PART II

GENETICS AND PERSONALIZED MEDICINE

7

Personalized and Precision Cardiovascular Medicine

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April 14, 2013 marked the 10th anniversary of the completion of the Human Genome Project. In just 10 years the field of genomics—the scientific study of genomes, their complete DNA sequences, and the functional interaction of their genes—has flourished as a result of high throughput technologies to generate, analyze, and interpret genome-derived data efficiently and cost-effectively. A broad aspiration of the Human Genome Project has been the concept of *personalized medicine*—a rapidly advancing field of health care that is informed by a person's unique clinical, genetic, genomic, and environmental information.[1] Personalized medicine seeks to couple established clinical-pathologic indices with state-of-the-art molecular profiling to create diagnostic, prognostic, and therapeutic strategies precisely tailored to each patient's requirements—hence the term *precision medicine*. Although this concept is not entirely new, many patients and providers have had great expectations that the genome would enable the development of novel diagnostic and predictive tests as well as therapies based on an individual's genetic information. This chapter presents a broad overview of the potential of personalized medicine. Subsequent chapters (**Chapters 8 to 10, and 42**) will elaborate specific approaches to various aspects of personalized medicine.

This decade also marks the 50th anniversary of the introduction of the term "factor of risk," coined by William Kannel, principal investigator of the Framingham Heart Study (FHS).[2] The risk factors for developing coronary artery disease (CAD)—male sex, hypertension, diabetes mellitus, increased low-density lipoprotein (LDL) cholesterol, tobacco use, and family history of heart disease—remain foundational for stratifying individuals to therapeutic strategies based on their risk of developing CAD. The FHS was among the first studies to illustrate the benefit of data integration to achieve refined risk classification. The massive and comprehensive collection of clinical and biologic data associated with the outcome of coronary disease enabled development of the Framingham predictive models[3] and the resulting Framingham risk score (FRS).[4] Today, it is anticipated that the inclusion of data that address the subtle distinctions in individuals revealed through genomic analyses might greatly enhance this prediction—a concept that has stimulated the development of genomic risk scores (GRSs) combined with the FRS (see later in this chapter) to enhance predictive accuracy. The opportunity for impact on clinical decision making offered by genome technologies lies in increased resolution: the potential to improve a person's placement on the complex, multidimensional risk spectrum based on detailed, individual molecular characteristics on a genomic scale. The FHS example emphasizes the value of making use of the full spectrum of available clinical and demographic data; the genomic era simply expands this view toward integrated approaches that embrace and exploit genomic data in conjunction with other data.

ASSESSMENT OF DISEASE RISK: FAMILY HEALTH HISTORY AND HEALTH RISK ASSESSMENTS

Several approaches to risk assessment for cardiovascular disease have emerged that, if routinely used, might impact our ability to tailor chronic disease prevention strategies to the individual and promote improved cardiovascular public health. These include the FRS,[4] the Reynolds risk score,[5] and the European Society of Cardiology score.[6] My colleague and I proposed a framework that includes family history assessment to identify high-risk persons for disease, thus enabling preventive and therapeutic interventions.[7] Family health history (FHH) is a simple yet invaluable tool for the delivery of personal health risk information. Reflecting the complex combination of shared genetic, environmental, and lifestyle factors, a thorough FHH can approximate genetic/genomic risk information for integration

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into patient care. FHH assessments can identify persons at higher risk for common chronic diseases, enabling preemptive and preventive steps including lifestyle changes, health screenings, testing, and early treatment as appropriate.

Systematic collection of FHH for cardiovascular risk assessment was recently implemented in 24 family practices in the United Kingdom using a pragmatic cluster randomized controlled trial design, and demonstrated a highly significant (40%) increase in the identification of individuals at high risk.[8] This was the first rigorously designed prospective study to show that the collection and use of FHH in a primary care setting can improve risk stratification for cardiovascular disease and health behaviors. Thus, ascertainment of FHH data is a feasible practice-level intervention that could improve cardiovascular risk assessment and help target patients who most need preventive interventions.

Family history and genomic testing are complementary techniques for evaluating health risks.[9] Rather than choosing between the two, an approach that incorporates both types of information, in addition to nongenetic risk factors, promises the most accuracy. The combination of detailed family history, medical history, clinical evaluation, and genome sequence information, as exemplified by the ClinSeq Project at the National Human Genome Research Institute (NHGRI),[10] may eventually provide the most accurate cardiac risk prediction.

A GENOMIC TOOLBOX FOR PERSONALIZED AND PRECISION MEDICINE

Several genome-wide technology platforms are now routinely available for the exploration of the impact of the genome and its expressed products on health and disease states (**see also Chapter 8**). Concurrently, several cohort studies with longitudinal clinical data and biologic specimens sponsored by the National Heart, Lung, and Blood Institute (NHLBI) provide the opportunity for molecular analyses, disease classification, and predictive modeling. These include the FHS, the Coronary Artery Risk Development in Young Adults (CARDIA) study, the Atherosclerosis Risk in Communities (ARIC) study, the Jackson Heart Study (JHS), the Women's Health Initiative (WHI) study, the Cardiovascular Health Study (CHS), and the Multi-Ethnic Study of Atherosclerosis (MESA). These powerful longitudinal studies and their clinical data and biospecimens can be accessed via the NHLBI's BioLINCC program (https://biolincc.nhlbi.nih.gov), which contains a vast catalog of biospecimens resources that can be used to facilitate population genomics, using the tools outlined below. The discovery and development of genome-based biomarkers requires high-quality biospecimens linked to exquisitely defined phenotypes, assayed using one or more genome-based technologies. Their translation to clinical application forms the basis for personalized medical care.

DNA Variation

Genome-wide association studies (GWASs) emerged in 2005 as an unbiased strategy to provide information on common DNA variants associated with complex phenotypes. GWASs are predicated on the common disease–common variant hypothesis, which postulates that common diseases result from many disease-influencing alleles that occur at relatively high frequencies in the population, but individually have little predictive value. Nineteen published GWASs on CAD are recorded in the NHGRI Catalogue of GWASs (http://www.genome.gov/26525384), the largest being a meta-analysis of 63,746 CAD cases and 130,681 control cases.[11] The total number of loci for CAD now exceeds 46. These loci encompass genes related to lipid metabolism and other CAD risk factors, but some novel loci—such as the region on chromosome 9 near the genes *CDKN2A/CDKN2B*—represent truly novel risk variants that will advance our understanding of the mechanisms underlying CAD. Together, these variants account for less than 10% of the heritability of CAD, suggesting the involvement of genetic factors beyond common variants.

Whole-Genome Sequencing

Advances in sequencing technologies have reduced costs such that a human genome can now be sequenced for less than $5000, and may be at the $1000 level in the coming year.[12] At this cost, sequencing a patient's genome will fall within the range of DNA-based diagnostic tests. More than 30,000 human genomes[13] have now been sequenced and applied to elucidation of the biology and diagnosis of malignancies, rare genetic diseases, and microbial infections.[14-16] Whole-genome sequencing also has advanced to the clinic, where it permits definitive diagnosis and even guides treatment.[17-19] Although these approaches have yielded success when applied to mendelian disorders and cancer, methods for identifying rare variants for common diseases such as CAD are still nascent.[20]

GENE EXPRESSION

The genome-wide study of RNA expression levels includes a spectrum of molecules from mRNA to noncoding RNAs. Microarrays and RNA sequencing now can assay the entire complement of RNA expressed in a cell, tissue, or biologic fluid. Clustering of co-expressed genes using parametric or nonparametric methods provides the foundation for generating a "pattern" or "signature" of gene expression that is associated with a phenotype or physiologic state. These methods have been applied to classify a disease or to predict future disease states; the same data may also serve to generate molecular pathway information for the biologic mechanisms underlying disease. Two recent reviews nicely summarize the emerging gene expression–derived biomarkers for clinical applications in cardiovascular medicine.[21,22]

A surprising feature of the transcriptome is the significance of noncoding RNAs in the regulation of genes. Of particular interest are the expression patterns of small interfering RNAs (siRNAs) and microRNAs (miRNAs). Whereas siRNAs interfere with transcription through degradation of the message RNA, miRNAs work differently. The latter are usually 22 nucleotides in length, and through an miRNA-induced silencing complex, they inhibit gene expression on a post-transcriptional level by binding to complementary 3′ untranslated regions (UTRs) of target mRNA.[23] The miRNAs play a role in several diseases and are advancing to clinical application in acute coronary syndromes,[24] acute myocardial infarction (MI),[25] cardiomyopathies,[26] type 2 diabetes,[27] hypertension,[28] and heart failure.[29] Most of these studies are small and require validation in larger populations.

Proteomics

Proteomics refers to the large-scale study of proteins, and the proteome often is considered to embody the full complement of proteins and their various derivatives (e.g., splice variants or post-translational modification) (**see also Chapter 10**). In the context of health and disease, proteomics seeks to define the full set of proteins associated with a particular physiologic state. Although this technology is relatively immature in its applications to human health and disease compared with RNA and metabolic profiling, application of these methods, combined with the development of mass spectroscopy technology, should advance proteomics to more routine use in disease classification and diagnosis, prognosis, and pharmacogenomics within the next several years.

Metabolomics

Metabolomics measures the approximately 5000 discrete small molecule metabolites and allows the identification of metabolic fingerprints for specific diseases. This technology may have practical use in the development of therapies, because metabolic changes immediately suggest enzymatic drug targets (**see also Chapter 10**). Similar to genomics and proteomics, metabolomics may be useful in disease diagnosis, prognosis, and drug development. Targeted

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mass spectroscopy–based metabolic profiling has been applied to cardiovascular disease to classify CAD and to predict ischemic events.[30,31]

PERSONALIZED AND PRECISION CARDIOVASCULAR MEDICINE: CLINICAL POTENTIAL

Hypertension

Genetic variants associated with blood pressure (BP) that robustly replicate have finally emerged from GWASs. The single-nucleotide polymorphisms (SNPs) discovered have mainly been common variants (minor allele frequency [MAF] of ≥5%), with small effect sizes (mostly ≤1 mm Hg for systolic BP [SBP] and ≤0.5 mm Hg for diastolic BP [DBP]), and they collectively have explained only a small proportion (3% to 4%) of BP heritability. A recent GWAS investigated associations with SBP, DBP, mean arterial pressure (MAP), and pulse pressure (PP) by genotyping some 50,000 SNPs that capture variation in approximately 2100 candidate genes for cardiovascular phenotypes in 61,619 persons of European ancestry from cohort studies in the United States and Europe. Novel associations were identified for SBP (chromosomal locus 3p25.3 in an intron of *HRH1*; and 11p15 in an intron of *SOX6*, previously associated with MAP) and for DBP (1q32.1 in an intron of *MDM4*). Ten previously known loci associated with SBP, DBP, MAP, or PP were confirmed (*ADRB1, ATP2B1, SH2B3/ATXN2, CSK, CYP17A1, FURIN, HFE, LSP1, MTHFR, SOX6;* $P < 2.4 \times 10^{-6}$).[32] These results represent a major advance in view of the fact that just a few years ago, almost no specific details were known about the genetic architecture of hypertension beyond the mendelian disorders. The results of ongoing fine-mapping studies of BP loci and sequence-based discovery of rare variants in extreme hypertensive cases and normotensive controls will provide further insights into the underlying genetic causes of BP, with the potential for improvement in the means for predicting and stratifying hypertension.

Coronary Artery Disease and Myocardial Infarction

As indicated previously, recent studies have identified a growing number of CAD-related and MI-related SNPs, and their results have stimulated additional studies to explore the value of these SNPs for risk prediction. Paynter and associates assessed the relationship of 101 SNPs to CAD in a cohort of 19,000 women, followed for 12 years, from the Women's Genome Health Study.[33] A GRS based on these 101 SNPs revealed a significant relationship between higher GRS and CAD, but failed to add incremental value to existing clinical models. Another GRS based on the counting of the number of "adverse" alleles influencing lipids has been shown to enhance risk prediction compared with measurement of lipids alone.[34] Clinical adoption of GRS for CAD risk prediction will require unequivocal evidence that genotype predicts CAD, even after adjustment for plasma lipids and other known CAD risk factors.

Accompanying the transformative discoveries on genetic susceptibility variants just described are additional predictive CAD and MI biomarkers emerging from the expressed genome. Rosenberg and colleagues found that the gene expression signature of 23 genes obtained from the peripheral blood of nondiabetic patients undergoing coronary angiography for acute chest pain permitted reclassification of the risk of having CAD, at a rate of approximately 20% of that for traditional clinical models.[35] The negative predictive value of 83% for the gene expression assay compared favorably with typically used clinical tests such as myocardial perfusion imaging. In addition, Voora and co-workers recently reported the development of an RNA signature associated with the platelet response to aspirin and the ability of that same signature to predict acute coronary syndromes in two cohorts.[36]

Heart Failure

Increasingly detailed characterization of gene expression from diseased tissues and circulating cells from animals and patients are providing new insights into the pathophysiology of heart failure (HF) that permit identification of novel diagnostic and therapeutic targets. Differential gene expression profiles for failing and nonfailing hearts have already identified types of HF with different causes.[37] Gene expression profiles usually compare matched pairs of samples, such as nonfailing versus failing hearts, ischemic versus nonischemic hearts, male versus female failing hearts, or atria versus ventricles of failing hearts. This approach identified cardiac myosin light chain kinase (*MLCK*) as an HF-related gene by correlating expression levels with the severity of HF; further investigations confirmed the importance of cardiac *MLCK* in HF. A robust gene expression signature composed of 27 genes emerged from analysis of four independent microarray data sets from evaluation of the failing myocardium of dilated cardiomyopathy.[38] Among these genes are several associated with mitochondrial dysfunction and oxidative phosphorylation, as well as three extracellular molecules, including periostin, pleiotrophin, and *SERPINA3*—some of which may become novel diagnostic and therapeutic targets for HF. Although the complexity of genomic and transcriptional profiling may be challenging to use in the clinic, advances in clinical information technology and user interfaces (see were later in this chapter) should permit greater individualization of prevention and treatment strategies to personalize the treatment of HF.[39]

Arrhythmias

Inherited arrhythmia syndromes and forms of structural heart disease cause arrhythmias and sudden cardiac death (**see also Chapters 9 and 33**). Genetic testing for these conditions is among the most clinically advanced areas of personalized and precision cardiovascular medicine. Tests for several arrhythmia syndromes are currently available through qualified laboratories including Correlagen, Familion/Transgenomic, GeneDx, and Partners Healthcare. A definitive genetic diagnosis for the cause of a rhythm disorder may help to direct clinical recommendations, which include periodic follow-up, avoiding medications that may exacerbate the condition, and avoiding strenuous activities such as competitive sports. In addition, specific genetic diagnoses may guide therapies, such as the use of beta blockers in long-QT syndrome and recommendations for an implantable cardioverter-defibrillator.

Current clinical practice guidelines recommend screening of asymptomatic first-degree relatives and all potentially symptomatic relatives of patients with a known inherited arrhythmia. Identification of a causative gene in a proband should prompt genetic screening of family members, although insurance carriers may not reimburse for the genetic screening of asymptomatic patients. The greatest use for genetic testing at present lies in the ability to define, in a family with an inherited condition of known genetic etiology, unaffected persons who therefore require no further clinical follow-up and cannot pass the condition to their children.

Cardiac Transplant Rejection

Profiling of patients after cardiac transplantation is altering clinical decision making and management of allograft rejection. Standard protocols after heart transplantation require patients to undergo serial endomyocardial biopsies as a means to monitor rejection and to guide immunosuppressive therapy. Horwitz and associates demonstrated that gene expression profiles of peripheral blood mononuclear cells (PBMCs) might provide an alternative approach to the diagnosis of allograft rejection.[40] Patients who subsequently developed acute rejection had a distinct genomic profile compared with patients without any rejection, and after treatment for rejection, a majority (98%) of differentially expressed genes returned to baseline. The CARGO (Cardiac Allograft Rejection Gene Expression Observational) study prospectively investigated gene expression analysis

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from PBMCs as a diagnostic tool to predict transplant rejection.[41] From the core group of 11 genes associated with immune response pathways, which were identified by quantitative real-time polymerase chain reaction (RT-PCR) and assigned weighted scores, the CARGO investigators were able to predict rejection with a sensitivity and specificity of 80% and 60%, respectively.[42] This study provided proof-of-concept that expression profiling of 11 genes in PBMCs could predict acute rejection pathways in cardiac transplant recipients. One important implication is that blood genomic profiling can provide a sensitive marker for transplant rejection,[41] potentially guiding surveillance and therapeutic management.

Pharmacogenetics

The use of genetic variation to identify subgroups of patients who may respond differently to certain medications represents the leading edge of personalized and precision medicine. Since its first description, the field of pharmacogenetics has expanded to study a broad range of cardiovascular drugs, and has become a mainstream research discipline (**see also Chapter 9**). Three principal classes of pharmacogenetic markers have emerged: (1) pharmacokinetic, (2) pharmacodynamic, and (3) underlying disease mechanism. Significant advances have identified markers in each class for a variety of therapeutics, some with the potential to improve patient outcomes (**Table 7-1**). Although ongoing clinical trials will determine the potential benefits of routine pharmacogenetic testing, current data support pharmacogenetic testing for certain variants on an individualized, case-by-case basis. Major pharmacogenetic variants have been identified that are associated with commonly used cardiovascular medications (**Tables 7-2 to 7-4**).[43] Presented next is an overview of the current status for the pharmacogenetics of statins, warfarin, and clopidogrel.

Statins (see Table 7-2). Genetic testing for statin *efficacy* is not likely to enter clinical care, because the magnitude of association is small (approximately 10% to 15% differences in LDL cholesterol lowering), and physicians can reasonably forecast the magnitude of LDL cholesterol lowering based on statin type, dose, and baseline LDL cholesterol. By contrast, statin-induced *side effects and nonadherence* are less predictable. The solute carrier organic anion transporter family, member 1B1 gene (*SLCO1B1,* also referred to as *SLC21A6, OATP-C,* or *OATP1B1*) harbors a genetic variant, the *5 variant (rs4149056, Val174Ala), that interferes with the localization of this transporter to the hepatocyte plasma membrane,[44] leading to higher plasma statin concentrations.[45-47] In candidate gene studies and GWASs, carriers of *5 have a fourfold to fivefold increased risk for severe, creatine kinase (CK)-positive simvastatin-induced myopathy, and a twofold to threefold increased risk for CK-negative myopathy.[48,49]

In trials of randomly assigned statins and in observational studies, the risk for myopathy with *5 depends on the statin type: The risk is greatest for simvastatin > atorvastatin > pravastatin, rosuvastatin, or fluvastatin.[49,50] These effects parallel the influence of the *5 allele on the clearance of these statins,[45,47] and thus appear to be statin-specific.

Clinical guidelines do not currently recommend prospective genotyping for *SLCO1B1*5* based on the current levels of evidence, but the test is currently offered on consumer-directed genotyping platforms,

TABLE 7-1 Sources of Pharmacogenetic Variation

CATEGORY	DESCRIPTION	TYPES OF GENES	EXAMPLE DRUGS WITH: GENES
Pharmacokinetic	Variability in the concentration of drug at the site of drug effect	Drug-metabolizing enzymes Drug transporters	Warfarin: *CYP2C9* Clopidogrel: *CYP2C19* Simvastatin: *SLCO1B1* Metoprolol: *CYP2D6*
Pharmacodynamic	Variability in the drug ability to influence its target	Transmembrane receptors Intracellular enzymes	Clopidogrel: *P2RY12* Simvastatin: *HMGCR* Metoprolol: *ADBR1*
Underlying disease	Variability in the disease being treated	Often downstream or independent of drug target	HCTZ: *ADD1* Simvastatin: *APOE*

HCTZ = hydrochlorothiazide.
Modified from Voora D, Ginsburg GS: Clinical application of cardiovascular pharmacogenetics. J Am Coll Cardiol 60:9, 2012.

TABLE 7-2 Genetic Associations with the Response to Statins

GENE	VARIANT(S)	STATIN RESPONSE	STATIN TYPE
APOE	ε2, ε3, and ε4 haplotypes defined by alleles at rs7412 rs429358	LDL cholesterol lowering	Class effect
HMGCR	H7 haplotype defined by alleles at rs17244841, rs17238540, and rs3846662	LDL cholesterol lowering	Simvastatin
SLCO1B1	rs4149056	Musculoskeletal side effects	Simvastatin, atorvastatin
SLCO1B1	rs4149056	Nonadherence	Simvastatin, atorvastatin

Modified from Voora D, Ginsburg GS: Clinical application of cardiovascular pharmacogenetics. J Am Coll Cardiol 60:9, 2012.

TABLE 7-3 Genetic Associations with the Response to Clopidogrel

GENE	VARIANT(S)	DRUG RESPONSE
CYP2C19	*2 (rs4244285)	Drug concentration, platelet function, recurrent MI, stent thrombosis
CYP2C19	*17 (rs3758581)	Drug concentration, platelet function, bleeding
ABCB1	T-T-T haplotype defined by T allele at C1236T (rs1128503), G2677T (rs2032582), and C3435T (rs1045642)	Drug concentration, platelet function, recurrent MI, stroke, death
P2RY12	F haplotype defined by following alleles: rs6798347, rs6787801, rs9859552, rs6801273, rs9848789, and rs2046934	Inhibition of platelet function

MI = myocardial infarction.
Modified from Voora D, Ginsburg GS: Clinical application of cardiovascular pharmacogenetics. J Am Coll Cardiol 60:9, 2012.

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and is being provided to clinicians as part of the National Institutes of Health (NIH) eMERGE program.[51] A potential strategy for prospective *SLCO1B1*5* testing might recommend pravastatin, rosuvastatin, or fluvastatin as first-line agents for carriers, because these drugs seem to depend the least on *SLCO1B1* for their clearance.

Clopidogrel (see Table 7-3). The *CYP2C19*2* allele is associated with a graded risk of death, MI, or stroke. Carriers of one allele (intermediate metabolizers) have an approximately 1.5-fold increased risk, and carriers of two alleles (poor metabolizers) experience a 1.8-fold increase. This pattern also extends to stent thrombosis, with an approximately 2.6-fold and 4-fold increased risk in persons with one and two *2 alleles, respectively.[52-57] The *CYP2C19* genetic associations with platelet function correlate with the clinical response to clopidogrel in the setting of percutaneous coronary intervention (PCI). These observations formed the foundation for the U.S. Food and Drug Administration's (FDA) updating the clopidogrel label to include pharmacogenetic information. Despite having an FDA "black box warning" for efficacy in individuals carrying the *CYP2C19* genetic variant, its adoption in practice has lagged.

Warfarin (see Table 7-4). The response to warfarin has strong genetic associations with *CYP2C9, VKORC1,* and *CYP4F2* variants. Commercial testing and algorithms (e.g., see www.warfarindosing.org) can assist in the interpretation of genotypes. Evidence to justify and tools to enable genotype-guided warfarin therapy are thus well recognized. Until large-scale trials demonstrate a benefit for routine testing, physicians may choose to pursue testing in selected patients in whom it may be beneficial, for (1) diagnosing those with complications from warfarin therapy (e.g., hemorrhage); (2) predicting dose for those at high risk of bleeding (e.g., "triple therapy" with aspirin, clopidogrel, and warfarin); or (3) weighing the costs of newer anticoagulants against warfarin.

For cardiovascular pharmacogenomics, the pace of genetic discovery has outstripped the generation of evidence justifying its clinical adoption for many of the findings to date. Until the evidentiary gaps are filled, however, clinicians may choose to target therapeutics to individual patients whose genetic backgrounds indicate that they stand to benefit the most from pharmacogenetic testing.

TABLE 7-4 Genetic Associations with the Response to Warfarin

GENE	VARIANT(S)	DRUG RESPONSE
CYP2C9	*2 (rs1799853) *3 (rs1057910)	Drug concentration, warfarin dose requirements, out-of-range INR values, hemorrhage
VKORC1	–1639 (rs9923231)	Warfarin dose requirements, out-of-range INR values
CYP4F2	rs2108622	Warfarin dose requirements

INR = international normalized ratio.
Modified from Voora D, Ginsburg GS: Clinical application of cardiovascular pharmacogenetics. J Am Coll Cardiol 60:9, 2012.

TABLE 7-5 Barriers and Solutions to Implementing Personalized and Precision Cardiovascular Medicine

CHALLENGE	ISSUE(S)	POTENTIAL SOLUTIONS
Evidentiary framework	Evidence of clinical validity and utility of genomic and predictive tests is key for FDA approval, insurance coverage, and physician uptake. Randomized clinical trials (RCTs), the current gold standard for demonstrating clinical validity and utility, are expensive and time-consuming.	• Public-private consortia of stakeholders to pool resources and validate genomic biomarkers, e.g., the Biomarker Consortium between government and pharmaceutical companies • Tailoring thresholds of evidence according to potential benefits and risks of the test • Conducting pragmatic clinical trials (PCTs) in circumstances in which RCTs are not feasible • Use of comparative effectiveness research to systematically evaluate data from real-world clinical practice setting
Diffusion of innovation	Health care providers need to know what tests are available and the evidence supporting their use.	• Access to Genetic Testing Registry (http://www.ncbi.nlm.nih.gov/gtr/), GAPPKB (http://www.hugenavigator.net/GAPPKB/home.do), and PharmGKB (http://www.pharmgkb.org/) for information on available genomic tests • CPIC (http://www.pharmgkb.org/page/cpic), EGAPP (http://www.egappreviews.org/), PloS Currents (http://currents.plos.org/) for systematically reviewing tests and developing guidelines and recommendations on their use
Clinical implementation	Integration of genomic testing into current systems of health care delivery requires fundamental changes to medical infrastructure, including broad access to CLIA-certified labs, different methods for tissue handling, and electronic health records with the capacity to access genomic data and deliver clinical decision support.	• Plug-and-play bioinformatic support tools developed by sequencing companies and commercial vendors of electronic health records • Self-management of genomic data in personal health records such as Microsoft Health Vault and Dossia • National, standardized technical architecture for integrating clinical decision support into electronic health records being developed by HL-7 • Open access clinical decision support repositories
Regulation	Define the evidence required for approval of genomic and predictive test.	See "Evidentiary framework" section of this table
Coverage and reimbursement	Define the evidence required for approval of genomic and predictive test.	See "Evidentiary framework" section of this table
Ethical issues	Return of incidental findings to patients is a new concern with next-generation sequencing.	• NHGRI-sponsored, ClinAction (http://www.genome.gov/27546546), to devise a plan for systematically evaluating and cataloguing genetic variants based on their clinical actionability • Position statements developed by professional organizations on return of incidental findings
Education	Need for physician training and knowledge in genomic medicine. Need for patient understanding of genomic tests.	• Genomic medicine courses, CME offered at many medical schools • Programs for primary and secondary education in genomics (e.g., GEON, GenEd)

CLIA = Clinical Laboratory Improvement Amendment; CME = continuing medical education; HL-7, Health Level Seven International [global authority on standards for interoperability of health information technology].
Modified from Manolio TA, Chisholm RL, Ozenberger B, et al: Implementing genomic medicine in the clinic: the future is here. Genet Med 10:157, 2013.

