# Atherosclerosis: Open Access

# Genetic Studies in Atherosclerosis: Unveiling Susceptibility and Personalized Risk Assessment for Heart Disease and Stroke

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## Abstract

Atherosclerosis, a condition affecting the large arteries, stands as the leading cause of heart disease and stroke. In westernized societies, it accounts for approximately 50% of all deaths. While epidemiological studies have identified crucial environmental and genetic risk factors linked to atherosclerosis, its etiological complexity has impeded progress in understanding the underlying mechanisms. Fortunately, advancements in investigative tools over the past decade, such as genetically modified mouse models of the disease, have shed light on the molecular interactions involved. These insights have revealed the intricate connections between altered cholesterol metabolism, other risk factors, and the formation of atherosclerotic plaque. We now comprehend that atherosclerosis is not merely an inevitable consequence of aging but rather a chronic inflammatory condition. This condition can turn into an acute clinical event when plaque rupture and thrombosis occur. With this improved understanding, we are better equipped to address the complexities of atherosclerosis and potentially develop more effective treatments to combat its devastating consequences.

**Keywords:** Genetic studies; Atherosclerosis; Stroke; susceptibility; Risk assessment; Blood pressure; DNA-based tests; Environmental influences; Personalized risk; Early intervention

## Introduction

Atherosclerosis is a progressive disease marked by the buildup of lipids and fibrous components within the large arteries. In its early stages, atherosclerosis manifests as subendothelial accumulations of cholesterol-engorged macrophages known as 'foam cells.' These initial lesions, called 'fatty streaks,' are commonly found in the aorta. Certain areas within the arteries are more prone to lesion formation due to variations in blood flow dynamics. These 'fibrous lesions' typically feature a 'fibrous cap' comprising SMCs and extracellular matrix, surrounding a lipid-rich 'necrotic core.' Over time, plaques can become increasingly complex, displaying calcification, ulceration at the luminal surface, and hemorrhage from small vessels that grow into the lesion from the blood vessel's media [1,2]. While advanced lesions can grow large enough to obstruct blood flow, the most critical clinical complication arises from acute occlusion caused by the formation of a thrombus or blood clot. This can lead to myocardial infarction or stroke. Typically, thrombosis is associated with the rupture or erosion of the lesion, triggering severe consequences. Animal models, such as rabbits, pigs, non-human primates, and rodents, have provided significant insights into the events of atherosclerosis. Among these models, mice deficient in apolipoprotein E (apoE) or the low-density lipoprotein (LDL) receptor have been extensively utilized in genetic and physiological studies, showcasing advanced lesion development. Upon feeding a high-fat, high-cholesterol diet, the first noticeable change in the artery wall is the accumulation of lipoprotein particles and their aggregates in the intima, particularly at sites predisposed to lesion formation [3-6]. Within a short period, monocytes adhere to the endothelial surface and subsequently transmigrate across the endothelial monolayer into the intima. Once in the intima, the monocytes proliferate, differentiate into macrophages, and uptake the lipoproteins, resulting in the formation of foam cells. Some fatty streaks progress further by accumulating smooth muscle cells (SMCs) that migrate from the medial layer. These SMCs secrete fibrous elements, leading to the development and enlargement of occlusive fibrous plaques. The continuous growth of the lesions is facilitated by the migration of new mononuclear cells from the bloodstream, entering at the vessel's shoulder. This migration is accompanied by cell proliferation, extracellular matrix production, and the accumulation of extracellular lipid. Atherogenesis can be seen as a 'response to injury,' with injurious agents such as lipoproteins or other risk factors playing a role in this process. Over the past five decades, epidemiological studies have unveiled a multitude of risk factors associated with atherosclerosis [7]. These factors can be categorized into those heavily influenced by genetics and those predominantly influenced by the environment. Among them, the relative abundance of various plasma lipoproteins emerges as a crucial factor, as elevated levels of atherogenic lipoproteins serve as a fundamental prerequisite for most forms of the disease. While gender and lipoprotein (a) levels are exceptions, each genetic risk factor involves multiple genes, leading to intricate complexities [8]. Such intricacies become apparent when studying genetic crosses in animals kept under similar environmental conditions. In the case of rodents, numerous genetic loci have been identified that contribute to lipoprotein levels, body fat, and other risk factors. Furthermore, there exists an additional layer of complexity due to interactions between these risk factors. Often, their combined effects are not merely additive. For example, the impact of hypertension on coronary heart disease (CHD) becomes significantly amplified when cholesterol levels are high. This demonstrates how a synergistic interplay between risk factors can influence the development and progression of atherosclerosis. Numerous family and twin studies have thoroughly investigated the role of genetics and the environment in human coronary heart disease (CHD). These studies have consistently demonstrated that within a given population, atherosclerosis has a high heritability, often surpassing 50%, indicating that genetics play a significant role in the

