentially linked to inflammatory responses and plaque stability. M1 macrophages are primarily involved in pro-

inflammatory responses and plaque destabilization, whereas M2 macrophages are primarily involved in anti-inflammatory responses and promoting tissue repair and plaque stability. Several enzymes counteract the M2 macrophage phenotype, including HDAC4, SIRT2, JMJD3, protein arginine methyltransferases (PRMT1), SET and MYN-domain containing protein 3 (SMYD3), HDAC3, and HDAC9. Conversely, M1 macrophage phenotypes are positively regulated by several chromatin modifiers, including HDAC1, SET7/UTX, JMJD2, and HDAC3. Inhibitors of histone modifications affecting M2 polarization include SMYD2, JMJD1A, SIRT1, and SIRT2 (16).

While lysine methylation and acetylation have been extensively studied, arginine residues are also subject to methylation and acetylation. The functional consequence of arginine methylation, whether it leads to gene repression or activation, depends on the specific arginine residue that is methylated (17,18).

### *DNA Methylation in Atherosclerosis*

Beyond X-chromosome inactivation, approximately 4% of DNA is methylated in normal blood cells. DNA methylation, an epigenetic process, involves the covalent addition of a methyl group to cytosine residues, especially at CpG dinucleotide sites. DNA methylation leads to transcriptional repression and gene silencing. This occurs through two main mechanisms: directly inhibiting the binding of methylation-dependent transcriptional activators, or indirectly by altering the binding affinity of chromatin remodeling proteins like methyl-CpG-binding protein 2 (MeCP2). MeCP2 is a reader protein that recognizes methylated CpG sites and recruits chromatin modifying enzymes (19).

DNMT1, DNMT3a, and DNMT3b catalyze DNA methylation, while TET1, TET2, and TET3 reverse this process. Specifically, DNMT1 adds methyl groups to hemimethylated DNA strands during replication, while DNMT3a and DNMT3b establish de novo methylation patterns by adding methyl groups to unmethylated DNA. DNA demethylation, mediated by TET3, is crucial for DNA damage repair and gene expression. Similarly, DNA demethylase TET2 regulates the expression of contractile genes, including SRF, MYOCD, and MYH11, in human VSMCs (20, 21).

DNMT expression levels are correlated with atherosclerosis severity in patients. Specifically, DNMT1 expression levels are independently associated with monocyte expression levels and DNA hypomethylation, which are linked to an increased risk of coronary artery disease Furthermore, DNMT3a restricts interleukin-13 (IL-13) expression in T helper cells, thereby generally suppressing the inflammatory response. It has been suggested that DNMT3b regulates macrophage polarization via DNA methylation at promoter regions. The expression of DNA demethylase TET1 increases in atherosclerotic plaques, leading to DNA hypomethylation. Specific DNA hypermethylation patterns are associated with disease progression in the vascular tissue of atherosclerotic aortas. This hypermethylation is associated with EC damage and is mediated by the nuclear factor kappa B/toll-like receptor 4 (NF-κB/TLR4) pathway. Under such conditions, the expression of endothelial-specific genes can be altered, and DNMT expression levels are elevated in cultured ECs exposed to oscillatory shear stress. Oscillatory shear stress in the carotid arteries can induce DNA hypermethylation, thereby contributing to EC damage, a process mediated by DNMTs. Various DNMTs are implicated in distinct aspects of atherosclerosis development (22). Disturbed blood flow has been observed to increase DNA methylation within the Krüppel-like factor 4 (KLF4) promoter, resulting in the suppression of KLF4 expression. This effect can be mitigated by DNMT inhibitors (23).

ATP-binding cassette transporter A1 (ABCA1) protein facilitates the transport of cholesterol and phospholipids across the interior of the cell membrane to its exterior. Promoter methylation levels of ABCA1 are associated with serum inflammatory mediators, such as C-reactive protein (CRP) and interleukin-1 beta (IL-1β), and with circulating extracellular DNA and neutrophil extracellular traps. These associations are observed in studies of premature coronary artery disease, and indicate that higher DNA methylation levels correlate with reduced

high-density lipoprotein (HDL) levels in older men (24).