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BARRIERS AND SOLUTIONS TO THE INTEGRATION OF GENOMICS INTO CARDIOVASCULAR MEDICINE

The implementation and adoption of personalized and precision cardiovascular medicine in practice will require several key strategies.[58] One such strategy is the development of enabling infrastructure at the level of specialized laboratory resources (i.e., coordinated biobanking linked to clinical data; informatics support and standards; easy access to genome-wide technologies and core laboratories). In addition, considerable bioinformatics and information technology development is required to make use of the deluge of data that is emerging from these resources: informatics and statistical specialists who can analyze complex multidimensional data; reliable, interoperable electronic health records linked to molecular data; integration of research, clinical, and molecular data; and clinical decision support. Moreover, there is a critical shortage of physicians trained in quantitative skills and decision analysis (understanding of human behaviors and decision making; elucidation of the biologic, psychological, and social factors in decision making). Further integration of personalized medicine into the clinical workflow requires overcoming several key barriers,[59] including the development of evidence to support personalized and precision medicine technology use in clinical care, provider understanding and acceptance of these technologies, implementation and integration into clinical workflows, standards for regulation and reimbursement, and education of patients and providers on the benefits and risks of genomic testing (**Table 7-5**).

FUTURE PERSPECTIVE: TOWARD PERSONALIZED AND PRECISION CARDIOVASCULAR MEDICINE

Cardiovascular medicine is poised to become more personalized and precise through the translation of genome-based discoveries to clinical practice. Several parallel approaches should speed the elucidation of the genomic basis of many cardiovascular diseases. Rare susceptibility variants will rapidly be identified through exome and whole-genome sequencing programs. Detailed cataloging of tissue-specific expression profiles—including the transcriptome, proteome, and metabolome—will yield important insight on the intrinsic biology of disease, along with the environmental and lifestyle impacts on disease. "Framingham 2.0" represents a model that incorporates genomics fully into longitudinal population studies with detailed environmental and geospatial data. The complete integration of genomics and electronic health records is another critical innovation required for a truly systems medicine approach that delivers genetic and genomic biomarkers for potential clinical use. The clinician, fully armed with the knowledge, informatics, and clinical decision support to interpret and use complex data, will be an essential facilitator of personalized and precision cardiovascular medicine and the improvement of cardiovascular public health.

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Principles of Cardiovascular Genetics

8

Kiran Musunuru and Sekar Kathiresan

As physicians, we seek to understand the root cause of human disease. Human genetics provides a unique tool for generating new hypotheses about the root causes of disease based on genome-wide searches in the human population that are unlimited by prior assumptions about the underlying pathophysiologic processes. Over the past several decades, application of the principles discussed here has successfully identified the causative genes for a range of cardiovascular diseases. This information has provided explanations to our patients, improved the ability to predict risk for disease, and most importantly, enabled understanding of the pathophysiology as a foundation for designing rational approaches to improving prevention and therapy.[1] This chapter reviews the principles of human genetics used to make gene discoveries and to translate these findings to improve patient care. We highlight these principles in the context of a clinical case presentation.

INHERITED BASIS FOR THE VARIATION IN RISK FOR CARDIOVASCULAR DISEASE

PATIENT CASE, PART I. A 44-year-old man (JS) is seen in a cardiologist's office for a follow-up visit after having suffered an ST-segment elevation myocardial infarction (STEMI) and undergone treatment consisting of primary angioplasty and placement of a drug-eluting stent. His cardiovascular risk factors before STEMI included a fasting low-density lipoprotein cholesterol (LDL-C) level of 235 mg/dL and active cigarette smoking. His body mass index (BMI) is 25 kg/m^2, he does not have a history of type 2 diabetes, and he is normotensive. His father died at 45 years of age as a result of myocardial infarction (MI), and his paternal uncle suffered an MI at 49 years of age. He has two brothers, 43 and 39 years old; both are free of clinical cardiovascular disease. The 43-year-old brother (KS) has an elevated LDL-C level (214 mg/dL). The 39-year-old brother (LS) has an LDL-C level of 130 mg/dL and a high-density lipoprotein cholesterol (HDL-C) level of 29 mg/dL. The pedigree of the family is shown in **Figure 8-1**.

Many cardiovascular diseases cluster within families, and studies of familial aggregation can determine the extent to which inherited DNA sequence variants contribute to these patterns. A family history of premature coronary heart disease (CHD) elevates the risk for CHD in offspring approximately threefold.[2] Family history is an important risk factor for almost every cardiovascular disease—including atrial fibrillation, congenital heart disease, and hypertension—but familial clustering of disease can reflect shared environment in addition to shared genetic sequence.

Heritability—the fraction of interindividual variability in risk for disease attributable to additive genetic influences—is a commonly used measure for isolating the role of shared genetic sequence. The remaining variability among individuals results from all other contributors: environmental influences on disease, nonadditive (*epistatic*) genetic effects (e.g., gene-gene interactions or gene-environment interactions), error in the measurement of relatedness or disease, and random chance. For most clinically important traits (diseases and risk factors), empiric estimates of heritability range from 20% to 80% (see Online Mendelian Inheritance in Man, available at www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=OMIM, for comprehensive information).

A BRIEF PRIMER ON MOLECULAR BIOLOGY

Genes are encoded in DNA, a polymeric molecule with two strands in a configuration known as a double helix. The "code" comprises four different DNA bases—adenine (A), cytosine (C), guanine (G), and thymine (T)—linked together in nonrandom order. The two strands contain redundant information by virtue of complementarity—an adenine on one strand is always paired with a thymine on the other strand, and a cytosine on one strand is always paired with a guanine on the other strand. Thus double-strand DNA can be considered to be a sequence of A-T, T-A, C-G, and G-C base pairs (**Fig. 8-2**).

Human DNA is organized into a total of 23 pairs of chromosomes, with each chromosome spanning millions of base pairs. The 46 chromosomes in total make up the genome. Each chromosome has numerous genes, which contain so-called coding DNA, separated by large stretches of noncoding DNA. A process called transcription copies the information in the DNA sequence into single-strand RNA, a polymer that is structurally similar to DNA but uses uracil (U) in place of thymine (T). Subsequently, the process of translation converts the RNA sequence into an amino acid sequence that makes up a protein, which can serve in a variety of roles (structural elements, enzymes, hormones, etc.). Thus genetic information flows from DNA to RNA to protein in what is classically known as the "central dogma" of molecular biology (**Fig. 8-3**).

One of the consequences of the central dogma is that a change in the DNA sequence in the genome, if it should occur in or near a gene, can result in a change in the protein encoded by the gene, which in turn can have important consequences on the phenotype of an organism. Phenotype refers to any observable characteristic in a human being. Changes in DNA sequence leading to phenotypic

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changes underlie most of the heritability of diseases that have a genetic component.

Epigenetics pertains to phenotypic changes caused by DNA-level modifications that do not involve the DNA sequence, typically structural modifications either of certain DNA bases or of the proteins (called histones) in which the DNA is packaged. These changes can result in altered levels of RNA being transcribed from the DNA, which in turn results in altered levels of protein. In some cases the epigenetic changes are transmitted from parents to offspring, and thus can represent an additional source of phenotypic heritability.

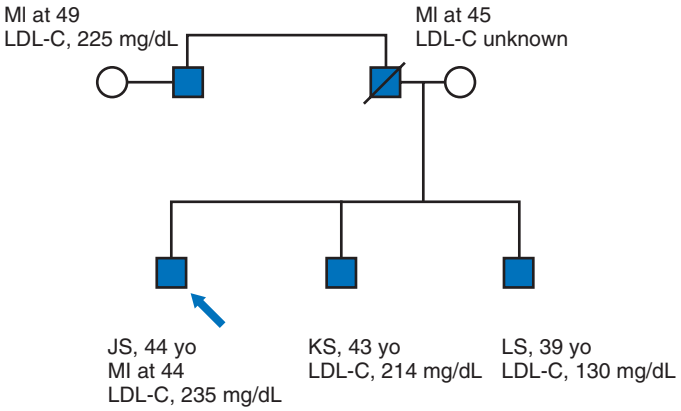

FIGURE 8-1 Pedigree of the case patient JS (indicated by the *arrow*), who had a STEMI when he was 44 years old (yo).

MODES OF INHERITANCE

The genetic architecture of a disease refers to the number and magnitude of genetic risk factors that exist in each patient and in the population, as well as their frequencies and interactions. Diseases can be due to a single gene (monogenic) in each family or to multiple genes (polygenic). Identifying genetic risk factors is easiest when only a single gene is involved and this gene has a large impact on disease in that family. In cases in which a single gene is necessary and sufficient to cause disease, the condition is termed a mendelian disorder because the disease tracks perfectly with a mutation (in the family) that obeys Mendel's simple laws of inheritance.

For monogenic disorders, modes of inheritance include autosomal dominant, autosomal recessive, and X-linked. In autosomal dominant disorders, a single defective copy of a gene (either the maternal or paternal copy for every autosomal gene) suffices to cause the phenotype. In autosomal recessive disorders, both copies need to be

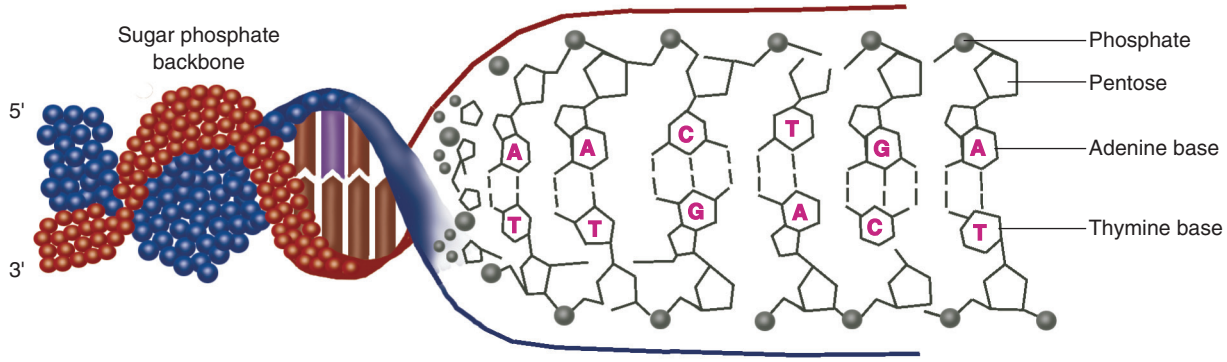

FIGURE 8-2 Schematic representation of the DNA double helix. The specificity of genetic information is carried in the four bases—guanine (G), adenine (A), thymine (T), and cytosine (C)—that extend inward from a sugar phosphate backbone and form pairs with complementary bases on the opposing strand.

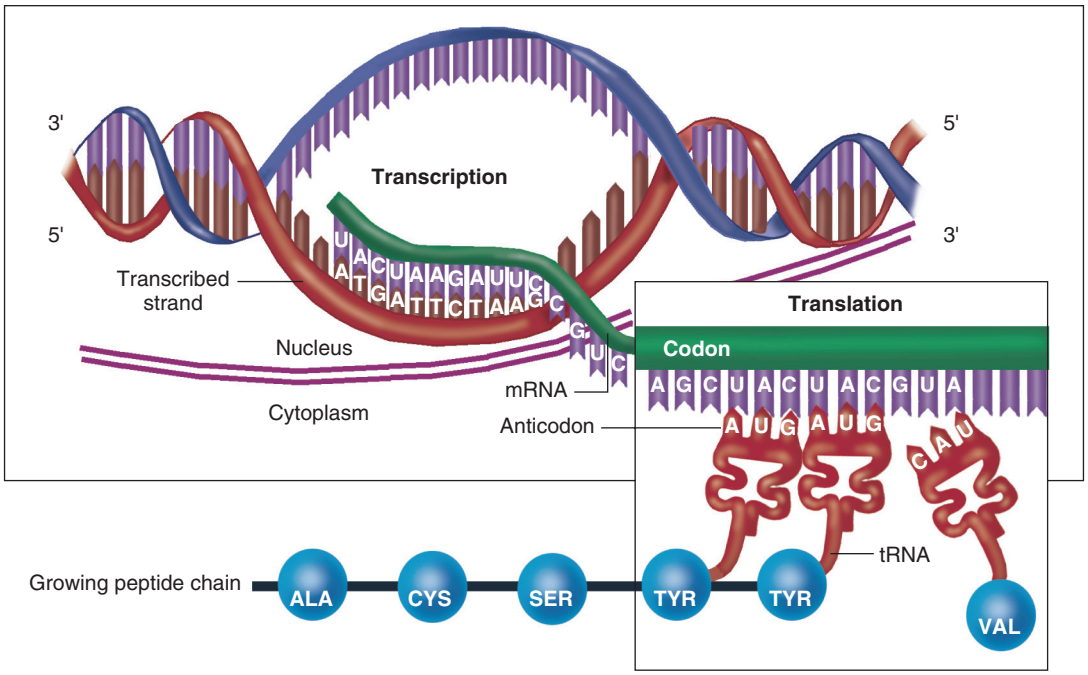

FIGURE 8-3 Flow of genetic information. Transcription in the nucleus creates a complementary RNA copy from one of the DNA strands in the double helix. mRNA is transported into the cytoplasm, where it is translated into protein.

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defective to lead to the phenotype. In X-linked disorders, the defective gene resides on the X chromosome. Given that men have only one X chromosome and women have two X chromosomes, men who carry the defective copy are affected with the disorder, whereas women are unaffected carriers.

Most common cardiovascular diseases, however, do not obey Mendel's simple laws of inheritance but rather are complex—the result of an interplay between multiple genes and the environment. For these polygenic disorders, variants in more than one gene are needed to cause a disease. Accordingly, in these cases it becomes difficult to understand a disease by studying a single family. A corollary is that each contributing gene variant may have a small phenotypic effect that is not obvious by comparing a few people with and without that variant. For these reasons, elucidating the genetic architecture of a complex disorder is more feasible by studying a large population.

The patient case presented earlier describes both discrete cardiovascular phenotypes (i.e., traits defined by their presence or absence based on a set of criteria) and quantitative phenotypes. MI is a discrete (also called dichotomous) phenotype, whereas blood pressure, LDL-C, HDL-C, and BMI are continuous cardiovascular traits. In the general population, most of these traits display a complex pattern of inheritance.

For many complex traits, however, some subtypes of the disease are monogenic in inheritance. In our patient case, the co-occurrence of high LDL-C, early-onset MI, and a family history of premature MI suggests a specific mendelian disorder, namely, familial hypercholesterolemia (FH).[3] In FH, the extremely high LDL-C level and MI result from defects in the LDL receptor gene. Severely high LDL-C and early MI can also be caused by defects in other genes, including proprotein convertase subtilisin/kexin type 9 (*PCSK9*) and apolipoprotein B (*APOB*). Other examples of monogenic subtypes of complex traits include extremely high or low blood pressure caused by rare mutations in genes involved in renal salt handling; extremely low LDL-C as a result of mutations in *APOB*, *PCSK9*, or *ANGPTL3*; and extreme obesity caused by mutations in *MC4R*.

APPROACHES TO DISCOVERING THE INHERITED BASIS FOR CARDIOVASCULAR DISEASE

Human Genetic Variation

The human genome contains about 6 billion base pairs across the 46 chromosomes. Approximately 1% of the genomic DNA is coding DNA, which comprises an estimated 20,000 genes.[4] Although most of the DNA in the genome is shared among all human beings, variations in the DNA sequence—occurring in both coding DNA and noncoding DNA—distinguish individuals from one another. These DNA sequence variants partly account for why a disease is more or less likely to develop in some individuals or why some respond more favorably or more adversely to a medication (**see also Chapters 7 and 9**).

As alluded to earlier, some DNA sequence variants have large phenotypic effects—meaning that they can cause disease singlehandedly. These DNA sequence variants tend to be rare (and sometimes unique to a single person or family) because natural selection weeds them out of a population. Classically, they cause monogenic disorders. Other DNA sequence variants commonly occur in a population and tend to have smaller phenotypic effects. Typically it is these variants, in combination, that cause polygenic disorders. Because of natural selection, in general there is an inverse relationship between the frequency of a DNA sequence variant and the phenotypic effect conferred by that variant. For example, such a relationship is observed for gene variants that affect LDL-C in the population (**Fig. 8-4**).[5-8]

Coding sequence variants potentially disrupt the function of genes and their protein products (**Fig. 8-5**).[9] Some coding variants do not affect the amino acid sequence of a protein; these are known as synonymous variants and do not usually have any phenotypic consequences. Other coding variants can cause a variety of alterations in a protein—substitution of a single amino acid in a protein with a

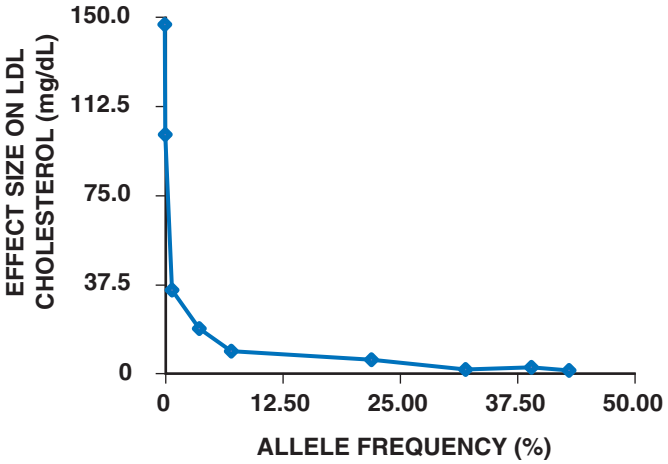

FIGURE 8-4 Effect sizes on LDL-C for DNA sequence variants at a range of allele frequencies. Gene, variant, frequency, and effect size on LDL-C are as follows: *NPC1L1*, rs217386,[5] 43%, 1.2 mg/dL; *HMGCR*, rs12916,[5] 39%, 2.5 mg/dL; *ANGPTL3*, rs2131925,[5] 32%, 1.6 mg/dL; *SORT1*, rs629301,[5] 22%, 5.7 mg/dL; *APOE*, rs429358/C130R,[6] 7.1%, 9.3 mg/dL; *APOE*, rs7412/R176C,[6] 3.7%, 18.8 mg/dL; *APOB*, R3500Q,[7] 0.08%, 100 mg/dL; *LDLR*, W23X or W66G or W556S,[8] 0.03%, 147 mg/dL.

Wild-Type Sequence						
…	AUG	GCC	TAC	GTT	CGA	CCC …
…	Met	Ala	Tyr	Val	Arg	Pro …
Missense						
	AUG	ACC	TAC	GTT	CGA	CCC
	Met	Thr	Tyr	Val	Arg	Pro
Nonsense						
	AUG	GCC	TAG	GTT	CGA	CCC
	Met	Ala	Stop			
Frameshift						
	AUG	GCC	TAC	•TTC	CGA	CCC
	Met	Ala	Tyr	Phe	Asp	…
Deletion						
	AUG	GCC	TAC	GTT	…	CCC
	Met	Ala	Tyr	Val	–	Pro
				OR		
	AUG	GCC	TA•	G	TT	CCC
	Met	Ala	Stop	⟶		
Insertion						
	AUG	GCC	AAA	TAC	GTT	CGA CCC
	Met	Ala	Lys	Tyr	Val	Arg Pro
				OR		
	AUG	GCC	ATA	CGT	TCG	ACC …
	Met	Ala	Ile	Arg	Ser	Thr …

FIGURE 8-5 Different types of mutations that alter the structure and expression of human genes.

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different amino acid (missense), premature truncation of a protein (nonsense), scrambling of the amino acid sequence past the site of the variant (frameshift), or insertion or deletion of amino acids. Any of these so-called nonsynonymous variants can have phenotypic effects ranging from negligible to profound, although nonsense and frameshift variants tend to be more deleterious than missense variants to protein function. Finally, sequence variants at splice sites (the first and second bases after the end of each exon and before the beginning of each exon) can lead to a severely disrupted protein product missing an entire exon.

Noncoding variants, although they do not directly affect the amino acid sequences of proteins, can cause phenotypic changes in other ways. For example, a noncoding variant near a gene might affect transcription of the gene and result in an increased amount of RNA being produced from a gene, and consequently an increased amount of the protein product.[10] Noncoding variants can affect the processing of RNA in several other ways.

In addition to genes, the genome harbors a number of expressed RNA molecules that do not code for protein; such RNA includes microRNA and large intergenic noncoding RNA (lincRNA). Both these categories of noncoding RNA have been demonstrated to interact with and modulate the activity of coding RNA, thereby regulating protein levels. For example, a given microRNA might physically bind to complementary sequences in a large number of coding RNA molecules and result in either suppression of RNA translation into proteins or degradation of the RNA. A noncoding variant that falls in the midst of a microRNA might impair (or enhance) its ability to interact with specific coding RNA and result in phenotypic changes.

DNA sequence variants, also known as polymorphisms (derived from Greek words meaning "multiple forms"), consist of three major classes. Single-nucleotide polymorphisms (SNPs) involve the alteration of a single DNA base pair in the genome. They are the most common and best cataloged of the DNA variants, with tens of millions having been identified to date across all human populations. Variable number tandem repeats (VNTRs) involve a variable number of repeats of a short DNA sequence at a genomic location; the number of repeats ranges from very few to thousands. Copy number variants (CNVs) involve a variable number of repeats of a long DNA sequence (more than 1000 base pairs), typically ranging from zero to one or a few repeats. An indel (an abbreviation of insertion/deletion) is a type of DNA variant in which a sequence is either present (insertion) or absent (deletion); it could be either a special type of a VNTR or a special type of a CNV, depending on the size of the involved sequence.

Characterizing Human Genetic Variation: Genotyping and Sequencing

In most cases a person has two copies of each DNA sequence because of the presence of paired chromosomes (the exceptions are DNA sequences on the X or Y chromosome in men, because these two chromosomes are entirely different). The two copies are known as alleles. For a DNA variant, the genotype is the identity of the two alleles at the site of the variant. The two alleles may be identical, in which case the person is said to be homozygous for the allele. If the two alleles are different, the person is heterozygous at the DNA variant. A haplotype is a series of genotypes at nearby sites of DNA variants. Because the haplotype is located on a single region of the chromosome, it tends to remain linked together as it passes from parents to offspring.

For polymorphisms that are primarily present in just two forms (typical of SNPs, i.e., one DNA base versus another DNA base, but not for VNTRs, which are usually found in at least a few forms, i.e., different numbers of repeats), the allele found more commonly in a given population is termed the major allele, with the less common allele being the minor allele. Common variants are so defined by virtue of the frequency of the minor allele being greater than 5% in the population. Low-frequency variants have a minor allele frequency of between 0.5% and 5%; rare variants have less than a 0.5% frequency. Rare variants are typically referred to as mutations. In some cases, mutations are so rare that they are found only in one individual or in one family.

Two types of methods can be used to determine genotypes at the sites of DNA variants. In the first type, a genotyping technology directly ascertains the genotype at a single location in the genome. In the second type, polymerase chain reaction (PCR) is used to amplify the region of DNA immediately surrounding the site of the DNA variant (**Fig. 8-6**). The PCR product is subjected to DNA sequencing, which indirectly determines the genotype. The first type is generally cheaper—indeed, fabricated "chips" can directly genotype millions of DNA variants at a time—but requires optimization beforehand. Thus direct genotyping is most useful for common and low-frequency variants that have already been cataloged. The second type is more expensive and can be used only at one location at a time, but it can be flexibly adapted to any location in the genome. This approach can be used to discover previously uncataloged rare DNA sequence variants.

In recent years, a third type of method has been devised to characterize a person's genetic variation. This method entails the use of any of a group of techniques known as next-generation DNA sequencing.[11] Although the operational details differ, these techniques share the ability to sequence billions of DNA base pairs at a time within a reasonable time frame and at a reasonable cost. The techniques have been applied successfully to efficiently sequence the entirety of a patient's coding DNA, known as the exome, which accounts for about 1% of the genome.[12,13] More recently, sequencing the entirety of a patient's genome for a few thousand U.S. dollars within 24 hours has become feasible, with the highly publicized "thousand-dollar genome" expected to emerge very soon.

Although performing DNA sequencing remains more expensive than direct genotyping, the decreasing cost of whole-genome sequencing will soon enable it to be performed in large cohorts of people. The advantage of whole-genome sequencing is that it determines genotypes at the locations of all known DNA sequence variants in a single experiment and, at the same time, identifies previously unknown DNA variants that are unique to the individual.

