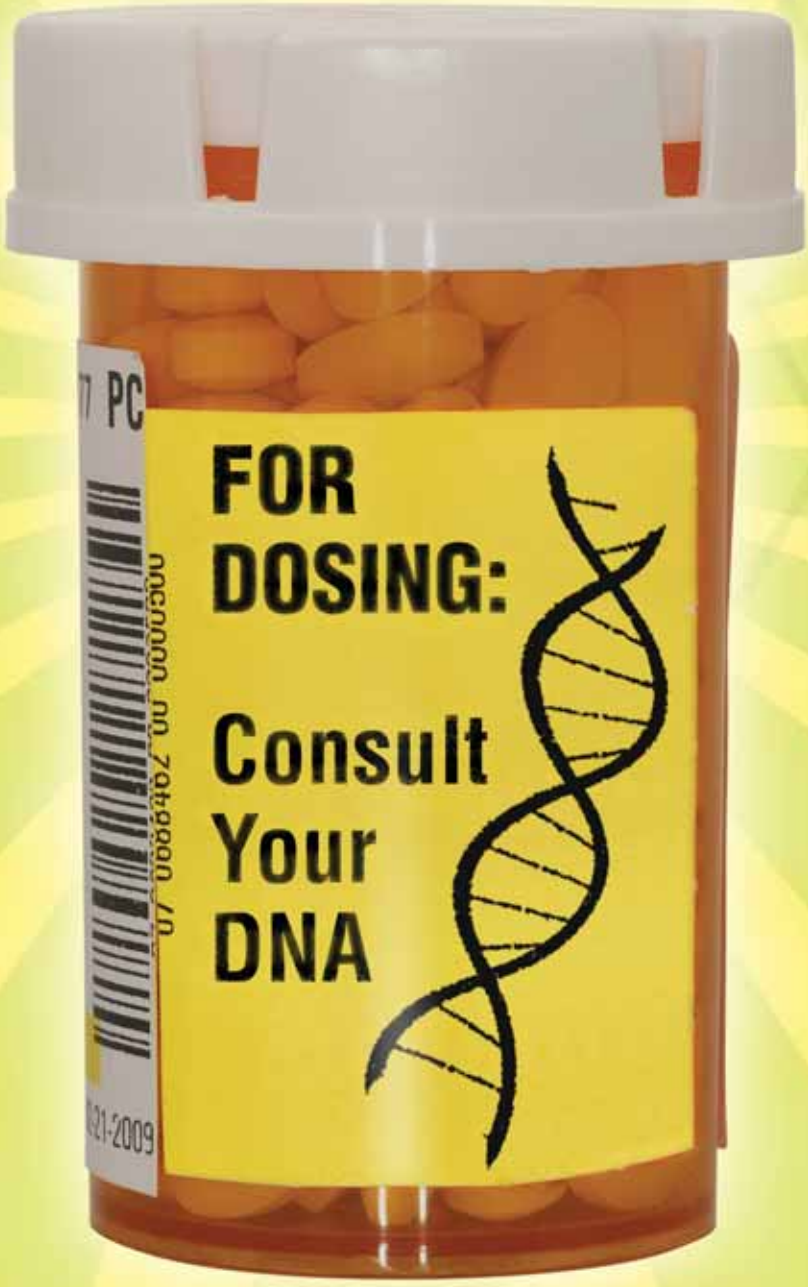
From SNPs to Can genes



By Chandra Shekhar, PhD

Prescriptions: predict drug response?

Decades of steady progress in pharmacogenetics have unearthed hundreds of associations between genes and drug response. But the field has to solve some theoretical and practical issues before it can deliver on the promise of personalized drug therapy.

As algorithms go, it's deceptively simple. Just add together eight weighted pieces of patient information—age, height, weight, race, data about two genes, and a pair of clinical parameters. Yet this straightforward linear equation could mark a watershed moment in medical science.

The algorithm in question helps physicians prescribe a safe and effective dose of the blood thinner warfarin. Currently, because warfarin's optimal dose varies tenfold among patients, physicians prescribe an intermediate dose and make adjustments over the course of several weeks to achieve the desired effect. But this approach carries huge risks: Too high a dose could trigger fatal bleeding while an insufficient dose might allow dangerous blood clots to form. And it's hard to tell how a patient will react. "If you gave warfarin to a huge football player and a tiny grandma, the football player could bleed uncontrollably at a dose much smaller than what you give grandma," says **Balaji Srinivasan, PhD**, a Stanford University statistician who worked with the International Warfarin Pharmacogenetics Consortium—an unprecedented collaboration of 21 research groups that jointly developed the new dosing algorithm.

"If you gave warfarin to a huge football player and a tiny grandma, the football player could bleed uncontrollably at a dose much smaller than what you give grandma," says Balaji Srinivasan.

According to the Consortium's study of about 5,000 patients from a variety of ethnic backgrounds, the new algorithm can significantly lower the risk of under- or over-dosing compared to using clinical information alone. It is nearly 50 percent better at identifying patients who need low doses and more than 3 times better at identifying those who need high doses. Given warfarin's wide use—up to two million new patients take it each year—the concrete benefits are obvious. But the greater significance is symbolic: the genetic dosing algorithm for warfarin could be a major milestone in the evolution of drug prescription from a trial-and-error strategy to an exact science.

"It's an amazing story at many levels," says Stanford University computational biologist **Russ Altman, MD, PhD**, one of the organizers of the Consortium and a senior author of its warfarin study. "You had 21 research groups in nine countries who pooled all their data together to come up with this algorithm. And we clearly show that genotype-based dosing can be a vast improvement over the guessing game physicians have to play now."

