Arteriosclerosis, Thrombosis, and Vascular Biology

BRIEF REVIEW

False Utopia of One Unifying Description of the Vulnerable Atherosclerotic Plaque: A Call for Recalibration That Appreciates the Diversity of Mechanisms Leading to Atherosclerotic Disease

Gerard Pasterkamp, Hester M. den Ruijter, Chiara Giannarelli

ABSTRACT: Atherosclerosis is a complex disease characterized by the formation of arterial plaques with a broad diversity of morphological phenotypic presentations. Researchers often apply one description of the vulnerable plaque as a gold standard in preclinical and clinical research that could be applied as a surrogate measure of a successful therapeutic intervention, despite the variability in lesion characteristics that may underly a thrombotic occlusion. The complex mechanistic interplay underlying progression of atherosclerotic disease is a consequence of the broad range of determinants such as sex, risk factors, hemodynamics, medications, and the genetic landscape. Currently, we are facing an overwhelming amount of data based on genetic, transcriptomic, proteomic, and metabolomic studies that all point to heterogeneous molecular profiles of atherosclerotic lesions that lead to a myocardial infarction or stroke. The observed molecular diversity implies that one unifying model cannot fully recapitulate the natural history of atherosclerosis. Despite emerging data obtained from -omics studies, a description of a natural history of atherosclerotic disease in which cell-specific expression of proteins or genes are included is still lacking. This also applies to the insights provided by genome-wide association studies. This review will critically discuss the dogma that the progression of atherosclerotic disease can be captured in one unifying natural history model of atherosclerosis.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: hemodynamics ■ myocardial infarction ■ proteomic ■ risk factors ■ transcriptomic

Atherosclerosis is a complex condition with many manifestations and underlying causes. Yet, the scientific community is striving for descriptions to reflect this complexity in one unifying model. This has led to widely appreciated classifications based on pathological descriptions of affected blood vessels obtained from patients who died of acute myocardial infarction in different age strata.[1,2] These classifications go back to the early 1990s and are still used as a gold standard in scientific studies that test new drugs in experimental models or the diagnostic potential of biomarkers for advanced atherosclerotic disease. Despite the fact that the histological characterization of late-stage atherosclerotic plaques has brought many insights into the mechanisms of atherosclerosis, research achievements over the last decades have revealed a diversity in cell-cell interaction that drives gene regulation, protein expression, and underlying subsequent pathology in atherosclerosis. The complex mechanistic interplay underlying progression of atherosclerotic disease is also a logical consequence of the broad range of characteristics that are of influence such as sex, risk factors, hemodynamic factors, medications, and the genetic landscape. The observed molecular diversity of atherosclerotic lesions implies that one unifying model may not fully recapitulate the natural history of atherosclerosis.

The observed variability in (epi)genetic and transcriptomic profiles in addition to histological classifications will provide in depth insight into the diverse phenotypes of plaques that give rise to clinical manifestations

Correspondence to: Gerard Pasterkamp, Prof, Central Laboratory Diagnostics, University Medical Center Utrecht, Room G03 538, Heidelberglaan 100, 3584CX Utrecht, The Netherlands, Email g.pasterkamp@umcutrecht.nl
For Sources of Funding and Disclosures, see page e93.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

CAD	coronary artery disease
GWAS	Genome-Wide Association Study
MMP	matrix metalloprotease

Highlights

- There is an overwhelming amount of data that all point to heterogeneous molecular profiles of atherosclerotic lesions that lead to a myocardial infarction or stroke.
- The use of one unifying concept and definition of the vulnerable plaque narrows our field of view and can lead to interpretations of experimental research that only covers part of the patient load.
- Novel insights could lead to diverse phenotypic descriptions of the plaque at risk for an adverse cardiovascular event that includes hereditary genetic, plaque transcriptomic, and pathological criteria.

of atherosclerotic disease (Figure). Appreciation of the significant variability of phenotypes that characterize the so-called vulnerable plaque will also ask for a careful consideration and recalibration of end points used in experimental studies.

THE DIVERSITY IN PATHOLOGY CLASSIFICATIONS OF PLAQUES THAT ASSOCIATE WITH MYOCARDIAL INFARCTION

Human studies have already convincingly shown that in addition to plaque rupture, fibrous plaques can also be the underlying pathological substrate for myocardial infarction because of plaque erosion.[3,4] Current concepts for potential mechanisms leading to plaque erosion have been recently reviewed in study by Kolte et al[5] and Libby et al.[6] Research on the mechanism of plaque erosion has implicated circulating and adherent neutrophil granulocytes[7] and T lymphocytes in both preclinical and clinical studies.[5] The endothelial layer gets disrupted by either apoptosis or cell detachment—because of gelatinases (MMP-2 [matrix metalloproteinase-2]) and collagenases that breakdown the subendothelial matrix—events prompt the formation platelet rich thrombi and adhesion of neutrophils. Triggers for an inflammatory response that specifically attract the polynuclear leucocytes are danger-associated molecules that, by binding the toll like receptor 2, activate the NFkB-dependent inflammatory

