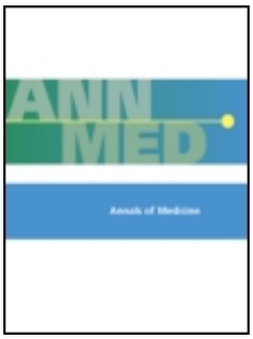

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REVIEW ARTICLE

Genetics, genomics and proteomics in atherosclerosis research

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Abstract
Atherosclerosis and its clinical manifestations are the leading cause of death in Western countries. Atherosclerosis is a multifactorial disease characterized by endothelial dysfunction, smooth muscle cell (SMC) proliferation and migration, inflammation, lipid and matrix accumulation and thrombus formation. Multiple genetic and environmental features and interactions between these factors influence the disease process. To understand fundamental pathobiological mechanisms in atherogenesis and to develop and target new therapies, information on genetic factors (atherogenetics), gene expression patterns (atherogenomics) and protein expression patterns (atheroproteomics) are needed. This review will summarize current knowledge in these areas of atherosclerosis research with a special emphasis on microarray technology.

Key words: *Atherosclerosis, gene expression, genetics, genomics, macrophages, proteomics*

Atherogenetics

Atherogenesis involves several cell types and physiological processes, such as inflammation and lipid accumulation (1). Thus, it is not surprising that the genetic basis of atherosclerosis is complex. The heritability component of cardiovascular diseases (CVD) in most populations has been estimated to be between 40% and 60% (2). Single-gene disorders, such as familial hypercholesterolemia resulting from defects in low density lipoprotein (LDL) receptor and Tangier disease resulting from defects in ATP-binding cassette (ABC) transporters, are the most dramatic examples of the genetic contribution to atherosclerosis. However, it is clear that only a minor portion of cardiovascular diseases are due to single-gene disorders and they are mainly influenced by complex multigene patterns with multiple environmental interactions. It is also worth noting that in addition to the disorders of lipid metabolism many of the common risk factors of atherosclerosis, such as elevated blood pressure and type 2 diabetes, have a strong genetic component (3). Additionally, it has been noticed that genetic factors may partly explain the effect of environmental factors on CVD development. For example, it has been shown that there is a link between the effect of smoking on CVD progression and the apolipoprotein E (ApoE) phenotype: smokers who carry ApoE E4 allele have a particularly high risk of CVD (4).

Classically, the genetic basis of atherosclerosis has been studied by association studies and linkage analyses, and several new candidate regions have been revealed which may contain novel and unknown candidate genes (3,5). For example, by meta-analysis of four linkage analyses, Chiodini et al. concluded that genetic regions 3q26–27 and 2q34–37, which also show linkages to type 2 diabetes and contain genes involved in glucose homeostasis and lipid metabolism, may contain novel susceptibility genes for CVD (5). As a drawback, these methods have shown very limited power, and the results have significantly differed between the researchers and study populations. However, the identification of hundreds of thousands of single nucleotide polymorphisms (SNPs) has entailed

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Abbreviations

ApoE	apolipoprotein E
CETP	cholesteryl ester transfer protein
CRP	C-reactive protein
CSF	colony stimulating factor
CVD	cardio vascular diseases
HMG-CoA	3-hydroxy-3-methylglutaryl-Coenxyme A
HPBM	human peripheral blood monocytes
IL	interleukin
LDL	low density lipoprotein
LMD	laser microdissection
LPS	lipopolysaccharide
MCP	mococyte chemotactic protein
MMP	matrix metalloproteinase
PAI	plasminogen activator inhibitor
PMA	phorbol 13-myristate 12-acetate
SMC	smooth muscle cells
SNP	single nucleotide polymorphism
SOD	superoxide dismutase
TNF	tumor necrosis factor

whole-genome association studies (6). For example, by whole-genome association study in a Japanese population, Ozaki et al. concluded that the most significant association of myocardial infarction occurred with genetic differences in an inflammatory response gene lymphotoxin-α (7). As an additional limitation of single-gene approach is that the knockout mice, although useful, are often very unphysiological, disturbing only one gene in a very large genetic network which may explain differences between results of the animal and human studies. It can be imagined that more physiological approaches, such as RNA interference may provide more reliable models of gene function (8).

