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MOLECULAR PATHWAYS AND GENE EXPRESSION PROFILES IN ATHEROSCLEROSIS DEVELOPMENT

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INTRODUCTION

Atherosclerosis is a chronic, progressive cardiovascular condition marked by the accumulation of lipid-laden plaques in the arterial walls, resulting in diminished blood flow, arterial rigidity, and an elevated risk of severe complications, including myocardial infarction, stroke, and peripheral artery disease. This disease process is influenced by a complex interaction of genetic, molecular, and environmental variables that affect endothelial dysfunction, lipid metabolism, inflammatory responses, immune system activation, and vascular remodelling(Linton et al. 2000; Morrison, Sullivan, and Aday 2023). The development of atherosclerosis begins with the degradation of the endothelium in blood vessels, often triggered by oxidative stress, high cholesterol levels (hyperlipidaemia), high blood pressure (hypertension), diabetes, smoking, and various other risk factors (Jebari-Benslaiman et al. 2022; Rafieian-Kopaei et al. 2014). Endothelial dysfunction results in heightened permeability of the arterial wall, promoting the infiltration and retention of low-density lipoprotein (LDL) cholesterol particles, which subsequently undergo oxidation to become oxidised LDL (ox-LDL). Oxidised LDL is pivotal in the onset and advancement of atherosclerosis because it triggers endothelial activation, facilitates inflammatory cell recruitment, and encourages foam cell production, which aids in plaque growth (Linton et al. 2000; Mundi et al. 2018). At a molecular level, many critical signalling pathways govern the development of atherosclerosis. The oxidative stress pathway, driven by reactive oxygen species (ROS), worsens endothelial dysfunction by reducing nitric oxide (NO) bioavailability, enhancing vascular inflammation, and elevating adhesion molecule expression. The nuclear factor kappaB (NF-κB) signalling pathway is a vital regulator that influences the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM1), intercellular adhesion molecule-1 (ICAM1), and endothelial selectin (E-selectin), which promote the adhesion and migration of monocytes into the subendothelial space (Batty, Bennett, and Yu 2022; Incalza et al. 2018). Upon entering the intima, monocytes develop into macrophages and phagocytiseoxidised LDL, converting it into lipid-laden foam cells. These foam cells facilitate plaque development and exacerbate the inflammatory response by releasing cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) (Moore, Sheedy, and Fisher 2013). Lipid metabolic pathways significantly contribute to the pathogenesis of atherosclerosis. Peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), and sterol regulatory element-binding proteins (SREBPs) govern cholesterol metabolism, lipid transport, and foam cell production (A. C. Li and Glass 2004; Fuior et al. 2023). Dysregulation of these pathways results in an imbalance in cholesterol homeostasis, leading to excessive lipid buildup in arterial plaques. The ATP-binding cassette (ABC) transporters, including ABCA1 and ABCG1, promote cholesterol efflux from macrophages, hence inhibiting foam cell production. In atherosclerotic circumstances, the diminished function or expression of these transporters leads to excessive lipid buildup, fostering plaque formation and instability (Pennings et al. 2006; Duan et al. 2022). Gene expression profiling studies have shown substantial alterations in the expression of genes associated with endothelial activation, immunological responses, vascular smooth muscle cell (VSMC) proliferation, and extracellular matrix remodelling. The transformation of vascular smooth muscle cells (VSMCs) from a contractile to a synthetic phenotype facilitates plaque propagation since synthetic VSMCs generate extracellular matrix constituents, such as collagen and proteoglycans, which affect plaque stability (Cao et al. 2022; Jang et al. 2025). Matrix metalloproteinases (MMPs), especially MMP-2 and MMP-9, damage the extracellular matrix, compromising the fibrous cap and increasing the

risk of plaque rupture and thrombus development. Moreover, the death of foam cells and vascular smooth muscle cells inside the plaque core facilitates the growth of the necrotic core, hence exacerbating the instability of atherosclerotic plaques. Epigenetic changes, such as DNA methylation, histone alterations, and non-coding RNAs, have become essential regulators of gene expression in atherosclerosis (T. Li et al. 2020; Olejarz, Łacheta, and Kubiak-Tomaszewska 2020). MicroRNAs (miRNAs) like miR-33, miR-155, and miR-21 regulate lipid metabolism, inflammation, and the activity of vascular smooth muscle cells. For instance, miR-33 suppresses cholesterol efflux by targeting ABCA1, while miR-155 enhances inflammation by modulating macrophage activation and cytokine synthesis (Aranda et al. 2013; Citrin, Fernández-Hernando, and Suárez 2021). Long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) significantly influence atherosclerosis by regulating endothelial function, immune cell activation, and lipid metabolism. The interaction between genetic predisposition and environmental variables greatly affects susceptibility to and development of atherosclerosis (Zhang, Salisbury, and Sallam 2018; Xie et al. 2025). Genome-wide association studies (GWAS) have found several genetic loci linked to atherosclerosis risk, including variations in genes such as APOE, PCSK9, and SORT1, which govern lipid metabolism and cholesterol transport. Additionally, single nucleotide polymorphisms (SNPs) in inflammatory genes including IL-6 and TNF-α have been associated with heightened cardiovascular risk (Sivapalaratnam et al. 2011; López Rodríguez, Arasu, and Kaikkonen 2023). Recent data indicates that lifestyle variables, including nutrition, physical exercise, smoking, and stress, influence gene expression via epigenetic pathways, underscoring the significance of gene-environment interactions in the progression of atherosclerosis. Progress in genomics, transcriptomics, and bioinformatics has elucidated the molecular underpinnings of atherosclerosis and found possible biomarkers for early identification and risk assessment (Alegría-Torres, Baccarelli, and Bollati 2011; Motsinger-Reif et al. 2024; H. Liu et al. 2025). Circulating microRNAs, inflammatory cytokines, and lipid metabolites are under investigation as non-invasive biomarkers for the diagnosis and monitoring of atherosclerosis (Pereira-da-Silva et al. 2021; Fazmin et al. 2020). Additionally, tailored treatment approaches designed to manipulate critical molecular pathways are being explored. Statins, which reduce LDL cholesterol levels, continue to be the foundation of atherosclerosis treatment; however, innovative strategies, including PCSK9 inhibitors, anti-inflammatory agents (such as IL-1β inhibitors), and RNA-based therapies targeting genes associated with atherosclerosis, are being developed to offer more targeted and effective interventions (Yilmaz et al. 2018; Barut et al. 2024)(Hetherington and Totary-Jain 2022). Atherosclerosis is a multifaceted disease influenced by numerous molecular pathways and alterations in gene expression that govern endothelial dysfunction, inflammation, lipid metabolism, and vascular remodelling. Understanding these pathways is essential for identifying innovative treatment targets and formulating personalised medicine strategies to address cardiovascular diseases (Jebari-Benslaiman et al. 2022; Ajoolabady et al. 2024). Future investigations, including multi-omics data, such as genomes, epigenomics, proteomics, and metabolomics, will augment our comprehension of atherosclerosis pathogenesis and facilitate the development of novel diagnostic and therapeutic approaches.

