Genomics and Drug Response

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PHARMACOGENOMICS IS THE STUDY OF THE ROLE OF INHERITED AND ACquired genetic variation in drug response.[1] Clinically relevant pharmacogenetic examples, mainly involving drug metabolism, have been known for decades, but recently, the field of pharmacogenetics has evolved into "pharmacogenomics," involving a shift from a focus on individual candidate genes to genomewide association studies. Such studies are based on a rapid scan of markers across the genome of persons affected by a particular disorder or drug-response phenotype and persons who are not affected, with tests for association that compare genetic variation in a case–control setting.[2] An example is provided in this issue of the *Journal*: McCormack and colleagues, testing for genomewide association, identified an HLA allele that is associated with hypersensitivity reactions to the anticonvulsant and mood-stabilizing drug carbamazepine in persons of European descent.[3] Pharmacogenomics facilitates the identification of biomarkers that can help physicians optimize drug selection, dose, and treatment duration and avert adverse drug reactions. In addition, pharmacogenomics can provide new insights into mechanisms of drug action and as a result can contribute to the development of new therapeutic agents.

In 2003, two reviews of pharmacogenetics were published the *Journal*.[4,5] Since then, both genomic science and its application to drug response have undergone major advances.[6] Here we review some of those advances, with an emphasis on discovery through genomewide association studies. We describe examples that highlight principles of pharmacogenomics that are relevant to a wide variety of drugs. The Food and Drug Administration (FDA) has altered drug labels and issued warnings about pharmacogenomic variation affecting drug response, raising the issue of the level of evidence required to show clinical utility[7] and the respective roles of regulatory agencies such as the FDA and of academic and professional societies in the evaluation of pharmacogenetic analyses for the clinic.

CARDIOVASCULAR DRUGS

Many drugs have proven efficacy in the treatment and prevention of cardiovascular disease. Not uncommonly, these drugs have narrow therapeutic indexes that are influenced by genetic variation — a hallmark of drugs for which pharmacogenomic approaches are likely to provide substantial clinical benefit. The anticoagulant agents warfarin and clopidogrel are high on the list of widely prescribed cardiovascular drugs with narrow therapeutic indexes. The pharmacogenomic features of these drugs illustrate the rapid evolution of our understanding of the role of inheritance in the variation in drug efficacy and the risk of adverse drug reactions. In the case of both agents, the application of classic candidate-gene pharmacogenetics