The role of epigenetic modifications of DNA in atherogenesis should be further clarified. Epigenetic regulation of transcription occurs by three mechanisms: DNA methylation, RNA-associated silencing and histone modification (9). There is evidence that for example DNA methylation may play a role in atherogenesis. Methylation status of the CpG islands of the promoter regions is proportional to the activity of the transcription. Several atherosclerosis-linked genes, such as matrix metalloproteinases and extracellular superoxide dismutase (EC-SOD) are at least partially regulated by DNA methylation (10). Today, microarray formats are available for the large-scale studies of DNA methylation. For example, bisulfite-treated and PCR-amplified DNA can be hybridized to oligonucleotide arrays to distinguish CpG dinucleotides that are methylated from those that are not (11).

Key messages

- The analysis of the genetic basis, gene expression and protein expression patterns are all needed for better understanding of the pathogenesis of atherosclerosis.
- Microarray analyses combined with laser microdissection provide effective tools for the analysis of cell-specific gene expression patterns in atherosclerotic lesions.

Before genetic tests for the susceptibility for CVD are suitable for clinical practice some obstacles have to be met. The screening of populations should be performed for CVD-risk genes by DNA tests in order to identify the value of the tests. One of the major problems is the huge number of genes that have been linked to CVD, and moreover, the number of mutations in single genes. For example, over 700 variations have been identified in the LDL receptor gene alone (12). So far, genetic testing of the asymptomatic relatives for LDL receptor mutations has not been cost-effective (13).

In addition to the disorders of single genes that affect the lipid metabolism, associations between polymorphisms in several groups of genes and CVD have been reported. Among those are lipid metabolism-related genes such as lipoprotein lipase, apoE, cholesteryl ester transfer protein (CETP), lipoprotein (a) and paraoxonases 1, 2 and 3. Also, genes related to the inflammatory response such as interleukin 6 (IL-6), stromelysin-1, tumor necrosis factor alpha (TNF-α) and its receptor 2, monocyte chomotactic protein 1 (MCP-1) and the chemokine receptor CX3CR1 have shown associations with CVD. Gene polymorphisms related to endothelial dysfunction including endothelial nitric oxide synthase, Mn superoxide dismutase (Mn-SOD) and cytochrome b558, and genes within the renin angiotensin cascade (angiotensinogen, angiotensin converting enzyme and angiotensin II receptor) have been shown to correlate with the risk of CVD (14).

Thrombosis is not only the final event leading to symptomatic CVD, but platelet aggregation, thrombin formation and clot dissolution are thought to participate also in the early events during the initial phase of the disease. However, none of the hemostatic proteins where a polymorphism has been found to be associated with CVD are generally acknowledged as being strong 'atherosclerosis' genes. Specifically, polymorphisms in

platelet receptors (Gp Ia-IIa, Gp Ib-V-IX, Gp IIb-IIIa), procoagulant factors (fibrinogen, FII, tissue factor, FV, FVII, FVIII, von Willebrand factor, FXIII), anticoagulant proteins (antithrombin III, protein C, protein S, thrombomodulin, endothelial protein C receptor, tissue factor pathway inhibitor) and proteins affecting fibrinolytic system (plasminogen activator inhibitor 1, tissue type plasminogen activator) have been shown to associate with CVD (15). Of special interest is the plasminogen activator inhibitor 1 (PAI-1): even though PAI-1 levels are significantly elevated in patients with previous myocardial infarction they are rather associated with insulin resistance and likely part of the 'metabolic syndrome' (16). In addition, the PAI-1 promoter polymorphism is strongly associated with a poor outcome from sepsis and PAI-1 knockout mice but not PAI-1 transgenic mice show increased tendency for restenosis (17). The findings with PAI-1 promoter polymorphism indicate that although a potential role of a protein in the development of CVD might be strongly suspected by its biological functions, results from clinical studies may remain not only controversial but also support an association with other pathologic conditions indirectly related to CVD (18).