Materials and Method

Data Collection

The primary data for this research was sourced from the Gene Expression Omnibus (GEO) collection. Keywords and selection criteria were used to get data from expression profile arrays of tissue or clinical specimens. Expression data for breast cancer was obtained using the accession ID GSE43292 from GEO. Tissue samples from 52 individuals were examined to identify differentially expressed genes, with each sample representing a unique patient, enabling a direct comparison between sick and normal gene expression patterns. The GEPIA2 web tool (http://gepia2.cancer-pku.cn/#index) was used to analyse differential gene expression in the dataset.

Data Pre-Processing:

The Series Matrix Files for GSE43292 were obtained from the GEO database for comprehensive investigation. Before analysis, the probe data in the dataset was transformed into standard gene symbols, matching gene identification with universally recognised nomenclature. The dataset was normalised to ensure uniformity and reduce technical biases. The robust multi-array average (RMA) approach was used in the R software environment (version 2.6.0) to standardise gene expression data, ensuring a uniform scale and distribution.

Identification of Differential Expressed Genes (DEGs)

This study used GEO2R to analyse differentially expressed genes (DEGs) in atherosclerosis. The tool produced a volcano plot, with the x-axis denoting the fold change in gene expression and the y-axis reflecting statistical significance (P-value). We used a stringent criterion to detect DEGs, establishing a p-value threshold of <0.05 and an absolute log fold change above 1. Furthermore, to augment our research, we acquired gene expression profiles for atherosclerosis from GEPIA2, using the same methods for DEG identification.

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Protein-protein interaction and hub gene identification

Protein-protein interaction (PPI) analysis was performed using the STRING database by entering a list of proteins to create a network of anticipated connections, including both physical and functional links. Interactions with a cumulative score over 0.08 were deemed significant, indicating the dependability of the found protein associations. The differentially expressed genes (DEGs) were used to develop and visualise the protein-protein interaction (PPI) network using Cytoscape software (version 3.5.1; http://www.cytoscape.org). In this network, protein interactions were shown as edges, with their widths reflecting the degree of interaction based on the aggregated score. Hub genes were found with the CytoHubba plugin in Cytoscape, categorising nodes with a degree above 10 as hub genes, hence underscoring their significance within the network. This integrated methodology provides a thorough framework for examining intricate protein interactions and pinpointing essential regulatory proteins in cellular mechanisms.

mRNA expression and survival analysis of hub genes

In silico techniques, including UALCAN, GEPIA, and KM plotter, were used to evaluate survival rates and gene expression relationships in patients with atherosclerosis. The Kaplan-Meier technique, supplemented by log-rank testing, enabled the survival analysis. A connection of statistical significance was identified between gene expression levels and patient survival, with a significance threshold of $P < 0.05$. Data from patients with atherosclerosis, obtained from The Cancer Genome Atlas, were used for expression validation. The data, expressed as transcripts per million (TPM) values, facilitated the establishment of two separate groups. The GEPIA database was used to visualise these categories, categorising patients with TPM levels below the top quartile into the low/medium expression group, while those with TPM values above the upper quartile were put into the high expression group.

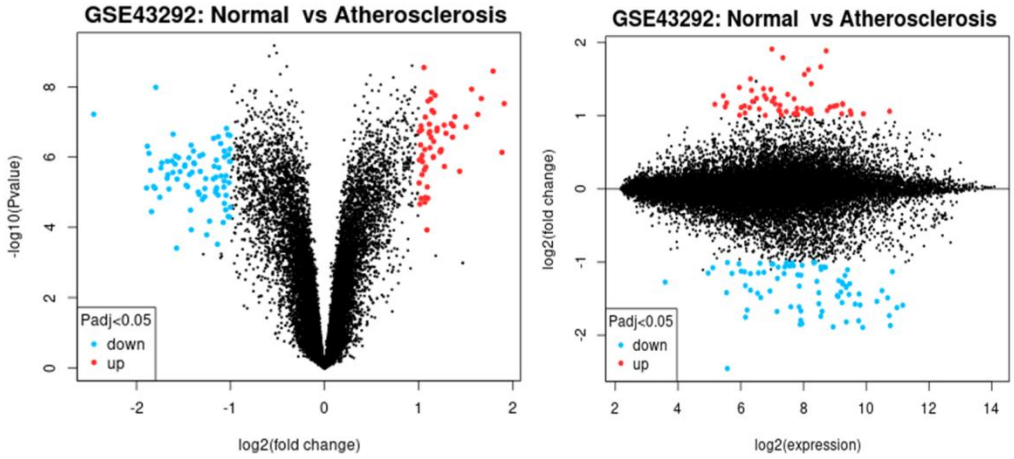
Gene ontology and pathway enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analyses of differentially expressed genes (DEGs) were performed using the Database for Annotation, Visualisation, and Integrated Discovery (DAVID, https://david.ncifcrf.gov/tools.jsp), with a significance threshold established at $P < 0.05$. Furthermore, KEGG pathway analysis was used to uncover highly enriched pathways related to DEGs. Pathway crosstalk analysis was conducted using particular criteria: a Benjamini-Hochberg adjusted P-value below 0.05 and a Jaccard coefficient together with an overlap coefficient, both over 50% (0.5), deemed statistically significant. This thorough evaluation of DEGs within pertinent pathways underscores their potential significance in essential biological processes and regulatory networks.