has identified important genomic markers of variation in efficacy and adverse reactions, observations that were subsequently confirmed in genomewide association studies. The FDA acted quickly on these data by relabeling warfarin and adding a warning box on the labeling for clopidogrel. Data supporting the clinical utility of routine use of pharmacogenetic testing for both these drugs are evolving.[8-10]
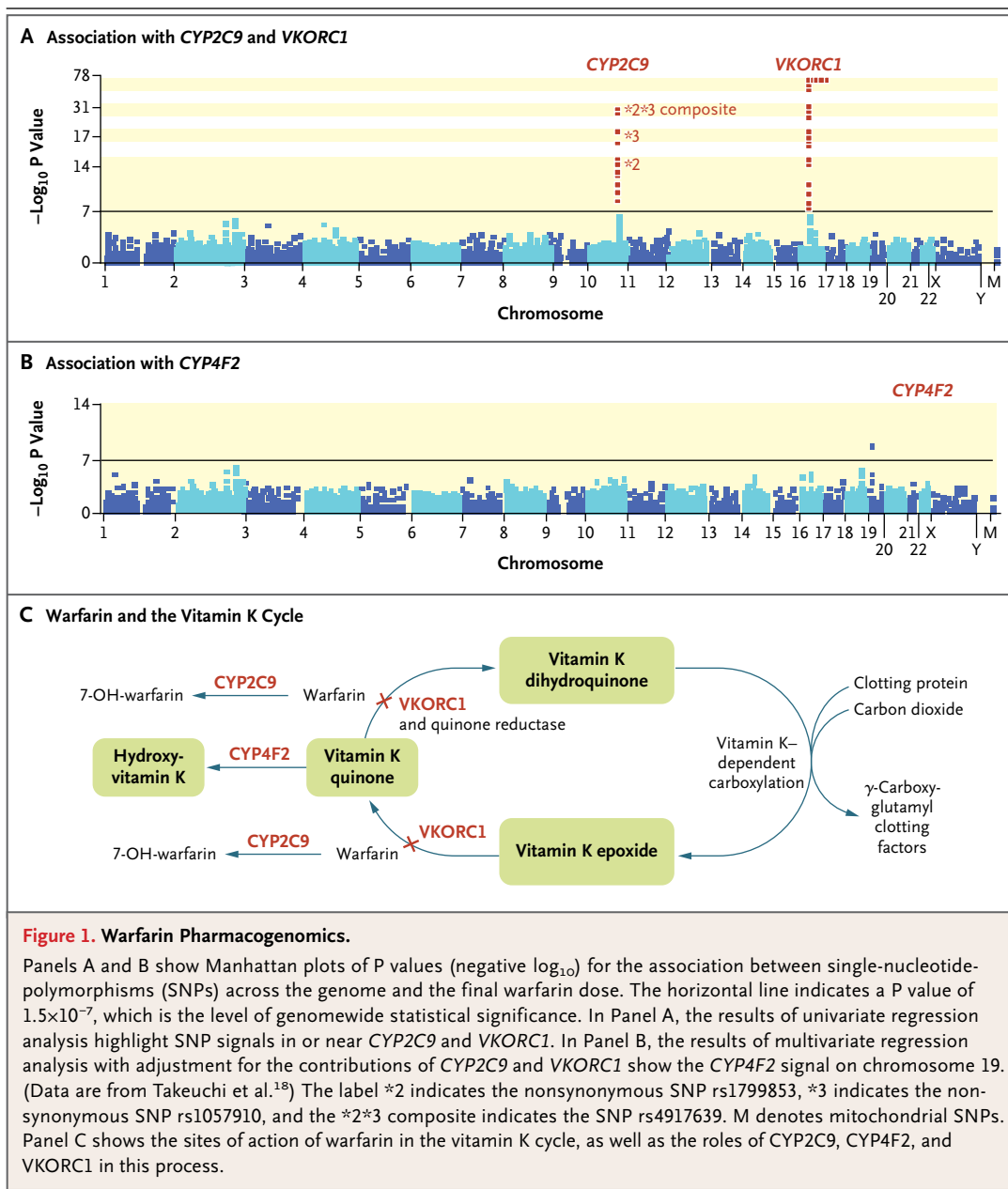
Warfarin is the most widely prescribed oral anticoagulant in North America and much of Europe.[11] Despite the availability of the international normalized ratio (INR), a laboratory test that is universally used to measure the anticoagulant effect of warfarin, serious adverse responses, including hemorrhage and undesired coagulation, continue to complicate therapy, making warfarin one of the drugs most often responsible for emergency room visits.[12,13] Chemically, warfarin is a racemic mixture (i.e., one that is composed of two enantiomorphic isomers). S-warfarin is three to five times as potent as R-warfarin as an anticoagulant, has a shorter half-life, and is metabolized predominantly by a cytochrome P-450 enzyme, CYP2C9.[11] Two common CYP2C9 allozymes (see Glossary) have only a fraction of the level of enzyme activity of the wild-type allozyme CYP2C9*1: 12% for CYP2C9*2 and 5% for CYP2C9*3.[11,14] More than a decade ago, it was reported that patients who required a low final dose of warfarin on the basis of INR values often carried one or two of these two common CYP2C9 variant alleles and were at increased risk for hemorrhage during warfarin therapy, presumably because they metabolized the drug more slowly.[14] Those observations were confirmed, but it quickly became clear that the presence of CYP2C9 polymorphisms did not explain most of the variation in the final warfarin dose.

Pharmacogenetic studies of warfarin changed dramatically in 2004 when the target for warfarin-based anticoagulants, vitamin K epoxide reductase complex subunit 1 (VKORC1), was identified,[15,16] and single-nucleotide polymorphisms (SNPs) in VKORC1 were shown to be associated with the dose of warfarin required to achieve a target INR value.[17] In 2009, a genomewide association study looked for associations between several hundred thousand SNPs and warfarin dose in about 1000 Swedish patients who were taking warfarin. The results showed two major signals in and around CYP2C9 and VKORC1 (Fig. 1A).[18] When the authors removed the effects of those signals through multiple regression adjustment, they observed an additional signal, implicating another cytochrome P450 gene (CYP4F2) (Fig. 1B). CYP4F2 was subsequently shown to catalyze vitamin K oxidation.[19] The variant CYP4F2 allozyme shows decreased ability to catalyze the reaction, and as a result persons who carry the relevant genetic variant in CYP4F2 might require an increase in the warfarin dose (Fig. 1C). CYP2C9, VKORC1, and CYP4F2 have also been implicated in a genomewide association study of the administration of acenocoumarol, an anticoagulant related to warfarin.[20]