Genetic polymorphisms also influence drug responses. It has been noticed that the magnitude of plasma lipid responses to drug therapies varies considerably among individuals. For example, the apoE genotype affects the lipid-lowering effects of statins: ApoE E2 carriers are the most responsive to statin therapy, and controversially, ApoE E4 carriers are more susceptible to dietary intervention (19). Additionally, the polymorphism of CETP gene has an effect on the statin therapy: pravastatin therapy slowed the progression of atherosclerosis in CETP B1B1 carriers, but not in B2B2 carriers (20). Therefore, genotyping may be used to identify individuals who would get the greatest benefit from different types of therapies.

Atherogenomics

Applications of microarrays in atherosclerosis research

Microarrays or 'gene chips' are powerful large-scale gene expression analysis methods, which enable analysis of gene expression patterns of thousands of genes in normal and diseased tissues/cells in a single experiment (see reference (21) for the basics of microarray technology). Since their development in the mid 1990s, microarrays have been applied in atherosclerosis research comprising studies using human samples, animal models and cell cultures (Table I). When human atherosclerotic lesions have been analyzed using microarrays, induction of for example early-growth response-1 (Egr-1) and Egr-1 inducible genes, SOD and intercellular adhesion molecule-1 (ICAM-1) (22), signal mediators, such as Janus kinase 1 and mitogen-activated protein kinase (MAP kinase) activator, and several inflammatory and immunological genes (23) have been noticed. However, the use of whole-mount atherosclerotic lesions or arteries presents many problems, since arterial tissue is a very heterogenous collection of cells. Even the intima contains several cell types: endothelial cells, SMCs and a few macrophages – not to mention atherosclerotic lesions which consist of fibrous cap, necrotic core and inflammatory cell-rich areas. Therefore, it is evident that gene expression findings which have been obtained using whole-mount lesions, have to be localized to certain cell types using *in situ* hybridization and/or immunohistochemistry. However, with the aid of laser microdissection (LMD) single cells or small cell populations can be isolated. We isolated macrophage-rich shoulder areas from human lesions using LMD, and found the induction of colony stimulating factor (CSF) receptors, interleukin receptors and integrins (24). Interestingly, the target for statin therapy, HMG-CoA reductase, was strongly upregulated in lesion macrophages. The applications of LMD in the field of atherosclerosis research are versatile – there are several distinct areas with different cellular contents in the lesions, and it has also been suggested that even single cell types, such as SMCs or macrophages may have different gene expression patterns and functions in different lesion microenvironments. For example, it can be imagined that macrophages in the shoulder areas might have more inflammatory properties than macrophages surrounding lipid-rich core areas, where lipid metabolism-related functions may be more prominently present.

Although the most relevant risk factors for atherosclerosis, such as high serum LDL-cholesterol, low serum high-density lipoprotein (HDL) cholesterol, smoking, hypertension, obesity and ageing, are epidemiologically well established, molecular mechanisms of their effects are not always fully understood. Recently, several animal and cell culture studies have been performed to clarify the mechanisms of maternal hypercholesterolemia, obesity, ageing, hypertension, cytomegalovirus infection (CMV) and C-reactive protein (CRP) (Table I). Maternal hypercholesterolemia induced expression of genes related to matrix components and intracellular proteins in the arteries of offspring instead of, for example, macrophage-related genes (25). The

Table I. Microarray studies of atherosclerosis.

