Atherosclerosis is a systemic disease of the arterial vessel wall. Although the mortality due to cardiovascular events is decreasing, the prevalence of atherosclerosis and its comorbidities, and the consequent heath care costs are expected to rise sharply in the near future.1

Because the precise cause and pathogenesis of this complex, multifactorial disease are still not fully understood, the clinical assessment of cardiovascular risk has been traditionally based on population risk factors (RFs).2 However, this approach still largely fails to capture the individual’s cardiovascular risk: most cardiovascular events occur in patients with 1 or few traditional RFs, whereas individuals classified as high risk may never experience clinical events.3

The past 10 years have seen a significant paradigm shift in our understanding of the mechanisms of atherogenesis. From being considered the mere result of passive lipid accumulation in the vessel wall, atherosclerosis is now classified as an active inflammatory condition.4–5 The presence of abundant, active inflammatory cells is a known hallmark of high risk, vulnerable atherosclerotic plaques.4,5 Many studies have identified several systemic proinflammatory conditions (such as lupus,6 rheumatoid arthritis,7–9 and primary cardiovascular events themselves10) as emerging, independent RFs for atherosclerosis. New evidence suggests that atherosclerosis arises from the complex influence of genetic, environmental, and behavioral variables on systemic and local inflammation through a complex network of molecules, cells, and organs.

Thanks to the recent technological advancements of high-throughput ‘-omics’, a plethora of the genes, proteins, and cells involved in the atherosclerotic cascade have already been identified. However, many steps still need to be taken to fully exploit this information, and improve patients’ risk stratification and antiatherosclerotic therapies. The mutual relationship between genetic and molecular key drivers, and their interplay in peripheral blood, atherosclerotic plaques and other organs still need to be established. Furthermore, quantitative methods to noninvasively measure these markers in the vessel wall and other tissues need to be developed and validated before they can be routinely used in the clinical practice.

In this review, we highlight novel work in high-throughput ‘-omics’, systems biology and noninvasive quantitative imaging of atherosclerosis, with specific emphasis on articles recently featured in ATVB. New advancements in these disciplines are discussed separately, as well as in their complementary applications, to showcase how these fields may be successfully integrated to improve cardiovascular risk prediction and patients’ stratification in the future clinical practice (Figure).

**Systems Biology and High-Throughput ‘-omics’ of Atherosclerosis**

Systems biology can be broadly defined as the combination of experimental and computational research used to understand complex biological systems.11 It involves the integration of data derived from high-throughput ‘-omics’ with computational/statistical tools to build comprehensive networks and predictive physiological models12 (Figure).

In recent years, high-throughput ‘-omics’ have been intensely applied to the study of atherosclerosis,13 with the aim to deepen our knowledge of this disease and refine our tools for cardiovascular risk assessment. For example, recent data14 from the Erasmus Rucphen Family and Rotterdam Study have shown that common genetic variants for total cholesterol and low-density lipoprotein cholesterol are, in combination, significantly associated with subclinical and clinical outcomes of atherosclerosis. Other investigations have suggested the association between soluble interleukin-2 receptor subunit α (regulating lymphocytes activation) and cardiovascular disease (CVD), and also uncovered the genetic determinants of its levels.15 These results substantiate our existing knowledge on the impact of long-life, cumulative exposure to modifiable and nonmodifiable RFs on cardiovascular risk.16,17 Although previous genome-wide association studies (GWAS)18 have reported only marginal improvements19,20 in risk stratification compared with the Framingham Risk Score,3,21,22 more recent investigations23,24 found that genetic risk scores from validated GWAS significantly improved prediction of cardiovascular events over traditional RFs, from 4 to 5%23 up to 12%.24 Extensive bioinformatics analyses25 of loci known to be associated with coronary artery disease (CAD) from GWAS, suggest that sequence variations mainly occur in noncoding regions of the genome and promote CAD risk by either affecting gene expression or by leading to amino acid changes. By extending the list of candidate genes likely linked to CAD, these studies suggest that bioinformatics analysis of GWAS may be beneficial in the context of atherosclerosis as well as other diseases.

By quantifying the expression levels of protein-coding genes, transcriptomic studies26–29 identified several promising biomarkers for CVD26 and vulnerable plaques.30 The Systems Approach to Biomarker Research (SABRe) study (launched as part of the Framingham Heart Study) recently found that 35 genes and 3 gene clusters (metagenes) were differentially

**Recent Highlights of ATVB**

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expressed in cases with coronary heart disease (CHD) versus controls, whereas GUK1 38 and other genes were differentially spliced in the 2 populations. More recently, transcriptomics has been extended to study noncoding, regulatory gene transcripts, such as microRNAs. Several types of circulating microRNAs have recently been implicated in CVD.32–45 Using transcriptomics, the prospective Bruneck study found that 3 miRNAs (miR-126, miR-223, and miR-197) originating from platelets improved patients’ risk classification for CHD compared with the Framingham Risk Score.46 Transcriptomics analyses have also directly implicated several miRNAs in the development of vascular inflammation as mediated by wall shear stress. Numerous flow-sensitive miRNAs (miR-10a, miR-633, miR-21, and miR-92a) have already been identified.48

