Sequencing of the human genome has ushered in prospects for individualizing cardiovascular health care. There is growing evidence that the practice of cardiovascular medicine might soon have a new toolbox to predict and treat disease more effectively. The Human Genome Project has spawned several important "omic" technologies that allow "whole genome" interrogation of sequence variation (re-sequencing, genotyping, comparative genome hybridization), transcription (expression profiling, tissue arrays), proteins (gas or liquid chromatography and tandem mass spectroscopy [MS]), and metabolites (MS or nuclear magnetic resonance profiling); deoxyribonucleic acid, ribonucleic acid, protein, and metabolic approaches all provide more exacting detail of cardiovascular disease mechanisms and, in some cases, are redefining its taxonomy. Pharmacogenomic approaches are emerging across broad classes of cardiovascular therapeutics to assist practitioners in making more precise decisions about which drugs to give to which patients to optimize the benefit–to–risk ratio. Molecular imaging is developing chemical and biological probes that can sense molecular pathway mechanisms that will allow us to monitor health and disease. Together, these tools will enable a paradigm shift from genetic medicine—on the basis of the study of individual inherited characteristics, most often single genes—to genomic medicine, which by its nature is comprehensive and focuses on the functions and interactions of multiple genes and gene products, among themselves and with their environment. The information gained from such analyses, in combination with clinical data, is now allowing us to assess individual risks and guide clinical management and decision-making, all of which form the basis for cardiovascular genomic medicine. (J Am Coll Cardiol 2005;46:1615–27) © 2005 by the American College of Cardiology Foundation

Today, large-scale sequencing and the ability to measure genotypes at thousands of loci can generate an individualized profile of health and/or disease. Deoxyribonucleic acid (DNA) microarrays, which generate a “molecular signature” defining a particular phenotype, are another approach whose power resides in its ability to measure expression levels of thousands of genes in parallel (1,2). Genomic complexity is also reflected in proteomics—the analysis of the entire protein content of a cell or tissue—a further approach that will be essential to bringing personalized health care to fruition (3,4). At the end of the biochemical pathway lies metabolomics, a breakthrough technology that allows visualization of the biological complexity of small molecule metabolites on a large scale in serum or other biological fluids (Table 1).

Linkage of genomic information with clinical data will result in predictive biosignatures (5) (Fig. 1) for use in identifying individuals predisposed to illness, classifying disease on a molecular basis to improve diagnostic and prognostic precision, developing predictive pharmacogenomics profiles, and developing non-invasive methods to detect disease and monitor response to therapy (6,7). As it becomes increasingly available, genomic medicine will result in a significant paradigm shift from acute intervention to prospective and personalized cardiovascular health care (Table 2). In this review, we highlight emerging knowledge and paradigms from a number of these technologies. Most had their genesis in genomic research; all are harbingers of the potential for personalized cardiovascular care.

DNA: GENOMICS AND SUSCEPTIBILITY TO VASCULAR DISEASE

For the first time in history, health and disease can be defined by genetic fingerprints enabled by the availability of the entire complement of the human genome. An approximate 0.1% difference in the nucleotide sequence of the approximately 22,500 genes in the human genome distinguishes individuals, often in the form of variations of a single base pair called single nucleotide polymorphisms (SNPs) that are expected to be key in diagnostics and symptomatic testing. In complex phenotypes of polygenic origin such as cardiovascular disease (CVD), SNPs
from multiple unlinked genes with weaker effects might be analyzed in parallel and used to generate quantitative risk assessments. The challenge remains to translate these differences into practical knowledge that will assist clinicians and improve patient outcomes. The promise for doing so stems from recent advances in technology, including high-throughput genotyping as well as lower costs and improved clinical study designs. In addition, investigators are now routinely banking DNA from large, well-phenotyped families with inherited disorders as well as from disease cohorts in anticipation of using large-scale genomic analyses to determine the gene(s) responsible for the observed traits.

Two major approaches are used to identify potential genes for further analysis (8). The first approach, linkage analysis via whole genome scans, is an unbiased approach in which no prior assumptions are made about which genes are causal. The second is association studies, using the “candidate gene” approach, in which knowledge of disease pathophysiology is used to develop hypotheses about the association of variants in a specific gene or group of genes with disease.

**Linkage analysis via genome-wide scanning in families.** To date, there are eight genome-wide linkage studies that have isolated candidate regions for coronary artery disease (CAD) or myocardial infarction (MI) (9–16) (Table 1). Identification of the first autosomal dominant gene for CAD and MI was reported within the past year (16). A deletion mutation in a member of the myocyte enhancer factor 2 transcription factor family (MEF2A) was discovered in a genome-wide analysis of a single large family in which 13 members had CAD, nine of whom had MI. Re-sequencing of MEF2A in cases with CAD and MI revealed three novel point mutations in exon 7 resulting in amino acid substitutions (17). No mutations were observed in the control population, but these novel mutations were present in nearly 2% of cases. Although these associations are compelling, further determination of the prevalence and clinical significance of these mutations are needed.