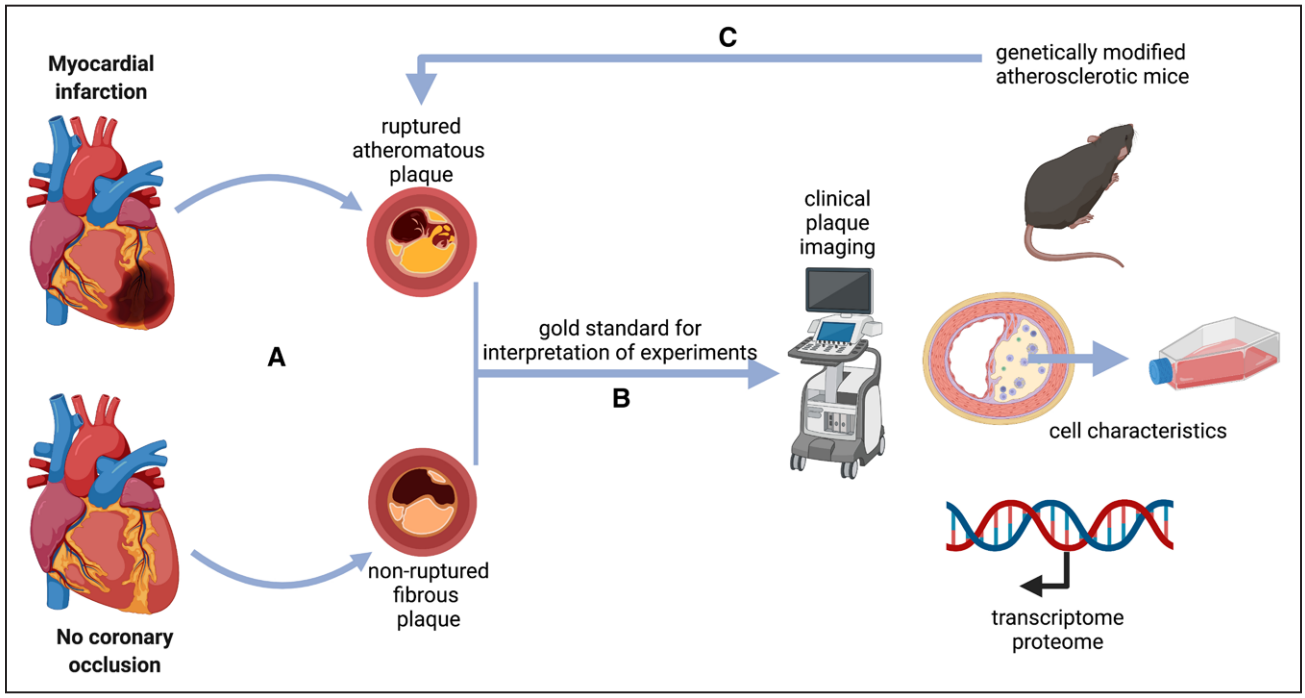

Figure 1. The most widely used workflow in search for determinants of progression of atherosclerotic disease.
A, Plaques are obtained from patients who suffered from a major ischemic event (MI or stroke) or who died at different age strata. Plaques have been characterized and the observed phenotype is used as a gold standard for future studies. **B**, The classical description of the vulnerable plaque is subsequently used. (1) As most important measure in search for genes, proteins and cell characteristics that may destabilize atherosclerotic lesions; (2) interpretation of imaged characteristics of human lesions. (3) Interpretations of phenotypic changes in the plaques observed in atherosclerotic genetically modified mice. **C**, The gene specific effects on lesion phenotypes in mice are also often translated to human disease by assessment of RNA and/or protein expression in human specimen that fulfill the classical description of the vulnerable plaque (the gold standard).

pathway.[8] Another proposed, less frequent, mechanism for plaque thrombosis is associated to the presence of calcified noduli,[9] where a lesion with fibrous cap disruption and thrombi is associated with eruptive, dense, calcific nodules. This plaque pathology could be the result of plaque healing.

Although the atheromatous inflammatory plaque is the most dominant end point used in preclinical and clinical studies, the prevalence of the fibrous plaque as pathological substrate of a clinical event is gaining ground because of drastic improvements in therapy and lifestyle changes in recent decades.[10,11] In addition, serial imaging of nontarget lesions showed that only in a minority of cases the traditionally described vulnerable atheromatous plaque resulted in a clinical event.[12] Accordingly, the PDAY STUDY revealed that 20% of young subjects <35 years old already present Type IV and V lesions according to the AHA classification.[13]

Pathology studies have also shown that the diversity in morphological presentation goes beyond a distinction in rupture, erosion or calcified nodules. The vessel wall is capable of a strong adaptive geometric response when atherosclerotic plaque volume changes. Geometric remodeling is a double-edged sword. Expansive remodeling is associated with initial preservation of the lumen area but is associated with local inflammation, MMPs activity, atheromatous plaques and clinically with the presentation of unstable angina, non ST-elevation myocardial infarction and stroke.[14] Constrictive remodeling typically results from fibrous smooth muscle cell and collagen rich lesions and is more associated with the presentation of stable angina and mild cerebral symptoms such as amourosis fugax.[15] This variety of geometric remodeling can be found in one patient and even in a single arterial segment.[16]

In summary, although pathological observations have been the hallmark for the current most widely used description of the natural history of atherosclerotic plaque destabilization, it also shows a large variability in underlying substrates that can lead to a clinical adverse cardiovascular event.

INSIGHTS BEYOND ATHEROSCLEROTIC PATHOLOGY, THE AWARENESS OF INCREASING DIVERSITY IN MECHANISMS

Screening the literature on the "natural history of atherosclerosis" tells us that our knowledge of the disease is mostly based on pathology[9] and imaging modalities.[17–20] Local hemodynamic changes have a significant impact on the progression of atherosclerotic disease by differentially affecting the size and composition of atherosclerotic plaques depending on their location in the arterial tree.[21,22] Despite emerging data obtained from -omics studies and a single-cell RNA seq study of atherosclerosis progression and regression in mice,[23] comprehensive studies that fully describe the natural history of atherosclerosis with cell-specific expression of proteins or genes are lacking. This also applies to the insights provided by genome wide association studies (GWAS) that have shown that genetic architecture contributes to up of 40% of the risk of heart attack.[24–26] The number of reports on potential new mechanisms for progression of atherosclerosis according to human GWAS and animal studies is enormous. Indeed, hereditary and acquired mutations in the DNA are associated with acute cardiovascular events.[27–29] It has become clear that, in addition to hereditary genetic variants, some specific somatic mutations increase the chance of having a cardiovascular event.[28] To make the field even more complex, cells present unique plasticity dictated by the plaque microenvironment. Cell populations previously considered homogeneously stabilizing can transdifferentiate and phenotypically have a deleterious effect on plaque composition.[30,31] Lineage tracing studies in mice show that smooth muscle cells can differentiate into cells that no longer express ACTA-2 but do express macrophage marker CD68, and the endothelium can undergo a phenotypic shift by differentiating into a mesenchymal cell.[31,32]

Also, environmental factor interactions with the aforementioned associations can influence the expression of genes and proteins via epigenetic and transcriptomic changes.[33] For example, established risk factors for atherosclerosis enhance the downstream transcription effect of genetic risk loci.[34] Although risk factors for atherosclerotic lesion development may vary, it appears that the common denominator is that they all promote a (vascular) inflammatory response.

Lifestyle and medication use have changed the pathological landscape of atherosclerotic disease.[35] Statins have pleiotrophic effects resulting in alterations in plaque characteristics.[36] Physical activity tones down hematopoietic stem and progenitor cell proliferation via modulation of their niche and reducing hematopoietic output of inflammatory leukocytes.[37] On the other hand, acute mental stress stimulates proatherogenic innate responses and plaque destabilization because of higher leucocyte vascular wall infiltration.[38] Chronic stress reprograms monocytes and to a hyperinflammatory phenotype[39] that could contribute to aggravated atherosclerosis following chronic stress in mice.[40]

These and many other new insights indicate the enormous complexity of mechanisms implicated in the development and progression of atherosclerotic disease over the lifespan. Hereditary and acquired DNA mutations as well as epigenetic changes may influence gene transcription and subsequent expression of proteins and metabolites. The differences in transcriptomic, protein, and metabolite expression between human atherosclerotic plaques may provide a basis for new and more

diverse classifications of human plaques that better stratify lesions and patients for risk for an ischemic event.

SEX-SPECIFIC CHARACTERISTICS OF ATHEROSCLEROTIC PLAQUE PHENOTYPES

Differences in atherosclerotic disease between women and men are widely acknowledged with women being more prone to plaque erosion and stable symptomatic lesions. While sex-stratification in biobanks is still limited, mainly because of the limited number of women in biobank studies, knowledge gaps are being addressed.