Proteomic studies49–56 collectively identified >150 potential single biomarkers of CVD.49,57,58 More recent studies have seen a shift from the analysis of single protein markers to simultaneously quantifying the combination of the levels of several proteins in multimarker panel analyses. A recent study in 135 myocardial infarction cases and matched controls59 found that both single and multimarker analysis of plasma proteins were associated with incidence of myocardial infarction, with multimarkers analysis providing higher discrimination. Similar results were found in a prospective study, where multimarkers analysis in 336 patients was found to be predictive of CVD (P<0.0001), with moderate improvement over traditional RFs (C-statistic of 0.69 versus 0.73).59

Although these and previous studies offer invaluable insights on the genetic, molecular, and metabolic basis of atherosclerosis, they still do not provide a cohesive, integrated approach to the study of this disease. Recent approaches have tried to overcome this obstacle by applying a combination of ‘omics’. For example, a combination of GWAS and transcriptomics is being used to investigate the recently suggested association between the haplogroup I of the Y male chromosome and an increased risk of CAD.60 New studies at the interface between metabolomics (lipidomics61–63) and proteomics have shed light on the complexity of the human lipoproteome, and helped characterizing >90 forms of high-density lipoprotein particles associated with different lipoproteins, with a diverse range of antiatherosclerotic properties, beyond the known effects on cholesterol efflux. A combined approach67 using adipose tissue transcriptomics, high-density lipoprotein lipidomics, and genotyping found a shift toward inflammatory high-density lipoprotein particle types in individuals with low high-density lipoprotein cholesterol, mirrored by an increase in inflammatory markers in adipose tissue and in the peripheral blood.

By taking these approaches a step forward, Shang et al68,69 used gene subnetworks profiling of the Stockholm Atherosclerosis Gene Expression (STAGE) study to find a candidate gene strongly correlated to leukocyte migration, and assess its association with clinical manifestation of disease (coronary angiography and carotid intima-media thickness [CIMT] by ultrasound). This work offers an example of how a more integrated systems biology approach may be used to better understand the process of atherogenesis, from the genetic determinants to the phenotypic manifestations of systemic and vascular inflammation. As part of the SABRe study mentioned above, Huan et al70 also use an integrated systems biology approach to find differential gene coexpression modules in the blood of subjects with CHD and matched controls. By integrating these results with previous GWAS
and single-nucleotide polymorphisms, the authors are able to draw a causal relationship between the differential gene coexpression modules and CHD in this cohort. With the further integration of Bayesian networks and protein–protein interaction, networks they also identify key drivers, regulatory genes important for the differential gene coexpression modules stability and therefore potential targets for novel drugs. This network-driven, integrated approach not only identifies genes related to CHD but also strives to build a network structure that informs on the molecular interactions of genes associated with CHD risk.

Despite the enormous potential of a panomic/systems biology approach to atherosclerosis, several obstacles have to be overcome so that ’omics’ can be successfully used in future clinical practice. First, a causal relationship between biomarkers and disease mechanisms has to be solidly established. Dissecting causal effects from confounders can prove challenging in cross-sectional studies, whereas prospective, causal studies with cardiovascular events as end points are costly and lengthy to perform. Furthermore, performing ’-omics’ analyses on direct tissue samples may not be always feasible, whereas peripheral blood analyses may only reflect transient changes in metabolites that do not necessarily inform on the overall disease activity.

Imaging

In recent years, medical imaging has made great strides in the evaluation of virtually every organ in the body, including atherosclerotic plaques. Modality-specific imaging traits (imaging phenotypes) emerge from the combination of tissues structure, physiology and function, and inform on organs physiology and pathology.71,72

Several imaging modalities have already found widespread use in the clinical practice to evaluate atherosclerotic burden. CIMT by surface ultrasound73,74 is one such technique, although its clinical usefulness to significantly improve risk prediction over traditional RFs has recently been questioned.75,76 Recently, CIMT was found to decrease in subjects consuming a Mediterranean diet supplemented with 30 g/d of mixed nuts, compared with a control, low-fat diet, thereby corroborating the results form the Primary Prevention of Cardiovascular Disease with a Mediterranean Diet (PREDIMED) trial.77 Other than CIMT, surface ultrasound can be used to measure other parameters related to plaque vulnerability, such as vascular strain or the extent of plaque microvasculature using nontargeted micro bubbles.78 Ultrasound with micro bubbles targeted to vascular cell adhesion molecule 1 and platelet glycoprotein Ibα was recently validated in genetically modified mice as being able to assess the anti-inflammatory properties of apocynin, before detectable changes in macrophages burden.79