Taken together, CYP2C9 and VKORC1 genotypes explain about 30 to 40% of the total variation in the final warfarin dose.[21] These observations raise the possibility that testing patients for variations in CYP2C9 and VKORC1 might provide information that could enhance clinical algorithms currently used to guide the administration of warfarin. To examine the potential clinical utility of testing for CYP2C9 and VKORC1 genotypes, in addition to INR monitoring and routine use of clinical algorithms, the International Warfarin Pharmacogenetics Consortium recently investigated the anticoagulant response to warfarin, as well as CYP2C9 and VKORC1 genotype data, for about 4000 persons of various ancestral origins. The investigators compared therapeutic outcomes with the application of standard clinical algorithms that included age, sex, and INR values and outcomes with the use of an algorithm that included CYP2C9 and VKORC1 genotype information and concluded that the addition of genotype information enhanced outcomes, especially for patients who required unusually high or low warfarin doses.[22] CYP4F2 was not included in this algorithm but has been included in several algorithms developed more recently.[23,24] Consistent with this conclusion are the results of a study comparing nearly 900 pa-

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Glossary

Allozyme: Alternate versions of an enzyme determined by genetic variants (alleles) present at a genetic locus.

Gene cluster: Two or more genes in close physical proximity in the genome that encode similar gene products.

Genomewide association study: An approach used in genetics research to look for associations between large numbers (typically hundreds of thousands) of specific genetic variations (most commonly single-nucleotide polymorphisms) and particular diseases.

Single-nucleotide polymorphism: A single-nucleotide variation in a genetic sequence; a common form of variation in the human genome.

Figure 1. Warfarin Pharmacogenomics.

Panels A and B show Manhattan plots of P values (negative $\log_{10}$) for the association between single-nucleotide-polymorphisms (SNPs) across the genome and the final warfarin dose. The horizontal line indicates a P value of 1.5×10^{-7}, which is the level of genomewide statistical significance. In Panel A, the results of univariate regression analysis highlight SNP signals in or near *CYP2C9* and *VKORC1*. In Panel B, the results of multivariate regression analysis with adjustment for the contributions of *CYP2C9* and *VKORC1* show the *CYP4F2* signal on chromosome 19. (Data are from Takeuchi et al.[18]) The label *2 indicates the nonsynonymous SNP rs1799853, *3 indicates the non-synonymous SNP rs1057910, and the *2*3 composite indicates the SNP rs4917639. M denotes mitochondrial SNPs. Panel C shows the sites of action of warfarin in the vitamin K cycle, as well as the roles of CYP2C9, CYP4F2, and VKORC1 in this process.

tients for whom genetic information on *CYP2C9* and *VKORC1* was made available to prescribing physicians with a matched historical control group of patients who were started on warfarin therapy without genetic information.[25] Six months after the initiation of warfarin therapy, hospitalizations for hemorrhage were 28% less common in the group of patients for whom genetic information on *CYP2C9* and *VKORC1* had been supplied to prescribing physicians than in the control group (Fig. 2).

The FDA revised the label on warfarin in February 2010, providing genotype-specific ranges of doses and suggesting that genotypes be taken into consideration when the drug is prescribed. The wide availability of *CYP2C9* and *VKORC1* genotyping and the release of both Web-based and personal decision-support tools have facilitated the clinical use of this information. Nevertheless, the clinical adoption of genotype-guided administration of warfarin has been slow, even though the evidence supporting such adoption is similar to

the evidence supporting currently used clinical variables, such as age, drug interactions, and ancestral origin. Some observers have expressed a need for prospective assessment of the value of this genetic information in warfarin therapy, and several prospective clinical trials are ongoing.[26] Alternative anticoagulant therapies are also being developed that might replace warfarin, perhaps in patients with genotypes associated with extreme variation in warfarin response.[27]