Result

Identification of DEGs in Breast cancer

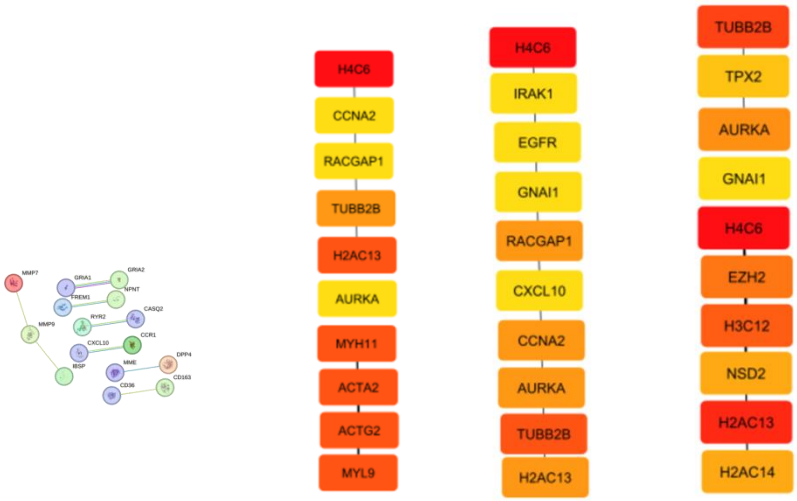
A total of 141 differentially expressed genes (DEGs) were found in the atherosclerosis dataset GSE43292. The identification was performed by GEO2R analysis with the Limma tool, employing stringent selection criteria of an adjusted p-value <0.05 and a log fold change >1. This approach facilitated the generation of volcano charts for each dataset.



PPI network construction and hub gene identification

A protein-protein interaction (PPI) network was established for proteins represented by 141 differentially expressed genes (DEGs) using STRING analysis. Additionally, five hub genes (KIT, LEF1, NGFR, EDNRB, INHBB) were

discovered using MCC, closeness, and degree centrality methodologies. All three hub genes exhibited an increase among the DEGs, indicating their potential involvement in the pathogenesis of atherosclerosis.



Rank	Name
1	H4C6
2	ACTA2
2	MYL9
2	MYH11
2	ACTG2
2	H2AC13
7	TUBB2B
8	CCNA2
8	RACGAP1
8	AURKA

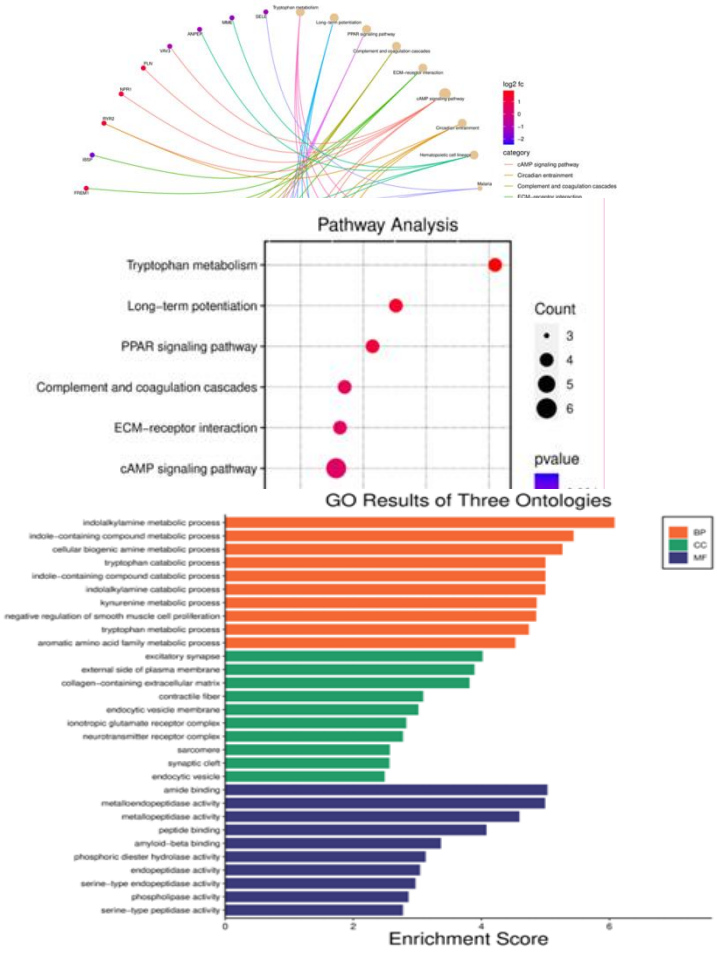
Rank	Name
1	H4C6
2	TUBB2B
3	CCNA2
3	RACGAP1
3	AURKA
3	H2AC13
7	EGFR
7	GNAI1
7	IRAK1
7	CXCL10

Rank	Name
1	H4C6
2	H2AC13
3	TUBB2B
4	H3C12
5	EZH2
6	AURKA
7	H2AC14
7	NSD2
9	TPX2
10	GNAI1

Gene ontology and KEGG pathway analysis of DEGs

A gene ontology study of atherosclerosis identifies critical biological processes, cellular components, and molecular activities closely linked to disease prognosis. The increased number of genes associated with cellular matrix organisation and cell-substrate adhesion indicates a significant involvement in the invasion of atherosclerotic plaques, which may result in cardiac problems. Epithelial cell proliferation indicates accelerated disease progression, a characteristic of atherosclerotic plaque formation that heightens the risk of stroke. Neutrophil activation in the immune response may influence the disease microenvironment, either facilitating or impeding disease progression based on the equilibrium of pro- and anti-inflammatory factors. From a biological component standpoint, the collagen-dense extracellular matrix and intercellular connections are essential for preserving tissue integrity, while their disturbance may promote disease advancement. Membrane rafts, microdomains, basolateral plasma membranes, and desmosomes facilitate signal transduction and cellular adhesion, which are essential for disease progression. Integrin binding, cadherin binding, and growth factor binding exemplify the intricate connections between cells and their surroundings, affecting migration and survival throughout disease progression. Peptidase regulators and inhibitors, by altering proteolytic processes, facilitate extracellular matrix remodelling, a crucial aspect of disease progression. Pathway enrichment analysis of differentially expressed genes in atherosclerosis highlights the complex nature of disease development. The ECM-receptor interaction and focal adhesion pathways are essential for cell migration and adhesion, enabling the separation and movement of atherosclerotic plaques in major blood arteries. The cell cycle and

