Predict, prevent and personalize: Genomic and proteomic approaches to cardiovascular medicine

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Genomic and proteomic approaches to cardiovascular medicine promise to revolutionize our understanding of disease initiation and progression. This improved appreciation of pathophysiology may be translated into avenues of clinical utility. Gene-based presymptomatic prediction of illness, finer diagnostic subclassifications and improved risk assessment tools will permit earlier and more targeted intervention. Pharmacogenetics will guide our therapeutic decisions and monitor response to therapy. Personalized medicine will require the integration of clinical information, stable and dynamic genomics, and molecular phenotyping. Bioinformatics will be crucial in translating these data into useful applications, leading to improved diagnosis, prediction, prognostication and treatment. The present paper reviews the potential contributions of genomic and proteomic approaches in developing a more personalized approach to cardiovascular medicine.

Key Words: *Biomarkers; Genes; Personalized medicine; Proteins*

Prédire, prévenir et personnaliser : Les approches génomiques et protéomiques de la médecine cardiovasculaire

Les approches génomiques et protéomiques de la médecine cardiovasculaire promettent de révolutionner la compréhension de l'apparition et de l'évolution de la maladie. Cette meilleure appréciation de la physiopathologie peut se traduire par des solutions d'utilité clinique. La prévision présymptomatique des maladies d'après les gènes, une sous-classification diagnostique plus précise et des outils d'évaluation du risque améliorés permettront d'effectuer des interventions plus précoces et plus ciblées. La pharmacogénétique orientera les décisions thérapeutiques et la surveillance des réponses aux thérapies. La médecin personnalisée exigera l'intégration d'information clinique, de génomique stable et dynamique et du phénotypage moléculaire. La bioinformatique sera essentielle pour traduire ces données en applications utiles, afin de parvenir à une amélioration du diagnostic, de la prédiction, de la pronostication et du traitement. Le présent article contient l'analyse des apports potentiels des approches génomiques et protéomiques pour mettre au point une démarche plus personnalisée de la médecine cardiovasculaire.

Human cardiovascular disease is a consequence of a complex interplay of genetic, epigenetic and environmental factors. The resulting phenotype has traditionally been described by a variety of clinical descriptors, as well as biochemical markers and imaging techniques. To date, conventional cardiology attempts to prevent disease by modifying established risk factors and to treat manifest disease, often after complications have occurred and irreversible tissue changes have taken place. Current therapies (eg, those for heart failure) are administered uniformly across a heterogeneous spectrum of disease etiology, severity and genetic background. As large-scale investigation platforms become more mainstream, we have the opportunity to expand our medical armamentarium to include molecular phenotypes that distinguish subtle subclassifications of disease and allow us to better tailor both prevention strategies and therapeutics. The present paper reviews the potential contributions of genomic and proteomic approaches in developing a more personalized approach to cardiovascular medicine.

PANOMIC APPROACHES TO BIOLOGY

The emergence of high-throughput molecular technologies has spurred a paradigm shift from a reductionist, single-molecule, biological approach to one that integrates vast networks of information into fluid, dynamic systems. 'Omics' encompasses comprehensive methodologies that attempt to capture the exhaustive output of an organism's genes (genomics), RNA (transcriptomics), metabolites (metabolomics) and proteins (proteomics). The whole is often greater than the sum of its parts, and by embracing the complexity of various profiling patterns, far more subtle information may be extracted than would have been otherwise available from single targets (1,2). The systems biology approach allows the study of networks of interactions, in addition to dissecting individual molecular components. This approach has a long history (3), but has only recently received renewed attention given the increasing availability of pathway data and advances in computational analyses (4).

Complementary to this global, nonhypothesis-driven methodology is the candidate gene approach, which evaluates genes and proteins based on a prior understanding of their roles in disease. Understanding a system's structure and dynamics has proven to be a multidisciplinary venture, requiring expertise in the fields of biology, engineering, chemistry and informatics. Its ultimate impact on cardiovascular medicine remains to be determined, but will likely be far-reaching, leading to innovations in many aspects of patient care. The present paper introduces the reader to the fundamentals of proteomics,

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and refers the reader to comprehensive reviews detailing genomic and transcriptomic methodologies (5,6).