Human atherosclerotic lesions		
Study design	**Sample**	**Reference**
Atherosclerosis	Fibrous cap *versus* media	(22)
	Advanced lesions *versus* normal arteries	(23)
	Macrophage-rich shoulder area (laser microdissected)	(24)
Apoptosis	Advanced lesions *versus* normal arteries	(66,67)
Unstable angina pectoris	Atherosclerotic lesions from patients with unstable angina *versus* stable angina	(68)
Animal studies		
Stimulus	**Animal species, tissue samples used**	**Reference**
Maternal hypercholesterolemia	LDL-R -/- mice, aortas	(25)
Oxidized phospholipids	apoE-/- mice, carotid artery	(40)
Hypertension	Fischer 334 rats, small arteries	(26)
	Fischer 334 rats, aortic media	(27)
Cytomegalovirus infection	ApoE-/-mice, aortas	(31)
Obesity	C57BL/6N mice, adipose tissue	(32)
Hyperhomocysteinemia	Rat	(69)
Ageing	Fischer 334 rats, coronary arteries	(29,30)
Cell culture studies		
Stimulus	**Cell line**	**Reference**
oxLDL	Macrophages	(46,47,70)
	Endothelial cells	(71,72)
	SMC	(73)
oxVLDL	Endothelial cells	(74)
TNF-α	SMC	(75,76)
IL-4	Endothelial cells	(77)
LPS	Macrophages	(78)
CRP	SMC	(33)
	Endothelial cells	(34)
Changes in shear stress	Endothelial cells	(79–81)
L-NAME	Endothelial cells	(28)

oxVLDL=oxidized very low density lipoprotein; L-NAME=N(omega)-nitro-L-arginine methyl ester. For other abbreviations, see text.

microarray studies of hypertension suggested that the RhoA/Rho kinase system plays a role in the transduction of altered flow stimulus, and leads to changes in cytoskeletal markers and in SMC phenotype (26), genes regulating cell proliferation, extracellular matrix remodeling, nitric oxide/cGMP (NO/cGMP) signaling pathway (27) and lectin-like oxidized low-density lipoprotein receptor (LOX-1) (28). The upregulation of LOX-1 implies that hypertension also facilitates lipoprotein trafficking in endothelium (28). Ageing-induced inflammatory and pro-apoptotic changes were studied in rats (29,30), and upregulation of TNF-α and caspase 9, and decreased bioavailability of NO, were detected, which altogether promote endothelial apoptosis and endothelial dysfunction. CMV infection leads to the upregulation of MCP-1 and interferon-γ-inducible genes – a finding that suggests that viral infection could accelerate the atherosclerotic process (31). Interestingly, study of gene expression in adipose tissue of normal mice and high-fat-diet-induced obese mice led to detection of high levels of MCP-1 mRNA (32), and it was suggested that by secreting MCP-1, adipose tissue activates monocytes and accelerates atherogenesis. Blaschke et al. demonstrated that CRP, in addition to being a predictor of coronary events, has direct proatherogenic effects (33). They detected upregulation of growth arrest- and DNA damage-inducible gene 153 (GADD153) and caspase-mediated apoptosis in CRP-treated SMCs. Additionally, CRP has an effect on genes functioning in cell growth and differentiation and vascular remodeling as studied in endothelial cells (34).

In addition to atherosclerosis, other processes such as aneurysm formation and vein graft remodeling have been studied. Nakahashi et al. found that genes functioning in oxidative stress and in matrix remodeling were induced in experimental rat

aneurysms (35). Vein grafts (36) and arterial grafts (37) have been studied in canines, and changes in matrix components, such as in collagens and in SMC phenotype, have been detected.

