The path to personalized medicine
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Advances in personalized medicine, or the use of an individual's molecular profile to direct the practice of medicine, have been greatly enabled through human genome research. This research is leading to the identification of a range of molecular markers for predisposition testing, disease screening and prognostic assessment, as well as markers used to predict and monitor drug response. Successful personalized medicine research programs will not only require strategies for developing and validating biomarkers, but also coordinating these efforts with drug discovery and clinical development.

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Abbreviation
SNP single nucleotide polymorphism

Introduction
The realization of personalized medicine, or the fine tailoring of the practice of medicine to an individual, is being fostered through numerous efforts aimed at characterizing individual differences in molecular processes underlying disease pathogenesis, disease progression and the response to therapeutics. Once these molecular differences are understood, therapeutic development will be enhanced by using the information to identify individuals more likely to benefit from a given intervention strategy. High-throughput genomic technologies are already providing the data that will serve as the foundation of personalized medicine. Here, we briefly describe the current state of these technologies and highlight the directions required to fully develop and integrate personalized medicine into practice. We draw upon a range of examples that demonstrate the relevance of molecular markers throughout the development and treatment of disease, including markers for disease predisposition, screening and progression, as well as markers for drug response and drug monitoring (Figure 1).

Individual differences in the development of disease and response to therapeutics
Clearly, for many common diseases, there is abundant evidence to suggest that the molecular underpinnings of disease susceptibility, and its natural history, differ markedly among individuals. For example, while it has been demonstrated in numerous investigations that the development of obesity, asthma, type 2 diabetes and cardiovascular disease are under genetic control [1–4], there is no evidence to suggest that the genetic basis is due to variation in just a single gene. Instead, the consensus has emerged that subtle genetic differences in one or many of several genes serve as risk factors for these illnesses. Thus, while genetic variants in the melanocortin-4 receptor may explain some risk for developing obesity [5], and polymorphisms in PPAR-gamma may correlate with the risk of developing type 2 diabetes [6•], these variants do not explain all of these genetic diseases. There are certainly more genetic variants, or predisposition markers, to uncover. In the context of personalized medicine, the ultimate goal of these types of studies is to provide a suite of markers that can be used to assess one’s lifetime risk of developing disease in the presence of various environmental (e.g. diet, lifestyle) variables.

As with disease predisposition, individual differences characterize disease progression. For example, some individuals with impaired glucose tolerance will proceed quite rapidly to type 2 diabetes, whereas others proceed slowly. Similarly, individuals diagnosed with rheumatoid arthritis may or may not develop erosive disease. In both of these cases, genetic variation, that is, variation measured at the DNA level, may be a good predictor of the individual differences that emerge as disease progresses. For example, Brinkman et al. [7] have demonstrated that a polymorphism in TNF-α correlates with erosive rheumatoid arthritis, but shows no association with non-erosive disease. Alternatively, variation in disease progression may be best predicted by a combination of genetic and environmental factors, the impact of which is indexed through changes in gene expression in relevant tissues, or changes in secreted protein levels in serum or synovial fluid. In our laboratories, we are using a range of genomics technologies to find markers for disease progression that are both stable (DNA) as well as dynamic (mRNA, protein), giving us the opportunity to evaluate the utility of both types of markers in prospective studies.

Given that individual variability in disease predisposition and progression exists and has the potential of being molecularly characterized, it is not at all surprising that such differences also characterize response to therapeutics (see Figure 1). Marked individual variation in the efficacy and toxicity of therapeutic compounds is common and can have a profound impact on the success of a pharmaceutical clinical development program. Clearly, molecular markers that predict the variation in these endpoints could be extremely useful in clinical trials, drug development and clinical practice, as they would allow the identification of patients who would benefit most from the drug.

Technological advances drive broad biomarker discovery
While the existence of individual differences in disease predisposition, progression and response to therapeutics is
far from a novel concept, our ability to comprehensively measure the molecular markers that track these processes, and draw proper inferences from large amounts of molecular data, is novel. Over the past decade, significant advancements have been made in technologies to discover variation at the mRNA, DNA and protein levels. Indeed, with the advent of glass and nylon microarray technologies for gene-expression studies, it is quite feasible to characterize the expression levels of 30,000 genes in tissue samples from dozens, if not hundreds, of individuals. Certainly, several years ago, although it would have been theoretically possible to assess this number of genes using northern blot analysis, it never would have been undertaken in a sample from even a single individual. In the same fashion, high-throughput technologies for DNA polymorphism discovery and single nucleotide polymorphism (SNP) genotyping, coupled with broad academic and commercial initiatives to characterize genetic variation genome-wide [8•], are resulting in catalogs of variants that can be used in large-scale experiments. To complement these efforts, searches for ‘haplotype blocks’, or correlated patterns of SNPs that can be adequately represented by fewer SNPs, are underway and have the promise of reducing the amount of genotyping required for genome-wide searches [9•,10•]. For protein-based discovery initiatives, traditional 2D electrophoresis experiments are used in conjunction with advanced mass spectrometry to discover protein markers in a range of complex fluids, including serum, plasma, synovial fluid and cerebral spinal fluid.