Using a linkage-based study, investigators from Iceland mapped another gene predisposing to MI to a region of chromosome 13 (15). The Icelandic population is particularly well suited for these studies, owing to its homogeneity, low incidence of migration, and infrastructure to support collection of health data and DNA on a nationwide scale. A large-scale case-control association study subsequently implicated a 4-SNP haplotype of the gene ALOX5AP in MI. The protein product of this gene, 5-lipoxygenase accessory protein (FLAP), operates in concert with 5-lipoxygenase in leukotriene biosynthesis (18). 5-lipoxygenase was initially found to contribute to atherosclerosis sensitivity in mice and later implicated in human atherosclerosis (19,20). In the Los Angeles Atherosclerosis study, individuals homozygous for the gene variant had heavier burdens of atherosclerosis by carotid artery intima-media thickness (21). In addition, the observed genetic effect was more pronounced among those with a diet more concentrated in n-6 polyunsaturated fatty acids (5-lipoxygenase’s substrate in leukotriene metabolism).

Appropriate validation studies will be required to support the widespread use of any of these genes in routine testing, but as with the BRCA1 and BRCA2 variants in breast cancer, MEF2A, FLAP, and 5-lipoxygenase gene variants might be the first of several clinically relevant genetic tools to identify individuals at high risk for CAD and MI.

**Association studies using the candidate gene approach.** Several genetic variants could be used as tools in clinical practice today. Factor V Leiden and Prothrombin G20210A are examples of two such variants consistently associated with venous thrombosis, with adjusted odds ratios of approximately 3.0. On the basis of these data, a recommendation for testing for these gene variants in certain cases of venous thrombosis has been incorporated in several consensus panel guidelines (22,23).

In contrast, most gene variants are not as consistently or strongly associated with arterial thrombosis and cardiovascular clinical outcomes. Numerous small case-control association studies in selected populations have identified genes potentially predictive of CAD or MI, but often with low strengths of association or without adequate adjustment for potential confounders. Frequently, these findings cannot be replicated in other populations.
Meta-analyses and large-scale association studies both attempt to address this issue and validate findings from these smaller studies—the latter is preferable and, now, more feasible. The most compelling associations are those that not only are highly significant but also have a plausible biological mechanism.

Meta-analyses that attempted to clarify the relationship of a number of genetic variants in candidate genes for CAD and MI are shown in Table 4. Publication bias against studies with negative results might have profound effects on the results of meta-analyses with a tendency to overestimate the true risk. That said, most meta-analyses reveal only modest associations of genetic variants with clinical end points with odds ratios ≤1.5. Apolipoprotein E (24), methylene tetrahydrofolate reductase (25), angiotensin-converting enzyme (ACE) (26, 27), apolipoprotein B (28), plasminogen activator inhibitor-1 (29–31), fibrinogen beta chain (29, 32), and nitric oxide synthase (33–35) genes have all been associated with intermediate phenotypes (e.g., lipid levels) or coronary events and await validation in large population studies.

Large case-control association studies. The development of high-throughput genotyping has enabled the study of candidate genes in larger populations (Table 5). In the GeneQuest study (36), 72 SNPs were analyzed in a case-control design (352 cases and 418 controls) for association with premature MI. Among >50,000 genotypes generated, a total of 11 SNPs in nine genes were differentially associated with CAD or MI. Three members of the thrombospondin family of proteins—matrix proteins putatively involved in vascular integrity and calcium signaling—were among them. This study was later expanded to 210 SNPs in an even larger-scale association study (36).

A second large-scale association study investigated >175,000 individual SNPs (37). A total of 112 polymorphisms in 71 candidate genes were identified from a screening population and then confirmed in a second, larger population. Among male subjects, the gap junction protein, connexin 37 (relative risk [RR] 1.4, 95% confidence interval [CI] 1.1 to 1.6) and the NAD(P)H component p22(phox) (RR 0.7, 95% CI 0.6 to 0.9) were associated with MI. Previously, connexin 37 was identified as a prognostic indicator of plaque development (38), was associated with presence of CAD (39), and was demonstrated to be differentially expressed in plaques from animal models of CAD (40). As a component of the NAD(P)H redox system, p22(phox) might play a role in the development of athero-

**Figure 1.** Clinico-genomic biosignatures to predict health, disease, and environmental/drug response. Data from a variety of sources will be integrated to develop patterns of information and models for disease prediction and drug response (5). SNPs = single nucleotide polymorphisms.