Clopidogrel inhibits adenosine diphosphate (ADP)–stimulated platelet activation by binding irreversibly to a specific platelet receptor of ADP, $P2Y_{12}$, thus inhibiting platelet aggregation.[28,29] Dual antiplatelet therapy — clopidogrel and aspirin — has been shown to decrease the risk of subsequent ischemic vascular events.[30-32] However, clopidogrel is a prodrug that requires metabolic activation in a reaction catalyzed by another cytochrome P-450 enzyme, CYP2C19. Like *CYP2C9*, *CYP2C19* is genetically polymorphic with a common SNP that results in a truncated protein product with little enzymatic activity.[33] Several studies have shown that genetic variation in *CYP2C19* resulting in a paucity of activity is associated with decreased clopidogrel metabolic activation, a decreased antiplatelet effect, and an increased likelihood of a cardiovascular event.[34,35] These observations have been confirmed in a genomewide association study.[34]

Early in 2010, the FDA added a boxed warning to prescribing information for clopidogrel, stating that persons with a *CYP2C19* variant encoding a form of the enzyme associated with a low rate of metabolism might require dose adjustment or the use of a different drug.[36] After this FDA action, the American Heart Association and the American College of Cardiology issued a joint endorsement of *CYP2C19* genotyping for patients at moderate or high risk for cardiovascular events who are treated with clopidogrel.[37] This genetic test is widely available in the United States. However, enthusiasm for its use has been muted, owing to a lack of clarity with regard to the optimal treatment of patients who carry a *CYP2C19* variant, as shown by data from two large, randomized trials in which *CYP2C19* genotyping did not have a significant effect on the incidence of cardiovascular events among patients with acute coronary syndromes or atrial fibrillation.[38,39] On the other hand, in a recent meta-analysis of data from nine pharmacogenetic studies of clopido-
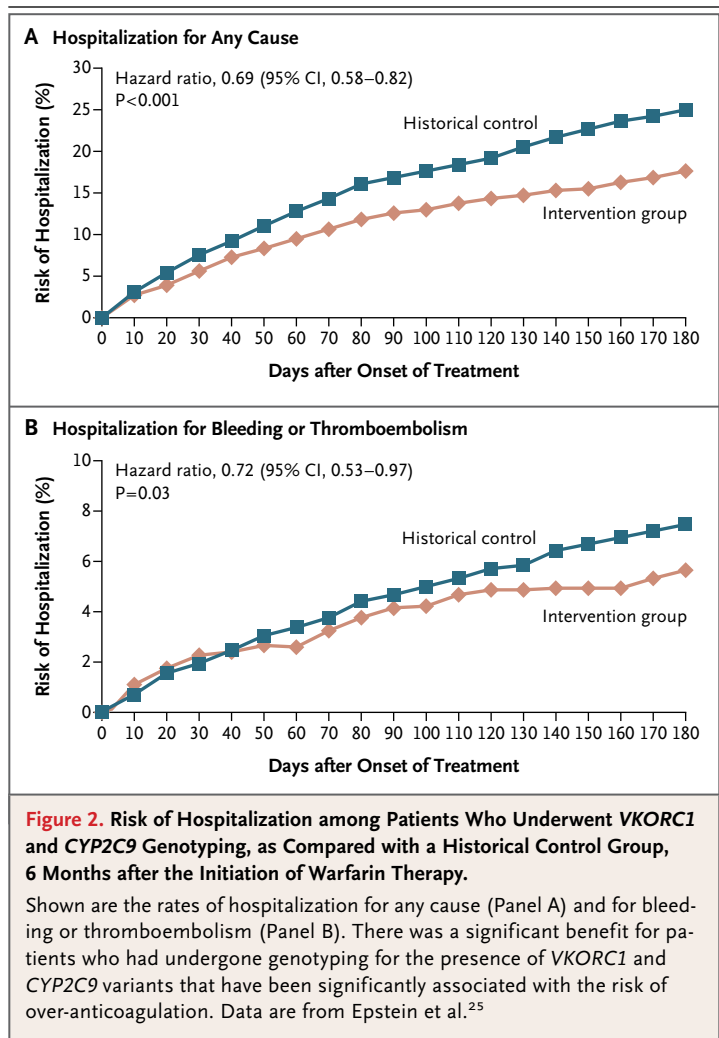

Figure 2. Risk of Hospitalization among Patients Who Underwent *VKORC1* and *CYP2C9* Genotyping, as Compared with a Historical Control Group, 6 Months after the Initiation of Warfarin Therapy.