It has been convincingly demonstrated that significant differences exist between sexes in the underlying pathology of atherosclerosis and its gene regulation.[41] Women develop more diffuse atherosclerotic disease with relatively more plaque erosion as an underlying cause of coronary artery disease (CAD), while men more often suffer from acute plaque rupture.[3,42] These nonruptured eroded plaques have specifically been observed in young (smoking) females and with an early menopause.[43] Recent outcomes of CAD GWAS studies showed that genetic loci can be associated with CAD risk in a sex specific manner.[29]

Transcriptomic studies using vascular specimen derived from females revealed a crucial involvement of gene regulation in endothelial cells and smooth muscle cells phenotype switching during development and progression of plaques.[41] The effect of sex on plaque development has also been shown in mouse models: XX sex chromosome complement promoted intestinal lipid absorption and subsequent bioavailability of dietary resulting in atherosclerosis progression.[44] In addition, escapees of X chromosomal transcription of the second X chromosome is another mechanism by which sex differences in cardiovascular disease can be explained[45]

DIVERSITY IN GENETIC RISK DETERMINANTS THAT INFLUENCE PLAQUE CHARACTERISTICS

Many genomic risk variants that contribute to CAD or stroke have been identified by GWAS. Combined, these loci explain an estimated 30% to 40% of heritability in CAD that account for ≈40% of all cases.[46] A significant number of GWAS risk loci are associated with lipid metabolism[47] or inflammation.[48] Many of these described GWAS risk loci are unrelated with known genes that have been extensively explored in the pathogenesis of atherosclerotic disease.[27] Moreover, a significant number of loci were observed in noncoding regions, which hampers the identification of their biological function and causality with CAD.[49] The functional context of many genes that can be linked with a risk locus has been studied in genetically modified mouse models. In most experimental studies, plaque area or classical pathology determinants of the "vulnerable plaque" (defined as lipid rich inflammatory plaques with thin fibrous cap) were applied as a surrogate end point for establishing a causal effect of individual genes on lesion development. A knowledge gap remains on how the genetic risk loci influence the characteristics of human advanced plaques. Such exercise requires large sample sizes to reach sufficient power because individual genetic loci likely confer only a small percentage in risk that likely corresponds to modest differences in plaque phenotypes.

Human lesion characterization with computed tomography angiography revealed that a polygenic risk based on GWAS increases CAD risk through an increased burden of coronary atherosclerosis rather than promoting specific plaque features.[50] The association of risk loci with wall thickness was also observed in a human carotid magnetic resonance imaging study where mutations in the SERPINA9 gene showed race-specific associations with characteristics of carotid atherosclerotic plaques.[51] Studies that associate genetic variants with human plaque pathological descriptions are even more scarce. For example, variants in HDAC9 have been associated with increased risk for stroke.[52] In mice, the proportion of Mac3-positive macrophages was higher in plaques from HDAC9(−/−)ApoE(−/−) mice.[53] Subsequent analysis of human atherosclerotic plaques revealed no association between the specific HDAC9 variant and specific plaque characteristics.[53] In the GWAS CARDIoGRAM study, risk loci (9p21 and 6p24) in the PHACTR1 gene were identified that associated with coronary calcium score.[54] However, plaque progression and calcification are highly correlated and therefore genetic association studies may be confounded by genes that mainly control atherosclerosis predisposition rather than calcification itself[55]

In the Athero-Express, human plaque biobank associations were found between loci described in CAD GWAS studies and pathological plaque characteristics. A total of 21 established risk variants nominally associated to a pathology-based plaque characteristic. Despite the limited power, one variant (rs12539895, risk allele A) at 7q22 associated to a reduction of intraplaque lipids.[56]

In recent years, there has been an explosive growth of atherosclerotic plaque biobanks, especially of carotid plaques. Leveraging existing tissue specimens for sufficiently powered genome-wide analyses will likely allow the identification of rare and common variants associated with various forms of pathological presentation of human advanced atherosclerosis.

In summary, the search for associations between common risk variants of CAD and human pathological characteristics may elucidate candidate mechanisms of atherosclerotic disease, but these studies will require increasing amounts of data.

SOMATIC MUTATIONS AND CLONAL EXPANSION

Somatic DNA mutation are acquired with aging and some specific mutations of hematopoietic stem cells in the bone marrow are known to increase the risk of cancer and cardiovascular disease.[28] In fact, in most cases, somatic mutations have little or no consequences, as most mutations have no effect on cellular function. However, a somatic mutation that confers competitive advantage to the mutant cell may lead to clonal expansion and detrimental consequences. Four genes (*DNMT3A*, *TET2*, *ASXL1*, and *JAK2)* are known to harbor somatic mutations with aging that increase the risk for hematologic cancer. The observation in large epidemiological studies of whole exome sequencing data that the mortality risk in the group patients with these somatic mutations was mainly attributed to cardiovascular death was a surprising incidental observation. Interestingly, *TET2* alters DNA demethylation, transcriptional activation and mediate transcriptional repression by recruiting histone deacetylases to gene promoters.[57] In atherosclerotic mice, the loss of *Tet2* resulted in clonal hematopoiesis and accelerated lesion development.[58] Macrophages lacking *Tet2* presented a higher expression of cytokines and chemokines and specifically the overproduction of IL-1β, suggesting that these cells can sustain a strong pro-inflammatory environment and plaque progression.[58] Transcriptomic studies of circulating leucocytes revealed that *DNMT3A* mutant cells exhibit a highly inflamed gene expression profile.[59]

Clonal expansion of cells in atherosclerotic lesion may not just be induced by mutations in somatic cells in the bone marrow. Recent observations in animal models indicate that clonal expansion in the vascular wall may also occur by other mechanisms that give cells a survival advantage. During atherogenesis, single smooth muscle cells gives rise to the clones of cells that coat the cap of atherosclerotic plaques, a process that is regulated by Integrin B3.[60] In another study using multicolor lineage tracing mouse models, it was observed that mature smooth muscle cell can give rise to a hyperproliferative cell which appears to promote inflammation via elaboration of complement-dependent anaphylatoxins.[32] Smooth muscle cells may escape immune surveillance of macrophages, a process that depends on the key antiphagocytic molecule CD47, thereby exacerbating its relative survival advantage.[32] The extent clonal expansion in plaques and its contribution to human vascular lesion development remains to be established. Moreover, it is not unlikely that future DNA sequencing studies of human plaques will demonstrate the occurrence of somatic mutations directly in human atherosclerotic plaques that influence cell survival, transdifferentiation, or clonal expansion and ultimately plaque pathology and fate.

VASCULAR-OMICS: WILL IT CONTRIBUTE FUTURE DETERMINANTS IN PLAQUE PHENOTYPING?

A considerable increase in –omics studies in human atherosclerotic plaques have been reported. Most associative analyses, however, still apply the traditional pathological description of vulnerable plaques as the gold standard in search for genes and proteins relevant for destabilization and thrombotic events. This can be regarded as a missed opportunity because when -omics derived data associate with clinical presentation these can theoretically also be implemented in novel (multiple) phenotypic descriptions of the lesions that give rise to myocardial infraction or stroke. Even more, it is expected that the examination of the outcomes of plaque derived -omics studies will reveal that the landscape of atherosclerotic disease progression is more complex and diverse than currently understood, making it virtually impossible to catch all destabilizing determinants in one unifying concept.

A study using micro array data of the BIKE study demonstrated the feasibility to associate the transcriptomic signature of plaques with CT angiography outcomes. The authors concluded that it would be possible to develop a virtual transcriptomics imaging protocol based on a combination of advanced morphological and molecular characterization of atherosclerotic plaques and machine learning and artificial intelligence methods to determine per-patient molecular level signatures.[61] Despite the progress being made, at present, a validated multi-omics measure of plaque destabilization, based on clusters or networks of genes and proteins, which can be used as surrogate end point in vascular biology research is still lacking.

To achieve this goal, future studies that merge genetic data, polymorphisms observed in the large GWAS studies, with RNA expression in vascular tissue will help identify gene-networks that links the observed genetic associations with biological functional differences between plaque types. Indeed, transcriptome-wide association studies integrate genome-wide association studies and gene expression datasets to identify gene-trait associations[62] and can point to causal genes at GWAS risk loci.