So is personalized drug therapy—prescribing the right drug at the right dose for an individual patient—about to become a reality?

Perhaps not right away. Pharmacogenetics, the study of genetic factors that influence drug response—and its younger sibling pharmacogenomics, which adopts large-scale genome-wide methods—are indeed hot research areas. (A PubMed search with the two terms brings up nearly 9,000 entries, most from this decade.) As with research in general, however, some studies in pharmacogenetics have turned out to be poorly designed. Others are well designed but don't give a biologically significant result. And still others make clear-cut biological predictions, but with limited clinical value. Finally, even interventions of proven worth are struggling to reach the clinic. So it may be a while before insurers stop using the dreaded "experimental" adjective when referring to these techniques.

Despite these challenges, the flood of pharmacogenetics results pouring in gives hope that at least a few will make it into everyday use. The need is obvious: nearly 90 percent of drugs don't work for half the people; worse, adverse reactions to drugs send millions of patients each year to the hospital and cause more than 100,000 deaths. In most cases, genetic factors seem to play a role. An editorial that accompanied the warfarin study in the February *New England Journal of Medicine* says, "A better understanding of individual differences in the response, either positive or negative, to medicines should be an overarching goal for pharmacotherapy over the next decade."

"You had 21 research groups in nine countries who pooled all their data together to come up with this algorithm. And we clearly show that genotype-based dosing can be a vast improvement over the guessing game physicians have to play now," Russ Altman says.

Beans to genes

To see where pharmacogenetics is headed, it is instructive to take a step back and see how it emerged. Although the field has gained much of its prominence this century, it has a long and eventful history. Some researchers credit Pythagoras, in the 6th century BC, with making the first contribution to it when he noted that eating fava beans made some people sick. (Two-and-half millenia later, scientists would discover the cause: a variant in a red blood cell enzyme that also causes abnormal responses to anti-malarial drugs.) One facetious researcher gives the credit to Karl Marx—"didn't he say to each according to his need?" A more serious claimant for the honor is chemist **Arthur Fox**. While working at the DuPont laboratories in 1931, Fox accidentally released a cloud of a chemical that he was working on. He felt nothing, but a colleague complained of a bitter taste sensation. Intrigued, Fox investigated this further and discovered that the ability to taste the compound is an inherited trait, later shown to be due to variants in a bitter taste receptor gene. In the 1950s, University of Toronto medical scientist **Werner Kalow, MD**, found that people who suffocated to death after getting certain muscle relaxants had inherited a variant of the gene for pseudocholinesterase, an enzyme involved in nerve function. Kalow would go on to pen a monograph in 1962 on pharmacogenetics, defining the term as the study of heredity and the response to drugs.

The work of Fox and Kalow set the template for pharmacogenetics that lasted until the mid-90s: identify a peculiarity in drug response and look for inherited variations in a relevant gene or enzyme. During the next few decades, researchers used this approach to explain atypical responses to the tuberculosis drug isoniazid, the malaria drug primaquine, and the heart arrhythmia drug sparteine. In 1964, even good old alcohol got a response enzyme; people with a variant of aldehyde dehydrogenase can get violently ill after even a tiny sip from the flask, an effect the alcohol aversion drug Antabuse achieves in other people by blocking the enzyme.

While these pioneering studies showed that drug response could be a hereditary trait, they dealt with relatively simple problems. "Classically, in pharmacogenetics the focus was on cases where we thought there's a single gene and single mutation that was going to explain drug response," explains **Marylyn Ritchie, PhD**, a geneticist at Vanderbilt University.

In most cases however, drug response is influenced by many genes that each have only a modest impact. These "pharmacogenes" come in two flavors: At the "front end" stand the so-called pharmacokinetic genes that determine how quickly the body breaks the drug down and eliminates it; at the "back end" lurk the so-called pharmacodynamic genes whose function the drug targets. Patients with a sluggish front end but touchy targets could have an excessive, even toxic, response—such as warfarin-induced bleeding—while those with an unusually brisk front end and apathetic tar-

gets could barely respond.

Another drawback of many early efforts was that they relied on family-based data to locate genetic markers that were inherited along with the trait being studied. But such studies require huge sample sizes to produce valid results—especially when the effect of the gene variant may be small, as is the case for many genetic drug responses. In a 1996 paper in *Science*, **Neil Risch, PhD**, and **Kathleen Merikangas, PhD**, showed one would need to look at 70,000 families in order to pinpoint a gene variant that occurs 10 percent of the time and increases the relative risk of a certain trait by 50 percent—which would be high by drug response standards. To detect genes of such modest influence, the authors recommend a different approach—the association study—"even if one needs to test every gene in the genome." This type of study compares the gene variants of patients who respond normally to a drug (controls) with those of patients who have an adverse reaction (cases). (If the drug response can be quantified, as with warfarin, the study may look at patients with a range of responses instead.)