Study Designs to Correlate Genotype with Phenotype

Approaches to correlate genotype with phenotype are highlighted in **Figure 8-7**. The *x* axis shows the frequency of the allele in the population, from rare to common; the *y* axis shows the size of the phenotypic effect conferred by the DNA sequence variant allele, from small to large. As described earlier, because of evolution and natural selection, an inverse relationship exists between allele frequency and effect size. Typically, to detect common DNA sequence variants of small to modest effect (e.g., increase in risk of 5% to 50%), genotyping characterizes the DNA sequence variation, and population-based association links genotype with phenotype. Rare variants with larger effect are discovered by sequencing to characterize their DNA sequence variation. One of two major approaches—family-based studies or extreme-phenotype studies—can be used to correlate rare variants with phenotype. Variants of low frequency (0.5% to 5%) can be approached by either genotyping or sequencing, and any of the three study designs may be useful in linking genotype with phenotype.

Family-Based Studies

PATIENT CASE, PART II. The cardiologist refers the 45-year-old patient (JS) who recently suffered an MI to a geneticist for evaluation. The geneticist suspects that the patient has FH and arranges for clinical sequencing of the *LDLR*, *APOB*, and *PCSK9* genes. These tests identify a mutation in the *PCSK9* gene: a T → A substitution in exon 2 at nucleotide 625, which predicts a substitution of arginine at codon 127 for the conserved serine (S127R). This mutation has been proved to lead to gain of PCSK9 function and cause autosomal dominant hypercholesterolemia.[14]

Two major study designs have been used to identify the gene mutations responsible for monogenic disorders. Both take advantage of

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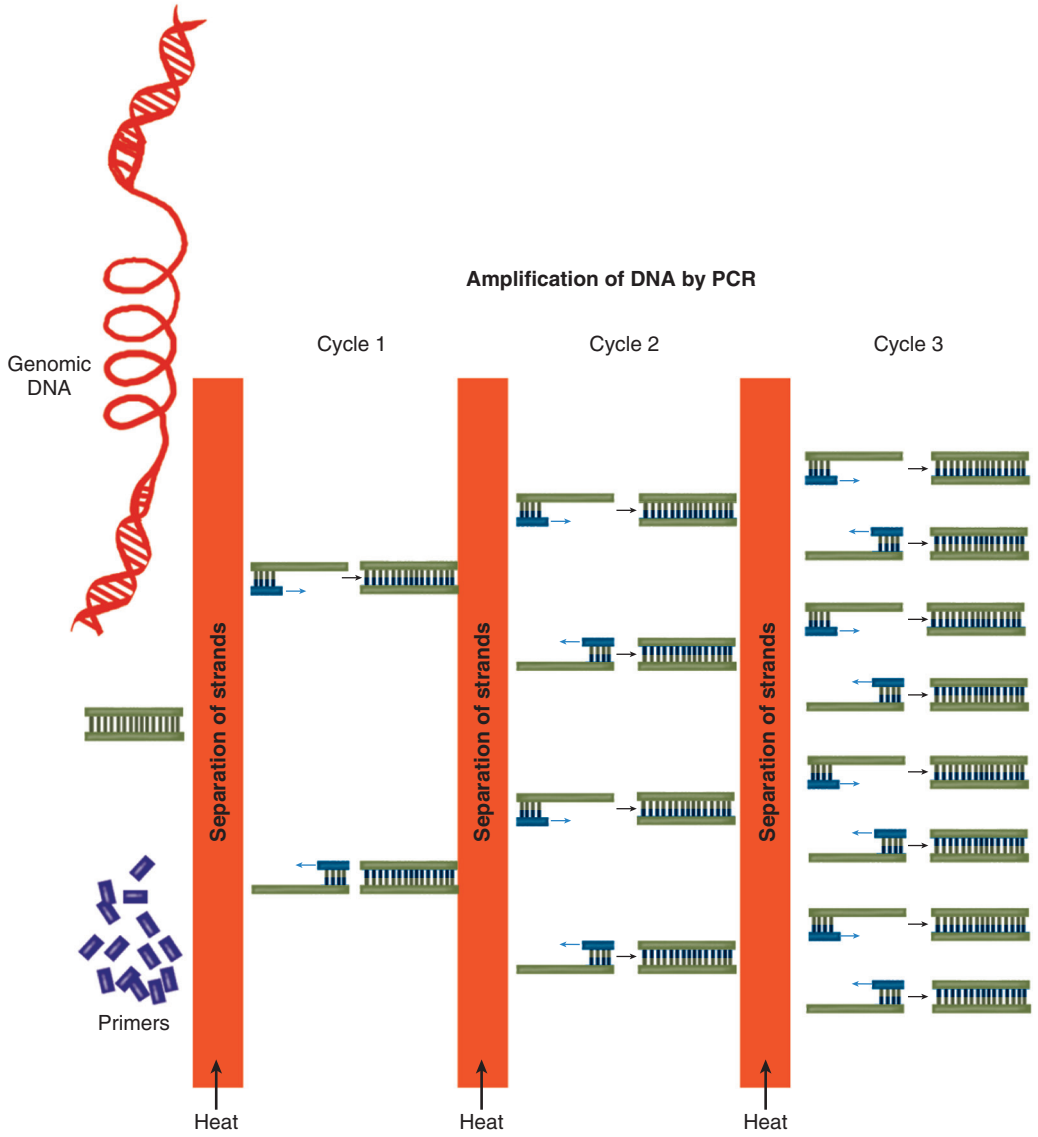

FIGURE 8-6 DNA amplification with PCR. Synthetic primers corresponding to the 5′ and 3′ ends of the DNA sequence are chemically synthesized. The double-stranded DNA is melted by heating to 92°C, followed by cooling to 72°C to anneal the primers. A heat-stable DNA polymerase amplifies each strand of the target sequence, which produces two copies of the DNA sequence. The process is repeated multiple times to achieve amplification of the target sequence.

family relationships. Classic linkage studies entail the genotyping of several hundred or thousand DNA variants (usually VNTRs with repeats that are two to six base pairs in length, also known as microsatellite markers) distributed across the genome. Linkage analysis identifies any markers that are strongly "linked" to the disease. For dominantly inherited disorders, linkage can be observed when one particular allele of the marker is found only in family members with the disease ("affecteds") and not in healthy family members ("unaffecteds"); for recessively inherited disorders, linkage is observed when two copies of a particular allele are found only in family members with the disease and not in healthy family members. The degree of linkage for each genomic marker with affected status is calculated to yield a metric known as the logarithm of odds (LOD) score. A LOD score higher than 3.0 is considered significant evidence of linkage.

As a practical matter, a high LOD score for a particular marker suggests that the causal disease mutation lies within several megabases (i.e., millions of base pairs) of the marker. This region of interest typically harbors dozens, if not hundreds, of candidate genes. The region can sometimes be narrowed further by genotyping a set of markers clustered around the original marker and assessing for linkage, a process called positional cloning. Identification of the disease mutation entails sequencing candidate genes in the hope of finding a rare coding variant. Traditionally, sequencing of a large number of genes was prohibitively expensive, and one would have to judiciously pick a limited number of candidate genes thought most likely to have the causal mutation—and often come up empty-handed.

The second study design has been made possible by advances in next-generation DNA sequencing technologies. Rather than sequencing a few candidate genes, one can now perform exome sequencing and capture the coding DNA of all approximately 20,000 human genes in a single, relatively affordable experiment. In this study design, one chooses a few affected family members, performs exome sequencing on their DNA samples, and filters through the sequencing data to identify the handful of rare variants that are shared by all affecteds.[15] This list of variants can be narrowed down further in several ways, such as confirming that a variant is not present in unaffecteds or simultaneously performing a linkage study and filtering for variants that are close to a marker with a high LOD score.

Once the rare gene variant thought most likely to be the causal mutation is selected, it can be confirmed by sequencing the gene in unrelated individuals who have the same disorder. If some of these individuals have mutations in the same gene (either the same rare

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variant or, more likely, different variants), it strongly argues that the gene is responsible for the disease.

Extreme-Phenotype Studies

Another approach to gene discovery is to identify individuals in a population who are at the extremes of a phenotype.[16] For a quantitative phenotype such as blood cholesterol level, this might entail finding a sizable number of people with extremely high cholesterol and people with extremely low cholesterol. For a discrete phenotype such as MI, the desired individuals might be young people with premature disease versus elderly people with multiple risk factors but no evidence of coronary artery disease (CAD).

DNA samples from these extreme cohorts undergo either candidate gene sequencing, exome sequencing, or even whole-genome sequencing. The analysis entails identifying genes that have a preponderance of rare variants in one group versus the other group. For example, if a particular gene were to display a significantly higher frequency of rare variants in young people with MI than in elderly people without CAD, it would argue for that gene being causal for MI. Conversely, if the gene had a higher frequency of rare variants in elderly people without CAD than in young people with MI, the gene might protect against disease.

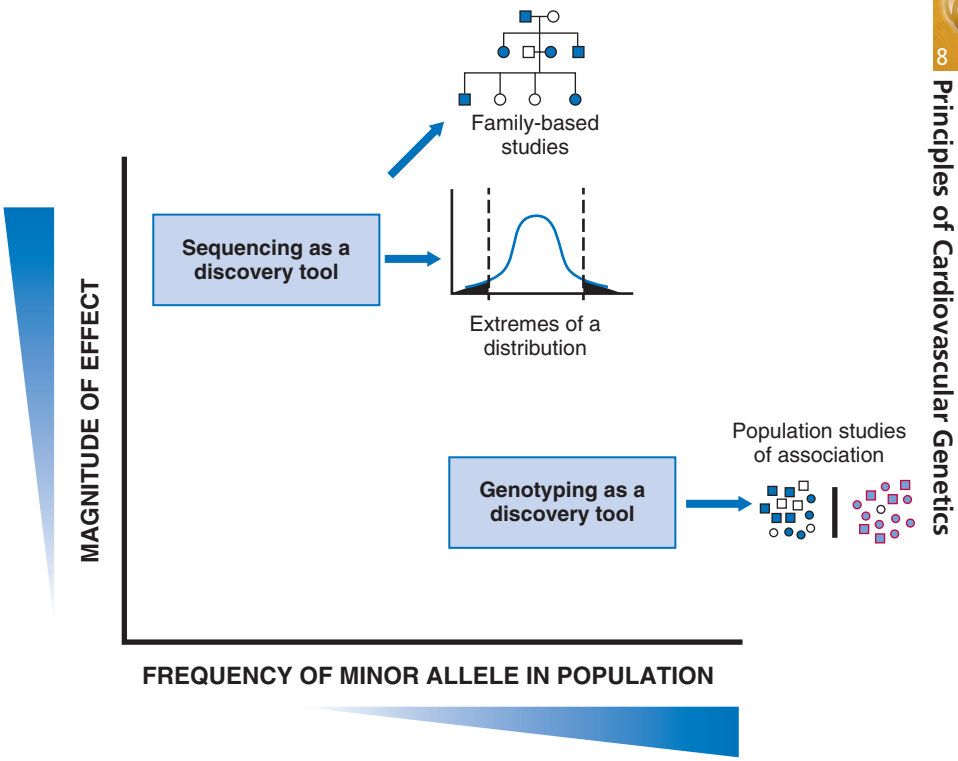

FIGURE 8-7 Approaches to correlate genotype with phenotype.

Population-Based Studies

Family-based studies are poorly suited to study polygenic disorders in which each contributing DNA variant has a small or moderate effect. Because these DNA variants tend to be common in a given population, population-based studies are better designed to detect their small effects with statistical rigor.

The genome-wide association study (GWAS) is the primary population-based study design.[17,18] In a GWAS, DNA samples from many unrelated individuals in a population—as many as hundreds of thousands of people—undergo genotyping of millions of SNP markers across the genome using chips. The analysis entails a search for SNPs that have robust statistical associations with the phenotype of interest. For a GWAS on a quantitative phenotype such as blood cholesterol level, each SNP is evaluated to determine whether individuals with one genotype at that SNP have on average a significant difference in cholesterol level from individuals with another genotype.

For a GWAS on a discrete phenotype such as MI, the study compares a group of individuals with the phenotype and a group of individuals without the phenotype (cases versus controls). Each individual SNP is evaluated to determine whether its minor allele frequency differs between the cases and controls (**Fig. 8-8**).

With any GWAS, because so many SNPs are being evaluated independently, the traditional statistical significance threshold of $P < 0.05$ is not valid and must be adjusted for the number of SNPs tested. The number of independent common SNPs tested in a single experiment is approximately 1,000,000. Accordingly, it is common practice with a GWAS to use a statistical significance threshold of $P < 5 \times 10^{-8}$ (i.e., Bonferroni correction of the traditional P value of 0.05 for 1,000,000 independent tests). The need to meet a very rigorous significance threshold, as well as the fact that most DNA variants contributing to a polygenic trait have small effects, often dictates studying very large numbers of people to carry out a GWAS successfully.

GWAS results are typically displayed in a "Manhattan plot," with the x axis representing each variant in chromosomal order and the y axis plotting $-\log_{10}$ of the P value associating each variant with the trait of interest. SNPs exceeding a P value threshold of $P < 5 \times 10^{-8}$ are considered "genome-wide significant" and are the least likely to be false-positive results. The Manhattan plot from a large-scale GWAS for CAD is displayed in **Figure 8-9**. A total of 25 chromosomal loci exceeded genome-wide significance in this study.

A GWAS uses a far more dense distribution of markers across the genome and data from far more people than a linkage study does. Furthermore, a GWAS takes advantage of the genome's discrete recombination hot spots, between which regions of DNA remain relatively intact as they are passed from parents to offspring. Consequently, the resolution of a GWAS is much higher than that of a linkage study; rather than megabases, the locus of interest is defined by flanking recombination hot spots, which on average occur just tens to hundreds of kilobases apart. For a given SNP with a positive association with a phenotype, this considerably narrows the number of candidate causal genes. Also in contrast to linkage studies, GWASs

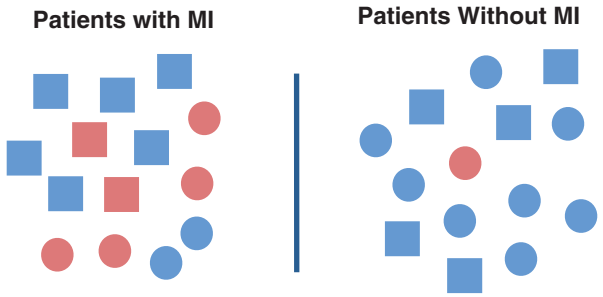

FIGURE 8-8 Analysis scheme for a GWAS involving a dichotomous phenotype. Step 1. Compare frequency of genetic variant in cases and controls.Carriers of a variant allele are shown in *pink*, and noncarriers are shown in *blue*. *Boxes* represent men and *circles* represent women. Here the variant allele is more frequent in cases compared with controls. Step 2. For each genetic variant (typically 300,000 to 1,000,000 in each experiment), generate P value for the difference in frequency being a chance observation.

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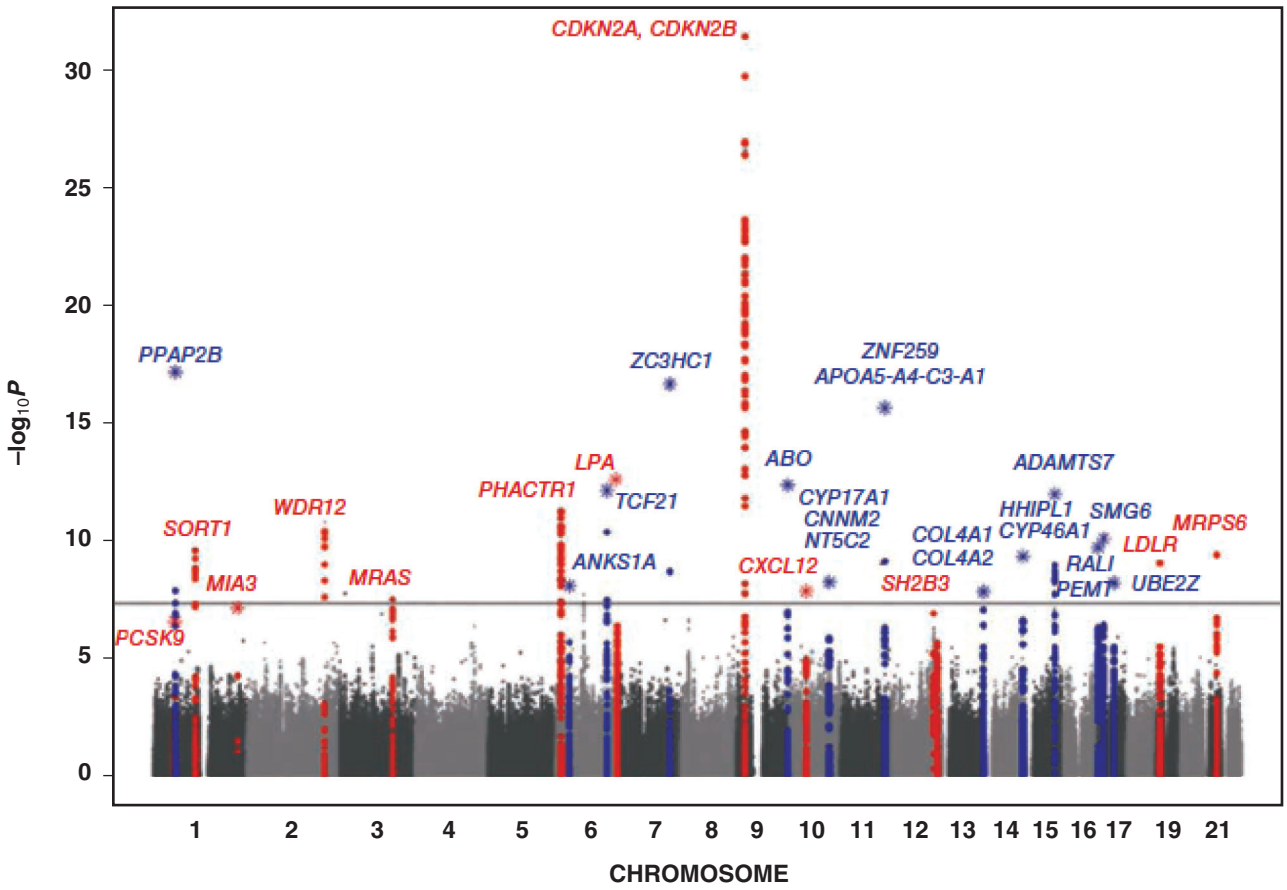

FIGURE 8-9 Graphic summary (Manhattan plot) of genome-wide association results. The *x* axis represents the genome in physical order; the *y* axis shows $-\log_{10} P$ for all SNPs. Data from the discovery phase are shown in *circles*, and data from the combined discovery and replication phases are shown in *stars*. Genes at the significant loci are listed above the signals. Known loci (before publication of this work) are shown in *red*, and newly discovered loci from this work are shown in *blue*. *(From Schunkert H, Konig IR, Kathiresan S, et al: Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 43:333, 2011.)*

have successfully pinpointed noncoding causal DNA variants that affect gene expression.

ILLUSTRATIVE EXAMPLES

In presenting examples of the various approaches described above, we focus on LDL-C—whether in the context of monogenic lipid disorders such as FH or in the context of the blood LDL-C level as a polygenic, quantitative trait.

Mendelian Disease Using Classic Linkage

FH is a monogenic disorder in which patients have extremely high blood LDL-C levels that result in abnormal deposition of cholesterol (xanthomas) and a severely increased risk for premature MI, as early as childhood. Initial studies in the 1970s and 1980s by Brown, Goldstein, and colleagues demonstrated that most cases of FH result from mutations in the LDL receptor gene (*LDLR*).[19] In 1989, a subset of cases were found to result from mutations in the apolipoprotein B gene (*APOB*).[20] Following these discoveries, other cases remained in which neither *LDLR* nor *APOB* mutations appeared to be responsible.

Boileau and coworkers identified French families affected by FH without apparent *LDLR* or *APOB* mutations and, in performing a linkage study, identified a region on chromosome 1 where markers had strong linkage to the disease.[14] Using positional cloning, they narrowed the region to an interval containing 41 genes. One gene, *PCSK9*, was a strong candidate because a similar gene had previously been reported to be involved in cholesterol metabolism. In sequencing *PCSK9*, they found two different rare variants in different families. Subsequent studies in mice confirmed that *PCSK9* is a bona fide regulator of blood cholesterol levels, and indicated that the mutations discovered were likely to be gain-of-function rather than loss-of-function mutations.[21]

Mendelian Disease Using Direct DNA Sequencing

Schonfeld and colleagues identified a family in which four siblings displayed extremely low blood LDL-C, HDL-C, and triglyceride levels—an apparently recessive disorder termed familial combined hypolipidemia.[22] A linkage study could not identify the causal gene because of the prohibitively large number of genes in the linkage region. Years later, following the advent of exome sequencing, DNA samples from two of the siblings were subjected to the technique. In a comparison of the siblings' exomes, only one gene harbored rare DNA variants in both alleles in both siblings—the angiopoietin-like 3 (*ANGPTL3*) gene, which had been implicated previously in the metabolism of triglycerides but not LDL-C. Of note, the siblings had two different mutations, each of which was a nonsense mutation, consistent with total loss of *ANGPTL3* function. Subsequent studies confirmed the presence of various *ANGPTL3* mutations in unrelated individuals with familial combined hypolipidemia.

Complex Trait Using Extremes in a Population

Shortly after the discovery of *PCSK9* as a causal gene in FH, Hobbs, Cohen, and colleagues hypothesized that loss-of-function variants in

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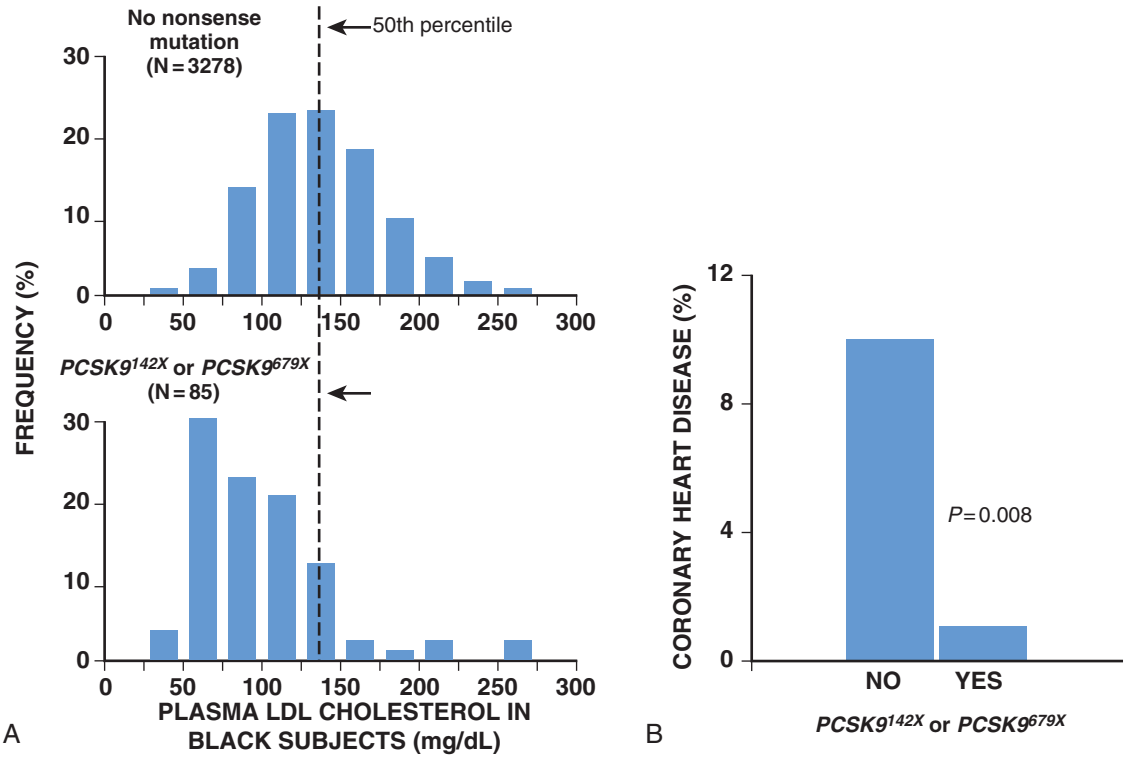

FIGURE 8-10 Distribution of LDL-C **(A)** and risk for CHD **(B)** in carriers versus noncarriers of nonsense mutations in the *PCSK9* gene. *(From Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH: Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med 354:1264, 2006.)*

PCSK9 may contribute to differences in blood cholesterol levels in the general population. Reasoning that individuals with low LDL-C levels were more likely to have such loss-of-function variants (because gain-of-function mutations cause increased LDL-C levels in FH), they sequenced *PCSK9* in individuals at the phenotypic extreme in the multiethnic Dallas Heart Study—those with the lowest LDL-C levels.[23,24] Several of these individuals had one copy of either of two different nonsense variants in the gene. The investigators then specifically genotyped at the sites of the two nonsense variants in the entire Atherosclerosis Risk in Communities study and found that together, 2.6% of the black subjects in the study had either of the two variants. These individuals had on average a 28% reduction in LDL-C when compared with those without *PCSK9* variants. Subsequent work demonstrated that individuals with *PCSK9* nonsense variants experience a significant reduction in the risk for incident CHD (**Fig. 8-10**). Notably, individuals with loss-of-function variants in *PCSK9* appear to suffer no adverse clinical consequences, thus suggesting that therapies directed against *PCSK9* would offer beneficial cardiovascular effects without any accompanying undesirable effects.

