

Translational medicine

Novel methodologies for biomarker discovery in atherosclerosis

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Received 20 February 2015; revised 7 May 2015; accepted 18 May 2015; online publish-ahead-of-print 6 June 2015

Identification of subjects at increased risk for cardiovascular events plays a central role in the worldwide efforts to improve prevention, prediction, diagnosis, and prognosis of cardiovascular disease and to decrease the related costs. Despite their high predictive value on population level, traditional risk factors fail to fully predict individual risk. This position paper provides a summary of current vascular biomarkers other than the traditional risk factors with a special focus on the emerging – omics technologies. The definition of biomarkers and the identification and use of classical biomarkers are introduced, and we discuss the limitations of current biomarkers such as high sensitivity C-reactive protein (hsCRP) or N-terminal pro-brain natriuretic peptide (NT-proBNP). This is complemented by circulating plasma biomarkers, including high-density lipoprotein (HDL), and the conceptual shift from HDL cholesterol levels to HDL composition/function for cardiovascular risk assessment. Novel sources for plasma-derived markers include microparticles, microvesicles, and exosomes and their use for current omics-based analytics. Measurement of circulating micro-RNAs, short RNA sequences regulating gene expression, has attracted major interest in the search for novel biomarkers. Also, mass spectrometry and nuclear magnetic resonance spectroscopy have become key complementary technologies in the search for new biomarkers, such as proteomic searches or identification and quantification of small metabolites including lipids (metabolomics and lipidomics). In particular, pro-inflammatory lipid metabolites have gained much interest in the cardiovascular field. Our consensus statement concludes on leads and needs in biomarker research for the near future to improve individual cardiovascular risk prediction.

Keywords

Atherosclerosis • Clinical biomarker • Risk prediction • Systems biology • Mass spectrometry • HDL • Micro-RNA

Biomarkers: state of the art

Preventive cardiovascular risk assessment relies on established risk factors, including smoking, hypertension, dyslipidaemia, and diabetes; however, approximately half of the people developing coronary heart disease (CHD) have been classified as having low or

intermediate risk based on current risk algorithms.^{1–4} Although biomarkers seem to be a rather novel research field the term ‘biomarker’ was already introduced in 1980.⁵ In fact, ‘biomarker’ in the broad sense, being ‘a characteristic that is objectively measured and evaluated as an indication of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention’,⁶

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covers also traditional risk factors that are used for the above-mentioned risk algorithms. Hence, 'biomarkers' will be used in the following as markers measured in biological specimens, such as cells or serum.

The clinical value of serological biomarkers for the diagnosis and prediction of clinical manifestations of atherosclerotic disease has been assessed in numerous clinical studies. Meta-analyses and reviews are widely available in international literature summarizing diagnostic and predictive properties of both, cardiac-specific markers (e.g. produced/released by the cardiac muscle and hence likely reflecting coronary atherosclerosis) and non-cardiac-specific markers (systemic markers such as lipids, creatinine, glycaemia and glycated haemoglobin, essentially reflecting metabolic risk factors). In spite of the large number of biomarkers that have been tested, the European Society of Cardiology (ESC) guidelines only recommend the use of troponin for the diagnosis and prognosis in the management of acute coronary syndromes (ACS),^{7,8} along with the assessment of lipid profile, creatinine, and glycaemia. The development of high-sensitivity (hs) assays for troponin I and T has improved the diagnostic sensitivity for acute myocardial infarction (MI), decreased the time to diagnosis and led to quicker rule-out of myocardial ischaemia. In addition, elevated hs-troponin has been associated with adverse outcomes in patients with stable CHD and in the general population. However, troponin does not have sufficient independent prognostic value to advise systematic measurements in patients with stable CHD.^{9–12} In fact, in this condition, the current guidelines do not recommend testing any biomarkers beyond lipids, creatinine, glycaemia and glycated haemoglobin, adding the organ specific BNP or NT-proBNP only if heart failure is suspected.⁹ Specifically, the use of hsCRP or any other novel biomarker is not recommended.^{7–9} However, the number of routinely measured biomarkers that can be used to predict MI or presence of clinically silent atherosclerotic disease is rather limited and systemic markers may have some limitations, because although atherosclerosis can be considered a systemic disease, in some cases it may be progressing at different rates in different arterial beds or individuals depending on variables such as age, ethnicity,^{13,14} etc. Thus, tentative new biomarkers need to be robust enough to be able to indicate progression of disease even if it happens in a relatively small part of the arterial tree.

Guidelines for biomarker use to assess presence of atherosclerotic disease in the absence of an acute event are scarce. The reasons for this may be found in our limited scientific understanding of biomarkers so far or the described limited added value of biomarkers on top of the predictive value of traditional risk factors for prediction of adverse events. This is in sharp contrast with biomarker guidelines for the diagnosis of heart failure where NTpro-BNP is an accepted standard.¹⁵

For the prediction of incident cardiovascular events, markers with strong potential are mainly associated with lipids and lipoproteins. For recurrent cardiovascular events, markers are mostly associated with ischaemia.¹⁵ There is an ongoing debate which biomarkers should be applied in patients with low, intermediate, and high 5- to 10-year risk for MI. For example, the National Academy of Clinical Biochemistry guidelines from 2009 discussed the use of the most often used commercially available biomarkers such as hsCRP and fibrinogen.¹⁶ Their conclusion was that there is no

need for further biomarker screening in low-risk patients and in case of intermediate risk much is left to the discretion of the medical practitioner.

Biomarkers that reflect the inflammatory state are not recommended for routine use in non-high-risk subjects. Recommendations for hsCRP screening slightly differ between US and EU standards. While both American Heart Association (AHA) and ESC recommend hsCRP measurements in patients with moderate or unusual CHD risk profile, asymptomatic high-risk patients, and patients with hypertension categorized as intermediate risk by Framingham criteria to assess 10-year CHD risk,^{4,17,18} the AHA also recommends screening of asymptomatic low-risk patients.¹⁷

The clinical value and appreciation of biomarkers may be hampered by many determinants such as intra-individual variability, lack of tissue specificity, inter-lab variability, analytical sensitivity and accuracy,¹⁹ age, weight, renal function, gender differences, or differences among ethnicities. Also, progression of atherosclerosis may not be homogeneous in different areas, and different degrees of peripheral artery disease have been described for similar degrees of CHD.¹⁴ This fact could limit theoretically the information given by a cardiac biomarker on the progression of atherosclerosis in other areas and vice versa. Finally, unmonitored and unaccounted differences in pre-analytical sample handling (e.g. time from collection to storage, isolation protocol, and room temperature) and marker stability may be additional limitations. This strongly depends on storage conditions; number of freeze–thaw cycles, etc., and is molecule specific and thus not generalizable.²⁰

Moreover, biomarkers with causal involvement (*Table 1*) are usually regarded more valuable for risk stratification as they may also be used in testing drug efficacy or applied as companion diagnostic. However, Mendelian randomization studies have shown that some of the most widely applied biomarkers for cardiovascular disease are not causally related with disease progression.^{21,22}

Thus, overall there is no consensus regarding the value of many current, mostly 'serological' biomarkers for risk prediction of MI or stroke. In the last years, new approaches are being used to search for novel biomarkers. These approaches have two distinctive features. First, they do not focus only in proteins, but they also assess other molecules (*Table 2*). Second, they are able to analyse large numbers of these molecules instead of a few of them, as happened with the traditional approaches. In fact, large EU-granted programs are using these new technologies to search novel biomarkers. Among these are the EPIC-CVD study²³ and the Biomarker for Cardiovascular Risk Assessment in Europe (BiomarCaRE). The latter is a European collaborative research project that integrates clinical and epidemiological biomarker research throughout Europe and validates the biomarker effectiveness in large, well-defined primary and secondary prevention cohorts from 13 European countries.²⁴ These studies will provide an overview of the clinical value of biomarkers including geographical differences of outcomes.

In the following, we will provide an overview of current and future biomarkers, changing paradigms and novel technologies, including their differences and pros and cons (*Table 2*). A particular focus will be on the discovery and measurements of biomarkers applying new –omics technologies. Although the relevance of genetic variants should not be underestimated, they cannot be held responsible

Table 1 Potential causal involvement in disease progression of discussed novel circulating biomarkers for atherosclerosis

Biomarker	Causality	Source or anticipated mechanism of action
(High-sensitivity) troponin	None	Consequence of myocardial necrosis
(NT-pro) BNP	None	Consequence of myocardial damage
HDLc	Causal factor?	Insufficient evidence that HDLc-raising therapeutic interventions are beneficial ¹⁸⁷
HDL-function	Likely causal factor	Capacity to exhibit cardioprotective effects (e.g. cholesterol efflux) ^{40,187}
Endothelial or leukocyte microparticles	Both	Consequence of cellular damage and/or regulated mechanism of inter-cellular communication ^{44,47–51}
Cardiac or endothelial miRNAs	Both	Consequence of cellular damage and/or regulated mechanism of inter-cellular communication ^{119,120}
HSP-27	Causal factor	Atheroprotective protein, low plasma levels are associated with cardiovascular disease ^{188–192}
sTWEAK	No evidence shown	Member of the tumor necrosis factor superfamily, low plasma levels are associated with cardiovascular disease ^{193–197}
Eicosanoids	Causal factor	Enhanced synthesis of inflammatory lipid mediators ^{198,199}
Polyunsaturated cholesteryl esters with long-chain fatty acids	Likely causal factor	Thought to contribute to foam cell formation and progression of atherosclerosis ²⁰⁰

Table 2 Comparison of novel circulating biomarkers for atherosclerosis and current technologies used for their identification

Type of biomarker	Screening technology	Sample throughput ^a	Limitations ^a	Future challenges
HDL	HPLC, NMR	Low to high	Single biomarker approach, limited predictive value	To identify predictive components of HDL (e.g. lipoproteins), shift towards alternative indexes such as particle size, subclass distribution, HDL functionality
Microparticles	Flow cytometry	Low	Lack of standardized measurement	To define measurement standards and develop novel analytical tools to reduce lower particle size limit of detection
miRNA	PCR-based array	Intermediate	Lack of validated internal controls for PCR-based approaches, sensitive to confounding factors, e.g. medications	To improve sensitivity of more specific technologies without amplification steps (e.g. probe-based arrays) for low-abundant miRNAs
Proteins	MS-based proteomics	Low	Requirement of large sample amount and complicated sample preparation	To improve sensitivity and simplify pre-analytical sample preparation steps
Metabolites	MS- or NMR spectroscopy-based metabolomics	Intermediate to high	High per-sample cost of detailed MS-based analysis detecting many metabolites vs. cost-effective NMR-based high-throughput analysis of abundant biomarkers	To improve cost-effectiveness and sample throughput of MS-based approaches, combination of NMR and MS in large population-based cohorts
Lipids	MS-based lipidomics	Intermediate	Mostly, targeted analysis of selective lipid classes and metabolites involved in known pathways	To perform holistic epidemiological studies including all lipid classes

MS, mass spectrometry; NMR, nuclear magnetic resonance spectroscopy.

^aReferring to currently used technologies.

for the vast majority of heredity (~90%) in cardiovascular medicine^{25,26} and are therefore beyond the scope of this review.

Before going into details of specific technologies, it should be noted that general –omics study design follows similar principles

as standard epidemiology. Current –omics technologies can be directly applied in existing large epidemiological sample collections. Quantitative –omics that can provide marker concentrations in physiological units can be analysed using the same statistical

methods, without requiring special knowledge on the underlying technology, as any other biomarker assay data.

High-density lipoprotein: shifting paradigms

The classically named high-density lipoproteins (HDLs) are clinically measured in plasma by analysing the amount of cholesterol contained in the particles, i.e. HDL cholesterol (HDLc). HDLc has been epidemiologically and clinically linked to cardiovascular disease presentation with low HDLc independently associated to high cardiovascular risk,^{27,28} whereas high levels were related to cardiovascular disease protection in the primary prevention setting.

High-density lipoprotein particles are complex lipoproteins and the pioneering studies evaluating the HDL vasculo-protective effects did not use HDL carrying high levels of cholesterol but used protein rich-HDL (nascent ApoA1-rich lipoprotein)^{29,30} or ApoA1 overexpression.³¹ These studies showed that HDL exerted atheroprotection. A key role of HDL is to promote cholesterol efflux and reverse cholesterol transport from the periphery to the liver. High-density lipoprotein cholesterol efflux capacity was recently shown to inversely correlate with the incidence of cardiovascular events in a population-based cohort, suggesting its potential use as a novel biomarker for atherosclerosis.³² A plethora of additional potential beneficial vascular properties have also been consistently attributed to HDL including antioxidant, anti-apoptotic, anti-inflammatory, anti-thrombotic/fibrinolytic, and vasodilatory effects.³³ The variable composition of HDL has emerged as the possible cause of these diverse functional effects. Indeed, the HDL particles not only contain ApoA1 and transport cholesterol but they are carriers of >85 proteins,^{34,35} numerous lipid species and small molecules.