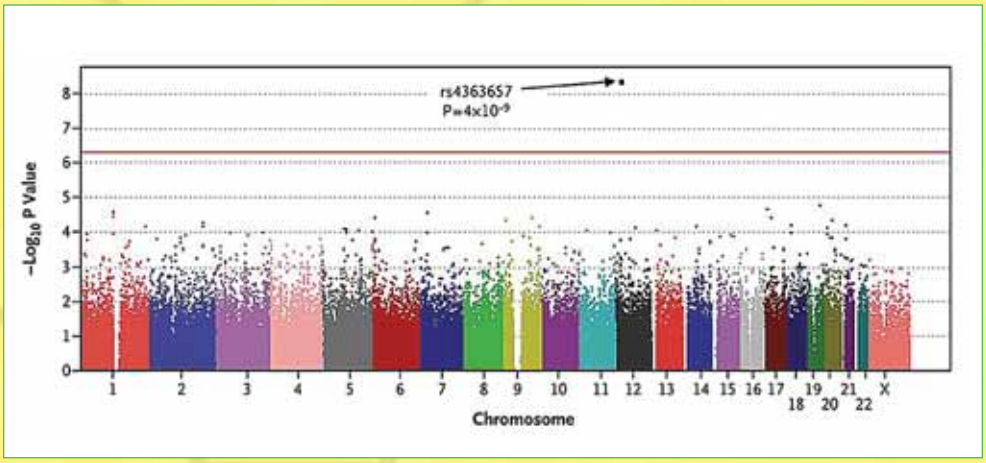
Early pharmacogenetic association studies didn't have the resources to look at the entire genome; instead, they focused on "candidate" genes—those suspected of modulating response to the drug. A 2002 effort comparing 18 cases and 167 controls taking the HIV drug abacavir identified a variant of an immune system-related gene (*HLA-B*) as a possible culprit in causing a toxic skin reaction. In another study, the cancer drug irinotecan was found to be toxic in patients with a variant in the promoter of a gene (*UGTIA1*) that helps the body break down and eliminate certain foreign compounds. Later studies have strongly validated both findings, and genetic testing for sensitivity to abacavir and irinotecan is now widely available.

Although the candidate gene study is a useful tool, it has an important limitation: "You make *a priori* assumptions about which genes may be important," says **Eileen Dolan, PhD**, a cancer pharmacologist at the University of Chicago interested in finding the genetic basis of toxicity to cancer drugs. "In the process, you risk missing some important ones."

Looking Far and Wide

To cast the net wider, many present-day association studies look at the entire genome, or a large fraction of it, using new high throughput platforms such as the "SNP-chip" that can simultaneously probe millions of single point mutations in the DNA (also called single nucleotide polymorphisms, or SNPs). This method has already paid off handsomely in disease studies—discovering a strong suspected link between age-related macular degeneration and a specific variant of a gene involved in inflammation. In pharmacogenetics too, useful results have started trickling in. In April last year a study of warfarin response that examined about half a million SNPs in 181 patients confirmed findings from earlier, smaller studies that the two genes included in the Consortium's dosing algorithm (*VKORC1* and *CYPC29*) are indeed the ones with the greatest impact. Another warfarin study published the same month hunted among about 1,200 markers within 170 pharmacogenes and caught a third gene of interest (*CYP4F2*). Knowing the patient's version of this gene can improve dosing accuracy by an extra five percent (about one milligram per day), the authors report. A study last August used a genome-wide scan of 175 subjects to find that a variant of a membrane transporter gene (*SLCO1B1*) is associated with toxic reactions to the

"In a genome-wide study you come with an open mind," says Eileen Dolan. "When you study that way, you often come up with genes that you didn't even conceptualize could be important to the disease or drug response."



***Statin Response Genes.** Some people who take statins to reduce cholesterol levels end up with a new problem: a type of muscular damage called myopathy. According to a 2008 genome-wide association study of 85 cases and 90 controls, this reaction is strongly associated with a variant of an anion transporter gene (SLCO1B1) on chromosome 12. In this map of 300,000 SNPs, the horizontal axis shows the genomic positions of SNPs grouped by chromosome, and the vertical axis shows the probability of error (p-value) for each SNP-response association. A SNP within SLCO1B1 (the dot above the horizontal line across the chart) had the strongest score of 4 x 10^{-9}. An individual with two copies of this variant has a 17-fold higher risk of statin-induced myopathy. Reprinted with permission from Massachusetts Medical Society:* New England Journal of Medicine *359:789-799 (2008).*

"It is hard to find large sample sizes of patients who are receiving pretty uniform treatment and for whom we have adequate follow-up," says Mary Relling. "That's really the rate-limiting step."

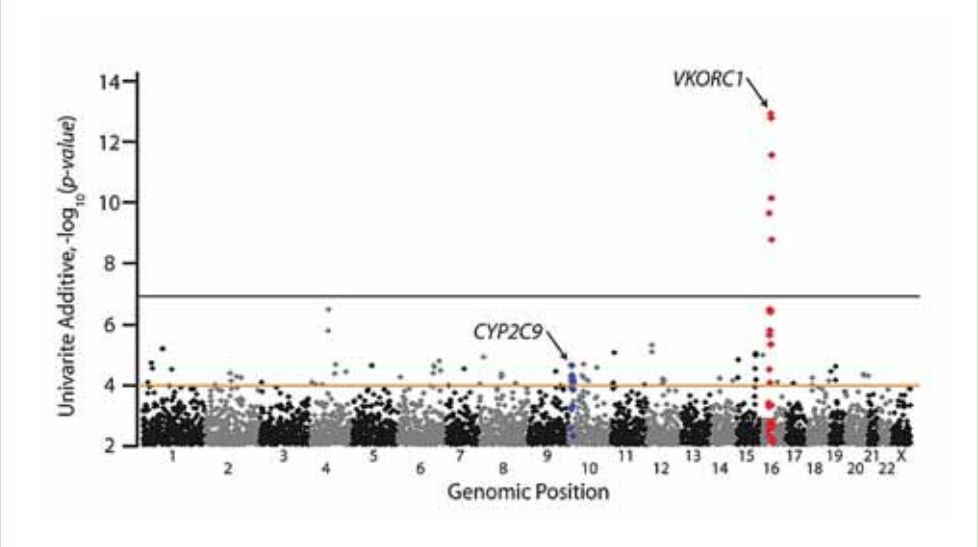
cholesterol-lowering drug simvastatin. "In a genome-wide study you come with an open mind," says Dolan. "When you study that way, you often come up with genes that you didn't even conceptualize could be important to the disease or drug response."