Coupled with the advent of these technologies have been extensive efforts to collect appropriate tissues and fluids for mRNA, DNA and protein analysis. These collections have been part of pharmaceutical clinical trials, as well as clinical studies established for the purpose of characterizing biomarkers. The latter studies may involve small numbers of patient samples for initial biomarker discovery efforts, as well as large-scale, disease registry initiatives designed to evaluate and, in some cases, prospectively validate, biomarkers in the relevant patient populations. Moreover, sample collections from general population cohorts, such as the Framingham Heart Study and Women’s Health Study, also offer opportunities to prospectively validate biomarkers for disease risk [11,12]. Recent reports on both of these cohorts demonstrate the utility of this approach by showing a significant relationship between C-reactive protein levels at baseline and vascular events (transient ischemic attack, ischemic stroke) at follow-up.

The predictive value of biomarkers

The impact that advanced genomic technologies and carefully designed biomarker studies will have on the personalization of medicine is foreshadowed in the current literature. For example, Mallal et al. [13•] conducted a pharmacogenetic investigation (i.e. a genetic study of drug response) of abacavir, an HIV-1 nucleoside reverse transcriptase inhibitor. They implicated MHC alleles that predict response to hypersensitivity among 5% of the HIV cases receiving the drug. Their findings suggest that screening patients for the presence of the predisposing MHC haplotype could reduce the prevalence of hypersensitivity to abacavir from 9% to 2.5%. While this study is small in scale in its characterization of genetic variation, it adds to the existing literature on several other variants (including those in MDR1, the multidrug transporter P-glycoprotein and CYP2D6, a cytochrome P450 isozyme) that correlate with the pharmacokinetic (drug clearance) characteristics of protease inhibitors and non-nucleoside reverse transcriptase inhibitors [14]. Additionally, genetic polymorphisms in chemokines and chemokine receptors (including RANTES, MIP-1α and CCR5) have been found to correlate with both the susceptibility to HIV-1 infection and the progression of disease [15•]. Taken together, these findings may lead to the development of a panel of polymorphisms that would personalize HIV therapy, by determining when to initiate therapy and how to choose compounds that will maximize efficacy and minimize adverse effects.
In addition to the abacavir example, pharmacogenetic efforts have also successfully characterized polymorphisms that correlate with response to asthma therapeutics. For example, Drazen et al. [16•] showed that a promoter polymorphism in 5-lipoxygenase, which alters transcription levels of the gene, also correlates with response to a derivative of the drug Zileuton, a 5-lipoxygenase inhibitor. Of the individuals who did not respond to Zileuton, 20% carried rare variant alleles at this locus. By contrast, all of the responders had wild-type alleles. Similarly in a study of genetic polymorphisms of the β-adrenergic receptor, Drysdale et al. [17•] demonstrated that a haplotype, or SNP signature across the gene, correlated strongly with asthma patients’ response to β-agonists. These two examples again demonstrate the possibility of using an individual’s genotype to suggest a therapeutic strategy that is more likely to be efficacious. Certainly, before such tests are incorporated into clinical practice, additional genetic markers would have to be coupled with the existing polymorphisms to make the resulting tests highly sensitive and specific.

In addition to these DNA-based strategies, recent applications of proteomics and expression profiling have generated a range of screening, prognostic and drug-response or ‘pharmacogenomic’ biomarkers. Many advances in the use of these technologies have been in oncology, where there is a tremendous need for serum-based screening markers and where tissue samples for expression profiling studies are easily obtained. For example, Petricoin et al. [18•] demonstrated that proteomic spectra, derived from a mass spectrometry analysis of serum, could be used to distinguish women with ovarian cancer from unaffected women. Indeed, the protein markers on a ‘training set’ of 100 samples and a validation set of 110 additional samples, had a sensitivity of 100% and a specificity of 94%. These encouraging results suggest that a serum-based protein assay may indeed become a viable mode of ovarian cancer screening in the general population. mRNA strategies for identifying prognostic markers for cancers have also proved successful. For example, in our collaborative studies [19•], we have shown that Melastatin, a melanocyte-specific gene identified through a genomics analysis of benign and malignant melanoma, is an effective prognostic marker for cutaneous malignant melanoma. In this work, uniform melastatin mRNA expression correlated strongly with disease-free survival, even after adjusting for other prognostic factors. In a similar fashion, mRNA strategies have generated pharmacogenomic markers for ovarian cancer. Hartmann et al. [20] studied the expression of 30 000 human genes in 51 tumors that were sensitive and resistant to platinum–paclitaxel chemotherapy and identified a subset of 10 markers that were highly predictive of outcome in an independent sample of tumors. Overall, these examples of biomarker studies in oncology demonstrate the broad application such markers will have for cancer screening, prognosis and response to therapeutics.

