REVIEW ARTICLE

The Changing Face of Atherosclerosis

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Abstract

BACKGROUND: Statins have been used for around three decades as the primary way to lower cholesterol and reduce the risk of atherosclerosis, but in some groups, statin resistance is common, and the danger of atherosclerosis is still high.

CONTENT: Atherosclerosis was once believed to be caused by cholesterol and thrombotic material passively accumulating in the walls of arteries. However, current knowledge shows that the immune cells and inflammatory processes are essential in the formation, progression, and consequences of atherosclerotic lesions, characterized by a persistent inflammatory response, including thrombotic complications. Study of genetic, creating risk score for atherosclerosis, add more information to create more new therapies targeting low density lipoprotein cholesterol (LDL-C) receptor, and shows prospect.

SUMMARY: Over time, atherosclerosis theories and treatment strategies have changed. While statins were widely used, they have now been supplanted by alternative options like the proprotein convertase subtilisin/kexin type 9 (PSCK9) inhibitor that manage atherosclerosis more effectively and comprehensive.

KEYWORDS: atherosclerosis, cholesterol, inflammation, immune system, metabolism

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Introduction

Despite significant advancement over the last 40 years, atherosclerotic cardiovascular disease (ASCVD) continues to be the world's leading cause of mortality.(1) Plasma cholesterol, in particular low-density lipoprotein cholesterol (LDL-C), has been implicated by a number of lines of evidence from genetic, experimental, epidemiological, and clinical research as the main factor in the development of the atherosclerotic plaque. The discovery and development of statin medications, which significantly reduce LDL-C and the incidence of morbidity and death due to ASCVD, has been one of the most important scientific advances throughout this time. With very minor incremental changes to the therapeutic toolbox, statins have remained the mainstay of treatment for more than 25 years. Fortunately, there has lately been a rebirth in the field of lipid therapies. Ezetimibe offers a better benefit compared to statin therapy in patients with acute coronary syndromes (ACS) have rekindled interest in the cholesterol hypothesis and the expectation that further LDL-C-lowering medications may reduce the risk of ASCVD. The proprotein convertase subtilisin kexin-9 (PCSK9) inhibitors are a new family of cholesterol-lowering medications that were created as a result of the quick translation of a unique biological route that was discovered. Trial with niacin shows a significant increases in high-density lipoprotein (HDL) levels, but have failed to show cardiovascular benefits, and studies of three cholesterol ester transfer protein inhibitors have likewise failed to lower the risk of ASCVD. Two sizable clinical studies are evaluating the impact of omega-3

polyunsaturated fatty acids (PUFA) on atherosclerotic events in hypertriglyceridemia, even if the efficacy of triglyceride-lowering treatments is yet unknown. The future looks bright for novel antisense medicines that target apolipoprotein C-III to lower triglycerides and Apo(a) to lower lipoprotein(a). Last but not least, two sizable clinical studies are formally testing the inflammatory concept of atherosclerosis and may present a fresh approach to lowering the risk of cardiovascular disease.(2) Many people believed that the success of statins and other preventative measures near the end of the 20th century, we would put an end to the ASCVD epidemic.(3) However, the burden of ischemic cardiovascular diseases has still increased as the leading cause of morbidity, mortality, and reduced of Quality of Life (QoL) globally.(1)

It has been acknowledged that aging-related processes such clonal hematopoiesis and senescence, as well as gut microbiota probably play a significant impact in atherosclerosis. The interaction of genetic and environmental risk factors for atherosclerosis and its link to cardiometabolic characteristics will be another promising developments in the field of diagnosis and treatment.(4)

There has also been an increase in interest in non-traditional causes of atherosclerosis, including poor sleep quality, sedentary lifestyle, gut microbiota, environmental toxins and stress. Leukocytes and inflammatory pathways play a role in both established and new risk factors, affecting the behavior of cells in arterial walls. Furthermore, bone marrow involvement in atherosclerosis, specifically clonal hematopoiesis, is a significant contributor to the risk of ASCVD, especially in older adults. This is due to somatic mutations in stem cells. The vulnerable plaque notion is no longer the primary way that the processes behind thrombotic complications of atherosclerosis.(5) In this review, the current perspectives in atherosclerosis pathophysiology, mechanisms, and provide an insight on potential novel management of ASCVD would be summarized.

From Lipid to Inflammation

A compelling clinical data shows that inflammation plays a critical role in atherogenesis and the pathophysiology of ischemic events, more than dyslipidaemia.(6) Instead of replacing or downgrading lipid risk, inflammation creates a number of pathways that connect lipids and other conventional risk factors to atherosclerosis. For instance, levels of C-reactive protein (CRP), a diagnostic of inflammation, are correlated with residual lipoprotein concentrations.(7) There is a lot of evidence linking inflammation to high blood pressure.(8) Experimental studies have demonstrated how innate and adaptive immunity contribute to atherosclerosis. Human biomarker studies have demonstrated that markers of inflammation independently of all conventional risk factors predict risk of ASCVD in a large population of people with or without apparent cardiovascular illness.(9) A recognized and therapeutically helpful indicator of an individual's general innate immune function in connection to ASCVD risk is the acute phase reactant, which may be evaluated using a highly sensitive assay (known as high-sensitive CRP (hs-CRP)).(10)

In the "Canakinumab Anti-inflammatory Thrombosis Outcomes Study" (CANTOS), patients with stable coronary artery disease who suffered previous myocardial infraction (MI) were randomly assigned to receive canakinumab, a proinflammatory cytokine interleukin (IL)-1β neutralizer antibody.(11) The subjects showed signs of inflammation despite of receiving regular standard of care (measured by a hsCRP >2 mg/L). The baseline LDL-C for the subjects was about 2 mM (81 mg/dL). Antiinflammatory treatment thus can decrease about 15% of the incidence of recurrent myocardial infarction (MI), stroke, or cardiovascular mortality. Individuals who responded to the neutralization of IL-1β achieved a greater reduction in hs-CRP than the median level. Additionally, they experienced a 26% decrease in the major endpoint and a decline in all-cause mortality. It was expected that CANTOS would demonstrate a slight increase in infections, including fatal infections, among those who received canakinumab due to the involvement of IL-1β in host defenses.(5)

The development of atherosclerosis is a multi-step process that involves both systemic and local components. Apolipoprotein-B (ApoB) is the most important lipoproteins among all. ApoB is a structural protein located on the membrane bilayer of LDL-C cholesterol, and acts as a ligand for receptor-mediated clearance. LDL-C is the primary ApoB that fuels the atherogenic process.(12) Particularly, plasma LDL-C reach the subendothelial compartment via penetrating the artery endothelial cell (EC) lining in vulnerable nonlaminar flow zones (bends, branch points).(13) The development of therapeutic and preventative measures is still hampered by the lack of a thorough understanding of the process by which ApoB move through the endothelial layer and travel along the arterial wall. The intimal ApoB are restrained in the extracellular matrix by binding to subendothelial proteoglycans, which contain sulfate groups with negative charges that interact with

arginine and lysine residues on ApoB possessing positive charges.(14)

Elie Metchnikoff and Ralph Steinman each received two Nobel Prizes in Physiology or Medicine, more than a century apart (1908 and 2011), for their work on the discovery of the dendritic cell (DC) and its function in adaptive immunity. The concept that persistent inflammation, marked by the attraction of monocytes, leads to the build-up of macrophages in atherosclerotic plaques has been firmly established through the fundamental principles of contemporary macrophage biology established by van Furth and Cohn. These principles include the understanding that these specialized cells that engulf and digest foreign substances are derived from monocytes in the bloodstream.(16)

The development of atherosclerosis depends on monocyte-derived macrophages. The lesion progression is also considerably influenced by in situ macrophage proliferation in addition to monocyte recruitment. Research on advanced atherosclerotic lesions in humans and rabbits showed evidence of macrophage growth.(17) Actually, this process starts in early lesions at the same time as increased monocyte recruitment.(18) Early lesions do not typically exhibit macrophage apoptosis as measured by terminal deoxynucleotidyl transferase 2′-Deoxyuridine, 5′-Triphosphate (dUTP) nick-end labeling, and increased apoptosis can be noticed at the 8-week time point when early lesions start to transform into advanced and complex lesions. Since macrophage foam cell egress into the circulation is thought to be an uncommon occurrence during atherogenesis, it is unlikely to have a significant effect on the buildup of macrophages.(19) Macrophage retention may be influenced by the synthesis and release of repellent neuroimmune guidance signals such netrin-1, ephrin-B, and semaphorins 3A and 3E.(20) By producing platelet-activating factor, hypercholesterolemia also hinders the migration of dermal DCs to local lymph nodes.(21) Oxidized LDL-C, as opposed to native LDL-C, obstructs toll-like receptor (TLR)4-triggered peritoneal macrophage outflow into the lymphatic system and *in vitro* migration via a mechanism that involves cluster of differentiation (CD)36, deactivation of Src homology 2-containing phosphotyrosine phosphatase, persistent activation of focal adhesion kinase, and changes in cytoskeletal dynamics.(22) Plaque regression may be aided by the upregulation of C-C motif chemokine receptor 7 (CCR7) in plaque macrophages during reversal of hypercholesterolemia or therapy with statins.(23) Collectively, these findings imply that the primary biological processes governing the development of foam cell lesions are macrophage proliferation and monocyte recruitment, rather than macrophage outflow.(24)

More studies indicates that leukocyte counts in circulation are also linked to atherosclerosis exhibit diurnal oscillations. Recent investigation on mouse have shown that part of the regulatory mechanisms have a role in the diurnal changes of immune cell counts.(25) Leukocyte counts in tissues such as bone marrow, skeletal muscle, and the heart vary in contrast to those in blood, with the highest levels observed at the onset of the active phase. Conversely, leukocyte counts in murine blood fluctuate and reach a peak during the inactive phase.(26,27) One of the main immune cell types recruited into atherosclerotic lesions are classical monocytes.(28) Through transcriptional adjustments controlled by the clock transcription factor basic helix-loop-helix ARNT like 1 (BMAL1), their quantities fluctuate in blood and tissue.(29) Monocyte-intrinsic BMAL1, a transcriptional repressor of chemokines involved in monocyte recruitment, such as CC-chemokine ligand-2 (CCL2), was found at the molecular level.(30)

The subendothelial retention of ApoB leads to the development of atherosclerosis, and cause persistent inflammatory response. The macrophage, whose main source is myeloid progenitor cells in bone marrow, is the main immune cell in atherosclerotic plaques. In mouse, myeloid progenitor cells mature into circulating monocytes, and in some circumstances, the spleen serves as a reservoir for monocytes infiltrating atherosclerotic lesions. It's interesting to note that several ASCVD risk factors, including hypercholesterolemia, stimulate the production of monocytes in the bone marrow, which results in monocytosis (an increase in the number of circulating monocytes), which is a risk factor for atherosclerotic disease on its own.(28,31) Circulating monocytes then attach to ECs lining the lumen of vulnerable arteries, which allows them to access regions of arterial hemodynamic stress.(32) The monocytes develop into lesional macrophages after they have reached the subendothelial area.(33)

Several distinct scavenger receptors (SRs) produced by macrophages, as well as macropinocytosis and phagocytosis, take up modified LDL-C, aggregated lipoproteins, and other compounds.(34) $ApoE^{-/-}$ mice that lack the SR-A28 or CD3629 genes in all tissues exhibit significantly reduced susceptibility to atherosclerosis. Several scavenger receptors, including SR type 1 (SR-A), CD36, SR-BI, LOX1, and LDL-C receptor-related protein 1, are involved in this process. However, when $ApoE^{-/-}$ $LDL\text{-}CR^{-/-}$ mice with SR-A or CD36 deficiencies were bred into the C57BL/6 background, there was no decline

in atherosclerosis, and numerous lesional macrophage foam cells were found. These findings suggest that the effects of SR-A and CD36 on atherosclerosis may be influenced by other factors or by the genetic background of the mouse. (35) Recent research has linked SR-A to the promotion of lesional macrophage proliferation, CD36 to the coordination of inflammasome activation, inhibition of migration and promotion of macrophage spreading and attachment, and SR-A and CD36 to the promotion of apoptosis, lesion necrotic core expansion, and inflammatory gene expression. The latter effects were noted without any impact on lesion size or foam cell development.(36) Therefore, in addition to encouraging lipid uptake, SR-A and CD36 appear to have significant signalling roles that influence the development of atherosclerosis.(37)

Since metabolism and inflammation are intricately intertwined, a macrophage's metabolic phenotype can affect its inflammatory phenotype and *vice versa*. We refer to the phenotype created by energy metabolism's substrates and intermediates as the macrophage metabolic phenotype where in advanced atherosclerosis the monocytes tend to differentiate into M1 macrophages (pro-inflammatory) rather than M2 (anti-inflammatory form) which help in resolution.(38) Additionally, deficiencies in efferocytosis, the process by which apoptotic macrophages are cleared, along with problems in macrophage egress from advanced lesions result in an enlarged necrotic core. Furthermore, it appears that the M2 phenotype is dependent on fatty acid oxidation, and the survival of activated macrophages M1 depends on glycolysis.(39) However, overexpression of the glucose transporter glucose transporter 1 (GLUT1) in myeloid cells increase glycolysis but it is not enough to promote atherosclerosis or inflammatory activation. (40) Reducing glycolysis can promote apoptosis in active macrophages, which might increase the production of necrotic cores.