Complex Trait Using Genome-Wide Association

Starting in 2007, GWASs were performed on collections of individuals of European descent to identify SNPs associated with blood LDL-C, HDL-C, triglycerides, and/or total cholesterol levels. Each year brought a successively larger study and culminated in a collaborative study involving approximately 100,000 people in 2010.[5] This study identified a total of 95 loci associated with one or more of the lipid phenotypes. Remarkably, a third of the loci have genes previously known to be involved in lipid metabolism; indeed, more than a dozen genes had formerly been found to harbor rare DNA variants responsible for monogenic lipid disorders, including *LDLR*, *APOB*, *PCSK9*, and *ANGPTL3*. The other two thirds of the loci presumptively harbor novel lipid-regulating genes, and considerable effort is now focused on characterizing the functions of some of these genes. Some examples include *GALNT2*, *SORT1*, and *TRIB1*.

CLINICAL APPLICATION OF GENETIC FINDINGS

Risk Prediction

PATIENT CASE, PART III. The two brothers of the patient JS are referred to a cardiologist for assessment of risk for MI. Both brothers are asymptomatic but are worried about their strong family history and that JS suffered a coronary event at a similarly young age. They inquire whether they have an increased risk for a coronary event, whether that risk can be quantified, and whether they should be changing their lifestyle or taking any medications. Both patients undergo DNA sequencing to determine whether they carry the *PCSK9* mutation responsible for disease in JS. The 43-year-old brother (KS) carries *PCSK9* S127R, but the 39-year-old brother (LS) does not.

Identifying individuals at increased risk for cardiovascular disease and implementing preventive interventions to reduce that risk are key goals of biomedicine (**see Chapters 7 and 42**). Genetic markers have long been considered a promising tool to discern patients at increased risk. The use of genetic markers to assess risk entails consideration of two scenarios.

The first is risk prediction in the context of a family that suffers from a mendelian disorder. Here, a single defective gene is responsible for disease in the family. The central question is whether the asymptomatic family member carries the causal mutation (or two mutations for a recessive disease). Direct DNA sequencing can determine mutation status and whether the mutation is present, which typically means a near-certain risk for disease. However, complexities may exist in even a single-gene disorder.[1] Among carriers of a mendelian mutation in a given family, some may exhibit the condition and others may not. Penetrance refers to the proportion of individuals with a given genotype who exhibit the phenotype associated with the genotype. In many mendelian cardiovascular conditions inherited in an autosomal dominant manner, evidence exists for incomplete penetrance. For example, Hobbs and colleagues reported that in a pedigree with FH caused by a point mutation in *LDLR*, only 12 of 18 heterozygotes had high LDL-C (>95th percentile), whereas some of the remaining 6 heterozygotes had LDL-C as low as the 28th percentile for the

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population.[25] The lack of a high-cholesterol phenotype given the same genotype may be due to modifier genes or environmental influences.

The second scenario uses genetics to predict risk for a common, complex disease. Here, disease results from the interplay of multiple genetic and nongenetic factors. The central questions are whether genetic markers can identify a subset of the population at higher risk for disease and whether effective interventions can be allocated to this subset of individuals to reduce their risk. For example, we commonly use a nongenetic marker, the presence of type 2 diabetes mellitus, to identify a subset of the population at higher risk for CHD (those with type 2 diabetes have a twofold increase in CHD).[26] We target statin intervention to this group to reduce their absolute risk for CHD.

Use of the GWAS approach has recently identified 45 common variants for CAD or MI, thereby permitting construction of a genetic risk score using mapped variants.[27] For the first 12 common variants mapped for CAD or MI using GWAS, a simple genetic risk score ranging from 0 to 24 alleles was generated (i.e., each individual can carry 0, 1, or 2 copies of the risk allele at each of these 12 sites), with 0 being ideal and 24 being the most unfavorable.[28] The distribution of this genetic risk score in the population approaches normal. Those in the top quintile of this distribution (the 20% of the population with the highest scores) had an approximately 1.7-fold increased risk for incident CHD, even after accounting for all other cardiovascular risk factors.

Will this information have clinical usefulness? Debated at present is whether young and middle-aged individuals (i.e., men 30 to 50 years of age and women 40 to 60 years of age) should be treated with a statin to prevent a first MI. Based on the genetic results presented earlier, one approach could be to use a genetic risk score to identify the subset of individuals at highest genetic risk and target statin treatment to these individuals. This hypothesis remains to be tested formally in randomized controlled trials.

Distinguishing Causal from Reactive Biomarkers

PATIENT CASE, PART IV. The 39-year-old brother of the patient JS has an HDL-C level of 29 mg/dL. Does his low HDL-C concentration causally contribute to risk for MI?

Hypotheses concerning causative agents for complex diseases have often initially come from observational epidemiology. In a 1961 paper titled "Factors of Risk in the Development of Coronary Heart Disease," William Kannel and colleagues in the Framingham Heart Study established an association of total plasma cholesterol with future risk for CHD.[29] Since then, hundreds of soluble biomarkers have similarly been associated with risk for CAD (**see also Chapter 10**). How many of these biomarkers directly cause CAD, how many simply reflect other causal processes, and why is this question important? Both causal and noncausal biomarkers may be helpful in predicting risk for future disease, but only a causal biomarker may be appropriate as a target of therapy. The ultimate proof of causality in humans is a randomized controlled trial testing whether a treatment that alters the biomarker will affect risk for disease. But, because clinical trials are expensive and time-consuming, having evidence in humans before engaging in a clinical trial would be helpful.

In a technique termed mendelian randomization, DNA sequence variants are used to address the question of whether an epidemiologic association between a risk factor and disease reflects a causal influence of the former on the latter.[30-32] In principle, if a DNA sequence variant is known to directly affect an intermediate phenotype (e.g., a variant in the promoter of a gene encoding a biomarker that alters its expression) and the intermediate phenotype truly contributes to the disease, the DNA variant should be associated with the disease to the extent predicted by (1) the size of the effect of the variant on the phenotype and (2) the size of the effect of the phenotype on the disease (**Fig. 8-11**). If the predicted association between the variant and disease was not observed in an adequately powered sample, it would argue against a purely causal role for the intermediate phenotype in pathogenesis of the disease.

The study design is akin to a prospective randomized clinical trial in that randomization for each individual occurs at the moment of conception—genotypes of DNA variants are randomly "assigned" to gametes during meiosis, a process that should be impervious to the typical confounders observed in observational epidemiologic studies. For example, a parent's disease status or socioeconomic status should not affect which of the parent's two alleles at a given SNP is passed to a child, with each allele having an equal (50%) chance of being transmitted via the gamete to the zygote. Thus mendelian randomization should be unaffected by confounding or reverse causation. Mendelian randomization has potential shortcomings, however, including that (1) the technique is only as reliable as the robustness of the estimates of the effect sizes of the variant on the phenotype and of the phenotype on disease, and that (2) it assumes that the DNA variant does not influence the disease by means other than the intermediate phenotype being studied (pleiotropy), which may not be true. In addition, a potential confounder of mendelian randomization is that, in certain situations, a disease might cause the allele of a DNA variant passed from a parent to an offspring to be expressed in a different way; for example, it could occur through inherited epigenetic effects. Nevertheless, mendelian randomization has the potential to be as informative as a traditional randomized clinical trial.

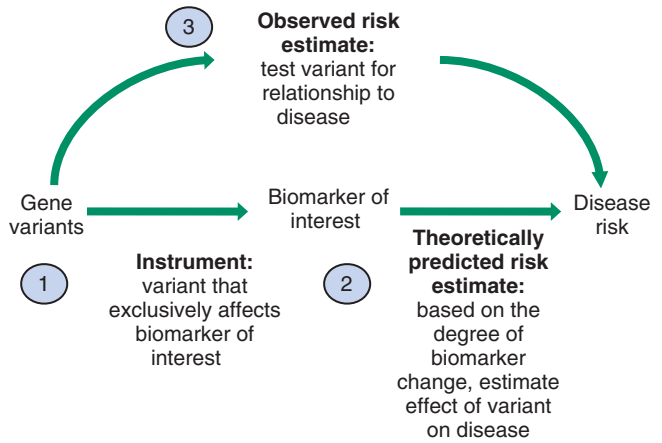

FIGURE 8-11 Design of a mendelian randomization study to test whether a biomarker causally influences risk for disease. The study design has three elements. First, one needs to identify a genetic variant, an instrument that exclusively alters the biomarker of interest. Second, one needs to derive a theoretically predicted estimate of disease risk for the instrument. This estimate is usually derived on the basis of (1) association of the gene variant to the biomarker (i.e., the degree of change in the biomarker conferred by the variant) and (2) association of the biomarker to disease in the population (i.e., the extent to which a given change in biomarker is expected to alter risk for disease in the population). Finally, one derives an observed disease risk estimate for the instrument after testing the instrument for association with disease in the population. If the observed risk estimate for the instrument is consistent with that predicted theoretically, this supports the notion that the biomarker causally influences risk for disease.

Several mendelian randomization studies have confirmed a causal relationship between LDL-C and CHD. Nonsense variants in the *PCSK9* gene that significantly reduce plasma LDL-C concentrations have been associated with a reduced incidence of CHD in a black cohort.[24] Similarly, in white subjects a low-frequency missense variant in *PCSK9* was found to be associated with lower LDL-C levels, as well as with a lower risk for MI. These observations suggest that lower LDL-C is sufficient to provide protection against CHD. Similar to LDL-C, several recent genetic studies have confirmed previous observations that plasma lipoprotein(a) (Lp[a]) is causally related to CHD.[33,34]

Unlike the results with plasma LDL-C and Lp(a) concentrations, a recent large mendelian randomization study of variants that affect plasma HDL-C, performed in more than 100,000 individuals, did not show an association between these variants and MI.[35] The investigators performed two mendelian randomization analyses. First, an SNP in the endothelial lipase gene (*LIPG* Asn396Ser) was used as an instrument, and this SNP was tested in 20 studies (20,913 MI cases, 95,407 controls). Second, a genetic score consisting of 14 common

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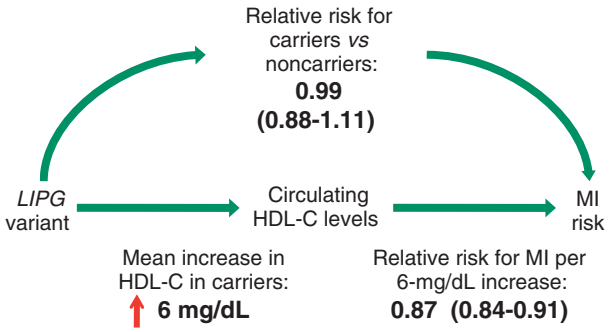

FIGURE 8-12 Mendelian randomization study for plasma HDL-C and risk for MI by using an instrument in the endothelial lipase (*LIPG*) gene. Individuals who carry the serine allele at amino acid 396 of the *LIPG* gene have about 6 mg/dL higher HDL-C. If HDL-C were a causal factor, carriers of the serine allele would be expected to be protected from risk for MI. After association testing in 116,320 individuals, the *LIPG* instrument was not associated with MI. Individuals who carried the HDL-boosting variants had the same risk for MI as did those who did not carry the variant.

SNPs that are exclusively associated with HDL-C was used as an instrument, and this score was tested in up to 12,482 MI cases and 41,331 controls. As a positive control, the investigators tested a genetic score of 13 common SNPs exclusively associated with LDL-C. Carriers of the *LIPG* 396Ser allele (2.6% frequency) had higher HDL-C (5.5 mg/dL higher, $P = 8 \times 10^{-13}$) but similar levels of other lipid and nonlipid risk factors for MI when compared with noncarriers. This difference in HDL-C was expected to decrease the risk for MI by 13% (odds ratio [OR], 0.87; 95% confidence interval [CI], 0.84 to 0.91), but the 396Ser allele was not associated with risk for MI (OR, 0.99; 95% CI, 0.88 to 1.11, $P = 0.85$) (**Fig. 8-12**). From observational epidemiology, an increase of 1 standard deviation (SD) in HDL-C is associated with a reduced risk for MI (OR, 0.62; 95% CI, 0.58 to 0.66). A 1-SD increase in HDL-C because of genetic score, however, was not associated with risk for MI (OR, 0.93; 95% CI, 0.68 to 1.26, $P = 0.63$). For LDL-C, the estimate from observational epidemiology (a 1-SD increase in LDL-C is associated with risk for MI; OR, 1.54; 95% CI, 1.45 to 1.63) agreed with that from the genetic score (OR, 2.13; 95% CI, 1.69 to 2.69, $P = 2 \times 10^{-10}$). The authors interpreted these findings as indicating that some genetic mechanisms that raise plasma HDL-C do not seem to lower the risk for MI. These data challenge the concept that raising plasma HDL-C therapeutically will uniformly translate into reductions in risk for MI.

A parallel line of clinical trial evidence also casts doubt on the notion that *any* intervention that raises HDL-C will reduce risk for MI. Dalcetrapib, an inhibitor of cholesterol ester transfer protein (CETP), raised HDL-C by approximately 30% in comparison to placebo. As a result, the dal-OUTCOMES trial randomly assigned more than 15,000 participants to test the hypothesis that CETP inhibition with dalcetrapib will reduce cardiovascular morbidity and mortality in patients with a recent acute coronary syndrome.[36] In May 2012, the data safety and monitoring board stopped the trial at a second interim analysis because of "lack of clinically meaningful efficacy" (**see also Chapters 42 and 45**). When combined, the dalcetrapib clinical trial results and the human genetic findings summarized here cast doubt on the notion that raising HDL-C in isolation will reduce risk for CHD. For several decades the biomedical research community has assumed that if an intervention raises HDL-C, that intervention will reduce risk for CHD. It seems prudent now to rethink this assumption and reevaluate the use of HDL-C as a biomarker predictive of CHD in intervention studies.

Overall, with the recent explosion in our ability to measure soluble biomarkers (including metabolites and proteins, **see Chapter 10**) and genetic variation, mendelian randomization will probably be used increasingly to distinguish causal biomarkers from noncausal ones.

Personalized Medicine

PATIENT CASE, PART V. Shortly after his clinic visit, the 43-year-old brother (KS) goes to the emergency department because of severe chest pain. He is found to be in the throes of STEMI. The cardiac catheterization team is activated to perform a percutaneous coronary intervention. The emergency department physician asks the cardiology consultant which antiplatelet agent besides aspirin should be administered to the patient at this time.

Just as genetic data can be used to predict a patient's risk for development of a disease, it can also be used to predict whether a patient will have a therapeutic response and/or an adverse response to a particular medication. Termed pharmacogenetics or, in broader terms, personalized medicine, its goal is to safely deliver the right therapy at the right dose to the right patient (**see also Chapters 7 and 9**).

One example of the emerging use of pharmacogenetics centers on use of the antiplatelet agent clopidogrel. Given routinely to patients after a coronary event, clopidogrel has reduced the risk for future coronary events and, in patients in whom coronary stents are placed, has decreased the risk for in-stent thrombosis. Common loss-of-function variants in the *CYP2C19* gene, which encodes an enzyme that metabolizes clopidogrel into its active form, have been shown to reduce the effectiveness of the medication, especially with respect to the prevention of in-stent thrombosis.[37,38] Accordingly, many institutions are evaluating whether *CYP2C19* genotyping should be performed at the point of care and used to guide the choice of therapy. Alternatives for patients found to have loss-of-function *CYP2C19* variants might be prescription of an increased dose of clopidogrel or the use of an alternative medication of the same drug class, such as prasugrel or ticagrelor, that *CYP2C19* function does not affect.

Therapeutic Targets: From Gene to Drug in a Decade

The example of *PCSK9* has emerged as a success story for the translation of cardiovascular genetics to the clinic in a relatively short time. The original report of the involvement of gain-of-function mutations in *PCSK9* in causing FH was published in 2003. Just 10 years later, several companies have developed antibody-based drugs targeting the PCSK9 protein that are being evaluated in clinical trials.[39,40] Development of these drugs was directly motivated by the finding that individuals with loss-of-function *PCSK9* mutations are genetically protected from CHD without suffering any known ill effects. Preliminary data from the clinical trials have demonstrated a large reduction in blood LDL-C levels with these agents, in some cases surpassing even the most potent statin drugs. Although the cholesterol-lowering effects of these agents are expected to result in a reduction in cardiovascular risk, definitive outcomes trials remain to be completed.

FUTURE DIRECTIONS

The last decade has witnessed remarkable advances in human genetics that have the promise of transforming our understanding of cardiovascular disease, as well as the approaches by which practitioners will prevent and treat disease. Although we are still largely in an information-gathering stage, the first practical applications of the information have begun to emerge—ranging from improvement in cardiovascular risk prediction, to the use of pharmacogenetics to tailor therapy for individual patients, to the development of novel therapies such as the PCSK9 antibody-based drugs. In the decade to come, we can expect substantial progress in all these domains.

Indeed, not too far in the future, the standard of cardiovascular care may look quite different from today's practices. Patients would undergo whole-genome sequencing at birth, thereby allowing so-called primordial prevention by assessing the genetic determinants of an individual's lifetime risk for cardiovascular disease and institution of appropriate counseling—starting with life-long exercise and dietary habits and, as the patient advances in age, individually tailored preventive medications and therapies that address all the individual's various validated, causal genetic risk factors for disease. If cardiovascular disease should nevertheless emerge at some point in the patient's life, he or she would receive the specific therapies that

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have been demonstrated to be most efficacious and safest for individuals with that genetic profile, both in the acute setting and in the long term for secondary prevention. This standard of care would represent an important step toward ensuring that people everywhere enjoy longer lives free of cardiovascular disease.

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Applications of Genetics to Lipid Disorders

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Personalized Medicine

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9 Drug Therapeutics and Personalized Medicine

Dan M. Roden

IMPORTANCE OF CORRECT DRUG USE

Drug treatment makes up a large fraction of total health care costs. In 2008, the total cost of healthcare in the United States was approximately $2.5 trillion, and more than 10% was spent on prescription drugs.[1] Cardiovascular disease makes up the largest subcategory in this spending: The American Heart Association estimated that the 2008 cost of care for cardiovascular disease was $298 billion, and total prescription drug costs for cardiovascular care were $33 billion.[2]

Patients vary in their responses to drug treatment, and multiple mechanisms can be invoked, such as poor compliance, variable impact of diverse disease mechanisms on drug actions, drug interactions, and an increasingly recognized role of genomic variation. Indeed, adverse drug reactions across all therapeutic categories are estimated to be the fourth to the sixth most common cause of death in the United States, costing $19 to $27 billion annually, and accounting directly for 3% to 6% of all hospital admissions.[3] This chapter outlines principles of drug action, the major mechanisms underlying variability in drug effects, and current and future approaches to enable the safest and most effective therapy for an individual patient.

THE KEY DECISION IN DRUG THERAPY: RISK VERSUS BENEFIT

The fundamental assumption underlying administration of any drug is that the real or expected benefit exceeds the anticipated risk. The benefits of drug therapy are initially defined in small clinical trials, perhaps involving several thousand patients, before a drug's marketing and approval. Ultimately, the efficacy and safety profiles of any drug are determined after the compound has been marketed and used widely in hundreds of thousands of patients.

When a drug is administered for the acute correction of a life-threatening condition, the benefits are often self-evident; insulin for diabetic ketoacidosis, nitroprusside for hypertensive encephalopathy, and lidocaine for ventricular tachycardia are examples. Extrapolation of such immediately obvious benefits to other clinical situations may not be warranted, however.

The efficacy of lidocaine to terminate ventricular tachycardia led to its widespread use as a prophylactic agent in cases of acute myocardial infarction, until it was recognized that in this setting, the drug does not alter mortality rates. The outcome of the Cardiac Arrhythmia Suppression Trial (CAST) highlights the difficulties in extrapolating from an incomplete understanding of physiology to chronic drug therapy. CAST tested the hypothesis that suppression of ventricular ectopic activity, a recognized risk factor for sudden death after myocardial infarction, would reduce mortality; this notion was highly ingrained in cardiovascular practice in the 1970s and 1980s. In CAST, sodium channel–blocking antiarrhythmics did suppress ventricular ectopic beats but also unexpectedly increased mortality threefold. Similarly, with the development of a first-generation cholesterol ester transport protein (CETP) inhibitor, the goal of elevation of high-density lipoprotein (HDL) levels was achieved, but with an accompanying increase in mortality. Thus, the use of arrhythmia suppression or of HDL elevation as a surrogate marker did not produce the desired drug action, reduction in mortality, probably because the underlying pathophysiology or full range of drug actions were incompletely understood.

Similarly, drugs with positive inotropic activity augment cardiac output in patients with heart failure but also are associated with an increase in mortality, probably as a consequence of drug-induced arrhythmias. Nevertheless, clinical trials with these agents suggest symptom relief. Thus, the prescriber and the patient may elect therapy with positive inotropic drugs to realize this benefit while recognizing the risk. This complex decision making is at the heart of the broad concept of personalized medicine that incorporates into the care of an individual patient not only genomic (or other) markers of variable drug responses but also factors such as patients' understanding of their disease and their willingness to tolerate minor or serious risks of treatment.

The risks of drug therapy may be a direct extension of the pharmacologic actions for which the drug is actually being prescribed. Excessive hypotension in a patient taking an antihypertensive agent and bleeding in a patient taking a platelet IIb/IIIa receptor antagonist are examples. In other cases, adverse effects develop as a consequence of pharmacologic actions that were not appreciated during a drug's initial development and use in patients. Rhabdomyolysis occurring with HMG-CoA reductase inhibitors (statins), angioedema developing during ACE inhibitor therapy, and torsades de pointes arising during treatment with noncardiovascular drugs such as thioridazine or pentamidine are examples. Of importance, these rarer but serious effects generally become evident only after a drug has been marketed and extensively used. Even rare adverse effects can alter the overall perception of risk versus benefit and can prompt removal of the drug from the market, particularly if alternate therapies thought to be safer are available. For example, withdrawal of the first insulin sensitizer, troglitazone, after recognition of hepatotoxicity was further spurred by the availability of other new drugs in this class.

The recognition of multiple cyclooxygenase (COX) isoforms led to the development of specific COX-2 inhibitors to retain aspirin's analgesic effects but reduce gastrointestinal side effects. However, one of these, rofecoxib, was withdrawn because of an apparent increase in cardiovascular mortality. The events surrounding the withdrawal of rofecoxib have important implications for drug development and utilization. First, specificity achieved by targeting a single molecular entity may not necessarily reduce adverse effects; one possibility is

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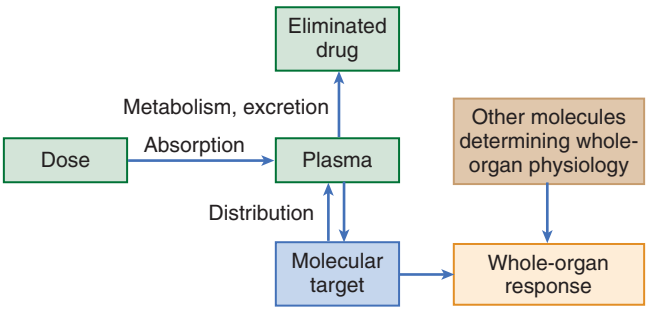

FIGURE 9-1 A model for understanding variability in drug action. When a dose of a drug is administered, the processes of absorption, metabolism, excretion, and distribution determine its access to specific molecular targets that mediate beneficial and toxic effects. The interaction between a drug and its molecular target then produces changes in molecular, cellular, whole-organ, and ultimately whole-patient physiology. This molecular interaction does not occur in a vacuum, but rather in a complex biologic milieu modulated by multiple factors, some of which are disturbed to cause disease. DNA variants in the genes responsible for the processes of drug disposition (*green*), the molecular target (*blue*), or the molecules determining the biologic context in which the drug-target interaction occurs (*brown*) all can contribute to variability in drug action.

that by inhibiting COX-2, the drug removes a vascular protective effect of prostacyclin. Second, drug side effects may include not only readily identifiable events such as rhabdomyolysis or torsades de pointes but also an increase that may be difficult to detect in events such as myocardial infarction that are common in the general population.

MECHANISMS UNDERLYING VARIABILITY IN DRUG ACTION

Two major processes determine how the interaction between a drug and its target molecule(s) can generate variable drug actions in a patient (**Fig. 9-1**). The first, *pharmacokinetics*, describes drug delivery to and removal from the target molecule and includes the processes of absorption, distribution, metabolism, and excretion—collectively termed drug disposition. The second process, *pharmacodynamics*, describes how the interaction between a drug and its molecular target(s) generates downstream molecular, cellular, whole-organ, and whole-body effects.

The framework shown in Figure 9-1 identifies a series of genes that mediate clinical drug actions, and in which variants may thus contribute to variable drug actions. These genes encode drug-metabolizing enzymes, drug transport molecules, drug targets, and molecules modulating the biology in which the drug-target interaction occurs. The latter include molecular perturbations that cause the disease being targeted. *Pharmacogenetics* describes the concept that individual variants in the genes controlling these processes contribute to variable drug actions, whereas *pharmacogenomics* describes the way in which variability across multiple genes, up to whole genomes, explains differences in drug response among individuals and populations. Presented next is an overview of broad principles of pharmacokinetics, pharmacodynamics, and pharmacogenomics, followed by more detailed discussions of the specific genes, their function, and important variants influencing cardiovascular drug responses.

Pharmacokinetic Principles

Administration of an intravenous drug bolus results in maximal drug concentrations at the end of delivery of the bolus, followed by a decline in plasma drug concentrations over time (**Fig. 9-2A**), generally due to drug elimination. The simplest case is one in which this decline occurs monoexponentially over time. A useful parameter to describe this decline is the half-life ($t_{1/2}$), the time in which 50% of the drug is eliminated; for example, after two half-lives, 75% of the drug has been eliminated, after three half-lives, 87.5%. A monoexponential process can be considered almost complete in four or five half-lives.