Even with this complex nature, HDLc is still a good biomarker in the primary prevention setting. A recent study in over 35 000 patients has shown that for every 5 mg/dL increase of HDLc the risk of hospitalization for cardiovascular disease decreased 6%. In addition, if the levels of HDLc increased 6.5 mg/dL cardiovascular disease decreased 8%; on the contrary, if HDLc decreased 6.5 mg/dL cardiovascular risk increased 11%.³⁶ In fact, in acute percutaneous coronary intervention studies for every 5 mg/dL increase in HDLc concentrations, the risk of periprocedural acute MI decreased by 20%.³⁷ However, in some patient populations with established chronic coronary artery disease or chronic kidney disease the inverse association between HDL cholesterol levels and cardiovascular events is attenuated.^{38,39}

Recent studies have revealed that the occurrence of coronary disease is associated with a reduction in HDL antioxidant and anti-inflammatory potential indicating the variable and complex nature of these particles⁴⁰ that are easily remodelled during their metabolic life span. Interestingly, recent data with drugs developed to specifically raise HDLc levels have questioned the assumed protective role of raising the HDL particles carrying high levels of cholesterol.⁴¹ Human genetic analysis has also shown that genetic alterations of HDL cholesterol levels are not uniformly associated with the risk of coronary disease.²²

In summary, HDL particles express a multitude of molecular complexity and the measurement of cholesterol levels in particles

alone is not the only biomarker of HDL function. Although HDLc is still used clinically in primary prevention, new tests to better measure HDL functionality⁴² including cholesterol efflux capacity³² are being actively sought.

Microparticles

Microparticles (MPs; often also called microvesicles) belong to the family of extracellular vesicles released from activated or apoptotic cells. Microparticles (~100–1000 nm in diameter) stem from the cellular plasma membrane, whereas exosomes, which are <100 nm, originate from intracellular multivesicular bodies (for reviews, Refs 43–46). However, MPs are not only surrogate markers of cellular injury as they can affect the function of target cells and therefore influence the course of cardiovascular diseases (for reviews, Refs 44,47–51).

Microparticles of different cellular origin, as well as exosomes, circulate in human plasma and other body fluids.^{52–54} The major fractions in plasma stem from platelets, red blood cells, and leukocytes, whereas in most studies circulating endothelial MPs are less abundant.⁵² So far, the cellular origin of circulating exosomes remains elusive, although their role in inter-cellular signalling and carriers of RNA, especially micro-RNAs (miRNAs), receives increasing attention.^{55,56}

Detection of MP subpopulations in human plasma has gained increasing interest in the past two decades for their potential as biomarker. Considered as remnants of parental cell injury, they are easily accessible for measurement in the circulation. Their identification relies on the presence of externalized phosphatidylserine and specific markers from the parental cell membrane.⁵⁷ Plasma MP levels increase in subjects with cardiovascular risk factors and in patients with atherosclerosis or other cardiovascular disorders (for reviews, Refs 58–61). Interestingly, local levels of circulating MPs increase in culprit coronary arteries of patients with ST-segment elevation MI,⁶² and even further in those from patients with sudden cardiac death,⁶³ suggesting that changes in circulating MP levels reflect an increased release of microvesicles in atherosclerotic vascular disease. Finally, patients on lipid-lowering treatment with statins have lower numbers of circulating MPs despite similar plasma cholesterol levels.⁶⁴

Microparticle quantification remains challenging; the pros and cons of each method have been reviewed previously.^{65,66} Standardized methods are being developed and will certainly foster the application of MP assays in clinical settings. Both the standardization of the pre-analytical steps and the sensitivity of MP flow cytometry analysis have greatly improved in the past decade^{67–75} resulting in numerous investigations of the potential benefit of using plasma MPs as biomarkers.

So far, two specific subpopulations of circulating MPs have received most attention for use as CHD biomarkers: endothelial and leukocyte MPs.

Plasma levels of endothelial MPs expressing either CD144 or CD31 inversely associate with the degree of endothelium-dependent vasodilation in humans.^{76–81} Therefore, they reflect acute or chronic endothelial dysfunction and vascular injury in general. Elevation of endothelial MP subsets predicts atherosclerotic plaque instability in patients undergoing endarterectomy.⁸²

Furthermore, endothelial MPs expressing either CD144, CD31 or CD62E are independent predictors of cardiovascular outcome in patients with heart failure,⁸³ coronary artery disease,^{83,84} end-stage renal failure,⁸⁵ stroke history,⁸⁶ or other cardiovascular diseases.^{83,87} Combining endothelial MP detection with classical biomarkers strategy improves risk stratification for cardiovascular events in patients at risk of CHD.⁸⁸ No data are available yet in the general population regarding the prognostic value of circulating endothelial MPs.

Levels of circulating CD11a expressing MPs associate with atherosclerotic plaque burden in asymptomatic patients.⁸⁹ In patients with ACS, CD11b⁺ MPs inversely associate with the early recurrence of cardiovascular events, possibly because these MPs are consumed during thrombus formation.⁹⁰ Recent studies demonstrate that plasma MPs might be useful to assess the composition and the vulnerability of atherosclerotic plaques. In patients with severe carotid stenosis (>70%), plasma levels of leukocyte CD11b⁺ CD66b⁺ MPs are associated with plaque instability.⁹¹ In patients with familial hypercholesterolemia, levels of CD45⁺CD3⁺ lymphocyte MPs help to discriminate lipid-rich plaques from fibrous lesions.⁹²

Taken together, these findings indicate that MPs from endothelial cells and leukocytes could provide useful tools to identify patients at high risk for future cardiovascular events. Furthermore, the complex MP composition (proteins, lipids, and nucleic acids) might be an interesting source for –omics.^{93–96}

Micro-RNA

Micro-RNAs are small non-coding RNAs that control gene expression by binding to target mRNAs, thereby inducing mRNA degradation or repression of protein translation. Besides their important intracellular functions and potential value as therapeutic targets,^{97–99} extracellular miRNAs have also been detected in various body fluids including the blood. The levels of circulating miRNAs are modulated in disease states and, therefore, yield potential value as cardiovascular disease biomarkers.

Initially, researchers found elevated levels of miRNAs that are highly expressed in the myocardium ('cardiac miRNAs') in acute MI patients, e.g. miRNA-1, miRNA-133, miRNA-208a/b, and miRNA-499.^{100–104} Meanwhile, multiple additional circulating miRNAs were shown to be enhanced following MI or angina pectoris.^{101,103,105–114} Several cardiac-enriched miRNAs accumulate in plasma early after MI or transcatheter ablation of septal hypertrophy¹¹⁵ with similar kinetics as conventional cardiac injury biomarkers.^{103,105,115} Their increase in transcatheter gradients of ACS patients¹⁰⁴ suggests that they are indeed released from damaged cardiac myocytes. Circulating cardiac miRNAs were proposed to improve the diagnostic value, when combined with traditional markers like high sensitive troponin T,¹¹⁶ and circulating cardiac miRNAs were associated with poor prognosis in first studies.¹¹⁷ More recently, combinations of miRNAs, such as miRNA-132, miRNA-150, and miRNA-186, were shown to facilitate the diagnosis of unstable angina¹¹⁸ and changes in miRNA-126, miRNA-223, and miRNA-197 expression predicted subsequent MI.⁹⁶ Recent studies revealed that miRNAs also circulate within microvesicles and may contribute to cardiac pathophysiology

by targeting vascular and cardiac cells^{119,120} and can predict subsequent heart failure.¹²¹

Less is known about the use of circulating miRNAs as biomarkers to detect early stages of atherosclerosis or atherosclerotic plaque characteristics. Patients with stable coronary artery disease exhibit reduced levels of the endothelial cell-enriched and vasculoprotective miRNA-126 and members of the miRNA-17-92a cluster.¹²² Lower levels of circulating miRNA-126 were also observed in patients with diabetes.¹²³ Moreover, the smooth muscle-enriched miRNA-145-5p was significantly lower in patients with CHD.¹²² Circulating miRNAs may thus have the potential to reflect endothelial function or provide non-invasive insights into plaque vulnerability. Recent evidence suggests that the passenger strands of miRNAs that normally undergo degradation also deserve attention as potential biomarkers, since they have been reported to regulate endothelial regeneration^{98,119} or circulate in fibroblast-derived exosomes to target cardiac cells.¹²⁴

However, miRNA measurements currently depend on PCR, making them sensitive for confounding factors and normalization is challenging due to the lack of valid house-keeping miRNAs. Furthermore, the origin of miRNAs cannot be unequivocally determined as many circulating miRNAs derive from platelets¹²⁵ and the discrimination between vessel wall- and platelet-derived miRNAs is difficult if not impossible. Moreover, several pharmacological interventions, such as platelet inhibitors and heparin were shown to affect miRNA measurements.^{125–127}

While circulating miRNAs may be promising CHD biomarkers, the field is still in its infancy and is challenged by confounding factors that interfere with miRNA measurement. Solutions to current methodological problems may include: (1) the use of multi-miRNA panels, (2) technical improvements in miRNA measurements (i.e. replacing PCR by hybridization technologies). Large-scale studies to document the ability of circulating miRNAs to detect early atherosclerosis or plaque vulnerability remain to be performed.

Proteomic technologies

Proteomic technologies allow comparing the expression of hundreds or thousands of proteins from two biological specimens, including fluids, tissue, or cells. For instance, arteries with and without atherosclerosis can be compared or the effect of different therapies can be assessed.¹²⁸ Over the past decade, proteomics analyses have evolved from protein separation by two-dimensional electrophoresis to mass spectrometry (MS)-based approaches.¹²⁸ At present, a variety of proteomic platforms are available. Their selection depends on the specimens and the type of proteins to explore (for reviews, Refs 128–130).

In general, there are two MS approaches: First, the untargeted discovery approach, in which samples are analysed without *a priori* assumptions and peptides are prioritized for fragmentation based on their relative abundance. This approach is limited by its bias towards abundant proteins since there is currently no technological platform to resolve the entire human plasma proteome.^{130,131} The result of an untargeted discovery proteomic experiment is a list of proteins, among which potential biomarker candidates are selected according to their highest statistical significance and relevance. Second, targeted MS offers an alternative approach, in which

a pre-selected panel of proteins is measured with high precision. This method is termed using multiple reaction monitoring and was selected by Nature Methods as technology of the year in 2012.¹³²

Although multiple reaction monitoring offers better sensitivity than untargeted proteomics, higher abundant plasma proteins are still more readily quantified and for now antibody-based methods remain the method of choice for the quantitation of less abundant proteins (i.e. cytokines, chemokines, growth factors, etc., which are present at picograms to nanograms/mL plasma). A combination of an untargeted with a targeted approach provides the most comprehensive strategy to discover new biomarkers by proteomics.

Blood proteomics

Blood is an excellent source for the discovery of new biomarkers in atherosclerosis, as it exchanges molecules with the arterial wall and several blood cell populations are causally involved in atherosclerosis. However, proteomic studies of plasma or serum are complicated by the presence of high-abundant proteins, such as albumin or immunoglobulins, that may mask important biomarker candidates present at lower concentrations.^{128,131} By using adequate methods to remove these high-abundant proteins, different candidate biomarkers have been identified.^{133,134} Nevertheless, this approach is not without risk as removing albumin from the sample may also remove albumin bound proteins with potential value.