One of the most tempting applications of the microarrays is their use in the clarification of drug responses. Recently, it has been shown that for, example, HMG-CoA reductase inhibitors, statins, have various pleiotropic effects on the treatment of atherosclerosis that are not related to their lipid-lowering effects: statins can attenuate the expression of CD68 and fatty acid binding protein 4 in oxLDL-treated macrophages (38) and they affect genes involved in coagulation, vascular constriction and cell growth in a cell-type specific manner in endothelial cells, SMCs and hepatocarcinoma cells (39). We also found that structurally defined oxidized phospholipids (OxPAPC) induced a set of atherosclerosis-related genes, including MCP-1 and keratinocyte-derived chemokine (KC), tissue factor (TF), IL-6, heme oxygenase 1 (HO-1), and Egr-1 in intact murine arteries of wild type mice. These genes were also found upregulated in ApoE-/- mice, and the OxPAPC-triggered rolling and firm adhesion of monocytes was P-selectin and KC-dependent (40). These results indicate that data from microarray studies can be used as a starting point for mechanistic experiments. Additionally, it has been shown that IL-1β, whose expression is elevated in peripheral blood monocytes in patients suffering from coronary artery disease, was downregulated by statins (41). The molecular responses of SMCs to curcumin- and paclitaxel-eluting stent materials have also been studied (42,43), and it was found that these stent materials prevented neointima formation by upregulating apoptotic, anti-proliferative and anti-inflammatory factors via MAP-kinase and protein kinase A (PKA) pathways. Hishikawa et al. studied the effects of an orally administered natural flavonoid, caffeic acid phenethyl ester (CAPE), in apoE-/- mice, and found that CAPE reduced activity of nuclear factor-κB (NF-κB), genes related to it and cytokines and growth factors in aortas (44).

Advantages and limitations of microarrays in atherosclerosis research

An advantage of microarray analysis is the possibility to perform time-scale analyses in cell cultures or in animal experiments, and with the aid of data clustering, for example, with self-organizing maps one can visualize time-dependency of gene expression patterns (45). For example, Shiffman et al. studied gene expression changes during foam cell formation and detected upregulated wave of expression in genes involved in migratory and adhesive functions (46). In turn, genes involved in cell cycle progression, nucleotide metabolism and DNA repair showed downregulated wave of expression. We analyzed monocyte-macrophage differentiation in PMA-stimulated THP-1 cells and found patterns of activation of different gene classes: early activation of transcription and translation factors (time-point of 3 h) were followed by the activation of genes functioning in cell proliferation, inflammation and lipid metabolism (time-points of 12–72 h) (47). When Kalish et al. studied gene expression after arterial grafting they detected activation of transcription factors and matrix-associated molecules 7 and 14 days after grafting, whereas genes involved in immune or inflammatory processes were activated later (36). Another approach is to analyze gene expression data as functionally related groups of genes. For example, Mootha et al. found that the expression of a set of genes involved in oxidative phosphorylation was coordinately decreased in human diabetic muscles (48).

In addition to the possibility to perform time-scale experiments, microarray analyses of cell culture experiments have certain advantages. The experiments can be easily standardized, and therefore, for example, the identification of regulatory pathways is possible. Moreover, the interpretation of results is not hampered by tissue heterogeneity. However, it is evident that understanding of the complex regulatory network including the effects of different environmental factors, cytokines and growth factors secreted by several cell types is difficult in *in vitro* experiments. When we compared the expression changes in *in vivo* LMD macrophages with *in vitro* macrophages (PMA-stimulated THP-1 cells), we noticed that the expression ratios were consistently higher in the *in vivo* situation (24). It has been questioned how much the cell culture models can resemble cells *in vivo*. Also, the malignant origin of many cell lines hinders the interpretation of results, especially when they concern cell proliferation. Kohro et al. systemically studied gene expression differences in THP-1-monocytes and THP-1-derived macrophages as compared to human peripheral blood monocytes (HPBM) and HPBM-derived macrophages induced by macrophage-CSF (M-CSF) or granulocyte-macrophage-CSF (GM-CSF) (49). Although many genes, such as apoE and MMP9 were induced similarly during monocyte-macrophage differentiation in THP-1-derived macrophages and HPBM-derived macrophages, there were also some differences. The correlation coefficient between the expression of individual

