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# Leducq Epigenetics of Atherosclerosis Network

Menno P.J. de Winther, Christopher K. Glass

The incidence of atherosclerosis-related cardiovascular diseases (CVDs) is still rising and is expected to further increase over the next decade, partly because of the global prevalence of obesity. LDL (low-density lipoprotein) cholesterol–lowering therapy by statins and more recently by anti-PCSK9 (proprotein convertase subtilisin-kexin type 9) has proven effective in reducing CVD risk by ≈35%.[1] However, this still leaves a major group of patients prone to develop clinical complications. Moreover, despite major advances in medical and surgical intervention, 10% to 25% of patients with CVD experience recurrent cardiovascular events within 3 to 5 years after the first manifestation.[2] To allow better treatment of this residual risk, it is essential to refine our view of the mechanisms underlying CVD. With our Leducq consortium, we aim to provide novel insights into the regulation of inflammatory mechanisms in CVD by investigating epigenetic processes that control atherosclerosis development. We will focus on macrophages, and their circulating precursors monocytes, to define critical inflammatory pathways in disease and identify novel approaches for diagnosis and treatment.

Funded by Fondation Leducq, we have been able to bring together an exciting group of scientists to study the epigenetic regulation of monocytes and macrophages in atherosclerosis. Our network is led by Christopher Glass (University of California, San Diego) as American coordinator and Menno de Winther (Amsterdam University Medical Center) as European coordinator. The other project leaders are Bart Staels (University of Lille, France), Sam Tsimikas (University of California, San Diego), Seppo Ylä-Herttuala (University of Eastern Finland, Kuopio, Finland), Ronald Evans (Salk Institute for Biological Studies), Udo Oppermann (Oxford University, Oxford, United Kingdom), and Frederic Geissmann (Memorial Sloan-Kettering Cancer Center, New York). We thus bring together leading scientist in the fields of transcriptional and epigenetic regulation of monocytes and macrophages in cardiometabolic disease, clinical CVD studies, pharmacology of epigenetic enzymatic function and ontology, and function of monocytes and macrophages.

Inflammation plays a prominent role in atherosclerosis development and associated CVD. This was elegantly demonstrated by the recent CANTOS trial (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study)[3] where blockade of the proinflammatory cytokine IL (interleukin)-1β by antibody therapy reduced recurrent cardiovascular events in patients who had experienced myocardial infarction. We and others have recently demonstrated that hypercholesterolemia or risk factors like Lp(a) can promote a proinflammatory state, reflected in activated circulating monocytes.[4,5] Moreover, inflammatory markers, such as high-sensitivity C-reactive protein, are predictive biomarkers of cardiovascular events independent of LDL cholesterol levels; patients with chronic inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus have an increased risk for CVD, and there is a vast body of evidence from basic research showing that interventions in inflammatory pathways can reduce atherosclerosis development. In atherosclerotic lesions, the abundance of immune cells, and more specifically macrophages (which originate from circulating monocytes), correlates with key features of atherosclerotic plaque instability and clinical sequelae. Understanding the inflammatory mechanisms through which monocytes and macrophages modulate atherosclerotic disease will open new avenues for better diagnosis of patients at risk and allow improved interventions.

Epigenetic processes govern many aspects of inflammation. They involve the complex regulation of gene programs through genomic DNA methylation and modification of histones by methylation or acetylation, all of which control chromatin accessibility and transcriptional use. Epigenetic mechanisms regulate cellular differentiation processes (eg, monocyte to macrophage differentiation) but also control direct responses of monocytes and macrophages to systemic and local inflammatory cues (eg, lipids, cytokines, damage-associated molecular patterns). The enzyme families that control epigenetic processes involve so-called writers, which deposit histone marks, erasers, which remove them, and reader, which can read epigenetic histone modifications. Several investigators from our network have been studying these epigenetic regulators and have identified epigenetic enzymes that influence macrophage function and thereby contribute to atherosclerosis development. For example, in a collaborative effort, de Winther and Glass could show that HDAC3 (histone deacetylase 3) is a major regulator of macrophage phenotype in atherosclerosis. By myeloid-specific deletion of HDAC3 in mice, macrophages gain anti-inflammatory and profibrotic features, and atherosclerotic plaques stabilize.[6] Moreover, the group of Glass have shown that particularly regulation of enhancers

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and their activity is important in controlling cellular identity and function.[7–9] Also endogenously derived molecules, including fatty acids and desmosterol, can impact on histone modifications required for inflammatory gene activation and thereby inhibit inflammatory responses.[10,11] Interestingly, it was recently established that clonal hematopoiesis of indeterminate potential (CHIP [chromatin immuno-precipitation]) is a major risk factor for coronary heart disease,[12] and the major causative genes in CHIP (eg, *TET2* and *DNMT3A*) are actually epigenetic enzymes. Somatic mutations in these genes contribute to atherosclerotic disease, particularly by affecting cells of the myeloid lineage. The group of Geissmann could show that a somatic mutation in BRAF strongly impacts on myeloid cell activation and thereby affects neuroinflammation in mice and human patients.[13] Relatively little is understood of the underlying mechanisms whereby CHIP contributes to CVD.