An association between the single nucleotide polymorphism rs4142986 within the collagen type XV alpha 1 chain (COL15A1) gene and atherosclerosis has been demonstrated. Methylation at this locus decreases COL15A1 expression and increases disease susceptibility. Treating SMCs with decitabine reversed the methylation effect and increased COL15A1 expression levels (25).

In atherosclerosis, DNA methylation can affect the binding of specific transcription factors, including hypoxia-inducible factor 1-alpha (HIF-1α), MYC, and CCCTC-binding factor (CTCF). CTCF is a protein that regulates gene expression and acts as a barrier against heterochromatin spreading. The CTCF binding site on DNA is methylated on paternal chromosomes, whereas CTCF regulates gene expression on maternal chromosomes (26).

Atherosclerosis pathogenesis involves the dual effects of epigenetic regulation on specific genes. Inducible nitric oxide synthase (iNOS), which plays a role in neointimal atherosclerotic plaque formation (an arterial response to injury), undergoes methylation in most tissues. Although hyperhomocysteinemia might decrease global DNA methylation in atherosclerosis patients, specific promoters, such as those of iNOS and fibroblast growth factor 2 (FGF2), can exhibit hypermethylation (27,28).

Different subsets of B and T lymphocytes exhibit varying pro-inflammatory and anti-inflammatory responses in atherosclerosis. Tregs exert atheroprotective functions by limiting the pro-inflammatory responses of T helper (TH) cells. FOXP3 gene hypermethylation has been proposed to suppress FOXP3 expression, thereby reducing Treg levels and increasing the risk of acute coronary syndrome. For instance, clinically, patients with end-stage kidney disease and chronic inflammation show increased DNA methylation in peripheral blood lymphocytes, which contributes to rising cardiovascular mortality in dialysis patients (29,30).

### *Acetylation*

Acetylation is a critical histone PTM and is associated with CVD progression. HATs and HDACs mediate histone acetylation and deacetylation, respectively. Acetylation can occur on lysine (K) residues of histones H3 (K9, K14, K18, K23, K27) and H4 (K5, K8, K12, K16, K20). This acetylation neutralizes the positive charge on histone tails, reducing their interaction with negatively charged DNA phosphate groups. As a result, chromatin structure becomes more relaxed, leading to enhanced transcriptional activity. HDAC activity, involving the removal of acetyl groups, leads to chromatin compaction and transcriptional suppression. Additionally, HDAC activity impacts non-histone proteins, such as the transcription factor p53, nuclear receptors, and cell cycle regulatory proteins, also known as lysine deacetylases (KDACs) (31).

HDAC3 is suggested to play a significant role in macrophage phenotypes within atherosclerotic lesions. Collagen deposition was observed in HDAC3-deficient mice, and this stabilized atherosclerotic plaques. In these mice, increased IL-4 gene expression was noted, and anti-inflammatory cytokine secretion by macrophages was prominent. Moreover, under hypertensive conditions, the expression of HDAC2, HDAC3, and HDAC5 is increased within the aortic arch of rats, promoting a pro-atherogenic EC phenotype (32).

Certain atherosclerosis-related factors, such as OxLDL, can modulate the activity of KDACs. In cultured ECs, OxLDL reduces the expression of KDAC1 and KDAC2, while statins restore global KDAC activity. LDL and Ox-LDL can initiate long-term epigenetic reprogramming of innate immune cells. This phenomenon, known as trained immunity, demonstrates that innate immune cells, including natural killer cells, can acquire non-specific immunological memory through epigenetic modulation, altering their reactions to subsequent stimuli (33).

### *Dietary Effects*

Established risk factors do not fully elucidate the epigenetic basis of CVDs. Environmental factors in early life can induce diverse epigenetic alterations that may influence metabolic processes. Epigenetics provides insight into how diet, environment, and lifestyle can influence disease

progression by modulating gene expression patterns throughout an individual's lifespan. For instance, environmental exposures have been associated with an increased incidence of Beckwith-Wiedemann and Angelman syndromes in infants. Furthermore, an alkaline diet rich in omega-3 polyunsaturated fatty acids (PUFAs) serves as an example of environmental factors influencing the epigenetic basis of CVDs, given that omega-3 PUFAs are known to modulate lipid metabolism and reduce inflammation, thereby promoting overall health (34).