FUTURE PERSPECTIVE: CELL TRANSCRIPTOMICS-BASED CATEGORIZATION OF ATHEROSCLEROTIC PLAQUES

The increased numbers with which bulk sequencing of atherosclerotic lesions is performed will allow to regroup lesions based on the associations with clinical presentation. Indeed, most systems genetics studies have been based on bulk RNA-seq that averages transcriptional expression across cells. Single-cell sequencing can

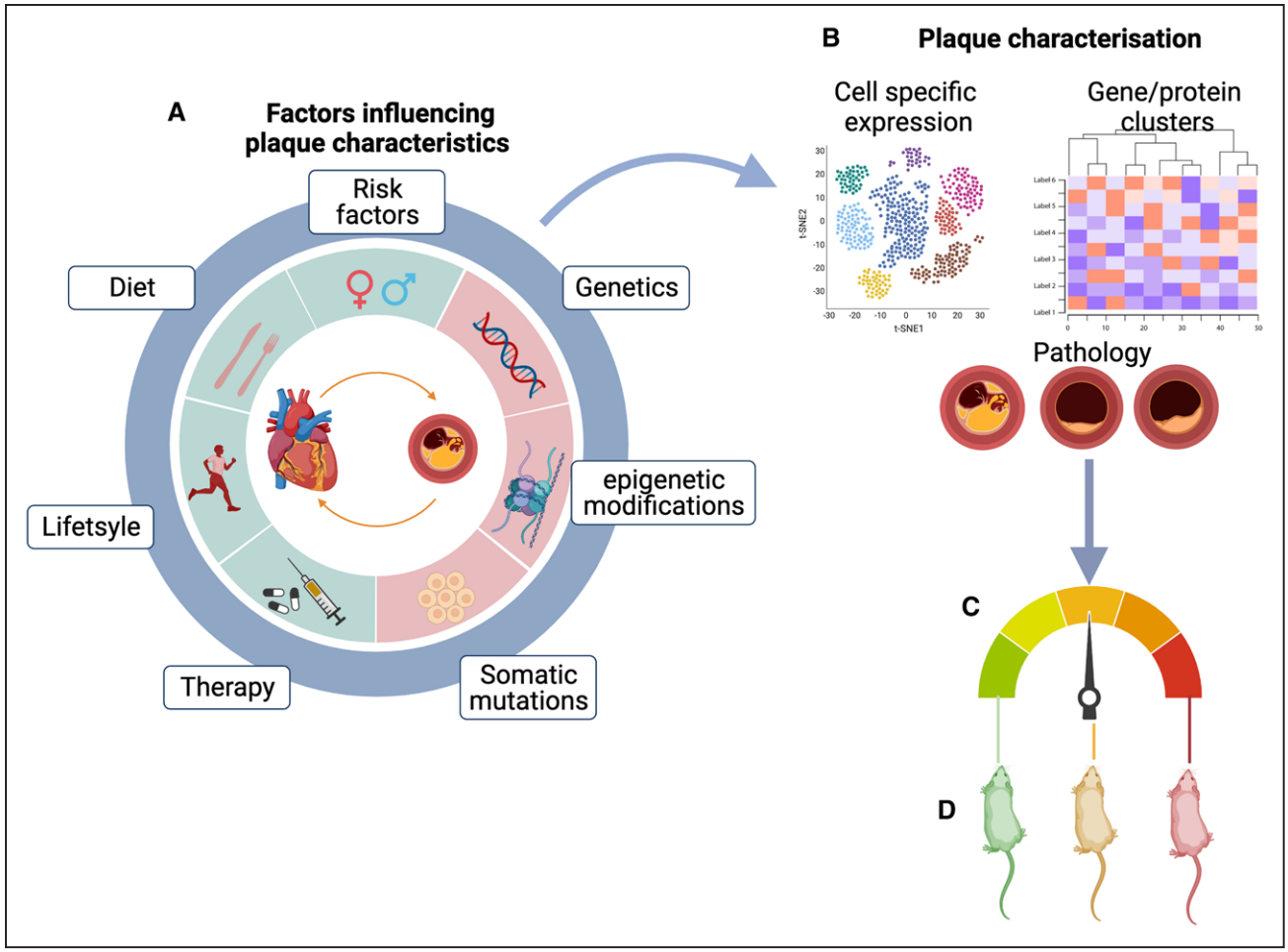

Figure 2. Future perspective.
A, A manifestation of ischemic adverse events is recognized by many underlying mechanisms and risk factors resulting in a broad diversity of atherosclerotic plaque phenotypes. **B**, Next to a pathology based description, future plaque phenotyping can be executed including bulk transcriptomics and proteomics as well as single cell analyses. These analyses need to be executed in a sex stratified manner. **C**, The analyses result in the appreciation of a diversity of lesion types that will ask for stratification by clustering. **D**, The novel combined pathology AND -omics based plaque clusters will influence the interpretation of experimental outcomes in mouse models and will ask for a search of the best stratified matches between mouse and men.

further differentiate plaque phenotypes based on cell specific characterization with regard to biological function, cell-cell interaction, and possibly cell differentiation. Therefore, single-cell technologies are ideal for uncovering new alterations of the complex hierarchical set of molecular and cellular networks that contribute to atherosclerotic cardiovascular disease and clinical outcomes. The identification of the complex cell type composition and functional states of cells in atherosclerotic plaques is crucial for the identification of the cellular contribution to disease and of new druggable cellular targets.[63]

An increasing number of publications in which human plaques have been characterized by single-cell sequencing indicate a wide variety of cell types that are indistinguishable on the basis of single epitopes.[64,65] Cell types that were previously considered to represent one homogeneous cluster seem to express gene networks that clearly point to differential functions and also to states of transdifferentiation.[31,41] The recent reports on single-cell sequencing of human plaques confirm the complexity and multiplicity of biological processes with spatial and temporal variation. Cells are clustered on the basis of gene expression and the gene-based cell clusters often overlap between patients. However, the expression patterns of individual genes in the clustered cell types differ greatly from patient to patient. The latter may be partly explained by the underrepresentation of cells that are less viable or lost during digestion procedures.

Single-cell analysis of gene expression is labor-intensive, time-consuming and expensive, disadvantages that make it difficult to obtain such large amounts of data to allow associative studies with risk factors, drug use, etc. It will take some time before the large numbers of patients required to stratify plaques and patient groups based on single-cell seq data that robustly associate with an acute cardiovascular event. The increase in the diversity of cell types found and the lack of knowledge of the cell-specific biological function in plaque destabilization

require large numbers of observations to contribute to the recalibration of the description of high-risk plaques. The plaque single-cell sequencing efforts does also facilitate the application of deconvolution methods that allows the dissection of cellular components found in whole plaque transcriptomes.[66]

The growth of single-cell transcriptomic studies will provide an efficient solution to identify the heterogeneous subpopulations and establish the spatiotemporal dynamic model of vascular biology and pathological cell composition.[67]

A PLETHORA OF MECHANISMS OF PROGRESSION OF ATHEROSCLEROTIC DISEASE: WHAT ARE THE IMPLICATIONS FOR THE INTERPRETATIONS OF CLINICAL AND EXPERIMENTAL RESEARCH?