An attractive feature of the epigenetic machinery is that DNA and histone modifications are reversible, and epigenetic enzymes are often amenable to pharmacological modulation. Therefore, their targeting represents a rapidly evolving therapeutic space. Not only in oncology but now considered in the broad scale of inflammatory diseases, therapeutic modulation of epigenetics is heavily investigated. For example, inhibitory small-molecule drugs that block epigenetic reader complexes were demonstrated to suppress activation of macrophages.[14] These have subsequently been developed into successful clinical molecules and are currently undergoing phase 2 clinical trials for efficacy in inflammation. Using similar small molecules targeting epigenetic reader complexes, it was also established

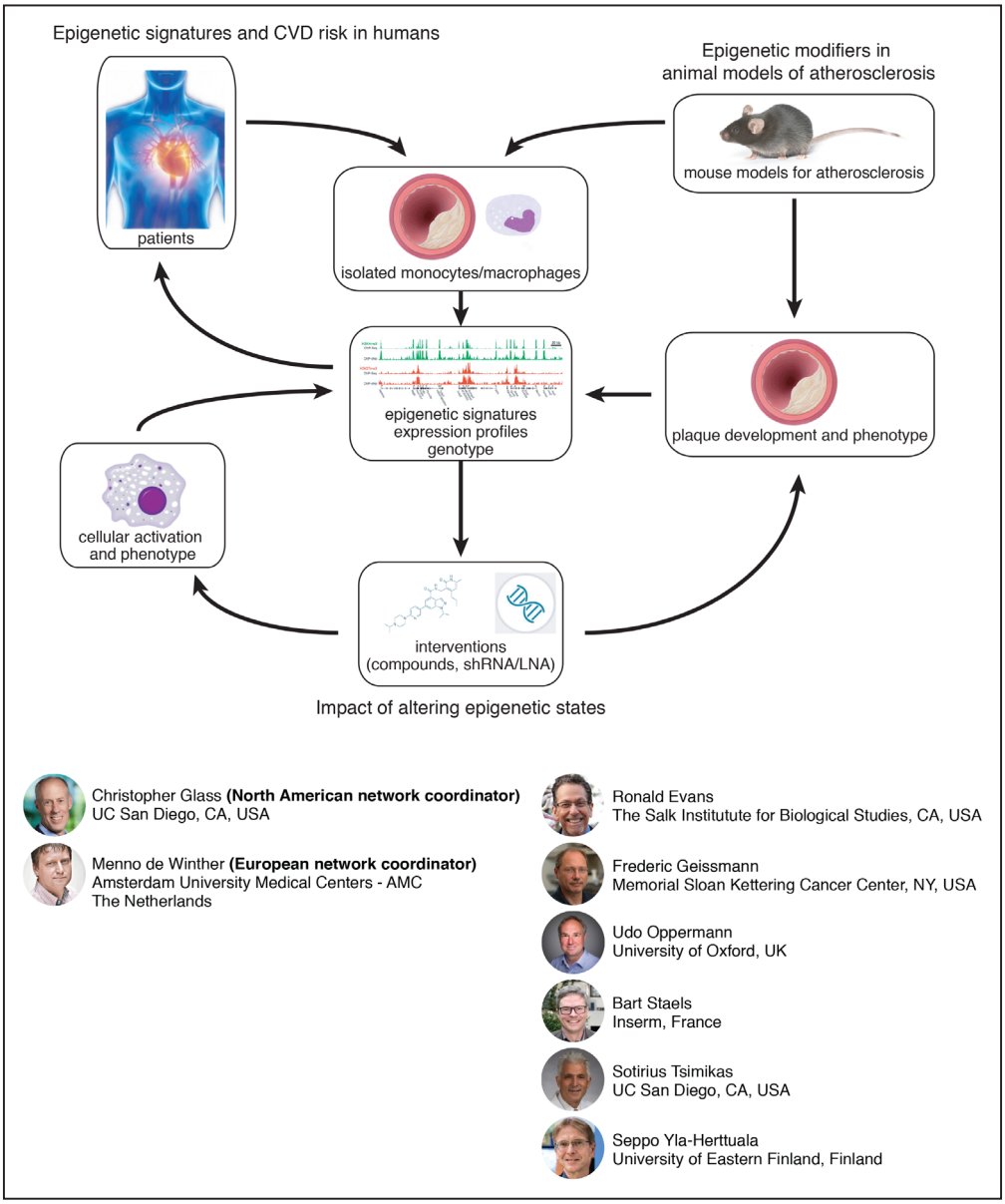


**Figure. The Leducq Epigenetics of Atherosclerosis Network has the ambition to define novel epigenetics-driven inflammatory mechanisms that control monocyte and macrophage function and thereby contribute to disease development.** We will characterize the relationship of epigenetic states in human monocytes and macrophages with cardiovascular disease (CVD) and CVD risk, identify underlying regulatory mechanisms by applying a combination of cell-based and animal model work, and finally assess novel interventional approaches to control disease. Indicated are the members of our network and their affiliations. LNA indicates locked-nucleic acid; and shRNA, short-hairpin RNA.