The proteome of circulating cells may yield relevant information comparing either patients with different clinical pictures of atherosclerosis or testing the effect of anti-atherosclerotic drugs in subjects with this disorder.^{135,136} The potential value of post-translational protein modifications as biomarkers of CVD can also be evaluated by proteomic analysis, as reported in blood of ACS patients.^{137,138} A different study used nuclear magnetic resonance (NMR)-based technology to identify a protein glycan that is associated with incident CHD.¹³⁹ Finally, urine may be a potential source of biomarkers as it contains proteins filtrated from plasma with low-molecular weight that can be analysed avoiding excessive manipulation.¹⁴⁰

Proteomics of atherosclerotic lesions

Another complementary approach is to study whole tissue specimens. For instance, patients with higher atheroma content of osteopontin are at increased risk of developing cardiovascular events.¹⁴¹ However, studying the whole tissue could result in identification of structural proteins not involved in atherosclerosis. In this case, the study of the secretome may be instrumental.¹⁴² In this procedure, a tissue specimen is cultured, allowing it to release molecules into the medium, mimicking the secretion of biomarkers from atheroma into the blood. Thereafter, the supernatant is analysed avoiding the presence of structural proteins. Comparing the secretome of carotid endarterectomy specimens and healthy arteries, low plasma levels of HSP-27 (Heat Shock Protein-27) and sTWEAK (soluble tumour-necrosis factor-like weak inducer of apoptosis) have been identified as diagnostic markers of atherosclerosis,^{143,144} although their prognostic value remains controversial.^{145–147} Another possibility is to assess the effect of drugs when added to the cultured tissue.¹⁴⁸ Moreover, retrieved coronary thrombi of MI patients have been

recently analysed by proteomics, revealing potential new candidates for biomarkers of atherothrombosis.^{149,150}

Proteomic approaches allow screening to detect differences in protein expression between different biological specimens. Although this approach is not free of limitations, it has considerably increased our ability to discover novel biomarkers of atherosclerosis as untargeted or targeted protein analysis can be performed by MS without *a priori* assumptions and without the need for the availability of good antibodies to a specific protein of interest.

Metabolomics

Detailed profiling of metabolic status, termed metabolite profiling or metabolomics, can provide insights into the molecular mechanisms underlying atherosclerosis.^{151–156} The quantification of large numbers of circulating metabolites across multiple pathways may also identify metabolic changes prior to the onset of overt disease, and hereby potentially lead to earlier and more accurate identification of individuals at high cardiovascular risk.^{156–158} Two technological platforms are used: nuclear magnetic resonance spectroscopy¹⁵⁶ and MS.^{158,159} The former has an advantage in throughput but is limited in sensitivity. Vice versa, MS can identify many more metabolites but often with limited throughput.

Coronary heart disease biomarkers

Technological improvements in sample throughput now allow for metabolite profiling of extensive epidemiological cohorts, rather than case–control settings, to enhance biomarker discovery and replication.^{160,161} Metabolite profiling has been successful in identifying biomarkers for the development of type 2 diabetes.^{162–167} Circulating biomarkers for diabetes are more directly associated with the disease than those for atherosclerosis.^{168,169} However, few metabolite biomarkers have been consistently associated with future cardiovascular events across multiple studies.^{154,156–158,170,171}

Many common systemic metabolites, such as amino acids show consistent associations in properly powered epidemiological studies. For example, it has been demonstrated that there are prospective associations of some amino acids with carotid intima-media thickness, a subclinical measure of atherosclerosis.^{156,172} Nuclear magnetic resonance-based metabolite profiling in large prospective cohorts recently identified phenylalanine, monounsaturated and polyunsaturated fatty acids as biomarkers for CHD risk.¹⁷¹ The association strengths of some of these new biomarkers, as of phenylalanine, are comparable with those of established risk factors, e.g. LDL cholesterol, and they remain predictive even when adjusted for standard lipids and glycaemic traits.^{156,171}

Key issues in metabolic profiling in epidemiology

Quantitative metabolic profiling can aid biomarker discovery in an unbiased and unsupervised manner by providing molecular information across multiple pathways: all metabolic measures can then be separately tested for the potential disease association or incidence. This should be followed by appropriate independent replication of the candidate biomarkers identified in the discovery cohort. Unfortunately, the promise of metabolomics in biomarker discovery has

not been fully realized; even though various papers have been published, there is very little consistency and rigor in the metabolomics works in this area as recently pointed out.¹⁷³ We call for a stringent attention to statistics and replication in the field of metabolomics to strengthen the scientific value of the work, irrespective of the analytical platform used. Particularly when aiming for clinical applications, recent frameworks are recommended to strengthen the methodological rigour and quality for the prediction models.^{174,175}

Lipidomics

Lipidomics is a specific subset of metabolomics, which refers to a systems-based study of all lipids.¹⁷⁶ This approach could potentially go beyond the analysis of cholesterol and triglycerides for assessment of cardiovascular risk and response to therapy. In addition, lipidomics can be used to detect several lipid mediators involved in cellular homeostasis as well as in inflammation initiation and resolution¹⁷⁷ and whose circulating levels may give insight into pathophysiological processes of atherosclerosis. However, the simultaneous detection of lipids is impeded by the heterogeneity of the lipid molecules reflecting different metabolic pathways originating

from structurally different lipid species. The theoretical number of different molecular lipid species has been estimated to 180 000.¹⁷⁸ To facilitate the lipid classification, the Lipid Maps consortium¹⁷⁹ categorized the human plasma lipidome into six major classes, namely fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, and prenol lipids.

In addition to structural diversity, it should also be considered that the concentrations may differ between different lipid classes, which also should be taken into account when using lipidomics for the identification of circulating biomarkers. For example, several of the eicosanoids are active at nanomolar concentrations at specific G-protein-coupled receptors,¹⁸⁰ and the local response to these lipid mediators at the site of an atherosclerotic lesion may not necessarily be reflected in their circulating levels. Selective lipidomic analysis of eicosanoids released by carotid atherosclerotic lesions has characterized the metabolites of 5-, 12-, and 15-lipoxygenase as the predominant eicosanoids produced by the atherosclerotic lesion, compared with cyclooxygenase products. For example, 12-hydroxyeicosatetraenoic was the most abundant eicosanoid, at levels ≈ 40 -fold greater compared with a prostacyclin metabolite.¹⁸¹

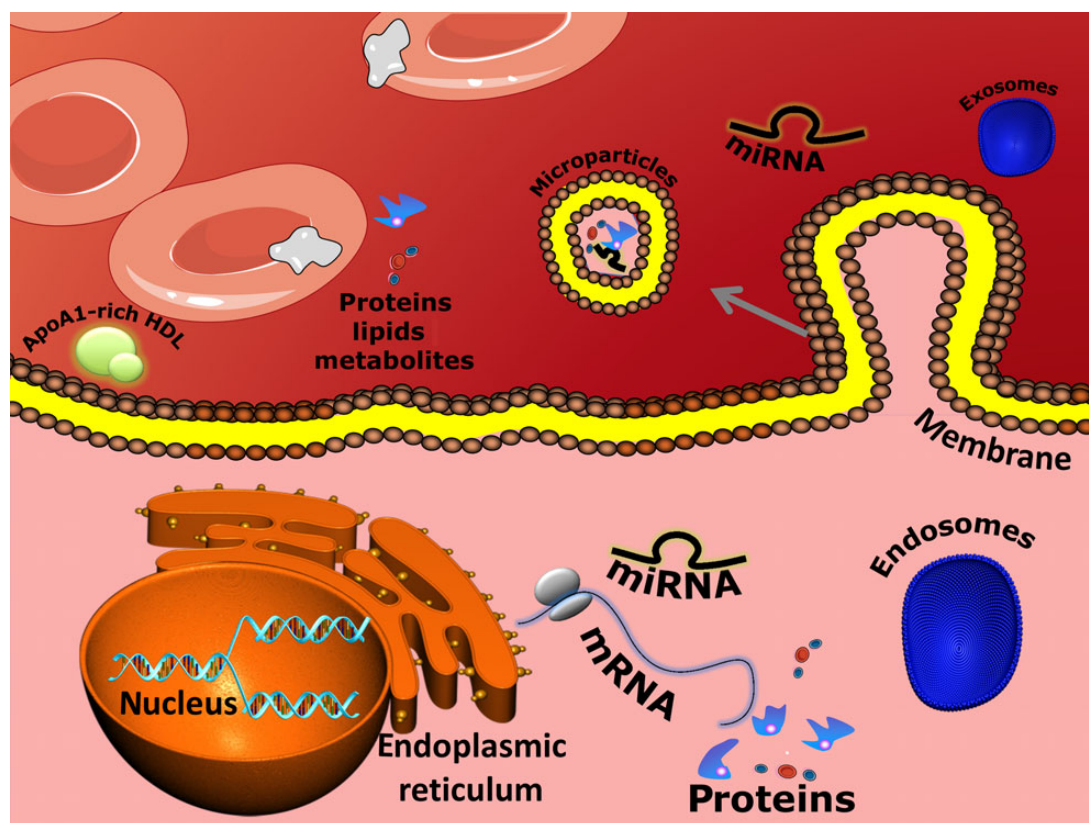


Figure 1 Circulating biomarkers for cardiovascular disease. The transcriptome, proteome, metabolome, and lipidome analysis of cells, blood, and serum is increasingly identifying circulating biomarkers for cardiovascular disease originating from the cellular and extracellular compartments. These include high-density lipoprotein particles and high-density lipoprotein-related proteins (e.g. apoA1), small circulating particles enveloped in cell membranes, such as microparticles (which stem from the cellular plasma membrane) and exosomes (which derive from intracellular multi-vesicular bodies such as endosomes), intracellular molecules such as short regulatory sequences of gene transcription (miRNA), as well as proteins, peptides and lipid metabolites. These might circulate freely or be carried by microparticles and exosomes.

Broader lipidomic analyses have identified members of several different lipid classes in human atherosclerotic lesions.^{182,183} Comparing the local lipidome of atherosclerotic lesion with that observed in plasma has revealed that polyunsaturated cholesteryl esters with long-chain fatty acids and certain sphingomyelin species exhibited the greatest relative enrichment in plaques compared with plasma and formed part of a lipid signature for vulnerable and stable plaque areas within the same lesion.^{158,184} Despite these differences in terms of lipid profiles within atherosclerotic plaques and the plasma lipidome, qualitative and quantitative characterization of circulating lipid species by means of lipidomics may offer additional tools for patient risk stratification. For example, the assessment of 305 different plasma lipid species added incremental value in classifying coronary patients and matched healthy controls.¹⁸⁵ In addition, multiple lipid species were shown to distinguish unstable from stable coronary disease.¹⁸⁵ However, some of the reported associations were rather unexpected, i.e. the inverse association of cholesteryl esters with unstable lesions, and could potentially have been confounded by the higher proportion of statin and heparin use in patients with unstable compared with stable coronary disease. Those initial findings in patient cohorts were recently extended by the assessment of 135 lipid species in a prospective population-based study.¹⁵⁸ The latter study highlighted that a shift in the fatty acid chain length of cholesteryl esters, sphingomyelins, and triacylglycerides exhibited the strongest and most consistent association with cardiovascular disease. A similar fatty acid chain length shift has previously been linked to the risk of type 2 diabetes.¹⁶³

The top scoring lipids significantly improved the risk discrimination for cardiovascular disease during a 10-year follow-up, hence providing a first piece of evidence for a predictive value of circulating biomarker identified by means of plasma lipidomics.¹⁵⁸ Finally, the possibility of using lipidomics to predict response to lipid-lowering therapy has received some support by studies of, for example, statins and fibrates.¹⁸⁶

Consensus statement

For decades, the endeavours to find new biomarkers for prediction, prevention, diagnosis, and prognosis of cardiovascular events have focused on a rather small number of molecules. Technological improvements in automated analytical methodologies, for example in terms of sensitivity and sample throughput, have revolutionized biomarker research in the past 10 years. Integration of multiple complementary platforms, such as micro-RNA, transcriptome, proteome, metabolome, and lipidome analysis, allows unprecedented biological details across multiple pathways and is leading to a conceptual shift from individual markers to multi-marker panels for cardiovascular risk prediction (Figure 1). However, the vast amount of data generated also calls for bioinformatics expertise to handle and combine the data. In the case of new technologies, it is also essential to critically assess their advantages and disadvantages, and keep their potential limitations in mind (Table 2). For example, the sensitivity of a method inherently affects the biomarker panel obtainable. Reliable interpretation of improved molecular details may also call for more stringent analytical and clinical sample quality standards—collection and storage conditions safe for traditional biomarkers may not be such for some new biomarkers

and, say, the molecular effects of diet and medication can be more noticeable than with traditional biomarkers. Explanatory research related to technical, analytical, and practical aspects of new technologies is therefore essential and expected to increasingly commence in the near future. Ultimately, the application and value of new candidate biomarkers will depend on their predictive power over traditional risk assessment, on their reproducibility in multiple cohorts and on the practicalities and the cost-effectiveness of their integration into clinical routines and laboratories.