**Table 2.** What Genomics Might Bring to Cardiovascular Medicine

- Clinical use of genomic variation to predict health and disease in individuals, communities, and whole populations
- Incorporation of the complex interplay of genes and the environment vis-à-vis patient care, whether that environment be cellular, industrial, or socioeconomic
- Integration of precise phenotypic data with equally precise genotypic data in a comprehensive and computationally robust clinical framework
- Development of state-of-the-art technologies that assess the activity of large portions of the genome, transcriptome, proteome, and metabolome
- Utilization of genomic information to streamline drug development and improve our understanding of drug safety, tolerance, and efficacy
- Discussion of the ethical, legal, and policy issues raised by the integration of the genome sciences into the practice of medicine
- Fundamentally alter the way in which health care is delivered and practiced in order to optimize care and reduce costs overall
produce an entirely new list of potential risk-promoting as should catalyze the next round of candidate gene studies and (47,48). Observations such as these lower risk of developing carotid atherosclerosis (RR 0.54, the toll-like receptor 4 gene variant was associated with a inflammation and atherosclerosis, it is not surprising that toll-like receptor 4 is associated with a poor host response to infection (45), and functional variation in the Toll receptor family has been well characterized with respect to innate immunity (45), and functional variation in toll-like receptor 4 is associated with a poor host response to sepsis (46). Given the now-established connection between inflammation and atherosclerosis, it is not surprising that the toll-like receptor 4 gene variant was associated with a lower risk of developing carotid atherosclerosis (RR 0.54, 95% CI 0.32 to 0.98) (47,48). Observations such as these should catalyze the next round of candidate gene studies and produce an entirely new list of potential risk-promoting as well as protective biomarkers for CAD and MI.

**Genome-wide association.** In genome-wide association studies, genetic variants or “markers” spaced evenly across the genome are evaluated individually for association with a disease. In one of the first such analyses, >65,000 different SNPs were analyzed in 94 cases with MI and 658 control subjects (49). Six million genotypes were generated, and SNPs identified as associated with MI were then typed in a separate case series. A significant association was observed between MI and a block of genes that included the lymphotoxin-alpha gene. These associations were supported by nearly identical associations when cases were compared with two different control groups (Table 5). The association of the lymphotoxin-alpha gene with MI in humans is pathophysiologically plausible, because lymphotoxin-alpha was implicated in atherosclerosis in animal models (50). Variant SNPs were found to not only influence transcriptional levels of the gene but also to be associated with elevated levels of C-reactive protein (CRP) and to profoundly influence the induction of cellular adhesion molecules, including vascular cell adhesion molecule (VCAM)-1 (51).

Data from meta-analyses, case-control studies, and genome-wide association studies all describe genes associated with, at most, a modestly increased risk for CAD and MI. This observation is consistent with the general paradigm that complex diseases have small contributions from multiple genes. No studies have yet investigated the association of a multiplex panel of several genetic variants with the risk for CAD or MI. Yet, it is likely that multiple genetic markers will be required to determine the risk of developing

### Table 3. Genome-Wide Linkage Studies in Families With CAD or MI

<table>
<thead>
<tr>
<th>Genome Scans</th>
<th>Outcome</th>
<th>Number and Type of Subjects</th>
<th>Population Demographics</th>
<th>Gene Isolated?</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hauser et al. (9)</td>
<td>Premature CAD</td>
<td>438 families (1,168 individuals)</td>
<td>U.S. and Europe</td>
<td>No</td>
<td>13</td>
</tr>
<tr>
<td>Wang et al. (10)</td>
<td>Premature MI</td>
<td>428 families (1,613 individuals)</td>
<td>U.S.</td>
<td>No</td>
<td>14</td>
</tr>
<tr>
<td>Pajukanta et al. (11)</td>
<td>Premature CAD</td>
<td>156 families (364 individuals)</td>
<td>Finland</td>
<td>No</td>
<td>15</td>
</tr>
<tr>
<td>Francke et al. (12)</td>
<td>CAD</td>
<td>99 families (535 individuals)</td>
<td>Northwestern India</td>
<td>No</td>
<td>16</td>
</tr>
<tr>
<td>Broeckel et al. (13)</td>
<td>CAD</td>
<td>513 families (1,406 individuals)</td>
<td>Germany</td>
<td>No</td>
<td>17</td>
</tr>
<tr>
<td>Harrup et al. (14)</td>
<td>ACS</td>
<td>61 families (161 individuals)</td>
<td>Australia</td>
<td>No</td>
<td>18</td>
</tr>
<tr>
<td>Helgadottir et al. (15)</td>
<td>MI</td>
<td>296 families (2,454 individuals)</td>
<td>Iceland</td>
<td>Yes*</td>
<td>19</td>
</tr>
<tr>
<td>Wang et al. (16)</td>
<td>CAD or MI</td>
<td>1 family (25 individuals, 19 available for genotyping)</td>
<td>U.S.</td>
<td>Yes</td>
<td>21</td>
</tr>
</tbody>
</table>

*Association study conducted in conjunction with linkage study.
ACS = acute coronary syndrome; CAD = coronary artery disease; MI = myocardial infarction.

