The Use of High-Throughput Technologies to Investigate Vascular Inflammation and Atherosclerosis

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Abstract—The greatest challenge of scientific research is to understand the causes and consequences of disease. In recent years, great efforts have been devoted to unraveling the basic mechanisms of atherosclerosis (the underlying pathology of cardiovascular disease), which remains a major cause of morbidity and mortality worldwide. Because of the complex and multifactorial pathophysiology of cardiovascular disease, different research techniques have increasingly been combined to unravel genetic aspects, molecular pathways, and cellular functions involved in atherogenesis, vascular inflammation, and dyslipidemia to gain a multifaceted picture addressing this complexity. Thanks to the rapid evolution of high-throughput technologies, we are now able to generate large-scale data on the DNA, RNA, and protein levels. With the help of sophisticated computational tools, these data sets are integrated to enhance information extraction and are being increasingly used in a systems biology approach to model biological processes as interconnected and regulated networks. This review exemplifies the use of high-throughput technologies—such as genomics, transcriptomics, proteomics, and epigenomics—and systems biology to explore pathomechanisms of vascular inflammation and atherosclerosis. (Arterioscler Thromb Vasc Biol. 2012;32:182-195.)

Key Words: atherosclerosis ■ coronary artery disease ■ vascular biology ■ high-throughput ■ systems biology

As the underlying pathology of cardiovascular disease, atherosclerosis entails a chronic inflammation of the vascular wall, which is initiated by vascular structural alterations and the subendothelial accumulation of low-density lipoprotein (LDL). On oxidation, oxidized LDL elicits the activation of endothelial cells (ECs), mediating leukocyte recruitment. Intimal neutrophils, T cells, and monocyte-derived macrophages contribute to sustained inflammation, causing early fatty streak lesions to progress into advanced fibroatheromatous plaques, which, on gradual thinning of the protective smooth muscle cell (SMC) cap, transform into rupture-prone lesions.1 Although atherosclerosis starts in childhood, it is mostly diagnosed only at a late stage. Optimized treatment requires the understanding of the disease-associated mechanisms. However, atherosclerosis is a multifactorial disease and results from an intricate interplay of multiple genes, signaling pathways, cells, and tissues. Also, many environmental factors, such as hyperlipidemia and hypertension, have been identified as risk factors for atherosclerosis, which further increases the complexity of the disease. This review discusses the use of high-throughput approaches to study atherosclerosis, and also briefly addresses cerebrovascular diseases (focusing on stroke) and peripheral artery disease (PAD). Although the latter is a direct consequence of atherosclerosis, cerebrovascular diseases comprise a variety of disorders (eg, vascular dementia or subarachnoid hemorrhage), with stroke being the most numerous. About 80% of strokes are ischemic, and atherosclerosis, but cardioembolism mostly triggered by atrial fibrillation—and small-vessel disease are also very common causes.2,3 The multifaceted pathomechanisms underlying all these diseases necessitate a holistic study approach. Therefore, in vitro experiments, which lack the complex organization of an organism, need to be combined with clinical and animal studies. In atherosclerosis research and many other disorders, mice are the gold standard, and although they do not meet all criteria of human diseases, they offer great advantages because of the relatively low cost, easy access, high fertility, and availability of numerous biological tools, including genetic modifications.4–6 However, not only mice but also other animals, such as rabbits, hamsters, pigs, and dogs, have been used to study atherosclerosis and vascular inflammation.7,8

Importantly, animal models provide the opportunity to apply high-throughput technologies, which enable the generation of large-scale data on DNA, RNA, and protein levels, to a broad variety and high number of cell and tissue samples, including those that are not available from humans (eg, genetically modified background). Furthermore, computational tools have been developed to integrate all these...
“omics”-based data for improved information extraction. In addition, biological processes are increasingly being approached from a systems biology point of view, aiming to model them as interconnected networks, and this has revealed information on disease-associated mechanisms that would never have been discovered with a classical approach.9

This review outlines the use of high-throughput technologies in the research field of vascular inflammation and cardiovascular disease. The widespread application of genomics, transcriptomics, and proteomics in biomedical research makes it impossible to address these techniques in a detailed way. Instead, we discuss selected studies to exemplify the context of their usage, their advantage versus classical small-scale approaches, and their current limitations. In addition, we address the rising field of epigenomics in cardiovascular disease.