References

- Catapano AL, Reiner Z, De Backer G, Graham I, Taskinen MR, Wiklund O, Agewall S, Alegria E, Chapman M, Durrington P, Erdine S, Halcox J, Hobbs R, Kjekshus J, Filardi PP, Riccardi G, Storey RF, Wood D. ESC/EAS Guidelines for the management of dyslipidaemias The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Atherosclerosis* 2011;**217**:3–46.
- Wang TJ. Assessing the role of circulating, genetic, and imaging biomarkers in cardiovascular risk prediction. *Circulation* 2011;**123**:551–565.
- Yla-Herttuala S, Bentzon JF, Daemen M, Falk E, Garcia-Garcia HM, Herrmann J, Hoefler I, Jauhainen S, Jukema JW, Krams R, Kwak BR, Marx N, Naruszewicz M, Newby A, Pasterkamp G, Serruys PW, Waltenberger J, Weber C, Tokgozlu L. Stabilization of atherosclerotic plaques: an update. *Eur Heart J* 2013;**34**:3251–3258.
- Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syvanne M, Scholte op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). *Eur Heart J* 2012;**33**:1635–1701.
- Paone JF, Waalkes TP, Baker RR, Shaper JH. Serum UDP-galactosyl transferase as a potential biomarker for breast carcinoma. *J Surg Oncol* 1980;**15**:59–66.
- Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001;**69**: 89–95.
- Hamm CW, Bassand JP, Agewall S, Bax J, Boersma E, Bueno H, Caso P, Dudek D, Gielen S, Huber K, Ohman M, Petrie MC, Sonntag F, Uva MS, Storey RF, Wijns W, Zahger D. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2011;**32**:2999–3054.
- Steg PG, James SK, Atar D, Badano LP, Blomstrom-Lundqvist C, Borger MA, Di Mario C, Dickstein K, Ducrocq G, Fernandez-Aviles F, Gershlick AH, Giannuzzi P, Halvorsen S, Huber K, Juni P, Kastrati A, Knuuti J, Lenzen MJ, Mahaffey KW, Valgimigli M, van't Hof A, Widimsky P, Zahger D. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur Heart J* 2012;**33**:2569–2619.
- Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, Budaj A, Bugiardini R, Crea F, Cuisset T, Di Mario C, Ferreira JR, Gersh BJ, Gitt AK, Hulot JS, Marx N, Opie LH, Pfisterer M, Prescott E, Ruschitzka F, Sabate M, Senior R, Taggart DP, van der Wall EE, Vrints CJ, Zamorano JL, Achenbach S, Baumgartner H, Bax JJ, Bueno H, Dean V, Deaton C, Erol C, Fagard R, Ferrari R, Hasdai D, Hoes AW, Kirchhof P, Knuuti J, Kolh P, Lancellotti P, Linhart A, Nihoyannopoulos P, Piepoli MF, Ponikowski P, Sirnes PA, Tamargo JL, Tenders M, Torbicki A, Wijns W, Windecker S, Knuuti J, Valgimigli M, Bueno H, Claeys MJ, Donner-Banzhoff N, Erol C, Frank H, Funck-Brentano C, Gaemperli O, Gonzalez-Juanatey JR, Hämilos M, Hasdai D, Husted S, James SK, Kervinen K, Kolh P, Kristensen SD, Lancellotti P, Maggioni AP, Piepoli MF, Pries AR, Romeo F, Ryden L, Simoons ML, Sirnes PA, Steg PG, Timmis A, Wijns W, Windecker S, Yldirim A, Zamorano JL. 2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology. *Eur Heart J* 2013;**34**:2949–3003.
- Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, Tjora S, Domanski MJ, Gersh BJ, Rouleau JL, Pfeffer MA, Braunwald E. A sensitive cardiac troponin T assay in stable coronary artery disease. *N Engl J Med* 2009;**361**: 2538–2547.

11. Ndrepepa G, Braun S, Mehilij J, Birkmeier KA, Byrne RA, Ott I, Hosl K, Schulz S, Fusaro M, Pache J, Hausleiter J, Laugwitz KL, Massberg S, Seyfarth M, Schomig A, Kastrati A. Prognostic value of sensitive troponin T in patients with stable and unstable angina and undetectable conventional troponin. *Am Heart J* 2011;**161**: 68–75.
12. Sherwood MW, Kristin Newby L. High-sensitivity troponin assays: evidence, indications, and reasonable use. *J Am Heart Assoc* 2014;**3**:e000403.
13. Ahmad N, Thomas GN, Chan C, Gill P. Ethnic differences in lower limb revascularisation and amputation rates. Implications for the aetiopathology of atherosclerosis? *Atherosclerosis* 2014;**233**:503–507.
14. Chaturvedi N, Coady E, Mayet J, Wright AR, Shore AC, Byrd S, Mc GTSA, Kooner JS, Schalkwijk CG, Hughes AD. Indian Asian men have less peripheral arterial disease than European men for equivalent levels of coronary disease. *Atherosclerosis* 2007;**193**:204–212.
15. Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA, Stromberg A, van Veldhuisen DJ, Atar D, Hoes AW, Keren A, Mebazaa A, Nieminen M, Priori SG, Swedberg K. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur Heart J* 2008;**29**:2388–2442.
16. van Holten TC, Waanders LF, de Groot PG, Vissers J, Hoefler IE, Pasterkamp G, Prins MW, Roest M. Circulating biomarkers for predicting cardiovascular disease risk: a systematic review and comprehensive overview of meta-analyses. *PLoS ONE* 2013;**8**:e62080.
17. Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kushner FG, Lauer MS, Shaw LJ, Smith SC Jr, Taylor AJ, Weintraub WS, Wenger NK, Jacobs AK, Smith SC Jr, Anderson JL, Albert N, Buller CE, Creager MA, Ettinger SM, Guyton RA, Halperin JL, Hochman JS, Kushner FG, Nishimura R, Ohman EM, Page RL, Stevenson WG, Tarkington LG, Yancy CW. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2010;**56**:e50–e103.
18. Mancia G, Laurent S, Agabiti-Rosei E, Ambrosioni E, Burnier M, Caulfield MJ, Cifkova R, Clement D, Coca A, Dominiczak A, Erdine S, Fagard R, Farsang C, Grassi G, Haller H, Heagerty A, Kjeldsen SE, Kiowski W, Mallion JM, Manolis A, Narkiewicz K, Nilsson P, Olsen MH, Rahn KH, Redon J, Rodicio J, Ruilope L, Schmieder RE, Struijker-Boudier HA, van Zwieten PA, Viigimaa M, Zanchetti A. Reappraisal of European guidelines on hypertension management: a European Society of Hypertension Task Force document. *J Hypertens* 2009;**27**:2121–2158.
19. Mestdagh P, Hartmann N, Baeriswyl L, Andreasen D, Bernard N, Chen C, Cheo D, D'Andrade P, DeMayo M, Dennis L, Derveaux S, Feng Y, Fulmer-Smentek S, Gerstmayer B, Gouffon J, Grimley C, Lader E, Lee KY, Luo S, Mouritzen P, Narayanan A, Patel S, Peiffer S, Ruberg S, Schroth G, Schuster D, Shaffer JM, Shelton EJ, Silveria S, Ulmanella U, Veeramachaneni V, Staedtler F, Peters T, Guettouche T, Wong L, Vandesompele J. Evaluation of quantitative miRNA expression platforms in the microRNA quality control (miRQC) study. *Nat Methods* 2014;**11**:809–815.
20. Poste G. Bring on the biomarkers. *Nature* 2011;**469**:156–157.
21. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med* 2008;**359**:1897–1908.
22. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, Hindy G, Holm H, Ding EL, Johnson T, Schunkert H, Samani NJ, Clarke R, Hopewell JC, Thompson JF, Li M, Thorleifsson G, Newton-Cheh C, Musunuru K, Pirruccello JP, Saleheen D, Chen L, Stewart A, Schillert A, Thorsteinsdottir U, Thorgeirsson G, Anand S, Engert JC, Morgan T, Spertus J, Stoll M, Berger K, Martinelli N, Girelli D, McKeown PP, Patterson CC, Epstein SE, Devaney J, Burnett MS, Mooser V, Ripatti S, Surakka I, Nieminen MS, Sinisalo J, Lokki ML, Perola M, Havulinna A, de Faire U, Gigante B, Ingelsson E, Zeller T, Wild P, de Bakker PI, Klungel OH, Maitland-van der Zee AH, Peters BJ, de Boer A, Grobbee DE, Kamphuisen PW, Deneer VH, Elbers CC, Onland-Moret NC, Hofker MH, Wijmenga C, Verschuren WM, Boer JM, van der Schouw YT, Rasheed A, Frossard P, Demissie S, Willer C, Do R, Ordovas JM, Abecasis GR, Boehnke M, Mohlke KL, Daly MJ, Guiducci C, Burt NP, Surti A, Gonzalez E, Purcell S, Gabriel S, Marrugat J, Peden J, Erdmann J, Diemert P, Willenborg C, Konig IR, Fischer M, Hengstenberg C, Ziegler A, Buyschaert I, Lambrechts D, Van de Werf F, Fox KA, El Mokhtari NE, Rubin D, Schrezenmeir J, Schreiber S, Schafer A, Danesh J, Blankenberg S, Roberts R, McPherson R, Watkins H, Hall AS, Overvad K, Rimm E, Boerwinkle E, Tybjaerg-Hansen A, Cupples LA, Reilly MP, Melander O, Mannucci PM, Ardissino D, Siscovick D, Elosua R, Stefansson K, O'Donnell CJ, Salomaa V, Rader DJ, Peltonen L, Schwartz SM, Altshuler D, Kathiresan S. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet* 2012;**380**:572–580.
23. EPIC-CVD. The EPIC-CVD study. <http://www.epiccvd.eu/frontpage/the-epic-cvd-study.html> (14 January 2015).
24. Zeller T, Hughes M, Tuovinen T, Schillert A, Conrads-Frank A, Ruijter HD, Schnabel RB, Kee F, Salomaa V, Siebert U, Thorand B, Ziegler A, Breek H, Pasterkamp G, Kuulasmaa K, Koenig W, Blankenberg S. BiomarcCaRE: rationale and design of the European BiomarcCaRE project including 300,000 participants from 13 European countries. *Eur J Epidemiol* 2014;**29**:777–790.
25. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, Ingelsson E, Saleheen D, Erdmann J, Goldstein BA, Stirrups K, Konig IR, Cazier JB, Johansson A, Hall AS, Lee JY, Willer CJ, Chambers JC, Esko T, Folkersen L, Goel A, Grundberg E, Havulinna AS, Ho WK, Hopewell JC, Eriksson N, Kleber ME, Kristiansson K, Lundmark P, Lytikainen LP, Rafelt S, Shungin D, Strawbridge RJ, Thorleifsson G, Tikkanen E, Van Zuydam N, Voight BF, Waite LL, Zhang W, Ziegler A, Absher D, Altshuler D, Balmforth AJ, Barroso I, Braund PS, Burgdorf C, Claudi-Boehm S, Cox D, Dimitriou M, Do R, Doney AS, El Mokhtari N, Eriksson P, Fischer K, Fontanillas P, Franco-Cereceda A, Gigante B, Groop L, Gustafsson S, Hager J, Hallmans G, Han BG, Hunt SE, Kang HM, Illig T, Kessler T, Knowles JW, Kolovou G, Kuusisto J, Langenberg C, Langford C, Leander K, Lokki ML, Lundmark A, McCarthy MI, Meisinger C, Melander O, Mihailov E, Maouche S, Morris AD, Muller-Nurasyid M, Nikus K, Peden JF, Rayner NW, Rasheed A, Rosinger S, Rubin D, Rumpf MP, Schafer A, Sivananthan M, Song C, Stewart AF, Tan ST, Thorgeirsson G, van der Schoot CE, Wagner PJ, Wells GA, Wild PS, Yang TP, Amouyel P, Arveiler D, Basart H, Boehnke M, Boerwinkle E, Brambilla P, Cambien F, Cupples AL, de Faire U, Dehghan A, Diemert P, Epstein SE, Evans A, Ferrario MM, Ferrieres J, Gauguier D, Go AS, Goodall AH, Gudnason V, Hazen SL, Holm H, Iribarren C, Jang Y, Kahonen M, Kee F, Kim HS, Klopp N, Koenig W, Kratzer W, Kuulasmaa K, Laakso M, Laaksonen R, Lee JY, Lind L, Ouweland WH, Parish S, Park JE, Pedersen NW, Peters A, Quertermous T, Rader DJ, Salomaa V, Schadt E, Shah SH, Sinisalo J, Stark K, Stefansson K, Tregouet DA, Virtamo J, Wallentin L, Wareham N, Zimmermann ME, Nieminen MS, Hengstenberg C, Sandhu MS, Pastinen T, Syvanen AC, Hovingh GK, Dedoussis G, Franks PW, Lehtimäki T, Metspalu A, Zalloua PA, Siegbahn A, Schreiber S, Ripatti S, Blankenberg SS, Perola M, Clarke R, Boehnke BO, O'Donnell C, Reilly MP, Marz W, Collins R, Kathiresan S, Hamsten A, Kooner JS, Thorsteinsdottir U, Danesh J, Palmer CN, Roberts R, Watkins H, Schunkert H, Samani NJ. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet* 2013;**45**:25–33.
26. Aavik E, Lumivuori H, Leppanen O, Wirth T, Hakkinen SK, Brasen JH, Beschornor U, Zeller T, Braspenning M, van Criekinge W, Makinen K, Yla-Herttuala S. Global DNA methylation analysis of human atherosclerotic plaques reveals extensive genomic hypomethylation and reactivation at imprinted locus 14q32 involving induction of a miRNA cluster. *Eur Heart J* 2015;**36**:993–1000.
27. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009;**302**:1993–2000.
28. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am J Med* 1977;**62**:707–714.
29. Badimon JJ, Badimon L, Fuster V. Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. *J Clin Invest* 1990;**85**:1234–1241.
30. Badimon JJ, Badimon L, Galvez A, Dische R, Fuster V. High density lipoprotein plasma fractions inhibit aortic fatty streaks in cholesterol-fed rabbits. *Lab Invest* 1989;**60**:455–461.
31. Paszty C, Maeda N, Verstuyft J, Rubin EM. Apolipoprotein AI transgene corrects apolipoprotein E deficiency-induced atherosclerosis in mice. *J Clin Invest* 1994;**94**: 899–903.
32. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland JJ, Yuhanna IS, Rader DR, de Lemos JA, Shaul PW. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med* 2014;**371**:2383–2393.
33. Badimon L, Vilahur G. LDL-cholesterol versus HDL-cholesterol in the atherosclerotic plaque: inflammatory resolution versus thrombotic chaos. *Ann N Y Acad Sci* 2012;**1254**:18–32.
34. Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, Byun J, Vuletic S, Kassim S, Singh P, Chea H, Knopp RH, Brunzell J, Geary R, Chait A, Zhao XQ, Elkon K, Marcovina S, Ridker P, Oram JF, Heinecke JW. Shotgun proteomics implicates protease inhibition and complement activation in the anti-inflammatory properties of HDL. *J Clin Invest* 2007;**117**:746–756.
35. Shah AS, Tan L, Long JL, Davidson WS. Proteomic diversity of high density lipoproteins: our emerging understanding of its importance in lipid transport and beyond. *J Lipid Res* 2013;**54**:2575–2585.