Expression profiling analyses, for the purpose of generating biomarkers, are also emerging outside of the field of oncology. An elegant example of this is given by...
Oestreich et al. [21*] in their study of the molecular classification of psoriasis. The investigators use a combination of cross-sectional expression studies on (untreated) psoriasis patients and controls, as well as longitudinal studies of treated psoriasis patients, to characterize a molecular profile of psoriasis and generate an understanding of how this profile changes with treatment by agents that antagonize calcineurin or the NF-kB pathway. These experiments have generated a range of markers that not only yield insight into the pathogenesis of psoriasis, but may also prove useful as both predictive markers for treatment outcome, as well as surrogate markers for disease endpoints.

Turning biomarker discoveries into personalized medicines

All of the examples cited above provide excellent demonstrations of the power of new technologies to deliver a range of biomarkers that index individual differences in disease predisposition, progression and response to therapeutics. Thus, they clearly form a basis for the 'personalization' of medicine. However, the discovery of these markers is not sufficient for the pharmaceutical industry to deliver personalized medicines. Indeed, the delivery of such medicines will require the careful integration of biomarker discovery and validation programs into drug discovery and clinical development programs (see Figure 2). This integration will serve two key purposes. First, and foremost, by initiating SNP, expression profiling and proteomics biomarker programs early on in the drug discovery process, one can carefully weave the discovery and validation of biomarkers into drug discovery and development timelines; the risk of ‘retro-fitting’ biomarker programs to a clinical trial would be avoided. As an example, consider a drug discovery program for rheumatoid arthritis for which surrogate markers for erosive rheumatoid arthritis is highly desirable. Discovery and validation programs for biomarkers must commence well in advance of a clinical trial in order for these markers to be useful in the trial. Similarly, any functional information that could be gained regarding SNPs in drug targets, or relevant drug metabolizing enzymes or drug transporters, before initiating a trial could reduce the SNP genotyping efforts within the trial and focus the exploration of genetic hypothesis regarding markers of toxicity or efficacy. The second compelling reason to integrate biomarker programs within a drug-discovery pipeline is that the pipeline itself will be enhanced by biomarker discovery efforts. As an example, consider the characterization of genetic variation in potential drug targets. These variants may not only ultimately serve as pharmacogenetic markers for the drug which targets the gene of interest, but may also be used at an earlier stage in target validation programs that seek to correlate genetic variants with disease. Similar pipeline synergies would be gained if expression profiling experiments that are conducted on ‘standard of care’ therapies not only identify markers that predict response to a drug, but also markers that correlate with non-response. This latter category of markers may generate opportunities to redefine the disease under study molecularly, develop new therapeutic targets, as well as identify groups of individuals that would benefit from novel intervention strategies.

Conclusions

Clearly, several challenges remain to achieve a successful integration of large-scale, biomarker studies with drug development. While there has been an incredible advance in high-throughput, molecular technologies, over the past several years, further improvements in technologies and validation strategies are required to capture the true extent of individual differences in molecular markers. For example, although it is plausible to consider screening the genome for SNPs or haplotypes that correlate with disease predisposition or drug response, the current cost of SNP genotyping makes this impractical. Additionally, bioinformatic and statistical advances are needed to extract the most relevant data from the wealth of molecular information generated by new technologies, and these advances must be effectively communicated to the health-care environment. Finally, and most importantly, plans must be in place to provide adequate validation for the enormous number of candidate biomarkers that will emerge from the studies. Validation will require access to large, and in some cases, prospective, collections of well annotated clinical samples with appropriate consent and security issues addressed. While these issues, as well as the commercial and regulatory considerations surrounding the development of personalized medicines, are indeed challenging, the successful execution of biomarker programs will have an enormous impact on our ability to tailor medical practice to the individual.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest ** of outstanding interest

Next-generation therapeutics

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