Maternal diet during pregnancy can exert a lasting impact on offspring health by influencing epigenetic modifications. For example, a high-fat diet during pregnancy has been associated with alterations in DNA methylation patterns in offspring, potentially increasing their risk of developing CVD later in life. Furthermore, paternal diet and lifestyle choices can influence the sperm epigenetic profile, potentially contributing to the transgenerational inheritance of CVDs. This highlights the crucial role of both parental health and dietary habits in shaping the health outcomes of future generations (35).

Dietary interventions and the consumption of specific nutrients or bioactive compounds can modulate epigenetic marks and may be associated with atherosclerosis. For example, studies have shown that dietary factors such as folate, vitamin B12, and omega-3 fatty acids can influence DNA methylation patterns and histone modifications, potentially reducing the risk of atherosclerosis. Understanding the role of diet in epigenetic modifications and transgenerational inheritance of CVDs is crucial for developing effective strategies for prevention and therapeutic intervention. By promoting healthy dietary and lifestyle choices, individuals can potentially lessen the risk of transmitting epigenetic alterations predisposing their offspring to CVDs (36).

### *Pharmacological Interventions*

Clinically, statins are commonly used to manage CVDs. Statins function as lipid-lowering agents and possess anti-inflammatory properties. However, a subset of patients exhibits suboptimal responses to statin therapy. Certain plant-derived compounds, recognized for their anti-atherosclerotic properties, such as resveratrol, curcumin, and epigallocatechin gallate, have shown promise in modulating epigenetic enzymes within vascular cells and atherosclerotic lesions (37,38).

Epigenetic drugs, comprising small molecules that target chromatin structure, offer a novel therapeutic strategy to combat atherosclerosis (Table 2). For example, 5-Aza-2′-deoxycytidine has demonstrated potential clinical efficacy in mitigating atherosclerosis. Furthermore, Vitamin C, an antioxidant agent, can enhance DNA demethylation processes mediated by TET enzymes, thus exhibiting beneficial anti-atherosclerotic effects (39,40).

### *Genetic Predisposition*

The genetic diversity associated with atherosclerotic CVD provides crucial insights into the pathophysiology of atherosclerosis. Numerous genes that contribute to susceptibility to atherosclerosis are present within the general population, each with a subtle effect, typical of complex diseases. Table 3 lists the top 10 genes most strongly associated with atherosclerosis (41).

**Table 2.** Some epigenetic drugs delay the progression of atherosclerosis**.**

| Drug | Epigenetic category |
|---|---|
| Ascorbic acid | TET2 activator |
| Epigallocatechin gallate | DNMT inhibitor |
| Statins | EZH2 inhibitor |
| Quercetin | DNMT inhibitor |
| Curcumin | Broad-spectrum epigenetic modulator |
| Resveratrol | SIRT1 activator |
| 5-Aaza2'-deoxycytidine | DNMT inhibitor |

## Materials and Methods

### *Protein-Protein Interaction Network Construction*

To construct a protein-protein interaction (PPI) network, we imported a set of 20 atherosclerosis-related genes identified through a review into the STRING database (https://string-db.org/). The PPI network was then visualized using Cytoscape v3.10.1. Five hub genes were identified using the CytoHubba application with the Maximal Clique Centrality (MCC) topological analysis method.

### *Gene Set Enrichment Analysis*

To investigate the biological pathways and gene ontology (GO) associations of these genes, we performed gene set enrichment analysis. Using the Metascape database (https://metascape.org/gp/index.html#/main/step1), we were able to gain insights into the GO terms linked to the genes of interest. This analysis played a key role in identifying potential functional annotations that are essential to our study and in elucidating the underlying biological mechanisms (Figure 5).