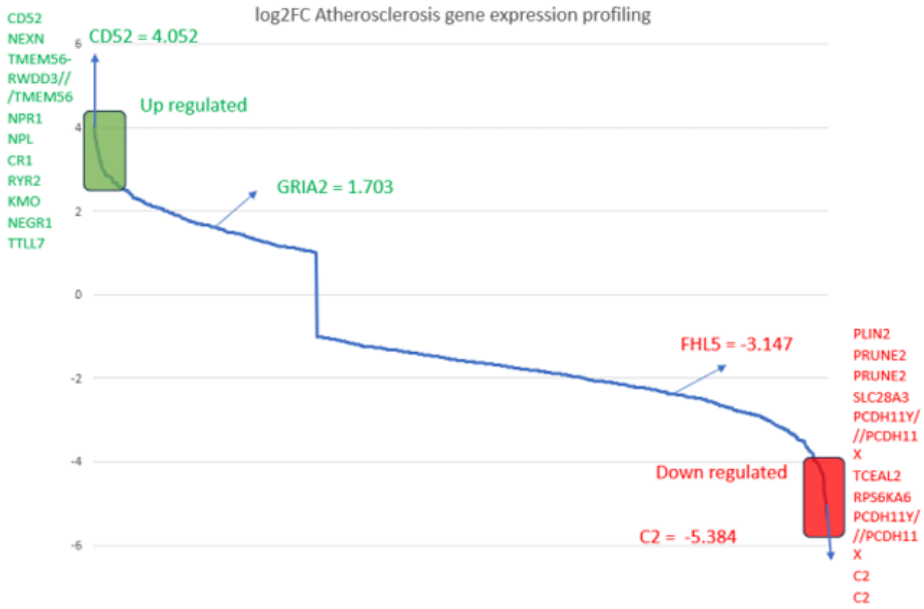
DNA replication pathways signify heightened cellular proliferation, while glycolysis demonstrates metabolic reprogramming that facilitates the survival and expansion of diseased cells. The relaxation signalling pathway affects the plaque microenvironment, leading to cardiovascular problems. The complement and coagulation cascades may augment the survival of circulating pathogenic cells, whereas phenylalanine metabolism contributes to the manufacture of chemicals that facilitate disease development. Collectively, these pathways provide insights into possible causes and treatment options for alleviating disease development in individuals with atherosclerosis. The thorough gene ontology study provides significant insights into the molecular processes behind atherosclerosis and suggests possible treatment targets.



Verification and survival analysis of hub genes in Atherosclerosis

A comparative investigation of mRNA expression for hub genes in atherosclerosis was performed using the GEPIA platform. The findings demonstrated a significant upregulation of all five hub genes, suggesting their potential role in disease progression. This persistent overexpression may influence the prognosis of atherosclerosis and its progression to serious cardiac problems, such as myocardial infarction, stroke, and cardiac arrest, the most prevalent outcomes of the condition. To further explore the relationship between hub gene expression and disease stages, correlation analysis was performed using the UALCAN platform. The identified gene upregulation in atherosclerosis signifies a robust correlation with disease advancement, implying an enhanced potential for disease infiltration and consequences. The constant expression patterns throughout multiple phases underscore the potential involvement of these genes in the underlying processes driving disease development, placing them as viable biomarkers for aggressive types of

atherosclerosis and as targets for therapeutic intervention. A survival analysis of atherosclerosis patients from the TCGA database was performed using UALCAN to assess the influence of hub gene expression. The findings demonstrated that elevated expression levels of KIT, NGFR, INHBB, and LEF1 were substantially associated with patient outcomes, as shown by p-values below 0.05. KIT (p = 8.2e-05) and NGFR (p = 1.2e-05) demonstrated significant correlations with survival, highlighting their potential as crucial indicators for disease progression. INHBB also demonstrated a significant relationship with prognosis (p = 0.0092), further reinforcing its relevance. In contrast, while LEF1 (p = 0.058) approached statistical significance, its precise impact on survival remains uncertain. Overall, our results underscore the crucial involvement of these hub genes in the pathogenesis of atherosclerosis, with KIT and NGFR emerging as especially attractive candidates for future exploration and possible therapeutic treatment. Utilising these findings, personalised therapy plans customised to individual genetic profiles may be established, facilitating precision medicine methods to reduce the risk of serious cardiac consequences in patients with atherosclerosis.