Figure 1 showed in the early lesion of fatty streak, monocytes and macrophages loaded with lipid from lipoproteins and become foam cells.(41) Macrophages metabolism changes, induced by some substrates and intermediates to generate ATP from nutrients, refer as metabolic phenotype and become more efficient in efferocytosis. When the lesion grow, this efferocytosis capability is impaired and death macrophages remnants accumulated. These contributes to the inflammatory process and plaque rupture. Once the lesion regressing, monocyte infiltration reduced, efferocytosis improved, and the remaining macrophages have altered gene expression, with less lipid load. Finally, the inflammation resolved.

Vasoconstriction and thrombosis components are added to atherosclerotic lesions in the ACS processes. Thrombosis typically remains undetected, but can be seen during an autopsy, angiography, or angioscopy. The vasospasm makes measurement become naturally difficult. Evidence for arterial spasm can be seen in the reduction of stenoses caused by nitrate administration (42) or the use of provocative manoeuvres (43). In fact, vasospasm can result from thrombosis. Serotonin, thromboxane A2, and thrombin are produced during local thrombus development. Each of these thrombosis-related mediators has the ability to constrict blood vessels downstream in addition to at the site of thrombosis. A proximal thrombus in an epicardial conduit

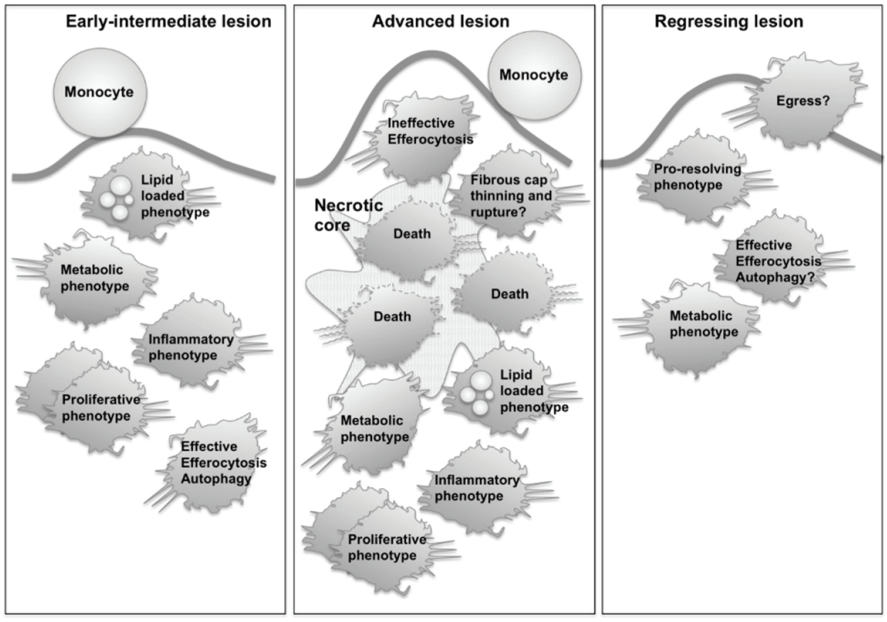

Figure 1. Macrophages altered roles in the lesion development.(41) (Adapted with permission from American Heart Association).

coronary artery may cause the distal, smaller arteries to spasm in this way.

New therapies built on a deeper comprehension of the processes behind plaque instability might lead to even better results. An unfavorable outcome in ACS is predicted by increased levels of circulating inflammatory markers, particularly CRP, regardless of how severe the atherosclerotic or ischemic load is, according to growing data. Thus, one potential new pathophysiological mechanism of the ACS that may offer such a new target for treatment is inflammation.(44)

Vascular Smooth Muscle Cells in Atherosclerosis

The ability of electron microscopy to detect smooth-muscle-like cells in the media of healthy arteries, the significant function of vascular smooth muscle cells (VSMCs) in atherosclerosis was established around 1960s.(45) These confirmed that the cells in atherosclerotic plaques have the characteristics of VSMC but with different behaviors.(46) A re-evaluation of the role of VSMCs in atherosclerosis is necessary because over the past 50 years, perceptions of how VSMCs contribute to atherosclerotic plaque development, remodelling, and stabilization have changed significantly, and many studies published in the last ten years have called into question long-held beliefs about the identity of cells in atherosclerotic plaques.

VSMCs are defined based on their anatomical location within the vasculature and functional characteristics. These cells are primarily located in the medial layer of healthy arteries, where they perform vital functions, such as maintaining compliance and elastic recoil in response to hemodynamic changes, as well as controlling arterial diameter and synthesizing extracellular matrix (ECM) proteins. VSMCs play a crucial role in determining the characteristics of vessels throughout the arterial tree. For instance, they are responsible for producing elastin in large elastic arteries like the aorta, which is critical for elastic recoil. In muscular arteries and arterioles, VSMC contraction largely determines arterial diameter, which is essential for systemic arterial resistance. The functional state of VSMCs is typically inferred through a combination of traits, including their morphology and expression of "VSMC-specific" function-associated markers such as proteins and glycosaminoglycans. In healthy arteries, VSMCs have a fusiform shape, express contractile proteins such as smooth muscle myosin heavy chain (SMMHC or MYH11) and α-smooth muscle actin (α-SMA), and secrete ECM macromolecules such as elastins, collagens, and proteoglycans.

In healthy arteries, VSMCs have a completely defined, functional phenotype yet nevertheless exhibit amazing adaptability. Reduced myofilament density and lesser expression of contractile proteins are characteristics of VSMCs that have undergone de-differentiation, modulation, or phenotypic flipping. Dedifferentiated VSMCs express pro-inflammatory cytokines, ECM components, and ECM-remodelling enzymes at greater quantities. They also exhibit larger amounts of secretory organelles.(47) As a result, VSMCs with altered phenotypes are frequently referred to as "synthetic," and VSMCs that express a lot of contractile proteins are normally referred to as "contractile". The synthetic, dedifferentiated state has also been linked to activation of VSMC proliferation and migration, although coordinated control of these activities has not been reported, and mitotic VSMCs with high quantities of contractile proteins have been seen.(48,49)

Multiple mechanisms, including DNA damage, mitochondrial degeneration, and oxidative stress, all of which occur during atherosclerosis, are believed to be responsible for VSMC senescence *in vivo*. Senescence of the VSMC can also be caused by loss of autophagy.(50) Because replicative senescence would most likely result from the creation of all the VSMC-derived cells in advanced plaques via clonal growth, replicative senescence is particularly significant in the context of atherosclerotic plaque VSMC clonality. According to this theory, the severity of the illness is correlated with the noticeably shorter telomeres of VSMCs found in human atherosclerotic plaques.(51) Senescence-associated secretory phenotypes (SASPs), are secreted proteins from the senescent cells which have altered behaviors and secreting many pro-inflammatory factors such as cytokines, chemokines, growth factors, etc.(52)

Senescent surveillance refers to the physiological function of SASPs as a molecular beacon that attracts and directs immune cells to eliminate senescent cells before additional mutation allows senescence bypass and, for example, the re-initiation of tumor development.(53) However, the number of senescent cells that are not eliminated by phagocytes increase with age and sickness (perhaps as a result of an immune system that isn't functioning properly or a suppressive environment) and encourage chronic inflammation that may cause atherosclerosis or aggravate the situation.(54)

Depending on the stage of atherogenesis, the function of VSMCs and the results of VSMC growth or loss vary. The

proliferation, migration, and demise of VSMCs determine VSMC number, which is negatively linked with plaque rupture (Figure 2).(55,56) Advanced atherosclerotic lesions in humans exhibit limited VSMC growth (57), but there is an increase in VSMC mortality by apoptosis and necrosis as compared to normal arteries (58,58,59), as well as in unstable vs stable plaques (60). In fact, it has been suggested that VSMC apoptosis is essential for plaque instability. (61) Insulin-like growth factor (IGF)1 and platelet-derived growth factor (PDGF) act as survival factors for VSMCs found in atherosclerotic plaques in humans. Compared to VSMCs in non-atherosclerotic arteries' medial layer, plaque VSMCs have lower expression levels of IGF1 receptors. (62) This research was pivotal in demonstrating that VSMCs from human atherosclerotic plaques spontaneously undergo apoptosis *in vitro*.(63) Similarly, N-cadherin-mediated cell-to-cell contact encourages VSMC survival.(64) In contrast, a variety of variables, including cell-directed killing (by macrophages, T lymphocytes, and mast cells), ROS, DNA damage, anoikis, and cholesterol, have been identified in atherosclerotic plaques that cause VSMC death.

Therefore, in all phases of atherosclerosis, VSMCs and cells generated from VSMCs constitute a significant source of plaque cells and extracellular matrix. The extracellular matrix-producing cells of the fibrous cap, macrophage-like cells, foam cells, mesenchymal stem cell-like cells, and osteochondrogenic cells are only a few of the numerous plaque cell morphologies that VSMCs contribute to. The relevance of the developmental origin, clonal growth, and phenotypic flipping of VSMCs in atherosclerosis has been highlighted by advances in determining the source of VSMCs and VSMC-derived cells in atherosclerotic plaques.(55)

Role of Innate and Adaptive Immune System in Atherosclerosis

The importance of the innate and adaptive immune systems in causing atherosclerosis-related chronic inflammation in arterial blood vessels has been established by extensive study in preclinical models and growing evidence in people. The tremendous variability of leukocyte subsets in the artery wall play pro-inflammatory or regulatory functions in atherogenesis.(65) Researchers are still trying to understand the intricate immunological responses that cause inflammation in the arterial wall in response to an atherosclerotic milieu. The survival, expansion, and functionality of immune cells are strongly influenced by changes in intracellular metabolic pathways, according to new insights into the molecular mechanisms underlying immunity and inflammation.(66)

Major adverse cardiovascular events (MACEs) were successfully reduced in the CANTOS Study, using immunomodulation by IL-1β suppression. It also offered the first significant proof that it is possible to treat the inflammatory component of atherothrombosis.(11) Colchicine lowered MACEs when it was administered in

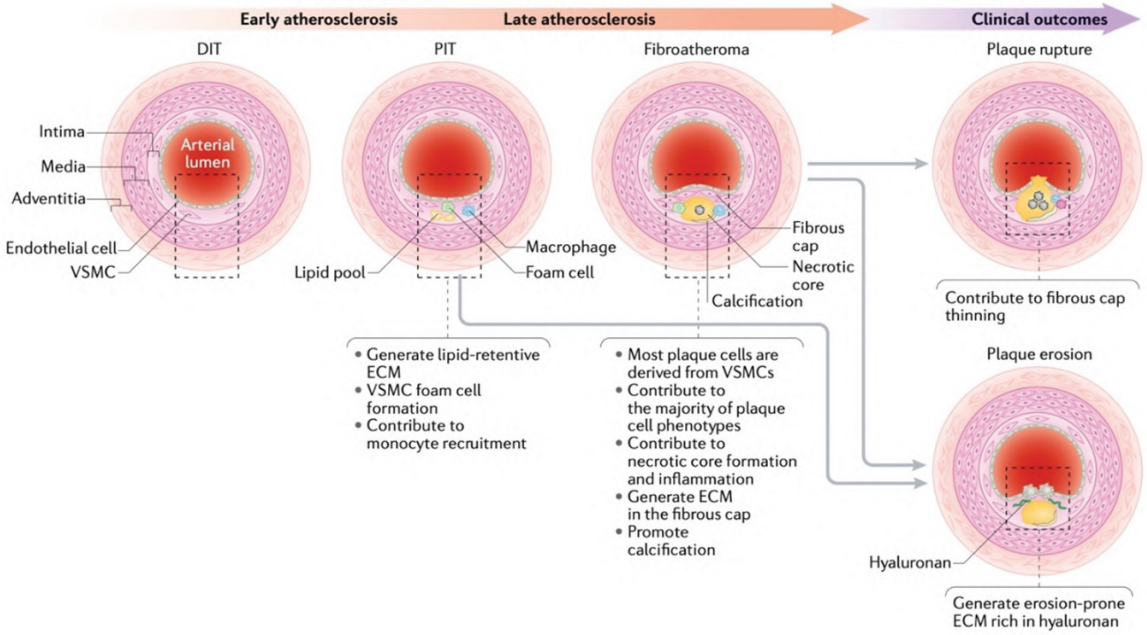

Figure 2. Overview of the role of VSMCs in atherosclerosis.(55) (Adapted with permission from Springer Nature).

the Colchicine Cardiovascular Outcomes Trial (COLCOT). (67) However, systemic immune suppression increased major adverse events and even fatalities in both studies by increasing the incidence of infections in the treatment arm compared to the placebo group. This emphasizes the requirement for the development of immune-targeted therapies that are effective, long-lasting, and safe that can modify atherosclerosis more accurately. An in-depth mechanistic understanding of the cellular and molecular mechanisms behind atherosclerosis will be necessary to selectively target particular immune network components that promote atherogenesis. Therefore, therapies that can selectively block the maladaptive inflammation linked to atherosclerosis or increase anti-atherogenic regulatory systems have the potential to improve patient outcomes through slower atherosclerosis development or faster atherosclerosis resolution.