PROTEOMICS: GENERAL CONSIDERATIONS

Proteomics is the study of the proteome, or the entire protein complement of a genome. The proteome consists of all proteins present in a cell or tissue at a given time and is far more complex than was originally proposed by the one-gene, one-transcript, one-protein hypothesis. On average, there are five to seven protein isoforms per open reading frame in the human genome. To date, it is apparent that the approximately 30,000 human genes encode for nearly one million proteins (7). Alternative splicing is a significant contributor to protein diversity, occurring in 35% to 60% of our genes (8). This is complemented by other mechanisms, such as the use of multiple transcription start sites, polyadenylation and specific editing of pre-messenger RNA. The diversity is further compounded by the post-translational modification of proteins, such as phosphorylation, sulphation, glycosylation, hydroxylation, N-methylation, carboxymethylation, acetylation, prenylation and N-myristylation.

Proteins are the effectors of nearly every cellular function and, as such, dictate the phenotype of a given cell, tissue or organ, representing not only the genetic composition of an individual, but gene-gene and gene-environment interactions. The proteome varies under all physiological conditions, with pronounced changes observed in aging, as well as acute or chronic stressors leading to disease states. Chronic illnesses often result in altered protein levels due to specific gene up- or downregulation, isoform switching or de novo protein synthesis. There is often insufficient time during an acute illness to perturb the synthetic machinery of proteins, and the most common mechanisms of protein alteration are, therefore, post-translational modifications (9).

Numerous techniques exist for the analysis of the proteome (10,11). The development of two-dimensional polyacrylamide gel electrophoresis (2-DE) more than 20 years ago permitted the simultaneous separation of large numbers of proteins first by charge (isoelectric point) and then by molecular mass. Difficulties arise with 2-DE when separating low-abundance or membrane-associated proteins, or those at the extremes of pH or molecular weight. A complementary approach uses isotope-coded affinity tags, which selectively label cysteine residues of peptide fragments following tryptic digest of the protein sample. This increases the sensitivity of the detection of smaller peptides and permits quantification of changes in protein composition. Gel-free methods include 'shotgun' approaches using tryptic digests of complex sets of proteins, followed by peptide sequencing in a tandem mass spectrometer for identification (12). More recently, state-of-the-art liquid chromatography-mass spectroscopy systems have been used to identify and track the relative abundance of thousands of proteins (12-14). Despite the rapid advances in proteomic technology, several areas continue to need improvement, particularly in the detection, quantification and identification of low-abundance proteins and assessment of protein distribution among cells and subcellular structures, as well as the characterization of post-translational modifications.

Biological samples subjected to proteomic analysis comprise three major types: tissues, cell populations and biological fluids. To date, most of the cardiovascular studies have focused on tissue obtained by endomyocardial biopsy or, more commonly, cardiac explantation at the time of transplantation. Endomyocardial biopsy carries a small but significant degree of risk inherent in the procedure. As well, the small sample volume is at the lower limit of detection for most high-throughput technologies today. It is, therefore, important to consider the utility of molecular signature analysis derived from peripheral blood leukocytes. Successful examples of this exist in the cancer literature (15,16), and more recently, peripheral blood molecular signatures correlated with biopsy-proven allograft rejection in cardiac transplant recipients (17). The dynamic range of proteins in the blood is formidable, ranging from 55,000,000,000 pg/mL (eg, albumin) to 5 pg/mL (eg, interleukin-6). Only four proteins (albumin, transferrin, haptoglobulin and immunoglobulins) make up more than 90% of the protein mass in the blood (7). Although techniques exist for depleting these proteins from the serum (18,19), challenges remain to assure their efficiency and specificity.

Essential aids to proteomic studies of the heart are the federated online databases of human cardiac proteins that have been established. These databases, known as HSC-2DPAGE (20), HEART-2DPAGE (21) and HP-2DPAGE (22), are widely accessible and display more than 6000 myocardial proteins separated by 2-DE. Open-access data sharing through such mechanisms will be crucial for the rapid advancement of the field and international collaboration. There are also international organizations attempting to provide standards and benchmarks for the increasing data being mined in proteomic studies. One organization, the Human Proteome Organisation, was created in 2001 with the objective of going beyond the Human Genome Project in understanding health and disease, while providing clear guidelines for proteomic studies (23). A second key organization is the Proteomics Division at the National Heart, Lung and Blood Institute (24).