In some cases, the decline of drug concentrations following administration of an intravenous bolus dose is multiexponential. The most common explanation is that drug is not only eliminated (represented by the terminal portion of the time-concentration plot) but also undergoes more rapid distribution to peripheral tissues. Just as elimination may be usefully described by a half-life, distribution half-lives also can be derived from curves such as those shown in Figure 9-2B.

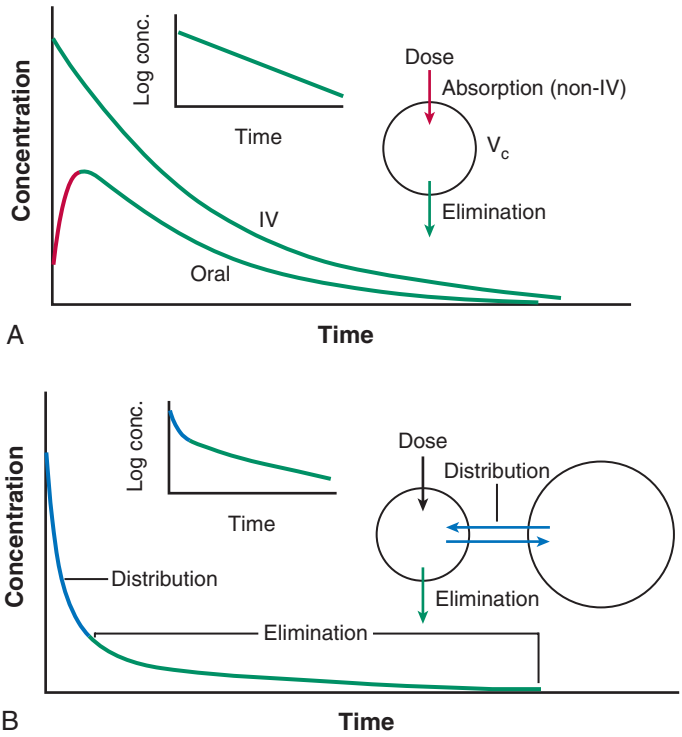

FIGURE 9-2 Models of plasma concentrations as a function of time after a single dose of a drug. **A,** The simplest situation is one in which a drug is administered as a rapid intravenous (IV) bolus into a volume (V_c), where it is instantaneously and uniformly distributed. Elimination then takes place from this volume. In this case, drug elimination is monoexponential; that is, a plot of the logarithm of concentration versus time is linear (*inset*). When the same dose of drug is administered orally, a distinct absorption phase is required before drug entry into V_c. Most absorption (shown here in *red*) is completed before elimination (shown in *green*), although the processes overlap. In this example, the amount of drug delivered by the oral route is less than that delivered by the intravenous route, assessed by the total areas under the two curves, indicating reduced bioavailability. **B,** In this example, drug is delivered to the central volume, from which it is not only eliminated but also undergoes distribution to the peripheral sites. This distribution process (*blue*) is more rapid than elimination, resulting in a distinct biexponential disappearance curve (*inset*).

The plasma concentration measured immediately after a bolus dose can be used to derive a volume into which the drug is distributed. When the decline of plasma concentrations is multiexponential, multiple distribution compartments can be defined; these volumes of distribution can be useful in considering dose adjustments in cases of disease but rarely correspond exactly to any physical volume, such as plasma or total body water. With drugs that are highly tissue-bound (e.g., some antidepressants), the volume of distribution can exceed total body volume by orders of magnitude.

Drugs are often administered by nonintravenous routes, such as oral, sublingual, transcutaneous, or intramuscular. Such routes of administration differ from the intravenous route in two ways (see Fig. 9-2A). First, concentrations in plasma demonstrate a distinct rising phase as the drug slowly enters plasma. Second, the total amount of drug that actually enters the systemic circulation may be less than that achieved by the intravenous route. The relative amount of drug entering by any route, compared with the same dose administered intravenously, is termed *bioavailability*. Some drugs undergo such extensive presystemic metabolism that the amount of drug required to achieve a therapeutic effect is much greater (and often more variable) than that required for the same drug administered intravenously. Thus, small doses of intravenous propranolol (5 mg) may achieve heart rate slowing equivalent to that observed with much larger oral doses (80 to 120 mg). Propranolol is actually well absorbed but undergoes extensive metabolism in the intestine and liver before entering the systemic circulation. Another example is that of amiodarone; its physicochemical characteristics make it only 30% to 50% bioavailable when administered orally. Thus, an intravenous infusion of 0.5 mg/min (720 mg/day) is equivalent to 1.5 to 2 g/day orally.

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Drug elimination occurs by metabolism followed by the excretion of metabolites and unmetabolized parent drug, generally by the biliary tract or kidneys. This process can be quantified as *clearance,* the volume that is cleared of drug in any given period. Clearance may be organ-specific (e.g., renal clearance, hepatic clearance) or whole-body clearance. Drug metabolism is conventionally divided into phase I oxidation and phase II conjugation, both of which enhance water solubility and, consequently, biliary or renal elimination.

The most common enzyme systems mediating phase I drug metabolism are those of the cytochrome P-450 superfamily, termed CYPs. Multiple CYPs are expressed in human liver and other tissues. A major source of variability in drug action is variability in CYP expression and/or genetic variants that alter CYP activity. **Table 9-1** lists CYPs and other drug-metabolizing enzymes important in cardiovascular therapy. Excretion of drugs or their metabolites into the urine or bile is accomplished by glomerular filtration or specific drug transport molecules, whose level of expression and genetic variation are only now being explored. One widely studied transporter is P-glycoprotein, the product of expression of the *MDR1* (or *ABCB1*) gene. Originally identified as a factor mediating multiple drug resistance in patients with cancer, P-glycoprotein expression is now well recognized in normal enterocytes, hepatocytes, renal tubular cells, the endothelium of the capillaries forming the blood-brain barrier, and the testes. In each of these sites, P-glycoprotein expression is restricted to the apical aspect of polarized cells, where it acts to enhance drug efflux. In the intestine, P-glycoprotein pumps substrates back into the lumen, thereby limiting bioavailability. In the liver and kidney, it promotes drug excretion into bile or urine. In central nervous system capillary endothelium, P-glycoprotein–mediated efflux is an important mechanism limiting drug access to the brain. Drug transporters play a role not only in drug elimination but also in drug uptake into many cells, including hepatocytes and enterocytes.

Pharmacodynamic Principles

Drugs can exert variable effects, even in the absence of pharmacokinetic variability. As indicated in Figure 9-1, this can arise as a function of variability in the molecular targets with which drugs interact to achieve their beneficial and adverse effects, as well as variability in the broader biologic context within which the drug-target interaction takes place. Variability in the number or function of a drug's target molecules can arise because of genetic factors (see later) or because disease alters the number of target molecules or their state (e.g., changes in the extent of phosphorylation). Simple examples of variability in the biologic context are high dietary salt, which can inhibit the antihypertensive action of beta blockers, and hypokalemia, which increases the risk for drug-induced QT prolongation. In addition, disease itself can modulate drug response. For example, the effect of lytic therapy in a patient with no clot is manifestly different from that in a patient with acute coronary thrombosis, or the vasodilating effects of nitrates, beneficial in patients with coronary disease with angina, can be catastrophic in patients with aortic stenosis. These examples highlight the requirement for precision in diagnosis to avoid situations in which risk outweighs potential benefit. One hope is that emerging genomic or other molecular approaches can add to this precision.

The targets with which drugs interact to produce beneficial effects may or may not be the same as those with which drugs interact to produce adverse effects. Drug targets may be in the circulation, at the cell surface, or within cells. Many newer drugs have been developed to interact with a specific drug target; examples of such targets are 3-hydroxy-3-methyl-glutaryl–coenzyme A (HMG-CoA) reductase, angiotensin-converting enzyme (ACE), G protein–coupled receptors (e.g., alpha, beta, angiotensin II, histamine), and platelet IIb/IIIa receptors. Such targets generally are identified in the course of basic mechanistic studies; a very appealing newer approach is to use modern genetic techniques to identify DNA variants associated with desired phenotypes, such as absence of myocardial infarction, as a clue to identify new drug targets.[4] On the other hand, many drugs widely used in cardiovascular therapeutics were developed when the technology to identify specific molecular targets simply was not available; digoxin, amiodarone, and aspirin are examples. Some, like amiodarone, have many drug targets. In other cases, however, even older drugs turn out to have rather specific molecular targets. The actions of digitalis glycosides are mediated primarily by the inhibition of Na^+,K^+-ATPase. Aspirin permanently acetylates a specific serine residue on the COX enzyme, an effect that is thought to mediate its analgesic effects and its gastrointestinal toxicity.

TABLE 9-1 Proteins Important in Drug Metabolism and Elimination

PROTEIN	SUBSTRATES
CYP3A4, CYP3A5	Erythromycin, clarithromycin; quinidine, mexiletine; many benzodiazepines; cyclosporine, tacrolimus; many antiretrovirals; HMG CoA reductase inhibitors (atorvastatin, simvastatin, lovastatin; not pravastatin); many calcium channel blockers
CYP2D6*	Some beta blockers—propranolol, timolol, metoprolol, carvedilol Propafenone; desipramine and other tricyclics; codeine†; tamoxifen†; dextromethorphan
CYP2C9*	Warfarin, phenytoin, tolbutamide, losartan†
CYP2C19*	Omeprazole, clopidogrel†
P-glycoprotein	Digoxin
N-acetyl transferase*	Procainamide, hydralazine, isoniazid
Thiopurine methyltransferase*	6-Mercaptopurine, azathioprine
Pseudocholinesterase*	Succinylcholine
UDP-glucuronosyltransferase*	Irinotecan†
SLCO1B1*	Simvastatin and other statins; methotrexate; troglitazone; bosentan

Full CYP listing is available at http://medicine.iupui.edu/flockhart.
*Clinically important genetic variants described; see text.
†Prodrug bioactivated by drug metabolism.

Time Course of Drug Effects

With repeated doses, drug levels accumulate to a *steady state*, the condition under which the rate of drug administration is equal to the rate of drug elimination in any given period. Drug accumulation to steady state is near-complete in four to five elimination half-lives (see **Fig. 9-3**). For many drugs, the target molecule is in or readily accessible from plasma, so this time course also describes the development of pharmacologic effects. However, in other cases, whereas steady-state plasma concentrations are achieved in four to five elimination half-lives, steady-state drug effects take longer to achieve and several explanations are possible. First, an active metabolite may need to be generated to achieve drug effects. Second, time may be required for translation of the drug effect at the molecular site to a physiologic endpoint; inhibition of synthesis of vitamin K–dependent clotting factors by warfarin ultimately leads to a desired elevation of the international normalized ratio (INR), but the development of this desired effect occurs only as levels of clotting factors fall. Third, penetration of a drug into intracellular or other tissue sites of action may be required before development of drug effect. One mechanism underlying such penetration is the variable function of specific drug uptake and efflux transport proteins that control intracellular drug concentrations.

Pharmacogenomic Principles

As described next, studies have exploited a range of experimental techniques to establish a role for both common and rare DNA polymorphisms in pharmacokinetic and pharmacodynamic pathways as mediators of variable drug actions. Rare disease-associated variants are traditionally termed *mutations*, whereas commoner variants (traditionally defined as minor allele frequency >1%) are termed *polymorphisms*. The commonest type is a single-nucleotide polymorphism (SNP); SNPs that change the encoded amino acid are termed

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nonsynonymous. The advent of modern sequencing technologies has demonstrated that most DNA variants in an individual are in fact rare,[5] so the distinction between mutation and polymorphism is blurred. Furthermore, polymorphism frequencies can vary strikingly by ethnicity; a common variant in persons of African ethnicities may be absent in whites.

One of the great success stories of modern cardiovascular genetics has been the use of linkage analysis in large families to identify disease-causing rare variants (mutations) in familial syndromes with highly unusual clinical phenotypes, such as familial hypercholesterolemia (**see Chapter 45**), hypertrophic cardiomyopathy (**see Chapter 66**), or the ion channelopathies (**see Chapter 32**). Linkage analysis has not been widely applied to study pharmacogenomics because large kindreds in which multiple individuals display extreme responses to drug exposure generally are not available. In the syndrome of malignant hyperthermia occurring in response to general anesthetics, it was possible to assign phenotype using functional studies in muscle biopsies and thus identify a linkage signal at chromosomal region 19q, which includes the gene encoding RYR1, the skeletal muscle calcium release channel in which mutations cause the disease.

When an extreme phenotype occurs in multiple family members, it is logical to invoke a genetic origin. It is now clear that DNA variation also contributes importantly to variability in common human traits, such as laboratory values or susceptibility to common disease. Methods are available to establish the extent to which that variability includes a heritable component, generally by examining twins, large families, or groups of families; evidence for heritability provides strong justification for pursuing studies to identify contributing genetic variation. Indeed, this general approach has established that common phenotypes such as LDL cholesterol, blood pressure, or susceptibility to atrial fibrillation are highly heritable. The extent to which rare and common variants contribute to this variability is only now being addressed. Across populations, it is very unusual for single *common* DNA polymorphisms to account for more than even 1% of variability in common traits. Variability in response to drug exposure presents a striking exception to this general rule, where even single common DNA polymorphisms may contribute substantially, 10% or more in many cases, to overall variability in drug response. It has been speculated that common variants with large effects on drug response can persist in a population because there is no evolutionary pressure against such variants since drug exposure is a recent event in human history. One mechanism accounting for this large effect is common SNPs in drug metabolism pathways that then result in very large fluctuations in drug concentration and corresponding effects. As described further on, common SNPs in drug target genes also can produce such large effects. Examples of specific cardiovascular phenotypes in which common SNPs have been associated with risk are presented in **Table 9-2** and discussed later on. Of note, rarer variants in these (or other) genes are only now being described, so their role in mediating drug response is much less well understood. In addition, virtually all studies to date have focused primarily on white populations, and data are only now being generated on specific polymorphisms mediating variable drug actions in other ancestries.

One technique to identify associations between DNA polymorphisms and drug response (or other) traits uses an understanding of the physiology of the trait under question to identify candidate genes modulating the trait. Thus, for example, an investigator

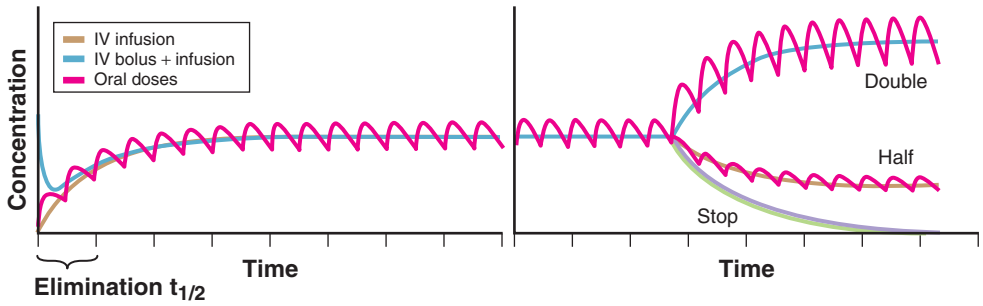

FIGURE 9-3 Time course of drug concentrations when treatment is started or dose changed. **Left,** The hash lines on the abscissa each indicate one elimination half-life ($t_{1/2}$). With a constant rate intravenous (IV) infusion (*gold*), plasma concentrations accumulate to steady state in four or five elimination half-lives. When a loading bolus is administered with the maintenance infusion (*blue*), plasma concentrations are transiently higher but may dip, as shown here, before achieving the same steady state. When the same drug is administered by the oral route, the time course of drug accumulation is identical (*magenta*); in this case the drug was administered at intervals of 50% of a $t_{1/2}$. Steady-state plasma concentrations during oral therapy fluctuate around the mean determined by intravenous therapy. **Right,** This plot shows that when dosages are doubled, or halved, or the drug is stopped during steady-state administration, the time required to achieve the new steady state is four or five $t_{1/2}$ and is independent of the route of administration.

TABLE 9-2 Examples of Common Single Nucleotide Polymorphisms Mediating Variable Drug Actions

DRUG EFFECT	PATHWAY	GENE	SNP*	DBSNP ID NUMBER	COMMENTS
Adverse outcomes during clopidogrel treatment for acute coronary syndrome	PK	*CYP2C19*	*CYP2C19*2*: truncation at P227	rs4244285	*2 results in defective clopidogrel bio-activation; this SNP contributes ~10% to variability in clopidogrel-mediated inhibition of ADP-induced platelet aggregation
Excess beta blocker effect: metoprolol, timolol	PK	*CYP2D6*	Many variants		
Warfarin steady-state dose	PK	*CYP2C9*	*CYP2C9*2*: R144C *CYP2C9*3*: I359L	rs1799853 rs1057910	VKORC1 and CYP2C9 variants account for ~50% of variability in warfarin steady-state dose
	PD	*VKORC1*	Promoter variant: –1639G>A	rs9923231	
	PD	*CYP4F2*	V433M	rs2108622	
Statin myotoxicity	PK	*SLCO1B1*	*SLCO1B1*5*: V174A	rs4149056	Risk of simvastatin myotoxicity increased 20-fold in homozygotes and 4-fold in heterozygotes
Response to beta blockers for hypertension, heart failure	PD (target)	*ADRB1* *ADRB2*	S49G R389G	rs1801252 rs1801253	
Beta blocker therapy in heart failure	PD (target)	*GRK5*	G41L	rs17098707	
Antihypertensive response during thiazides	PD	*ADD1*	G460W	rs4961	
Torsades de pointes	PD	*KCNE1*	D85N	rs1805128	8% allele frequency in patients with torsades versus ~2% in control subjects (odds ratio ~10)

*Trivial name (e.g., *2, *3) and amino acid change provided.
dbSNP = National Center for Biotechnology Information's SNP database; PD = pharmacodynamic; PK = pharmacokinetic.

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interested in variability in the PR interval might invoke polymorphisms in calcium channel genes, or an investigator interested in blood pressure might invoke variation in the ACE gene. The association between polymorphisms in these candidate genes and the phenotype under study is then examined in persons with well-characterized phenotypes. The candidate gene approach is intuitively appealing because it takes advantage of what is known about underlying physiology. Despite this appeal, however, the method is now recognized to carry with it the great potential for false-positive associations, especially when small numbers of subjects are studied. An important exception has been in pharmacogenomics, where the candidate gene approach has yielded important and clinically reproducible associations between single common polymorphisms and drug response. This exception probably reflects the unusually high contribution of SNPs to overall variability in drug response mentioned above.

Another approach to identifying polymorphisms contributing to variable human traits is the genome-wide association study (GWAS). Here, study subjects are genotyped at hundreds of thousands or millions of sites known to harbor common SNPs across the genome. Because the GWAS platforms focus on common SNPs, effect sizes are often small and difficult to identify and validate unless very large numbers of subjects, thousands or more, are studied. In addition, the SNPs associated with the trait usually are not themselves functional but rather serve as markers for loci that harbor truly functional variants. The great advantage of the method is that it makes no assumptions about underlying physiology, and one of its major accomplishments has been to identify entirely new pathways underlying variability in human traits.[6] The GWAS approach has been applied to study drug response phenotypes[7] and even in relatively small sets has occasionally been successful in identifying associated common variants. Sometimes these are known from candidate gene studies. In other cases, notably drug hypersensitivity reactions,[8] GWASs involving even a few dozen cases have identified strong signals that have then been replicated.

The GWAS paradigm is enabled by technology to generate the dense genotype datasets. New technologies being developed to generate other types of high-dimensional data similarly hold the promise of elucidating new biologic pathways in disease and drug response. Rapid, extremely high-throughput and increasingly inexpensive sequencing technologies are detecting rare DNA sequence variants whose contribution to disease is only now being appreciated.[5] RNA sequencing ("RNA-Seq") using these technologies is replacing microarray analysis as the method of choice for cataloguing RNA transcript profiles and abundance by specific cellular subtype and disease. Advances in mass spectrometry are similarly enabling development of catalogs (proteomic and metabolomic profiling) of all proteins or of small-molecule metabolites of cellular processes, including drug metabolites, by cell and disease. Other sources of high-dimensional data include electronic medical record (EMR) systems, discussed further later on, and high-density digital images. Integrating these diverse data types into a comprehensive picture of the perturbations that result in disease or variable drug responses is the goal of the evolving discipline of systems biology and pharmacology. It has been proposed that future drug development would be better served by a focus on pathways identified by systems approaches rather than single targets.[9]

MOLECULAR AND GENETIC BASIS FOR VARIABLE DRUG RESPONSE

Many factors contribute to variable drug responses—the patient's age, the severity of the disease being treated, presence of disease of excretory organs, drug interactions, and poor compliance, to name but a few. This section describes major pathways leading to variable drug responses.

When a drug is metabolized and excreted by multiple pathways, absence of one of these, because of genetic variants, drug interactions, or dysfunction of excretory organs, generally does not affect drug concentrations or actions. By contrast, if a single pathway plays a critical role, the drug is more likely to exhibit marked variability in plasma concentration and associated effects, a situation that has been termed high-risk pharmacokinetics.[10]

One high-risk scenario is that involving bioactivation of a drug—that is, metabolism of the drug to active and potent metabolites that mediate pharmacologic action. Decreased function of such a pathway reduces or eliminates drug effect. Bioactivation of clopidogrel by CYP2C19 is an example; persons with reduced CYP2C19 activity (caused by genetic variants or possibly by interacting drugs; see Tables 9-1 and 9-2) have an increased incidence of cardiovascular events following coronary stent placement.[11] Similarly, the widely used analgesic codeine undergoes CYP2D6-mediated bioactivation to an active metabolite, morphine, and patients with reduced CYP2D6 activity display reduced analgesia. A small group of individuals with multiple functional copies of *CYP2D6*, and hence increased enzymatic activity, has been identified; in this group, codeine may produce nausea and euphoria, presumably because of rapid morphine generation. A third example is the angiotensin receptor blocker losartan, which is bioactivated by CYP2C9; reduced antihypertensive effect is a risk with common genetic variants that reduce CYP2C9 activity or with coadministration of CYP2C9 inhibitors, such as phenytoin.

A second high-risk pharmacokinetic scenario is one in which a drug is eliminated by only a single pathway. In this case, absence of activity of that pathway will lead to marked accumulation of drug in plasma, and for many drugs, such accumulation results in a high risk of drug toxicity. A simple example is the dependence of sotalol or dofetilide elimination on renal function; failure to decrease the dosage in a patient with renal dysfunction leads to accumulation of these drugs in plasma and an increased risk for drug-induced QT prolongation and torsades de pointes. Similarly, administration of a wide range of P-glycoprotein inhibitors will predictably elevate plasma concentration of digoxin, which is eliminated primarily by P-glycoprotein–mediated efflux into bile and urine (see Table 9-2).

Administration of CYP2D6-metabolized beta blockers, including metoprolol and carvedilol, to patients with defective enzyme activity may produce exaggerated heart rate slowing. The weak beta-blocking actions of the antiarrhythmic propafenone also are increased in patients with reduced CYP2D6 activity. Some antidepressants are CYP2D6 substrates; for these drugs, cardiovascular adverse effects are more common in poor metabolizers (PMs) of CYP2D6, whereas therapeutic efficacy is more difficult to achieve in ultrarapid metabolizers.

The risk of aberrant drug responses due to CYP variants is greatest in persons who are homozygous (i.e., PMs). However, for drugs with very narrow therapeutic margins (e.g., warfarin, clopidogrel), even heterozygotes may display unusual drug sensitivity. Although PMs make up a minority of subjects in most populations, many drugs in common use can inhibit these enzymes (see Table 9-3) and thereby "phenocopy" the PM trait. Omeprazole and possibly other proton pump inhibitors block CYP2C19 and have been associated with an increase in cardiovascular events during clopidogrel therapy.[12] Similarly, specific inhibitors of CYP2D6 and CYP2C9 can phenocopy the PM trait when coadministered with substrate drugs (**Table 9-3**).

An example of variant drug transporter function mediating variable drug actions is provided by *SLCO1B1*, encoding a drug uptake transporter in liver. A common nonsynonymous SNP in this gene has been associated by candidate studies with variability in simvastatin pharmacokinetics and by GWASs with a markedly increased risk for simvastatin-induced myopathy.[13]

The heart rate slowing and blood pressure effects of beta blockers and beta agonists have been associated with polymorphisms in the *drug targets*, the beta-1 and beta-2 receptors. A common variant in *ADRB1*, encoding the beta-1 receptor, has been implicated as a mediator of survival during therapy with the beta blocker bucindolol in heart failure. Variability in warfarin dose requirements has been clearly associated with variants in both CYP2C9, which mediates elimination of the active enantiomer of the drug, and VKORC1, part of the vitamin K complex that is the drug target. Indeed, these common variants account for up to half of the variability in warfarin dose requirement,[14] illustrating the large impact that common SNPs can exert on drug response phenotypes. Furthermore, allele frequencies vary strikingly by ancestry, probably accounting for the fact that warfarin dose requirements are low in Asian subjects and high in African subjects compared with whites.[15]

An example of a variant modulating biologic context in which the drug acts is susceptibility to stroke in patients receiving diuretics; this has been linked to a polymorphism in the alpha-adducing gene whose product plays a role in renal tubular sodium transport.