Gene Symbol	Gene title	Log2 (fold change)	Log 10 (p value)	Chromosome location
CD52	CD52 molecule	-1.263	5.386	chr1:26,317,958-26,320,523
NEXN	nexilin F-actin binding protein	1.027	6.886	chr1:77,888,513-77,943,895
TMEM56	TMEM56-REDD3 transmembrane protein 56	1.144	7.325	chr1:95,092,517-95,197,607
NPR1	natriuretic peptide receptor 1	1.077	5.716	chr1:153,678,688-153,693,992
NPL	N-acetylneuraminate pyruvate lyase	-1.066	6.173	chr1:182,789,293-182,830,384

CR1	complement component 3b/4b receptor 1 (Knops blood group)	-1.011	4.293	chr1:207,496,147-207,641,765
RYR2	ryanodine receptor 2	1.241	6.212	chr1:237,042,184-237,833,988
KMO	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	-1.124	5.699	chr1:241,532,134-241,595,642
NEGR1	neuronal growth regulator 1	1.101	7.595	chr1:71,395,943-72,282,539
TTLL7	tubulin tyrosine ligase like 7	1.026	5.951	chr1:83,865,024-83,999,150

DISCUSSION

Atherosclerosis is a significant worldwide health issue because of its correlation with various problems. This disorder, characterised by plaque formation in the arteries, is pivotal for the onset of cardiovascular illnesses (CVDs), including coronary artery disease (CAD), stroke, and peripheral arterial disease (PAD). Cardiovascular diseases (CVDs) continue to be the predominant cause of global mortality, resulting in over 17.9 million fatalities each year, according to the World Health Organisation (WHO). Atherosclerosis may result in myocardial infarction by inducing the production of thrombi in the coronary arteries, hence diminishing blood supply to the cardiac muscle. According to the Centres for Disease Control and Prevention (CDC), around 1.5 million heart attacks occur annually in the United States. Atherosclerosis impacting the cerebral arteries may lead to ischaemic strokes, the second foremost cause of mortality globally, accounting for about 6 million deaths each year (Pahwa and Jialal 2025; Ojha and Dhamoon 2025). Moreover, when atherosclerosis impacts the peripheral arteries, it may result in peripheral artery disease (PAD), a disorder marked by diminished blood flow to the extremities, causing discomfort, reduced movement, and tissue damage. The significant challenges highlight the need for preventative measures and efficient treatment techniques to mitigate the worldwide impact of atherosclerosis and its related conditions (Zemaitis, Boll, and Dreyer 2025; Nordanstig et al. 2023). This study's examination of the atherosclerosis dataset revealed 141 differentially expressed genes (DEGs). Five hub genes (KIT, LEF1, NGFR, EDNRB, and INHBB) were identified as elevated in atherosclerosis, suggesting their possible role in cancer development. KIT, known as CD117, has garnered interest for its possible involvement in the advancement of atherosclerosis (Qiu et al. 2022). Although it is largely acknowledged for its role in haematopoiesis and oncogenesis, recent data indicates its participation in vascular pathology. KIT is expressed in several cell types pertinent to atherosclerosis, including endothelial cells, smooth muscle cells, and immune cells. KIT signalling seems to govern critical events in atherosclerosis, including inflammation, immune response regulation, and smooth muscle cell activity (Zigmond et al. 2021; Poller, Nahrendorf, and Swirski 2020). The activation of KIT receptors on immune cells, including macrophages and mast cells, may affect cytokine production and the recruitment of immune cells, thereby influencing the inflammatory milieu inside atherosclerotic plaques. Furthermore, KIT signalling may influence smooth muscle cell proliferation, migration, and phenotypic transitions essential processes in plaque formation and disease progression (Xu and Shi 2012; Dileepan et al. 2023). While the exact processes connecting KIT to atherosclerosis are still being explored, a more comprehensive understanding of its function may provide innovative treatment approaches for atherosclerotic vascular disease. Additional hub genes, such as LEF1 (Lymphoid Enhancer-binding Factor 1), NGFR (Nerve Growth Factor Receptor, often referred to as CD271), EDNRB (Endothelin Receptor Type B), and INHBB (Inhibin Beta B), exhibit many biological activities; however, their role in atherosclerosis is still being investigated (J. Liu et al. 2022; Tasouli-Drakou et al. 2025). LEF1, a transcription factor in the Wntsignalling system, may contribute to atherosclerosis by regulating

cellular proliferation and differentiation, both of which are critical for plaque formation. NGFR, a receptor for nerve growth factor (NGF), may have a role in arterial remodelling and angiogenesis, both of which are essential in the pathophysiology of atherosclerosis (Santiago et al. 2017). EDNRB, an endothelin receptor, is associated with endothelial dysfunction and smooth muscle cell activity, possibly affecting plaque development. Although little is researched about atherosclerosis, INHBB, a constituent of the inhibin complex, has pleiotropic properties that may indirectly influence vascular biology (Davignon and Ganz 2004; Ivey, Osman, and Little 2008). The precise functions of these genes in atherosclerosis are not yet understood; however, more study may uncover their potential as biomarkers or therapeutic targets for the condition. Pathway enrichment analysis offers significant insights into the molecular processes of atherosclerosis and emphasises possible treatment targets(Rosati et al. 2024) . Focal adhesion kinase (FAK) signalling is recognised as a key regulator of cellular processes associated with atherosclerosis development. FAK is a cytoplasmic tyrosine kinase that mediates signalling from integrins transmembrane receptors that connect the extracellular matrix to the cytoskeleton,thereby regulating cell adhesion, migration, and survival (Tan et al. 2023; Zhao and Guan 2011). Dysregulated FAK signalling in atherosclerosis leads to endothelial dysfunction, smooth muscle cell migration, and inflammation. The activation of FAK enhances endothelial permeability and dysfunction; hence, it commences the preliminary phases of atherosclerotic lesion development (Gimbrone and García-Cardeña 2016; Finney et al. 2017). Moreover, FAK activation in vascular smooth muscle cells facilitates their migration from the media to the intima, thereby contributing to plaque formation and disease progression. Moreover, FAK signalling contributes to the modulation of inflammatory responses inside atherosclerotic plaques, intensifying local inflammation (Finney et al. 2017; Louis and Zahradka 2010). Analysing the role of FAK in atherosclerosis using route enrichment analysis offers a compelling justification for exploring FAK inhibitors or modulators as prospective therapeutic strategies to ameliorate disease progression and associated cardiovascular consequences (Tan et al. 2023).

CONCLUSION

This study investigated the underlying molecular mechanisms of atherosclerosis. By analyzing gene expression patterns, we identified 141 differentially expressed genes (DEGs) related to atherosclerosis, potentially playing crucial roles in disease spread. Functional analysis revealed enrichment in processes like cell adhesion, matrix remodeling, and epithelial proliferation, all linked to cardiac complications. Further network analysis pinpointed five hub genes exhibiting upregulation in atherosclerosis. Notably, increased expression of these hub genes correlated with advanced stages of atherosclerosis and decreased patient survival, particularly KIT and NGFR, suggesting their potential as prognostic biomarkers. Overall, this work provides insight into the complex molecular mechanisms of atherosclerosis, identifying several genes and pathways that could be potential targets for future diagnosis, therapy, and personalized medicine strategies.