Immune cells develop from hematopoietic stem cells (HSCs) by the haematopoiesis process. To maintain homeostatic levels, healthy humans manufacture ≈4–5×1011 new blood cells daily.(68) All blood cell types are produced by HSCs, which have a physiological home in the HSC niche, a specific bone marrow milieu.(69,70) Mature leukocytes leave the bone marrow in response to diverse stimuli as a consequence of a strictly regulated proliferation and differentiation process. They then enter the bloodstream and travel to their target tissues via adhesion and diapedesis. While CVD development depends on the hematopoietic supply of inflammatory immune cells, CVD itself has a significant impact on hematopoiesis. Hematopoietic processes are significantly altered by common cardiovascular risk factors such hyperlipoproteinemia, arterial hypertension, and diabetes mellitus. Similar to atherosclerosis, myocardial infarction also has a significant impact on hematopoiesis.

The supply of inflammatory leukocytes from the bone marrow niche and extramedullary locations led to the understanding that atherosclerosis is a multifactorial inflammatory disease.(71) Neutrophils in particular, as well as other innate immune cells like monocytes and macrophages, play a critical role in the development, progression, and destabilization of atherosclerotic plaques.

Localized macrophages that are part of the healthy artery wall reside in the adventitia, where they regenerate by local proliferation. In mice, circulating monocytes, many of which concentrate soon after birth, are the primary source of arterial resident macrophages. Studies in mice using $CD11c^{+}$ cells for labelling show that dendritic cells also dwell in the artery wall and heart valves, although it is still unclear how these cells differ from macrophages.(72,73) Although it is probable that arterial resident macrophages perform generic macrophage activities such tissue homeostasis and pathogen clearance, our understanding of their artery-specific roles is still developing.(74)

Recent studies have shown that the supply of inflammatory leukocytes from the bone marrow niche is critical for the development of atherosclerosis, from its early stages to its severe consequences. When a ASCVD occurs, such as a myocardial infarction, the hematopoietic system reacts quickly. As a result, bone marrow myeloid progenitors and monocytes grow (75), and circulating leukocytes are drawn to the ischemic myocardium. While after a stroke, myelopoiesis rises whereas abnormalities in B lymphopoiesis are caused by the hypothalamic-pituitary-adrenal axis.(76,77) Both neuronal healing and reperfusion damage may be facilitated by myeloid cells that have been attracted to the wounded brain.(78,79) Blood monocyte numbers gradually rise during the low-grade, chronic inflammation that results from atherosclerosis, as seen in $ApoE^{-/-}$ mice with atherosclerosis.(80) Neutrophils and lymphocytes are among the immune cells that live inside the atherosclerotic plaque and help it expand and become unstable. Lesional neutrophils are quite uncommon, hence their contributions have long been undervalued. However, recent research has shown their existence and contribution to the pathophysiology of atherosclerosis.(81)

Macrophages, DCs, monocytes, mast cells, and neutrophils are only a few examples of the many innate immune cells that are important for the development of atherosclerosis.(82) Recent research has also emphasized the role of non-cytotoxic innate lymphoid cells and natural killer (NK) cells. Type I interferon-inducible cells (83), a second fraction that also derived from monocytes, are characterized by elevated expression of a number of interferon-inducible genes, including as interferon induced protein with tetratricopeptide repeats 3 (Ifit3), interferon regulatory factor 7 (Irf7), and interferon-stimulated gene 15 (Isg15) (84). This subgroup plays a pro-inflammatory role through the generation of type I interferon and is exclusively present during atherosclerosis.(85) Mice with both early and severe atherosclerotic lesions were shown to have foam cells that expressed triggering receptor expressed on myeloid cells 2 (TREM2).(85) Embryonic precursors or circulating monocytes may both be used to produce TREM2hi macrophages.

Mouse and human arteries, as well as atherosclerotic lesions, have been detected with conventional DCs (cDCs)

and plasmacytoid DCs (pDCs).(86) $CD103^+$ type 1 cDCs are related to lymphoid-resident $CD8^+$ DCs and are derived from fms-like tyrosine kinase 3 $(FLT3)^+$ migratory pre-cDCs.(87) The most prevalent subset of aortic DCs are $CD11b^+$ type 2 cDCs, which also include DCs generated from monocytes. In draining lymph nodes or the spleen, cDCs pick up antigens from the vessel wall and stimulate naïve T cells that are specific for the antigen. Although DCs may be found in the intimal layers of healthy mouse and human arteries, during atherosclerotic circumstances, their numbers are considerably increased and their morphologies are noticeably changed.(86)

Atherosclerosis is largely regulated by adaptive immunity. A new preclinical results demonstrate the significance of MHC class II-mediated activation of $CD4^+$ T cells by different APCs as well as the proportional contributions of the most extensively researched subclasses of $CD4^+$ T cells. Many studies described the functions of $CD8^+$ T cells, lipid antigen-specific NK T cells, and T cells in atherosclerosis as well as the functions of B cells and antibodies in.(88) T lymphocytes can be seen in the adventitia of healthy wild-type mouse arteries (89) and in mouse models which is genetically prone to atherosclerosis (90). At all phases of atherosclerotic disease, aortic T cells have been identified by scRNA-seq studies.(86) The infiltration of T lymphocytes into the plaque is mediated by chemokine receptors such as CCR5 and CXCR6.(89,91) Atherosclerotic lesions contain large amounts of CCL5, the primary ligand for CCR5.(91)

Macrophages, T cells, B cells, dendritic cells, neutrophils, and mast cells, whose makeup changes throughout atherogenesis, are among the immune cells that atherosclerotic plaques attract.(90,92) Through the production of cytokines, chemokines, proteases, pro-thrombotic factors, and other bioactive molecules, they work together to decide the advancement of atherosclerotic plaque. The ratio of pro-inflammatory to anti-inflammatory reactions in the plaque will determine how quickly the illness progresses as well as how big and complicated the lesions are. Large atherosclerotic lesions with unresolved inflammation, significant matrix remodeling, substantial necrotic cores, and thin fibrous caps are susceptible to rupture, which might result in rapid thrombosis and arterial blockage.(82)

Immune cells are required to fulfil a range of metabolic requirements in an inflammatory environment. Upon activation, immune cells can undergo metabolic reprogramming, transitioning between distinct metabolic states to adapt to changes in environmental signals such as oxygen, nutrition, and growth hormones, as well as energy and biosynthesis demands. Seven significant cellular metabolic pathways have been identified in immune cells, including glycolysis, the pentose phosphate pathway (PPP), the tricarboxylic acid cycle (TCA), oxidative phosphorylation (OXPHOS), mitochondrial fatty acid β-oxidation (FAO), fatty acid synthesis, and amino acid metabolism (Figure 3). These interconnected pathways regulate the survival, expansion, and activation of immune cells.(93)

Although immune regulatory cells such as M2 macrophages and Tregs have a mixed metabolism that includes glycolysis, fatty acid oxidation (FAO), and oxidative phosphorylation (OXPHOS), activated macrophages and T cells exhibit a stronger preference for aerobic glycolysis over mitochondrial metabolism.(94) Figure 3 showed how immunometabolism involved in atherosclerosis. The alterations of immune cells metabolism can influence the plaque stabilization and progression. Plaque with pro-inflammatory M1 type macrophages, together with Th1 and Th17 create a catabolic metabolism and higher risk of plaque rupture, while the opposite anti-inflammatory M2 type macrophages with Treg promote a plaque stabilizing response.(66)

The functional significance of immunometabolic pathways in CVD has been demonstrated by research in experimental atherosclerosis models, that hematopoietic GLUT1-deficient atherosclerotic mice have a lower glycolytic flux in their bone marrow and atherosclerotic plaques, leading to a reduction in atherosclerosis. Similarly, a lack of glucose-6-phosphate dehydrogenase, a crucial enzyme in the PPP, lowered atherosclerosis and vascular superoxide levels.(95) The metabolism of amino acids has turned shown to be significant in atherosclerosis.(96) In $ApoE^{-/-}$ mice, deletion of indoleamine 2,3-dioxygenase (IDO)-dependent Trp metabolism significantly increases vascular inflammation and speeds up atherosclerosis. (97,98) According to this information, IDO induction has been associated with enhanced plaque stability and atheroprotection.(99) Nevertheless, changes in the gut microbiota that may diminish IDO's anti-inflammatory and anti-atherosclerotic actions appear to be sensitive to its involvement in health and illness.(100)

Recent experimental data has brought attention to the possibility of systemic or distant inflammation causing local effects within the arterial wall. Two sources of data from 1990 suggest this to be the case. The first experiment involved mouse atheromata, where acute myocardial damage led to increased inflammation, leukocyte recruitment, and

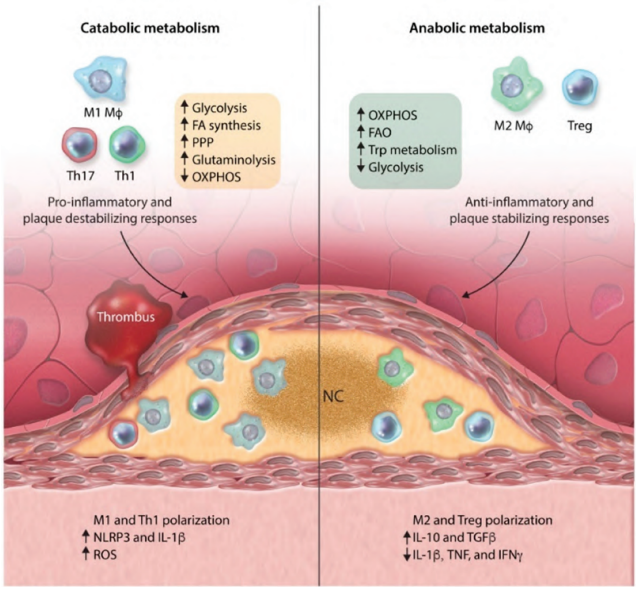

Figure 3. The role of immunometabolism in atherosclerosis.(66) (Adapted with permission from European Society of Cardiology).

activation. The second experiment explored the concept of "trained immunity" and how exposure to a proinflammatory stimulus could result in an amplified reaction. This anamnestic reaction, which has long been acknowledged in the concept of adaptive immunity, may have specific relevance to atherosclerosis.(41) The anamnestic reaction inherent in the idea of "trained immunity," though long acknowledged in the concept of adaptive immunity, may apply specifically to atherosclerosis. These various considerations offer further connections between systemic inflammation (the fluid phase of blood), localized inflammation in extra-arterial sites, and increased atherogenesis, as well as the triggering properties of preformed plaques (the solid state of the lesion itself) that can cause thrombotic complications.

