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Pharmacogenomics: a systems approach

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Abstract

Pharmacogenetics and pharmacogenomics involve the study of the role of inheritance in individual variation in drug response, a phenotype that varies from potentially life-threatening adverse drug reactions to equally serious lack of therapeutic efficacy. Pharmacogenetics-pharmacogenomics represents a major component of the movement to `individualized medicine'. Pharmacogenetic studies originally focused on monogenic traits, often involving genetic variation in drug metabolism. However, contemporary studies increasingly involve entire `pathways' that include both pharmacokinetics (PKs)—factors that influence the concentration of a drug reaching its target(s)—and pharmacodynamics (PDs), factors associated with the drug target(s), as well as genome-wide approaches. The convergence of advances in pharmacogenetics with rapid developments in human genomics has resulted in the evolution of pharmacogenetics into pharmacogenomics. At the same time, studies of drug response are expanding beyond genomics to encompass pharmacotranscriptomics and pharmacometabolomics to become a systems-based discipline. This discipline is also increasingly moving across the `translational interface' into the clinic and is being incorporated into the drug development process and governmental regulation of that process. The article will provide an overview of the development of pharmacogenetics-pharmacogenomics, the scientific advances that have contributed to the continuing evolution of this discipline, the incorporation of transcriptomic and metabolomic data into attempts to understand and predict variation in drug response phenotypes as well as challenges associated with the `translation' of this important aspect of biomedical science into the clinic.

Pharmacogenetics is the study of the role of inheritance in individual variation in drug response. Pharmacogenetics has been heralded as one of the first major clinical applications of the striking advances that have occurred in human genomic science.[1] The context within which both basic and translational pharmacogenomic science have developed includes a revolution in drug therapy that took place during the latter half of the 20th century and continues into the 21st century.[2] As a result, diseases that range from childhood leukemia to hypertension, from depression to viral infections, have been cured or controlled for the first time in human history. The development of these potent and effective medications represents an advance as striking as that which has occurred in genomics. However, these advances in pharmacology carry with them a responsibility to develop ways in which to maximize drug efficacy, minimize toxicity, and select responsive patients. Although many factors can influence drug response, it has become clear during the last 50 years that inheritance can be a very important factor.[3,4] That realization led to the birth of the discipline of pharmacogenetics. It is now becoming equally clear that the union of transcriptomic and metabolomic with genomic data will accelerate the process of understanding mechanisms responsible for variable response to the powerful therapeutic agents used in 21st century medicine.

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The conceptual basis for pharmacogenetics was laid more than 50 years ago.[5] The science that underlies pharmacogenetics has contributed both to basic understanding of molecular mechanisms responsible for inherited variation in drug response and to the translation of that understanding to the bedside and into the drug development process. In parallel with those developments, advances in genomic technology resulted in the evolution of pharmacogenetics into `pharmacogenomics'. Pharmacogenomics has many definitions, but the transition from studies of monogenic to polygenic traits, and the rapid integration of genomic science to make genome-wide studies possible have served as hallmarks of that evolutionary process. Pharmacogenetic-pharmacogenomic effects are often classified as those which alter factors that influence the concentration of a drug reaching its target, so-called pharmacokinetic (PK) factors, and those that involve the target itself, so-called pharmacodynamic (PD) factors. When a drug is administrated to a patient, it must be absorbed, distributed to its site of action, interact with its targets, undergo metabolism, and finally be excreted.[6] Absorption, distribution, metabolism, and excretion can all influence PKs. However, functionally important genetic variation also occurs in the drug target itself, or in signaling cascades downstream from the target. The ability to take all of the factors that can influence drug response in the cell into account would help us to better understand mechanisms involved in variation in drug response and to better treat patients, moving toward the goal of truly individualized medicine. On a scientific level, this approach would also lead us into the network-based analyses required for such a complex system. In subsequent paragraphs, the development of pharmacogenetic-pharmacogenomic science will be described—its evolution from a focus on relatively simple monogenic traits, most often traits that involve drug metabolism, to polygenic traits that incorporate both PK and PD factors—as well as recent attempts to apply genome-wide techniques. We will also briefly address factors of importance for the translation of this area of genomic science into clinical medicine, a process that has not occurred as rapidly as many had originally hoped. Finally, the recent incorporation of additional high-throughput phenotyping technologies such as metabolomics has the potential to add another `layer' of data beyond traditional PK and PD information, requiring the use of a `systems' approach (Figure 1).

Subsequent paragraphs will trace the development of pharmacogenetics from its `origins' half a century ago, origins based on single gene effects on drug metabolism, through a transition that incorporated molecular genomic techniques, to entire PK and PD pathways to the present emphasis on genome-wide approaches and, most recently, the incorporation of transcriptomics and metabolomics—with an increasing requirement for a truly systems perspective.