We sincerely apologize to all scientists whose important contributions to the field could not be cited because of space constraints.

Genomics
Genomics involves the genome-wide sequencing and annotation of an organism’s DNA, including the identification of open reading frames and regulatory elements, such as transcription factor binding sites. The sequencing of the complete human genome and the rise of high-throughput technologies as DNA microarray multiplex genotyping, enabling genome-and population-wide screens of single-nucleotide polymorphisms (SNPs) or copy number variations, has triggered an explosion of genome-wide association studies (GWASs), aiming to identify disease-associated genetic loci using disease quantitative trait locus (QTL) mapping.10 Furthermore, metaanalyses of multiple GWAS data sets have considerably increased the power to detect genetic disease associations.10

In this way, many susceptibility loci have been identified for coronary artery disease (CAD) and myocardial infarction,1,11–13 as discussed in more detail in another review of this series. Also, chromosomal loci associated with stroke, PAD, and serum lipid levels have been revealed.14–17 However, many of the identified loci still require independent replication to avoid false-positive results.18–20 Studies examining PAD-associated chromosomal loci to unravel disease mechanisms are scarce.21–23 Notably, several approaches have been made to open up the way for successful GWAS in addressing PAD with the help of electronic medical records, including the storage of DNA and plasma samples, as well as demographic data and laboratory values.24,25

Despite the recent explosion of GWAS-generated disease-associated susceptibility loci, single genetic loci or even their combination can only explain a small fraction of the risk for multifactorial diseases, such as atherosclerosis.9 In addition, GWASs have the considerable drawback that the identified susceptibility loci mostly do not reveal direct information on disease genes or affected pathways, which is crucial for therapeutic drug discovery. Therefore, human GWAS-generated susceptibility loci have been combined with corresponding mouse disease QTLs in a cross-species comparative genomics approach to narrow down susceptibility intervals.26 In addition, complementation of clinical trait QTL data with transcriptomics17,27,28 and expression QTL (eQTL) mapping29–32, which aims to map chromosomal loci that are strongly associated with RNA expression (expression SNPs), can considerably facilitate candidate disease gene identification. For example, combination of GWAS and mouse clinical QTLs identified L3q1, a locus on mouse chromosome 7, to be responsible for the infarct volume of ischemic stroke in mice, and the risk allele of SNP rs4783244 is significantly associated with decreased plasma adiponectin levels, pointing to an increased risk of ischemic stroke.33,34 Furthermore, a genome-wide SNP scan and atherosclerosis QTL mapping in intercrosses of mouse strains with different atherosclerosis-susceptibility were combined with macrophage gene expression profiling and transcription factor binding site analysis to reveal genes and promoter elements associated with atherosclerosis susceptibility. The genes Wbscr and Aps, which were differentially expressed in macrophages from mice with high versus low atherosclerosis susceptibility, mapped close to a specific atherosclerosis QTL on chromosome 5, indicating these genes as potential candidates influencing atherosclerosis risk.35 In a comparable approach, QTL mapping for atherosclerotic plaque size in a segregating mouse population with variable atherosclerosis susceptibility was combined with transcriptome analysis of liver and adipose tissue and subsequent eQTL mapping, allowing a prioritization of the atherosclerosis QTL-associated candidate genes based on colocalization with cis-acting eQTLs.29 Similarly, eQTLs affecting gene expression in human liver were screened for correlation with CAD-associated QTLs identified by GWASs, revealing the genes SORT1, PSRC1, CELSR2, and SYPL2 on chromosome 1p13.3 as potential candidates for CAD development. Transcriptional analysis of these genes in the liver of a segregating mouse population with varying atherosclerosis risk furthermore indicated expression levels of Sort1, PSRC1 and CELSR2, but not SYPL2, to be significantly associated with plasma LDL cholesterol levels, a risk factor for CAD. Combination of genotypic and transcriptomic data enabled the modeling of a genome-wide gene network for both human and mouse and revealed the 3 genes to colocalize in a species-conserved subnetwork causally associated with cholesterol levels and atherosclerosis.31