APPLICATION OF GENOMICS AND PROTEOMICS TO CARDIOVASCULAR DISEASE

Genomic and proteomic approaches provide complementary insights into cardiovascular disease pathways. Genetic disorders are traditionally thought of as early-onset, single-gene entities that are transmitted in a Mendelian fashion to offspring, such as cystic fibrosis or Marfan syndrome. Hypertrophic cardiomyopathy is known as a disease of the sarcomere and is inherited as an autosomal dominant trait in at least one-half of all cases. The first causal mutation in the beta-myosin heavy chain was described in a large French-Canadian family in 1990 (25). Monogenic causes have been identified for many other cardiovascular disorders, including dyslipidemia (26), coronary disease (27), dilated cardiomyopathy (28), long QT syndrome (29), arrhythmogenic right ventricular cardiomyopathy (30) and atrial fibrillation (31).

Individuals are distinguished from one another by a 0.1% difference in the nucleotide sequence of the human genome. These differences are often in the form of variations of a single base pair, called single nucleotide polymorphisms (SNPs). Individual SNPs often cause only a modest change in the resulting protein concentration or function. It is, therefore, the concurrent presence of a number of SNPs that determines susceptibility to disease development and progression, particularly for polygenic diseases.

There is increasing evidence for a genetic basis to many complex diseases without monogenic transmission, including heterogeneous conditions such as heart failure, myocardial infarction and atherosclerosis. Heart failure among the Framingham study cohort was recently shown to confer a significantly increased risk

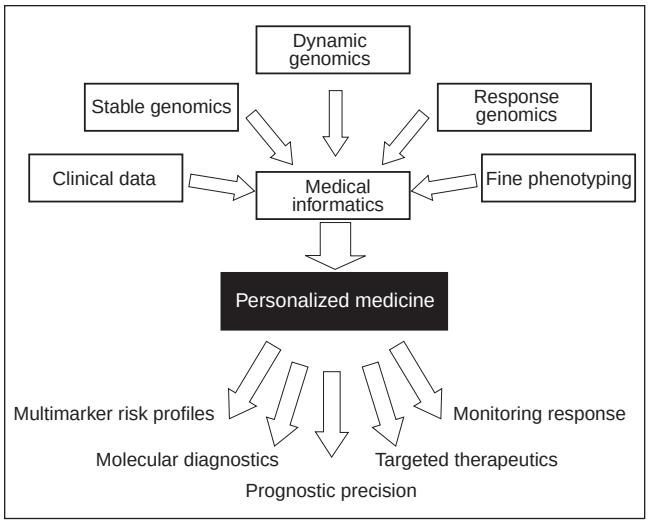

Figure 1) *Personalized medicine will require the integration of clinical information, stable and dynamic genomics, and molecular phenotyping. Bioinformatics will be crucial in translating these data into useful applications, leading to improved diagnosis, prediction, prognostication and treatment*

of the condition in their offspring (32). In complex genetic diseases, the genotype confers a susceptibility to disease, but the development of disease is dependent on interactions of genes with the environment and with other genes. The genotype-phenotype correlation is often not straightforward. The incorporation of transcriptomic and/or proteomic data will allow for a more complete picture that accounts for epigenetic and environmental influences to be elucidated.

Microarrays have been used to examine the transcriptional profiles of the adult heart and the developing heart in normal and pathological conditions (33,34). In the field of heart failure, many studies have performed binary comparisons of gene expression, such as failing and nonfailing heart (35-38), dilated and hypertrophic cardiomyopathy (39), and before and after left ventricular assist device placement (40-42). Other studies have included three-way comparisons: ischemic and nonischemic cardiomyopathy relative to nonfailing hearts (43,44), or failing and left ventricular assist device-supported hearts relative to nonfailing hearts (45).

Proteomic studies have confirmed and extended the findings of their stable and functional genomic investigations. To identify novel markers of cardiac allograft rejection, proteomic analysis was performed on cardiac biopsy samples, and upregulated proteins, including tropomyosin and alphaB-crystallin, were confirmed in patients' sera (46). Several studies (47-49) of ischemia-reperfusion models have identified alterations in several functional groups: sarcomeric and cytoskeletal proteins, redox regulation, energy metabolism and the stress response. To further understand the preconditioned phenotype, proteomic analysis of pharmacological protection to ischemia-reperfusion injury revealed that the majority of the proteins involved are involved in mitochondrial energetics (50). Myocardial subproteome analysis of a Rac-1-expressing mouse with lethal hypertrophy identified differential expression of the creatine kinase M-chain, tubulin beta-chain, manganese superoxide dismutase and malate dehydrogenase (51). More recently, the first comprehensive characterization of cardiac 26S proteasomes was reported, providing critical information fundamental to our understanding of this essential protein degradation system in the myocardium (52).