To be able to discriminate reliably between true and false associations, a study that looks at a large number of genetic markers needs a very large number of subjects. This is hard enough to achieve in a disease study—the biggest ones to date boast of a few thousand subjects at most. For drug response studies, a few hundred would be a luxury; follow-up studies to replicate results may have even more difficulty finding subjects. "Not only do you need people with the same disease, you need people treated the same way with the same drug," says Ritchie. And unlike a disease study, Ritchie points out, "all your controls should have the disease as well—they are actually cases, too." As a result, most genome-wide studies tend to be under-powered from a statistical perspective. "It is hard to find large sample sizes of patients who are receiving pretty uniform treatment and for whom we have adequate follow-up," says **Mary Relling, PharmD**, who uses pharmacogenetics to improve drug therapy for children with leukemia at St. Jude's Children's Research Hospital in Memphis. "That's really the rate-limiting step."

One way to gain statistical power is to choose samples carefully. For instance, a study to predict the degree of response to a drug could be better off with 300 low responders and 300 high responders rather than 200 each of low, medium, and high responders. This trick, however, doesn't apply to studies of drug toxicity, where a person has either a normal or an adverse reaction. And for rare, potentially serious adverse responses to drugs, finding enough samples gets even harder. Examples include Stevens-Johnson syndrome, a serious skin rash; rhabdomyolysis, a muscle-destroying condition; QT prolongation, an abnormal heart condition; and drug-induced liver injury. These reactions may strike as few as one patient among several tens of thousands. "Because of the low incidence of such events, it is almost impossible to study them within a single academic institution or at individual pharma companies," says **Andrea Califano, PhD**, a professor of biomedical informatics at Columbia University.

Califano is the head of the data analysis and coordination center of the International Serious Adverse Event Consortium, a pharmaceutical company and Wellcome Trust funded effort to identify the genetic determinants of rare adverse drug reactions. For their Serious Skin Rash study in 2007, the Consortium set up about 20 centers in the United States, the United Kingdom and Canada to enroll study subjects. "Even this massive effort yielded only 71 cases and 135 matched controls," says Califano.

Finding enough samples for a genome-wide study that may examine up to a million SNPs is one challenge; making sense of the huge amount of data generated is another. "It is the typical sorting the wheat from the chaff problem," says **Howard McLeod, PharmD**, of the University of North Carolina at Chapel Hill. "Where there's a whole lot of chaff, there's got to be some wheat in it somewhere." From a statistical per-



***Warfarin Response Genes.** This figure from the genome-wide study by Cooper and his colleagues shows the strength of association of about 500,000 genetic markers with warfarin response. The horizontal axis shows the genomic positions of SNPs grouped by chromosomes. SNPs lying within 500 kilobases of the* **VKORC1** *and* **CYP2C9** *genes are shown in red and blue, respectively. The vertical axis shows the probability of error (p-value) of each SNP-response association. Overall, after accounting for all related SNPs and data from all replication experiments,* **VKORC1** *scored a stunning 4.7 x 10^{-34},* **CYP2C9** *a more modest, but still convincing 6.2 x 10^{-12}. This research was originally published in Blood. Cooper GM, et al., "A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose,"* **Blood** *112: 1022-1027 (2008). © American Society of Hematology.*

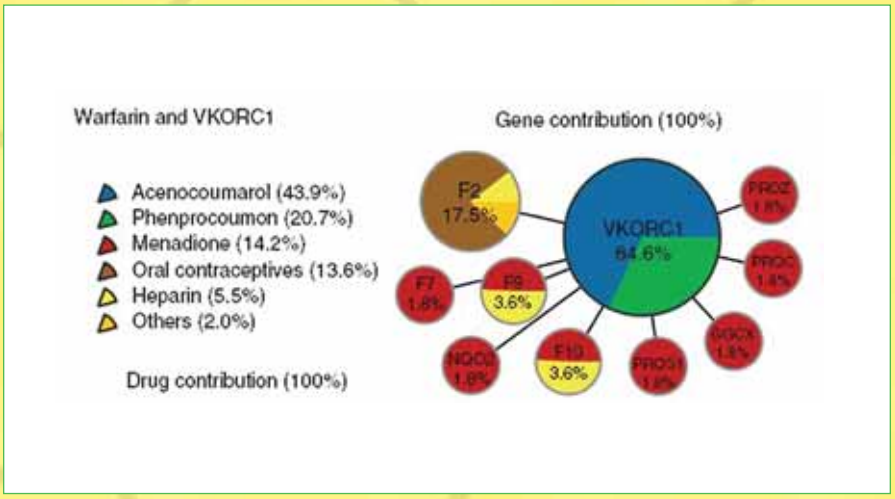
spective, this is a classic multiple-comparison problem with a high risk of false positives—chaff can masquerade as wheat. Several high-profile failures attest to this risk, such as a 2005 study of Parkinson's disease that later studies could not replicate. "The problem with whole genome studies is dealing with extremely wide data matrices," says Srinivasan. "Unless the signal is so ridiculously strong that it jumps out at you, there are strong theoretical reasons to think that such datasets will be highly under-determined."