### Table 4. Case-Control Association Studies: Meta-Analyses of Candidate Genes for CAD or MI

<table>
<thead>
<tr>
<th>Gene</th>
<th>Risk Allele</th>
<th>Outcome</th>
<th>Comparative Risk (95% CI)</th>
<th>Studies (Cases/Controls)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apolipoprotein E</td>
<td>e4/e4</td>
<td>CAD</td>
<td>1.42 (1.26–1.61)</td>
<td>48 (15,492/32,965)</td>
<td>24</td>
</tr>
<tr>
<td>Methylene tetrahydrofolate reductase</td>
<td>C677T</td>
<td>CAD or MI</td>
<td>1.21 (1.06–1.39)</td>
<td>72 (12,193/11,945)</td>
<td>25</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme insertion/deletion</td>
<td>DD</td>
<td>CAD</td>
<td>1.16 (1.08–1.25)</td>
<td>18 (4,442/11,008)</td>
<td>26</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme insertion/deletion</td>
<td>DE</td>
<td>MI</td>
<td>1.21 (1.11–1.32)</td>
<td>19 (2,848/10,256)</td>
<td>27</td>
</tr>
<tr>
<td>Apoipoprotein B</td>
<td>Ins/Del (DD)</td>
<td>CAD or MI</td>
<td>1.19 (1.05–1.35)</td>
<td>22 (6,007/5,609)</td>
<td>28</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>Gln451Lys (GG)</td>
<td>CAD or MI</td>
<td>1.32 (1.14–1.54)</td>
<td>15 (1,816/2,054)</td>
<td>28</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor-1</td>
<td>4G/5G</td>
<td>MI</td>
<td>1.20 (1.04–1.37)</td>
<td>10 (1,515/1,866)</td>
<td>29</td>
</tr>
<tr>
<td>Fibrinogen beta-chain</td>
<td>G-455A</td>
<td>MI</td>
<td>0.68 (0.46–0.99)</td>
<td>4 (745/816)</td>
<td>29</td>
</tr>
<tr>
<td>Endothelial nitric oxide</td>
<td>Gln298Asp</td>
<td>CAD or MI</td>
<td>1.31 (1.13–1.51)</td>
<td>14 (6,036/6,106)</td>
<td>30</td>
</tr>
<tr>
<td>Endothelial nitric oxide</td>
<td>Intron-4</td>
<td>CAD or MI</td>
<td>1.34 (1.03–1.75)</td>
<td>16 (6,212/6,737)</td>
<td>30</td>
</tr>
</tbody>
</table>

CAD = coronary artery disease; CI = confidence interval; MI = myocardial infarction.
diseases such as atherosclerosis or MI, and studies now can be carried out to develop these as clinically useful diagnostics. At present, however, there are no data strong enough to support the use of tests for individual polymorphisms in routine clinical practice to predict the presence or extent of CAD or to risk-stratify patients for MI.

RNA: DEFINING MOLECULAR PHENOTYPES AND PATHWAYS

Although study of gene variants and their associations with disease is important, presence of gene variants does not necessarily specify that a disease phenotype will be observed clinically. Ribonucleic acid is an intermediate gene product that results from the transcription of DNA sequence. Whereas DNA is a fixed set of information, RNA is dynamic and might change in response to various intracellular and extracellular stimuli and environmental factors (e.g., dietary, stress, hormonal, growth factors, smoking) in both health and disease. It is now possible to interrogate the entire expressed genome of approximately 25,000 transcripts (the transcriptome) using microarrays (“gene chips”). The “expression pattern” or RNA profile might serve as an additional means to subcategorize disease and/or predict its response to various therapies as well as to define groups of genes associated with a clinical outcome. Furthermore, it is poised to become a valuable tool for candidate gene discovery for use in DNA-based association studies (52) as well as for identifying protein markers in cases where the protein product of highly expressed transcripts are secreted or shed into serum.

Studies of RNA samples from cancers have been particularly revealing of “gene signatures” that correlate with treatment response and survival. In CVD, transcriptomes from relevant target tissues (e.g., coronary arteries) are more difficult to access. Recently, transcriptional profiling of atherosclerotic material from human and mouse aortas identified potential genes and biological pathways involved in the molecular pathophysiology of atherosclerotic development and progression as well as robust gene expression patterns that are highly predictive for lesion severity (53). In addition, several novel genes were identified that were not previously associated with atherogenesis. Although it is unlikely that aortic material will be used clinically, it is valuable because: 1) several expressed genes from aortic tissue might also be measured in serum; it might be possible to develop enhanced diagnostic capabilities for atherosclerosis; 2) the genes identified in these expression signatures might also be used to identify SNPs for large-scale analyses; and 3) important new mechanisms of disease might be revealed (54).

Expression profiling of RNA from explanted hearts and endomyocardial biopsies has also been used to subclassify heart failure (55,56). Gene expression signatures were developed that highlighted important mechanistic information that could be used to identify new therapeutic targets.
about the underlying pathophysiology of ischemic cardiomyopathy versus cardiomyopathy of infectious etiology. More recently, gene expression profiles were used to develop prognostic indexes for patients with congestive heart failure undergoing left ventricular assist device placement. The profiles accurately predicted the etiology of cardiomyopathy (56), providing a good argument for including these assays in studies aimed at developing prognostic indicators or biomarkers of response to therapy. Expression signatures from cardiac tissue are also being investigated as predictors of future arrhythmias in the post-bypass surgery setting or with automatic defibrillator implantation (57).

Expression profiling of RNA from blood mononuclear cells might also serve as a proxy for diseased tissue. This could be a breakthrough use of the technology to develop predictive markers where the diseased tissue is inaccessible (such as vascular tissue). This is also an appealing approach for disease processes in which the monocyte might be a participant, as is the case in atherosclerosis and transplant rejection. In one study, molecular signatures from peripheral blood expression profiles correlated well with biopsy-proven allograft rejection (58). If these results are validated in larger studies, it would pave the way for a blood-based screening test instead of the more invasive endomyocardial biopsy.