ORIGINS

The process by which pharmacogenetics has developed mirrors the overall development of medical genetic-genomic science. The conceptual basis for pharmacogenomics can be traced to Sir Archibald Garrod's `1939 Inborn Factors of Disease'.[7] However, the earliest experimentally validated demonstrations of an effect of inheritance on drug response were first reported half a century ago, in the 1950s and 1960s. Those examples grew out of clinical observations of large differences among patients in their response to `standard' drug doses, often coupled with individual variations in plasma or urinary drug or drug metabolite concentrations. Two `classical' examples of pharmacogenetics involved genetic variation in the enzymatic hydrolysis of the short-acting muscle relaxant succinylcholine and the enzymatic acetylation of drugs such as the antituberculosis isoniazid. Both behaved as monogenic traits and both involved PK variation, variation because of inherited differences in drug metabolism. Specifically, some patients treated with succinylcholine experienced prolonged muscle paralysis, a serious and potentially lethal adverse response that resulted from the inheritance of an `atypical' form of the enzyme butyrylcholinesterase (BCHE).[8–10]

Subsequently, it was demonstrated that the *BCHE* allele encoding the most common atypical form of the enzyme included a nonsynonymous coding single nucleotide polymorphism (cSNP), G209 > A, resulting in an Asp70 > Gly change in the encoded amino acid that altered the active site of the enzyme.[11] Atypical BCHE had less ability to catalyze the hydrolysis of succinylcholine and was resistant to inhibition by the compound dibucaine.[12] That latter phenomenon is depicted graphically in Figure 2(a) in which the inhibition of plasma BCHE by dibucaine is plotted as `dibucaine number,' with subjects homozygous for the atypical allozyme shown at the far left.[12] Those subjects would be at a greatly increased risk for prolonged paralysis after treatment with standard doses of succinylcholine.

At almost the same time that pharmacogenetic variation in succinylcholine hydrolysis was described, isoniazid, one of the first effective drugs for the treatment of tuberculosis, was developed.[13] It was quickly reported that plasma concentrations of isoniazid showed a bimodal frequency distribution after the administration of identical doses to different subjects [Figure 2(b)].[14] This trait was shown by segregation analysis to be inherited and to result from genetic variation in *N*-acetylation,[14] a reaction catalyzed, in the case of isoniazid, by the enzyme *N*-acetyltransferase 2 (NAT2).[15] We now know that the bimodal frequency distribution shown in Figure 2(b) is because of the genetic polymorphisms in the *NAT2* gene.[15] Those polymorphisms also influence the PKs of drugs as disparate as the antihypertensive agent hydralazine[16] and the antiarrhythmic drug procainamide.[17] Subjects who are genetically `slow' acetylators of hydralazine and procainamide are at increased risk for the occur-rence of autoimmune disorders after exposure to those drugs.[18,19] Hydralazine is rarely used today in the treatment of hypertension other than in pregnant patients with preeclampsia,[20] but it recently reemerged as one of the two active components in BiDil, a combination of hydralazine and a nitrate that is used to treat patients with serious congestive heart failure.[21] BiDil is the only drug that the Food and Drug Administration (FDA) has approved for use in only one ethnic group, in this case, patients of African ancestry,[22] presumably because of ethnically-dependent genetic differences in response to this drug.

There are many other early examples of pharmacogenetic alterations in drug metabolism and, as a result, inherited differences in PKs. At approximately the same time that the pharmacogenetics of BCHE and NAT2 were being studied, a slightly different research strategy was being applied that reinforced the conclusion that genetics could influence PKs. In that case, plasma drug half-life values were measured in monozygotic and dizygotic twin pairs, and those data were used to calculate heritability, demonstrating that inheritance could significantly influence individual variation in drug half-life.[3,4] The striking differences observed in variance between monozygotic and dizygotic twin-pair half-life values for the anticoagulant dicumerol and the antiinflammatory agent antipyrine are shown in Figure 3.[3,4]

These early examples of the effects of inheritance on drug response served as a stimulus for the discovery of a series of additional examples, some of which became `poster children' for pharmacogenetics. Most of the second generation examples continued to involve drug metabolism, so they were associated with PKs. Most of them also began with clinical observations—mainly adverse drug reactions. At that time, this research involved the application of a phenotype-to-genotype research strategy that started with a clinical observation, followed by characterization of the gene involved and identification of genetic variation in the gene which was responsible for the original clinical observations. Two prototypic examples of this era that have become pharmacogenetic `icons,' examples that also bridged the transition from biochemical to molecular pharmacogenetics, were the cytochrome P450 2D6 (CYP2D6) and thiopurine S-methyltransferase (TPMT) genetic polymorphisms.

CLASSICAL EXAMPLES AND CLINICAL IMPLICATIONS

One early example of pharmacogenetics that has had significant implications for both basic and clinical pharmacogenetics involved the enzyme TPMT. TPMT catalyzes the S-methylation of thiopurine drugs such as 6-mercaptopurine (6-MP).[24] These drugs are used to treat acute lymphoblastic leukemia of childhood, inflammatory bowel disease and organ transplant recipients.[25,26] Like many other cytotoxic agents, thiopurines have a `narrow therapeutic index', that is, the difference between the dose required to achieve the desired therapeutic effect and that causing toxicity is small.[25,27] The major toxicity associated with the use of thiopurines is myelo-suppression (bone-marrow suppression) that can be life-threatening.[25,27] Early studies in which TPMT enzyme activity was measured in red blood cells from a large population of randomly selected individuals demonstrated that variation in TPMT enzyme activity is inherited (Figure 4).[28,29] Individuals with genetically lower levels of enzyme activity have a significantly increased risk for the development of myelosuppression when treated with standard dose of thiopurines.[26,30] Later it was demonstrated that common alleles in the gene encoding TPMT are responsible for decreased TPMT enzyme activity and protein levels. The most common *TPMT* variant allele in Caucasians is *TPMT***3A*, an allele primarily responsible for the trimodal frequency distribution shown in Figure 4, with a frequency of approximately 5% in Caucasian populations.[31] This variant allele has two nonsynonymous cSNPS, single nucleotide polymorphisms (SNPs) that change the encoded amino acids (Figure 5). However, *TPMT***3A* is rarely observed in East Asian populations. *TPMT**3*C*, with only the exon 10 SNP (Figure 5), is the most common variant allele in those populations, with a frequency of approximately 2%.[32,33] The clinical manifestations of *TPMT**3*C* are much less severe than those for *3*A*. Subjects homozygous for *TPMT***3A* have no detectable TPMT protein or enzyme activity. That is because of the rapid degradation of the TPMT*3A protein.[34] Mechanistic studies demonstrated that the TPMT*3A protein misfolds and, as a result, either aggregates to form aggresomes in cells or is removed from the cell through the ubiquitin-proteasome-mediated degradation pathway (Figure 6).[35] Recently, it was demonstrated that autophagy can also contribute to TPMT*3A degradation.[36] Because of its significant clinical implications, TPMT was the first example for which the FDA held hearings on the incorporation of pharmacogenetics into drug relabeling.[37] The molecular genetic characterization and striking clinical implications of *TPMT* polymorphisms make this one of the most striking examples of pharmacogenetics—both for translational science and as a model system to study pharmacogenomic mechanisms.