Studies often overcompensate by being excessively cautious. Consider one that compares *n* genetic markers between cases and controls. To keep the overall probability of making a wrong association below *p*, the study would typically try to keep the risk of getting a false positive from a single comparison below *p/n*. To ensure that a study with 100,000 SNPs has an error rate of 0.01, the error rate for individual SNP comparisons has to be 1.0×10^{-7}—each test would need to be 99.99999 percent reliable. Known as the Bonferroni adjustment, this method assumes the worst-case scenario of the tests being independent. In reality, however, SNPs are usually inherited in bunches and so the tests are not independent. The Bonferroni adjustment ends up being too harsh on the type of moderate-effect association that typifies most gene-drug ties. Alternative scoring schemes exist, but none is adept at the delicate balancing act of finding true gene-drug associations while avoiding false matches. "The statistical tools out there are pretty crude," says McLeod. "They're geared towards preventing false positives, whereas initially what we need is some method that enriches true positives, or helps minimize false negatives."

For GWAS studies, says Howard McLeod, "The statistical tools out there are pretty crude. They're geared towards preventing false positives, whereas initially what we need is some method that enriches true positives, or helps minimize false negatives."

Getting it right: Adding known biological data

One way to enrich true positives is to bring in prior biological information, says Altman. As he points out, a vast amount of data about biological pathways and mechanisms of drug action already exists. To use this information, Altman makes some common-sense assumptions about the interactions of genes and drugs. For instance, genes whose proteins interact with each other are more likely to interact with the same small molecule drugs. At the same time, drugs that have a similar chemical structure, or drugs that are



***Biological Priors.** For a given query drug and indication, Altman and his colleagues use pre-existing biological knowledge in the form of gene-drug, drug-target, and gene-gene interactions to rank genes in the order of pharmacogenetic relevance. This preliminary ranking can then help a genome-wide study focus on important candidates and avoid making false associations. For the blood-thinning drug warfarin, pre-existing knowledge from other vitamin K agonists (acenocoumarol, phenprocoumon), a vitamin K2 precursor (menandione), other blood thinners (heparin), and important genes in the anticoagulation pathway (F2, F9, etc.) leads to a high ranking for VKORC1. The figure shows how different drugs as well as different genes contribute to this ranking. Oral contraceptives may seem out of place, but Altman explains: "Oral contraceptives can cause clotting….and have distant structural similarity to warfarin, and that's what gets them on the list." Reprinted by permission from MacMillan publishers:* **Clinical Pharmacology & Therapeutics,** *Hansen, NT, et al., Generating Genome-Scale Candidate Gene Lists for Pharmacogenomics, (2009).*

chemically dissimilar but treat the same disease, are likely to interact with the same genes. For a given query drug and indication, Altman uses these principles to rank-order all the 12,000 or so genes whose interactions are reasonably well understood. For a blood pressure drug such as nadolol, for instance, his method would prioritize genes that interact directly or indirectly with other beta blockers as well as with other blood pressure medications such as ACE inhibitors or calcium channel blockers. Altman then combines this result with data from a genome-wide study. Doing so helps to move the analysis from the purely statistical realm to one that takes biological mechanisms into account, he says. "Now you are beginning to tell a story other than 'they are correlated.'"

Altman and his team get their prior biological information from three public databases—PharmGKB, DrugBank, and InWeb—for gene-drug, drug-target, and gene-gene interactions, respectively. (It turns out that each gene interacts with about 25 other genes on average while a typical pharmacogene interacts with about 3 drugs.) Based on the prior biological information alone, the now-famous warfarin gene duo (*VKORC1* and *CYP2C9*) ranked 11 and 16, respectively. Based purely on raw experimental data from a genome-wide association study, the genes would rank 25 and 34, says Altman. Combining the prior scores with the experimental data boosted the rankings to 1 and 2. (The genome-wide study too got these final rankings, but using other, less general criteria.)

Altman and his colleagues get similarly encouraging results for some other common drugs using the same approach. "It works amazingly well," says Altman. The key, he says, is to avoid infusing any bias into the analysis of the association data. "You should get your prior, put it in an envelope, and get it time-stamped by the post office," he says. "Otherwise you are going to have a rough idea of what genes are involved, and that is going to poison your prior."

"You should get your prior, put it in an envelope, and get it time-stamped by the post office," says Russ Altman. "Otherwise you are going to have a rough idea of what genes are involved, and that is going to poison your prior."