Human atherosclerosis develops over decades, and progression and rupture are followed by healing and stabilization,[68,69] all of which complicates studies on the mechanisms underlying plaque destabilization in humans.

Genetically modified mice are the most widely applied models to study mechanisms of atherosclerotic disease. A large number of treatments have been shown over the past decades to be effective in mice but never reached a clinical stage because of lack of successful translation to human disease. Many genes that have significant effect on the cardiovascular phenotype in atherosclerotic mice fail to pass a test involving human genetic risk loci.[70]

Multiple triggers and mechanisms can underlie the (also diverse) pathological substrates of myocardial infarction and stroke but in experimental research we still follow the oversimplified concept of the gold standard for lesion destabilization: the lipid rich, smooth muscle cell poor, and inflammatory plaque with a thin fibrous cap. It has already been demonstrated in mouse models and humans that differential changes in shear stress can result in lesion growth with different plaque morphologies.[71,72] The same local hemodynamic determinants can be observed in the vascular system of human species that together with genetic diversity and risk factors can influence lesion phenotype. Unlike mouse models of disease, the human species is characterized by genetic diversity, acquired risk factors, and environmental triggers that together influence lesion phenotype. In preclinical research, the genetic background and environmental factors are intentionally harmonized, and it can be questioned what mouse strain reflects the human disease at best? As stated in a recent review paper, "we must and should continue to mine studies on mice for the incredibly valuable mechanistic insight they provide. Yet, we as a community could consider more carefully some of the barriers to glib extrapolation of the results of experiments in mice to human disease".[73]

The genetic background of the strain of wild-type mice being used should be taken into account, as this can significantly affect the development of atherosclerosis.[74] The diverse processes promoting atherosclerosis in humans demand for different mouse models. A roadmap has been proposed that should facilitate current and future researchers to choose an adequate mouse model for their studies.[75] A nice example of the way forward was the study in 100 inbred mouse strains that provided a comprehensive systems genetic analysis of traits relevant to atherosclerosis.[76] This study showed that many of the factors associated with atherosclerosis in human populations were replicated in mice strains and provided a rich resource for studies of the complex genetic and metabolic interactions that underlie the disease. The study in the 100 inbred mouse strains also showed significant differences in atherosclerotic lesion development between male and female mice in most identical strains.[76] It is, therefore, important to use both male and female mice in studies that are designed to translate human (transcriptome based) strata of atherosclerotic disease to experimental animal models.

The reported and ongoing bulk and single cell sequencing efforts of human plaques will facilitate the search for stratified matches of mice models with human atherosclerotic disease (Figure [B]). The integration of human and mouse single-cell datasets could help achieve a dual goal by providing information on the relevance in humans of mechanisms identified in experimental models and on the most suitable experimental model to study in vivo the shared mechanisms across species.[63] The first reports already emerge that correlate single cell transcriptomic characteristics of mice and men.[77]

A surrogate marker for plaque stabilization is considered a holy grail in clinical pharmaceutical studies that aim to decrease the risk of myocardial infarction. Numerous imaging modalities and circulating biomarkers have been tested for their associative and predictive value of a major adverse cardiovascular event because of progressive atherosclerosis. Atherosclerosis is a chronic inflammatory process and many studied biomarkers are based on this concept that inflammatory cells and proteolysis complicate disease. For example, C-reactive protein is an indicator of augmented inflammation and in the presence of a thin fibrous atheromatous plaque high levels (>10 mg/L) increase the chance of an adverse event.[78] Appreciating the diversity and complexity of atherosclerotic disease progression and complication can enforce the demand for multimarker panels that represent the diverse mechanisms of disease and enable determination of large numbers of proteins in small sample volumes.[79] But then, first we will have to face a major challenge: unravel what blood-derived genes or proteins reflect the differential gene networks that are currently being discovered and explored in plaques that, via different mechanisms, lead to an adverse event.

In summary, the widely used pathology-based classification of atherosclerosis is in need of revision. The enormous increase in insights into mechanisms of arteriosclerosis and the associated strong variation in cell-specific expression of genes and proteins indicate a plethora of natural histories of arteriosclerosis. The use of one unifying concept and definition of the vulnerable plaque narrows our field of view and can lead to interpretations of experimental research that only covers part of the patient load. The increase of -omics data in growing cohorts of patients may facilitate the fine tuning of the definitions of the vulnerable plaque. We foresee that these novel insights could lead to a phenotypic description of the plaque at risk for an adverse cardiovascular event that includes a description of hereditary genetic, plaque transcriptomic, and pathological criteria. However, this will have a downside: the disease will show more diverse and complex end stages in human plaques and the idea of one unifying concept of atherosclerotic disease progression will be abandoned. This will also have implications for mouse-to-human translation and will require human-to-mouse transcriptomic validation to map which animal model best matches which plaque type.

ARTICLE INFORMATION

Received September 28, 2021; accepted January 20, 2022.

Affiliations

Circulatory Health Laboratories (G.P., H.M.d.R.), Central Diagnostics Laboratories (G.P.), and Laboratory of Experimental Cardiology (H.M.d.R.), University Medical Center Utrecht, the Netherlands. NYU Cardiovascular Research Center (C.G.) and Department of Pathology (C.G.), New York University Grossman School of Medicine.

Sources of Funding

None.

Disclosures

None.