A second `classical' example of pharmacogenetics, one for which the FDA has also held public hearings, is CYP2D6. The cytochromes P450 (CYPs) are a family of microsomal drug-metabolizing enzymes,[39] and CYP2D6 is the family member that has been studied for the longest time and most intensively from a pharmacogenetic perspective. The *CYP2D6* polymorphism was originally described by two laboratories studying two different probe drugs, the antihypertensive debrisoquine and the agent sparteine.[40,41] The frequency distribution of the debrisoquine urinary metabolic ratio, the ratio of the parent drug to oxidized metabolite, is shown in Figure 7. Northern European populations include a group of debrisoquine poor metabolizers (PMs) (shown, counterintuitively, at the far right in Figure 7, subjects with two inactive copies of the gene), a group of extensive metabolizers (EMs), and a small number of ultrarapid metabolizers (UMs),[42] some of whom have been shown to have multiple copies of the *CYP2D6* gene.[43] The cloning and characterization of the *CYP2D6* gene made it possible to identify copy number variation as well as functional SNPs in this gene.[44,45] Although *CYP2D6* gene duplication is relatively infrequent among Northern Europeans, in East Africa the frequency of alleles with duplication of *CYP2D6* is as high as 29%.[43,46] An updated list of the *CYP2D6* alleles and haplotypes—as well as those for other human CYPs—can be found at http://www.imm.ki.se/CYPalleles. Subjects who

have multiple functional copies of *CYP2D6* display significantly increased metabolism of scores of drugs, including tricyclic antidepressants such as nortriptyline and antihypertensive and antiarrhythmic beta-blockers such as metoprolol,[47–49] which can result in lack of efficacy. On the other hand, patients with *CYP2D6* deletion or with low activity alleles will have impaired ability to metabolically activate prodrugs such as codeine and the selective estrogen receptor modulator (SERM), tamoxifen, to form active drug metabolites.[50–53] Tamoxifen is first-line hormonal therapy for women with estrogen receptor positive breast cancer. It is also one of the few drugs that have been approved by the FDA for the prevention of breast cancer in high-risk women.[54] A series of clinical studies have demonstrated that CYP2D6 genotype can significantly influence breast cancer outcome, i.e., disease-free survival or overall survival, after the treatment of women with breast cancer with tamoxifen[55–58] (Figure 8). As a result, tamoxifen is another drug for which the FDA held hearings on the incorporation of pharmacogenetic information into drug labeling. CYP2D6 is also involved in the bioactivation of another prodrug, codeine to morphine, as mentioned previously. Patients who are CYP2D6 UMs are at a greater risk for codeine-related toxicity.[59,60] Thus far, four examples of pharmacogenetics have been highlighted by the FDA in this fashion. A third example involves the glucuronidation of an anticancer drug, irinotecan (CPT-11), by a member of the UDP-glucuronosyltransferase (UGT) enzyme family.

Glucuronidation is a major phase II (conjugating) drug-metabolizing reaction in humans. This reaction is also involved in protection against environmental toxicants, carcinogens, dietary toxins and participates in the homeostasis of numerous endogenous molecules, including bilirubin, steroid hormones and bile acids.[61] Irinotecan is a topoisomerase I inhibitor and is `activated' by hydrolysis to form SN-38, which is then inactivated by glucuronidation catalyzed by UGT1A1.[62] A thymine-adenine (TA) repeat polymorphism in the promoter for *UGT1A1* (*UGT1A1*28*), the gene encoding the enzyme that catalyzes the glucuronidation of bilirubin and irinotecan, is responsible for most cases of Gilbert's syndrome in Caucasians and is also associated with severe irinotecan side effect such as neutropenia and diarrhea.[62–64] The *UGT1A1*28* polymorphism involves a TA insertion in the TATA box of the *UGT1A1* promoter. `Wild type' *UGT1A1*1* has six TA repeats, but the *28 variant allele has seven TA inserts, resulting in reduced promoter activity and reduced UGT1A1 activity.[65,66] In a Scottish population, the frequency of 6/6 homozygotes was 40%, 48% for heterozygous 6/7 subjects and 12% for homozygous 7/7 subjects.[66] African-American populations have a higher 7/7 genotype frequency (23% in one population studied), with an even smaller percentage who have eight TA repeats.[64] However, Chinese and Japanese populations have a lower 7/7 genotype frequency than do Caucasians.[67] The fact that *UGT1A1* genotype is ethnically-dependent might influence clinical response to irinotecan in different ethnic groups.