genes in THP-1-monocytes and HPBM-monocytes was 0.78, and between THP-1-derived macrophages and HPBM-derived macrophages from 0.80 to 0.88 depending on the time-point and CSF used. Additionally, it is not clear what would be the best model for cells in atherosclerotic lesions. For example, when we compared *in vivo* LMD macrophages with PMA-stimulated monocytes, monocyte-macrophages and oxLDL-stimulated macrophage foam cells, it became evident that *in vivo* LMD macrophages from lesion shoulder areas share more similarities with PMA-stimulated monocytes than with the oxLDL-stimulated macrophages (24,47). In LMD macrophages, genes that function in macrophage differentiation, inflammation and immune response, such as CSF and interleukin receptors were activated but, on the contrary, there were only minor changes in lipid metabolism-related genes.

Microarrays are prone to various problems which could arise from: 1) study design, 2) experimental samples, 3) microarray technology, and 4) interpretation, confirmation, and reporting of the results (Table II) (50,51). The choice of microarray types, the non-linearity of correlation between mRNA and protein expression; and the problems in reporting of the results will be discussed below. The expression measurements made by cDNA and oligonucleotide arrays have been compared, and it was found that the correlation from different types of arrays is highly dependent on the array design (52). One can also choose between 'whole-genome' microarrays and gene-group specific microarrays, such as cardiovascular gene-specific microarrays. In addition to the difference in the price of the arrays and the number of probes on the arrays, the array types also differ in respect to the number of unknown expressed sequence tags (EST)-sequences. The use of gene group-specific arrays could also be misleading to some extent, for example if one uses apoptosis-specific arrays, and, therefore, finds only apoptosis-related genes.

The fact that the correlation between mRNA and protein expression is not linear poses many challenges to genomic analyses. This discrepancy arises from various posttranscriptional and posttranslational modifications, functional interactions of proteins, and gene regulation, which could function solely at the mRNA level, at the protein level, or at both levels (53). The correlation between mRNA and protein species has been found to be poor ($r=0.4$ for yeast (54)), and it has also been noticed that the linearity of the correlation differs in different gene groups: the linearity is poor in structural genes and genes functioning in protein synthesis. On the contrary, there is a better correlation, for example, in transcription factors (55). Thus, it is evident that the microarray findings have to be confirmed with independent methods both at mRNA and at protein level.

Table II. Limitations of microarray analyses.

Source of error	**Problem**
Study design	Choice of an optimal animal/cell culture model Choice of an optimal control Number of replicates: Biological & technical Time-points: early response genes (minutes–hours), long-lasting changes (weeks–months) Choice of the array type: gene group specific arrays, oligonucleotide/cDNA arrays
Experimental sample	Inter-individual differences (drugs, age, sex etc.) Treatment of experimental animals (e.g. stress) Tissue preservation, post-mortem changes of autopsy samples Tissue heterogeneity Quality of RNA Amount of RNA (linearity of amplification)
Microarray technology	Choice of optimal probe (crosshybridization of nearly similar sequences, mRNA splice variants, incomplete genome sequences available for probe design) Problems in manufacturing the arrays (e.g. printing errors, contamination of cDNA clone collections) Differences between oligonucleotide-based and cDNA-based microarrays
Performing the microarray analysis	Different efficiencies of dye incorporation if Cy3/Cy5 labelling is used Hybridization errors: dust, non-uniform hybridization Inter-array variations
Data analysis	Choice of the normalization method (mean/ median centralization or more sophisticated methods, e.g. Lowess) Use of statistics and clustering methods, choice of methods and software
Reporting the results	Gene lists/pathways/single genes