Despite the small number of samples and the heterogeneity of experimental protocols in these studies, several pathways emerge as being consistently dysregulated in heart failure. Functionally related groups of genes are often identified, suggesting coordinated regulation of multiple genes. Recurrent themes include cytoskeletal and extracellular matrix remodeling, inflammation, calcium handling, oxidative stress pathways, and cell survival and death. These investigations have both confirmed the importance of existing targets and suggested novel mechanisms to explore.

PROTEOMICS AND PERSONALIZED MEDICINE

Personalized medicine was defined by Francis Collins, head of the Human Genome Project, as "using information about a person's genetic makeup to tailor strategies for the detection, treatment, or prevention of disease" (53). By applying new strategies, we can intervene at an earlier stage of disease progression and implement tailored therapies designed for the individual (Figure 1). The field of oncology currently leads the way in personalized medicine, due largely to years of molecular research and more ready access to human tissue samples in this discipline. The addition of molecular signatures to traditional pathological and imaging studies promises to refine our ability to care for our patients.

Biomarker discovery and validation

A strategy to develop novel biomarkers in cardiovascular medicine was recently outlined (54). In brief, the goal of the program is to discover novel biomarkers for heart failure, particularly those that might be specific for early, middle and late stages of the disease. To this end, multiple genetic and acquired animal models of heart failure have been used. The informatic analysis from these models is then integrated with tissue analysis from patients or explanted hearts with advanced forms of heart failure. This approach generates a large number of biomarker candidates in a biological, temporal and clinically relevant context, and permits the validation of these candidates in the serum or plasma of appropriately selected patient cohorts in replicates.

The challenges for the next decade have been identified by the National Heart, Lung, and Blood Institute as the development of systematic and adequately powered approaches to validate the associations of protein biomarkers with disease state, risk or treatment effect that are identified from genomic and proteomic approaches; and the development of informatics and analytical platforms for grouping putative markers of risk into panels that are clinically useful.

The ideal biomarker or multimarker panel would facilitate diagnosis, provide independent prognostic information and reflect ongoing changes in the patient's clinical condition. Also, it would have maximum discriminatory power and have been reliably derived and validated in representative target populations. It would be reproducible across laboratories and reliable across differences in age, sex, ethnicity and storage. Finally, an ideal biomarker would guide patient management and improve clinical outcomes.

It is likely that most useful biomarkers will be shown to have biological relevance to the disease condition. Examples to date include brain natriuretic peptide for heart failure, which is released directly from the myocardium, and troponin I or T, which are released from ischemically damaged myocytes.

Therefore, convergence of proteomic patterns observed from both relevant animal models of the disease and robust corresponding clinical phenotypes would be anticipated. Potential biomarkers can be identified with timed evaluation of animal models of disease development. The subsequent validation of these panels of candidate biomarkers is critical to distinguish those that have clinical utility from those that do not provide adequate sensitivity, specificity or biological insight into disease progression.

Clinical utility: Prevention, diagnosis and prognosis

Opportunities abound for gene-based presymptomatic prediction of illness. The goal will be to identify genetically high-risk (susceptible) subgroups that may benefit disproportionately from screening or therapeutic interventions. The response to the preventive intervention may also be predicted from their molecular phenotype. For example, considerable evidence exists that smoking initiation, dependence and response to cessation therapies have a genetic basis (55). Subgroups with high-risk polymorphisms may benefit from early, targeted counselling.

The segmentation of complex diseases into simpler subclassifications through the use of novel biomarkers and signature profiles will improve our understanding of common illnesses. Diseases that are more similar mechanistically will be separated into distinct categories earlier in their pathophysiological progression.

The greatest limitation in personalizing cardiovascular medicine today is our inability to accurately predict risk for patients. Current risk assessment tools are derived from large-scale epidemiological studies assessing the association of certain measurable traits with a given outcome. Most are multivariable models, comprising a combination of demographic and clinical factors that have a given predictive accuracy in certain populations. Our risk assessment tools of the future will be a form of molecular triage, extending familiar models, such as the Framingham risk score, to include genomic and proteomic profiles. Only by integrating all forms of data will we improve our ability to predict outcomes across a broad range of health and disease (Figure 2).