REFERENCES

1. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W Jr, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*. 1994;89:2462–2478. doi: 10.1161/01.cir.89.5.2462
2. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W Jr, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler Thromb*. 1994;14:840–856. doi: 10.1161/01.atv.14.5.840
3. Arbustini E, Dal Bello B, Morbini P, Burke AP, Bocciarelli M, Specchia G, Virmani R. Plaque erosion is a major substrate for coronary thrombosis in acute myocardial infarction. *Heart*. 1999;82:269–272. doi: 10.1136/hrt.82.3.269
4. Farb A, Burke AP, Tang AL, Liang TY, Mannan P, Smialek J, Virmani R. Coronary plaque erosion without rupture into a lipid core. A frequent cause of coronary thrombosis in sudden coronary death. *Circulation*. 1996;93:1354–1363. doi: 10.1161/01.cir.93.7.1354
5. Kolte D, Libby P, Jang IK. New insights into plaque erosion as a mechanism of acute coronary syndromes. *JAMA*. 2021;325:1043–1044. doi: 10.1001/jama.2021.0069
6. Libby P, Pasterkamp G, Crea F, Jang IK. Reassessing the mechanisms of acute coronary syndromes. *Circ Res*. 2019;124:150–160. doi: 10.1161/CIRCRESAHA.118.311098
7. Gregory F. Role of mechanical stress and neutrophils in the pathogenesis of plaque erosion. *Atherosclerosis*. 2021;318:60–69. doi: 10.1016/j.atherosclerosis.2020.11.002
8. Quillard T, Araújo HA, Franck G, Shvartz E, Sukhova G, Libby P. TLR2 and neutrophils potentiate endothelial stress, apoptosis and detachment: implications for superficial erosion. *Eur Heart J*. 2015;36:1394–1404. doi: 10.1093/eurheartj/ehv044
9. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol*. 2000;20:1262–1275. doi: 10.1161/01.atv.20.5.1262
10. Pasterkamp G, den Ruijter HM, Libby P. Temporal shifts in clinical presentation and underlying mechanisms of atherosclerotic disease. *Nat Rev Cardiol*. 2017;14:21–29. doi: 10.1038/nrcardio.2016.166
11. van Lammeren GW, den Ruijter HM, Vrijenhoek JE, van der Laan SW, Velema E, de Vries JP, de Kleijn DP, Vink A, de Borst GJ, Moll FL, et al. Time-dependent changes in atherosclerotic plaque composition in patients undergoing carotid surgery. *Circulation*. 2014;129:2269–2276. doi: 10.1161/CIRCULATIONAHA.113.007603
12. Stone GW, Maehara A, Lansky AJ, de Bruyne B, Cristea E, Mintz GS, Mehran R, McPherson J, Farhat N, Marso SP, et al; PROSPECT Investigators. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med*. 2011;364:226–235. doi: 10.1056/NEJMoa1002358
13. Zieske AW, Malcom GT, Strong JP. Natural history and risk factors of atherosclerosis in children and youth: the PDAY study. *Pediatr Pathol Mol Med*. 2002;21:213–237. doi: 10.1080/15227950252852104
14. Pasterkamp G, Schoneveld AH, van der Wal AC, Haudenschild CC, Clarijs RJ, Becker AE, Hillen B, Borst C. Relation of arterial geometry to luminal narrowing and histologic markers for plaque vulnerability: the remodeling paradox. *J Am Coll Cardiol*. 1998;32:655–662. doi: 10.1016/s0735-1097(98)00304-0
15. Smits PC, Pasterkamp G, Quarles van Ufford MA, Eefting FD, Stella PR, de Jaegere PP, Borst C. Coronary artery disease: arterial remodelling and clinical presentation. *Heart*. 1999;82:461–464. doi: 10.1136/hrt.82.4.461
16. Pasterkamp G, Schoneveld AH, van Wolferen W, Hillen B, Clarijs RJ, Haudenschild CC, Borst C. The impact of atherosclerotic arterial remodeling on percentage of luminal stenosis varies widely within the arterial system. A postmortem study. *Arterioscler Thromb Vasc Biol*. 1997;17:3057–3063. doi: 10.1161/01.atv.17.11.3057
17. Andrews J, Puri R, Kataoka Y, Nicholls SJ, Psaltis PJ. Therapeutic modulation of the natural history of coronary atherosclerosis: lessons learned from serial imaging studies. *Cardiovasc Diagn Ther*. 2016;6:282–303. doi: 10.21037/cdt.2015.10.02
18. Antoniadis AP, Stone PH. Evolving understanding of the heterogeneous natural history of individual coronary artery plaques and the role of local endothelial shear stress. *Curr Opin Cardiol*. 2017;32:748–754. doi: 10.1097/HCO.0000000000000459
19. Brown AJ, Teng Z, Evans PC, Gillard JH, Samady H, Bennett MR. Role of biomechanical forces in the natural history of coronary atherosclerosis. *Nat Rev Cardiol*. 2016;13:210–220. doi: 10.1038/nrcardio.2015.203
20. Papadopoulou SL, Neefjes LA, Garcia-Garcia HM, Flu WJ, Rossi A, Dharampal AS, Kitslaar PH, Mollet NR, Veldhof S, Nieman K, et al. Natural history of coronary atherosclerosis by multislice computed tomography. *JACC Cardiovasc Imaging*. 2012;5(Suppl 3):S28–S37. doi: 10.1016/j.jcmg.2012.01.009
21. White SJ, Newby AC, Johnson TW. Endothelial erosion of plaques as a substrate for coronary thrombosis. *Thromb Haemost*. 2016;115:509–519. doi: 10.1160/TH15-09-0765
22. Pedrigi RM, Mehta VV, Bovens SM, Mohri Z, Poulsen CB, Gsell W, Tremoleda JL, Towhidi L, de Silva R, Petretto E, et al. Influence of shear stress magnitude and direction on atherosclerotic plaque composition. *R Soc Open Sci*. 2016;3:160588. doi: 10.1098/rsos.160588
23. Lin JD, Nishi H, Poles J, Niu X, McCauley C, Rahman K, Brown EJ, Yeung ST, Vozhilla N, Weinstock A, et al. Single-cell analysis of fate-mapped macrophages reveals heterogeneity, including stem-like properties, during atherosclerosis progression and regression. *JCI Insight*. 2019;4:e124574. doi: 10.1172/jci.insight.124574