The combination of clinical trait QTL mapping with expression profiling or eQTL mapping in a network-based framework is called systems genetics or integrative genetics, and it has also been successfully applied to identify genes and pathways underlying other complex diseases, such as obesity.36,37

Network modeling is further discussed below, but a more comprehensive application of comparative genomics is outlined in a recent review.26 Whether genetic screening will also be useful beyond cardiovascular disease gene identification (eg, for disease classification, outcome prediction, or therapy guidance) remains unclear.17,38,39

Transcriptomics
Transcriptomics entails the genome-wide study of RNA expression, mostly through microarray analysis. It is a widely applied technique for studying mechanisms underlying diseases, and it has also extensively been used to study expression profiles of atherosclerotic-prone or atherosclerotic ves-
sels, predominantly in human and mouse, but also in rat, monkey, and pig. Macromolecule and laser-capture microdissection and laser-capture microdissection have furthermore enabled scientists to isolate and analyze specific subregions within atherosclerotic vessels or lesions, such as, e.g., plaque area versus media and adventitia, fibrous cap, lesion shoulder, or adventitial aortic tertiary lymphoid organs. In addition, these methods have allowed the enriched isolation of particular cell types, such as ECs, SMCs, and hepatocarcinoma cells treated with orvastatin or pitavastatin showed statins to affect genes involved in coagulation, cell growth, and vascular constriction, providing insight into statins’ cellular effects.

Finally, profiling of microRNAs, which are major regulators of mRNA expression, can also offer insights in atherosclerosis-related mechanisms, and has been performed on injured vascular walls, plasma, and vascular cell types. For instance, Fichtlscherer et al detected a significant reduction in the plasma concentration of vascular and inflammatory cell-derived microRNAs in patients with CAD, whereas cardiac muscle-microRNAs showed an increased trend. Whether specific microRNAs could also be used as biomarkers to predict the course of disease or classification remains to be further examined.

In the context of cerebrovascular disease and stroke, transcriptomic analysis was mainly set up to gain insight in the blood transcriptome after injury. Stroke-associated gene expression changes, for example, may be reflected within this transcriptome and therefore mediate cerebral inflammatory changes. In addition, animal models comparing changes in gene expression in cerebral tissue revealed an important role for blood microRNAs, which have also gained interest as biomarkers for brain injury. As an example, microRNA-124 has recently been described as biomarker for cerebral infarction. Another study explored the transcriptomic profiling of cellular pathways being regulated after an in vivo transient cerebral ischemic stroke in mice mediated in the presence and absence of glutathione peroxidase 1. Glutathione peroxidase 1 is an enzyme that is important in reduction of oxidative stress, and its lack has been implicated in neurodegenerative disorders and stroke. Here, the authors were able to show that glutathione peroxidase 1 plays a crucial role in the protection against oxidative stress and inflammation during ischemia-reperfusion injury.

Regarding PAD, Zhao et al performed a microarray analysis of 30 femoral artery samples identifying, for example, MAP4K4 as being differentially upregulated in intermediate and advanced lesions. Surprisingly, many of the differentially regulated genes in this study have not been reported to be related to atherosclerosis before. A similar approach was used by Evans et al, who classified 335 genes as differentially regulated between normal and diseased arteries. Moreover, they were able to acknowledge a gene expression signature for PAD and an associated diabetic phenotype in PAD.

**Proteomics**

Dynamic proteins are key regulators of biological functions. The total protein complement of an organism and its genome is called proteome. Methods trying to unravel protein structure, expression, modification, localization and protein-protein interactions are referred to as proteomics. Most important proteomic methods comprise 2-dimensional gel electrophoresis (2-DE) or 2D differential in-gel electrophoresis, which is an improved 2-DE, including the fluorescent labeling of the different protein suspensions investigated. Moreover, gel–liquid chromatography–tandem mass spec-
trometry and shotgun (gel-free) proteomics are used to explore proteomic functions. Using gel-based proteomics, proteins are first separated by 1- or 2-DE before enzymatic digestion and mass spectrometry (MS) analysis. Shotgun proteomics uses the digested protein mixture directly without the need for gel separation.79,82