TPMT, CYP2D6 and *UGT1A1* pharmacogenetics all involve a single gene and only a few SNPs within that gene. They also all encode drug-metabolizing enzymes that influence PKs. Therefore, all of these examples behave as monogenic Mendelian traits, as do many other examples from pharmacogenetics. The discovery and description of these relatively simple, monogenic traits was probably necessary to help make the point that inheritance must be considered among factors that contribute to variation in the drug response phenotypes. However, it should be emphasized that pharmacogenetics and pharmacogenomics have now moved beyond monogenic traits and beyond a focus on PKs to include functionally and clinically significant variation in the drug target itself, i.e., PDs, as well as multiple genes that influence both the PKs and PDs of a drug. The examples highlighted in subsequent paragraphs were specifically chosen to illustrate the extension of pharmacogenetics and pharmacogenomics beyond single genes to pathways involving multiples genes responsible

for both PK and PD effects and, ultimately, to include pharmacotranscriptomics and pharmacometabolomics.

BEYOND SINGLE GENES

As knowledge of both human genomics and therapeutics has grown, it became clear that drug response phenotypes are complex traits, involving genes not just in PK pathways but also pathways that include drug targets and downstream signaling from targets. One recent example, warfarin, illustrates that point. Warfarin is the most widely prescribed oral anticoagulant drug in North America and Europe.[68] However, in spite of a laboratory test that is used universally to follow its effect on coagulation [the International Normalized Ratio (INR)], serious adverse reactions—involving both hemorrhage and undesired coagulation—continue to complicate warfarin therapy. Warfarin is a racemic mixture, and S-warfarin is three to five times more potent than R-warfarin as an anticoagulant, and is metabolized predominantly by a genetically polymorphic cytochrome P450 isoform, CYP2C9.[69] Two common *CYP2C9* polymorphisms, polymorphisms that result in Arg144>Cys (*CYP2C9*2*) and Ile358>Leu (*CYP2C9*3*) alterations in the encoded amino acid sequence, are associated with approximately 12% and 5%, respectively, of the level of enzyme activity observed with the wild type allele (*CYP2C9*1*).[69,70] The frequencies of *CYP2C9*2* and *CYP2C9*3* vary between 8–12% and 6–10%, respectively, in Caucasians, with lower frequencies in subjects from Africa or Southeast Asia. In 1999, it was reported that patients who required a `low' warfarin dose after dose adjustment based on INR values carried one or more of the two common *CYP2C9* variant alleles more often than did subjects in a randomly selected population sample [odds ratio (OR) 6.2].[70] Those same subjects also had an increased risk of hemorrhage during warfarin therapy.[70] A series of follow-up studies generally confirmed those observations,[71–74] but it was clear that this pharmacogenetic variation in PK failed to explain most of the variance in the final dose of warfarin in patients anticoagulated with this very useful, but potentially dangerous, drug. As a result, pharmacogenetic testing for *CYP2C9* genotype during warfarin therapy never found a wide level of acceptance. That was true in spite of the life-threatening nature of adverse responses to warfarin therapy. This situation changed after PD pharmacogenetic data were added to the equation.

The target for coumadin-based anticoagulant drugs such as warfarin had remained elusive and was not identified until 2004. The gene encoding that target, vitamin K epoxide reductase complex 1, *VKORC1*, was cloned in 2004.[75,76] Several groups then `resequenced' *VKORC1* and, although no nonsynonymous cSNPs were observed, a series of haplotypes were found to be associated with the final dose of warfarin—a dose determined on the basis of INR values.[68] In one study, the average warfarin maintenance dose in patients with *VKORC1* haplotypes associated with low dose requirement was approximately half of that for subjects with haplotypes associated with a requirement for a high maintenance dose. In that same study, the combination of *VKORC1* haplotyping and genotyping for *CYP2C9* explained approximately 25% of the variance in warfarin dose on the basis of the *VKORC1* haplotype and 6–10% for the *CYP2C9* genotype.[68] Two other studies conducted in 2005 reported similar results.[68,77,78]

Since 2005, numerous studies of warfarin pharmacogenomics have been performed in North America, Europe and Asia.[79–82] However, the number of patients in most studies was numbered in the hundreds and, therefore, the data were inadequate to result in the incorporation of genotyping into clinical practice. The Pharmacogenetics Knowledge Base (PharmGKB), a data base supported by the National Institutes of Health (NIH) and a part of the NIH Pharmacogenetics Research Network (PGRN), recently initiated a consortium to consolidate warfarin pharmacogenetic data from studies performed across the world. As a

result, data from studies performed in different locations could be pooled, significantly increasing the power to test predictive models that included genotyping in addition to clinical predictors.[83] Because of the significant clinical implications of warfarin pharmacogenetics, the FDA also held public hearings on warfarin pharmacogenetics and, as a result, warfarin labeling was changed to include that information.

Warfarin provides a striking example of a situation in which PK pharmacogenetic data were inadequate for clinical translation because those data explained too little of the dose variance, followed by an appreciation for the contribution of PD genetic variation (because of the *VKORC1* haplotype). When *CYP2C9* polymorphisms and *VKORC1* haplotypes were both determined, it was possible to assess genetic variation in both drug metabolism and in the drug target and, as a result, to move beyond the monogenic pharmacogenetics represented by *NAT2, CYP2D6*, and *TPMT*. This situation, with warfarin as an example, is depicted schematically in Figure 9. The `warfarin' example represents, probably in simplified form, the type of polygenic model that many investigators expect to observe with increasing frequency in the future.