Role of Innate and Adaptive Immune System in Atherosclerosis

Inflammation may be separated into two stages: initiation and resolution, which correspond to the host defense response to infections and tissue injury. Acute inflammation can be a protective process if it is carried out in a timely and appropriate manner.(101) Although the catabolism of pro-inflammatory mediators and diffusion of chemotactic gradients were previously assumed to be involved in the resolution of inflammation, we now understand that the resolution programme is a highly coordinated, dynamic process.(101) Specialized pro-resolving mediators (SPMs), such as resolvins, lipoxins, maresins, and protectins, along with endogenous gases like nitric oxide (NO), hydrogen sulfide (H_2S), and carbon monoxide (CO), as well as the vagus nerve and regulatory T cells (including proteins like IL-10 and annexin A1), are all responsible for resolution of inflammation, which reduces leukocyte recruitment, promotes efferocytosis, restores tissue damage, and replenishes blood and lymphatic channels when carried out properly. In an infection, decreasing inflammation also aids in the removal of pathogens. On the other hand, persistent tissue damage results when a flaw in the resolution program manifests. It is now known that many chronic inflammatory illnesses, such as atherosclerosis, are promoted by failure to trigger inflammation resolution.(101,102)

The balance between pro-inflammatory and inflammation-resolving pathways determines the final clinical outcome in atherosclerosis, a lipid-driven inflammatory disease of the artery intima. Atherosclerotic lesions begin to form as a result of lipid-filled foam cells being formed as a result of intimal infiltration, modification of plasma-derived lipoproteins, and their primary uptake by macrophages. The progression of lesions is then maintained by insufficient efferocytotic removal of foam cells and apoptotic cells.(103)

The resolution response is not merely a reduction of the inflammatory reaction, but rather a separate and dynamic process. During this process, small lipids, proteins, and signalling gases, become increasingly important and begin to appear as early as the initial inflammatory response, in preparation for the critical resolution phase that follows. Although pus and oedema fluid are significant sources of resolution mediators, pro-resolving mediators, including those found in bodily fluids like tears and human milk, may also play a role in the basal or preventive resolution of inflammation.(104) Pro-resolving mediators work to limit tissue damage and enable the repair of injured tissue by activating cell-surface receptors, blocking inflammatory cell influx and promoting inflammatory cell egress, modulating pro-inflammatory T cell responses, and promoting clearance of both pathogens and dead cells (efferocytosis).(105,106)

Many chronic inflammatory diseases, including advanced atherosclerosis, are characterized by an imbalance between pro-resolving and pro-inflammatory mediators, such as leukotrienes (LTs), leading to impaired resolution of inflammation, tissue damage, and inflammation mediated by damage-associated molecular patterns (DAMPs).(107) Specifically, the formation of a necrotic lipid core, weakening of the protective collagen cap, poor efferocytosis, and DAMP-mediated inflammation are all characteristic

features of clinically dangerous atherosclerotic plaques. (108) In humans, a low ratio of resolvin D1 (RvD1) to leukotriene B4 in saliva can predict carotid intimal thickness, while advanced atherosclerotic plaques have lower ratios of pro-resolving lipid mediators to leukotrienes. Similarly, pro-resolving lipid mediators are absent in advanced atherosclerotic lesions in mice.(109)

Fatty acids can support both pro-inflammatory and pro-resolution signaling in atherosclerosis in a variety of ways. For instance, certain free fatty acid receptors (FFARs) such as FFAR4 that have been linked to protection against the inflammatory response to vascular damage can be directly activated by omega-3 fatty acids themselves.(110) On the other hand, lipid aldehydes like 4-hydroxy-2-nonenal (HNE), which are produced from fatty acids, are vascular toxin.(111) The production of bioactive lipid mediators including leukotrienes, prostaglandins, and thromboxane regulates the inflammatory and thrombotic reactions in atherosclerosis.

SPMs are a distinct class of bioactive lipids produced from fatty acids.(105) The SPMs formed from eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA) include compounds like maresins and protectins, as well as E-series and D-series resolvins, which are produced from EPA and DHA, respectively. The class of lipoxins includes the SPMs produced from the omega-6 fatty acid arachidonic acid. The metabolism of SPM precursors is altered by the aspirin-induced acetylation of the enzyme cyclooxygenase 2. Aspirin-triggered resolvins and lipoxins are the consequent R-epimers, which have similar biological action but are more metabolically stable than the S-epimers.(101)

Activation of a multiprotein complex known as the inflammasome is easily induced by the build-up of lipids produced from lipoproteins in macrophages or dendritic cells.(112,113) Nod-like receptor protein (NLRP)3 has received the greatest research attention among the several inflammasomes. Major NLRP3 components are expressed more often in human atherosclerotic lesions (114), and ApoE-/- mice with NLRP3 inflammasome suppression have less atherosclerosis (115). A functional NLRP3 inflammasome is regulated by a two-hit process. First signal primes the transcription of the inflammasome and the second one activates the inflammasome and causes the release of the pro-inflammatory cytokines IL-1 and IL-18. (116,117) TLR ligands, such as bacterial lipopolysaccharide (LPS), can trigger the priming stage, with oral and intestinal infections both being possible sources of LPS in the artery intima.(118,119) Endogenous cues can prime the inflammasome in addition to microbial components, with these stimuli likely dominating NLRP3 priming in sterile inflammatory disorders like atherosclerosis.(116)

The bioactive lipid mediators mentioned earlier are generated by various cells in response to inflammation, and the main producers are neutrophils and macrophages. Enzymes like 5-lipoxygenase (5-LOX), 12-LOX, and 15-LOX play a significant role in producing these lipid mediators. Of these, the regulation of 5-LOX is particularly fascinating. In neutrophils, mast cells, and macrophages, 5-LOX is situated on the nuclear membrane, where it converts arachidonic acid to the pro-inflammatory leukotriene B4. However, in macrophages, 5-LOX present in the cytoplasm tends to synthesize the pro-resolving mediator lipoxin A4 (LXA4) from arachidonic acid.(120) Through the phosphorylation of 5-LOX by the enzyme MK2 (also known as MAPKAPK2), the nuclear (pro-inflammatory) location of 5-LOX is mediated.(121) The calcium-activated kinase Calcium-calmodulin-dependent protein kinase II (CaMKII), as part of a pro-resolving amplification loop, and activators of the efferocytosis receptor tyrosine-protein kinase MER (MERTK) can both activate and inhibit this pro-inflammatory MK2 pathway.(122-124) These regulatory systems are crucial in the development of atherosclerosis. Figure 4A showed that omega-6 PUFA arachidonic acid was metabolized into lipoxin A4, while omega-3 PUFAs docosahexaenoic acid and eicosapentaenoic acid was metabolized into resolvins. Together these resolvins will decrease inflammatory cytokines, reduced oxidative stress and improved efferocytosis in macrophages. While Figure 4B described the ratio of Resolvin:leukotriene correlated with macrophages phenotype as the character of net inflammation or net resolution, and this will affect the plaque.(103)

The NLRP3 inflammasome can be deactivated by SPMs in mouse macrophages, inhibiting the priming stage as well. In a mouse model of peritonitis, RvD2 supported the inflammation resolution process and led to self-resolution with minimal inflammation *in vivo*. RvD2 also greatly decreased the release of mature IL-1 by mouse macrophages *in vitro*. This is a significant finding as IL-1 can initiate an inflammatory cascade, similar to the inflammatory response caused by an infection or injury, eventually leading to a cytokine storm, a systemic event that is imitated on a microscale inside the developing atherosclerotic plaque.(125)

Peptide and lipid pro-resolving mediators use specific G protein-coupled receptors (GPCRs) to communicate, such as N-formyl peptide receptor 2 (ALX/FPR2; also known as

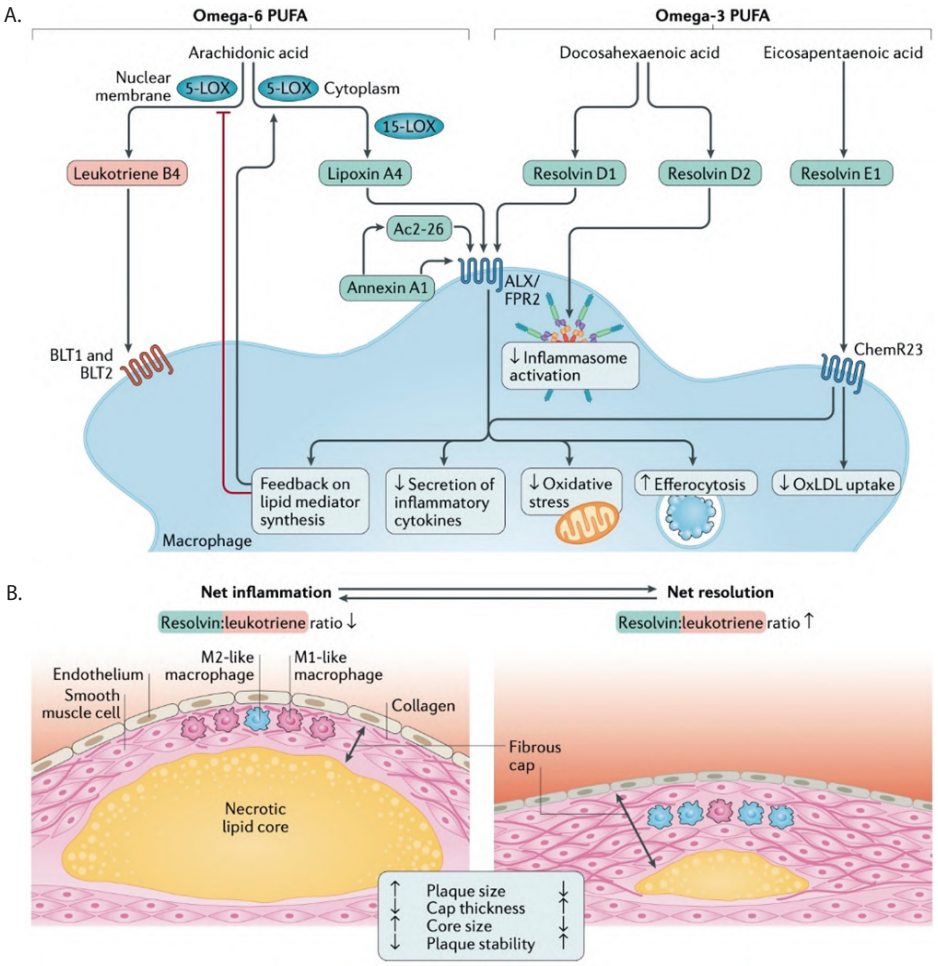

Figure 4. Ligands and receptors transducing pro-resolving signalling in macrophages. A: Specialized pro-resolving lipid mediators (in blue); B: Atherosclerotic plaques with net inflammation (left) and with net resolution of inflammation (right). A net resolution show better and stable atherosclerotic plaque while a net inflammation increase the risk of plaque rupture.(103) (Adapted with permission from Springer Nature).