In the past decade, several attempts were made to exploit the VSMC83–85 and EC86–88 proteomes in different species; however, identification of signaling networks is complex.80 For example, proteomic studies, including Western array analysis, screened human carotid artery specimens for key proteins and identified numerous genes which are differentially expressed under atherosclerotic conditions, eg, apoptosis linked gene 2 (ALG2) or Annexin 1.89,90 Furthermore, metabolic and proteomic investigations of SMCs from Apoe−/− mice under normoxic versus hypoxic conditions showed a differential regulation of key enzymes in glucose metabolism, resulting in faster glucose consumption under hypoxia and a compensatory reduction in baseline interleukin (IL)-6 secretion, which was associated with a marked upregulation of insulin-like growth factor binding protein 3.91 However, direct conclusions identifying signaling pathways remain elusive in the above-mentioned studies. Nevertheless, other studies, addressing more specific questions, did investigate signaling pathways by proteomic approaches: 2-DE identified the correlation of the strongly anti-apoptotic heat shock protein (HSP)-27 phosphorylation level with cardiovascular diseases, by identifying LDL as an important regulator of HSP27 phosphorylation involving p38–mitogen-activated protein kinase signaling in VSMCs.92 Moreover, 2-DE and MS revealed that paracrine secretion of platelet-derived growth factor–BB by ECs is implicated in the pathophysiological vascular remodeling induced by low shear stress, involving the phosphorylation of extracellular signal-regulated kinases (ERK1 and 2, which belong to the mitogen-activated protein kinase family.93 In addition, the importance of ERK1 and ERK2 phosphorylation was shown in vitro in bovine aortic ECs after stimulation with oxidized phospholipids using liquid chromatography-mass spectrometry analysis.94 Supplementally, proteomics has also been proven to be of emerging interest in atrial fibrillation–associated stroke,3 and tobacco use.98,99

Importance of ERK1 and ERK2 phosphorylation was shown in the development of metabolic diseases.108–110 Furthermore, metabolomics were also used to study and introduce metabolic biomarkers of stroke and cerebrovascular diseases. Jung et al were able to demonstrate, that plasma of stroke patients profiles did more accurately predict the diagnosis of multiple sclerosis than anticipated cerebrovascular disease.112 Hence, metabolomics will provide further insight into the mechanisms of cardiovascular physiology and may lead to improved therapeutic approaches.

A main risk factor for atherosclerosis is hypertlipidemia, caused by an imbalanced lipid metabolism of multifactorial
origin. The lipidome of an organism comprises all lipid molecules, which are additionally divided into lipid classes, and their associated interacting factors. Therefore, lipidomics is of outstanding interest for atherosclerotic research, also with regard to lipid distribution within cells and biochemical mechanisms mediated by lipids. The most important methods to enable high-throughput and high-quality lipid analysis are MS and lipid biochemistry, which make simultaneous identification and quantification of hundreds of molecular lipids within 1 species possible.113,114 For example, comparing fractionized serum samples of diabetic patients versus healthy controls by liquid chromatography-mass spectrometry indicated that the serum concentrations of specific triacylglycerol acids are more accurate markers for insulin resistance and abdominal obesity than total serum triacylglycerol concentrations. Thus, fatty acid characteristics may become important markers identifying individual risk profiles for various diseases.115 Furthermore, 1 example of how lipids may affect pathway signaling has been nicely discussed by Wheelock et al, who revealed 77 biochemical pathways to be differentially affected between low and high fat cholesterol diet in mice, by combining liver transcriptomics and lipidomics data and mapping them onto a metabolic pathway database.116 System biology approaches like those introduced by Wheelock et al116 are very helpful to process high-throughput data, which is further discussed below. In addition, changes in lipid profiles, including phospholipids, ceramides, sphingomyelin, cerebrosides, cholesterol, and their oxidized derivatives, may also affect or be indicative of ischemic stroke, as indicated by Fonteh and Fisher117 and by Fonteh et al.118