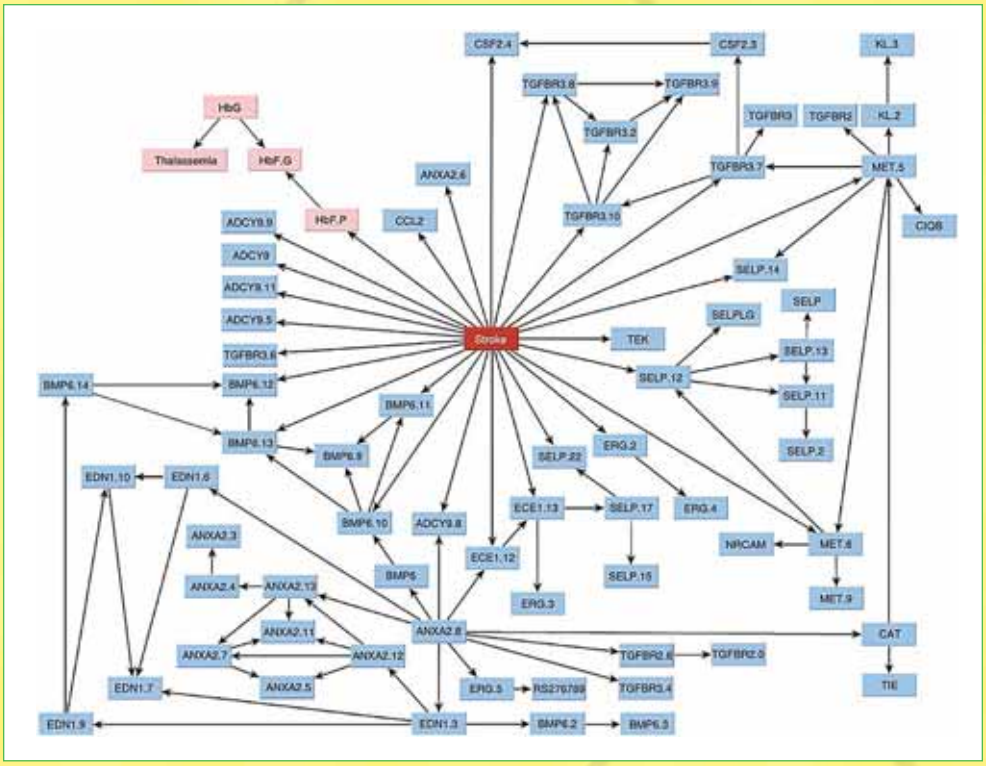
Getting it right: Addressing complexity

While serious drug reactions such as Stevens-Johnson syndrome may have a simple genetic cause, normal drug response may arise from a more complex interplay of genetic factors. Association studies that compare individual SNPs could miss these factors. "The problem is that most of these SNPs and genes by themselves explain only a small amount of the variation," says **Scott Weiss, MD**, a Harvard Medical School researcher who studies the genetic basis of response to asthma drugs. "I am a firm believer that modeling epistasis—gene-gene interactions—is going to be necessary." UCLA systems biologist **Steve Horvath, PhD**, agrees. "Often the different SNPs in a drug response pathway each have only a small effect, and would be terrible biomarkers," he says. "It is only when they interact together that the effects add up to clinical relevance."

However, a naive accounting for SNP interactions would be disastrous. If the SNP-by-SNP study is error prone, imagine what happens when you compare SNP pairs rather than single SNPs—the 100,000-SNP example would now entail nearly 5 billion such tests. The Bonferroni adjustment would run haywire—associations less than 99.9999999998% reliable would be rejected. Compare triplets, quadruplets, or larger SNP cliques, and the combinatorics get hairier, and the reliability requirement even more unreasonable. Goodbye, subtle pharmacogenetic associations! If one does manage to find the causative SNPs or genes, the problems don't end—one now needs to fit them to a model to predict drug response. Standard methods for achieving this, such as logistic regression, wind up with a huge number of possible solutions. Overfit happens.

"Often the different SNPs in a drug response pathway each have only a small effect, and would be terrible biomarkers," says Steve Horvath. "It is only when they interact together that the effects add up to clinical relevance."

To tackle this challenge, some researchers are turning to a classic computer science formalism, the Bayes network. This approach provides an elegant way of exploring a space of gene interaction networks to find the one that best predicts disease or drug response based on the association data. "Bayes methods are almost a hundred years old, but they're designed to handle exactly this kind of problem," says **Marco Ramoni, PhD**, who directs the Biomedical Cybernetics Laboratory at Harvard Medical School. In 2005, Ramoni and his team used this type of analysis to determine which sickle-cell anemia patients were at a high risk for stroke. About one patient in 10 has a



Stroke Risk in Sickle Cell Anemia. This Bayesian network shows how the risk of stroke among people with sickle cell anemia depends on 69 SNPs (blue nodes) in 20 genes and four clinical variables (pink nodes). Twenty-five SNPs on 11 different genes have a direct connection to the trait. By accurately capturing the interaction between SNPs, the network achieves high accuracy (98.2 percent) in predicting risk. Ramoni suggests a similar approach to predict gene-drug associations instead of the SNP-by-SNP comparison between cases and controls that many genome-wide studies employ. Reprinted by permission from MacMillan Publishers LTD, James F Meschia & V Shane Pankratz, Defining stroke risks in sickle cell anemia,* Nature Genetics*, 37: 435-400 (2005).

stroke before they reach 25 years old, but doctors don't know why, and typically medicate everyone. "So 90 percent of them unnecessarily get the therapy, and it's not a pleasant one." Using Bayesian analysis on genetic association data, Ramoni's group found a network of 25 SNPs and 4 clinical factors that could predict the risk with 98.2 percent accuracy. Recently, they used the same method to find a network of 37 SNPs in 20 genes that is 86 percent accurate in predicting the risk of a common type of stroke in the general population. While Bayesian analysis can easily incorporate previous biological knowledge, Ramoni for one doesn't use any. "We make the greatest effort to minimize the amount of prior information that we get," he says. "The process is entirely and happily data driven."

Some researchers are questioning the rationale for going genome-wide in the first place. They point out that such studies implicitly assume that a handful of common gene variants account for most of the differences in disease (or drug response) susceptibility. There is increasing evidence that this "common disease/common variant" hypothesis is not valid, even for classic "single gene" disorders such as phenylketonuria (for which 531 genetic variants have been found so far). Drug response, being a complex trait, is likely to be even more diverse. "Most studies tend to ignore rare variants completely," says **Robert Elston, PhD**, a biostatistician at Case Western Reserve University. "I find that unrealistic."

Finding rare variants may need a different strategy, one that leverages prior biological information to look at specific areas of the genome at a resolution 10-fold or greater than current genome-wide studies—trading breadth for depth. Made possible by dramatic recent improvements in the speed and cost of DNA sequencing, this "deep resequencing" strategy is rapidly emerging as one of the most exciting tools for pharmacogenetics. In a sense it is a return to candidate gene approach, but with more powerful technology. "It is cheaper and more accurate than doing genome-wide studies," says Duke University researcher **Allen Roses, MD**, who has used this method to find genetic variants linked to Alzheimer's disease. "We are getting spectacular results from it."