Recently, the focus in pharmacogenomic studies is increasingly on `pathways,' pathways that include both PKs and PDs. However, there are still very few clinically relevant examples that have taken an entire pathway into account—followed by the functional validation of significant SNPs identified. One such story involves a study of the possible contribution of the glutathione pathway to variation in response to platinum drugs in lung cancer patients.[84] Platinum compounds such as cisplatin are widely used in the treatment of many forms of cancer and they are a mainstay in the therapy of lung, testicular, ovarian, head and neck, and bladder cancer.[85–87] However, there are large interindividual variations in response to treatment with these agents, with response rates of only 30% for cisplatin combination chemotherapy of lung cancer.[88] Platinum compounds can be inactivated by glutathione conjugation. The `glutathione pathway' (Figure 10) includes enzymes responsible for glutathione synthesis and redox status, as well as glutathione *S*-transferases and transporters that remove glutathione conjugates from cells. Many of the genes encoding these proteins are genetically polymorphic. In one recent study, the authors genotyped 973 consecutive lung cancer patients for 290 glutathione pathway SNPs, as well as deletion of the *GSTT1* and *GSTM1* genes, to assess the possible influence of inherited variation in this pathway on response to therapy with platinum compounds, using survival as a response phenotype.[84] Compared to subjects with no copies of *GSTT1*, subjects with a single copy had a 21% increased risk of death (hazard ratio, [HR] 95% confidence interval, CI = 0.96–1.54) and subjects with two copies of *GSTT1* had a 40% increased risk of death (HR 95% CI = 1.10–1.76). In addition, three tag SNPs, one each in *GSTA5* (rs4715354), *GSTM4* (rs560018), and *ABCC4* (rs7984157), were associated with survival, although after correction for multiple comparisons, these SNPs were not statistically significant.[84] These association studies were followed by functional validation using 100 immortalized lymphoblastoid cell lines from unrelated Caucasian subjects, a model system that will be discussed in detail subsequently, as well as lung cancer cell lines.[84] These functional studies added biological plausibility to the results obtained during the clinical association studies. The functional validation of any SNPs or genes identified during clinical genomic association studies is crucial to help us understand the underlying biology responsible for the association. That is especially important in an era of genome-wide association studies (GWASs), as described subsequently.

GENOME-WIDE AND SYSTEMS BIOLOGY STUDIES

Both candidate gene and candidate pathway-based pharmacogenomic studies are dependent on our current level of knowledge. Without prior knowledge, we are unable to select genes

or pathways that might be involved in drug response to study the influence of genetic variation. In order to make it possible to conduct an `unbiased' query across the entire genome, we are now applying whole genome, high-throughput techniques to pharmacogenomics. These high-throughput technologies include genomics, transcriptomics, and metabolomics. Proteomics has not yet been widely applied to study drug response in an integrated fashion even though there have been attempts to incorporate proteomics into the drug discovery process. Although the application of genome-wide techniques to perform `pharmacogenomic' studies is only in its infancy, genomics, transcriptomics, and even metabolomics are beginning to be applied to integrated studies of drug response, highlighting the need for a systems biology approach. These developments promise to significantly enhance our functional and mechanistic understanding of causes for variation in drug response phenotypes and to help us move toward individualized drug therapy. Subsequent paragraphs will highlight examples of both clinical genome-wide studies in pharmacogenomics as well as the application of genomics and transcriptomics to identify and functionally characterize candidate genes and/or SNPs identified in the course of those studies. In addition, we will also describe initial studies using metabolomics as an intermediate phenotype to identify changes in a large number of metabolites, followed by mapping those metabolites to pathways to help us better understand biochemical processes associated with drug response. GWASs of drug response include both clinical studies and studies performed with pharmacogenomic model systems. Model systems are being used increasingly for candidate gene identification and hypothesis generation, for the functional validation of candidates identified during clinical studies and to make it possible to explore pharmacogenomic mechanisms. As a result, there are currently more examples of the application of genome-wide techniques to pharmacogenomic model systems than there are of clinical studies—a situation that will undoubtedly change rapidly in the future.

One clinical pharmacogenomic study that was reported recently suggests that the application of genome-wide techniques might make it possible to identify polymorphisms of practical value for therapeutic decision making (i.e., clinical biomarkers). HMG-CoA reductase inhibitors, the `statins', are among the best-selling drugs worldwide. These agents are generally safe and effective. However, they do have side effects. The most serious of those side effects is myopathy, which can be life-threatening in a small number of patients.[89] A recent GWAS identified an SNP associated with risk for statin-induced myopathy.[90] Specifically, 6031 patients enrolled in the SEARCH trial who were treated with an 80-mg dose of the commonly used statin, simvastatin, were surveyed to identify patients who had suffered serious myopathy. Eighty-five myopathy patients with adequate deoxyribonucleic acid (DNA) for genotyping were identified, a number that usually would be considered inadequate for a GWAS based on power calculations for OR values like those observed in studies of disease risk. However, these investigators persevered and performed a GWAS with these 85 patients and 90-matched controls from the same group of patients treated with simvastatin. An SNP in the transporter gene *SLCO1B1*, encoding the organic anion-transporting polypeptide OATP1B1, had a *P*-value of 4×10^{-9}, with an OR of 16.9 for subjects homozygous for the variant allele, and 4.5 for heterozygous subjects [Figure 11(a)]. The authors then replicated their finding in an independent study that included 10,269 patients who had been treated with 40 mg of simvastatin.[91] The replication study showed an OR value of 2.6 per copy of the variant allele for statin-induced myopathy. The authors estimated that more than 60% of statin-induced myopathy cases in their study could be attributed to this single variant allele [Figure 11(b)]. Whether this example will prove to be representative of pharmacogenomic traits associated with adverse drug reactions remains to be determined, pending the application of genome-wide techniques to study many drug reaction phenotypes. However, the size of the clinical trials required, as well as the expense and difficulty associated with gaining access to clinical samples suitable for this type of study, highlights the need for the development of genome-wide model systems that might be