FPR2 or ALX) and ChemR23 (also known as chemokine-like receptor 1 (CMKLR1)). While annexin A1, RvD1 derived from DHA, and lipoxins have receptors called ALX/FPR2, EPA-derived resolvins communicate their pro-resolving activities through the ChemR23 receptor. Human aortic, coronary, and carotid atherosclerotic lesions express these receptors, indicating that their pro-resolving ligands have local effects on the vascular wall. Significantly, exogenous treatment can overcome the deficiency of pro-resolving mediators in advanced atherosclerotic lesions. Notably, intraperitoneal injections of RvD1 into Western-diet-fed LDL-CR-knockout mice with mid-stage atherosclerotic lesions restored RvD1 levels in the lesions, increased efferocytosis, decreased oxidative stress in the lesions, and promoted a more stable plaque phenotype, which inhibited the progression of mid-stage lesions to more advanced ones.(126)

Macrophages play a significant role in the resolution of local inflammation and, more importantly, the regression of an atherosclerotic lesion. The majority of the cells in both human and animal atherosclerotic plaques are macrophages. (90,127) The major role of the intimal macrophages is to remove these lipoproteins from the forming lesions by their absorption and destruction since the accumulation and infiltration of atherogenic plasma lipoproteins is what drives atherogenesis. Intimal macrophages may initially slow the growth of lesions by internalizing and destroying lipoproteins retained beneath the endothelium, as the accumulation of atherogenic plasma lipoproteins is the main factor driving the development of atherosclerosis. The many combinations of pathogenic and protective roles that macrophages can perform in humans are made possible by the phenotypic variability of macrophage subtypes. Additionally, each macrophage has the capacity to transform its phenotype dynamically, affecting how the macrophage functions.(128,129) It is noteworthy that pro-inflammatory macrophages and less-inflammatory macrophages are localized in separate regions in human atherosclerotic plaques, and this finding shows the role of plaque microenvironment.(130)

Several SPM family members promote the polarization of macrophages towards an M2-like or intermediate phenotype.(131) SPM production is also increased in response to efferocytosis and is greater in M2-like macrophages than in M1-like macrophages. This rise in SPM production with efferocytosis may be caused, in part, by apoptotic cells activating the efferocytosis receptor MERTK. (124) A biological explanation for the deficient inflammation resolution characteristics of advanced atherosclerotic lesions is provided by the surprisingly low ratio between specific pro-resolving mediators and pro-inflammatory lipids (in particular leukotrienes). Atherosclerosis and the activity of lesional macrophages may be impacted by the balance of proinflammatory and pro-resolving mediators. How to increase the local concentrations of pro-resolving mediators in the atherosclerotic lesion is one therapeutic challenge for promoting the resolution of inflammation in atherosclerosis and the prospect of collagen-type-IV-targeted nanoparticles.(132)

Defective Efferocytosis in Atherosclerosis

Adult human's cells experience 0.4% cell death every day. Apoptotic cells (ACs) are not often found even in tissues with fast cell turnover, indicating very high AC clearance efficiency and capacity. Efferocytosis, the process by which ACs are cleared, is crucial for maintaining tissue homeostasis in healthy physiology and for re-establishing it after illness.(103,133) Efferocytosis malfunctions in a number of chronic inflammatory disorders that do not go away, causing a build-up of dead cells.(134) Secondary necrosis of the dead cells may result in tissue necrosis, pathological inflammation, and autoimmune.(135,136) To better understand how efferocytosis is effectively carried out in healthy physiology and how it becomes deficient in illness, a significant area of biomedical study is now emerging.

Organogenesis and development can proceed properly when apoptotic cells were eliminated, and anti-inflammatory and antitumor responses are conserved and in homeostasis. (137) Efferocytosis is therefore a crucially important cellular effector arm of the inflammation resolution response, especially when it occurs in macrophages. According to recent research, a biological route of the inflammation resolution response, which is regulated by regulatory T cells, also increases efferocytosis. This mechanism is influenced by both SPMs and IL-10.(138) The pro-efferocytic cytokine IL-10 activates macrophages as part of the process of the regulatory T cell response by stimulating IL-13 release. (139) It's interesting to note that during atherosclerosis, IL-13 encourages additional resolving activities, such as boosting the formation of lesional collagen, decreasing monocyte recruitment, and shifting macrophages towards a pro-resolving phenotype.(140)

Many illnesses are caused by the ineffective removal of apoptotic cells, such as atherosclerosis which is primarily caused by poor efferocytosis.(141,142) As previously mentioned, ApoB-containing lipoproteins are maintained in the sub-endothelial matrix of medium to large arteries during early atherosclerosis and trigger a potent inflammatory response that results in the production of chemotactic molecules that promote leukocyte recruitment. This inflammatory response fails to settle appropriately as atherogenesis progresses, which sustains leukocyte recruitment even more. Moreover, cells in the atherosclerotic lesion undergo apoptosis due to both external agents, such as cytokines, and internal processes, such as endoplasmic reticulum stress. Although phagocytic monocyte-derived macrophages are the main kind of cell in growing atherosclerotic plaques, the ability of these cells to carry out efferocytosis is compromised as atherosclerosis progresses.(134)

When efferocytosis performed well and intact, the apoptotic cells were cleared successfully and the inflammation was resolved. When it was defective, for example in the macrophage when A disintegrin and metalloprotease 17 (ADAM17) cut off the MERTK. This can induce apoptotic cells release CD47 and CD47-signal regulatory protein α (SIRPα) interaction. The inflammation is not resolved but on the contrary promote even more pro-inflammatory cytokine and induce secondary necrosis, in the end the cell engulfment does not happens and accumulated in necrotic core (Figure 5). Pro-inflammatory processes include pyroptosis and necroptosis.(143) Pyroptosis associated with inflammasome activation and requires the activity of caspase 1. Pyroptotic cell death is very pro-inflammatory because it causes the production of cytokines like IL-1 and IL-18 as well as the release of intracellular contents to the extracellular fluid. While necroptosis and necrosis share many characteristics with apoptosis, necroptosis is a controlled form of necrosis that does not entail caspase activation. Plasma membrane breakdown during necroptosis causes the release of numerous DAMPs as well as the contents of the cell. It has been established that necroptosis plays a role in atherosclerosis and, more significantly, that unstable atherosclerotic lesions in people exhibit this kind of cell death.(144)

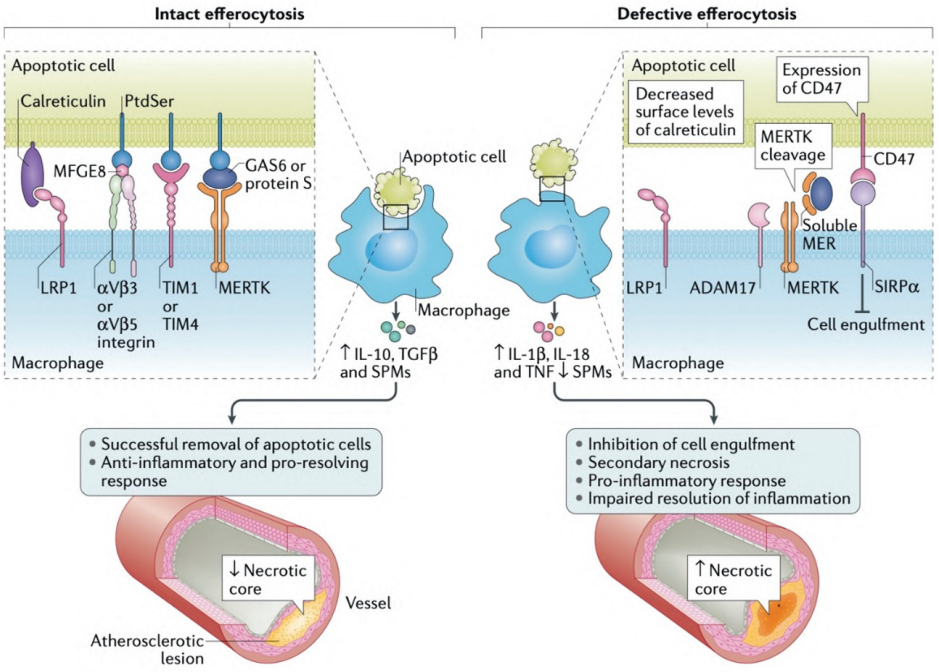

Figure 5. Defective efferocytosis drives necrotic core formation in atherosclerosis. LRP1: LDL-C receptor-related protein 1; MFGE8: lactadherin; PtdSer: phosphatidylserine; SPM: specialized pro-resolving mediator; TIM: T cell immunoglobulin mucin receptor.(103) (Adapted with permission from Springer Nature).

The capability of phagocytes to absorb several ACs within a brief period of time, known as constant efferocytosis, which is the significant aspect of efferocytosis. When the AC-to-phagocyte ratio is large, the ability of efferocytes is crucial (for example, after an acute inflammatory response). Phagocytes that continuously take up AC must overcome a number of obstacles. First, each AC uptake event internalizes a significant quantity of plasma membrane when the phagosome enters the cell, necessitating a fast restoration of cell surface area during future rounds of efferocytosis.(145) Second, the huge volumes of metabolic cargo produced by phagolysosomal breakdown of ACs, such as amino acids, lipids, and nucleic acids, must be handled in a way that is both safe and effective.(145)

The synthesis of lipid mediators, which are essential for controlling inflammation, is also affected by efferocytosis. The expression of LCFA-derived lipids or SPMs, such as lipoxin A4 and resolvins D1, D2, and E2, is increased in macrophages when they are incubated with apoptotic neutrophils or neutrophil microparticles, while the expression of proinflammatory prostaglandins and leukotriene B is simultaneously decreased. Further efferocytosis is enhanced by lipoxin A4 and resolvin D1, which create a loop whereby they increase their own production.(146) There are a number of methods through which the generation of SPM increases. First, SPM precursors are present in ACs and their microparticles, and when ACs are consumed by macrophages, the SPM precursors are transformed into mature lipid mediators. Efferocytosis also increases the production of 12/15-lipoxygenase, a crucial SPM synthesis enzyme.(147) By boosting the cytoplasmic-to-nuclear ratio of 5-lipoxygenase, another crucial enzyme in SPM manufacture, MERTK-extracellular signal-regulated kinase (ERK) signalling encourages SPM synthesis.(123,124)

Numerous cell death mechanisms exist in atherosclerotic lesions, and the inflammatory response to each of these cell death mechanisms varies considerably. (148) Apoptosis is defined by the formation of apoptotic bodies, a shrinking nucleus, and deteriorated cellular contents with an intact plasma membrane. Caspase 8 initiates apoptosis, and when this protease is suppressed, receptor-interacting protein (RIP)1 and RIP3 kinases become phosphorylated and form a complex known as the necrosome.(148) These apoptotic bodies include parts of the dying cells, limiting the release of intracellular DAMPs from the dying cells. Additionally, the apoptotic cells' signals to eat and find theirself induce their efferocytosis. Excessive autophagy is also linked to cell death, despite the fact that it is thought to be a stress response that is pro-survival. (149) Cellular macromolecules are targeted by autophagy to autophagosomes, which combine with lysosomes to create autolysosomes, where they are hydrolyzed. Interestingly, the ingestion of oxidated (oxLDL-C), aggregated LDL-C, or VLDL induces autophagy in macrophage foam cells created *in vitro* and enhances cholesterol efflux to HDL.(150)

Modified lipoproteins build up in the sub-endothelial layer of arteries, causing an inflammatory response that causes leukocyte influx into the artery wall and the

formation of atherosclerotic plaques. While early in the development of the lesion are effectively cleaned, many of these apoptotic leukocytes, as the plaque progresses, fail the efferocytosis and cause a buildup of secondary necrotic cells in the necrotic core region of the plaque result in a large necrotic core.(58,151,152) These are linked to myocardial infarction and stroke.(153) Therefore, a key goal of atherosclerosis research is to understand the causes of defective efferocytosis.(134)

Why does efferocytosis fails in advanced atherosclerosis and lesional apoptotic cells cannot trigger efferocytosis? It is doubtful that excessive lesional apoptosis is the root cause of efferocytosis because it is a high-capacity mechanism (154), human atherosclerotic plaque cells exhibit a large increase in CD47 expression, most likely through a TNFα-dependent mechanism. However, lesional efferocytes have difficulty internalizing these cells. (155) In line with this idea, CD47-blocking antibodies were given to atheroprone animals, and the results included better lesional efferocytosis and smaller necrotic cores. According to another research, lesions' dead cells have reduced levels of the "eat-me" signal calreticulin.(156)

Competition for apoptotic cells binding may potentially affect efferocytosis. Oxidized phospholipids increased due to lesions' accumulation of lipids and reactive oxygen species (ROS) as atherosclerosis advances. These lipids may compete with one another to be recognized as an apoptotic cell by efferocytosis receptors.(157) Similar to this, autoantibodies to oxLDL and other oxidized phospholipids have the ability to attach to and maybe obscure "eat-me" ligands on the surface of dying cells in lesions.(158,159) Additionally, oxLDL enhances TLR4 expression and signalling, which increases the release of pro-atherogenic cytokines tumor necrosis factor (TNF)-α and IL-1β while decreasing the release of anti-inflammatory cytokines transforming growth factor (TGF)-β and IL-10.(160) As will be detailed below, this pro-inflammatory milieu reduces the expression of many important efferocytosis molecules, impairing efferocytosis, and encourages higher lipid absorption at the expense of phagocytosis.(161)

The atherosclerotic lesion becomes secondary necrotic cells and forms a highly inflammatory necrotic core as a result of dead cells removal failure.(141,162) Efferocytosis has three overall effects in addition to secondary necrosis prevention: it stops inflammatory reactions, encourages self-tolerance, and activates pro-resolving pathways. These processes are disrupted when efferocytosis is defective, and progress the disease, inflammation, and result in poor resolution.(163) It's interesting to note that traditional macrophages and dendritic cells formed from monocytes are different from the macrophage-like cells derived from vascular smooth muscle cells.(103)