PROTEINS: BIOMARKERS OF RISK IN ATHEROSCLEROTIC CVD

In cardiology, there has been rapid escalation in the search for and identification of circulating protein biomarkers for use in establishing diagnosis, determining prognosis, and selecting treatment. We are now confronted with a perplexing variety of biomarkers of process and risk, from development of atherosclerosis to plaque instability and rupture, to arterial thrombosis associated with ischemia and myocardial necrosis. As demonstrated with the recent publication of data from the INTERHEART study (59) showing ApoB/ApoA1 ratio as a measure of lipid abnormalities as strong predictor of MI, even our fundamental constructs regarding biomarkers of risk for CAD events are being continually reshaped. This and other recent examples highlight the burgeoning potential of incorporating new protein biomarker assessments in risk stratification. Moreover, with the advent of mass spectroscopy (MS) as a tool to comprehensively examine the proteome—the full complement of proteins in serum—there will undoubtedly emerge many novel candidate markers for CVD risk.

Troponins as the prototypical protein biomarker for personalized medicine. Protein biomarkers of myocardial necrosis are perhaps the best example of the evolution from diagnostic tool to prognostic instrument to utility in treatment selection. Until the early 1990s, biomarkers of myocardial necrosis, predominantly creatine kinase (CK) and its myocardial-specific isoform, CK-MB, were used largely to confirm the diagnosis of MI. With the development of sensitive and specific cardiac troponin assays, measurable release of these proteins became not only a marker of the occurrence of MI, but also a powerful risk stratification tool for subsequent adverse clinical outcomes (60–62). In meta-analyses, the odds ratios for death or MI in patients with elevated troponin measurements relative to those without ranged from 2.5 to 4.9 and were independent of type of troponin (I or T) measured (63,64). Not only do these biomarkers identify patients at increased risk, they contribute independent information to risk stratification in the context of readily identifiable clinical characteristics (65); however, the potential of this protein biomarker was most fully realized when the association between troponin elevation and benefit from potent antiarrhythmic and antiplatelet therapies and invasive management in acute coronary syndromes (ACS) became evident (66–72). Furthermore, analyses showed that for both glycoprotein IIb/IIIa inhibitors and the early invasive strategy, treatment benefit was maximal among the highest risk ACS patients (positive troponins), regardless of gender, helping to explain observations of differences in response to these therapies among male and female subgroups in randomized clinical trials (73,74). Thus, troponins provide one of the first examples of the potential for personalized treatment of CVD, providing a framework that focuses on directing use of effective but expensive therapies in ways that can potentially avoid adverse effects to patients.

Evolution of protein biomarker testing for personalized cardiovascular care. Despite the potential of troponin testing as an agent for personalized treatment, its story also highlights one of the challenges facing the development of protein biomarker approaches to risk stratification and disease management. Data on the use of troponins and most protein markers reflect population averages; thus, they are not a perfect tool for either risk stratification or guiding treatment selection at the individual patient level. Even among troponin-negative patients, there are those at increased risk for adverse events who might benefit from a particular therapy if they could be identified. Conversely, among troponin-positive patients there are individuals for whom benefit from a given treatment might be less substantial or less certain (66,75). This conundrum fuels the exploration for additional protein biomarkers to further risk-stratify troponin-positive and -negative patients with CVD and improve the overall desired sensitivity and specificity.

Table 6 displays a partial list of protein biomarkers for disease state and risk and the general pathophysiological process with which they are associated. Included are potential markers of plaque rupture or ischemia, hemodynamic stress, and biomarkers that reflect the role of inflammation in the atherosclerotic process. At present, many are in early stages of development (i.e., “emerging”), have not been validated in the clinical setting, lack a commercially available, standardized, and validated assay for widespread use, or lack data supporting their independent clinical utility in guiding treatment and management decisions, whereas others that have these characteristics are “in clinical use.”
Inflammation. Since the landmark observations of Ross (76), the role of inflammation in development and progression of atherosclerosis has been increasingly recognized. Circulating markers of inflammation correlate with presence of atherosclerotic disease and risk for clinical events in both stable subjects with and without known CAD and those with ACS (77–86), and several existing therapies are associated with decreases in circulating markers of inflammation (84,87–90). It is less clear, however, that measurement of inflammatory markers is useful in targeting treatment selection to specific groups of patients (77,91).

The potential for using markers of inflammation to both assess risk and guide therapy was first explored with high-sensitivity C-reactive protein (hs-CRP) in a substudy of the Cholesterol and Recurrent Events (CARE) trial of CAD secondary prevention with statin therapy. Patients with higher hs-CRP levels had more cardiovascular events than those without evidence of inflammation (92); pravastatin use was associated with a reduction of hs-CRP (87), a finding subsequently shown to be common among statins (92–95), and patients with greater evidence of inflammation by hs-CRP had a greater relative treatment benefit. Similar observations were made in subanalyses of the Air Force/Texas Coronary Atherosclerosis Prevention Study (AF-CAPS/TexCAPS) and in ACS patients in the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction 22 (PROVE IT-TIMI 22) study (89,96); in these studies, treatment benefit was observed even in patients with lipid levels below treatment guidelines. An ongoing randomized trial, Justification for the Use of statins in Primary prevention: an International Trial Evaluating Rosuvastatin (JUPITER), is testing the hypothesis that patients with low-density lipoprotein cholesterol below current treatment guidelines, but with evidence of inflammation on the basis of elevated hs-CRP, might benefit from statin therapy directed primarily at reducing inflammation (97).