## Results

### *PPI Network Analysis*

As described in the methods, the PPI network was constructed using the STRING database. An interesting observation from the PPI network analysis is the notable absence of SMN1 gene interactions. Furthermore, using the CytoHubba application, five significant hub genes were identified: HDAC1, HDAC9, HDAC3, SIRT1, and HDAC6 (Figure 6). HDAC1, HDAC9, and HDAC3 function to suppress gene expression by removing acetyl groups from histones, enabling histones to bind DNA more tightly.

**Table 3.** Genes associated in atherosclerosis development and progression.

| Gene symbol | Gene name | Location |
|---|---|---|
| WNT5A | Wnt family member 5A | 3p14.3 |
| TLR2 | Toll-like receptor 2 | 4q31.3 |
| TLR4 | Toll-like receptor 4 | 9q33.1 |
| TLR9 | Toll-like receptor 9 | 3p21.2 |
| MYD88 | MYD88 innate immune signal transduction adaptor | 3p22.2 |
| SYK | spleen associated tyrosine kinase | 9q22.2 |
| IRF7 | interferon regulatory factor 7 | 11p15.5 |
| CXCL8 | C-X-C motif chemokine ligand 8 | 4q13.3 |
| IL7 | Interleukin-7 | 8q21.13 |
| IL15 | Interleukin-15 | 4q31.21 |

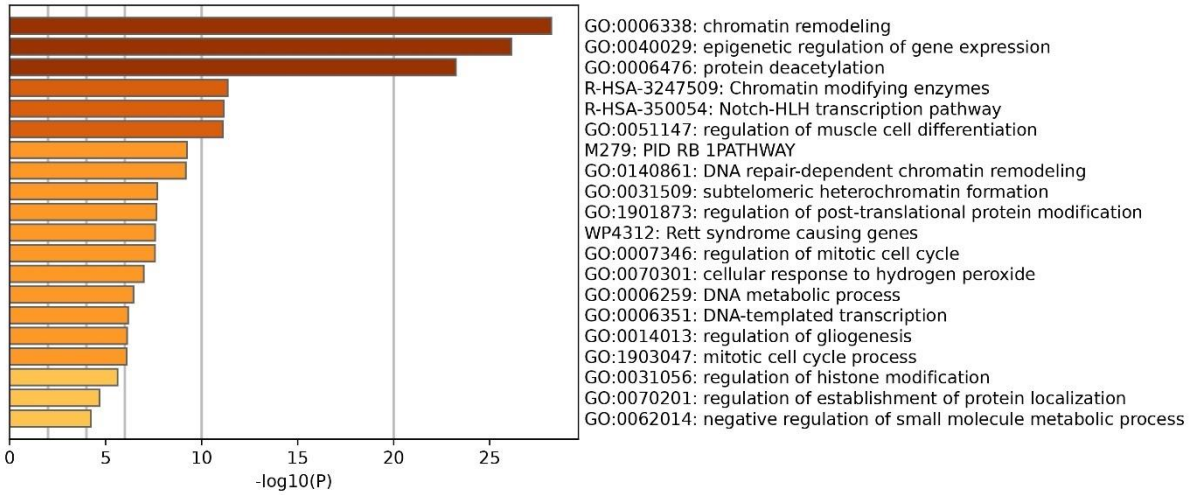


**Figure 5.** Functional enrichment analysis involves the use of bar graphs obtained through Metascape to display the top enriched terms of final genes.

These hub genes are implicated in inflammation, cell proliferation, and atherosclerotic plaque formation (42).

SIRT1, a deacetylase, contributes to cellular longevity, stress resistance, and metabolic processes. SIRT1 exerts diverse roles in atherosclerosis: it can prevent plaque formation by promoting cell survival and suppressing inflammation. However, SIRT1 may also promote the formation of senescent cells, which can exacerbate atherosclerosis. HDAC6, a tubulin deacetylase, influences cellular motility and structure. By preventing SMCs from migrating away from atherosclerotic plaques, HDAC6 contributes to plaque stabilization. However, HDAC6 may also contribute to inflammation and cell death, potentially exacerbating thrombosis, and plaque rupture.