The bulk of treatment for atherosclerotic disease involves lowering LDL-C levels in the blood, and there is evidence to suggest that this kind of treatment may indirectly halt plaque processes like inflammation and oxidative stress that may eventually lead to impaired efferocytosis. However, there is a place for complementary treatments to the degree that many at-risk people are unable to reduce their LDL-C to a level low enough to totally suppress atherosclerotic disease.(164) For instance, the recent CANTOS trial result showed that reducing inflammation by giving patients an anti-IL-1β antibody effectively decreased recurrent cardiovascular events without the need for cholesterol reduction.(11) Antibodies that inhibit CD47 are one sort of novel strategy that may be helpful in treating faulty efferocytosis. Anti-CD47 antibodies, however, also contribute to anemia because they improperly remove red blood cells.(155) One approach to improve efferocyte function is to inhibit the proteolysis of efferocytosis receptors, such as MERTK, by blocking ADAM17-mediated cleavage. Another method is to enhance efferocytes' ability to eliminate multiple apoptotic cells, for instance, by improving the macrophages' capacity to effectively ingest apoptotic cells that they encounter subsequently, through the mitochondrial fission-calcium mechanism.(123,165) The SPM:leukotriene ratio might be shifted to favor SPM generation, for example, by administering RVD1, which has been demonstrated to improve macrophage-apoptotic cell contacts and to raise lesional efferocytosis.(165,166) Finally, because glucocorticoids produce anti-inflammatory molecules, they are frequently utilized in the treatment of inflammatory illnesses. An example of a glucocorticoid drug is annexin A1, which improves efferocytosis, reduces inflammation, and slows the development of atherosclerosis in mice.(167) In fact, the most promising treatment approach to treat atherosclerotic cardiovascular disease may include boosting efferocytosis while concurrently restoring resolution mediators in lesions.(134)

Intestinal Microbiota in Cardiovascular Health and Disease

Another major factor affecting the cardiovascular health that may be modified is nutrition. The foods we eat also support the gut bacteria metabolism. As a result, a more comprehensive understanding of metabolism is taking

shape. Our overall metabolism and interindividual differences in our metabolic profiles are influenced by a mix of gut microbiota and host metabolic transformations. Our biggest environmental exposure, what we eat, is filtered by intestinal bacteria. The gut microbiome also performs as a significant endocrine organ that is sensitive to food intake because several metabolites produced by the intestinal microbiota are physiologically active and alter host phenotypes.(168)

In a healthy physiological ecology, the human gut supports billions of microbial cells. The term "microbiota" and the term "microbiome" are frequently used to refer to these communities of bacteria, fungi, archaea, and viruses. The majority of the bacteria in the phyla make up the known gut microbial population. Firmicutes, particularly those of the genus Clostridia, Actinobacteria, Proteobacteria, and Verrucomicrobia.(169)

We do not yet fully understand the extent to which an individual's microbiota changes over time. There are significant environmental impacts on a person's gut microbial makeup, activity, and metabolism starting at delivery that can either directly or indirectly alter host metabolism. Intestinal microbiota continue to activate the immune system under healthy settings, particularly through intestinal-associated lymphoid tissues. Additionally, intestinal microbiota regulate the mucosal synthesis of immunoglobulins, particularly immunoglobulin A, and are involved in the activation and differentiation of a variety of T and B lymphocytes.(169)

The situation of having an unbalanced microbial community on or in the body is referred to as dysbiosis. Over the past ten years, the majority of human microbiome research investigations have concentrated on changes in intestinal microbial composition related to the presence of various illnesses and/or phenotypes. It is intriguing to think of being able to pinpoint certain microbiota compositional patterns that, over time, are linked to increased disease susceptibilities. Bacteroidetes and Firmicutes make up the majority of the bacterial species in healthy intestines, and their ratio is frequently used as a proxy for the state of the intestinal microbiome. In several case-control investigations using fecal samples from individuals with diverse morphologies, distinct microbial compositional alterations in the context of atherosclerotic coronary artery disease (CAD) have been identified. Uncertainty exists over whether these are risk factors for the development of CAD or microbiome taxa linked to CAD in contrast to medicines. Undigested nutrients including resistant starch, dietary fiber, and different complex polysaccharides are fermented anaerobically to create short-chain fatty acids (SCFAs), which are fatty acids with 1 to 6 carbon chains.(170) SCFAs act as signaling molecules to body systems, including the control of autonomic systems and systemic blood pressure, as well as inflammatory reactions and other cellular processes, even though they only contribute 5% to 10% of the energy for the human host. Inhibition of histone deacetylases, control of chemotaxis and phagocytosis, generation of reactive oxygen species, cell proliferation, and change of intestinal barrier integrity are only a few of the physiological effects of SCFAs.(170) Recent animal research in a mouse model of myocardial infarction with or without antibiotics showed that gut microbiotaderived SCFAs are essential for the host immune response and heart healing potential.(171) Direct evidence of these effects in human CVD is still few, though.

Data have been collecting over the past few years that point to a significant connection between the gut microbiota and CVD. By metabolizing food components that result in the generation of SCFAs, some of which are expected to induce significant positive cardiovascular effects, it is now obvious that the microbiome plays a critical role at the junction of nutrition and CVD. The intestinal microbiota filter the nutrient intake in two ways (Figure 6), metabolism-dependent by generate the microbial metabolites including SCFA, trimethylamine and choline/carnitine, and by metabolism-independent way result in lipopolysaccharides and peptidoglycans, which all metabolites affect the vasculars like in cardiovascular or kidney.(172) We now understand that metabolism that depends on the microbiome may also result in the creation of compounds with potentially harmful cardiovascular consequences, such as trimethylamine-N-oxide (TMAO), which may encourage atherosclerosis and increase thrombosis risks. These findings offer a fantastic chance to create and test brand-new therapeutic approaches that focus on the gut microbiota for the prevention and management of CVD. Probiotics and/or prebiotics, tailored dietary treatments, or nonlethal microbial inhibitors that, once discovered, target certain pathways are some of the methods that can be used (*e.g.*, TMA production). Additionally, it would be anticipated that drugs that target the TMAO pathway would have a wide range of other potential therapeutic advantages, such as slowing the course of HF, renal functional decline, and unfavorable outcomes in a variety of high-risk groups (those with type 2 diabetes, CKD, and HF).

To support this innovative therapy strategy, well-powered prospective intervention trials are required. Furthermore, it's critical to emphasize that TMAO is

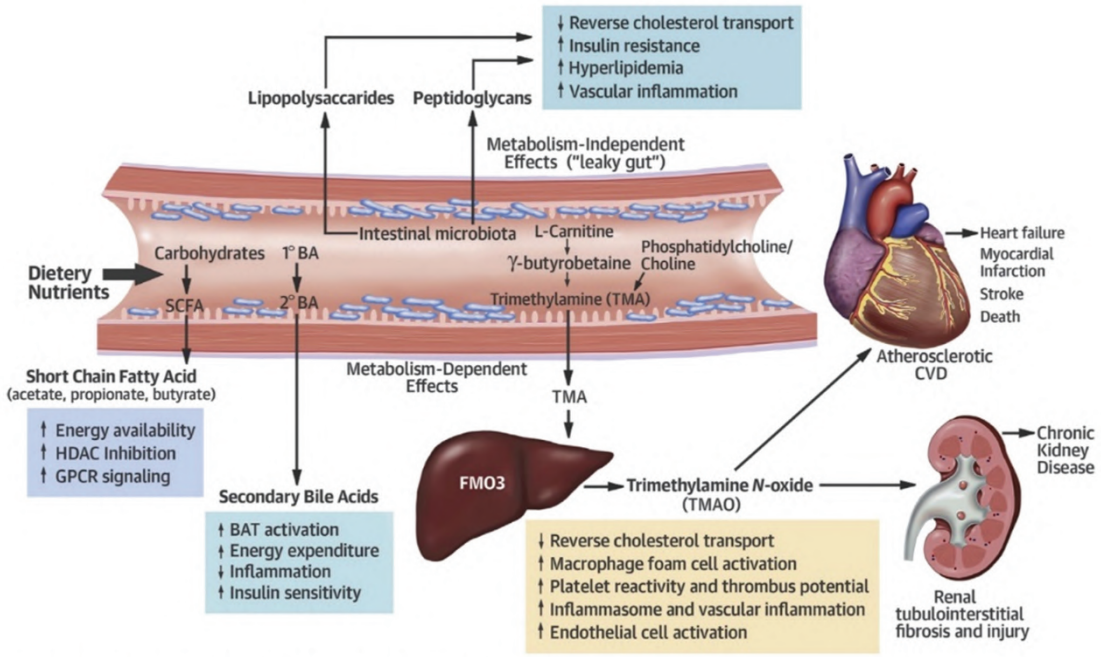

Figure 6. Intestinal microbiota and its metabolic contributions to cardiovascular health and disease.(172) (Adapted with permission from The American College of Cardiology Foundation).

probably simply the tip of the iceberg in terms of the number of metabolites that likely contribute to cardiometabolic disorders. These metabolites may each have varying degrees of influence depending on an individual's sensitivity.(172)

Genetics of Coronary Artery Disease

Important genetic foundations for CAD are seen as being on par with environmental influences.(173) Participants in the Framingham Offspring Study who had a family history of early illness saw an age-specific incidence of CAD that was less than 2-fold higher after controlling for traditional CAD risk variables.(174) The heritability of fatal CAD events was estimated by the Swedish Twin registry using data from around 21000 participants followed up for more than 35 years to be 0.57 for men and 0.38 for women, respectively. It's important to highlight that heritable effects are more obvious in younger people.(175) This is consistent with earlier information showing that the genetic component is more important for early-onset CAD occurrences.(176)

Single-nucleotide polymorphisms (SNPs) can be used to predict and investigate people with risk. This strategy has a lot of drawbacks even if it was economically advantageous. Studies are by definition restricted to genes having a known or suspected function in defining a certain phenotype and do not offer fresh information about the molecular processes that underlie illness. Additionally, candidate gene connections typically did not replicate for a variety of reasons, such as insufficient statistical power due to sample size, heterogeneity in causation, and population stratification.(177) There isn't much evidence to date supporting the incident of CVD with only single-gene involvement without another risk factors related to lipid metabolism.

The found CAD susceptibility variations have tiny individual impacts, but together, they have independent, additive effects. A genetic risk score (GRS), which consists of the number of risk alleles adjusted for their individual impact sizes, might include these. People who belong to the top quintile of an LDL-C GRS consisting of 23 SNPs have a greater likelihood of developing coronary artery disease compared to those in the bottom quintile (WHII: OR=1.43; BWHHS: OR=1.31), according to an analysis done in two prospective cohorts, Whitehall II and the British Women's Heart and Health Study.(178) This connection was totally diminished in WHII but not in BWHHS after correcting for LDL-C levels. With more recent analysis using a more extensive list of recently discovered CAD loci, the utility of a GRS for CAD risk prediction has increased beyond prior research evaluating a small number of risk variants (56,179) beyond those associated to plasma lipid characteristics (180-182).

Given the known accumulation of CAD and its aftereffects of MI in families, particularly when illness initiation occurs early in life, the heredity of ASCVD has long been hypothesized.(183-186) In the Framingham Heart Study Offspring Cohort, it was found that a parent's history of premature CAD was associated with a two-fold increased risk of incident cardiovascular disease after accounting for conventional clinical risk factors. This suggests that there is a distinct heritable basis for susceptibility to cardiovascular disease. The heritability of CAD has been estimated to be between 40% to 60% through studies of high-risk families and twin populations. However, it is possible that familial aggregation may indicate the presence of shared harmful DNA sequence variants or non-genetic factors, such as health-related behaviors, access to food, parental income, and neighborhood. Individually, those whose CAD develops early are likely to have the highest relative contribution of inherited over acquired risk factors.(175,187)

A polygenic score is a single, normally distributed quantitative component that encapsulates the combined genetic impacts of several common genetic variations, and can be used to reflect the genetic variables influencing an individual's propensity for a complex characteristic. When assessing the genetic predisposition to a binary disease outcome like CAD, the polygenic risk score (PRS) can be a useful tool. This score takes into account several common genetic variations and reflects an individual's overall susceptibility to the illness, based on the cumulative effect of those variations. Although the underlying heritability of a disease limits the predictive accuracy of any PRS, a number of other factors, such as the accuracy of common variant association estimates from GWAS, the particular populations in which the PRS is developed and used, as well as various methodological considerations when assembling a PRS, have a significant impact on PRS performance (Figure 7).