Therapeutics: Pharmacogenomics and pharmacoproteomics

The greatest area of immediate clinical application of genomic technologies is pharmacotherapy, in which the ultimate goal is to maximize response and minimize toxicity. The 'one drug fits all' concept has shifted to the paradigm of 'the right drug for the right patient at the right dose and time'.

Both drug efficacy and drug safety have the potential to be improved by genotype-based pharmacotherapy (56). Molecularly targeted therapies could address responsive subgroups more directly. For instance, the authors' oncology colleagues have developed trastuzumab (Herceptin, Hoffmann-La Roche Limited, Canada), a monoclonal antibody directed against the HER2 receptor, which was recently approved for a subtype of breast cancer (57). Women may be tested for HER2 protein overexpression or gene amplification and be eligible for receptor-directed therapy accordingly. Many similar examples exist in the cancer literature.

Along with therapies directed at individual molecules, proteomic profiles may reveal multiple protein nodes along several interconnected pathways that are responsible for the aberrant signalling that leads to disease. This information may lead to diverse pharmacological targets to shut down the aberrant signalling in the causal pathways (58). Redundancies typically present in biological systems could be addressed by this multipronged approach. Thus, the entire pathway involved, rather than the individual gene or protein, may be the most attractive target in the future.

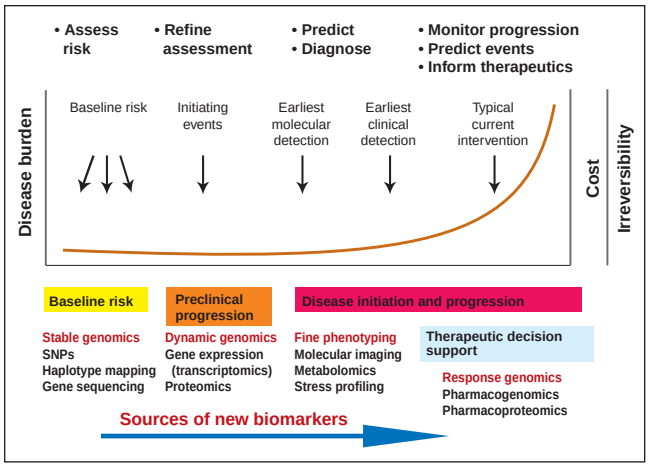

Figure 2) *Gene-based presymptomatic prediction of illness, finer diagnostic subclassifications and improved risk assessment tools will permit earlier and more targeted intervention. Pharmacogenetics will guide therapeutic decisions and monitor response to therapy. SNPs Single nucleotide polymorphisms*

Adverse drug reactions may also be predicted or monitored more closely by genomic and proteomic profiling. The examples are numerous, and in December 2004, the United States Food and Drug Administration approved the first genotyping test for drug metabolism. Information from the AmpliChip Cytochrome P450 test (Roche Molecular Systems Inc, USA) may determine the presence of mutations in a gene that metabolizes many types of drugs, including antidepressants, antipsychotics and beta-blockers.

Warfarin is the mainstay of anticoagulant therapy for the treatment and prevention of thromboembolic disease. It is also one of the best examples of the potential benefits of personalized medicine (59). The drug's narrow therapeutic index and wide variability in patient response makes dosing unpredictable and difficult to manage. Poor initial control carries a substantial risk of serious adverse events, including bleeding and recurrent thromboembolism. Polymorphisms in genes influencing metabolism (*CYP2C9*) and pharmacodynamic response (*VKORC1*) are strongly associated with warfarin responsiveness. Two common variants of *CYP2C9*, *CYPC9*2* and *CYPC9*3*, encode enzymes that are less efficient in metabolizing warfarin than the more common allele. In the Caucasian population, 22% are heterozygous carriers of the *CYP2C9*2* allele and 15% carry the *CYPC9*3* allele. Carriage of variant alleles is associated with an increased risk of bleeding and low warfarin dosage requirements (60). Similarly, two common haplotypes in the *VKORC1* gene are associated with lower expression of *VKORC1* and lower warfarin requirements (61). The empiricism of current warfarin dosing algorithms and the potential improvement in anticoagulation-related outcomes drive an emerging argument for prospective genotyping of warfarin patients. Incorporating the known genetic and environmental factors will allow individualization and optimization of oral anticoagulation therapy.