36. Nichols GA, Vupputuri S, Rosales AG. Change in high-density lipoprotein cholesterol and risk of subsequent hospitalization for coronary artery disease or stroke among patients with type 2 diabetes mellitus. *Am J Cardiol* 2011;**108**:1124–1128.
37. Sattler KJ, Herrmann J, Yun S, Lehmann N, Wang Z, Heusch G, Sack S, Erbel R, Levkau B. High high-density lipoprotein-cholesterol reduces risk and extent of percutaneous coronary intervention-related myocardial infarction and improves long-term outcome in patients undergoing elective percutaneous coronary intervention. *Eur Heart J* 2009;**30**:1894–1902.
38. Angeloni E, Paneni F, Landmesser U, Benedetto U, Melina G, Luscher TF, Volpe M, Sinatra R, Cosentino F. Lack of protective role of HDL-C in patients with coronary artery disease undergoing elective coronary artery bypass grafting. *Eur Heart J* 2013;**34**:3557–3562.
39. Zewinger S, Speer T, Kleber ME, Scharnagl H, Woitas R, Lepper PM, Pfahler K, Seiler S, Heine GH, Marz W, Silbernagel G, Fliser D. HDL cholesterol is not associated with lower mortality in patients with kidney dysfunction. *J Am Soc Nephrol* 2014;**25**:1073–1082.
40. Riwanto M, Rohrer L, Roschitzki B, Bestler C, Mocharla P, Mueller M, Perisa D, Heinrich K, Altwegg L, von Eckardstein A, Luscher TF, Landmesser U. Altered activation of endothelial anti- and proapoptotic pathways by high-density lipoprotein from patients with coronary artery disease: role of high-density lipoprotein-proteome remodeling. *Circulation* 2013;**127**:891–904.
41. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, Chaitman BR, Holme IM, Kallend D, Leiter LA, Leitersdorf E, McMurray JJ, Mundt H, Nicholls SJ, Shah PK, Tardif JC, Wright RS. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med* 2012;**367**:2089–2099.
42. Martin SS, Khokhar AA, May HT, Kulkarni KR, Blaha MJ, Joshi PH, Toth PP, Muhlestein JB, Anderson JL, Knight S, Li Y, Spertus JA, Jones SR. HDL cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: the Lipoprotein Investigators Collaborative. *Eur Heart J* 2015;**36**:22–30.
43. Sluijter JP, Verhage V, Deddens JC, van den Akker F, Doeveandans PA. Microvesicles and exosomes for intracardiac communication. *Cardiovasc Res* 2014;**102**:302–311.
44. Loyer X, Vion AC, Tedgui A, Boulanger CM. Microvesicles as cell-cell messengers in cardiovascular diseases. *Circ Res* 2014;**114**:345–353.
45. Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ Res* 2010;**107**:1047–1057.
46. EL Andaloussi S, Mager I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat Rev Drug Discov* 2013;**12**:347–357.
47. Hulsmans M, Holvoet P. MicroRNA-containing microvesicles regulating inflammation in association with atherosclerotic disease. *Cardiovasc Res* 2013;**100**:7–18.
48. Buzas EI, Gyorgy B, Nagy G, Falus A, Gay S. Emerging role of extracellular vesicles in inflammatory diseases. *Nat Rev Rheumatol* 2014;**10**:356–364.
49. New SE, Aikawa E. Role of extracellular vesicles in de novo mineralization: an additional novel mechanism of cardiovascular calcification. *Arterioscler Thromb Vasc Biol* 2013;**33**:1753–1758.
50. Kleinjan A, Boing AN, Sturk A, Nieuwland R. Microparticles in vascular disorders: how tissue factor-exposing vesicles contribute to pathology and physiology. *Thromb Res* 2012;**130** Suppl 1:S71–S73.
51. Andriantsitohaina R, Gaceb A, Vergori L, Martinez MC. Microparticles as regulators of cardiovascular inflammation. *Trends Cardiovasc Med* 2012;**22**:88–92.
52. Viera AJ, Mooberry M, Key NS. Microparticles in cardiovascular disease pathophysiology and outcomes. *J Am Soc Hypertens* 2012;**6**:243–252.
53. Rautou PE, Vion AC, Amabile N, Chironi G, Simon A, Tedgui A, Boulanger CM. Microparticles, vascular function, and atherothrombosis. *Circ Res* 2011;**109**:593–606.
54. Owens AP 3rd, Mackman N. Microparticles in hemostasis and thrombosis. *Circ Res* 2011;**108**:1284–1297.
55. Boon RA, Vickers KC. Intercellular transport of microRNAs. *Arterioscler Thromb Vasc Biol* 2013;**33**:186–192.
56. Khalyfa A, Gozal D. Exosomal miRNAs as potential biomarkers of cardiovascular risk in children. *J Transl Med* 2014;**12**:162.
57. Morel O, Jesel L, Freyssonnet JM, Toti F. Cellular mechanisms underlying the formation of circulating microparticles. *Arterioscler Thromb Vasc Biol* 2011;**31**:15–26.
58. Amabile N, Rautou PE, Tedgui A, Boulanger CM. Microparticles: key protagonists in cardiovascular disorders. *Semin Thromb Hemost* 2010;**36**:907–916.
59. Tushuizen ME, Diamant M, Sturk A, Nieuwland R. Cell-derived microparticles in the pathogenesis of cardiovascular disease: friend or foe? *Arterioscler Thromb Vasc Biol* 2011;**31**:4–9.
60. Shantsila E, Kamphuisen PW, Lip GY. Circulating microparticles in cardiovascular disease: implications for atherogenesis and atherothrombosis. *J Thromb Haemost* 2010;**8**:2358–2368.
61. Burnier L, Fontana P, Kwak BR, Angelillo-Scherer A. Cell-derived microparticles in haemostasis and vascular medicine. *Thromb Haemost* 2009;**101**:439–451.
62. Min PK, Kim JY, Chung KH, Lee BK, Cho M, Lee DL, Hong SY, Choi EY, Yoon YW, Hong BK, Rim SJ, Kwon HM. Local increase in microparticles from the aspirate of culprit coronary arteries in patients with ST-segment elevation myocardial infarction. *Atherosclerosis* 2013;**227**:323–328.
63. Empana JP, Boulanger CM, Tafflet M, Renard JM, Leroyer AS, Varenne O, Prugger C, Silvain J, Tedgui A, Cariou A, Montalescot G, Jouven X, Spaulding C. Microparticles and sudden cardiac death due to coronary occlusion. The TIDE (Thrombus and Inflammation in sudden DEath) study. *Eur Heart J Acute Cardiovasc Care* 2015;**4**:28–36.
64. Suades R, Padro T, Alonso R, Mata P, Badimon L. Lipid-lowering therapy with statins reduces microparticle shedding from endothelium, platelets and inflammatory cells. *Thromb Haemost* 2013;**110**:366–377.
65. Arraud N, Linares R, Tan S, Gounou C, Pasquet JM, Mornet S, Brisson AR. Extracellular vesicles from blood plasma: determination of their morphology, size, phenotype and concentration. *J Thromb Haemost* 2014;**12**:614–627.
66. van der Pol E, Coumans FA, Grootemaat AE, Gardiner C, Sargent IL, Harrison P, Sturk A, van Leeuwen TG, Nieuwland R. Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing. *J Thromb Haemost* 2014;**12**:1182–1192.
67. Witwer KW, Buzas EI, Bemis LT, Bora A, Lasser C, Lotvall J, Nolte-’t Hoen EN, Piper MG, Sivaraman S, Skog J, Thery C, Wauben MH, Hochberg F. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *J Extracell Vesicles* 2013;**2**. <<http://www.journalofextracellularvesicles.net/index.php/jev/article/view/20360>>.
68. Lacroix R, Judicone C, Poncelet P, Robert S, Arnaud L, Sampol J, Dignat-George F. Impact of pre-analytical parameters on the measurement of circulating microparticles: towards standardization of protocol. *J Thromb Haemost* 2012;**10**:437–446.
69. Robert S, Lacroix R, Poncelet P, Harhour K, Bouriche T, Judicone C, Wischhusen J, Arnaud L, Dignat-George F. High-sensitivity flow cytometry provides access to standardized measurement of small-size microparticles – brief report. *Arterioscler Thromb Vasc Biol* 2012;**32**:1054–1058.
70. Robert S, Poncelet P, Lacroix R, Raoult D, Dignat-George F. More on: calibration for the measurement of microparticles: value of calibrated polystyrene beads for flow cytometry-based sizing of biological microparticles. *J Thromb Haemost* 2011;**9**:1676–8; author reply 1681–2.
71. Chandler WL, Yeung W, Tait JF. A new microparticle size calibration standard for use in measuring smaller microparticles using a new flow cytometer. *J Thromb Haemost* 2011;**9**:1216–1224.
72. Chandler WL. Microparticle counts in platelet-rich and platelet-free plasma, effect of centrifugation and sample-processing protocols. *Blood Coagul Fibrinolysis* 2013;**24**:125–132.
73. Ayers L, Kohler M, Harrison P, Sargent I, Dragovic R, Schaap M, Nieuwland R, Brooks SA, Ferry B. Measurement of circulating cell-derived microparticles by flow cytometry: sources of variability within the assay. *Thromb Res* 2011;**127**:370–377.
74. van der Pol E, van Gemert MJ, Sturk A, Nieuwland R, van Leeuwen TG. Single vs. swarm detection of microparticles and exosomes by flow cytometry. *J Thromb Haemost* 2012;**10**:919–930.
75. Erdbrugger U, Rudy CK, MEE, Dryden KA, Yeager M, Klibanov AL, Lannigan J. Imaging flow cytometry elucidates limitations of microparticle analysis by conventional flow cytometry. *Cytometry A* 2014;**85**:756–770.
76. Heiss C, Amabile N, Lee AC, Real WM, Schick SF, Lao D, Wong ML, Jahn S, Angeli FS, Minasi P, Springer ML, Hammond SK, Glantz SA, Grossman W, Balmes JR, Yeghiazarians Y. Brief secondhand smoke exposure depresses endothelial progenitor cells activity and endothelial function: sustained vascular injury and blunted nitric oxide production. *J Am Coll Cardiol* 2008;**51**:1760–1771.
77. Amabile N, Guerin AP, Leroyer A, Mallat Z, Nguyen C, Bodaert J, London GM, Tedgui A, Boulanger CM. Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. *J Am Soc Nephrol* 2005;**16**:3381–3388.
78. Esposito K, Ciotola M, Schisano B, Gualdiero R, Sardelli L, Misso L, Giannetti G, Giugliano D. Endothelial microparticles correlate with endothelial dysfunction in obese women. *J Clin Endocrinol Metab* 2006;**91**:3676–3679.
79. Werner N, Wassmann S, Ahlers P, Kosiol S, Nickenig G. Circulating CD31+/annexin V+ apoptotic microparticles correlate with coronary endothelial function in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 2006;**26**:112–116.
80. Koga H, Sugiyama S, Kugiyama K, Watanabe K, Fukushima H, Tanaka T, Sakamoto T, Yoshimura M, Jinnouchi H, Ogawa H. Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. *J Am Coll Cardiol* 2005;**45**:1622–1630.
81. Lacroix R, Robert S, Poncelet P, Kasthuri RS, Key NS, Dignat-George F, Workshop IS. Standardization of platelet-derived microparticle enumeration by flow cytometry with calibrated beads: results of the International Society on