24. Björkegren JLM, Kovacic JC, Dudley JT, Schadt EE. Genome-wide significant loci: how important are they? Systems genetics to understand heritability of coronary artery disease and other common complex disorders. *J Am Coll Cardiol*. 2015;65:830–845. doi: 10.1016/j.jacc.2014.12.033
25. Musunuru K, Kathiresan S. Genetics of common, complex coronary artery disease. *Cell*. 2019;177:132–145. doi: 10.1016/j.cell.2019.02.015
26. Won HH, Natarajan P, Dobbyn A, Jordan DM, Roussos P, Lage K, Raychaudhuri S, Stahl E, Do R. Disproportionate contributions of select genomic compartments and cell types to genetic risk for coronary artery disease. *PLoS Genet*. 2015;11:e1005622. doi: 10.1371/journal.pgen.1005622
27. Erdmann J, Kessler T, Munoz Venegas L, Schunkert H. A decade of genome-wide association studies for coronary artery disease: the challenges ahead. *Cardiovasc Res*. 2018;114:1241–1257. doi: 10.1093/cvr/cvy084
28. Jaiswal S, Natarajan P, Silver AJ, Gibson CJ, Bick AG, Shvartz E, McConkey M, Gupta N, Gabriel S, Ardissino D, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med*. 2017;377:111–121. doi: 10.1056/NEJMoa1701719
29. Aragam KG. Discovery and systematic characterization of risk variants and genes for coronary artery disease in over a million participants. *MedRXiv*. 2021; doi: 10.1101/2021.05.24.21257377
30. Shankman LS, Gomez D, Cherepanova OA, Salmon M, Alencar GF, Haskins RM, Swiatlowska P, Newman AA, Greene ES, Straub AC, et al. KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis. *Nat Med*. 2015;21:628–637. doi: 10.1038/nm.3866
31. Alencar GF, Owsiany KM, Karnewar S, Sukhavasi K, Mocci G, Nguyen AT, Williams CM, Shamsuzzaman S, Mokry M, Henderson CA, et al. Stem cell pluripotency genes Klf4 and Oct4 regulate complex SMC phenotypic changes critical in late-stage atherosclerotic lesion pathogenesis. *Circulation*. 2020;142:2045–2059. doi: 10.1161/CIRCULATIONAHA.120.046672
32. Wang Y, Nanda V, Direnzo D, Ye J, Xiao S, Kojima Y, Howe KL, Jarr KU, Flores AM, Tsantilas P, et al. Clonally expanding smooth muscle cells promote atherosclerosis by escaping efferocytosis and activating the complement cascade. *Proc Natl Acad Sci USA*. 2020;117:15818–15826. doi: 10.1073/pnas.2006348117
33. Siemelink MA, van der Laan SW, Haitjema S, van Koeverden ID, Schaap J, Wesseling M, de Jager SCA, Mokry M, van Iterson M, Dekkers KF, et al. Smoking is associated to DNA methylation in atherosclerotic carotid lesions. *Circ Genom Precis Med*. 2018;11:e002030. doi: 10.1161/CIRCGEN.117.002030
34. Saleheen D, Zhao W, Young R, Nelson CP, Ho W, Ferguson JF, Rasheed A, Ou K, Nurnberg ST, Bauer RC, et al. Loss of cardioprotective effects at the ADAMTS7 locus as a result of gene-smoking interactions. *Circulation*. 2017;135:2336–2353. doi: 10.1161/CIRCULATIONAHA.116.022069
35. Libby P. The changing landscape of atherosclerosis. *Nature*. 2021;592:524–533. doi: 10.1038/s41586-021-03392-8
36. Crisby M, Nordin-Fredriksson G, Shah PK, Yano J, Zhu J, Nilsson J. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. *Circulation*. 2001;103:926–933. doi: 10.1161/01.cir.103.7.926
37. Frodermann V, Rohde D, Courties G, Severe N, Schloss MJ, Amatullah H, McAlpine CS, Cremer S, Hoyer FF, Ji F, et al. Exercise reduces inflammatory cell production and cardiovascular inflammation via instruction of hematopoietic progenitor cells. *Nat Med*. 2019;25:1761–1771. doi: 10.1038/s41591-019-0633-x
38. Hinterdobler J, Schott S, Jin H, Meesmann A, Steinsiek AL, Zimmermann AS, Wobst J, Müller P, Mauersberger C, Vilne B, et al. Acute mental stress drives vascular inflammation and promotes plaque destabilization in mouse atherosclerosis. *Eur Heart J*. 2021;42:4077–4088. doi: 10.1093/eurheartj/ehab371
39. Barrett TJ, Corr EM, van Solingen C, Schlamp F, Brown EJ, Koelwyn GJ, Lee AH, Shanley LC, Spruill TM, Bozal F, et al. Chronic stress primes innate immune responses in mice and humans. *Cell Rep*. 2021;36:109595. doi: 10.1016/j.celrep.2021.109595
40. Giannarelli C, Rodriguez DT, Zafar MU, Christoffel D, Vialou V, Peña C, Badimon A, Hodes GF, Mury P, Rabkin J, et al. Susceptibility to chronic social stress increases plaque progression, vulnerability and platelet activation. *Thromb Haemost*. 2017;117:816–818. doi: 10.1160/TH16-10-0817
41. Hartman RJG, Owsiany K, Ma L, Koplev S, Hao K, Slenders L, Civelek M, Mokry M, Kovacic JC, Pasterkamp G, et al. Sex-stratified gene regulatory networks reveal female key driver genes of atherosclerosis involved in smooth muscle cell phenotype switching. *Circulation*. 2021;143:713–726. doi: 10.1161/CIRCULATIONAHA.120.051231
42. Vrijenhoek JE, Den Ruijter HM, De Borst GJ, de Kleijn DP, De Vries JP, Bots ML, Van de Weg SM, Vink A, Moll FL, Pasterkamp G. Sex is associated with the presence of atherosclerotic plaque hemorrhage and modifies the relation between plaque hemorrhage and cardiovascular outcome. *Stroke*. 2013;44:3318–3323. doi: 10.1161/STROKEAHA.113.002633
43. Burke AP, Farb A, Malcom GT, Liang Y, Smialek J, Virmani R. Effect of risk factors on the mechanism of acute thrombosis and sudden coronary death in women. *Circulation*. 1998;97:2110–2116. doi: 10.1161/01.cir.97.21.2110
44. AlSiraj Y, Chen X, Thatcher SE, Temel RE, Cai L, Blalock E, Katz W, Ali HM, Petriello M, Deng P, et al. XX sex chromosome complement promotes atherosclerosis in mice. *Nat Commun*. 2019;10:2631. doi: 10.1038/s41467-019-10462-z
45. Balaton BP, Brown CJ. Escape artists of the X chromosome. *Trends Genet*. 2016;32:348–359. doi: 10.1016/j.tig.2016.03.007
46. Nelson CP, Goel A, Butterworth AS, Kanoni S, Webb TR, Marouli E, Zeng L, Ntalla I, Lai FY, Hopewell JC, et al; EPIC-CVD Consortium; CARDIoGRAMplusC4D; UK Biobank CardioMetabolic Consortium CHD working group. Association analyses based on false discovery rate implicate new loci for coronary artery disease. *Nat Genet*. 2017;49:1385–1391. doi: 10.1038/ng.3913
47. Borén J, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ, Daemen MJ, Demer LL, Hegele RA, Nicholls SJ, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J*. 2020;41:2313–2330. doi: 10.1093/eurheartj/ehz962
48. Mäkinen VP, Civelek M, Meng Q, Zhang B, Zhu J, Levian C, Huan T, Segrè AV, Ghosh S, Vivar J, et al; Coronary ARtery DIsease Genome-Wide Replication And Meta-Analysis (CARDIoGRAM) Consortium. Integrative genomics reveals novel molecular pathways and gene networks for coronary artery disease. *PLoS Genet*. 2014;10:e1004502. doi: 10.1371/journal.pgen.1004502
49. Mauersberger C, Schunkert H, Sager HB. Inflammation-related risk loci in genome-wide association studies of coronary artery disease. *Cells*. 2021;10:440. doi: 10.3390/cells10020440
50. Christiansen MK, Nissen L, Winther S, Møller PL, Frost L, Johansen JK, Jensen HK, Guðbjartsson D, Holm H, Stefánsson K, et al. Genetic risk of coronary artery disease, features of atherosclerosis, and coronary plaque burden. *J Am Heart Assoc*. 2020;9:e014795. doi: 10.1161/JAHA.119.014795
51. Tang W, Morrison A, Wasserman BA, Folsom AR, Sun W, Campbell S, Kao WH, Boerwinkle E. Association of SERPINA9 gene variants with carotid artery atherosclerosis: the Atherosclerosis Risk in Communities (ARIC) Carotid MRI Study. *Int J Mol Epidemiol Genet*. 2013;4:258–267.
52. Traylor M, Farrall M, Holliday EG, Sudlow C, Hopewell JC, Cheng YC, Fornage M, Ikram MA, Malik R, Bevan S, et al; Australian Stroke Genetics Collaborative, Wellcome Trust Case Control Consortium 2 (WTCCC2); International Stroke Genetics Consortium. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies. *Lancet Neurol*. 2012;11:951–962. doi: 10.1016/S1474-4422(12)70234-X
53. Azghandi S, Prell C, van der Laan SW, Schneider M, Malik R, Berer K, Gerdes N, Pasterkamp G, Weber C, Haffner C, et al. Deficiency of the stroke relevant HDAC9 gene attenuates atherosclerosis in accord with allele-specific effects at 7p21.1. *Stroke*. 2015;46:197–202. doi: 10.1161/STROKEAHA.114.007213
54. O'Donnell CJ, Kavousi M, Smith AV, Kardia SL, Feitosa MF, Hwang SJ, Sun YV, Province MA, Aspelund T, Dehghan A, et al; CARDIoGRAM Consortium. Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction. *Circulation*. 2011;124:2855–2864. doi: 10.1161/CIRCULATIONAHA.110.974899
55. Sakamoto A, Virmani R, Finn AV. Coronary artery calcification: recent developments in our understanding of its pathologic and clinical significance. *Curr Opin Cardiol*. 2018;33:645–652. doi: 10.1097/HCO.0000000000000558
56. van der Laan SW, Siemelink MA, Haitjema S, Foroughi Asl H, Perisic L, Mokry M, van Setten J, Malik R, Dichgans M, Worrall BB, et al; METASTROKE Collaboration of the International Stroke Genetics Consortium. Genetic susceptibility loci for cardiovascular disease and their impact on atherosclerotic plaques. *Circ Genom Precis Med*. 2018;11:e002115. doi: 10.1161/CIRCGEN.118.002115
57. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, Zhang Y. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science*. 2011;333:1300–1303. doi: 10.1126/science.1210597