Shown are the rates of hospitalization for any cause (Panel A) and for bleeding or thromboembolism (Panel B). There was a significant benefit for patients who had undergone genotyping for the presence of *VKORC1* and *CYP2C9* variants that have been significantly associated with the risk of over-anticoagulation. Data are from Epstein et al.[25]

grel involving 9685 patients who had an acute coronary syndrome or were undergoing percutaneous coronary intervention, there was a significant association between homozygosity or heterozygosity for *CYP2C19* reduced-function alleles and an increased risk of death from cardiovascular causes, myocardial infarction, or stroke.[9] At present, it is unclear whether genotyping to predict the response to clopidogrel is clinically useful. Several studies are under way to assess the effect of dose adjustment for clopidogrel in patients who carry *CYP2C19* variant alleles.[8]

AGENTS USED FOR INFECTIOUS DISEASES

Genomewide association studies have confirmed the identity of genetic variants in previously im-

plicated candidate genes that contribute to clinically important outcomes, including severe idiosyncratic adverse reactions and variation in drug efficacy. In the next set of examples, the results of pharmacogenomic studies were unanticipated.

Hepatotoxicity is the most common reason for the termination of clinical trials investigating the efficacy of new drugs, accounting for approximately 33% of such terminations, and is a major reason for postmarketing drug withdrawal.[40] Floxacillin, an antibiotic used in Europe and Australia to treat staphylococcal infections, has been associated with an unusual form of cholestatic hepatitis, with an estimated incidence of approximately 8.5 cases per 100,000 patients.[41-44] A multicenter genomewide association study, reported in 2009, analyzed the genotypes of 51 persons with floxacillin-induced hepatic injury and 282 matched controls.[45] A SNP in the major histocompatibility complex and closely linked with HLA-B*5701 showed very strong association with hepatic injury. The association between the presence of HLA-B*5701 and hypersensitivity reactions to abacavir, a nucleoside analogue used to treat human immunodeficiency virus type 1 infection, had already been reported,[46-48] which resulted in the FDA modification of the abacavir label to include a recommendation that patients undergo genotyping for HLA-B*5701 before the initiation of therapy.[49] Rare but severe adverse events represent a major reason why drugs are withdrawn after FDA approval. Although it was possible to attempt a replication of the association between the variant in HLA-B*5701 and floxacillin-induced hepatitis,[45] it is often difficult to gather enough cases of rare adverse drug reactions to apply genomewide techniques.

This situation presents a challenge for regulators. To date, the FDA has generally chosen to include pharmacogenetic information relevant to rare severe adverse events on drug labels — even when the association between the variant and drug response has not been replicated — so as to warn prescribers of potential risk.[50] This approach places a burden on clinicians to use their own judgment regarding the need for pharmacogenetic testing before prescribing a drug. In contrast with unreplicated tests for association are prospective trials of genotyping to avoid adverse pharmacogenetic effects. One such study is reported in this issue of the *Journal*,[51] in which investigators observed no instances of the Stevens–Johnson syndrome or toxic epidermal necrolysis in

a sample of nearly 5000 Taiwanese candidates for carbamazepine therapy, among whom carbamazepine had been withheld from carriers of the HLA-B*1502 allele, which has been reported to be associated with the Stevens–Johnson syndrome in Han Chinese.[52]

Another pharmacogenomic example involving agents used to treat infectious diseases concerns the treatment of chronic infection with hepatitis C virus (HCV), which develops in approximately 80% of patients who are infected with the virus and is a major cause of liver failure.[53,54] Successful treatment of chronic HCV infection involves a sustained virologic response, which is defined by an undetectable level of HCV RNA in plasma. Unfortunately, only 40 to 50% of patients who are infected with HCV genotype 1 have a sustained virologic response when receiving the current standard of care for the treatment of chronic HCV infection — injections of pegylated interferon alfa together with oral ribavirin for 48 weeks.[53,54]

The ability to identify patients with a differential response to pegylated interferon alfa is important in the current era of new anti-HCV drugs because pegylated interferon alfa remains the backbone of therapy, to which many of these new agents are added. Recently, in three independent genomewide association studies[55-57] involving patients with chronic HCV infection who were treated with pegylated interferon alfa and ribavirin, there was an association between a variant in *IL28B*, the gene encoding interleukin-28B, and the drug response. In one of these studies, peripheral-blood mononuclear cells from patients carrying the variant allele that was associated with a poor response had comparatively low levels of IL28B expression.[56] *IL28B* encodes a protein that is thought to be involved in suppressing the replication of a number of viruses, including HCV.[55-58] This example shows how pharmacogenomic genomewide association studies not only have identified biomarkers of response to pegylated interferon alfa but also have provided insights that might be used to determine therapeutic approaches to this chronic infection and to select a drug target for therapeutic development.