used to screen for candidate genes that play a role in drug sensitivity or resistance. That is particularly true for drugs with a narrow therapeutic index—drugs for which the toxic dose is similar to the therapeutic dose—or drugs like the statins for which adverse reactions can be life-threatening. It is for that reason that more genome-wide pharmacogenomic data are currently available for model systems than for clinical pharmacogenomics studies.

In vitro model systems such as cell lines from large numbers of individuals represent an attractive and cost-effective approach that has been used to help identify genes associated with variation in drug response by the application of genome-wide techniques. Observations made with such model systems can be used for both functional validation and replication of studies performed with clinical samples, as outlined schematically in Figure 12. These cell line model systems can be used to perform pharmacogenomic studies with a variety of phenotypes in a setting that is much more highly controlled than the clinical environment. An additional advantage is that many of these model systems use cell lines for which genomic data are publicly available. That is an important advantage practically because genome-wide genotyping is very expensive and, all too often, more time is spent negotiating access to clinical samples than in actually performing the clinical GWAS and analyzing the data. Finally, unlike the situation that exists with clinical studies, information obtained with cell lines is cumulative, i.e. as new genomic and phenotypic assays are developed, they can be applied to the same `patients', i.e. the same cell lines. Therefore, *in vitro* cell line model systems are well suited for the generation of pharmacogenomic hypotheses during the `discovery phase', followed by both functional validation—as outlined subsequently—and by the translation of laboratory-based observations into the clinic. Conversely, these same *in vitro* cell line systems can also be used to functionally validate significant `hits' observed in the course of clinical genome-wide studies, as was mentioned earlier for the glutathione pathway study of lung cancer.

The *in vitro* cell lines that have been used most often in pharmacogenomic studies include HapMap and Human Variation Panel lymphoblastoid cell lines—both of which are publicly available from the Coriell Institute. These cell lines are Epstein–Barr virus (EBV)-transformed lymphocytes immortalized from hundreds of individuals of different ethnic groups. The use of this type of model system focuses on common variation present in germline DNA and can be contrasted with the use of tumor cell lines such as the National Cancer Institute (NCI)-60 cells in which cell lines of multiple tumor types—but obtained from only a few individuals each—are studied.[93–95] The samples used in the original International HapMap Project included four populations,[96–99] and the Human Variation Panel cell lines were collected in the United States from unrelated individuals of different ethnicities (http://ccr.coriell.org/ Sections/Collections/NIGMS/Populations.aspx?PgId1/4177&coll1/4GM). A great deal of data, including genome-wide SNP and expression array data, are available for most of these cell lines which makes it possible to begin to integrate different `omics' in the same system to generate and/or test hypotheses.[99–107] For example, the genome-wide SNP data required to perform a GWAS are publicly available for many of these cell line-based model systems. Studies performed with HapMap samples have demonstrated that variation in gene expression or in drug response phenotypes such as cytotoxicity in these cells is regulated, in part, by inheritance.[103] A variety of drug response phenotypes can be tested, with cytotoxicity being the most common endpoint that has been studied thus far. The use of GWAS with drug cytotoxicity studies could potentially identify candidate genes that lie outside of our current range of knowledge. Those studies could also provide novel insight into mechanisms of drug action. For example, Huang et al., Shukla et al. and Duan et al. have used HapMap cell lines to perform GWAS that included SNPs, expression array data and cytotoxicity phenotypes to study the antineoplastic drugs cisplatin, daunorubicin and etopside.[108–111] Those investigators identified a series of SNPs that were associated with drug-induced cytotoxicity as a result of their influence on gene expression.

Li et al. used nearly 200 Human Variation Panel cell lines to identify genes with expression levels that were significantly associated with sensitivity to two antineoplastic cytidine analogues, gemcitabine and cytosine arabinoside (AraC).[107] These studies provide examples of the way in which this type of cell line model system can be used for pharmacogenomic hypotheses generation.