Putting it to work

Thanks to these rapid advances in pharmacogenetics, we could soon have the technology to predict drug response more accurately and for a wider range of medications. However making this technology available for routine clinical use could be a challenge.

Consider the poster child of drug response prediction, warfarin. The marriage between the gene pair (*VKORC1* and *CYPC29*) and warfarin response is blessed with near-perfect statistical scores, clear-cut biological explanations, consensus among researchers, a simple dosing scheme, and the FDA's approval. Moreover, a 2008 report by the American Enterprise Institute-Brookings Joint Center estimates that routine genetic testing prior to warfarin therapy would prevent 85,000 serious bleeding events and 17,000 strokes, saving $1.1 billion a year. It's a pharmacogenetic dream scenario.

Yet the concrete achievement—an increase in predictive value from 30 percent to 50 percent—doesn't impress everyone. Some feel that this is not worth the additional cost of genotyping each patient (about $400 currently), disputing the bullish prediction in the Brookings report. For instance, a study in the *Annals of Internal Medicine* this January estimates that pharmacogenetics-based warfarin dosing would cost more than $170,000 per quality-adjusted life year gained, or QALY, for patients with a certain type of heart condition. (Medical interventions that cost more than about $50,000 per QALY are typically not considered cost-effective.) "Genetic Testing for Warfarin Dosing? Not Yet Ready for Prime Time" argues another paper published in *Pharmacotherapy* in May 2008. The authors point out that the theoretical benefits of genotype-based warfarin dosing have yet to be backed up by clinical data that demonstrate practical

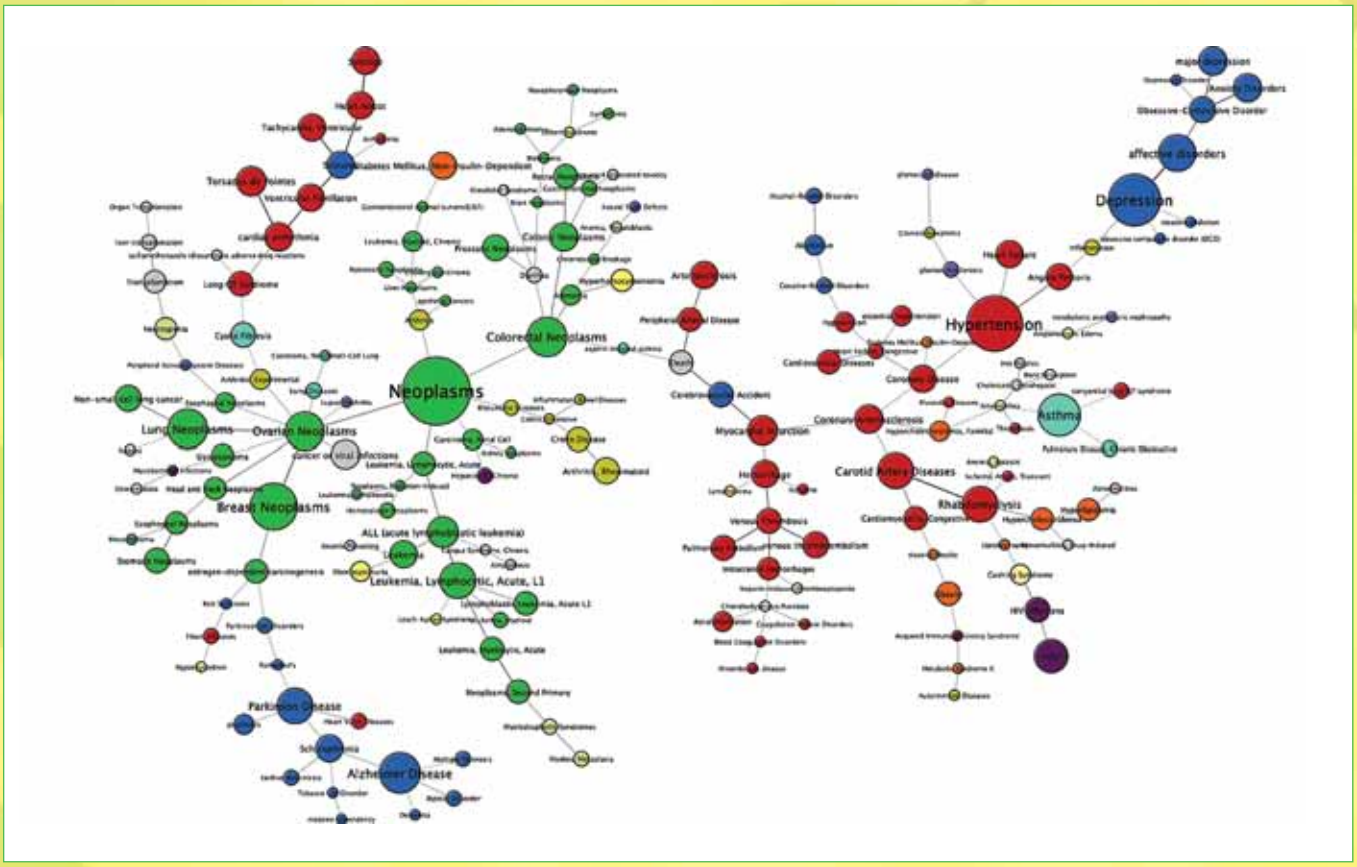
benefit. They express concern that clinicians may place blind faith in the new dosing scheme and ignore the time-tested methods of monitoring the patients' response to the drug.