The use of a PRS to direct preventative screening for subclinical coronary or carotid atherosclerosis may be a pertinent practical use of these genetic correlations. A CAD PRS (of 163 CAD risk loci, Figure 7) substantially predicted non-zero coronary artery calcium (CAC) in a recent investigation of more than 6000 people from the MESA observational cohort, increasing the accuracy of screening cardiovascular computed tomography (CT).

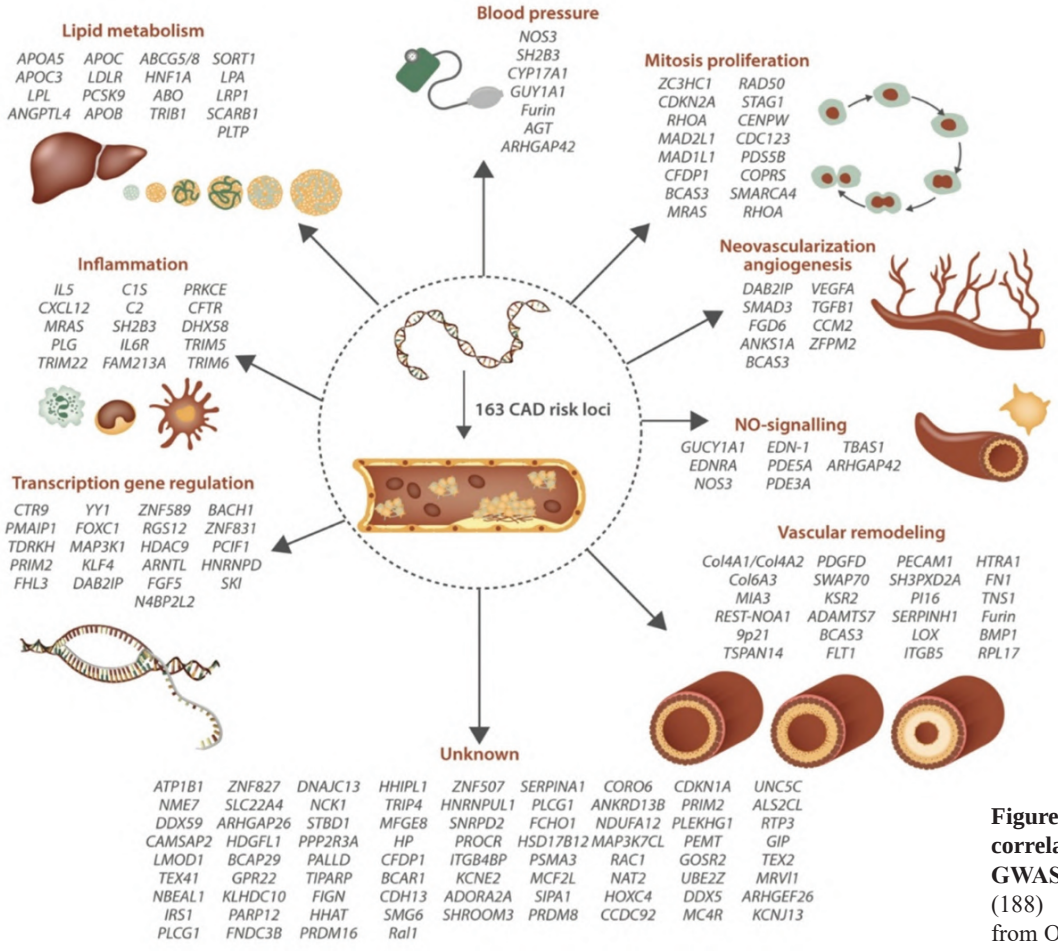

Figure 7. Genetic risk loci correlated with CAD based on GWAS and mechanistic pathway. (188) (Adapted with permission from Oxford University Press).

(188) Therefore, a CAD PRS may permit earlier and more focused imaging-based evaluations of those with high PRS for CAD, for whom the presence of CAC may have a significant impact on when statin therapy should be started.

There are now three areas that are being examined for the potential use of genetic data in clinical atherosclerosis management. The first, familial hypercholesterolemia, is the most convincing example of the use of genetic testing for diagnosis and maybe for therapy guidance. Genetic testing is already used to confirm familial hypercholesterolemia and to cascade screen relatives, but it is anticipated to become more widely used to aid make diagnosis and advance research on the most effective treatments, including novel drugs like PCSK9 inhibitors. It is not yet advised to integrate genetic data to cardiovascular risk prediction for primary prevention. The combination of known variations has not yet shown enough improvement in prediction for integration into widely used risk scores, even if the detection of new variants may significantly enhance prediction in the future. Pharmacogenetics, the third field, is useful for several modern medicines. According to the types of pharmaceuticals and therapeutic approaches that are now on the market, pharmacogenetics' future value will increase or decrease.(189)

The rapid advances in complex trait biology and CAD genetics over the past two decades have enhanced our understanding of disease processes and led to the creation of reliable polygenic predictors that can plot lifetime trajectories of CAD risk. This will be made possible by the growing body of research on methods to mitigate this inherited susceptibility as well as the more readily available and affordable array-based genotyping.(190)

Somehow it is still early to use genetic testing to predict and treat atherosclerosis, whether through a child's screening, or direct-to-consumer testing, the need to include genetics into clinical treatment might become more urgent. The issue shifts from whether to include genetic markers to how to effectively handle the genetic information that is currently accessible when this information becomes more readily available.(191)

MicroRNA Resolution of Atherosclerosis

The best investigated group of non-coding RNAs is microRNAs (miRs). The endonucleases Drosha and Dicer break them down into single-stranded short RNAs, which bind to mRNAs and prevent translation or cause destruction. Numerous miRNAs are involved in the control of vessel development, remodeling, and inflammation in the vasculature. The endothelial-enriched miR-126, which is converted into miR-126-3p and miR-126-5p, is an example of such a miRNA. It's interesting to note that both arms are crucial for regulating EC activities and preventing the development of atherosclerotic lesions.(192) MiR-92a, on the other hand, increases the development of atherosclerotic lesions, inhibits angiogenesis, and promotes EC dysfunction.(193)

Key signaling and molecular regulatory mechanisms involved in the onset and development of atherosclerotic plaques have been identified via research conducted over the past three decades. New molecular insights into the role miRNAs play in these pathways in atherosclerosis have been revealed by the recent discovery of miRNAs as significant regulators of pathophysiological processes, including cellular adhesion, proliferation, lipid uptake and efflux, and production of inflammatory mediators. These findings have also revealed new therapeutic targets. MiRNAs may also be used as biomarkers for diagnosis, prognosis, or in response to cardiovascular treatments as a result of the knowledge that they may be found extracellularly, even in circulating blood.

Changes in cellular or systemic cholesterol levels are linked to metabolic disorders, and maintaining a healthy amount of cholesterol is crucial for cellular function. Cholesterol is transported in the bloodstream by lipoproteins, which can transport (such as LDL-C) or remove (such as HDL) cholesterol from cells and tissues to regulate cholesterol levels. Atherosclerosis is accelerated by imbalances that encourage the build-up of cellular cholesterol, such as high levels of LDL-C and low levels of HDL-C. Our knowledge of the regulatory networks controlling plasma lipoprotein levels has been improved as a result of the recent discovery of miRNAs that regulate LDL-C and HDL abundance and functionality.(194)

It has also been revealed that LDL-C receptor (LDL-R) targeting by miRNA regulates plasma levels of LDL-C. Plasma cholesterol levels are significantly influenced by the liver's production of the LDL-CR protein, which increases the removal of circulating LDL-C particles. Two recent studies have suggested that inhibiting miR-148a in mice led to increased clearance of circulating tagged LDL-C and a decrease in plasma LDL-C levels. These studies have identified miR-148a as a negative regulator of LDL-R expression and function.(195,196) Notably, single-nucleotide polymorphisms in the promoter region of

miR-148A are linked to changed LDL-C in people (196), indicating that dyslipidemias may result from altered production of this miRNA. In fact, examination of genome-wide association study data revealed three additional miRNAs (miR-128-1, miR-130b, and miR-301b) expected to target the LDL-R and located adjacent to human SNPs linked to aberrant plasma lipid levels. Similar to miR-148a, miR-128-1 was boosted in mice by blocking it using locked nucleic acid antisense oligonucleotides, increasing hepatic LDL-R expression and LDL-C clearance. ATP-binding cassette transporter A1, 5' adenosine monophosphate-activated protein kinase a1, carnitine palmitoyltransferase 1a (CPT1a), salt-inducible kinase 1 (SIK1), and insulin receptor substrate 1 are among the genes that miR-148a and miR-128-1 target in addition to LDL-R, indicating that these miRNAs play important roles in.(196)

Additionally, it has been discovered that miRNAs play a crucial role in regulating cholesterol export and high density lipoprotein cholesterol (HDL-C) synthesis. These routes regulate plasma HDL-C levels as well as the reverse cholesterol transport system, which transports extra cholesterol to the liver for elimination. The ATP-binding cassette transporter (ABCA1) plays a critical role in regulating cholesterol efflux from peripheral cells, particularly macrophages in atherosclerotic plaques, by facilitating the transfer of cholesterol across the cell membrane onto lipid-poor ApoA1. This process is essential for both hepatic HDL-C biogenesis and the removal of excess cholesterol. However, several miRNAs, including miR-33, miR-75, miR-26, miR-106, miR-144, as well as miR-128-118 and miR-148a mentioned earlier, have been found to target ABCA1 and inhibit cholesterol efflux to ApoA1 *in vitro*. *In vivo* tests have also revealed that inhibiting the miR-33, miR-144, miR-128-1, and miR-148a increases plasma levels of HDL-C in mice or monkeys. (197) Hepatic clearance through the scavenger receptor BI, which has been demonstrated to be a target of miR-223, miR-455-5p, miR-96, miR-185, and miR-125a, also regulates the levels of HDL-C in the blood. Only miR-223 has been altered *in vivo* and demonstrated to affect plasma HDL-C levels, though.(198)

One of the initial signs of developing plaques is the early stimulation of adhesion molecule expression, which promotes leukocyte recruitment to the artery wall through the production of molecules including E-selectin, intracellular adhesion molecule-1, and vascular adhesion molecule-1. Because they may specifically target these molecules' 3′-UTRs, a number of miRNAs, including miR-17-3p (which targets intracellular adhesion molecule-1) and miR-31 (which targets E-selectin), have been linked to atherogenesis.(199) However, it is still unclear how both of these miRNAs operate in experimental atherosclerosis. One important mechanism that activates not just these proadhesive molecules but also a variety of other proinflammatory and prothrombotic factors is nuclear factor (NF)-κB signaling. Two cytokine-responsive miRNAs, miR-181b and miR-146a, control several NF-κB signaling components and have atheroprotective properties.(200)

MiRNAs may be used as diagnostic or prognostic markers in a variety of illness situations, according to growing body of research. Circulating miRNAs may be found in peripheral blood, saliva, and urine, hence the expression of these molecules may serve as early indicators of CAD at different stages, from asymptomatic atherosclerotic disease to acute coronary syndromes. Here, we provide a summary of the profiling of several research that relate certain miRNAs to the prevalence of atherosclerotic disease as primary diagnostic indicators. Further research will be needed to determine the prognostic importance of these miRNAs in CAD.(194)

Long non-coding RNAs are the most abundant functional non-coding RNAs and have the ability to modulate the availability of miRNAs or the stability of mRNAs. Over the past decade, miRNAs, a type of non-coding short RNA, have emerged as evolutionarily conserved regulators that fine-tune a wide range of molecular signaling pathways and pathological cellular effects involved in atherosclerosis. Figure 8 described the summary of how long non-coding RNAs regulates the vascular function. The relevance of miRNAs in controlling important signaling and lipid homeostasis pathways that change the balance of atherosclerotic plaque growth and reversal is becoming more clear as research mount.(194)