Although cell line-based model systems have already proved useful for the identification of pharmacogenomic candidate genes, there are also significant potential limitations associated with their use. First, these lymphoblastoid cells are not derived from tumor tissue and they are not tumor cell lines. Obviously, tumor DNA mutations are important for cancer drug response, but it is clear that germline polymorphisms are also important, as described in the previous sections. Second, only about 50% of human genes are expressed in lymphoblastoid cells, and the EBV transformation required to immortalize these cells is known to influence sensitivity to some drugs.[112–114] Therefore, like any genome-wide association data, results obtained with these *in vitro* systems must be functionally validated to provide biological plausibility and—ultimately—results obtained with these model systems must be replicated during clinical studies. For example, in the study performed by Li et al., the authors functionally validated the two top candidate genes that they identified by using siRNA `knockdown' performed with tumor cell lines, followed by cytotoxicity and other functional assays.[107] Figure 13 shows an example in which Li et al. demonstrated that knockdown of a nucleotidase candidate gene (*NT5C3*) identified using the Human Variation Panel cell lines shifted dose–response curves for AraC in cancer cell lines to the left, as anticipated.[107] These investigators also showed that expression of NT5C3 mRNA was inversely correlated with the active AraC phosphorylated metabolite levels in lymphoblastoid cells[107] (Figure 13(b)). Although no data are currently available which demonstrate that novel candidates identified with these cell line model systems can predict clinical drug response in patients, this type of approach could significantly narrow the list of candidate genes or SNPs to be tested during clinical studies.

These model systems also made it possible to combine genomic and transcriptomic data with drug-exposure related phenotypes. Although the assay of drug cytotoxicity is one way to assess drug response, can only be applied to cytotoxic agents such as anticancer chemotherapeutic drugs, but other phenotypes can also be directly related to drug response. One of those phenotypes is level of metabolites, not drug metabolites, but rather all small molecules that can be accurately assayed in the cells. These thousands of small molecules constitute the metabolome, which can also be altered by drug exposure and, as a result, alter drug response. Therefore, metabolomics is another potential drug response predictor that is beginning to be integrated with pharmacogenomic data to help identify biological pathways and proteins involved in variation in drug response. The addition of metabolomic data clearly brings us to a systems approach. Although the application of metabolomics in pharmacogenomics is still in its infancy, its promise is significant.

Metabolomics is the study of the metabolome, the repertoire of small molecules present in cells, tissues, organs, and biological fluids.[115–127] The con-centrations and fluxes of these compounds result from a complex interplay among gene expression, protein expression, and the environment—an environment that includes drug exposure. In contrast to classical biochemical approaches that often focus on single metabolites, single biochemical reactions and their kinetic properties, and/or defined sets of linked reactions, metabolomics generates quantitative data for a large number of metabolites in an attempt to understand metabolic dynamics associated with conditions such as drug exposure.[128] Metabolomic information complements data obtained from genomics and transcriptomics—adding another `layer' of data to make it possible to begin to apply a systems approach to study individual variation in drug response. Many analytical platforms are used in metabolomics, including gas

chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), and nuclear magnetic resonance (NMR). The selection of a specific platform is dependent on the nature of the metabolites to be assayed. For example, GC–MS, LC–MS, and NMR-based metabolomics platforms are best suited for mapping global biochemical changes, while LC–electrochemistry array metabolomic platforms are better for mapping neurotransmitter pathways and pathways involved in oxidative stress.[129]

One important potential application of metabolomics involves the identification of pathways that contribute to drug response phenotypes—`pharmacometabolomics'. That type of study could provide information with regard to mechanisms of drug action, as well as an insight into the contribution of specific pathways to individual variation in drug response. The application of metabolomics is already teaching us that metabolomic signatures can help identify biochemical pathways that may play an important role in variation in drug response. For example, one study focused on metabolic profiles of antipsychotic drugs and used a specialized lipidomics platform to measure more than 300 lipid metabolites to evaluate global lipid changes in schizophrenia after treatment with three commonly prescribed atypical antipsychotics, olanzapine, risperidone, and aripiprazole.[130] A major side effect associated with the use of these drugs is weight gain. In this particular study, lipid profiles were obtained for 50 patients with schizophrenia before and after 2–3 weeks of treatment with olanzapine (*N* 20), risperidone ($N = 14$), or aripiprazole ($N = 16$).[130] Prior to drug treatment, major changes were noted in two phospholipid classes, phosphotidylethanolamine (PE) and phosphotidylcholine (PC), as compared to control subjects, suggesting that phospholipids which play a key role in membrane structure and function may be impaired in patients with schizophrenia.[130] The omega 3 and omega 6 subclasses in PE and PC before and after drug exposure were also mapped, as well as shifts between saturated and polyunsaturated fatty acids.[130] Effects of the three antipsychotic drugs on lipid biochemical pathways were then evaluated by comparing metabolic profiles at baseline with post treatment assays.[130] PE concentrations were elevated after treatment with all three drugs. Olanzapine and risperidone affected a much broader range of lipid classes than did aripiprazole, with approximately 50 lipids that were increased after exposure to these drugs, but not after aripiprazole therapy[130] (Figure 14). Aripiprazole induced minimal changes in the `lipidome' (Figure 14), consistent with its limited metabolic side effect profile.

Another example of the application of metabolomics to study drug response involved the use of experimental animals to perform a study of drug-induced hepatotoxicity. Hepatotoxicity is a common and potentially serious adverse reaction to drugs such as acetaminophen (paracetamol).[132,133] In this metabolomic study, male Sprague-Dawley rats were treated with acetaminophen, and both preand postdrug exposure urine samples were subjected to NMR analysis.[134] A model was then developed that used predrug metabolomic data to predict both ratios of acetaminophen glucuronide conjugate to parent drug and postacetaminophen hepatotoxicity (class 1, no or minimal hepatic necrosis, to class 3, moderate necrosis). The major predrug urinary compounds that were associated with postacetaminophen hepatotoxicity were taurine, trimethylamine-N-oxide (TMAO), and betaine. A higher predrug urinary taurine level was associated with more class 1 than class 3 hepatic histology whereas higher combined predrug concentrations of TMAO and betaine were associated with more class 3 than class 1 histology after drug exposure.[134]