More invasive procedures involving intravascular ultrasound or transesophageal ultrasound80 can also be performed. For example, transesophageal ultrasound was recently used in mongrel dogs to quantify changes in aortic area and elastic properties from velocity-vector imaging with aging.80 Another study used a combination of multivessel intravascular ultrasound and novel near-infrared spectroscopy81,82 to evaluate features of vulnerability in fibroatheromas of diabetic/hypercholesterolemic pigs.83 Longitudinally, intravascular ultrasound demonstrated a progressive increase in plaque and media areas, with the appearance of necrotic cores and regions of positive vascular remodeling. Compared with histological samples, near-infrared spectroscopy–positive lesions exhibited features of high-risk fibroatheromas, such as large plaque size, necrotic cores, thin fibrous cap, and abundant presence of inflammatory cells. A more recent study using intravascular ultrasound demonstrated a greater progression in patients with CAD classified as statin responders, compared with individuals who exhibited low-density lipoprotein cholesterol reductions of >15% from baseline.84

Coronary calcium score evaluated by noncontrast enhanced computed tomography (CT),85,86 is another noninvasive measure of overall atherosclerotic burden, which has been described to predict the risk of future clinical events.77,78 A recent follow-up study of the Multi-Ethnic Study of Atherosclerosis (MESA) trial found that abdominal aortic calcium and coronary calcium score were predictors of CHD and cardiovascular events independent of one another, with only abdominal aortic calcium being independently associated with cardiovascular mortality, and showing a stronger association than coronary calcium score with overall mortality.89 Recent studies80 suggest that this measure could be complemented by cardiac computed tomographic angiography51 with the use of a iodinated contrast agent to provide additional information on the degree and distribution of coronary plaque stenosis, vessel wall positive remodeling, and plaque composition (such as presence of low-attenuation plaques and spotty calcification), which have been identified as markers of vulnerability.92

Other modalities, such as magnetic resonance imaging (MRI) and positron emission tomography (PET), are actively being investigated in both the preclinical and the clinical research arenas for their potential translation into clinical practice. With its superior soft tissue contrast compared with CT, and the possibility to image large segments of the vasculature with high-spatial resolution, noncontrast–enhanced MRI has been extensively investigated as a method to characterize atherosclerotic plaques components, such as lipid core, fibrous cap, intraplaque hemorrhage, and presence of thrombi.93,94 A recent study95 performed on 1016 individuals from the Framingham Heart Study Offspring cohort investigated the prevalence and RF correlates for aortic plaque detected by MRI and CT. The study found that while aortic plaque by both imaging modalities is associated with smoking and increasing age, the association with other RFs differs between calcified plaques detected by CT and noncalcified lesions detected by MRI. The study postulates that the relative predictive value of aortic plaque detected by MRI and CT still needs to be investigated. Combined with the use of nonspecific gadolinium-based contrast agents, MRI has been also used to interrogate plaque physiology and quantify the extent of microvascular permeability95,96 another important hallmark of plaque vulnerability. Other physiological parameters, such as carotid arterial strain and distensibility calculated from MRI, have been shown to predict the future incidence of cerebral microbleeds in 2512 patients recruited as part of the prospective, population-based Age, Gene/Environment Susceptibility (AGES)-Reykjavik study,100 PET, combined with the anatomic information from

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CT or, more recently, MRI has been extensively validated to quantify vascular inflammation itself and its changes on therapeutic intervention, both in humans and in animals using the tracer $^{18}$F fluoroiodoxyglucose (FDG). Recently, $^{18}$F-FDG PET was used to demonstrate a decrease in vascular inflammation in Ldlr−/− atherosclerotic mice after treatment with melatonin peptides. Other PET tracers are now being investigated, such as sodium fluoride targeting microcalcification, or tracers targeted to specific molecules, such as $\alpha\beta3$, vascular cell adhesion molecule 1, 68Ga-Fucoidan for P-selectin (abundantly expressed in vulnerable, but not stable plaques), $^{64}$Cu-FBP8 for thrombus detection and fibrin quantification, and $^{84}$ Cu-DOTATATE to selectively quantify plaque macrophages via the somatostatin receptor subtype-2.

Among emerging imaging modalities, optical coherence tomography is gaining increasing interest because of its ability to provide high-resolution images of tissues microstructure. Recently, optical coherence tomography was first validated in atherosclerotic rabbits and then used in a prospective study in patients to evaluate vascular healing after the implantation of drug-eluting stents, where it was found to be able to discriminate between immature and mature (healed) neointimal tissue. Another study used optical coherence tomography to study the composition of coronary segments after stimulus with acetylcholine. The study found that the segments showing the presence of macrophages and microchannels (microvasculature), exhibited a more prominent change in the diameter of coronary arteries, indicating higher endothelial dysfunction. Recently, novel, in vivo multiphoton laser scanning microscopy was used to study plaque microvasculature and confirmed that plaque-associated vasa vasorum exhibit increased permeability, and increased leucocyte adhesion and extravasation.