Novel Anti-atherosclerosis Therapies

Statin treatment has significantly reduced the burden of atherosclerotic cardiovascular disease, which has been of great value to society. Nevertheless, the leading cause of mortality worldwide continues to be atherosclerotic CVD. Innovative therapeutic targets to reduce LDL-C as well as other harmful lipids and lipoproteins have been discovered thanks to technological advancements, such as those in the field of genomics, which have changed drug discovery and development. Atherosclerotic CVD is prevented by therapeutic LDL-C lowering, with the extent of the impact being inversely related to the absolute LDL-C decreases and the duration of exposure. This knowledge supports the idea that a major treatment goal should be lowering cumulative

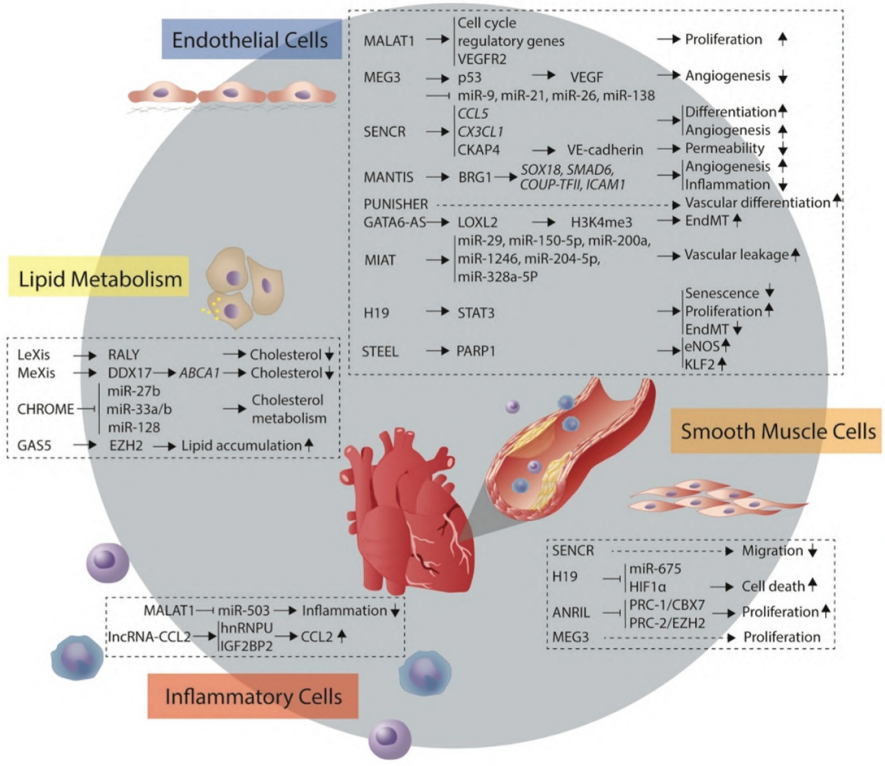

Figure 8. Summary of mechanisms by which long noncoding RNAs regulate vascular cell functions.(193) (Adapted with permission from American Heart Association).

LDL-C exposure. Monoclonal antibodies that inhibit PCSK9 have the potential to lower LDL-C to extremely low levels. By combining effective cholesterol reduction with infrequent dosage schedules, novel therapeutic platforms like RNA inhibition provide the chance to introduce medicines with vaccine-like features. The outcomes of ongoing clinical studies will likely decide the position of lipid-lowering medicines that target substances other than LDL-C, such as residual cholesterol, triglyceride-rich lipoproteins (TRL), and lipoprotein(a). According to recent research, lowering lipoprotein(a) or TRLs may help certain patients' risk of developing atherosclerotic CVD. The mechanics, effectiveness, and safety of the most recent treatment advancements are highlighted in this study.(201)

Characterizing patients with extreme phenotypes is a strategy to therapeutic target identification that is becoming more and more popular like the inherited hypolipidemias (PCSK9). A synthetic single-strand ApoB antisense oligonucleotide (ASO) is called mipomersen.(202) RNase H is responsible for the ASO's destruction once it binds to the appropriate ApoB mRNA. The reader is referred to in-depth reviews of this general subject because the specifics of antisense technology and pharmacology are outside the purview of this review.(203,204) Patients with homozygous familial hypercholesterolemia (HoFH) were the only ones for whom the drug Mipomersen was created (HoFH). In a phase III clinical study, weekly subcutaneous injections of mipomersen lowered LDL-C by 25% and lipoprotein(a) by 32% in 51 individuals with HoFH.(205)

The levels of ApoB and lipoprotein (a) decreased by 49% and 13%, respectively. The majority of the side effects of lomitapide therapy are gastrointestinal issues brought on by enterocytes' elevated triglyceride content. (206) A circulatory protein called angiopoietin like 3 (ANGPTL3) inhibits the enzymes lipoprotein lipase (LPL) and endothelial lipase. This gene's loss-of-function (LOF) mutations improve the metabolism of HDL-C and very low density lipoprotein particles.(207) Clinically, this shows up as low triglyceride, HDL-C, and LDL-C levels. Therapeutic approaches are being researched for this target since LOF mutations in ANGPTL3 are not linked to comorbidities.(208)

PCSK9 is another gene that, through a gain-of-function mutation, causes autosomal dominant hypercholesterolemia.(209) This discovery sparked a remarkable sequence of studies that pinpointed PCSK9 as the primary regulator of plasma LDL-C trafficking. The LDL-R is targeted for lysosomal eradication by PCSK9 once it binds to it. While PCSK9 function raises LDL-R, lowers LDL-C levels, and significantly lowers risks of ASCVD, PCSK9 action decreases LDL-CR and results in hypercholesterolemia.(210-212) Surprisingly, only a small number of people without circulating PCSK9 have been identified, and they exhibit normal health and reproductive

ability, exceptionally low LDL-C levels (15 mg/dL), and no signs of neurological or cognitive damage.(213,214) Together, these findings formed the basis for the creation of PCSK9's therapeutic antagonist. The potential benefits of PCSK9 inhibition are enormous. The success of this novel pharmaceutical strategy will depend on the findings of the big clinical outcomes studies, despite the fact that mAbs targeting PCSK9 are currently available on the market. The effects of PCSK9 antagonistism over the long run, however, need to be seen because the physiological function of this protein is not entirely understood.

In addition to playing a significant role in the transfer of reverse cholesterol, HDL-C possesses antioxidant, antiplatelet, anti-inflammatory, and antiapoptotic characteristics. Additionally, it plays a significant role in the innate immune system and positively influences glucose metabolism.(215) Different particles with varying compositions, sizes, and functions are included in the HDL spectrum. Differences in its proteome and lipidome are likely to account for a large portion of the functional variability.(216,217) Structure-function interactions are not well understood, despite the area's high level of attention. The scientific community is still invested in this subject despite the numerous setbacks, and various new compounds are now being developed.

The lack of interest in triglyceride management for the prevention of ASCVD has historically been a result of conflation of epidemiology and conflicting findings from clinical trials using triglyceride-lowering treatments However, given the failure of randomized controlled trials using treatments to raise HDL-C and the genetic evidence suggesting that triglycerides, rather than HDL, are the cause of ASCVD, there is growing interest in triglycerides.(218,219) Independent of HDL-C, Mendelian randomization and genome-wide association studies have repeatedly shown a link between higher triglyceride levels and an increased risk of ASCVD. In fact, a recent genetic analysis looked at 44 single nucleotide polymorphisms that mainly affect triglyceride levels (with little effect on LDL-C) in more than 86,000 subjects and showed that their impact on triglycerides was related to the level of ASCVD risk.(220)

At therapeutic dosages of 2 to 4 g per day, the marine omega-3 PUFAs, EPA, and docosahexaenoic acid are efficient triglyceride-lowering medications. Marine-derived omega-3 PUFAs offer theoretical advantages beyond decreasing triglycerides, such as improved endothelial function, vasodilation, decreased platelet aggregability, and decreased myocyte excitability. However, the use of omega-3 PUFAs to lower CVD risk has yielded conflicting results. Early research using EPA/docosahexaenoic acid or EPA by itself revealed a cardiovascular advantage.(221) These original findings have been called into doubt by recent trials and meta-analyses. Because these drugs have been investigated in various populations using various dosages, formulations, and concentrations of EPA and docosahexaenoic acid, it is rather challenging to draw strong conclusions.(222) It is also more challenging for any experimental therapy to show incremental value because recent studies have been conducted in cohorts on modern background medical and interventional medications, including as aspirin, statins, β-blockers, angiotensin-converting enzyme inhibitors, and coronary stents.

Lifestyle modifications should be the cornerstone of cardiovascular risk management for primary prevention. Although we don't have enough data to say that lifestyle treatments enhance cardiovascular outcomes in primary prevention, changing one's lifestyle has numerous clearly observable advantages. However, secondary prevention has entered a new age that focuses on reducing recurring occurrences by targeting nonlipid risk variables.(223)

Targeting oxidative pathways has not been successful. LDL-C that has been oxidatively changed is almost always cited as a major initiating component in theories about the pathophysiology of atherosclerosis.(224,225) Different phospholipases can release lipid moieties from oxidized lipoproteins that can activate leukocytes and vascular cells in atheromata to perform harmful tasks. However, several attempts to block phospholipases in order to stop the production of these allegedly harmful mediators have failed. Inhibition of a lipoprotein-associated phospholipase A2 (LpPLA2) was evaluated in the most extensive program in individuals with acute coronary syndromes or in the stable phase of atherosclerosis.(226) This class of enzymes has been dropped as a treatment target for atherosclerosis as a result of these findings and those from smaller research focusing on other phospholipases.

Results have also been poor when directly addressing oxidative stress and lipoprotein oxidation. Clinical research on a number of antioxidants have not produced positive results. In clinical trials that were properly powered and executed, the vitamins C, D, and E and beta carotene failed to lower events.(227) Sucinobucol, a potent antioxidant, partitions into lipoprotein particles and successfully inhibits LDL-C oxidation *in vitro*. However, the major Aggressive Reduction of Inflammation Stops Events (ARISE) study failed to achieve its main objective.(67) The use of antioxidant vitamins and direct oxidation inhibitors as

therapeutic targets in atherosclerosis has lost some of its initial appeal, similar to what happened with phospholipases. Inflammatory pathways have been implicated in the etiology of atherosclerosis and its clinical consequences in both laboratory and clinical investigations. A number of studies targeting various inflammatory pathways in the secondary prevention of atherosclerotic events have been completed or are now in development as a result of this body of research. A number of stressors, including oxidative stress, pro-inflammatory cytokines, and pathogen-associated molecular patterns like bacterial lipopolysaccharide, are mediated by the mitogen-activated protein kinases (MAPKs). A cogent set of preclinical and clinical investigations employing the inhibitor losmapimod led to a concentrated interest in the p38 MAPK. Treatment with losmapimod reduced the inflammatory biomarker CRP as evaluated by a highly sensitive test in individuals with acute coronary syndromes. A research examining the impact of losmapimod on fluorodeoxyglucose absorption in people was unable to achieve its main objective. Losmapimod was the subject of a significant clinical trial with the working title Losmapimod to Inhibit p38 MAP Kinase as a Therapeutic Target and Modify Outcomes After an Acute Coronary Syndrome (LATITUDE), which discouraged future investigation of this target.(228)

The DNA building blocks purines and pyrimidines, which are synthesized by methotrexate, appear to be prevented from doing so, thereby reducing inflammation. Adenine nucleotides and adenosine are released from cells as a result of methotrexate treatment. The G protein-coupled adenosine receptors that the adenosine can activate include the A2A receptor, which is associated with subsequent anti-inflammatory effects.

Conclusion

Advanced research and clinical studies have revolutionized our understanding of atherosclerosis and how to manage the associated risks. Recent studies have identified failed resolution as a critical factor in the development of the most clinically significant advanced plaques, suggesting that immunosuppressive therapy may address clinical plaque progression. This has prompted a new perspective on the comprehensive management of cardiometabolic illness, with resolution therapy as a fresh approach. Technological advancements such as next-generation sequencing and bulk and single-cell RNA sequencing have significantly advanced human genetics studies, while the toolkit for genetic modification of mice continues to evolve with gene-editing and induced pluripotent stem cells. Successful control of atherosclerosis will require a multidisciplinary effort, involving risk factor management, behavioral psychology, public health initiatives, the use of currently available treatments, and the development and validation of new therapeutic modalities.

Authors Contribution

AM drafted, wrote, and edited the manuscript. AW proposed the manuscript topic, supervised, and edited the manuscript. All authors had agree with the final manuscript.

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