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TABLE 9-3 Drug Interactions: Mechanisms and Examples

MECHANISM	DRUG	INTERACTING DRUG	EFFECT
Decreased bioavailability	Digoxin	Antacids	Decreased digoxin effect secondary to decreased absorption
Increased bioavailability	Digoxin	Antibiotics	By eliminating gut flora that metabolize digoxin, some antibiotics may increase digoxin bioavailability; NOTE: some antibiotics also interfere with P-glycoprotein (expressed in the intestine and elsewhere), another effect that can elevate digoxin concentration
Induction of hepatic metabolism	*CYP3A substrates*: Quinidine Mexiletine Verapamil Cyclosporine	Phenytoin Rifampin Barbiturates St. John's wort	Loss of drug effect secondary to increased metabolism
Inhibition of hepatic metabolism	*CYP2C9*: Warfarin Losartan	Amiodarone Phenytoin	Decreased warfarin requirement Diminished conversion of losartan to its active metabolite, with decreased antihypertensive control
	CYP3A substrates: Quinidine Cyclosporine HMG-CoA reductase inhibitors: lovastatin, simvastatin, atorvastatin; not pravastatin cisapride, terfenadine, astemizole	Ketoconazole Itraconazole Erythromycin Clarithromycin Some calcium blockers Some HIV protease inhibitors (especially ritonavir)	Increased risk for drug toxicity
	CYP2D6 substrates: Beta blockers (see Table 9-2) Propafenone Desipramine Codeine	Quinidine (even ultralow dose) fluoxetine, paroxetine	Increased beta blockade Increased beta blockade Increased adverse effects Decreased analgesia (due to failure of biotransformation to the active metabolite morphine)
	CYP2C19: Clopidogrel	Omeprazole, possibly other proton pump inhibitors	Decreased clopidogrel efficacy
Inhibition of drug transport	*P-glycoprotein transport*: Digoxin	Amiodarone, quinidine verapamil, cyclosporine itraconazole, erythromycin	Digoxin toxicity
	Renal tubular transport: dofetilide	Verapamil	Slightly increased plasma concentration and QT effect
	Monoamine transport: guanadrel	Tricyclic antidepressants	Blunted antihypertensive effects
Pharmacodynamic interactions	Aspirin + warfarin		Increased therapeutic antithrombotic effect; increased risk of bleeding
	Nonsteroidal anti-inflammatory drugs	Warfarin	Increased risk of gastrointestinal bleeding
	Antihypertensive drugs	Nonsteroidal anti-inflammatory drugs	Loss of blood pressure lowering
	QT-prolonging antiarrhythmics	Diuretics	Increased torsades de pointes risk secondary to diuretic-induced hypokalemia
	Supplemental potassium	ACE inhibitors	Hyperkalemia
	Sildenafil	Nitrates	Increased and persistent vasodilation; risk of myocardial ischemia

Torsades de pointes during QT-prolonging drug therapy has been linked to polymorphisms not only in the ion channel that is the drug target but to other ion-channel genes; a large candidate gene survey reported that a nonsynonymous SNP in KCNE1, a subunit for the slowly-activating potassium current I_{Ks}, conferred an odds ratio of approximately 10 for torsades risk.[16] In addition, this adverse effect sometimes occurs in patients with clinically latent congenital long-QT syndrome, emphasizing the interrelationship among disease, genetic background, and drug therapy (**see Chapters 32 and 35**). Drugs also can bring out latent Brugada syndrome (see www.brugadadrugs.org).

The anticancer drug trastuzumab is effective only in patients with cancers that do not express the Her2/neu receptor. Because the drug also potentiates anthracycline-related cardiotoxicity, toxic therapy can be avoided in patients who are receptor-negative (**see Chapter 85**).

OPTIMIZING DRUG DOSES

The goals of drug therapy should be defined before the initiation of drug treatment. These may include acute correction of serious pathophysiology, acute or chronic symptom relief, or changes in surrogate endpoints (e.g., blood pressure, serum cholesterol, international normalized ratio [INR]) that have been linked to beneficial outcomes in target patient populations. The lessons of CAST and of positive inotropic drugs should make prescribers skeptical about such surrogate-guided therapy in the absence of controlled clinical trials.

When the goal of drug therapy is to acutely correct a disturbance in physiology, the drug should be administered intravenously in doses designed to achieve a therapeutic effect rapidly. This approach is best justified when benefits clearly outweigh risks. Large intravenous drug boluses carry with them a risk of enhancing drug-related toxicity; therefore, even with the most urgent of medical indications, this

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approach is rarely appropriate. An exception is adenosine, which must be administered as a rapidly delivered bolus because it undergoes extensive and rapid elimination from plasma by uptake into almost all cells. As a consequence, a slow bolus or infusion rarely achieves sufficiently high concentrations at the desired site of action (the coronary artery perfusing the atrioventricular node) to terminate arrhythmias. Similarly, the time course of anesthesia depends on anesthetic drug delivery to and removal from sites in the central nervous system.

The time required to achieve steady-state plasma concentrations is determined by the elimination half-life (see earlier). The administration of a loading dose may shorten this time, but only if the kinetics of distribution and elimination are known beforehand in an individual subject and the correct loading regimen is chosen. Otherwise, overshoot or undershoot during the loading phase may occur (see Fig. 9-3). Thus, the initiation of drug therapy by a loading strategy should be used only when the indication is acute.

Two dose-response curves describe the relationship between drug dose and the expected cumulative incidence of a beneficial effect or an adverse effect (**Fig. 9-4**). The distance along the *x*-axis describing the difference between these curves, often termed the *therapeutic ratio* (or index or window), provides an index of the likelihood that a chronic dosing regimen that provides benefits without adverse effects can be identified. Drugs with especially wide therapeutic indices often can be administered at infrequent intervals, even if they are rapidly eliminated (see Fig. 9-4A, C).

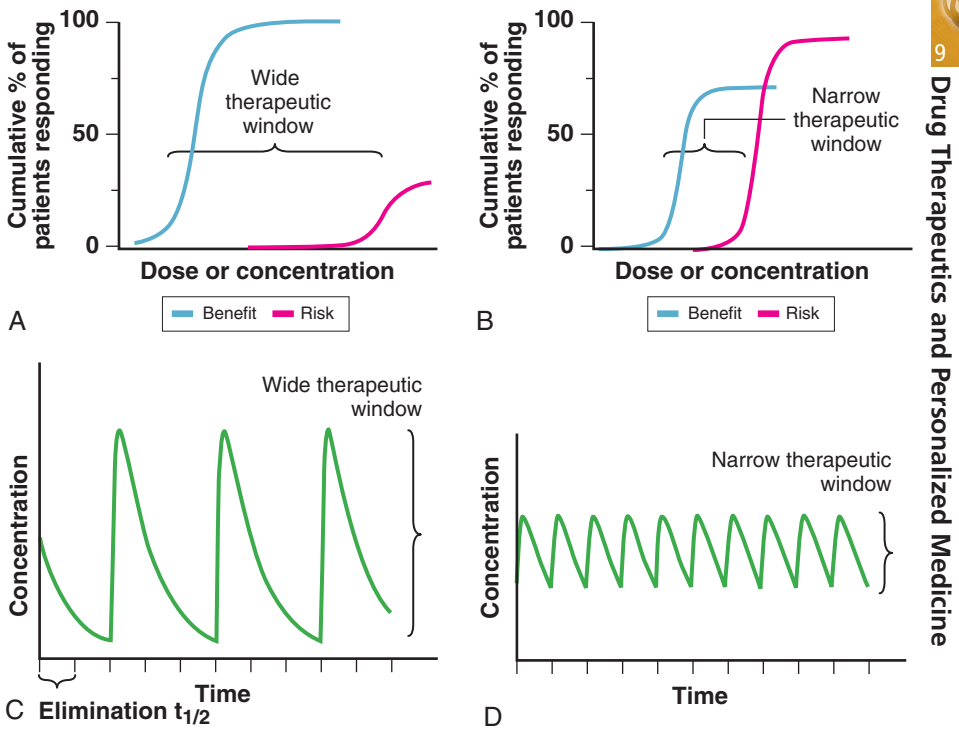

FIGURE 9-4 The concept of therapeutic ratio. **A, B,** Two dose- (or concentration-) response curves. The *blue* lines describe the relationship between dose and cumulative incidence of beneficial effects, and the *magenta* line depicts the relationship between dose and dose-related adverse effects (risk). As depicted in **A,** a drug with a wide therapeutic ratio displays separation between the two curves, a high degree of efficacy, and low degree of dose-related toxicity. Under these conditions, a wide therapeutic ratio can be defined. In **B,** conversely, the curves describing cumulative efficacy and cumulative incidence of adverse effects are positioned near each other, the incidence of adverse effects is higher, and the expected beneficial response is lower. These characteristics define a narrow therapeutic ratio. **C, D,** Steady-state plasma concentrations with oral drug administration as a function of time with wide (*left*) and narrow (*right*) therapeutic ratios. The hash marks on the abscissae each indicate one elimination half-life. **C,** When the therapeutic window is wide, drug administration every three elimination half-lives can produce plasma concentrations that are maintained above the minimum for efficacy and below the maximum beyond which toxicity is anticipated. **D,** The opposite situation is illustrated. To maintain plasma concentrations within the narrow therapeutic range, the drug must be administered more frequently.

When anticipated adverse effects are serious, the most appropriate treatment strategy is to start at low doses and reevaluate the necessity for increasing drug dosages once steady-state drug effects have been achieved. This approach has the advantage of minimizing the risk of dose-related adverse effects but carries with it a need to titrate doses to efficacy. Only when stable drug effects are achieved should increasing drug dosage to achieve the desired therapeutic effect be considered. An example is sotalol: Because the risk of torsades de pointes increases with drug dosage, the starting dose should be low.

In other cases, anticipated toxicity is relatively mild and manageable. It may then be acceptable to start at dosages higher than the minimum required to achieve a therapeutic effect, accepting a greater than minimal risk of adverse effects; some antihypertensives can be administered in this fashion. However, the principle of using the lowest dose possible to minimize toxicity, particularly toxicity that is unpredictable and unrelated to recognized pharmacologic actions, should be the rule.

Occasionally, dose escalation into the high therapeutic range results in no beneficial drug effect and no side effects. In this circumstance, the prescriber should be alert to the possibility of noncompliance or drug interactions at the pharmacokinetic or pharmacodynamic level. Depending on the nature of the anticipated toxicity, dose escalation beyond the usual therapeutic range may occasionally be acceptable, but only if anticipated toxicity is not serious and is readily manageable.

Plasma Concentration Monitoring

For some drugs, curves such as those shown in Figure 9-4A and B relating drug concentration to cumulative incidence of beneficial and adverse effects can be generated. With such drugs, monitoring plasma drug concentrations to ensure that they remain within a desired therapeutic range (i.e., above a minimum required for efficacy and below a maximum likely to produce adverse effects) may be a useful adjunct to therapy. Monitoring drug concentrations also may be useful to ensure compliance and to detect pharmacokinetically based drug interactions that underlie unanticipated efficacy and/or toxicity at usual dosages. Samples for measurement of plasma concentrations generally should be obtained just before the next dose, at steady state. These trough concentrations provide an index of the minimum plasma concentration expected during a dosing interval.

On the other hand, patient monitoring, whether by plasma concentration or other physiologic indices, to detect incipient toxicity is best accomplished at the time of anticipated peak drug concentrations. Thus, patient surveillance for QT prolongation during therapy with sotalol or dofetilide is best timed for 1 to 2 hours after the administration of a dose of drug at a steady state.

A lag between the time courses of drug in plasma and drug effects may exist (see earlier). In addition, monitoring plasma drug concentrations relies on the assumption that the concentration measured is in equilibrium with that at the target molecular site. Of note, it is only the fraction of drug not bound to plasma proteins that is available to achieve such equilibration. Variability in the extent of protein binding can therefore affect the free fraction and anticipated drug effect, even in the presence of apparently therapeutic total plasma drug concentrations. Basic drugs such as lidocaine and quinidine are not only bound to albumin but also bind extensively to alpha-1 acid glycoprotein, an acute-phase reactant whose concentrations are increased in a variety of stress situations, including acute myocardial infarction. Because of this increased protein binding, drug effects may be blunted, despite achieving therapeutic total drug concentrations in these situations.

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Dose Adjustments

Disease and Concomitant Drugs

Polypharmacy is common in patients with varying degrees of specific organ dysfunction. Although treatment with an individual agent may be justified, the practitioner should also recognize the risk of unanticipated drug effects, particularly drug toxicity, during therapy with multiple drugs.

The presence of renal disease mandates dose reductions for drugs eliminated primarily by renal excretion, including digoxin, dofetilide, and sotalol. A requirement for dose adjustment in cases of mild renal dysfunction is dictated by available clinical data and the likelihood of serious toxicity if drug accumulates in plasma because of impaired elimination. Renal failure reduces the protein binding of some drugs (e.g., phenytoin); in this case, a total drug concentration value in the therapeutic range may actually represent a toxic value of unbound drug.

Advanced liver disease is characterized by decreased hepatic drug metabolism and portacaval shunts that decrease clearance, particularly first-pass clearance. Moreover, affected patients frequently have other profound disturbances of homeostasis, such as coagulopathy, severe ascites, and altered mental status. These pathophysiologic features of advanced liver disease can profoundly affect not only the dose of a drug required to achieve a potentially therapeutic effect but also the perception of risks and benefits, thereby altering the prescriber's assessment of the actual need for therapy.

Heart disease similarly carries with it a number of disturbances of drug elimination and drug sensitivity that may alter the therapeutic doses or the practitioner's perception of the desirability of therapy on the basis of evaluation of risks and benefits. Patients with left ventricular hypertrophy often have baseline QT prolongation, so risks associated with use of QT-prolonging antiarrhythmics may increase; most guidelines suggest avoiding QT-prolonging antiarrhythmics in such patients (**see Chapters 35, 86, and 88**; see also www.torsades.org).

In heart failure (**see Chapter 25**), hepatic congestion can lead to decreased clearance with a corresponding increased risk for toxicity with usual doses of certain drugs, including some sedatives, lidocaine, and beta blockers. On the other hand, gut congestion can lead to decreased absorption of orally administered drugs and decreased effects. In addition, patients with heart failure may demonstrate reduced renal perfusion and require dose adjustments on this basis. Heart failure also is characterized by a redistribution of regional blood flow, which can lead to reduced volume of distribution and enhanced risk for drug toxicity. Lidocaine probably is the best-studied example; loading doses of lidocaine should be reduced in patients with heart failure because of altered distribution, whereas maintenance doses should be reduced in heart failure and liver disease because of altered clearance.

Age also is a major factor in determining drug doses, as well as sensitivity to drug effects. Doses in children generally are administered on an mg/kg body weight basis, although firm data to guide therapy are often not available. Variable postnatal maturation of drug disposition systems may present a special problem in the neonate. Older persons often have reduced creatinine clearance, even those with a normal serum creatinine level, and dosages of renally excreted drugs should be adjusted accordingly (see Chapter 76). Diastolic dysfunction with hepatic congestion is more common in older adults, and vascular disease and dementia are common, which can lead to increased postural hypotension and risk of falling. Therapies such as sedatives, tricyclic antidepressants, or anticoagulants should be initiated only when the practitioner is convinced that the benefits of such therapies outweigh this increased risk.

Drug Interactions

As a result of therapeutic successes not only in heart disease but also in other disease areas, cardiovascular physicians are increasingly encountering patients receiving multiple medications for noncardiovascular indications. Table 9-3 summarizes mechanisms that may underlie important drug interactions. Drug interactions may be based on altered absorption, distribution, metabolism, or excretion. In addition, drugs can interact at the pharmacodynamic level. A trivial example is the coadministration of two antihypertensive drugs, leading to excessive hypotension. Similarly, coadministration of aspirin and warfarin leads to an increased risk for bleeding, although benefits of the combination also can be demonstrated.

The most important principle in approaching a patient receiving polypharmacy is to recognize the high potential for drug interactions. A complete medication history should be obtained from each patient at regular intervals; patients will often omit topical medications such as eye drops, health food supplements, and medications prescribed by other practitioners unless specifically prompted. Each of these, however, carries a risk of important systemic drug actions and interactions. Even high dosages of grapefruit juice, which contains CYP3A and P-glycoprotein inhibitors, can affect drug responses. Beta blocker eye drops can produce systemic beta blockade, particularly with CYP2D6 substrates (e.g., timolol) in patients with defective CYP2D6 activity. St. John's wort induces CYP3A and P-glycoprotein activity (like phenytoin and other drugs) and thus can markedly lower plasma concentrations of substrate drugs such as cyclosporine. As with many other interactions, this may not be a special problem so long as both drugs are continued. However, if a patient stabilized on cyclosporine stops taking a concomitantly administered CYP3A inducer, plasma concentrations of the drug can rise dramatically and toxicity can ensue. Similarly, initiation of an inducer may lead to markedly lowered cyclosporine concentrations and a risk of organ rejection. A number of natural supplements have been associated with serious drug toxicity that has resulted in withdrawal from the market; phenylpropanolamine-associated stroke is an example.

Incorporating Pharmacogenetic Information into Prescribing

The identification of polymorphisms associated with variable drug responses naturally raises the question of how these data could or should be used to optimize drug doses, to avoid drugs likely to be ineffective, and to avoid drugs likely to produce major toxicities. Indeed, in 2007, the U.S. Food and Drug Administration (FDA) began systematically including pharmacogenetic information in drug labels.[17] Despite the intuitive appeal of a pharmacogenetically guided approach to drug therapy, however, practitioners wishing to adopt genetic testing to guide drug therapy encounter substantial practical barriers; these include cost, varying levels of evidence supporting a role for genetics, and implementation issues such as how fast and accurately a genetic test result can be delivered. It is the nature of pharmacogenetic variation that most patients will display average responses to most drugs, so systematically testing every patient in the hopes of finding the minority likely to display aberrant responses is cumbersome and seems time- and cost-inefficient unless the benefit for individual patients is large. An example of a large benefit is that routine genotyping of all patients receiving the antiretroviral agent abacavir is now standard of care because it avoids a potentially life-threatening skin reaction in 3% of patients.[18] In cardiovascular medicine, initial results of clinical trials suggest either no effect or a modest effect of genotyping to keep anticoagulation therapeutic during warfarin therapy.

A difficulty with such drug-specific approaches is that the benefit of the genotype data must be large to justify the cumbersomeness and cost of testing all exposed subjects. Although the probability is small that genetic variation plays an important role in predicting the response of an individual patient to a specific drug, it is likely that when many drugs are prescribed for a population of patients, each patient will display genetically determined aberrant responses to some drugs. This reasoning underlies the concept of preemptive genotyping, in which many genetic variants relevant to many variable drug responses are assayed in subjects who have not yet been exposed to the drugs.[19] These data are then stored in EMR systems with advanced point-of-care decision support capabilities that deliver instantaneous advice when a drug is prescribed to a patient with known genomic variants.[20] Several technological developments enable this vision, and these include advanced EMRs and multiplexed inexpensive genotyping assays that interrogate many

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polymorphisms for the same cost as a handful relevant to one drug. The concept is now being tested at a handful of medical centers with the goal of testing the idea, establishing its cost and benefit, and optimizing this approach to implementing pharmacogenomic information into health care.

FUTURE CHALLENGES

The past 25 years have seen dramatic advances in the treatment of heart disease, in no small part because of the development of highly effective and well-tolerated drug therapies such as with HMG-CoA reductase inhibitors, ACE inhibitors, and beta blockers. These developments, along with improved nonpharmacologic approaches, have led to dramatically enhanced survival of patients with advanced heart disease. Thus, polypharmacy in an aging and chronically ill population is becoming increasingly common. In this milieu, drug effects become increasingly variable, reflecting interactions among drugs, underlying disease and disease mechanisms, and genetic backgrounds. Furthermore, despite advances in the Western world, cardiovascular disease is emerging as an increasing problem worldwide as infectious diseases, formerly predominant contributors to morbidity and mortality, are coming under control and smoking continues to increase. Understanding the way in which genetic background plays into disease susceptibility and responses to drug therapy, concepts largely tested in only white populations to date, represents a major challenge in cardiovascular medicine.

More generally, an important point is that genomic science is still in its infancy, so reported associations require independent confirmation and assessment of clinical importance and cost-effectiveness before they can or should enter clinical practice. Importantly, most pharmacogenomic studies reported to date have focused on common variants with relatively large effects on phenotypes like drug concentrations or drug responses. However, application of modern sequencing technologies has revealed that the vast majority of polymorphisms are uncommon (minor allele frequencies under 1%), and *CYP* and other genes relevant to pharmacogenomics are no exception. Developing approaches to establish the clinical impact of such rare variants on drug responses is an emerging challenge.

This challenge is all the more acute because the cost of sequencing has fallen drastically since the completion of the first-draft human genome in 2000, and the sub-$1000 whole-genome sequence is likely to be a reality in 2014. This may be enabling for the preemptive pharmacogenomic strategy just outlined, as well as a broader vision of genome-guided healthcare but presents major challenges in data storage and mining.

The relationship between the prescriber and the patient remains the centerpiece of modern therapeutics. An increasingly sophisticated molecular and genetic view of response to drug therapy should not change this view, but rather complement it. Each initiation of drug therapy represents a new clinical experiment. Prescribers must always be vigilant regarding the possibility of unusual drug effects, which could provide clues about unanticipated and important mechanisms of beneficial and adverse drug effects.

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Biomarkers, Proteomics, Metabolomics, and Personalized Medicine

10

Peter Libby, Robert E. Gerszten, and Paul M Ridker

We use biomarkers daily in the practice of cardiovascular medicine. Moreover, the use of biomarkers has the potential to continue to improve our ability to provide clinically effective and cost-effective cardiovascular medicine in the years to come. Appropriate risk stratification and targeting of therapies should not only help improve patient outcomes but also assist in responding to the urgent need to "bend the cost curve" of medical care. In particular, excessive use of imaging biomarkers increases the cost of medical care and can jeopardize patient outcomes (for example, radiation exposure or complications of administering contrast material or investigating incidental findings). Inappropriate use or interpretation of blood biomarkers (e.g., cardiac troponin levels) can lead to unnecessary hospitalization or procedures as well.

Despite the current usefulness of biomarkers, their future promise, and the critical need to use them appropriately, a great deal of misunderstanding surrounds their current clinical application. In addition, contemporary technologies have the potential to greatly expand the gamut of biomarkers relevant to cardiovascular practice. Emerging genetic, proteomic, metabolomic, and molecular imaging strategies will surely transform the landscape of cardiovascular biomarkers (**see also Chapters 7, 8, 9, and 42**). This chapter provides a primer on cardiovascular biomarkers by defining some terms and discussing how the application of biomarkers can assist in clinical care, in addition to exploring some emerging technologies. Finally, we discuss an approach to the rigorous evaluation of the clinical usefulness of biomarkers. Advances in cardiovascular biology and the application of novel technologies have identified a plethora of novel cardiovascular biomarkers of potential clinical usefulness—begging the question of whether a novel biomarker adds value to existing and often better-validated biomarkers. Thus clinicians need tools to evaluate these emerging biomarkers, adoption of which may elevate clinical practice and improve patient outcomes.

WHAT IS A BIOMARKER?

For regulatory purposes, the U.S. Food and Drug Administration (FDA) first defined a *biomarker* in 1992 as "a laboratory measure or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful end point that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the therapy." At that time the FDA considered a *surrogate endpoint* as "reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence to predict clinical benefit."[1] The National Institutes of Health (NIH) convened a working group in 1998 that offered some parallel operating definitions to guide the biomarker field (**Table 10-1**).[2] They defined a biologic marker—biomarker for short—as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." Thus the NIH definition embraces not only soluble biomarkers in circulating blood but also "bedside biomarkers" such as anthropomorphic variables obtainable with a blood pressure cuff or a tape measure at the point of care. This broad definition encompasses not only measurements of biomarkers in blood (**Fig. 10-1A**) but also those derived from a variety of techniques, including measurements from imaging studies (Fig. 10-1B). Imaging biomarkers can include those derived from classic anatomic approaches. Imaging modalities now offer functional information, such as estimates of ventricular function, myocardial perfusion, and the like. Molecular imaging has the potential to target specific molecular processes. A functional classification of biomarkers helps sort through the plethora encountered by the clinician inasmuch as biomarkers can reflect a variety of biologic processes or organs of origin. For example, as a first approximation, cardiac troponin reflects myocardial injury, brain natriuretic peptide reflects cardiac chamber stretch, C-reactive protein (CRP) reflects inflammation, and the estimated glomerular filtration rate reflects kidney function (see Fig. 10-1B).

The NIH working group also provided further definitions relevant to the field of biomarkers. They defined a "surrogate endpoint" as "a biomarker intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm), or lack of benefit (or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence." (Note that the NIH definitions do not include the commonly used term "surrogate marker.") (Table 10-1). Thus a surrogate endpoint is a biomarker that has been "elevated" to surrogate status. This distinction has particular importance in the regulatory aspects of cardiovascular medicine. For example, the FDA previously accepted a certain degree of reduction in hemoglobin A1c (HbA1c) as a criterion for registration of a novel oral hypoglycemic agent—thus HbA1c was considered a biomarker accepted as a surrogate endpoint. Current FDA guidance now requires a cardiovascular safety study for the registration of new medications that target diabetes.[3] This policy indicates doubts about the fidelity of a drop in HbA1c as a surrogate endpoint for reduced cardiovascular risk in the eyes of regulatory authorities despite its value as a biomarker of glycemia.