- Thrombosis and Haemostasis SSC Collaborative workshop. *J Thromb Haemost* 2010;**8**:2571–2574.
82. Wekesa AL, Cross KS, O'Donovan O, Dowdall JF, O'Brien O, Doyle M, Byrne L, Phelan JP, Ross MD, Landers R, Harrison M. Predicting Carotid Artery Disease and Plaque Instability from Cell-derived Microparticles. *Eur J Vasc Endovasc Surg* 2014;**48**:489–495.
 83. Nozaki T, Sugiyama S, Sugamura K, Ohba K, Matsuzawa Y, Konishi M, Matsubara J, Akiyama E, Sumida H, Matsui K, Jinnouchi H, Ogawa H. Prognostic value of endothelial microparticles in patients with heart failure. *Eur J Heart Fail* 2010;**12**:1223–1228.
 84. Sinning JM, Losch J, Walenta K, Bohm M, Nickenig G, Werner N. Circulating CD31+/Annexin V+ microparticles correlate with cardiovascular outcomes. *Eur Heart J* 2011;**32**:2034–2041.
 85. Amabile N, Guerin AP, Tedgui A, Boulanger CM, London GM. Predictive value of circulating endothelial microparticles for cardiovascular mortality in end-stage renal failure: a pilot study. *Nephrol Dial Transplant* 2012;**27**:1873–1880.
 86. Lee ST, Chu K, Jung KH, Kim JM, Moon HJ, Bahn JJ, Im WS, Sunwoo J, Moon J, Kim M, Lee SK, Roh JK. Circulating CD62E+ microparticles and cardiovascular outcomes. *PLoS ONE* 2012;**7**:e35713.
 87. Amabile N, Heiss C, Chang V, Angeli FS, Damon L, Rame EJ, McGlothlin D, Grossman W, De Marco T, Yeghiazarians Y. Increased CD62e(+) endothelial microparticle levels predict poor outcome in pulmonary hypertension patients. *J Heart Lung Transplant* 2009;**28**:1081–1086.
 88. Nozaki T, Sugiyama S, Koga H, Sugamura K, Ohba K, Matsuzawa Y, Sumida H, Matsui K, Jinnouchi H, Ogawa H. Significance of a multiple biomarkers strategy including endothelial dysfunction to improve risk stratification for cardiovascular events in patients at high risk for coronary heart disease. *J Am Coll Cardiol* 2009;**54**:601–608.
 89. Chironi G, Simon A, Hugel B, Del Pino M, Garipey J, Freysson JM, Tedgui A. Circulating leukocyte-derived microparticles predict subclinical atherosclerosis burden in asymptomatic subjects. *Arterioscler Thromb Vasc Biol* 2006;**26**:2775–2780.
 90. Faillle D, Frere C, Cuisset T, Quilici J, Moro PJ, Morange PE, Bonnet JL, Alessi MC. CD11b+ leukocyte microparticles are associated with high-risk angiographic lesions and recurrent cardiovascular events in acute coronary syndromes. *J Thromb Haemost* 2011;**9**:1870–1873.
 91. Sarlon-Bartoli G, Bennis Y, Lacroix R, Piercecchi-Marti MD, Bartoli MA, Arnaud L, Mancini J, Boudes A, Sarlon E, Thevenin B, Leroyer AS, Squarcioni C, Magnan PE, Dignat-George F, Sabatier F. Plasmatic level of leukocyte-derived microparticles is associated with unstable plaque in asymptomatic patients with high-grade carotid stenosis. *J Am Coll Cardiol* 2013;**62**:1436–1441.
 92. Suares R, Padro T, Alonso R, Lopez-Miranda J, Mata P, Badimon L. Circulating CD45+/CD3+ lymphocyte-derived microparticles map lipid-rich atherosclerotic plaques in familial hypercholesterolaemia patients. *Thromb Haemost* 2014;**111**:111–121.
 93. Kanhai DA, Visseren FL, van der Graaf Y, Schoneveld AH, Catanzariti LM, Timmers L, Kappelle LJ, Uiterwaal CS, Lim SK, Sze SK, Pasterkamp G, de Kleijn DP. Microvesicle protein levels are associated with increased risk for future vascular events and mortality in patients with clinically manifest vascular disease. *Int J Cardiol* 2013;**168**:2358–2363.
 94. de Hoog VC, Timmers L, Schoneveld AH, Wang JW, van de Weg SM, Sze SK, van Keulen JK, Hoes AW, den Ruijter HM, de Kleijn DP, Mosterd A. Serum extracellular vesicle protein levels are associated with acute coronary syndrome. *Eur Heart J Acute Cardiovasc Care* 2013;**2**:53–60.
 95. Mayr M, Grainger D, Mayr U, Leroyer AS, Leseche G, Sidibe A, Herbin O, Yin X, Gomes A, Madhu B, Griffiths JR, Xu Q, Tedgui A, Boulanger CM. Proteomics, metabolomics, and immunomics on microparticles derived from human atherosclerotic plaques. *Circ Cardiovasc Genet* 2009;**2**:379–388.
 96. Zampetaki A, Willeit P, Tilling L, Drozdov I, Prokopi M, Renard JM, Mayr A, Weger S, Schett G, Shah A, Boulanger CM, Willeit J, Chowienczyk PJ, Kiechl S, Mayr M. Prospective study on circulating MicroRNAs and risk of myocardial infarction. *J Am Coll Cardiol* 2012;**60**:290–299.
 97. Hinkel R, Penzkofer D, Zuhlke S, Fischer A, Husada W, Xu QF, Baloch E, van Rooij E, Zeiher AM, Kupatt C, Dimmeler S. Inhibition of microRNA-92a protects against ischemia/reperfusion injury in a large-animal model. *Circulation* 2013;**128**:1066–1075.
 98. Schober A, Nazari-Jahantigh M, Wei Y, Bidzhikov K, Gremse F, Grommes J, Megens RT, Heyll K, Noels H, Hristov M, Wang S, Kiessling F, Olson EN, Weber C. MicroRNA-126–5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. *Nat Med* 2014;**20**:368–376.
 99. Thum T. MicroRNA therapeutics in cardiovascular medicine. *EMBO Mol Med* 2012;**4**:3–14.
 100. D'Alessandra Y, Devanna P, Limana F, Straino S, Di Carlo A, Brambilla PG, Rubino M, Carena MC, Spazzafumo L, De Simone M, Micheli B, Biglioli P, Achilli F, Martelli F, Maggolini S, Marenzi G, Pompilio G, Capogrossi MC. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. *Eur Heart J* 2010;**31**:2765–2773.
 101. Cheng Y, Tan N, Yang J, Liu X, Cao X, He P, Dong X, Qin S, Zhang C. A translational study of circulating cell-free microRNA-1 in acute myocardial infarction. *Clin Sci (Lond)* 2010;**119**:87–95.
 102. Ji X, Takahashi R, Hiura Y, Hirokawa G, Fukushima Y, Iwai N. Plasma miR-208 as a biomarker of myocardial injury. *Clin Chem* 2009;**55**:1944–1949.
 103. Wang GK, Zhu JQ, Zhang JT, Li Q, Li Y, He J, Qin YW, Jing Q. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J* 2010;**31**:659–666.
 104. De Rosa S, Fichtlscherer S, Lehmann R, Assmus B, Dimmeler S, Zeiher AM. Transcoronary concentration gradients of circulating microRNAs. *Circulation* 2011;**124**:1936–1944.
 105. Ai J, Zhang R, Li Y, Pu J, Lu Y, Jiao J, Li K, Yu B, Li Z, Wang R, Wang L, Li Q, Wang N, Shan H, Li Z, Yang B. Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. *Biochem Biophys Res Commun* 2010;**391**:73–77.
 106. Adachi T, Nakanishi M, Otsuka Y, Nishimura K, Hirokawa G, Goto Y, Nonogi H, Iwai N. Plasma microRNA 499 as a biomarker of acute myocardial infarction. *Clin Chem* 2010;**56**:1183–1185.
 107. Corsten MF, Dennert R, Jochems S, Kuznetsova T, Devaux Y, Hofstra L, Wagner DR, Staessen JA, Heymans S, Schroen B. Circulating MicroRNA-208b and MicroRNA-499 reflect myocardial damage in cardiovascular disease. *Circ Cardiovasc Genet* 2010;**3**:499–506.
 108. Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, Watanabe S, Baba O, Kojima Y, Shizuta S, Imai M, Tamura T, Kita T, Kimura T. Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. *Circ Cardiovasc Genet* 2011;**4**:446–454.
 109. Wang R, Li N, Zhang Y, Ran Y, Pu J. Circulating microRNAs are promising novel biomarkers of acute myocardial infarction. *Intern Med* 2011;**50**:1789–1795.
 110. Zile MR, Mehurg SM, Arroyo JE, Stroud RE, DeSantis SM, Spinale FG. Relationship between the temporal profile of plasma microRNA and left ventricular remodeling in patients after myocardial infarction. *Circ Cardiovasc Genet* 2011;**4**:614–619.
 111. Long G, Wang F, Duan Q, Yang S, Chen F, Gong W, Yang X, Wang Y, Chen C, Wang DW. Circulating miR-30a, miR-195 and let-7b associated with acute myocardial infarction. *PLoS ONE* 2012;**7**:e50926.
 112. Eitel I, Adams V, Dieterich P, Fuernau G, de Waha S, Desch S, Schuler G, Thiele H. Relation of circulating MicroRNA-133a concentrations with myocardial damage and clinical prognosis in ST-elevation myocardial infarction. *Am Heart J* 2012;**164**:706–714.
 113. Li C, Fang Z, Jiang T, Zhang Q, Liu C, Zhang C, Xiang Y. Serum microRNAs profile from genome-wide serves as a fingerprint for diagnosis of acute myocardial infarction and angina pectoris. *BMC Med Genomics* 2013;**6**:16.
 114. Vogel B, Keller A, Frese KS, Kloos W, Kayvanpour E, Sedaghat-Hamedani F, Hassel S, Marquart S, Beier M, Giannitis E, Hardt S, Katus HA, Meder B. Refining diagnostic microRNA signatures by whole-miRNome kinetic analysis in acute myocardial infarction. *Clin Chem* 2013;**59**:410–418.
 115. Liebetrau C, Mollmann H, Dorr O, Szardien S, Trold C, Willmer M, Voss S, Gaede L, Rixe J, Rolf A, Hamm C, Nef H. Release kinetics of circulating muscle-enriched microRNAs in patients undergoing transcatheter ablation of septal hypertrophy. *J Am Coll Cardiol* 2013;**62**:992–998.
 116. Oerlemans MI, Mosterd A, Dekker MS, de Vrey EA, van Mil A, Pasterkamp G, Doevendans PA, Hoes AW, Sluijter JP. Early assessment of acute coronary syndromes in the emergency department: the potential diagnostic value of circulating microRNAs. *EMBO Mol Med* 2012;**4**:1176–1185.
 117. Widera C, Gupta SK, Lorenzen JM, Bang C, Bauersachs J, Bethmann K, Kempf T, Wollert KC, Thum T. Diagnostic and prognostic impact of six circulating microRNAs in acute coronary syndrome. *J Mol Cell Cardiol* 2011;**51**:872–875.
 118. Zeller T, Keller T, Ojeda F, Reichlin T, Twerenbold R, Tzikas S, Wild PS, Reiter M, Czyz E, Lackner KJ, Munzel T, Mueller C, Blankenberg S. Assessment of microRNAs in patients with unstable angina pectoris. *Eur Heart J* 2014;**35**:2106–2114.
 119. Zernecke A, Bidzhikov K, Noels H, Shagdarsuren E, Gan L, Denecke B, Hristov M, Koppel T, Jahantigh MN, Lutgens E, Wang S, Olson EN, Schober A, Weber C. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci Signal* 2009;**2**:ra81.
 120. Hergenreider E, Heydt S, Treguer K, Boettger T, Horrevoets AJ, Zeiher AM, Scheffer MP, Frangakis AS, Yin X, Mayr M, Braun T, Urbich C, Boon RA, Dimmeler S. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nat Cell Biol* 2012;**14**:249–256.
 121. Matsumoto S, Sakata Y, Suna S, Nakatani D, Usami M, Hara M, Kitamura T, Hamasaki T, Nanto S, Kawahara Y, Komuro I. Circulating p53-responsive microRNAs are predictive indicators of heart failure after acute myocardial infarction. *Circ Res* 2013;**113**:322–326.
 122. Fichtlscherer S, Zeiher AM, Dimmeler S. Circulating microRNAs: biomarkers or mediators of cardiovascular diseases? *Arterioscler Thromb Vasc Biol* 2011;**31**:2383–2390.

123. Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, Mayr A, Weger S, Oberhollenzer F, Bonora E, Shah A, Willeit J, Mayr M. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ Res* 2010;**107**:810–817.
124. Bang C, Batkai S, Dangwal S, Gupta SK, Foinquinos A, Holzmann A, Just A, Remke J, Zimmer K, Zeug A, Ponomaskin E, Schmiedl A, Yin X, Mayr M, Halder R, Fischer A, Engelhardt S, Wei Y, Schober A, Fiedler J, Thum T. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. *J Clin Invest* 2014;**124**:2136–2146.
125. Willeit P, Zampetaki A, Dudek K, Kaudewitz D, King A, Kirkby NS, Crosby-Nwaobi R, Prokopi M, Drozdov I, Langley SR, Sivaprasad S, Markus HS, Mitchell JA, Warner TD, Kiechl S, Mayr M. Circulating microRNAs as novel biomarkers for platelet activation. *Circ Res* 2013;**112**:595–600.
126. Kaudewitz D, Lee R, Willeit P, McGregor R, Markus HS, Kiechl S, Zampetaki A, Storey RF, Channon KM, Mayr M. Impact of intravenous heparin on quantification of circulating microRNAs in patients with coronary artery disease. *Thromb Haemost* 2013;**110**:609–615.
127. Boeckel JN, Thome CE, Leistner D, Zeiher AM, Fichtlscherer S, Dimmeler S. Heparin selectively affects the quantification of microRNAs in human blood samples. *Clin Chem* 2013;**59**:1125–1127.
128. Tunon J, Martin-Ventura JL, Blanco-Colio LM, Lorenzo O, Lopez JA, Egido J. Proteomic strategies in the search of new biomarkers in atherothrombosis. *J Am Coll Cardiol* 2010;**55**:2009–2016.
129. Arab S, Gramolini AO, Ping P, Kislinger T, Stanley B, van Eyk J, Ouzounian M, MacLennan DH, Emili A, Liu PP. Cardiovascular proteomics: tools to develop novel biomarkers and potential applications. *J Am Coll Cardiol* 2006;**48**:1733–1741.
130. Langley SR, Dwyer J, Drozdov I, Yin X, Mayr M. Proteomics: from single molecules to biological pathways. *Cardiovasc Res* 2013;**97**:612–622.
131. Anderson L. Candidate-based proteomics in the search for biomarkers of cardiovascular disease. *J Physiol* 2005;**563**(Pt 1):23–60.
132. Marx V. Targeted proteomics. *Nat Methods* 2013;**10**:19–22.
133. Ramos-Mozo P, Rodriguez C, Pastor-Vargas C, Blanco-Colio LM, Martinez-Gonzalez J, Meilhac O, Michel JB, Vega de Ceniga M, Egido J, Martin-Ventura JL. Plasma profiling by a protein array approach identifies IGFBP-1 as a novel biomarker of abdominal aortic aneurysm. *Atherosclerosis* 2012;**221**:544–550.
134. Cubedo J, Padro T, Badimon L. Coordinated proteomic signature changes in immune response and complement proteins in acute myocardial infarction: the implication of serum amyloid P-component. *Int J Cardiol* 2013;**168**:5196–5204.
135. Barderas MG, Tunon J, Darde VM, De la Cuesta F, Duran MC, Jimenez-Nacher JJ, Tarin N, Lopez-Bescos L, Egido J, Vivanco F. Circulating human monocytes in the acute coronary syndrome express a characteristic proteomic profile. *J Proteome Res* 2007;**6**:876–886.
136. Barderas MG, Tunon J, Darde VM, De la Cuesta F, Jimenez-Nacher JJ, Tarin N, Lopez-Bescos L, Egido J, Vivanco F. Atorvastatin modifies the protein profile of circulating human monocytes after an acute coronary syndrome. *Proteomics* 2009;**9**:1982–1993.
137. Cubedo J, Padro T, Garcia-Moll X, Pinto X, Cinca J, Badimon L. Proteomic signature of Apolipoprotein J in the early phase of new-onset myocardial infarction. *J Proteome Res* 2011;**10**:211–220.
138. Cubedo J, Padro T, Badimon L. Glycoproteome of human apolipoprotein A-I: N- and O-glycosylated forms are increased in patients with acute myocardial infarction. *Transl Res* 2014;**164**:209–222.
139. Akinkuolie AO, Buring JE, Ridker PM, Mora S. A novel protein glycan biomarker and future cardiovascular disease events. *J Am Heart Assoc* 2014;**3**:e001221.
140. Rodriguez-Suarez E, Sivy J, Zurbig P, Mischak H. Urine as a source for clinical proteome analysis: from discovery to clinical application. *Biochim Biophys Acta* 2014;**1844**:884–898.
141. de Kleijn DP, Moll FL, Hellings WE, Oszarlak-Sozer G, de Bruin P, Doevendans PA, Vink A, Catanzariti LM, Schoneveld AH, Algra A, Daemen MJ, Biessen EA, de Jager W, Zhang H, de Vries JP, Falk E, Lim SK, van der Spek PJ, Sze SK, Pasterkamp G. Local atherosclerotic plaques are a source of prognostic biomarkers for adverse cardiovascular events. *Arterioscler Thromb Vasc Biol* 2010;**30**:612–619.
142. Martin-Ventura JL, Blanco-Colio LM, Tunon J, Gomez-Guerrero C, Michel JB, Meilhac O, Egido J. Proteomics in atherothrombosis: a future perspective. *Expert Rev Proteomics* 2007;**4**:249–260.
143. Martin-Ventura JL, Duran MC, Blanco-Colio LM, Meilhac O, Leclercq A, Michel JB, Jensen ON, Hernandez-Merida S, Tunon J, Vivanco F, Egido J. Identification by a differential proteomic approach of heat shock protein 27 as a potential marker of atherosclerosis. *Circulation* 2004;**110**:2216–2219.
144. Blanco-Colio LM, Martin-Ventura JL, Munoz-Garcia B, Orbe J, Paramo JA, Michel JB, Ortiz A, Meilhac O, Egido J. Identification of soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) as a possible biomarker of subclinical atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007;**27**:916–922.
145. Kardys I, Rifai N, Meilhac O, Michel JB, Martin-Ventura JL, Buring JE, Libby P, Ridker PM. Plasma concentration of heat shock protein 27 and risk of cardiovascular disease: a prospective, nested case-control study. *Clin Chem* 2008;**54**:139–146.
146. Carrero JJ, Ortiz A, Qureshi AR, Martin-Ventura JL, Barany P, Heimbürger O, Marron B, Metry G, Snaedal S, Lindholm B, Egido J, Stenvinkel P, Blanco-Colio LM. Additive effects of soluble TWEAK and inflammation on mortality in hemodialysis patients. *Clin J Am Soc Nephrol* 2009;**4**:110–118.
147. Tunon J, Blanco-Colio L, Cristobal C, Tarin N, Higuera J, Huelmos A, Alonso J, Egido J, Asensio D, Lorenzo O, Mahillo-Fernandez I, Rodriguez-Artalejo F, Farre J, Martin-Ventura JL, Lopez-Bescos L. Usefulness of a combination of monocyte chemoattractant protein-1, galectin-3, and N-terminal pro-brain natriuretic peptide to predict cardiovascular events in patients with coronary artery disease. *Am J Cardiol* 2014;**113**:434–440.
148. Duran MC, Martin-Ventura JL, Mohammed S, Barderas MG, Blanco-Colio LM, Mas S, Moral V, Ortega L, Tunon J, Jensen ON, Vivanco F, Egido J. Atorvastatin modulates the profile of proteins released by human atherosclerotic plaques. *Eur J Pharmacol* 2007;**562**:119–129.
149. Alonso-Organza S, Moreno-Luna R, Lopez JA, Gil-Dones F, Padial LR, Moreu J, de la Cuesta F, Barderas MG. Proteomic characterization of human coronary thrombus in patients with ST-segment elevation acute myocardial infarction. *J Proteomics* 2014;**109**:368–381.
150. Ramaola I, Padro T, Pena E, Juan-Babot O, Cubedo J, Martin-Yuste V, Sabate M, Badimon L. Changes in thrombus composition and profilin-1 release in acute myocardial infarction. *Eur Heart J* 2015;**36**:965–975.
151. Inouye M, Kettunen J, Soininen P, Silander K, Ripatti S, Kumpula LS, Hamalainen E, Jousilahti P, Kangas AJ, Mannisto S, Savolainen MJ, Jula A, Leiviska J, Palotie A, Salomaa V, Perola M, Ala-Korpela M, Peltonen L. Metabonomic, transcriptomic, and genomic variation of a population cohort. *Mol Syst Biol* 2010;**6**:441.
152. Inouye M, Ripatti S, Kettunen J, Lyytikäinen LP, Oksala N, Laurila PP, Kangas AJ, Soininen P, Savolainen MJ, Viikari J, Kahonen M, Perola M, Salomaa V, Raitakari O, Lehtimäki T, Taskinen MR, Jarvelin MR, Ala-Korpela M, Palotie A, de Bakker PI. Novel Loci for metabolic networks and multi-tissue expression studies reveal genes for atherosclerosis. *PLoS Genet* 2012;**8**:e1002907.
153. Quehenberger O, Dennis EA. The human plasma lipidome. *N Engl J Med* 2011;**365**:1812–1823.
154. Shah SH, Kraus WE, Newgard CB. Metabolomic profiling for the identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function. *Circulation* 2012;**126**:1110–1120.
155. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011;**472**:57–63.
156. Wurtz P, Raiko JR, Magnussen CG, Soininen P, Kangas AJ, Tynkynen T, Thomson R, Laatikainen R, Savolainen MJ, Laurikka J, Kuukasjarvi P, Tarkka M, Karhunen PJ, Jula A, Viikari JS, Kahonen M, Lehtimäki T, Juonala M, Ala-Korpela M, Raitakari OT. High-throughput quantification of circulating metabolites improves prediction of subclinical atherosclerosis. *Eur Heart J* 2012;**33**:2307–2316.
157. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med* 2013;**368**:1575–1584.
158. Stegmann C, Pechlaner R, Willeit P, Langley SR, Mangino M, Mayr U, Menni C, Moayyeri A, Santer P, Rungger G, Spector TD, Willeit J, Kiechl S, Mayr M. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. *Circulation* 2014;**129**:1821–1831.
159. Suhre K, Shin SY, Petersen AK, Mohny RP, Meredith D, Wagele B, Altmaier E, CardioGram, Deloukas P, Erdmann J, Grundberg E, Hammond CJ, de Angelis MH, Kastenmuller G, Kottgen A, Kronenberg F, Mangino M, Meisinger C, Meitinger T, Mewes HW, Milburn MV, Pohn C, Raffler J, Ried JS, Romisch-Margl W, Samani NJ, Small KS, Wichmann HE, Zhai G, Illig T, Spector TD, Adamski J, Soranzo N, Gieger C. Human metabolic individuality in biomedical and pharmaceutical research. *Nature* 2011;**477**:54–60.
160. Auro K, Joensuu A, Fischer K, Kettunen J, Salo P, Mattsson H, Niironen M, Kaprio J, Eriksson JG, Lehtimäki T, Raitakari O, Jula A, Tiitinen A, Jauhainen M, Soininen P, Kangas AJ, Kahonen M, Havulinna AS, Ala-Korpela M, Salomaa V, Metspalu A, Perola M. A metabolic view on menopause and ageing. *Nat Commun* 2014;**5**:4708.
161. Fischer K, Kettunen J, Wurtz P, Haller T, Havulinna AS, Kangas AJ, Soininen P, Esko T, Tammesoo ML, Magi R, Smit S, Palotie A, Ripatti S, Salomaa V, Ala-Korpela M, Perola M, Metspalu A. Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. *PLoS Med* 2014;**11**:e1001606.
162. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnell CJ, Carr SA, Mootha VK, Florez JC, Souza A,