Recent reports of the association of myeloperoxidase, placental growth factor, pregnancy-associated plasma protein-A (PAPP-A), CD40 ligand, interleukin-6, interleukin-10, monocyte chemotactrant protein-1, and other inflammatory markers, including hs-CRP, with risk and in some cases, treatment effect, in ACS highlight the growing recognition of the critical importance of inflammation in CVD (98–111). Whether specific therapies directed at specific components of the inflammatory process or targeted more generally at reducing inflammation will confer improved outcomes remains to be proved.

**Hemodynamic stress.** Brain (or B-type) natriuretic peptide (BNP), a neurohormone produced in increasing amounts by ventricular myocardium in response to dilatation and pressure overload, has proved useful in diagnosis and risk stratification of heart failure patients. Particular benefits have come in the emergency room when the cause of dyspnea was unclear (112–114). Brain natriuretic peptide and immunoreactive amino-terminal pro-brain natriuretic peptide also appear to have utility in risk stratification of asymptomatic individuals without known CVD, those with stable CAD and those post-ACS (115–119). They might also rapidly detect ischemia in the absence of infarction (120), but their role in guiding clinical management is less clear. In one study, BNP predicted sudden cardiac death after MI, even in patients with ejection fractions >30% (121). Although this finding must be substantiated, it suggests that BNP could potentially be used to determine which non–Multicenter Automatic Defibrillator Implantation Trial (MADIT) II patients might benefit from a defibrillator. In Treat Angina with Aggrastat and determine Cost of Therapy with an Invasive or Conservative Strategy–Thrombolysis In Myocardial Infarction (TACTICS-TIMI) 18, a randomized study of early invasive management compared with conservative care of ACS patients, BNP levels identified patients at increased risk for mortality but did not differentiate patients more likely to benefit from early invasive management (122). Thus, as with markers of inflammation, additional work remains to establish these markers as fundamental components of personalized cardiovascular care.

**Multimarker strategies: a forerunner to clinical proteomics.** Incremental information might be obtained by simultaneously measuring biomarkers of varying types or subtypes in determining prognosis for a particular population. Several multimarker strategies have been published, ranging from using a multimarker approach to improve MI diagnosis and risk stratification in low-risk chest pain patients (123) to

<table>
<thead>
<tr>
<th>Table 6. Protein Biomarkers of Disease State and Risk: In Clinical Use and Emerging</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myocardial necrosis</strong></td>
</tr>
<tr>
<td>Troponin I/T</td>
</tr>
<tr>
<td>Creatine kinase-MB</td>
</tr>
<tr>
<td>Myoglobin</td>
</tr>
<tr>
<td><strong>Ischemia</strong></td>
</tr>
<tr>
<td>Unbound free fatty acids</td>
</tr>
<tr>
<td>Ischemia modified albumin</td>
</tr>
<tr>
<td>Whole blood choline</td>
</tr>
<tr>
<td>B-type natriuretic peptide</td>
</tr>
<tr>
<td><strong>Inflammation/plaque stability</strong></td>
</tr>
<tr>
<td>C-reactive protein</td>
</tr>
<tr>
<td>Interleukin-6</td>
</tr>
<tr>
<td>Interleukin-10</td>
</tr>
<tr>
<td>Interleukin-18</td>
</tr>
<tr>
<td>Pregnancy-associated plasma protein-A</td>
</tr>
<tr>
<td>CD40 ligand</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
</tr>
<tr>
<td>Placental growth factor</td>
</tr>
<tr>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>Serum amyloid A</td>
</tr>
<tr>
<td>Monocyte chemotactrant protein-1</td>
</tr>
<tr>
<td>Matrix metalloproteinases</td>
</tr>
<tr>
<td>Lipoprotein-associated phospholipase A2</td>
</tr>
<tr>
<td><strong>Hemodynamic stress</strong></td>
</tr>
<tr>
<td>B-type natriuretic peptide</td>
</tr>
<tr>
<td>N-terminal proB-type natriuretic peptide</td>
</tr>
</tbody>
</table>

Clinical use = test available commercially and has demonstrated diagnostic or prognostic utility in clinical studies; see text for references.
combining markers of necrosis, inflammation, and hemodynamic stress to enhance risk stratification in patients with ACS (65,107–110). In each example, the information provided by groups of markers was additive to conventional risk assessment tools such as electrocardiography or clinical variables such as age and renal function.