The NIH working group defined a "clinical endpoint" as "a characteristic or variable that reflects how a patient feels, functions, or survives" (Table 10-1). Pivotal or phase III cardiovascular trials aspire

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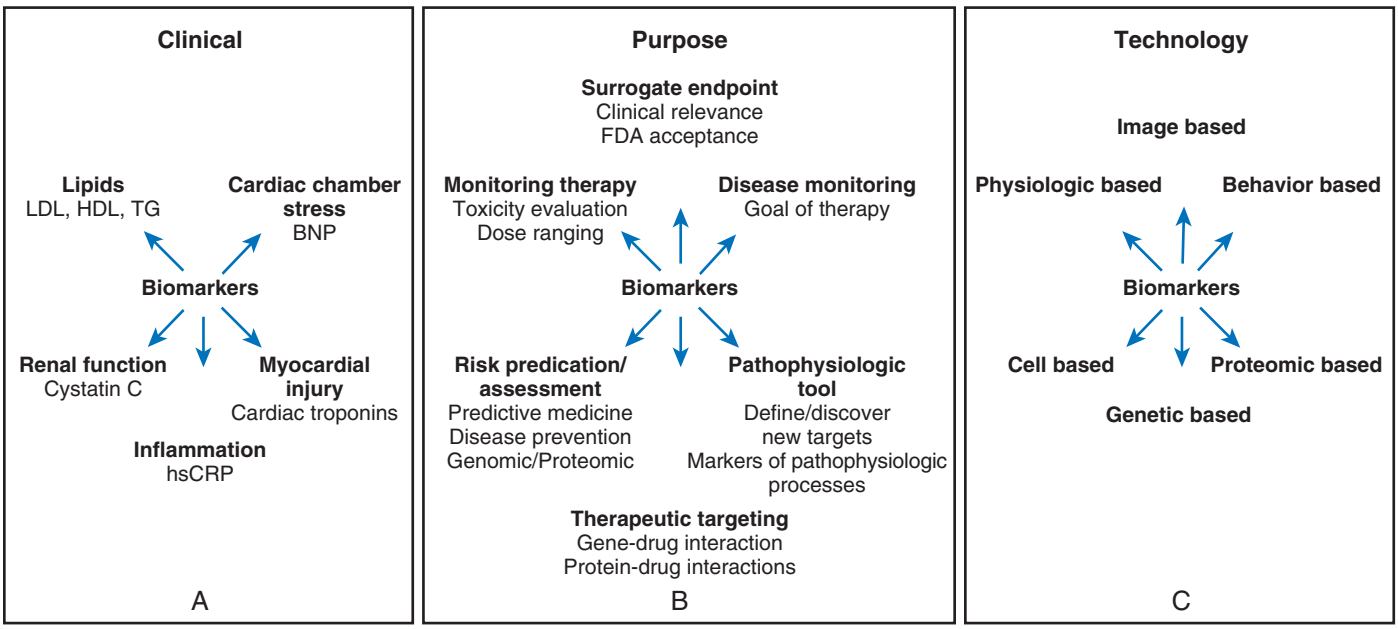

FIGURE 10-1 Examples of commonly used clinical biomarkers for cardiovascular disease **(A),** as well as research-oriented biomarkers categorized according to purpose **(B)** and technology **(C).** BNP = brain natriuretic peptide; TG = triglyceride.

TABLE 10-1 National Institutes of Health Biomarkers Definition Working Group (1998)

Biologic Marker (Biomarker)
A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.
Surrogate Endpoint
A biomarker intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence.
Clinical endpoint
A characteristic or variable that reflects how a patient feels, functions, or survives.

to use clinical endpoints as defined above. The distinction between biomarkers, surrogate endpoints, and clinical endpoints has crucial implications as practitioners, regulators, and payers increasingly demand evidence of improvements in actual clinical outcomes rather than mere manipulation of biomarkers as a criterion for adoption of a treatment in clinical practice.

Clinical Applications of Cardiovascular Biomarkers

Much of the prevailing confusion regarding biomarkers involves framing the question that one wants to answer with the use of a biomarker (Fig. 10-1C). We can classify the goals of application of cardiovascular biomarkers into several rubrics.

1. Diagnosis: The use of biomarkers for cardiovascular diagnosis has daily familiarity to practitioners of cardiovascular medicine. The current universal definition of myocardial infarction, for example, requires elevation of a biomarker of myocyte injury, such as cardiac-specific isoforms of troponin.
2. Risk stratification: Familiar examples of biomarkers used in risk stratification in cardiovascular medicine include systolic blood pressure or low-density lipoprotein (LDL) cholesterol. These biomarkers reliably predict future risk for cardiovascular events on a population basis.
3. Goals for therapy: Our contemporary guidelines often specify cut points for targets of treatment—for example, a specific level of a biomarker such as systolic blood pressure or LDL cholesterol in a particular group of individuals. Practitioners of cardiovascular medicine commonly use the biomarker international normalized ratio (INR) to titrate the dosage of warfarin administered to an individual patient. Abundant data support the clinical benefit of maintaining the INR within a certain range in various patient groups—an example of a widely used biomarker that has proven clinical usefulness as a goal for therapy.
4. Targeting of therapy: In clinical practice, using biomarkers to target therapy has great usefulness and promise as we move toward a more comprehensive "personalized medicine" approach to practice (**see Chapter 8**). Examples of biomarkers used to target therapy include troponin measurements to triage patients with acute coronary syndromes for early invasive management or measurement of high-sensitivity C-reactive protein (hsCRP) to allocate statin treatment to individuals with below-average LDL cholesterol.
5. Drug development, evaluation, and registration: Biomarkers have critical importance in the development of new pharmacologic agents. Biomarkers can provide early signals of efficacy that will help prioritize agents more likely to provide benefit on clinical endpoints in large-scale trials. Inappropriate dose selection represents a major mode of failure of clinical trials. Judicious use of biomarkers can help in selecting an appropriate dose of an agent to study in a large endpoint trial. Finally, biomarkers accepted as surrogate endpoints prove useful to regulatory agencies in granting approval for novel therapies.

Clinical use of cardiovascular biomarkers requires a clear understanding of *how* they should be used. Many biomarkers provide clinically useful information when measured once at "baseline." A baseline measurement of high-density lipoprotein (HDL) cholesterol, for example, indubitably correlates inversely with future risk for cardiovascular events. Yet serial measurement of biomarkers to document a change does not always guarantee a clinical benefit. In the case of HDL, recent large-scale trials that have measured clinical endpoints have cast doubt on the fidelity of a rise in HDL cholesterol as a predictor of clinical benefit (**see Chapter 45**).

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Biomarkers require rigorous validation before adoption into clinical practice. In cardiovascular medicine, LDL cholesterol has high reliability as a biomarker; it satisfies the modified Koch postulates. LDL levels prospectively predict cardiovascular risk, and drops in LDL generally correlate with improved outcomes. Not all biomarkers, though, have proved as faithful in predicting clinical events. In the 1960s and 1970s, for example, most of the cardiovascular community considered ventricular premature depolarizations on the electrocardiogram as important biomarkers for lethal arrhythmias. Numerous strategies have been aimed at suppressing ventricular ectopy. CAST (Cardiac Arrhythmia Suppression Trial), however, showed that drugs capable of suppressing ventricular premature depolarizations actually worsened clinical endpoints. The short-term improvements in indices of cardiac contractility produced by inotropic agents similarly led to worsened clinical outcomes, including increased mortality. These examples illustrate the necessity of rigorous validation of biomarkers before adoption into clinical practice.

Another important consideration in the use of cardiovascular biomarkers involves the question of causality. LDL cholesterol exemplifies a causal biomarker, one that clearly participates in the pathogenesis of atherosclerosis. Its levels prospectively correlate with risk for cardiovascular events and the development of atherosclerotic lesions identified by a variety of imaging modalities. A variety of independent manipulations of LDL levels correlate with clinical outcomes. Finally, very strong genetic evidence based on mendelian disorders (e.g., familial hypercholesterolemia) and unbiased genome-wide association scans, as well as mendelian randomization analyses, has established LDL cholesterol as a causal risk factor in atherosclerotic cardiovascular disease and as a generally valid surrogate endpoint offering great value in clinical practice (**see Chapter 45**).[4,5] For a biomarker that has a causal role, the expected random population distribution of a polymorphism that determines high or low biomarker concentrations would be skewed in individuals, depending on their disease status.

Other biomarkers, although clearly clinically useful, do not participate in the causal pathway for disease. For example, fever has served since antiquity as an important biomarker of infection. Resolution of fever correlates with successful resolution of infectious processes. Yet fever does not participate causally in the pathogenesis of infection but merely serves as a biomarker of the host defenses against the infectious process. Similarly, the use of hsCRP measurements improves the prediction of cardiovascular risk, and reductions in CRP correlate with clinical benefit in many cases. Yet, evidence supporting a causal role for CRP in the pathogenesis of cardiovascular disease lacks strength.[6]

These examples illustrate how a biomarker does not have to reside in the causal pathway of a disease to have clinical usefulness. A clear and early exposition of the uses and pitfalls in the application of biomarkers emerged from the landmark work of Fleming and DeMets (**Fig. 10-2**).[7] Biomarkers have the greatest potential for validity when there is one causal pathway and when the effect of intervention on true clinical outcomes is mediated directly through the biomarker surrogate (Fig. 10-2A). But, biomarker development can fail when the biomarker turns out not to be in the causal pathway, when the biomarker is insensitive to the specific intervention's effect, or when the intervention of interest has a mechanism of action (or a toxicity) that is independent of the pathway described by the biomarker (Fig. 10-2B-E). These examples do not mean that biomarkers lack value. Quite the contrary, few—if any—novel biologic fields could develop without biomarker discovery and validation. Yet surrogate endpoints probably will not replace large-scale randomized trials that address whether interventions reduce actual event rates.

Novel Technologies in the Identification of Biomarkers

The limitations of currently available biomarkers for screening or prognostic use underscore the importance of identifying "uncorrelated" or "orthogonal" biomarkers associated with novel disease pathways. Most current biomarkers have been developed as an extension of targeted physiologic studies investigating known pathways such as tissue injury, inflammation, or hemostasis. By contrast, emerging technologies now enable the systematic, unbiased characterization of variation in proteins and metabolites associated with disease conditions.

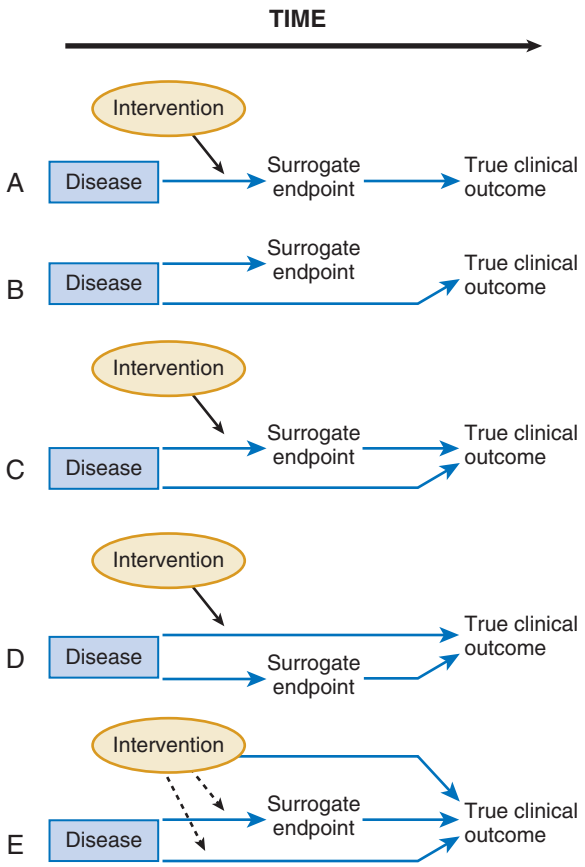

FIGURE 10-2 Biomarkers as surrogate endpoints in clinical research. **A,** The setting that provides the greatest potential for the surrogate endpoint to be valid. **B,** The surrogate is not in the causal pathway of the disease process. **C,** Of several causal pathways of disease, the intervention affects only the pathway mediated through the surrogate. **D,** The surrogate is not in the pathway of the intervention's effect or is insensitive to its effect. **E,** The intervention has mechanisms of action independent of the disease process. *Dotted lines* represent possible mechanisms of action. *(Modified from Fleming TR, DeMets DL: Surrogate end points in clinical trials: Are we being misled? Ann Intern Med 125:605, 1996.)*

INTRODUCTION TO PROTEOMICS AND METABOLOMICS

Of the emerging platforms for biomarker discovery, perhaps none have garnered more recent attention than proteomics and metabolomics. Proteomics aims to catalogue the entire protein products of the human genome. By contrast, metabolomics attempts to systemically capture smaller biochemical compounds, including simple amino acids and related amines, as well as lipids, sugars, nucleotides, and other intermediary metabolites. Although still in their infancy with respect to other approaches, proteomics and metabolomics offer insight into the full complexity of a given disease phenotype (**Fig. 10-3**). Because proteins and metabolites are downstream of genetic variation and transcriptional changes, they provide instantaneous "snapshots" of the state of a cell or organism. They can rapidly change in response to environmental stressors such as exercise or directly by the ingestion of foods or other compounds. A growing body of literature suggests unanticipated roles of small proteins and metabolites in the control of biologic functions such as blood pressure and energy homeostasis.[8,9] Thus metabolomics and proteomics may not

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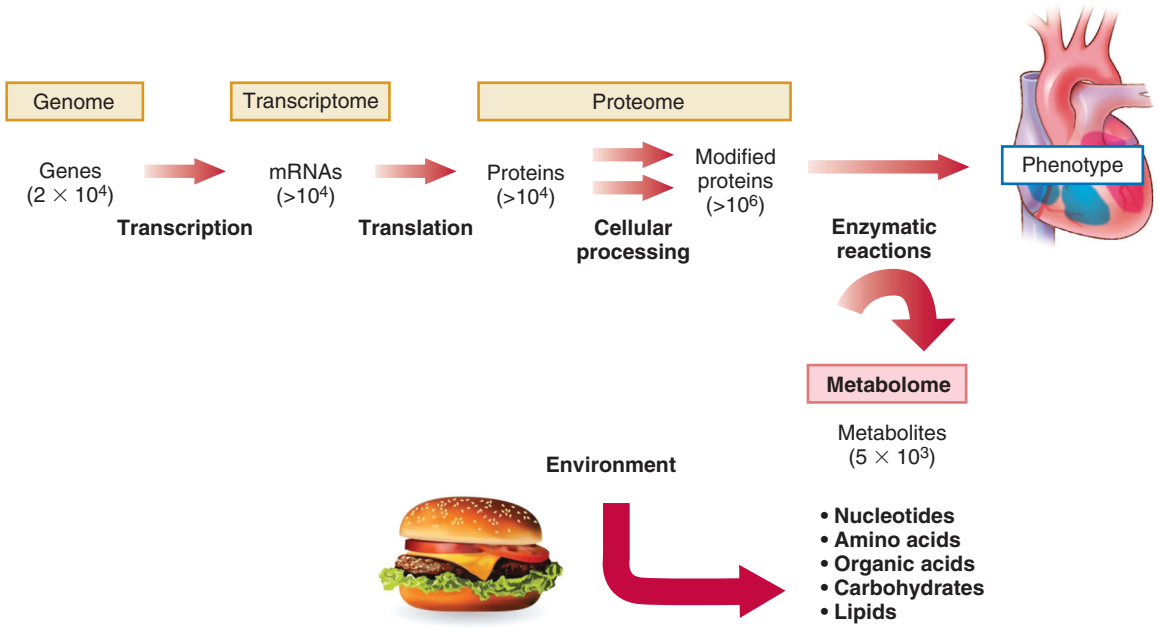

FIGURE 10-3 The conceptual relationship of the genome, transcriptome, proteome, and metabolome. Informational complexity increases from genome to transcriptome to proteome. The estimated number of entities of each type of molecule in humans is indicated in parentheses.

only identify novel biomarkers but also provide information on biology and highlight potential therapeutic targets.

The term *proteome* was coined in the 1990s with the increasing realization that although all cells of a given organism contain an equivalent genomic content, their protein content does not represent all possible proteins that the genome can express. Selective gene expression during development and differentiation and in response to external stimuli results in each cell expressing only a subset of the encoded proteins at any given time. One can speak not only of the general human proteome but also more specifically about the proteome of tissues such as the heart, of specific cells such as cardiac myocytes, and even of subproteomes that correspond to particular organelles or biologic compartments, such as mitochondria.

The proteome provides information beyond the messenger RNA (mRNA) expression profile of a particular genome. Studies suggest that gene expression often correlates poorly with protein levels.[10] Protein expression depends not only on transcription but also on mRNA stability and rates of protein synthesis and degradation, so the presence or absence of mRNA may not accurately reflect levels of the corresponding protein. Following transcription and translation, proteins may undergo one or more of dozens of potential post-translational modifications (such as phosphorylation, glycosylation, acetylation, or sulfation) at multiple sites. Subsequent enzymatic and nonenzymatic alterations greatly expand the number of simultaneously existing protein species.

When compared with proteomics techniques, metabolomics technologies focus on smaller compounds, generally less than 2 kDa in size. Metabolites are usually easily separated from protein constituents by simple extraction techniques and precipitation and removal of the proteins. As early as the 1970s, Arthur Robinson and Linus Pauling postulated that the quantitative and qualitative pattern of metabolites in biologic fluids reflected the functional status of the complex biologic system from which they were derived.[11] The term "metabolic profiling" was introduced to describe data obtained from gas chromatographic analysis of a patient sample.[12] This emerging approach to quantitative metabolic profiling of large numbers of small molecules in biofluids was ultimately termed "metabonomics" by Nicholson and colleagues[13] and "metabolomics" by others. Recently, more focused analyses of specific metabolite families or subsets have given rise to new terms such as "lipidomics." In terms of applications to human diagnostics, seminal studies of inborn errors of metabolism in infants have served as a key springboard. Millington and colleagues pioneered the use of mass spectrometry (MS)-based methods for monitoring fatty acid oxidation, as well as organic and selected amino acids. Their work has culminated in neonatal screening for metabolic disorders,[14] thereby enabling the identification of infants with fatty acid oxidation disorders, organic acidemias, and aminoacidopathies. In certain situations, rapid identification of these disorders triggers intervention in the form of dietary modulation, with beneficial therapeutic effects. A global metabolomic or proteomic analysis of more common complex diseases might similarly spotlight pathways for dietary or drug modulation.

Analytic Challenges for Proteomics and Metabolomics

The many classes of proteins and chemicals present analytic challenges, particularly as applied to searching for biomarkers in blood. Many different types of cells contribute to the plasma proteome and metabolome, thus increasing their complexities and presenting challenges to interpretation of the data that emerge. In the case of the proteome, the 22 most abundant proteins, including albumin and the immunoglobulins, account for 99% of the total proteome mass (**Fig. 10-4**). Many of the biologically interesting molecules relevant to human disease occur in low abundance. Cardiac markers such as troponin circulate in the nanomolar range, insulin in the picomolar range, and tumor necrosis factor in the femtomolar range. Plasma contains tens of thousands of unique protein species in concentrations spanning a range of more than 10 orders of magnitude. Indeed, some suggest that the plasma proteome might encompass the entire set of human polypeptide species resulting from splice variants and post-translational modifications[15] because the protein content of plasma unexpectedly includes proteins of all functional classes and from apparently all cellular localizations. Most low-abundance proteins in plasma are intracellular or membrane proteins that are present in plasma as a result of cellular turnover.[16] By contrast, recent estimates suggest that the human metabolome may include approximately 5000 small molecules[17] and thus may be somewhat more tractable to analyze and systematize than the human proteome.

Several features contribute critically to the success of proteomic or metabolomic technologies. First, the technique must have the

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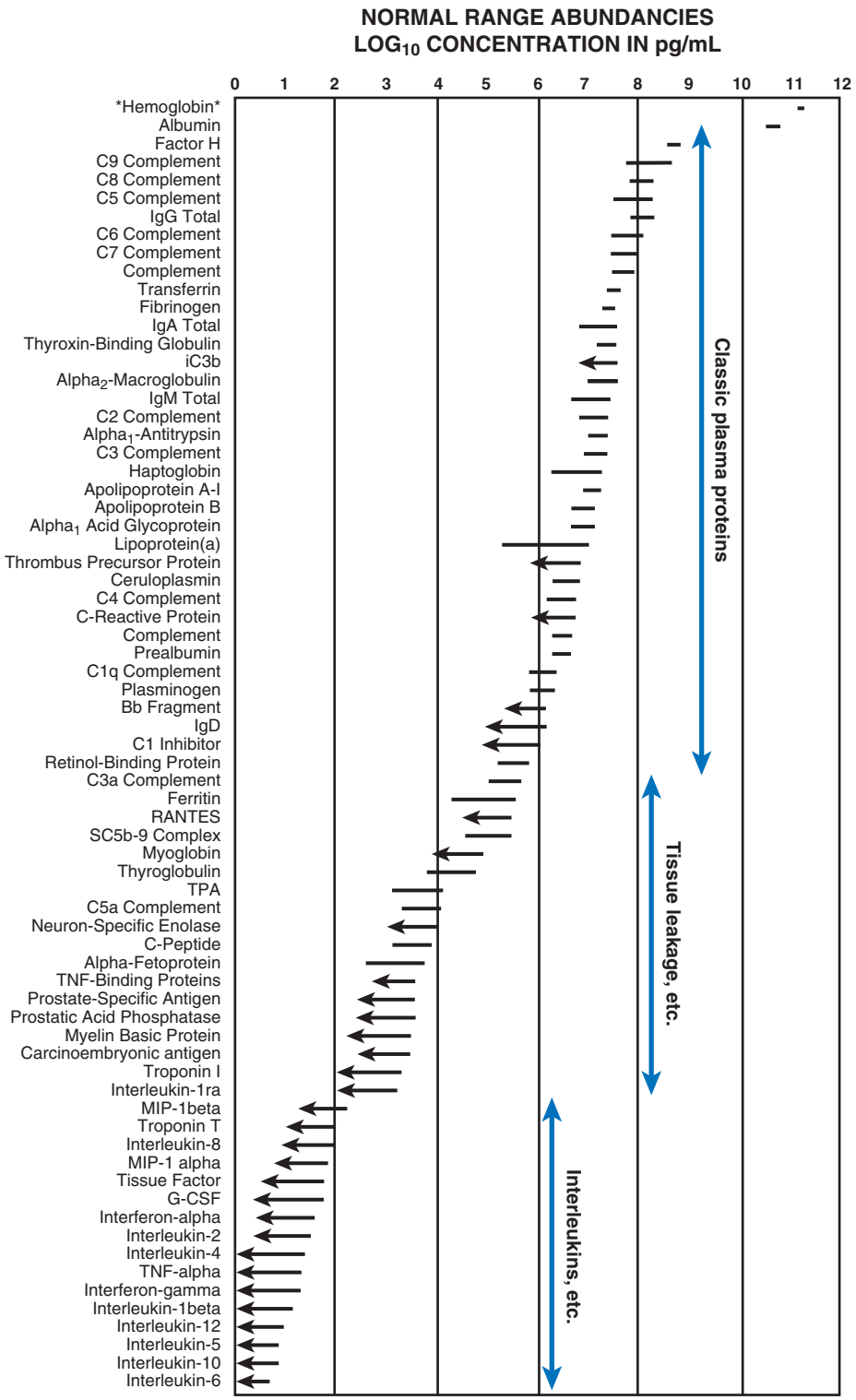

FIGURE 10-4 Reference concentration for representative protein analytes in plasma. Protein abundance is plotted on a log scale spanning 12 orders of magnitude. When only an upper limit is quoted, the lower end of the interval line shows an *arrowhead*. The classic plasma proteins are clustered to the left (high abundance), the tissue leakage markers (e.g., enzymes and troponins) are clustered in the center, and the cytokines are clustered to the right (low abundance). TPA = tissue plasminogen activator; G-CSF = granulocyte colony-stimulating factor; MIP = macrophage inflammatory protein; RANTES = regulated on activation, T cell expressed and secreted; TNF = tumor necrosis factor; TPA = tissue plasminogen activator. *(From Anderson NL, Anderson NG: The human plasma proteome: History, character, and diagnostic prospects. Mol Cell Proteomics 2:50, 2003.)*

capability of identifying a wide breadth of proteins or metabolite analytes within complex biologic samples across a broad range of physical characteristics, including size and charge. Second, the technologies must be sensitive enough to probe the proteome or metabolome to adequate "depths"—that is, to provide resolution of biologically active compounds of the lowest abundance. Frequently, the least abundant entities play critical regulatory roles in the response to physiologic stressors. Third, tools must also work across a broad dynamic range, a notion underscored in Figure 10-4—they must be able to simultaneously identify both more abundant and less abundant proteins in the same complex mixture. Unfortunately, most analytic techniques apply well only across concentrations of several orders of magnitude. Finally, the ideal technology should be stable and reproducible, an attribute necessary for minimizing artifacts during initial discovery, validation, and testing for clinical applications.

Robust, searchable databases for validation of identified proteins or metabolites represent an increasingly crucial support for biomarker discovery. The scope of investigation addressable by these techniques has widened immeasurably since completion of the Human Genome Project. At present, the human databases are the largest and easiest to use, which will help accelerate translational investigation. Genomic databases collectively provide a catalog of all known or theoretical proteins expressed in organisms for which databases exist. Software that can search through databases for identification of candidates has proved essential to interpretation of the data; much of this software is available on the Internet. Collaborative efforts have recently begun to catalog both the human proteome and the plasma metabolome.

OVERVIEW OF THE DISCOVERY PROCESS

Figure 10-5 summarizes the essential elements of the discovery approach by using a proteomics experiment as an example. Biologic samples consist of a complex mixture containing intact and partially degraded proteins and metabolites of various molecular weights, modifications, and solubility. The chance of identifying proteins or metabolites in a mixture increases as the complexity of the mixture decreases. As suggested by Liebler,[18] the problem of complexity and how to deal with it resembles the process of printing a book. Printing all the words on a single page could be accomplished quickly, but the resulting page would be illegibly black with ink; dividing the text into multiple pages reduces the complexity to reveal organized text. Samples can be analogously enriched for certain components through fractionation or affinity depletion columns, but all preparative procedures—including solubilization, denaturation, and reduction processes—should be compatible with the constraints of subsequent analysis steps. The quest to reduce complexity requires careful balance against the possibility that each additional step might also introduce undesired protein or metabolite modifications or loss.