ANTINEOPLASTIC DRUGS

The field of cancer pharmacogenomics is complicated by the fact that two genomes are involved: the germline genome of the patient and the somatic genome of the tumor. Obviously, the tumor

genome plays a critical role in the variation in response to antineoplastic therapy. Prominent examples include *HER2* overexpression or amplification in patients with breast cancer and the response of these tumors to trastuzumab[59,60] and increased sensitivity to the epidermal growth factor receptor (EGFR) antagonist gefitinib among patients with non–small-cell lung cancer who have activating mutations in the gene encoding EGFR.[61,62] A recent example involves melanoma and a mutation in *BRAF* encoding a serine–threonine protein kinase. Since a specific inhibitor, PLX4032, targets the mutant activated kinase, there is a pharmacogenetic effect in that PLX4032 prolongs survival in patients carrying the mutation. This clinical finding was based on the discovery of a *BRAF* mutation through the sequencing of a large number of kinase genes in tumors.[63,64] On the other hand, germline SNPs in the gene encoding the enzyme thiopurine S-methyltransferase (*TPMT*) can result in increased sensitivity to mercaptopurine as a result of decreased metabolism,[1,4,5] whereas the number of TA dinucleotide repeats in the promoter of *UGT1A1* in germline DNA can increase the toxic effects of irinotecan, also as a result of decreased metabolism.[1,65] There are now many examples of pharmacogenetic tests paired with anticancer drugs that are considered part of routine oncologic care (Table 1). The fact that clinically relevant pharmacogenomic variation in both the tumor genome and the patient's germline genome can influence the response to antineoplastic therapy is illustrated in Figure 3, with gefitinib and irinotecan as examples.

AROMATASE INHIBITORS

Genetic polymorphisms in a patient's germline genome can also play an important role in variation in the response to cancer therapy. Endocrine therapy of breast cancer[66,67] offers a striking example of how a genomewide association study has lead to the identification of a mechanism that would seem to be responsible for a serious drug-induced adverse reaction that limits therapeutic options for some patients.

The tumors of approximately 70% of postmenopausal women with breast cancer express the estrogen receptor. The blockade of this receptor with tamoxifen or the blockade of estrogen synthesis through the inhibition of aromatase (which catalyzes estrogen synthesis) halves the recur-

Table 1. Anticancer Drugs Approved by the Food and Drug Administration (FDA) with Labeling Regarding Pharmacogenomic Biomarkers.*

Type of Biomarker and Associated Drug
Biomarker with pharmacokinetic effect
TPMT
Mercaptopurine
Thioguanine
UGT1A1
Irinotecan
Nilotinib
Biomarker with pharmacodynamic effect
EGFR
Cetuximab
Erlotinib
Gefitinib
Panitumumab
KRAS
Cetuximab
Panitumumab
ABL
Imatinib
Dasatinib
Nilotinib
C-Kit (*KIT*)
Imatinib
HER2/neu (*ERBB2*)
Lapatinib
Trastuzumab
Estrogen receptor
Tamoxifen

* Data are from the FDA's pharmacogenetics Web site (www.fda.gov/Drugs/ScienceResearch/ResearchAreas/ Pharmacogenetics/ucm083378.htm). The biomarkers have been separated into pharmacokinetic effect (drug metabolism) and pharmacodynamic effect (drug target). Biomarkers for cytogenetic alterations have been excluded.