As we progress into the era of genomics and proteomics, we increasingly will be presented with new putative biomarkers of disease state and risk not only from biomarker, genetic, or RNA approaches but also from large-scale proteomic strategies (124). The National Heart Lung and Blood Institute recently recognized (125), as the challenges for the next decade: 1) development of systematic and adequately-powered approaches to validate the associations of protein biomarkers with disease state, risk, or treatment effect that are identified from genomic and proteomic approaches; and 2) development of informatics and analytical platforms for grouping putative markers of risk into “panels” that are clinically useful.

**METABOLICMS: A SYSTEMS BIOLOGY APPROACH TO BIOMARKERS OF HEALTH AND DISEASE**

Metabolomics—the study of small molecule (non-protein) profiles in biological samples—resides at the end of the biological road that starts with DNA (Table 1). We expect that metabolomics will be an integral part of the broader spectrum of personalized medicine (Fig. 1). Efforts are underway in several centers to catalogue the complete human metabolome (estimated to contain approximately 5,000 molecules) in both health and disease (126,127). The goal of metabolomics is to relate physiological consequences of disease (i.e., the metabolites associated with pathological processes) to the genomic origins of those diseases. Metabolite profiles can provide insight into cellular physiology and the environment in which that physiology takes place.

Although limited mammalian metabolic data have been amassed to date, there has been definite progress in using this approach to understand individual responses to diet and therapies aimed at metabolic disorders (128–131). In one study, the dietary effects of poly-unsaturated fatty acids (PUFAs) were correlated with gene expression and lipid metabolite level to understand the signaling pathways involved in PUFA-mediated impact on human health (130). In another study, a therapeutic agent for diabetes and obesity, roziglitazone, was studied in a mouse model that provided many of the metabolomic hallmarks found in humans with these conditions (elevated serum glucose, insulin, and triglycerides) as the animals became obese on specific diets. Receiving high doses of roziglitazone, the animals responded with changes to the three biomarkers. At the same time, the animals developed hepatic lipidosis, which could only be explained though quantitative fatty acid analysis of all lipid classes in liver, blood, and muscle (132). This type of study might permit identification of subsets of patients who could benefit from specific (personalized) dietary intervention or nutritional supplementation for disease prophylaxis. Lastly, 1H nuclear magnetic resonance (NMR) plasma analyses appear to differentiate between patients with and without CAD and might provide non-invasive methods for following disease burden and activity (133). Without question, the most mature metabolomics application is the use of biomarkers as diagnostics and indicators of disease progression. Serum cholesterol measurement is the best example of a limited form of metabolomics currently in clinical use. It provides proof-of-principle that one can measure a metabolite and offer useful advice to a patient otherwise deemed healthy: it is truly prospective in nature (134).

The two principal methods of examining metabolites, NMR and MS, are well established and already standard in most hospitals. Nuclear magnetic resonance requires minimal sample processing, preserves sample integrity, and can rapidly identify and localize abundant analytes in a sample. Mass spectroscopy can quantify individual components within a sample and allows simple spectral analysis when coupled with a chromatograph. Newer time-of-flight MS technology has reduced analysis times to just a few minutes. The challenge for metabolomics—as for proteomics—will be to move to the realm of multiplex testing and high-throughput screening of hundreds or even thousands of metabolites, just as microarrays are now used to examine the activity of thousands of genes simultaneously.

**PHARMACOGENOMICS: SAFETY AND EFFICACY IN CARDIOVASCULAR THERAPIES**

The field of pharmacogenomics—the use of genomic variation to predict efficacy and toxicity of drug therapy—might be the most promising area for near-term clinical application of genomic information. Commonly used medications such as lipid-lowering therapy, anti-hypertensives, anti-arrhythmics, and anti-coagulants have differential effects on the basis of individual genetic variation. No fewer than 40 pharmacogenomic variants have been associated with drug response (135). Ultimately, both prospective data and proof of causality will be necessary to make clinical pharmacogenetic tests robust.

Recently, associations were found between lipid response to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors and variants in the HMG-CoA reductase gene (136) and a variant of the estrogen receptor and HDL response in women on estrogen replacement (137). In hypertension, variation in the alpha-adducin gene was associated with renal sodium resorption and salt-sensitive hypertension in animal models and humans (138,139). A broad initial study found no association with MI (140), but a focused follow-up study revealed a specific benefit (RR 0.49, 95% CI 0.32 to 0.77) for those with the alpha-adducin variant (141). Thus, variation in this gene might identify which hypertensive patients would most benefit from diuretic therapy.
Beta-blockers and ACE inhibitors are standard treatments for heart failure (142). Genetic variation in the beta_{1}-adrenergic receptor has been well characterized. The Arg389Gly polymorphism results in differential stimulation in response to agonists; thus, it produces a different response to blockade (143). Recently, an analysis of DNA from 1,040 individuals in the Beta-Blocker Evaluation of Survival Trial (BEST), in which there was no survival benefit with bucindolol in the overall population, showed that homozygotes for the Arg389 variant had a 36% reduction in death and hospitalizations at two years (144). This study illustrates the importance of collecting biological specimens to stratify patients by genotype and the potential for genetic heterogeneity to confound the findings of randomized controlled trials designed around traditional and imprecise clinical attributes.