REFERENCE

1. Ajoolabady, Amir, Domenico Pratico, Ling Lin, Christos S. Mantzoros, Suhad Bahijri, Jaakko Tuomilehto, and Jun Ren. 2024. “Inflammation in Atherosclerosis: Pathophysiology and Mechanisms.” Cell Death & Disease 15 (11): 817. https://doi.org/10.1038/s41419-024-07166-8.
2. Alegría-Torres, Jorge Alejandro, Andrea Baccarelli, and Valentina Bollati. 2011. “Epigenetics and Lifestyle.” Epigenomics 3 (3): 267–77. https://doi.org/10.2217/epi.11.22.
3. Aranda, Juan F., Julio Madrigal-Matute, Noemi Rotllan, and Carlos Fernández-Hernando. 2013. “MicroRNA Modulation of Lipid Metabolism and Oxidative Stress in Cardiometabolic Diseases.” Free Radical Biology & Medicine 64 (September):31–39. https://doi.org/10.1016/j.freeradbiomed.2013.07.014.
4. Barut, Zerrin, Fatma Tuba Akdeniz, Orcun Avsar, and Ayca Turer Cabbar. 2024. “Investigation of miRNA-199a-5p Expression and Its Clinical Association with LDL Cholesterol Levels in Atherosclerosis.” In Vivo (Athens, Greece) 38 (6): 2656–64. https://doi.org/10.21873/invivo.13742.
5. Batty, Matthew, Martin R. Bennett, and Emma Yu. 2022. “The Role of Oxidative Stress in Atherosclerosis.” Cells (Basel, Switzerland) 11 (23): 3843. https://doi.org/10.3390/cells11233843.
6. Cao, Genmao, Xuezhen Xuan, Jie Hu, Ruijing Zhang, Haijiang Jin, and Honglin Dong. 2022. “How Vascular Smooth Muscle Cell Phenotype Switching Contributes to Vascular Disease.” Cell Communication and Signaling: CCS 20 (1): 180. https://doi.org/10.1186/s12964-022-00993-2.
7. Citrin, Kathryn M., Carlos Fernández-Hernando, and Yajaira Suárez. 2021. “MicroRNA Regulation of Cholesterol Metabolism.” Annals of the New York Academy of Sciences 1495 (1): 55–77.

https://doi.org/10.1111/nyas.14566.

8. Davignon, Jean, and Peter Ganz. 2004. “Role of Endothelial Dysfunction in Atherosclerosis.” Circulation 109 (23 Suppl 1): III27–32. https://doi.org/10.1161/01.CIR.0000131515.03336.f8.
9. Dileepan, Kottarappat N., Vineesh V. Raveendran, Rishi Sharma, Harita Abraham, Rajat Barua, Vikas Singh, Ram Sharma, and Mukut Sharma. 2023. “Mast Cell-Mediated Immune Regulation in Health and Disease.” Frontiers in Medicine 10 (August):1213320. https://doi.org/10.3389/fmed.2023.1213320.
10. Duan, Yajun, Ke Gong, Suowen Xu, Feng Zhang, Xianshe Meng, and Jihong Han. 2022. “Regulation of Cholesterol Homeostasis in Health and Diseases: From Mechanisms to Targeted Therapeutics.” Signal Transduction and Targeted Therapy 7 (1): 265. https://doi.org/10.1038/s41392-022-01125-5.
11. Fazmin, Ibrahim T., Zakaria Achercouk, Charlotte E. Edling, Asri Said, and Kamalan Jeevaratnam. 2020. “Circulating microRNA as a Biomarker for Coronary Artery Disease.” Biomolecules 10 (10): 1354. https://doi.org/10.3390/biom10101354.
12. Finney, Alexandra C., Karen Y. Stokes, Christopher B. Pattillo, and A. Wayne Orr. 2017. “Integrin Signaling in Atherosclerosis.” Cellular and Molecular Life Sciences: CMLS 74 (12): 2263–82. https://doi.org/10.1007/s00018-017-2490-4.
13. Fuior, Elena Valeria, Evangelia Zvintzou, Theodosios Filippatos, Katerina Giannatou, Victoria Mparnia, Maya Simionescu, Anca Violeta Gafencu, and Kyriakos E. Kypreos. 2023. “Peroxisome Proliferator-Activated Receptor α in Lipoprotein Metabolism and Atherosclerotic Cardiovascular Disease.” Biomedicines 11 (10). https://doi.org/10.3390/biomedicines11102696.
14. Gimbrone, Michael A., Jr, and Guillermo García-Cardeña. 2016. “Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis.” Circulation Research 118 (4): 620–36. https://doi.org/10.1161/CIRCRESAHA.115.306301.
15. Hetherington, Isabella, and Hana Totary-Jain. 2022. “Anti-Atherosclerotic Therapies: Milestones, Challenges, and Emerging Innovations.” Molecular Therapy: The Journal of the American Society of Gene Therapy 30 (10): 3106–17. https://doi.org/10.1016/j.ymthe.2022.08.024.
16. Incalza, Maria Angela, Rossella D’Oria, Annalisa Natalicchio, Sebastio Perrini, Luigi Laviola, and Francesco Giorgino. 2018. “Oxidative Stress and Reactive Oxygen Species in Endothelial Dysfunction Associated with Cardiovascular and Metabolic Diseases.” Vascular Pharmacology 100 (January):1–19. https://doi.org/10.1016/j.vph.2017.05.005.
17. Ivey, Melanie E., Narin Osman, and Peter J. Little. 2008. “Endothelin-1 Signalling in Vascular Smooth Muscle: Pathways Controlling Cellular Functions Associated with Atherosclerosis.” Atherosclerosis 199 (2): 237–47. https://doi.org/10.1016/j.atherosclerosis.2008.03.006.
18. Jang, Boeun, Dongfeng Zhang, Zhao Ma, Xueyao Yang, Libo Liu, Haoran Xing, Lanxin Feng, et al. 2025. “MicroRNAs in Vascular Smooth Muscle Cells: Mechanisms, Therapeutic Potential, and Advances in Delivery Systems.” Life Sciences 364 (123424): 123424. https://doi.org/10.1016/j.lfs.2025.123424.
19. Jebari-Benslaiman, Shifa, Unai Galicia-García, Asier Larrea-Sebal, Javier Rekondo Olaetxea, Iraide Alloza, Koen Vandenbroeck, Asier Benito-Vicente, and César Martín. 2022. “Pathophysiology of Atherosclerosis.” International Journal of Molecular Sciences 23 (6): 3346. https://doi.org/10.3390/ijms23063346.
20. Li, Andrew C., and Christopher K. Glass. 2004. “PPAR- and LXR-Dependent Pathways Controlling Lipid Metabolism and the Development of Atherosclerosis.” The Journal of Lipid Research 45 (12): 2161–73. https://doi.org/10.1194/jlr.R400010-JLR200.
21. Linton, Macrae F., Patricia G. Yancey, Sean S. Davies, W. Gray Jerome, Edward F. Linton, Wenliang L. Song, Amanda C. Doran, and Kasey C. Vickers. 2000. “The Role of Lipids and Lipoproteins in Atherosclerosis.” In Endotext. South Dartmouth (MA): MDText.com, Inc. https://www.ncbi.nlm.nih.gov/books/NBK343489/.
22. Li, Tiewei, Xiaojuan Li, Yichuan Feng, Geng Dong, Yuewu Wang, and Junmei Yang. 2020. “The Role of Matrix Metalloproteinase-9 in Atherosclerotic Plaque Instability.” Mediators of Inflammation 2020 (October):3872367. https://doi.org/10.1155/2020/3872367.
23. Liu, Hanxiang, Yuchen Zhang, Yueyan Zhao, Yuzhen Li, Xiaofeng Zhang, Lingyu Bao, Rongkai Yan, et al. 2025. “Research Progress and Clinical Translation Potential of Coronary Atherosclerosis Diagnostic Markers from a Genomic Perspective.” Genes 16 (1). https://doi.org/10.3390/genes16010098.
24. Liu, Jiaqi, Qing Xiao, Jiani Xiao, Chenxi Niu, Yuanyuan Li, Xiaojun Zhang, Zhengwei Zhou, Guang Shu, and Gang Yin. 2022. “Wnt/β-Catenin Signalling: Function, Biological Mechanisms, and Therapeutic Opportunities.” Signal Transduction and Targeted Therapy 7 (1): 3. https://doi.org/10.1038/s41392-021-