rence rate.[66-68] However, the administration of an aromatase inhibitor can also result in severe musculoskeletal pain that leads women (10 to 20% in some studies) to terminate therapy.[67] In a genome-wide association study that used DNA samples from a large clinical trial of aromatase inhibitors to treat women with breast cancer (called MA.27) (ClinicalTrials.gov number, NCT00968214), there was an association between musculoskeletal pain and variants in the gene cluster encoding T-cell leukemia–lymphoma (TCL) proteins. The marker showing the strongest (although not significant) association created a new estrogen-response element close to *TCL1A*.[69] Functional studies showed that the markers that were associated with susceptibility to musculoskeletal pain were also as-
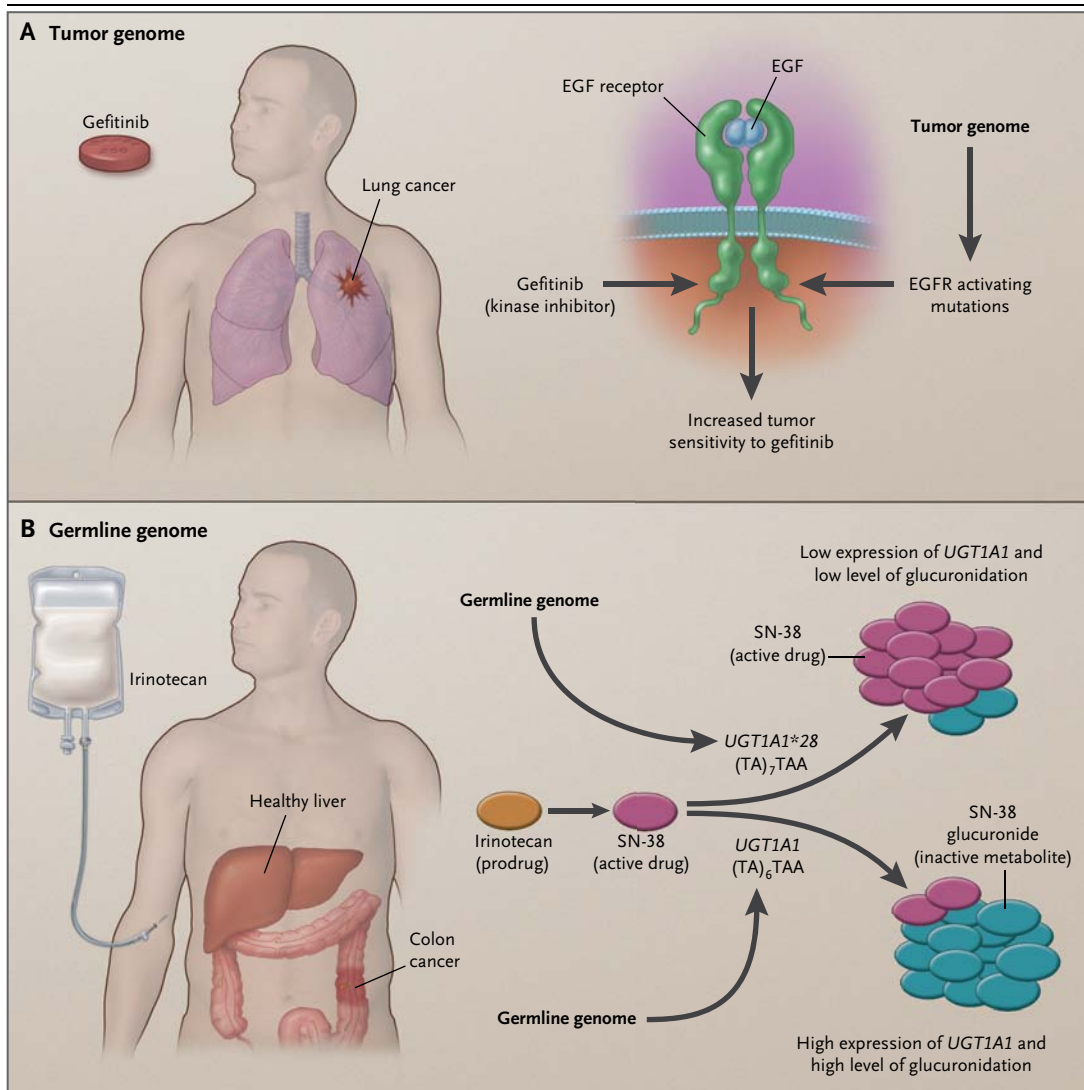
Figure 3. Cancer Pharmacogenomics and Tumor and Germline Genomes.