Protein microarrays are an emerging tool, competing with traditional gel-based methods (eg, 2-DE), offering a higher reproducibility and the possibility of high-throughput approaches. These microarrays mostly consist of thousands of immobilized proteins spotted to a glass slide and enable simultaneous analysis of various protein-protein interactions at once.119,120 Another example are cytokine antibody arrays, which use membrane-spotted capture antibodies to investigate the cytokine profile of biological fluids, a technique that became famous in cancer research to detect tumor markers.121 An example of its utility in vascular inflammation, the use of a cytokine array detecting 36 proteins released from vascular endothelial growth factor (VEGF)–stimulated ECs but not

RNA Interference

The term RNA interference (RNAi) describes an RNA-dependent gene silencing process, which is initiated by short double-stranded RNA, resulting in a reduction of targeted mRNA levels. RNAi molecules comprise sets of double-stranded RNA, short hairpin (sh)RNA, silencing RNAs (siRNAs) or endoribonuclease-prepared siRNAs. These small molecules are able to induce a reduction-of-gene-function, which may lead to a phenotypically detectable variation. In turn, this variation may be monitored by specific assays. RNAi offers the possibility of performing both small-scale studies targeting 1 gene or high-throughput approaches investigating numerous genes and their function at once, though of course careful data interpretation is needed.126 Although there are recent reviews126–129 and original studies130,131 summarizing and investigating high-throughput RNAi screening to unravel cellular signaling in general, studies addressing signaling cascades in vascular inflammation rather concentrated on knockdown of specific gene functions instead of investigating on large-scale basis. For example, silencing of bone morphogenetic proteins/Sma and Mad related proteins signaling in human aortic ECs,132 downregulation of tumor necrosis factor–dependent nuclear factor-κB activation in ECs,133 or silencing of P13-AKT signaling in human umbilical vein ECs134 was shown to have atheroprotective effects on the vascular endothelium. Furthermore, it was shown that simultaneous downregulation of the VEGF receptor 2/3-, CXCR4-, and CCR7-dependent signaling axes via AKT, ERK1/2, and p38 signaling pathways had a greater inhibitory effect on angiogenesis and cell growth than the blockade of each individual axis.135 These results may be of special interest regarding atherosclerosis because the CXCR4/CXCL12 axis in ECs seems to play a protective role in atherosclerosis.136,137 One other recent study of outstanding interest identified candidate genes involved in cellular cholesterol metabolism by transcriptional profiling of sterol-depleted HeLa cells, literature mining and a “multiple-gene” RNAi screen, which combined reverse siRNA transfection on siRNA microarrays with high-content screening microscopy and quantitative image analysis software to analyze effects on total cholesterol levels and LDL uptake efficiency.138 Thus, these findings may offer subsequent therapeutic approaches in terms of atherosclerosis or even Niemann-Pick disease type C, which is an inherited lipid storage disorder, characterized by a defect in intracellular trafficking of exogenous cholesterol and glycosphingolipids, and in which regulators of cellular cholesterol accumulation have also been studied through an RNAi screen.139 In addition, RNAi was helpful to unravel mechanisms important in cerebral ischemia-induced vascular and brain injury. Here, Yin et al. were able to show that Pparα expression in mouse aortic VSMCs can prevent ischemic brain injury by inhibition of matrix metalloproteinase-9 activation and attenuation of postischemic inflammation in mice. RNAi-mediated knockdown of matrix metalloproteinase-9 in Pparα knockout mice phenocopied these effects, rendering pharmacological activation of Pparα a promising therapeutic tool for stroke-induced vascular and neuronal damage.140 Similarly to matrix metalloproteinase-9, silencing of caspase-3 in mice by siRNA
treatment also exhibited beneficial effects in the cure of stroke. Another approach, investigating the plasma concentrations of circulating angiogenesis-related factors like VEGF, placenta growth factor, and thrombospondin-1 in 184 PAD patients and in 330 paired healthy controls demonstrated an increase of TSP-1 plasma levels in the disease group. Additional in vitro studies, knocking down TSP-1 with siRNA in human endothelial colony-forming cells, unraveled a potential mechanism by which increased TSP-1 levels might be responsible for inadequate neovascularization. Hence, targeting TSP-1 ex vivo or in vivo might have the potential to modulate angiogenesis in PAD patients. Overall, RNAi is a powerful tool to investigate inflammatory networks and cellular signaling, although it is still mostly used on a small scale in the context of vascular inflammation and cardiovascular disease.