development of the disease [9]. However, when examining variations in disease incidence between different populations through population migration studies, it becomes evident that the environment accounts for a substantial portion of this variation. As a result, the common forms of CHD arise from a combination of factors, including an unhealthy environment, genetic susceptibility, and the impact of our increased lifespan (5). These elements together contribute to the complex interplay that underlies the onset and progression of CHD in human populations [10,11].

## Interactions at the cellular and molecular levels

Pathological investigations have revealed a well-defined sequence of changes in the blood vessels during atherogenesis, highlighting the crucial involvement of blood-derived inflammatory cells, particularly monocytes/macrophages. In parallel, studies conducted using tissue cultures involving vascular cells and monocytes/macrophages have suggested potential pathways for disease initiation and progression. These investigations have provided compelling evidence for the pivotal role of the endothelium in triggering inflammation and have implicated the accumulation of oxidatively modified LDL in the intima as a significant factor in recruiting monocytes and promoting foam-cell formation. Over the past decade, the understanding of molecular mechanisms underlying atherogenesis has undergone a revolutionary shift, largely driven by studies in transgenic and gene-targeted mice [12]. These advanced studies have enabled in vivo testing of hypotheses, although it is essential to acknowledge that there are significant species differences between mice and humans. Consequently, reliable mouse models for thrombosis involving lesion rupture have not yet been developed. Nonetheless, these animal studies have yielded invaluable insights into the intricate processes driving atherogenesis and have paved the way for potential therapeutic interventions. The endothelium serves as a selectively permeable barrier with intercellular tight junctional complexes, separating blood from tissues. Functioning with both sensory and executive roles, it can produce effector molecules that regulate thrombosis, inflammation, vascular tone, and vascular remodeling. For instance, if the endothelium is removed, smooth muscle cell (SMC) migration and proliferation increase significantly, but this effect subsides upon endothelium regeneration. Among the significant physical forces impacting endothelial cells (ECs), fluid shear stress plays a crucial role and influences EC morphology [13,14]. In areas of arteries with uniform and laminar blood flow, such as the tubular regions, ECs take on an ellipsoid shape and align in the direction of flow. On the other hand, in regions with arterial branching or curvature where flow is disturbed, ECs exhibit polygonal shapes and lack a specific orientation. In these latter areas, permeability to macromolecules like LDL increases, making them preferred sites for lesion formation.

The rapid uptake of native LDL by macrophages is insufficient to generate foam cells, leading to the proposal that LDL undergoes some form of modification within the vessel wall. Subsequent studies have confirmed that trapped LDL does undergo modifications, including oxidation, lipolysis, proteolysis, and aggregation. These alterations contribute not only to foam-cell formation but also to inflammation. Among these modifications, lipid oxidation plays a significant role in the early formation of lesions, triggered by exposure to the oxidative waste products of vascular cells. The initial result of such modifications is the creation of "minimally oxidized" LDL species, which exhibit pro-inflammatory activity but might not be adequately modified to be recognized by macrophage scavenger receptors. Studies in mice lacking 12/15-lipoxygenase have shown a considerable reduction in