that pharmacological intervention with this reading machinery could be used to suppress atherosclerosis development in mice.[15] The group of Oppermann, functioning in the Structural Genomics Consortium, is actively working on the development and testing of such small-molecule epigenetic inhibitors. They could show that blockade of specific H3K27 (histone 3 lysine 27) histone demethylases could be used to suppress macrophage activation.[16] Collectively, we think that there are compelling arguments to investigate epigenetic mechanisms as novel targets for diagnosis and treatment of CVD.

Our overarching hypothesis is that altered epigenetic states underlie pathogenic gene programs in monocytes and macrophages that contribute to CVD. With our network, we aim to address this hypothesis by characterizing the relationship of epigenetic states in human monocytes and macrophages with CVD risk, identification of underlying regulatory mechanisms, and by assessing novel interventional approaches to control disease (Figure).

We will study a series of cohorts from large cardiovascular clinics in San Diego (Tsimikas), Kuopio (Yla-Herttual), and Lille (Staels) to define monocyte and macrophage epigenetic profiles that associate with CVD. Rigorously standardized protocols will be used for isolation and purification of monocytes and macrophages to allow comparison of genetic and epigenetic data across network laboratories. Purified cells will be subjected to standardized and well-controlled sets of analyses: chromatin accessibility assays (ATAC-seq [assay for transposase-accessible chromatin-sequence]), genome-wide analysis of histone modifications (ChIP-seq), and gene expression analysis (RNA-seq). Furthermore, we will be applying single-cell approaches to study cell populations in atherosclerotic plaques and determine their chromatin state. We will study isolated monocytes from patients with or without confirmed coronary artery disease and also analyze cells from patients carrying risk factors for CVD such as elevated Lp(a) (lipoprotein(a)). These cohorts will allow association of monocyte and macrophage (epigenetic) signatures with atherosclerotic disease and risk for disease. Additionally, we will isolate macrophages from atherosclerotic lesions from patients allowing correlation of monocyte signatures with those of atherosclerotic macrophages and definition of macrophage characteristics in their tissue (ie, lesional) environment. Moreover, we will study patient groups by assessing monocyte changes in patients undergoing cardiac surgery and prospectively assess epigenetic marks associated with postoperative atrial fibrillation. Because of the strong link between CHIP and epigenetics, we have the ambition to investigate clonal hematopoiesis in our patient cohorts and link it to cellular functional changes by modulating CHIP relevant enzymes in iPS (induced pluripotent stem cell)-derived macrophages. These studies will define epigenetic and transcriptomic signatures that associate with disease and CVD risk factors in patients.

To understand the impact of interventions in the epigenetic states on the functional repertoire of monocytes and macrophages, we will take several approaches. Human monocyte-derived macrophages, but also cell lines, will be treated in vitro with atherosclerosis-related stimuli combined with libraries of small-molecule drugs, RNA knockdown molecules, or CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat–associated 9) libraries, all targeting epigenetic enzymes and their associated regulatory machinery. Particularly, the Oxford-based small-molecule library offers a unique approach to pharmacologically intervene with epigenetic processes in monocytes and macrophages. Specific activation-state gene signatures and activation markers will serve as screening readouts and will subsequently be linked to consequences for cellular function in relation to disease. Genome-wide analysis will be performed to define the consequences on epigenetic signatures and transcriptional responses. Parallel studies will be performed in mouse macrophages to substantiate findings and to allow further validation in animal models. These studies will identify relevant pathways and epigenetic patterns and enable selection of candidates for further in vivo analysis.

Using mouse models for atherosclerosis, including LDL receptor–deficient mice, we will use our findings into in vivo models for diseases. We will determine the effect of dietary interventions on the transcriptomic and epigenetic signatures of monocytes and macrophages in relation to disease development. Moreover, promising modulators that were defined in our previous aims will be studied in vivo by approaches including (cell specific) knockouts for epigenetic enzymes, small-molecule drug interventions, or administration of epigenome modulatory factors. These studies will be combined with detailed epigenetic characterizations and provide important insights into the relationship between specific epigenetic states, cellular function, and disease phenotypes. We hereby aim to identify epigenetic profiles that could serve as diagnostic markers and define specific epigenetic modulators as targets for therapeutic intervention.

With our Leducq Epigenetics of Atherosclerosis network, we have the ambition to progress the field of epigenetics in CVD. Key to our research is also the training of young investigators. We have established a network where these young investigators will easily navigate between groups to master innovative technologies and to apply the range of expertise of our network to the integrative projects that we will be pursuing. Through these interactions, it is our utmost belief that by completion of our program, we will have defined novel mechanisms in CVD that may lead to innovative therapeutic applications.

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## Disclosures

None.

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