Beta-blocker therapy is standard care for patients with heart failure. Genetic variants of the $beta_1$-adrenergic receptor have

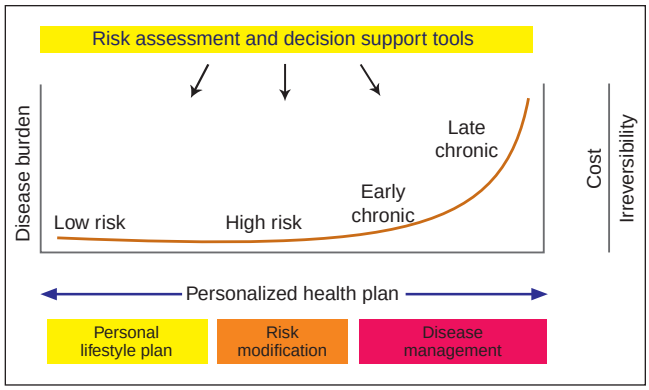

Figure 3) *Opportunities exist at every stage of disease initiation and progression to develop a personalized health plan addressing lifestyle, risk modification and disease management*

been well described. In particular, the Arg389 and Gly389 polymorphisms result in a differential response to both agonism and blockade (62). A recent analysis of the Beta-Blocker Evaluation Survival Trial (BEST) (63) found that although bucindolol conferred no survival advantage in the overall population, subjects who were homozygotes for the Arg389 polymorphism had a 38% reduction in death at two years. This finding highlights the added value of genetic information to clinical phenotypes in clinical trials.

Polymorphisms in the angiotensin-converting enzyme (ACE) pathway were initially described by McNamara et al (64) who demonstrated that the ACE D allele was associated with significantly poorer transplant-free survival in patients with heart failure and systolic dysfunction. In a follow-up study from the same group (65), patients with the DD polymorphism had worse outcomes on low-dose ACE inhibitor therapy than did patients on high-dose therapy. In addition, high-dose ACE inhibitors and beta-blockers had the greatest impact in those with the DD variant (P=0.001) and the least impact in those with ID and II genotypes (P=0.38).

Improved understanding of the genetic variations responsible for differences in pharmacodynamics and pharmacokinetics is a crucial step toward personalizing therapies.

Patient interaction of the future

A 43-year-old, asymptomatic businessman presents to your office for evaluation. His family history is significant for heart failure as well as ischemic heart disease. History and physical examination reveal borderline hypertension in an otherwise healthy, active gentleman. You obtain a genome/proteome-based profile describing his cardiovascular risk and create a probabilistic health history. His genotype is positive for thrombospondin-1,4 N700S/A387P and matrix-metalloproteinase-9 S185P, conferring a fivefold risk of myocardial infarction and a threefold risk of heart failure, respectively. His biosignature places him at high risk for developing the disease phenotype, and you decide to treat him prophylactically. You develop an individualized prevention plan that includes behavioural counselling, as well as pharmacotherapy directed at his aberrant signalling pathway. You follow him yearly with noninvasive methods to detect disease and monitor his response to therapy. You reduce his disease burden by intervening before overt manifestations of his genotype, and by tailoring your therapeutic strategy to avoid adverse events and optimize efficacy (Figure 3).

CONCLUSION

Genomic and proteomic approaches to cardiovascular medicine promise to revolutionize our understanding of disease initiation and progression. This improved appreciation of pathophysiology may be translated into avenues of clinical utility. Specifically, we can refine our ability to predict future disease, classify illness on a molecular basis for better diagnostic and prognostic precision, and design personalized therapies tailored to the individual.

A key advantage offered by a systems biology strategy is that it permits an unbiased characterization of the genes and proteins of interest, thereby allowing a characterization of gene and protein interactions in a systematic fashion. By combining this approach with knowledge of gene variants, we can optimize our capacity for identifying key targets in disease pathways.

Extending and integrating these new approaches into clinical medicine will necessitate rigorous data collection and validation in large-scale epidemiological research. The repositories of genomic and proteomic profiles that are generated by these studies will require linkages to detailed clinical and long-term follow-up data. Many challenges remain to realizing the benefits of personalized medicine, but thoughtful, well-designed investigations with currently available methodologies may help us to overcome them.

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