atherosclerosis, indicating that this enzyme likely plays a crucial role as a source of reactive oxygen species in LDL oxidation. Lipoxygenases are responsible for introducing molecular oxygen into polyenoic fatty acids, generating molecules like hydroperoxyeicosatetraenoic acid (HPETE), which are likely transferred across the cell membrane to "seed" the extracellular LDL. These modifications further contribute to the intricate processes underlying atherosclerosis development. HDL, commonly known as high-density lipoprotein, exhibits robust protection against atherosclerosis. Its protective effects stem from multiple mechanisms, with a key role being the removal of excess cholesterol from peripheral tissues. Additionally, HDL offers protection by inhibiting lipoprotein oxidation. These antioxidant properties of HDL are partly attributed to serum paraoxonase, an esterase present on HDL that can break down specific biologically active oxidized phospholipids. This interplay of HDL's cholesterol-clearing abilities and its antioxidative properties contributes to its beneficial impact in preventing atherosclerosis and promoting cardiovascular health. Atherosclerosis is characterized by the recruitment of monocytes and lymphocytes to the artery wall, while neutrophils are not involved in this process [15]. The accumulation of minimally oxidized LDL serves as a triggering event, prompting the endothelial cells (ECs) above to produce various pro-inflammatory molecules, including adhesion molecules and growth factors like macrophage colony-stimulating factor (M-CSF). The phospholipid fraction of minimally oxidized LDL contains the primary biological activity, and researchers have identified three active oxidation products resulting from the scission or rearrangement of unsaturated fatty acids. Furthermore, oxidized LDL has the ability to inhibit the production of nitric oxide (NO), a chemical mediator with multiple anti-atherogenic properties, including vasorelaxation. Studies in mice lacking endothelial NO synthase have shown increased atherosclerosis, partially attributed to elevated blood pressure. Diabetes may also promote inflammation, partially through the formation of advanced end products of glycation that interact with endothelial receptors. The interplay of these factors contributes to the intricate process of inflammation and atherosclerosis development. The recruitment of specific types of leukocytes into the artery wall is facilitated by adhesion molecules and chemotactic factors. When cultured endothelial cells (ECs) are exposed to oxidized LDL, they are capable of binding to monocytes but not neutrophils. The initial step in adhesion involves the "rolling" of leukocytes along the endothelial surface, a process mediated by selectins that bind to carbohydrate ligands on leukocytes. Studies involving mice deficient in P- and E-selectins or the cell adhesion molecule ICAM have shed light on the crucial role of these adhesion molecules in atherosclerosis. The firm adhesion of monocytes and T cells to the endothelium can be achieved through the integrin VLA-4 present on these cells, which interacts with both VCAM-1 on the endothelium and the CS-1 splice variant of fibronectin. Both in vitro and in vivo investigations have indicated that these interactions play a significant role in atherosclerosis. Lastly, studies on mice lacking monocyte chemotactic protein (MCP-1) or its receptor CCR2 have shown a considerable reduction in atherosclerotic lesions, suggesting that the interaction between MCP-1 and CCR2 is involved in monocyte recruitment during atherosclerosis. These findings highlight the critical role of adhesion molecules and chemotactic factors in orchestrating the recruitment and adhesion of leukocytes in the development of atherosclerosis. The cytokine M-CSF plays a vital role in stimulating the proliferation and differentiation of macrophages, and it also influences several macrophage functions, including the expression of scavenger receptors. Studies involving mice with a spontaneous null mutation of M-CSF have demonstrated a remarkable reduction in lesions, indicating that macrophages play an

essential and obligatory role in the formation of these lesions.

## Discussion

Catheterization is currently considered the gold standard for diagnosing atherosclerosis, but it comes with significant expenses and risks. As a result, there is an urgent need for reliable noninvasive diagnostic methods. Some biochemical markers, such as C-reactive protein, and certain noninvasive procedures like extravascular ultrasound and ultrafast computerized tomography show promise in diagnosing the disease, but they also have limitations. As our understanding of the genetic aspects of atherosclerosis advances, genetic diagnosis will become increasingly crucial. The anticipated "biallelic map" of the genome is expected to drive the development of new technologies for gene screening. These technologies will range from high-throughput, genome-wide methods to testing for specific gene variants in individuals. One significant application of genetic screening will be to differentiate between different forms of the disease, allowing for more targeted pharmacological interventions. Atherosclerosis is a heterogeneous condition, and the most suitable therapy will depend on the specific type of the disease. Currently, clinical classification groups patients based on the risk factors they display, but genetic testing is likely to greatly expand the subdivisions of the disease, providing a more personalized and precise approach to treatment.

## Conclusion

Genetic studies offer another potential advantage - testing for susceptibility to diseases like CHD and stroke. Since these conditions primarily affect adults, having knowledge of an individual's predisposition to the disease could be available long before clinical symptoms manifest, enabling early intervention. Testing for factors like LDL, HDL, and blood pressure has long been recommended to identify individuals at higher risk, and newer risk indicators have also emerged. Once the genes responsible for common forms of the disease, along with specific mutations, are identified, DNA-based tests may significantly enhance our ability to assess risk. However, due to the significant impact of environmental factors and the complex genetic nature of atherosclerosis, efficient screening procedures may not be readily available in the near future.

## Acknowledgement

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## Conflict of Interest

Author declares no conflict of interest.

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