Both the tumor genome (e.g., in the case of gefitinib therapy) and the patient's germline genome (e.g., in the case of irinotecan therapy) can contribute to pharmacogenomic variation in response to antineoplastic drugs. The tumor genome plays a critical role in the response to gefitinib (Panel A), since the sensitivity of non–small-cell lung cancer to this drug is enhanced by activating mutations in the kinase domain of the gene encoding epidermal growth factor receptor (*EGFR*).[58,59] Tumor *EGFR* encoding activating mutations within the kinase domain results in enhanced tumor sensitivity to gefitinib. The rate of toxic effects associated with irinotecan (diarrhea and myelosuppression) is increased in patients with seven TA dinucleotide repeats rather than the more common six repeats in the promoter region of *UGT1A1* encoding a UDP-glucuronosyltransferase in germline DNA, resulting in lower enzyme activity and a decreased rate of drug metabolism (Panel B).[1,62]

sociated with increased *TCL1A* expression after estrogen exposure. TCL1A regulates the expression of interleukin-17 receptor A, an experimental target for the treatment of patients with rheumatoid arthritis.[70] These observations, if confirmed, may provide new insight into the relationship between estrogens and joint pain.

This example illustrates several challenges and opportunities associated with pharmacogenomic studies and their application to clinical practice. First, associations that are uncovered by genome-wide association studies require replication if there are appropriate sample sets. However, MA.27 is a large clinical trial of aromatase inhibitors, span-

ning 8 years at a cost of more than $35 million. Therefore, identifying a large and appropriate sample to test for replication will be difficult. In cases in which replication samples are not available or are difficult to obtain, pharmacogenomic studies may benefit from the use of functional validation to help verify the results of genomewide studies. For example, the biologic plausibility that is provided by the functional data (i.e., the association between phenotype-associated markers and *TCL1A* expression) increases confidence that the genetic association is driven by biology rather than chance. A final consideration is the clinical context. Because aromatase inhibitors have only a slight benefit over tamoxifen in the treatment of breast cancer, and tamoxifen is much less expensive than aromatase inhibitors, a clear therapeutic alternative is available for patients at increased risk for musculoskeletal pain. Therefore, a genetic test with sufficient predictive power to identify such patients might be clinically useful.

CLINICAL TRANSLATION

The use of genotyping to inform clinical decisions about drug use is not widely practiced. The slow pace of the clinical application of pharmacogenomics has many causes. Obviously, the most important issue is the need to establish clinical utility in order to support the value of genotyping. In the absence of such evidence, payers will be unlikely to provide reimbursement for routine use of pharmacogenetic testing, and tests will remain inaccessible to the majority of patients. There seems to be little consensus on the level or nature of data required to establish clinical utility.[7]

No matter what level of evidence is required for each situation, it will be necessary to develop simple clinical algorithms to aid physicians in their interpretation and use of genetic data. This goal may be best achieved through the development of point-of-care tools embedded in electronic medical record systems. Even with such tools, physicians and other health care providers need to be aware of this area of biomedical science in order to apply the information clinically. A major effort will be required to educate all members of the health care team about clinical genomics.

In recent years, the FDA has aggressively pursued drug-label modification when excess risk can be convincingly linked to a genetic marker. Several of the examples have been described here; many more are listed in the FDA's Table of Pharmacogenomic Biomarkers in Drug Labels (www .fda.gov/Drugs/ScienceResearch/ResearchAreas/ Pharmacogenetics/ucm083378.htm). Warnings that the FDA has issued about the prescription of clopidogrel and abacavir without testing of the relevant genotype are examples of the agency's increasingly activist stance.

CONCLUSIONS

There has been a good deal of comment in the scientific literature[71-74] and the popular press[75] about the slow pace of the application of genomics to clinical medicine. We hope that we have provided some reassurance that advances resulting from the application of genomic science to drug therapy may be helpful in drug selection and administration and reduce the odds of adverse drug reactions. Challenges that are associated with the replication of study findings and the development of proof of the clinical significance of implicated variants underscore the importance of functional experiments to test for biologic plausibility and to extend our understanding of drug mechanisms. Finally, a blend of scientific, regulatory, and psychological factors must be addressed if pharmacogenomic tests are to become a routine part of clinical practice. The FDA-mandated incorporation of pharmacogenomic information in drug labeling will remain an important step in the acceptance of pharmacogenomics in clinical practice. Perhaps equally important will be the willingness of physicians to reexamine suboptimal pharmacologic management programs.

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