## Conclusion

Epigenetic modifications play a critical role in the pathogenesis of atherosclerosis. By dysregulating key processes such as inflammation, lipid metabolism, and vascular function, epigenetic alterations, including DNA methylation and histone modifications, significantly contribute to disease progression. Importantly, the reversible nature of epigenetic changes positions them as highly attractive therapeutic targets. Targeting key epigenetic enzymes like HDAC1, HDAC3, HDAC6, HDAC9, and SIRT1, which were identified as central hubs in our network analysis, along with broader epigenetic targets like DNMTs, using novel drugs combined with dietary and lifestyle interventions such as exercise and smoking cessation to modify epigenetic marks, holds great promise for the future prevention and treatment of atherosclerosis.

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This study did not receive any financial support.

## Conflict of Interest

There is no conflict of interest among the authors.

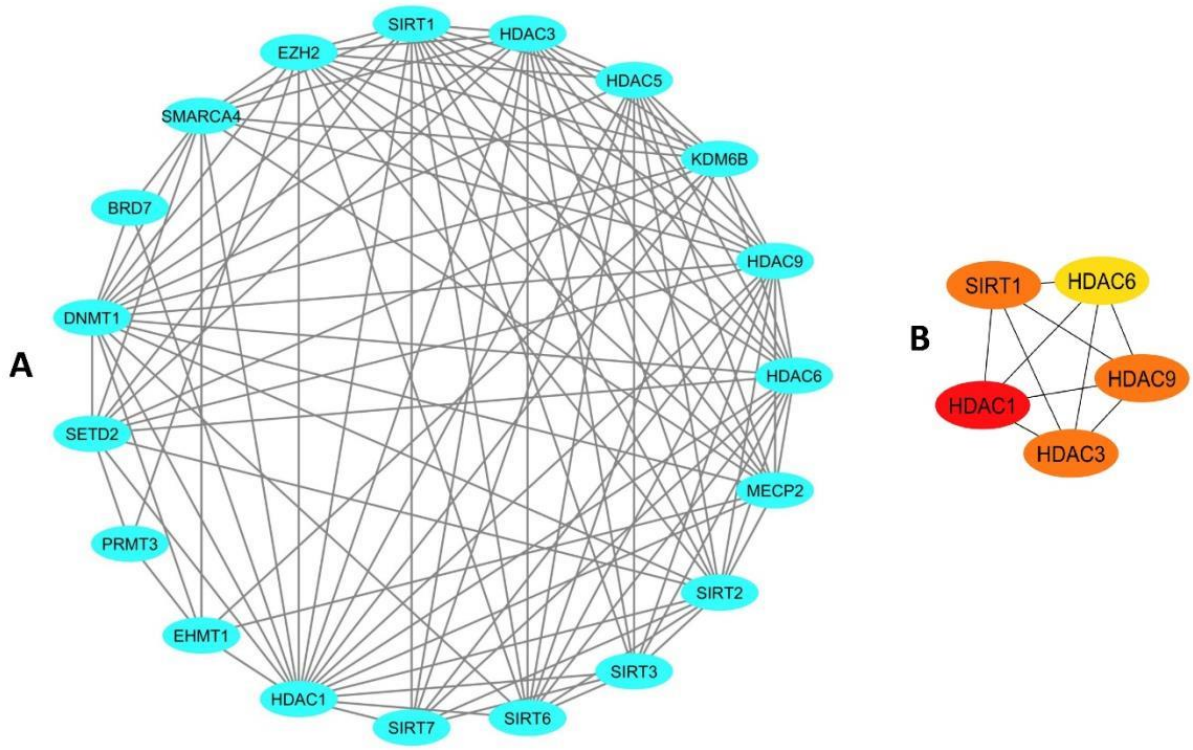


**Figure 6.** A) The STRING protein-protein interaction network comprises final genes. B) Hub genes are categorized according to a color gradient ranging from yellow to red, wherein red denotes the highest importance.

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