- Melander O, Clish CB, Gerszten RE. Metabolite profiles and the risk of developing diabetes. *Nat Med* 2011;**17**:448–453.
163. Rhee EP, Cheng S, Larson MG, Walford GA, Lewis GD, McCabe E, Yang E, Farrell L, Fox CS, O'Donnell CJ, Carr SA, Vasan RS, Florez JC, Clish CB, Wang TJ, Gerszten RE. Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. *J Clin Invest* 2011;**121**:1402–1411.
 164. Floegel A, Stefan N, Yu Z, Mühlenbruch K, Drogan D, Joost HG, Fritsche A, Haring HU, Hrabě de Angelis M, Peters A, Roden M, Prehn C, Wang-Sattler R, Illig T, Schulze MB, Adamski J, Boeing H, Pischon T. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. *Diabetes* 2013;**62**:639–648.
 165. Mahendran Y, Vangipurapu J, Cederberg H, Stancakova A, Pihlajamaki J, Soininen P, Kangas AJ, Paananen J, Civelek M, Saleem NK, Pajukanta P, Lusa AJ, Bonnycastle LL, Morken MA, Collins FS, Mohlke KL, Boehnke M, Ala-Korpela M, Kuusisto J, Laakso M. Association of ketone body levels with hyperglycemia and type 2 diabetes in 9,398 Finnish men. *Diabetes* 2013;**62**:3618–3626.
 166. Mahendran Y, Cederberg H, Vangipurapu J, Kangas AJ, Soininen P, Kuusisto J, Uusitupa M, Ala-Korpela M, Laakso M. Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes in Finnish men. *Diabetes Care* 2013;**36**:3732–3738.
 167. Stancakova A, Civelek M, Saleem NK, Soininen P, Kangas AJ, Cederberg H, Paananen J, Pihlajamaki J, Bonnycastle LL, Morken MA, Boehnke M, Pajukanta P, Lusa AJ, Collins FS, Kuusisto J, Ala-Korpela M, Laakso M. Hyperglycemia and a common variant of GSKR are associated with the levels of eight amino acids in 9,369 Finnish men. *Diabetes* 2012;**61**:1895–1902.
 168. Ala-Korpela M. Critical evaluation of ¹H NMR metabolomics of serum as a methodology for disease risk assessment and diagnostics. *Clin Chem Lab Med* 2008;**46**:27–42.
 169. Ala-Korpela M, Sipola P, Kaski K. Characterization and molecular detection of atherothrombosis by magnetic resonance – potential tools for individual risk assessment and diagnostics. *Ann Med* 2006;**38**:322–336.
 170. Roberts LD, Gerszten RE. Toward new biomarkers of cardiometabolic diseases. *Cell Metab* 2013;**18**:43–50.
 171. Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, Ghorbani A, Artati A, Wang Q, Tiainen M, Kangas AJ, Kettunen J, Kaikkonen J, Mikkilä V, Jula A, Kahonen M, Lehtimäki T, Lawlor DA, Gaunt TR, Hughes AD, Sattar N, Illig T, Adamski J, Wang TJ, Perola M, Ripatti S, Vasan RS, Raitakari OT, Gerszten RE, Casas JP, Chaturvedi N, Ala-Korpela M, Salomaa V. Metabolite Profiling and Cardiovascular Event Risk: A Prospective Study of Three Population-Based Cohorts. *Circulation* 2015;**131**:774–785.
 172. Magnusson M, Lewis GD, Ericson U, Orho-Melander M, Hedblad B, Engstrom G, Ostling G, Clish C, Wang TJ, Gerszten RE, Melander O. A diabetes-predictive amino acid score and future cardiovascular disease. *Eur Heart J* 2013;**34**:1982–1989.
 173. Xia J, Broadhurst DI, Wilson M, Wishart DS. Translational biomarker discovery in clinical metabolomics: an introductory tutorial. *Metabolomics* 2013;**9**:280–299.
 174. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multi-variable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. *Circulation* 2015;**131**:211–219.
 175. Steyerberg EW, Vergouwe Y. Towards better clinical prediction models: seven steps for development and an ABCD for validation. *Eur Heart J* 2014;**35**:1925–1931.
 176. Watson AD. Thematic review series: systems biology approaches to metabolic and cardiovascular disorders. Lipidomics: a global approach to lipid analysis in biological systems. *J Lipid Res* 2006;**47**:2101–2111.
 177. Yang R, Chiang N, Oh SF, Serhan CN. Metabolomics-lipidomics of eicosanoids and docosanoids generated by phagocytes. *Curr Protoc Immunol* 2011; Chapter 14:Unit 14.26.
 178. Yetukuri L, Ekroos K, Vidal-Puig A, Oresic M. Informatics and computational strategies for the study of lipids. *Mol Biosyst* 2008;**4**:121–127.
 179. Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH, Bandyopadhyay S, Jones KN, Kelly S, Shaner RL, Sullards CM, Wang E, Murphy RC, Barkley RM, Leiker TJ, Rietz CR, Guan Z, Laird GM, Six DA, Russell DW, McDonald JG, Subramaniam S, Fahy E, Dennis EA. Lipidomics reveals a remarkable diversity of lipids in human plasma. *J Lipid Res* 2010;**51**:3299–3305.
 180. Back M, Dahlen SE, Drazen JM, Evans JF, Serhan CN, Shimizu T, Yokomizo T, Rovati GE. International Union of Basic and Clinical Pharmacology. LXXXIV: leukotriene receptor nomenclature, distribution, and pathophysiological functions. *Pharmacol Rev* 2011;**63**:539–584.
 181. Liu HQ, Zhang XY, Edfeldt K, Nijhuis MO, Idborg H, Back M, Roy J, Hedin U, Jakobsson PJ, Laman JD, de Kleijn DP, Pasterkamp G, Hansson GK, Yan ZQ, NOD2-mediated innate immune signaling regulates the eicosanoids in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2013;**33**:2193–2201.
 182. Manicke NE, Neffliu M, Wu C, Woods JW, Reiser V, Hendrickson RC, Cooks RG. Imaging of lipids in atheroma by desorption electrospray ionization mass spectrometry. *Anal Chem* 2009;**81**:8702–8707.
 183. Stegemann C, Drozdov I, Shalhoub J, Humphries J, Ladroue C, Didangelos A, Baumert M, Allen M, Davies AH, Monaco C, Smith A, Xu Q, Mayr M. Comparative lipidomics profiling of human atherosclerotic plaques. *Circ Cardiovasc Genet* 2011;**4**:232–242.
 184. Ravandi A, Leibundgut G, Hung MY, Patel M, Hutchins PM, Murphy RC, Prasad A, Mahmud E, Miller YI, Dennis EA, Witztum JL, Tsimikas S. Release and capture of bioactive oxidized phospholipids and oxidized cholesteryl esters during percutaneous coronary and peripheral arterial interventions in humans. *J Am Coll Cardiol* 2014;**63**:1961–1971.
 185. Meikle PJ, Wong F, Tsorotes D, Barlow CK, Weir JM, Christopher MJ, MacIntosh GL, Goudey B, Stern L, Kowalczyk A, Haviv I, White AJ, Dart AM, Duffy SJ, Jennings GL, Kingwell BA. Plasma lipidomic analysis of stable and unstable coronary artery disease. *Arterioscler Thromb Vasc Biol* 2011;**31**:2723–2732.
 186. Stock J. The emerging role of lipidomics. *Atherosclerosis* 2012;**221**:38–40.
 187. Rader DJ, Hovingh GK. HDL and cardiovascular disease. *Lancet* 2014;**384**:618–625.
 188. Cuerrier CM, Chen YX, Tremblay D, Rayner K, McNulty M, Zhao X, Kennedy CR, de BelleRoche J, Pelling AE, O'Brien ER. Chronic over-expression of heat shock protein 27 attenuates atherogenesis and enhances plaque remodeling: a combined histological and mechanical assessment of aortic lesions. *PLoS One* 2013;**8**:e55867.
 189. Garcia-Arguinzon M, Padro T, Lugano R, Llorente-Cortes V, Badimon L. Low-density lipoproteins induce heat shock protein 27 dephosphorylation, oligomerization, and subcellular relocalization in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2010;**30**:1212–1219.
 190. Martin-Ventura JL, Nicolas V, Houard X, Blanco-Colio LM, Leclercq A, Egido J, Vranckx R, Michel JB, Meilhac O. Biological significance of decreased HSP27 in human atherosclerosis. *Arterioscler Thromb Vasc Biol* 2006;**26**:1337–1343.
 191. Raizman JE, Chen YX, Seibert T, Hibbert B, Cuerrier CM, Salari S, Zhao X, Hu T, Shi C, Ma X, Simard T, Caravaggio J, Rayner K, Bowdish D, Moore K, O'Brien ER. Heat shock protein-27 attenuates foam cell formation and atherogenesis by down-regulating scavenger receptor-A expression via NF-kappaB signaling. *Biochim Biophys Acta* 2013;**1831**:1721–1728.
 192. Rayner K, Chen YX, McNulty M, Simard T, Zhao X, Wells DJ, de BelleRoche J, O'Brien ER. Extracellular release of the atheroprotective heat shock protein 27 is mediated by estrogen and competitively inhibits acLDL binding to scavenger receptor-A. *Circ Res* 2008;**103**:133–141.
 193. Chorianopoulos E, Jarr K, Steen H, Giannitsis E, Frey N, Katus HA. Soluble TWEAK is markedly upregulated in patients with ST-elevation myocardial infarction and related to an adverse short-term outcome. *Atherosclerosis* 2010;**211**:322–326.
 194. Fernandez-Laso V, Sastre C, Valdivielso JM, Fernandez E, Martin-Ventura JL, Egido J, Blanco-Colio LM. Soluble TWEAK levels predict the presence of carotid atherosclerotic plaques in subjects free from clinical cardiovascular diseases. *Atherosclerosis* 2015;**239**:358–363.
 195. Moreno JA, Munoz-Garcia B, Martin-Ventura JL, Madrigal-Matute J, Orbe J, Paramo JA, Ortega L, Egido J, Blanco-Colio LM. The CD163-expressing macrophages recognize and internalize TWEAK: potential consequences in atherosclerosis. *Atherosclerosis* 2009;**207**:103–110.
 196. Richter B, Rychli K, Hohensinner PJ, Berger R, Mortl D, Neuhold S, Zorn G, Huber K, Maurer G, Wojta J, Pacher R, Hulsman M, Niessner A. Differences in the predictive value of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in advanced ischemic and non-ischemic heart failure. *Atherosclerosis* 2010;**213**:545–548.
 197. Urbonaviciene G, Martin-Ventura JL, Lindholt JS, Urbonavicius S, Moreno JA, Egido J, Blanco-Colio LM. Impact of soluble TWEAK and CD163/TWEAK ratio on long-term cardiovascular mortality in patients with peripheral arterial disease. *Atherosclerosis* 2011;**219**:892–899.
 198. Riccioni G, Back M, Capra V. Leukotrienes and atherosclerosis. *Curr Drug Targets* 2010;**11**:882–887.
 199. Back M, Hansson GK. Anti-inflammatory therapies for atherosclerosis. *Nat Rev Cardiol* 2015;**12**:199–211.
 200. Saito R, Matsuzaka T, Karasawa T, Sekiya M, Okada N, Igarashi M, Matsumori R, Ishii K, Nakagawa Y, Iwasaki H, Kobayashi K, Yataoh S, Takahashi A, Sone H, Suzuki H, Yahagi N, Yamada N, Shimano H. Macrophage Elovl6 deficiency ameliorates foam cell formation and reduces atherosclerosis in low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 2011;**31**:1973–1979.