**Epigenomics**

Epigenomics involves the study of phenotypic or gene expression changes caused by inheritable mechanisms independent of DNA sequence, eg, DNA methylation and histone posttranslational modifications. Also, noncoding RNA alterations can be classified as epigenetic mechanisms, as they have been shown to mediate epigenetic DNA and histone modifications. Epigenetic marks are increasingly being recognized as important elements underlying phenotypic variation, biological processes, inflammation, and diseases such as cancer, and large-scale epigenome projects have been set up in an attempt to create reference human epigenome maps. High-throughput techniques enabling epigenetic analysis on a genome-wide scale include, eg, shotgun bisulfite sequencing and pyrosequencing to analyze DNA methylation, and genome-scale chromatin immunoprecipitation with antibodies recognizing specific histone modifications or DNA methylation, followed by microarray analysis or high-throughput sequencing. Alternative methods to examine DNA methylation are differential methylation hybridization, which encompasses methylation-sensitive DNA restriction followed by microarray analysis or high-throughput sequencing. Epigenetic marks are increasingly being recognized as important elements underlying phenotypic variation, biological processes, inflammation, and diseases such as cancer, and large-scale epigenome projects have been set up in an attempt to create reference human epigenome maps.

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Analysis and Integration of High-Throughput Data: Network Modeling and Systems-Based Biology

All these “-omics” techniques generate an enormous wealth of data, urging computational methods to extract all con-
Systems biology aims to model biological processes as networks, generating graphical maps consisting of nodes and edges representing the individual system components and their relations, respectively. For a protein network, for example, edges can represent physical protein interactions, functional similarities, or the regulation of one protein by another. A high internal connection between a subset of nodes results in their clustering into modules, which represent key control points within the network. By combining multiple high-throughput data sets generated by in vitro and in vivo studies, with intensive literature mining and biomolecular databases of, eg, gene classification, protein interactions and pathways, network analysis has the strength to reveal disease-associated components and connections that would not have been discovered with classical approaches. Furthermore, mathematical models can be developed to simulate network behavior on perturbations. Iterative optimization of the model by additional, hypothesis-driven system perturbations and data integration is essential to increase its precision and its ability to accurately predict the dynamics of the system studied (for example, a patient’s response to drugs, or disease progression) (Figure). Although the development of such mathematical, disease-predicting models is still in its infancy, network analysis of high throughput data sets has already proven a powerful tool to increase our insights into complex diseases.

In the context of vascular inflammation and atherosclerosis, network analysis combining transcriptomics and literature mining revealed candidate gene sets involved in human in-stent restenosis and coronary and carotid atherosclerosis. Transcriptional profiling of lesions in atherosclerosis-prone mice with a human-like hypercholesterolemia and a genetic switch to lower plasma LDL cholesterol revealed a set of cholesterol-responsive atherosclerosis genes, of which a subset could be modeled into a gene regulatory network of foam cell formation based on additional expression profiling of acetylated LDL-exposed macrophages targeted with specific siRNAs. In the same context, network modeling and pathway analysis of transcriptomics data from oxidized LDL-treated macrophages identified biological processes relevant to foam cell formation, such as regulation of macrophage differentiation and lipoprotein catabolic processes. A pathway analysis approach has also been successfully applied on CAD-associated GWAS data, identifying perturbations in...
networks and identify modules that were significantly associated with lipoprotein metabolism were used to model gene coexpression in inbred mouse strains with dramatic differences in high-density lipoprotein metabolism. The latter furthermore exemplifies that the integration of the modeled network with CAD-associated GWAS data sets. The latter furthermore exemplifies that the integration of expression profiling or eQTL mapping with GWAS data increases the power to detect disease-associated genes, as also discussed above, and the simultaneous analysis of genotype, transcriptome, and clinical trait can even enable the construction of networks that can predict causal relationships between genes and disease.