**Imaging as a Tool for Systems Biology**

From the account above, it emerges that through the noninvasive characterization of tissues anatomy and physiology, medical imaging may be an ideal complement to ‘-omics’ technologies for a comprehensive systems biology approach to CVD. Several approaches are currently being explored to successfully integrate imaging in this framework.

Although confined to preclinical investigations, molecular imaging already reports on specific biological processes (optical imaging and fluorescence imaging), and can even directly quantify gene expression (bioluminescence) or cells (macrophages) development, migration and presence in tissues throughout the body. Among translatable modalities, molecular imaging with MRI and PET can also similarly be used for this purpose. Aside from the increasing number of MRI contrast agents being developed to target specific biomarkers, MRI with ultrasmall superparamagnetic iron oxide particles has been widely validated as a tool to detect plaque macrophages content in atherosclerotic plaques in both animals and patients. Recent studies in mice have shown the successful integration of proteomics, metabolomics, and quantitative and anatomic MRI to phenotype transgenic mice in regard to creatinine and phosphocreatinine cardiac metabolism. In addition to the quantification of plaque local inflammation, the use of $^{18}$F-FDG PET was recently extended to study the interplay between local and systemic inflammation and to substantiate the existence of a cardioprotective axis in humans (implicated from animal models in the high incidence of secondary cardiovascular events in patients with previous myocardial infarction). Similarly, another study has recently demonstrated increased vascular inflammation by $^{18}$F-FDG PET in patients with psoriasis, independent of cardiovascular RFs. These studies show an example of the integration of $^{18}$F-FDG PET in a systems physiology approach. Similarly, several clinical imaging modalities are currently being investigated to quantify noninvasively the extent (CT and MRI) and metabolic activity ($^{18}$F-FDG PET) of visceral and subcutaneous body fat, regarded as a potential marker and risk predictor of CVD.

Some studies have already focused on investigating the genetic and molecular correlates of imaging traits. A recent study identified the genetic variations influencing the effect of smoking on CIMT, thereby exemplifying how the study of gene–environment interactions may explain the interindividual variation in both cardiovascular events and surrogate measures of cardiovascular risk. The Genetic Loci and the Burden of Atherosclerotic Lesions (GLOBAL) study (NCT01738828) brings this concept to a different level by aiming to comprehensively integrate plaque phenotype by cardiovascular imaging, with a panomic approach including genomic, transcriptomic, proteomic, metabolomics, and lipodomic in a systems biology framework. The study plans to examine single-omic and multi-omic associations with each imaging phenotype evaluated (coronary calcium score and CT angiography) in training and validation data sets.

**Final Remarks**

In this report, we have reviewed the most recent advances in ‘-omics’/systems biology and noninvasive medical imaging applied to atherosclerosis and CVD, with specific focus on articles recently published in ATVB.

The combination of ‘-omics’ and systems biology is nowadays used more and more frequently to elucidate mechanisms of disease, and it is also being investigated as a complement to clinical data to improve patients risk stratification. Examples of these approaches are GWAS, the study of coding and noncoding RNAs using transcriptomics, as well as tissue and blood proteomics. More recently, sophisticated analyses aim to integrate this information in a comprehensive, systems biology approach to build a network structure of the molecular interactions in CVD.

Medical imaging is also making tremendous advancements in the diagnosis and characterization of CVD. Modalities such as ultrasound, CT, and MRI already allow for the accurate quantification of plaque burden and lesion characterization. Other approaches, such as PET and MRI with contrast report on relevant physiological parameters, that is, plaque permeability and inflammation, whereas molecular imaging techniques can shed light on specific molecular/cellular processes.

Although taken separately both ‘-omics’ and medical imaging can already tremendously contribute to our understanding of CVD and to our ability to stratify patients’ risk, their successful integration may bring additional, significant benefits.
Similarly to what is being recently proposed in oncology,123–129 the first step in integrating these 2 disciplines will be establishing the association130 between imaging phenotypes and specific genetic, molecular and cellular signatures in atherosclerotic plaques and other organs involved in atherogenesis. Once these correlations will be robustly established, the use of imaging phenotypes may be extended to function as predictors124,125 of plaques genetic and molecular makeup123–129 in both the preclinical and the clinical arenas. In this scenario, the integration of imaging and ‘omics’ in a systems biology framework may be better positioned to improve risk stratification and assessment of therapeutic response of atherosclerotic patients in the future clinical practice (Figure).

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