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Several analytic techniques can serve to identify metabolites or proteins, although MS instrumentation offers an unrivaled ability to provide several layers of complementary information, which has benefited tremendously from whole-genome analysis and the genomics revolution. MS provides accurate mass detection of peptides from proteolytic digests of complex protein mixtures or small metabolites derived from tissues or blood. The set of peptide or metabolite mass measurements can be searched in databases to obtain definitive identification of the parent proteins or metabolites of interest. Favorably compared against other proteomics and metabolomics technologies, MS offers high sensitivity and amenability to automation, thus promoting high-throughput processing. MS has a wide range of applicability and not only detects metabolites and proteins but also characterizes any post-translational modifications.

Mass spectrometers are composed of modular elements, including an ion source, mass analyzer, and a detector/recorder (**Fig. 10-6**). MS instruments are classified according to the ionization source and mass analyzer used, but all process samples as gas-phase ions, the movements of which are precisely measured within an electromagnetic field. An ion source generates these gas-phase ions from the analyte through a variety of available techniques, from either the solid state by matrix-assisted laser desorption/ionization (MALDI) or directly from the liquid phase by electrospray ionization (ESI). A coupled chromatographic separation step fractionates complex sample mixtures before ESI spectroscopic analysis. The gas-phase ions then enter the mass analyzer, which resolves the peptides based on their mass-to-charge (m/z) ratio. Examples of commonly used mass analyzers include the quadrupole mass filter, ion trap mass analyzer, and time-of-flight mass analyzer. Finally, the detector records the ions via an electronic multiplier and records ion intensity versus the m/z value to create the resulting MS spectra.

These technologies can be used to characterize biologic fluids either in a targeted manner or in a pattern discovery manner. In the former, the investigator targets a predefined set of analytes to be quantitated. For example, libraries of metabolites can be purchased and their chromatographic and MS characteristics determined empirically by "spiking" reference standards into plasma. Endogenous metabolites can then be quantified based on the information ascertained from the known standards. The targeted approach now readily permits assay of several hundred metabolites in as little as tens of microliters of plasma. In the pattern discovery experiment, by contrast, the investigator confronts a complex pattern of peaks, many of which are anonymous—the molecular identities of the species that give rise to the peaks are not generally known. Although the targeted approach is more limiting, the analysis is more straightforward because the analytes yielding the signals are already known. The untargeted or "fingerprint" approach has less inherent bias, but unambiguous identification of the peaks can prove laborious and difficult. In clinical samples, considerable care must be taken to rule out spurious associations—for example, confounding related to drug treatment.

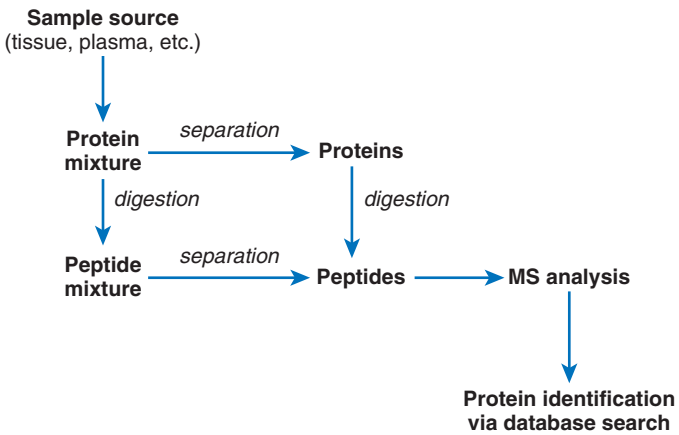

FIGURE 10-5 Overview of a proteomics experiment.

Applications of Mass Spectrometry–Based Discovery to Cardiometabolic Disease

In an initial proof-of-principle study using a targeted metabolite profiling approach, Newgard and colleagues profiled obese versus lean humans to gain a broad understanding of the metabolic and physiologic differences in these two disparate groups.[19] Their studies identified a branched-chain amino acid signature that correlated highly with the metrics of insulin resistance. Complementary studies in two large population-based cohorts demonstrated that branched-chain and aromatic amino acid concentrations associate significantly with incident type 2 diabetes up to 12 years before the onset of overt disease.[20] Adjustment for established clinical risk factors did not substantially attenuate the strength of these associations. Furthermore, the branched-chain amino acid signature also predicts atherosclerosis even after adjusting for the metrics of insulin resistance and diabetes.[21] For those in the top quartile of branched-chain amino acid levels, the odds for development of cardiometabolic disease exceeded any single-nucleotide polymorphism identified to date. Taken together, these findings have disclosed dysregulation of amino acid metabolism very early in the development of cardiometabolic diseases. Ongoing studies are examining the relative genetic versus environmental contributions to these findings.

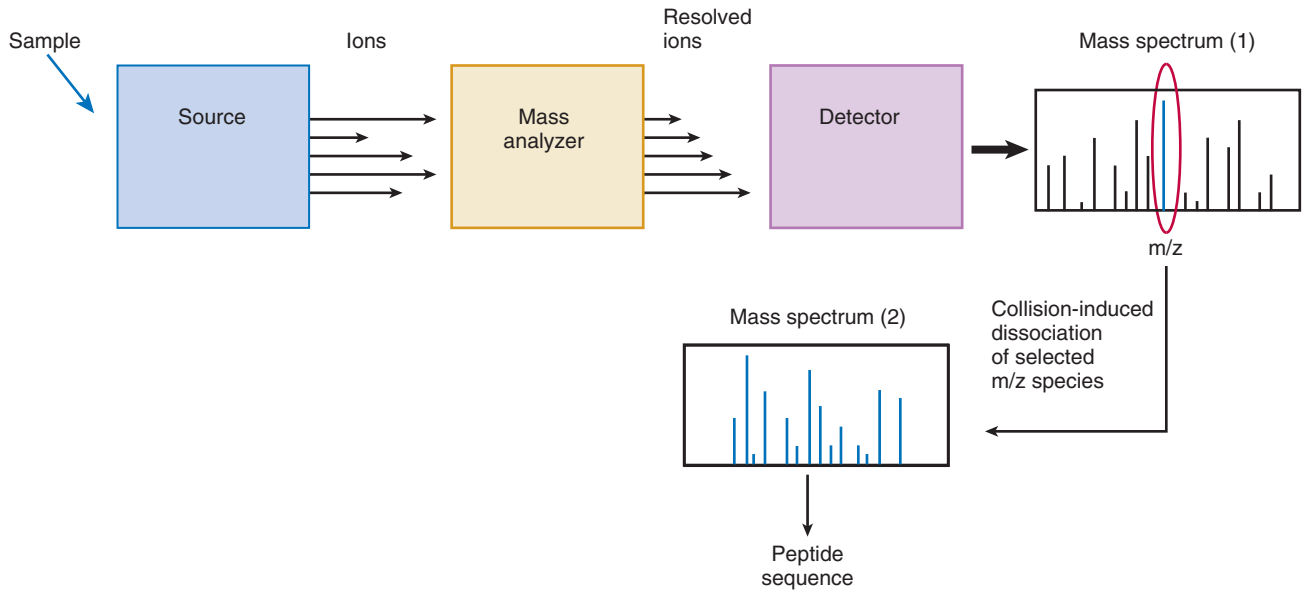

FIGURE 10-6 Schematic of tandem MS. m/z = mass-to-charge ratio.

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In a translational study using nontargeted liquid chromatography–MS–based metabolite profiling applied to cardiovascular disease, Wang and associates first profiled the plasma of 75 individuals from a hospital-based cohort who experienced a myocardial infarction, stroke, or death in the ensuing 3 years and 75 age- and sex-matched controls who did not.[20] Of 18 analytes that differed significantly between cases and controls, 3 demonstrated significant correlations among one another, thus suggesting a potential common biochemical pathway. Using complementary analytic methods, these metabolites were identified as betaine, choline, and trimethylamine-*N*-oxide, all metabolites of dietary phosphatidylcholine. Dietary supplementation of choline was sufficient to promote atherosclerosis in mice, and suppression of the intestinal bacteria responsible for the conversion of phosphatidylcholine to choline inhibited this atherogenesis. In addition to reinforcing the interaction between diet, gut bacteria, and the metabolome, this study demonstrated how metabolomic biomarker discovery can elucidate novel pathways to disease.

Future Directions in Biomarker Discovery

Identification of new biomarkers for cardiovascular disease depends on the complementary power of genetics, transcriptional profiling, proteomics, and metabolomics. As discussed in the next section, the clinical usefulness of new biomarkers will require rigorous evaluation of their ability to improve the prediction of risk or to direct and monitor management in an individual, the ultimate goal of personalized medicine. In addition to risk biomarkers, diagnostic biomarkers could help in making challenging acute diagnoses such as reversible myocardial ischemia, pulmonary embolism, and aortic dissection. The evolution of a clinical biomarker requires a long journey and an arduous transition from the research environment to clinical practice. Emerging technologies such as those described above have the potential to permit systematic assessment of variation in genes, RNA, proteins, and metabolites for identification of "uncorrelated" or "orthogonal" biomarkers that probably would not emerge with a focus on candidates from well-studied pathways.

CLINICAL MEASURES OF BIOMARKER PERFORMANCE

When considering any biomarker in a clinical setting for risk prediction, physicians should ask two interrelated questions. First, is there clear evidence that the biomarker of interest predicts future cardiovascular events independent of other already measured biomarkers? Second, is there clear evidence that those identified by the biomarker of interest will benefit from a therapy that they otherwise would not have received?[22] If the answer to both these questions is not a clear "yes," an argument can be made that measuring the biomarker will not probably have sufficient usefulness to justify its cost or unintended consequences. Such judgments require clinical expertise and will vary on a case-by-case basis.

Biomarker evaluation also typically involves repeated testing in multiple settings that include varied patient populations and that use different epidemiologic designs. Prospective cohort studies (in which the biomarker or exposure of interest is measured at baseline, when individuals are healthy, and then related to the future development of disease) provide a much stronger form of epidemiologic evidence than do data from retrospective case-control studies (in which the biomarker of interest is measured after the disease is present in the case subjects).

After discovery by the technologies described above or identification by a candidate approach, a novel biomarker typically requires development in a translational laboratory for refinement of its assay to address issues of interassay and intra-assay variation before any clinical testing begins. Focused studies in specific patient populations typically follow and eventually broaden to encompass the population of greatest clinical interest. Beyond simple reproducibility, biomarkers under development for diagnostic, screening, or predictive purposes require further evaluation with a standard set of performance measures that include sensitivity, specificity, positive and negative predictive value, discrimination, calibration, reclassification, and tests for external validity. These terms and their use in clinical biomarker development are outlined below.

TABLE 10-2 Summarizing the Results of Screening, Diagnostic, or Predictive Tests

	DISEASE PRESENT	DISEASE ABSENT	
Test positive	a	b	a + b
Test negative	c	d	c + d
Total	a + c	b + d	
Sensitivity = a/(a + c)			
Specificity = d/(b + d)			
Positive predictive value = a (a + b)			
Negative predictive value = d/(c + d)			

a = number of individuals for whom the screening test is positive and the individual actually has the disease (true positives); b = number of individuals for whom the test is positive but the individual does not have the disease (false positives); c = number of individuals for whom the test is negative but the individual actually has the disease (false negatives); d = number of individuals for whom the test is negative and the individual does not have the disease (true negatives).

Sensitivity, Specificity, and Positive and Negative Predictive Value

The validity of a screening or diagnostic test (or one used for prediction) is initially measured by its ability to correctly categorize individuals who have preclinical disease as "test positive" and those without preclinical disease as "test negative."[23] A simple two-by-two table is commonly used to summarize the results of a screening test by dividing those screened into four distinct groups (**Table 10-2**). In this context, sensitivity and specificity provide fundamental measures of the test's clinical validity. Sensitivity is the probability of testing positive when the disease is truly present and is defined mathematically as a/(a + c); as sensitivity increases, the number of individuals with disease who are missed by the test decreases, so a test with perfect sensitivity will detect all individuals with disease correctly. In practice, tests with ever-higher sensitivity tend to also classify as "diseased" many individuals who are not actually affected (false positives). Thus the specificity of a test is the probability of screening negative if the disease is truly absent and is defined mathematically as d/(b + d). A test with high specificity will rarely be positive when disease is absent and will therefore lead to a lower proportion of individuals without disease being incorrectly classified as test positive (false positives). A simple way to remember these differences is that sensitivity is "positive in disease" whereas specificity is "negative in health."

A perfect test has both very high sensitivity and specificity and thus low false-positive and false-negative classifications. Such test characteristics are rare, however, because there is a tradeoff between sensitivity and specificity for almost every screening biomarker, diagnostic, or predictive test in common clinical use. For example, although high LDL cholesterol levels commonly serve as a biomarker for atherosclerotic risk, up to half of all incident cardiovascular events occur in those with LDL cholesterol levels well within the normal range, and many events occur even when LDL cholesterol levels are low. If the diagnostic cutoff criterion for LDL cholesterol is reduced so that more people who actually have high risk for disease will be test positive (i.e., increase sensitivity), an immediate consequence of this change will be an increase in the number of people without disease in whom the diagnosis is made incorrectly (i.e., reduced specificity). Conversely, if the criterion for diagnosis or prediction is made more stringent, a greater proportion of those who test negative will actually not have the disease (i.e., improved specificity), but a larger proportion of true cases will be missed (i.e., reduced sensitivity).

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In addition to sensitivity and specificity, the performance or yield of a screening, diagnostic, or predictive test also varies depending on the characteristics of the population being evaluated. Positive and negative predictive values are terms used in epidemiology that refer to measurement of whether an individual actually has (or does not have) a disease, contingent on the result of the screening test itself.

The positive predictive value (PPV) is the probability that a person has the disease of interest, given that the individual tests positive, and is mathematically calculated as PPV = a/(a + b). High PPV can be anticipated when the disease is common in the population being tested. Conversely, the negative predictive value (NPV) is the probability that an individual is truly disease free, provided that the test has a negative result, and is mathematically calculated as NPV = d/(c + d). High NPV can be anticipated when the disease is rare in the population being tested. Although sensitivity and specificity are largely performance characteristics of the test itself (and thus tend to be fixed values), PPV and NPV depend in part on the population being tested (and thus tend to vary).[23]

Discrimination, C-Statistics, and the Receiver Operative Characteristic Curve

Discrimination is the ability of a test (or prognostic model) to separate those with disease or at high risk for disease (cases) from those without disease or at low risk for disease (controls). The most common method used to measure discrimination has been the area under the receiver operating characteristic (ROC) curve, which relates sensitivity (on the *y* axis) to (1 – specificity) (on the *x* axis) across a full range of cutoff values for the test or screening algorithm of interest (**Fig. 10-7**).

Given a population of individuals being evaluated, the area under the ROC curve—also called the C-statistic—equals the probability of correctly ranking risk for individuals by using the test or model under evaluation. A random test with no clinical usefulness would have a C-statistic (or area under the ROC curve) of 0.5, which corresponds to the diagonal line in Figure 10-7. A perfect test that completely discriminates individuals with disease from those without disease would have a C-statistic that approaches 1.0. As the C-statistic increases from 0.5 to 1.0, model fit (or test accuracy) improves—thus the change in the C-statistic has been used historically to judge whether a new biomarker can "add" significantly to those already in use. This approach permits direct comparison of the relative efficiency of multimarker panels. For example, using comparative C-statistic analyses, investigators in the Emerging Risk Factors Collaboration recently found that the incremental clinical usefulness of CRP has similar magnitude as that of total and HDL cholesterol.[24] Thus when change in the C-statistic can be demonstrated and the overall power to do so is adequate, this test can aid understanding of the impact that novel pathways and novel risk biomarkers have on prediction and prevention.

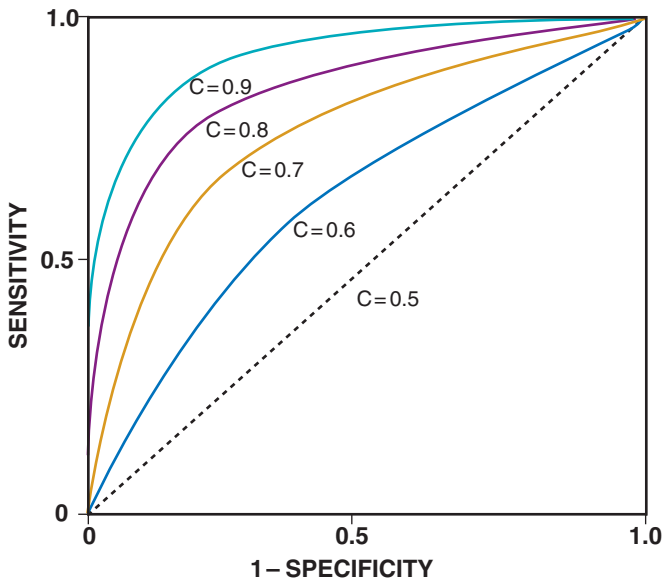

FIGURE 10-7 ROC curves for a series of biomarkers or risk prediction models with incremental improvement. The diagonal line corresponds to a random effect (C-statistic = 0.5), whereas the increasing C-statistic corresponds to improving model discrimination.

Unfortunately, as Cook has shown in several settings,[25,26] the traditional C-statistic approach is limited in that biomarkers with large associations may have little effect on the area under the ROC curve. For example, a predictor (or set of predictors) would need an odds ratio as high as 16 (>2 SD) to lead to a substantial increase in the C-statistic.[27] Almost no test in common use for risk prediction or prognostication in cardiovascular medicine has an odds ratio in this range; high cholesterol, smoking, high blood pressure, and diabetes are all associated with odds ratios of less than 2 and thus have little individual impact at all on the area under the ROC curve. Consequently, sole reliance on the C-statistic as a method for developing and evaluating new biomarkers, at least in the setting of risk prediction, is insufficient.

Accuracy and Calibration

Discrimination is only one measure of model accuracy. The other important measure is calibration, or the ability of a predictive model to assign risk estimates accurately in comparison to the actual observed risk in the population being tested. Unlike discrimination, which is based solely on relative rankings of risk, calibration compares the risk predicted from a model or test with that actually observed.

For binary outcomes (such as disease or no disease), calibration is often evaluated with the Hosmer-Lemeshow test, which places individuals within categories of estimated risk by using the test biomarker or multivariable model and compares these estimates with the proportions actually observed. These "predicted" and "observed" probabilities can be compared with standard goodness-of-fit tests across categories of risk (e.g., across estimated quintiles or estimated deciles of risk). Calibration becomes particularly important when addressing a biomarker in different populations from the one in which it was originally developed. A biomarker may calibrate well in men but not in women or among whites but not among blacks. This consideration also applies to multimarker panels—such as the Framingham Risk Score, which calibrates well in whites but less well in other population groups. Newer risk models such as the Reynolds Risk Score (www.reynoldsriskscore.org) show improved calibration, as well as discrimination, when compared with the traditional Framingham model.[28]

Risk Reclassification

To address the shortcoming of biomarker validation via the C-statistic alone, contemporary biomarker development programs for risk prediction now use a series of "reclassification statistics," as initially developed by Cook and colleagues[29,30] and refined by Pencina and associates.[31] Rather than addressing whether a new biomarker of interest adds to the area under the ROC curve, reclassification addresses whether the biomarker can shift overall risk estimates upward or downward in a clinically meaningful way. Specifically, reclassification methods compare risk strata formed from prediction models with and without the new biomarker of interest and then determine which model leads to the most accurate classification of risk. Risk reclassification is particularly useful when actionable and clinically relevant risk categories already exist. For example, in primary cardiovascular prevention, 10-year estimated risk is often categorized as being less than 5%, 5% to 10%, 10% to 20%, or greater than 20%, and those above or below these cut points are frequently targeted for interventions such as aspirin and statin therapy. Thus a biomarker that reclassifies a proportion of individuals upward (or

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downward) might well be highly effective for targeting (or avoiding) drug therapy, even if the overall effect on discrimination is modest.

Mere reclassification of an individual by a given biomarker does not provide sufficient evidence to support clinical use. Rather, an effective biomarker should correctly reclassify risk higher or lower and thus lead to more accurate overall risk assessment. The reclassification calibration (RC) statistic is a tool that tests how well the average predicted risk within a given cell agrees with the observed risk of individuals who actually experience the event. Accordingly, the RC statistic addresses whether the predicted risk estimates after reclassification (using the new biomarker) are more accurate than before reclassification (without the new biomarker). Superior reclassification occurs when the new prediction model places case individuals into higher-risk categories and places control individuals into lower-risk categories and when the net shift in these two effects is in the overall correct direction. This characteristic can be addressed by using the net reclassification index (NRI), analogous to a test of discrimination (the ability to separate cases from controls) in the context of a reclassification table.[31] Broadly, the NRI does not depend as much on the actual predicted probabilities as on movement across a categorical risk border that is the result of the new probabilities predicted. When reclassification is not addressed across categories, an alternative measure called the integrated discrimination improvement (IDI) is used; the IDI is based on the Yates slope, or the difference in predicted probabilities among case and control individuals.[32]

Despite their relatively recent introduction, reclassification statistics have rapidly become the standard for clinical evaluation of emerging biomarkers and alternative multibiomarker prediction panels.

External Validation and Impact Studies

External validation is a final but important test for any biomarker or biomarker panel when used for prognostication. External validation refers to the ability of the panel to function with clinically acceptable levels of sensitivity, specificity, discrimination, and calibration in external populations, distinct from the population used for generation of the panel. As Moons and coworkers pointed out, prognosis research and prognostic biomarkers differ from those used in diagnosis and screening.[33] Prognostic research involves three distinct phases in the development of multivariable prediction models. The first phase includes identification of relevant predictors, assignment of weights to the model, estimation of predictive performance, and optimization of fit. The second phase involves validation or formal testing of calibration and discrimination in new patient groups, which can be similar to those used in the development stage or purposely different. Finally, the third phase involves impact studies to quantify directly whether use of a prognostic model in daily practice actually changes physician behavior and decision making and whether this occurs in a net positive manner and is cost-effective. Prognostic impact studies also focus on the incremental usefulness of a given biomarker beyond simple clinical and nonclinical characteristics. Such studies tend to be less biologically driven than biomarker discovery work is and recognize that prediction does not necessarily involve a causal pathway.

A Practical Example: High-Sensitivity C-Reactive Protein, Lipids, and the Reynolds Risk Score

The use of hsCRP in clinical practice is an example of how biomarker development programs can move from pathophysiologic principles to clinical use and onward to multinational trials evaluating novel targets for vascular risk reduction. In 1997, hsCRP was shown in a prospective cohort of initially healthy individuals to predict future risk for a heart attack and stroke in men,[34] an observation externally validated and quickly extended to women.[35] Assay systems underwent rapid improvement such that by 2004, multiple commercial hsCRP assays—reproducible, internally calibrated, and externally validated to improve assay precision—were clinically available. Multiple studies have shown that statins reduce hsCRP in a manner largely independent of reduction of LDL cholesterol,[36] thus suggesting that statins have both lipid-lowering and anti-inflammatory effects.[37] In 2006, Cook and coauthors reported the ability of hsCRP to correctly reclassify patients into improved vascular risk categories.[29] The addition of hsCRP to the family history and HbA1c was formally incorporated into the Reynolds Risk Score in 2008. This score was subsequently externally validated and shown to have superior calibration, discrimination, and reclassification over the more traditional Framingham Risk Score.[28] Using hsCRP to define a high-risk population in need of treatment, JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) reported in 2008 that statin therapy (versus placebo) in those with elevated hsCRP but low levels of LDL cholesterol resulted in a 50% reduction in myocardial infarction and stroke and a 20% reduction in all-cause mortality.[38] By 2010, more than 50 prospective cohort studies evaluating hsCRP were subjected to a meta-analysis in which it was affirmed that the magnitude of vascular risk associated with a change of 1 SD in hsCRP was at least as large as that of a comparable change in cholesterol or blood pressure.[39] In an updated 2012 meta-analysis that evaluated clinical usefulness and risk prediction, found the change in C-statistic associated with hsCRP to be similar to the change in C-statistic associated with the use of total and HDL cholesterol.[24] On this basis, several national guidelines incorporated hsCRP screening in primary and secondary prevention,[40] and the FDA approved a labeling claim for the use of statin therapy in those with elevated hsCRP levels.

CRP itself, however, probably does not cause atherothrombosis but rather serves as a biomarker for the underlying inflammatory process. Thus as a direct outcome of the hsCRP development program, two randomized trials have been initiated to directly test whether lowering inflammation per se can reduce vascular risk. These two trials—the NIH-funded CIRT (Cardiovascular Inflammation Reduction Trial), which evaluated low-dose methotrexate, and CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study), which evaluated interleukin-1beta inhibition—are ongoing and, when complete, will have involved more than 18,000 patients worldwide.[41]

CONCLUSION

We use biomarkers in our daily clinical practice, and cardiovascular journals contain numerous reports regarding biomarkers, new and old, that purport to show how they may aid clinical practice. Moreover, many cardiovascular trials use biomarkers—hence the current practice of cardiovascular medicine requires a firm foundation in understanding and evaluating biomarkers. The road map to the field of biomarkers provided in this chapter—including their use, development, and methods for evaluating their usefulness for various specific applications—should give practitioners tools to sort out the various uses of biomarkers encountered in practice and in the cardiovascular literature.

Informed use of biomarkers can aid in decision making in daily patient care. Biomarkers should provide a key for personalized management by directing the right therapy to the right patient at the right time. They can also shed mechanistic insight on human pathophysiology that is difficult to obtain in other ways. Rigorous and careful use of biomarkers can aid in the development of novel therapies to address the residual burden of cardiovascular risk.

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Evaluation of the Clinical Performance of Biomarkers

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