58. Fuster JJ, MacLauchlan S, Zuriaga MA, Polackal MN, Ostriker AC, Chakraborty R, Wu CL, Sano S, Muralidharan S, Rius C, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science*. 2017;355:842–847. doi: 10.1126/science.aag1381
59. Abplanalp WT, Cremer S, John D, Hoffmann J, Schuhmacher B, Merten M, Rieger MA, Vasa-Nicotera M, Zeiher AM, Dimmeler S. Clonal hematopoiesis-driver DNMT3A mutations alter immune cells in heart failure. *Circ Res*. 2021;128:216–228. doi: 10.1161/CIRCRESAHA.120.317104
60. Misra A, Feng Z, Chandran RR, Kabir I, Rotllan N, Aryal B, Sheikh AQ, Ding L, Qin L, Fernández-Hernando C, et al. Integrin beta3 regulates clonality and fate of smooth muscle-derived atherosclerotic plaque cells. *Nat Commun*. 2018;9:2073. doi: 10.1038/s41467-018-04447-7
61. Buckler AJ, Karlöf E, Lengquist M, Gasser TC, Maegdefessel L, Perisic Matic L, Hedin U. Virtual transcriptomics: noninvasive phenotyping of atherosclerosis by decoding plaque biology from computed tomography angiography imaging. *Arterioscler Thromb Vasc Biol*. 2021;41:1738–1750. doi: 10.1161/ATVBAHA.121.315969
62. Wainberg M, Sinnott-Armstrong N, Mancuso N, Barbeira AN, Knowles DA, Golan D, Ermel R, Ruusalepp A, Quertermous T, Hao K, et al. Opportunities and challenges for transcriptome-wide association studies. *Nat Genet*. 2019;51:592–599. doi: 10.1038/s41588-019-0385-z
63. Fernandez DM, Giannarelli C. Immune cell profiling in atherosclerosis: role in research and precision medicine. *Nat Rev Cardiol*. 2022;19:43–58. doi: 10.1038/s41569-021-00589-2
64. Depuydt MAC, Prange KHM, Slenders L, Örd T, Elbersen D, Boltjes A, de Jager SCA, Asselbergs FW, de Borst GJ, Aavik E, et al. Microanatomy of the human atherosclerotic plaque by single-cell transcriptomics. *Circ Res*. 2020;127:1437–1455. doi: 10.1161/CIRCRESAHA.120.316770
65. Fernandez DM, Rahman AH, Fernandez NF, Chudnovskiy A, Amir ED, Amadori L, Khan NS, Wong CK, Shamailova R, Hill CA, et al. Single-cell immune landscape of human atherosclerotic plaques. *Nat Med*. 2019;25:1576–1588. doi: 10.1038/s41591-019-0590-4
66. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015;12:453–457. doi: 10.1038/nmeth.3337
67. Fu M, Song J. Single-cell transcriptomics reveals the cellular heterogeneity of cardiovascular diseases. *Front Cardiovasc Med*. 2021;8:643519. doi: 10.3389/fcvm.2021.643519
68. Peeters W, Hellings WE, de Kleijn DP, de Vries JP, Moll FL, Vink A, Pasterkamp G. Carotid atherosclerotic plaques stabilize after stroke: insights into the natural process of atherosclerotic plaque stabilization. *Arterioscler Thromb Vasc Biol*. 2009;29:128–133. doi: 10.1161/ATVBAHA.108.173658
69. Zhang M, Zhou SH, Li XP, Shen XQ, Fang ZF. A novel hypothesis of atherosclerosis: EPCs-mediated repair-to-injury. *Med Hypotheses*. 2008;70:838–841. doi: 10.1016/j.mehy.2007.06.041
70. Pasterkamp G, van der Laan SW, Haitjema S, Foroughi Asl H, Siemelink MA, Bezemer T, van Setten J, Dichgans M, Malik R, Worrall BB, et al. Human validation of genes associated with a murine atherosclerotic phenotype. *Arterioscler Thromb Vasc Biol*. 2016;36:1240–1246. doi: 10.1161/ATVBAHA.115.306958
71. Cheng C, Tempel D, van Haperen R, van der Baan A, Grosveld F, Daemen MJ, Krams R, de Crom R. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. *Circulation*. 2006;113:2744–2753. doi: 10.1161/CIRCULATIONAHA.105.590018
72. Bourantas CV, Zanchin T, Torii R, Serruys PW, Karagiannis A, Ramasamy A, Safi H, Coskun AU, Koning G, Onuma Y, et al. Shear stress estimated by quantitative coronary angiography predicts plaques prone to progress and cause events. *JACC Cardiovasc Imaging*. 2020;13:2206–2219. doi: 10.1016/j.jcmg.2020.02.028
73. Libby P. Murine "model" monotheism: an iconoclast at the altar of mouse. *Circ Res*. 2015;117:921–925. doi: 10.1161/CIRCRESAHA.115.307523
74. Paigen B, Ishida BY, Verstuyft J, Winters RB, Albee D. Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. *Arteriosclerosis*. 1990;10:316–323. doi: 10.1161/01.atv.10.2.316
75. Oppi S, Lüscher TF, Stein S. Mouse models for atherosclerosis research-which is my line? *Front Cardiovasc Med*. 2019;6:46. doi: 10.3389/fcvm.2019.00046
76. Bennett BJ, Davis RC, Civelek M, Orozco L, Wu J, Qi H, Pan C, Packard RR, Eskin E, Yan M, et al. Genetic architecture of atherosclerosis in mice: a systems genetics analysis of common inbred strains. *PLoS Genet*. 2015;11:e1005711. doi: 10.1371/journal.pgen.1005711
77. Zernecke A, Erhard F, Weinberger T, Schulz C, Ley C, Saliba AE, Cochain C. Integrated scRNA-seq analysis identifies conserved transcriptomic features of mononuclear phagocytes in mouse and human atherosclerosis. *BioXriv*. 2020; doi: 10.1101/2020.12.09.417535
78. Kelly CR, Weisz G, Maehara A, Mintz GS, Mehran R, Lansky AJ, Parise H, de Bruyne B, Serruys PW, Stone GW. Relation of C-reactive protein levels to instability of untreated vulnerable coronary plaques (from the PROSPECT Study). *Am J Cardiol*. 2014;114:376–383. doi: 10.1016/j.amjcard.2014.04.048
79. Lind L, Gigante B, Borné Y, Feldreich T, Leppert J, Hedberg P, Östgren CJ, Nyström FH, Sundström J, Ärnlöv J, et al. Plasma protein profile of carotid artery atherosclerosis and atherosclerotic outcomes: meta-analyses and mendelian randomization analyses. *Arterioscler Thromb Vasc Biol*. 2021;41:1777–1788. doi: 10.1161/ATVBAHA.120.315597