The latest blow is a rejection from Medicare. While acknowledging that "there is good evidence that persons who have these variant [*CYP2C9* and *VKORC1*] alleles have heightened warfarin responsiveness," the Centers for Medicare and Medicaid Services ruled on May 4, 2009, that "the evidence for improved health outcomes attributable to pharmacogenomic testing to determine warfarin responsiveness fails (as of this writing) to meet the standards of evidence to establish a basis for coverage."

Altman finds this attitude "disappointing." He feels that new medical advances with great potential to save lives should not be held back due to lack of clinical data. He points out that many medical practices are based on sense and evidence, not on randomized clinical trials (RCTs). Blood transfusion, for instance, is a standard medical procedure that has never been validated in a controlled trial. "I don't think pharmacogenomics should be held to the RCT standard unless the rest of medicine is willing to be based on that," Altman says. As for cost, he feels that with increasing use, genotyping will get cheaper and cheaper and eventually be almost free, "which means benefit/cost = infinity." Many other experts agree. One study on HIV patients at a UK clinic, for instance, found that genetic testing for hypersensitivity to abacavir could save about 22,000 Euro (about $30,000) per hypersensitivity reaction that was avoided. "Pre-prescription pharmacogenetic testing for this appears to be a cost-effective use of health care resources," the authors conclude.

While the medical community continues to debate pharmacogenetic testing, regulatory agencies seem more positive. During the past few years, the FDA has approved genetic tests for several medications including warfarin and the cancer drug irinotecan. "We're seeing some development towards point-of-care testing, with results available in 45 minutes or so of the patient giving a sample," says McLeod. However, he points out, clinical medicine doesn't yet have the tools to put this type of lab result to good use. For instance, he asks, if a test shows that a patient has the *CYP2C9*3* gene variant, is that good or bad? What does it mean for drug dosing? "Most practitioners don't have a clue," he says. "Just because we have a test doesn't mean we're smarter."

For warfarin, researchers have created a web site, http://warfarindosing.org, that does answer some of these questions; McLeod believes it is high time biologists created more tools like this so that we can begin to reap the benefits of pharmacogenetics. "We have all the genetic information we need at birth," he says. "In the ideal world we'll carry that with us and use it when needed." □



Pharmacogenetic Tree. Many diseases share pharmacogenetic interactions with the same drugs. Here, each node represents a disease and is shown proportional in size to the number of drugs available to treat it. Each disease is connected to another with which it shares the maximum number of drug-gene interactions. This information comes from the pharmacogenetic database PharmGKB housed at Stanford University. According to PharmGKB project director Teri Klein, PhD, the database contains information on about 650 drugs with gene-dependent responses and 1,890 genes known to modulate drug response—of which 39 are rated as Very Important Pharmacogenes, or VIP genes, because of their broad impact. Reprinted by permission from MacMillan publishers:* Clinical Pharmacology & Therapeutics, *Hansen, NT, et al., Generating Genome-Scale Candidate Gene Lists for Pharmacogenomics, (2009).

FDA-Approved Drug Warnings with Pharmacogenomic Information

Abacavir
(Treats HIV-1)

FDA Warning:
"WARNING: RISK OF HYPERSENSITIVITY REACTIONS . . . Patients who carry the HLA-B*5701 allele are at high risk for experiencing a hypersensitivity reaction to abacavir. Prior to initiating therapy with abacavir, screening for the HLA-B*5701 allele is recommended; . . ."

Variants listed in drug label: HLA-B*5701

FDA Requirements: Testing recommended, not required

Azathioprine
(Immunosuppressant)

FDA Warning:
"It is recommended that consideration be given to either genotype or phenotype patients for TPMT."

Variants listed in drug label:
TPMT*2, TPMT*3A, TPMT*3C

FDA Requirements:
Testing recommended, not required

Carbamazepine
(Treats epilepsy and neuralgia)

FDA Warning:
"Patients with ancestry in genetically at-risk populations should be screened for the presence of HLA-B*1502 prior to initiating treatment with Tegretol. Patients testing positive for the allele should not be treated with Tegretol unless the benefit clearly outweighs the risk . . ."

Variants listed in drug label:
HLA-B*1502

FDA Requirements:
Testing recommended, not required

Dasatinib
(Treats leukemia)

FDA Warning:
The FDA requires testing for Philadelphia chromosome-positive status and resistance or intolerance to prior therapy prior to initiating treatment of acute lymphoblastic leukemia (ALL) with dasatinib.

Variants listed in drug label:
BCR-ABL

FDA Requirements:
Testing required

Irinotecan
(Treats cancer)

FDA Warning:
"When administered in combination with other agents, or as a single-agent, a reduction in the starting dose by at least one level of CAMPOSTAR should be considered for patients known to be homozygous for the UGT1A1*28 allele..."

Variants listed in drug label:
UGT1A1*28

FDA Requirements:
Testing recommended, not required

Imatinib
(Treats cancer)

FDA Warning:
The decision of whether to treat patients with imatinib is based on the presence of genetic biomarkers, including BCR-ABL (the Philadelphia chromosome), KIT, and PDGFR gene rearrangements.

Variants listed in drug label:
BCR-ABL, KIT:D816V

FDA Requirements:
Testing required

Warfarin
(Treats blood pressure)

FDA Warning:
"The lower initiation doses should be considered for patients with certain genetic variations in CYP2C9 and VKORC1 enzymes . . ."

Variants listed in drug label:
VKORC1:G-1639A (rs9923231), CYP2C9*2 (rs1799853), CYP2C9*3 (rs1057910), CYP2C9*5 (rs28371686), CYP2C9*6 (rs9332131), CYP2C9*9 (rs2256871), CYP2C9*11 (rs28371685)

FDA Requirements:
Testing recommended, not required