Reporting the microarray results is a very challencing task. Should one concentrate only on single findings, report gene lists, or try to find regulatory pathways? Using the whole-genome microarrays it might even be possible to hypothesize new pathways: for example, Nguyen et al. proposed a new model for the effects of curcumin on SMC proliferation (42) and by using that approach and cluster analysis to group genes according to their expression profiles, and analyzing the respective promoters for regulatory elements that were over-represented in specific clusters, we could identify several potentially novel DNA binding sites and transcription factors (56). Some microarray data analysis software (e.g. GeneSpring, Silicon Genetics) utilizes, for example, KEGG database (http://www.genome.ad.jp/kegg) and helps to create pathways from microarray data. One should also decide if he/she strictly follows the rules of statistical significances and pre-defined expression ratios even though the (patho)biologically most relevant genes may have much lower expression ratios with higher expression levels instead of higher expression ratios with lower expression levels. Although the rules for reporting the microarray data have been proposed (57) there is still very much variability in the accuracy of microarray study reports.

Atheroproteomics

As the DNA microarrays have become a valuable tool in large scale RNA expression analyses, similar high-throughput protein microarrays have also been developed besides two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and mass spectrometry (58). Different array formats, such as peptide arrays, antigen-antibody arrays and protein arrays have been applied to studies of antigen-antibody, protein-protein as well as enzyme-substrate and ligand-receptor interactions (58). Recently, Huang et al. treated HPBM with leukotriene B_4, a product of 5-lipoxygenase, and using an immuno-protein array containing 79 cytokines they found that MCP-1 was strongly induced (59). Additionally, Martinet et al. used a new large-scale protein expression analysis method 'Western array' in the analysis of atherosclerotic artery samples, and detected upregulation of apoptosis-linked gene 2 (ALG-2) (60). We analyzed oxLDL-stimulated THP-1 macrophages by antibody array that contained 384 proteins. When we compared the results from protein array and DNA microarray, we found that the expression changes at the protein level were in general smaller than changes at the mRNA level. The scale of ratios obtained by protein array analysis was also narrower varying from 0.7 to 1.9 compared to the ratios obtained by cDNA arrays from 0.05 to 37.8 (47). This bias could be anticipated from the non-linear correlation between mRNA and protein abundancies as discussed above, and at least partly by the technical limitations of the protein arrays, such as problems in the specificity (58).

In addition to DNA and protein microarrays, tissue microarrays comprising small tissue samples have been introduced (61). The role of matrix metalloproteinases (MMPs) in thoracic aortic aneurysms and aortic dissections was studied using tissue microarray containing samples of aneurysms and dissections from 47 patients (62). In another study taking advantage of tissue microarrays it was found that intimal macrophage content was higher and the network of vasa vasorum was denser in patients with symptomatic atherosclerosis as compared to patients who had not suffered from the complications of atherosclerosis (63).

Future aspects of atherogenetics, atherogenomics and atheroproteomics

It is evident that for better understanding of the molecular mechanisms of atherogenesis, human and animal samples and cell culture models are all needed. Atherogenetics could give us information about SNPs, linkage of genes and activity of genes, such as the methylation patterns of genes, whereas atherogenomics together with atheroproteomics provide us with effective large-scale expression tools and enable the analysis of gene regulatory networks (64). There is also a growing demand for analysis of metabolic parameters, such as enzyme activities in large scale and bioinformatics to develop biostatistics, analysis methods and information databases to handle the huge amounts of data.

An example of the use of these new techniques is the CardioGene Study, which utilizes genetics, genomics and proteomics in the research of in-stent restenosis in 350 patients (65). Genetics, e.g. genome-wide scans are used in association studies to find susceptibility genes, and to find SNPs of predictive value. Transcriptional and translational analyses are performed from blood samples of patients to provide global assessment of gene activity after vascular injury. Furthermore, data from sequence variation and global gene expression profiles will be integrated to assess co-regulated sets of genes and functional cellular networks. It is anticipated that significant new information about the pathogenesis of atherosclerosis and its treatment and clinical manifestations can be derived from these studies.

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