00762-6.

25. López Rodríguez, Maykel, Uma Thanigai Arasu, and Minna U. Kaikkonen. 2023. “Exploring the Genetic Basis of Coronary Artery Disease Using Functional Genomics.” Atherosclerosis 374 (June):87–98. https://doi.org/10.1016/j.atherosclerosis.2023.01.019.
26. Louis, Sherif F., and Peter Zahradka. 2010. “Vascular Smooth Muscle Cell Motility: From Migration to Invasion.” Experimental and Clinical Cardiology 15 (4): e75–85. https://www.ncbi.nlm.nih.gov/pubmed/21264073.
27. Moore, Kathryn J., Frederick J. Sheedy, and Edward A. Fisher. 2013. “Macrophages in Atherosclerosis: A Dynamic Balance.” Nature Reviews. Immunology 13 (10): 709–21. https://doi.org/10.1038/nri3520.
28. Morrison, Amanda M., Alexander E. Sullivan, and Aaron W. Aday. 2023. “Atherosclerotic Disease: Pathogenesis and Approaches to Management.” The Medical Clinics of North America 107 (5): 793–805. https://doi.org/10.1016/j.mcna.2023.04.004.
29. Motsinger-Reif, Alison A., David M. Reif, Farida S. Akhtari, John S. House, C. Ryan Campbell, Kyle P. Messier, David C. Fargo, et al. 2024. “Gene-Environment Interactions within a Precision Environmental Health Framework.” Cell Genomics 4 (7): 100591. https://doi.org/10.1016/j.xgen.2024.100591.
30. Mundi, Santa, Marika Massaro, Egeria Scoditti, Maria Annunziata Carluccio, Victor W. M. van Hinsbergh, Marial Luisa Iruela-Arispe, and Raffaele De Caterina. 2018. “Endothelial Permeability, LDL Deposition, and Cardiovascular Risk Factors-a Review.” Cardiovascular Research 114 (1): 35–52. https://doi.org/10.1093/cvr/cvx226.
31. Nordanstig, J., C. A. Behrendt, A. W. Bradbury, G. J. de Borst, Fgr Fowkes, J. Golledge, A. Gottsater, R. J. Hinchliffe, S. Nikol, and L. Norgren. 2023. “Peripheral Arterial Disease (PAD) - A Challenging Manifestation of Atherosclerosis.” Preventive Medicine 171 (107489): 107489. https://doi.org/10.1016/j.ypmed.2023.107489.
32. Ojha, Niranjan, and Amit S. Dhamoon. 2025. “Myocardial Infarction.” In StatPearls. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK537076/.
33. Olejarz, Wioletta, Dominika Łacheta, and Grażyna Kubiak-Tomaszewska. 2020. “Matrix Metalloproteinases as Biomarkers of Atherosclerotic Plaque Instability.” International Journal of Molecular Sciences 21 (11): 3946. https://doi.org/10.3390/ijms21113946.
34. Pahwa, Roma, and Ishwarlal Jialal. 2025. “Atherosclerosis.” In StatPearls. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK507799/.
35. Pennings, Marieke, Illiana Meurs, Dan Ye, Ruud Out, Menno Hoekstra, Theo J. C. Van Berkel, and Miranda Van Eck. 2006. “Regulation of Cholesterol Homeostasis in Macrophages and Consequences for Atherosclerotic Lesion Development.” FEBS Letters 580 (23): 5588–96. https://doi.org/10.1016/j.febslet.2006.08.022.
36. Pereira-da-Silva, Tiago, Patrícia Napoleão, Marina C. Costa, André F. Gabriel, Mafalda Selas, Filipa Silva, Francisco J. Enguita, Rui Cruz Ferreira, and Miguel Mota Carmo. 2021. “Circulating miRNAs Are Associated with the Systemic Extent of Atherosclerosis: Novel Observations for miR-27b and miR-146.” Diagnostics (Basel, Switzerland) 11 (2): 318. https://doi.org/10.3390/diagnostics11020318.
37. Poller, Wolfram C., Matthias Nahrendorf, and Filip K. Swirski. 2020. “Hematopoiesis and Cardiovascular Disease.” Circulation Research 126 (8): 1061–85. https://doi.org/10.1161/CIRCRESAHA.120.315895.
38. Qiu, Xue, Jinyan Lin, Yanbing Chen, Bixiao Liang, and Lang Li. 2022. “Identification of Hub Genes Associated with Abnormal Endothelial Function in Early Coronary Atherosclerosis.” Biochemical Genetics 60 (4): 1189–1204. https://doi.org/10.1007/s10528-021-10139-7.
39. Rafieian-Kopaei, Mahmoud, Mahbubeh Setorki, Monir Doudi, Azar Baradaran, and Hamid Nasri. 2014. “Atherosclerosis: Process, Indicators, Risk Factors and New Hopes.” International Journal of Preventive Medicine 5 (8): 927–46. https://pmc.ncbi.nlm.nih.gov/articles/PMC4258672/.
40. Rosati, Diletta, Maria Palmieri, Giulia Brunelli, Andrea Morrione, Francesco Iannelli, Elisa Frullanti, and Antonio Giordano. 2024. “Differential Gene Expression Analysis Pipelines and Bioinformatic Tools for the Identification of Specific Biomarkers: A Review.” Computational and Structural Biotechnology Journal 23 (December):1154–68. https://doi.org/10.1016/j.csbj.2024.02.018.
41. Santiago, Larion, Garrett Daniels, Dongwen Wang, Fang-Ming Deng, and Peng Lee. 2017. “Wnt Signaling Pathway Protein LEF1 in Cancer, as a Biomarker for Prognosis and a Target for Treatment.” American Journal of Cancer Research 7 (6): 1389–1406. https://www.ncbi.nlm.nih.gov/pubmed/28670499.
42. Sivapalaratnam, S., M. M. Motazacker, S. Maiwald, G. K. Hovingh, J. J. P. Kastelein, M. Levi, M. D. Trip,