A systems biology approach has also been used to meet the complexity of the proangiogenic function of the VEGF family, not only in terms of CAD, but also for stroke and PAD. In addition, a predictive network model, evaluating the transcriptome of whole blood from a mouse model of neuroprotection in ischemic stroke, was described, and it proved to be able to precisely predict mammalian system behavior under novel conditions.

Far from being complete, these examples demonstrate how network modeling has contributed to the analysis of high-throughput data sets and the identification of new cardiovascular disease-associated genes and pathways that would not have been discovered with the traditional, single-analysis approaches. In this way, networks could trigger the development of new diagnostic markers (eg, of atherosclerosis) by revealing targets that accurately predict lesion vulnerability. Accordingly, systems biology and predictive network modeling may be used to develop concrete clinical applications helping to improve, for example, patient selection or monitoring of stroke preventive intervention. In addition, the subdivision of networks of multifactorial diseases in subnetworks or modules indicates that multiple genes and pathways probably need to be tackled together to treat the disease as efficient as possible, and could offer help in the design of a combination therapy. For more detailed information on network modeling, and an overview of currently available tools and databases, we refer to previous in-depth reviews.

**Evaluation and Conclusions**

High-throughput technologies comprising all omics techniques, as well as RNAi, offer great advantages to profile the complex pathophysiology of human cardiovascular disease. The combination of clinical trait QTLs and eQTLs enables the matching of a specific chromosomal locus to a phenotypic trait through gene expression. In addition, proteomic techniques such as 2-DE or LC/MS offer the possibility of identifying altered or differentially expressed proteins, which influence cellular signaling cascades or protein phosphorylation. In the same context, protein arrays explore protein contents of biological fluids. Moreover, metabolomics permits insight into dynamic and unique aspects of cellular homeostasis and general metabolism, similar to lipidomics, which helps to understand imbalances in lipid metabolism, the latter being of outstanding interest in atherosclerosis. In addition, approaches using RNAi arrays enable the specific knockdown of numerous target genes, leading to the detection of signaling pathways and subsequent phenotypic changes. Finally, epigenomics is rapidly gaining interest. It is not only expected to fill the gaps in our knowledge of cardiovascular disease risk but also represents an interesting area for drug discovery, given the reversible nature of epigenetic patterns.

It is expected that the refinement, expansion, and cost reduction of high-throughput methods in all these fields will increase data generation even more. However, the main challenge will be to extract all hidden information to enhance our insights in the pathophysiological processes of cardiovascular disease, and to trigger clinical application. For example, genomic information is not yet widely applied in clinical cardiology, in contrast to clinical practice in oncology. One reason could be the missing heritability of GWAS-identified risk SNPs, which may be due to low-frequency variants and epigenetics. Another reason is the lack of functional annotations of these SNPs and their links with cardiovascular disease. To fulfill the requirements of a clinical use, for example as a biomarker, these polymorphisms would have to meet the criteria of a preclinical marker, which is able to indicate pathogenic mechanisms for concrete diagnostic or...
interventional approaches. Likewise, epigenetic changes require translation into associated alterations in gene regulation and cellular signaling. Therefore, integration of all omics techniques is of extreme importance to extract functional links between omics-generated data sets and pathological processes, and to open the way for novel drug discovery. In this context, combination of genomics with transcriptomics has indeed proven to be very successful in identifying disease-associated genes and pathways. Enhanced integration of multidimensional data sets will unequivocally require systems biology approaches. Ultimately, it is hoped that accurate network modeling of diseases in such systems biology approach can be used to predict therapeutic responses and potential side effects and to trigger personalized medicine by integrating complex genetic and environmental aspects of multifactorial diseases, such as cardiovascular disease. Given these extreme challenges, the future success of systems biology requires a continuous expansion and refinement of software for high-throughput data acquisition, storage and integrative analysis through network-based approaches. Obviously, clear ethical guidelines how to deal with results, also in terms of, for example, life insurance, will be indispensable. Overall, high-throughput techniques are promising tools to unravel the complex biological networks underlying vascular inflammation and atherosclerosis.

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