Polymorphisms in the ACE pathway also provide an example of the possibility of pharmacogenomics in practice. In a population of chronic heart failure patients, the ACE DD polymorphism was significantly associated with death or need for transplant, but only those with ACE DD treated with beta-blockers had improved survival compared with those not on beta-blockers (145). In another study from the same group, patients with the DD polymorphism had worse outcomes on low-dose ACE inhibitor therapy compared with high-dose (146). In addition, high-dose ACE inhibitors and beta-blockers had the greatest association with transplant-free survival in those with the DD variant.

One area with great potential for pharmacogenomic applications is arrhythmias. The long QT syndrome (LQT) causes sudden death and has traditionally been diagnosed using clinical criteria (147). Long QT syndromes are clinically homogeneous but genetically heterogeneous, and studies over the last 10 years have yielded better molecular characterization of these disorders and subsequently better targeted therapy (148). It is now known that LQT-1 is caused by a potassium channel defect (KVLQT1 gene) and LQT-3 by a sodium channel defect (SCN5A); therefore, the choice of therapeutic strategy is now dictated by genotype. Long QT syndrome-1 responds better to beta-blockers than LQT-3, which responds more favorably to sodium channel agents, such as flecainide, and might be worsened by beta-blockers (149,150). It is likely that forms of acquired LQT, such as those induced by medications, might have a similar molecular basis.

Variation in drug metabolism has implications for pharmacogenomics. Two variations within the gene CYP2C9, the principal enzyme that metabolizes warfarin, are associated with increased risk of over-anticoagulation and bleeding events (151). The variations are common in the population and accurately predict the mean warfarin dose required for a therapeutic international normalized ratio (INR). With such a clearly defined phenotype for a gene with common alleles, one could conceive of an algorithm for use in anti-coagulation clinics to dose warfarin on the basis of a patient’s CYP2C9 genotype. Validation of such a strategy could easily be accomplished in a clinical trial, with “time to therapeutic INR” and safety measures as key end points.

The holy grail of pharmacogenomics is to deliver the “right drug for the right patient” by accurately predicting both therapeutic response and safety before prescribing it. To fulfill this promise, new “smart trials” are needed with stratification of entry according to this new type of information. When trials are not possible, appropriate registries that couple clinical and biological information can help generate this needed knowledge. The recent release of the Food and Drug Administration’s guidance on pharmacogenomics (152) is a significant step toward the development of personalized medicine and a statement of the U.S. government’s official advocacy of this paradigm for the future of drug development and health care.

**MOLECULAR IMAGING OF THE CARDIOVASCULAR SYSTEM: THE CONVERGENCE OF GENOMICS AND ENGINEERING**

The development of rationally designed chemical or biological probes and imaging reporter agents that sense molecular pathway mechanisms now allow us to monitor the activity of those pathways in health and disease. Use of genomic information and development of imaging systems in small animals is resulting in a rapid evolution of molecular imaging to display and quantify molecular and cellular targets in vivo.

Optical coherence tomography (OCT) is being used to measure the macrophage content of arterial plaques. These quantitative measures might aid in determining prognosis and guiding therapy (153). Radiolabeled antibodies against epitopes on low-density lipoprotein such as I-125 MDA2 have been used in animal models as a means of assessing disease severity (154). Magnetic resonance imaging, fluorescence imaging, bioluminescence imaging, positron emission/single-photon emission computed tomography (PET/SPECT), and ultrasound are techniques that take advantage of molecular probes designed to image enzymes (e.g., cathepsins B,D,K,S, and MMPs), receptors (e.g., GPCRs, integrins), and endothelial cells (e.g., E-selectin, VCAM) as well as the biological processes of apoptosis (phosphatidylserine), angiogenesis (VCAM), and thrombosis (fibrin, thrombin) (155). Magnetic resonance nanoparticles might be targeted directly to specific molecular species such as fibrin or integrins, allowing the visualization of clot formation and angiogenesis (156). Molecular imaging is already being applied in cancer and inflammatory diseases as well as atherosclerosis, heart failure, and thrombosis to: 1) detect disease earlier, 2) monitor disease progression, and 3) monitor response to therapies (155).

Although many of these techniques remain in the preclinical evaluation stage, the prospects of imaging the physiologic plaques and identifying those with vulnerable physiology might soon be realized. Used as an adjunct to anatomic imaging and standard functional imaging, these will be powerful tools for identifying the highest-risk patients...
and providing the appropriate pathway-specific therapies for them and the best means of monitoring their response.

CONCLUSIONS

A paradigm shift is taking place in cardiovascular care, driven by the capability to perform routine genomic analysis of individuals. In principle, this will include susceptibility screening, comprehensive expression analyses, proteomic and/or metabolomic testing, and probabilistic relation of those results to discrete clinical end points. Ideally, genomic medicine will be predictive and seamlessly integrated into patient care. In the coming years, we expect that the addition of genome-level testing and sophisticated analyses of genomic and environmental risk will further refine individualized approaches to care in CVD patients. Genomics will become increasingly pervasive in cardiovascular medicine. It is imperative that the cardiology community be comfortable with and embraces it so that patients can realize its full potential.

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