and G. M. Dallinga-Thie. 2011. “Genome-Wide Association Studies in Atherosclerosis.” Current Atherosclerosis Reports 13 (3): 225–32. https://doi.org/10.1007/s11883-011-0173-4.

43. Tan, Ximin, Yuheng Yan, Bin Song, Shuangli Zhu, Qi Mei, and Kongming Wu. 2023. “Focal Adhesion Kinase: From Biological Functions to Therapeutic Strategies.” Experimental Hematology & Oncology 12 (1): 83. https://doi.org/10.1186/s40164-023-00446-7.
44. Tasouli-Drakou, Vasiliki, Ian Ogurek, Taha Shaikh, Marc Ringor, Michael V. DiCaro, and Kachon Lei. 2025. “Atherosclerosis: A Comprehensive Review of Molecular Factors and Mechanisms.” International Journal of Molecular Sciences 26 (3). https://doi.org/10.3390/ijms26031364.
45. Xie, Xuena, Meiwen Huang, Shudong Ma, Qiqi Xin, Yuying Wang, Lantian Hu, Han Zhao, et al. 2025. “The Role of Long Non-Coding RNAs in Cardiovascular Diseases: A Comprehensive Review.” Non-Coding RNA Research 11 (April):158–87. https://doi.org/10.1016/j.ncrna.2024.12.009.
46. Xu, Jia-Ming, and Guo-Ping Shi. 2012. “Emerging Role of Mast Cells and Macrophages in Cardiovascular and Metabolic Diseases.” Endocrine Reviews 33 (1): 71–108. https://doi.org/10.1210/er.2011-0013.
47. Yilmaz, Seda Gulec, Selim Isbir, Atike Tekeli Kunt, and Turgay Isbir. 2018. “Circulating microRNAs as Novel Biomarkers for Atherosclerosis.” In Vivo (Athens, Greece) 32 (3): 561–65. https://doi.org/10.21873/invivo.11276.
48. Zemaitis, Michael R., Julia M. Boll, and Mark A. Dreyer. 2025. “Peripheral Arterial Disease.” In StatPearls. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK430745/#:~:text=Patients%20with%20peripheral%20arterial%20disease,blood%20flow%2C%20necessitating%20emergent%20surgery.
49. Zhang, Zhengyi, David Salisbury, and Tamer Sallam. 2018. “Long Noncoding RNAs in Atherosclerosis: JACC Review Topic of the Week.” Journal of the American College of Cardiology 72 (19): 2380–90. https://doi.org/10.1016/j.jacc.2018.08.2161.
50. Zhao, Xiaofeng, and Jun-Lin Guan. 2011. “Focal Adhesion Kinase and Its Signaling Pathways in Cell Migration and Angiogenesis.” Advanced Drug Delivery Reviews 63 (8): 610–15. https://doi.org/10.1016/j.addr.2010.11.001.
51. Zigmond, Zachary M., Lei Song, Laisel Martinez, Roberta M. Lassance-Soares, Omaida C. Velazquez, and Roberto I. Vazquez-Padron. 2021. “C-Kit Expression in Smooth Muscle Cells Reduces Atherosclerosis Burden in Hyperlipidemic Mice.” Atherosclerosis 324 (May):133–40. https://doi.org/10.1016/j.atherosclerosis.2021.03.004.