These two examples illustrate the use of metabolomics to explore mechanisms of variation in drug response. The ultimate goal of this type of approach would be the union of genomic, transcriptomic, proteomic, and metabolomic data to identify networks, proteins, and genes responsible for drug effect and/or variation in drug effect. We have already described a lymphoblastoid cell line model system in which genomic and transcriptomic data from the same `patients' (cell lines) has been used to gain insight into SNPs, genes and pathways

associated with variation in drug response phenotypes. It would require only a short step to add metabolomic information to those datasets to help identify and highlight additional pathways of importance for variation in drug response. However, even if the use of a true `systems' approach might allow us to identify novel biomarkers for variation in drug response phenotypes, we would still have to translate that knowledge into clinical practice. The history of pharmacogenetics-pharmacogenomics teaches us that that step may be challenging.

CLINICAL TRANSLATION

Pharmacogenomics is a major component of individualized therapy. Many successful examples already exist in which pharmacogenomic testing has been incorporated into clinical decision making. Besides the four examples for which FDA held public hearings, there are others that are now being used in the clinic to guide the treatment decisions, for example Her2 status and Herceptin.[135–138] However, even though the conceptual basis for pharmacogenetics has existed for over half century, even though recent developments in genomic science have made it possible for pharmacogenetics to evolve into pharmacogenomics, and even though we have begun to apply a systems approach to the study of drug response phenotypes, the translation of pharmacogenomics into the clinic has been slow. Except for the examples highlighted in previous sections, relatively few pharmacogenomic tests are currently used in the clinical setting, and even those examples are used less frequently than indicated. Unfortunately, a series of countervailing pressures have slowed the translation of pharmacogenomics into the clinic. Included among them is the need for increasingly large and complex studies to test pharmacogenomic hypotheses in the clinical setting; economic disincentives for the pharmaceutical industry to enthusiastically accept the implications of individualized drug response; and the relatively measured pace of the incorporation of this type of science into the drug evaluation process by regulatory agencies. However, recently there has been an increasing awareness by the FDA of the necessity to integrate genomic data into regulatory review. Guidance for Industry: Pharmacogenomic Data Submission (2005) and Companion Guidance (2007) have been published by the FDA and regulatory information is now available on-line (http://www.fda.gov/cder/genomics/). In parallel, the FDA also initiated a Voluntary Exploratory Data Submission (VXDS) program to help industry to share exploratory data with FDA.

Finally, we will also need to educate health care providers and patients to accept and utilize genomic data. Therefore, if pharmacogenomics is to be translated into individualized drug therapy, a concerted effort will have to be directed to the `genomic' education of all healthcare professionals—physicians, dentists, nurses, and physician's assistants. Furthermore, significant ethical issues will have to be addressed if the science underlying pharmacogenomics is to have its full potential impact on the practice of medicine and if patients and physicians are to embrace this new science enthusiastically. An important step in this process was passage in the United States of the Genetic Information Nondiscrimination Act (GINA) in April 2008. In many ways, these translational challenges are just as great as are the scientific challenges, but we owe it to patients who are being treated with the powerful drugs available today to face and overcome both types of challenges.

CONCLUSION

Pharmacogenetics and pharmacogenomics hold out the promise of helping to achieve the goal of individualized drug therapy. Many factors other than inheritance contribute to individual variation in drug response, but recent developments in genomic and pharmacological science have raised the possibility that we might be able to provide the

physician with objective information that might help make it possible to tailor drug selection and/or dose to the likely response of that particular patient to that class of drug, that specific agent or that dose on the basis of their individual genetic makeup. The future development of pharmacogenetics and pharmacogenomics, and the impending incorporation of pharmacotranscriptomics, pharmacoproteomics, and pharmacometabolomics into this area of science will require integrated teams of investigators with complementary and overlapping areas of expertise. Ultimately, the application of pharmacogenomics, pharmacoproteomics, and pharmacometabolomics to patient care promises to help make it possible to treat each patient as the complex, unique, and fascinating individual who they are. Finally, there is little doubt that the application of an integrated, systems approach will significantly advance our ability to individualize drug therapy in the 21st century.

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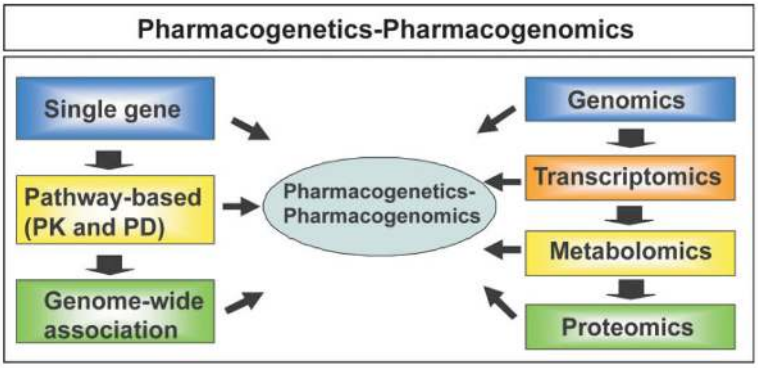

FIGURE 1.

The evolution of pharmacogenetics and pharmacogenomics. Pharmacogenomics has evolved from a single gene approach to incorporate pathway-based and genome-wide approaches (left side of the diagram). In parallel, it has increasingly incorporated a variety of high-throughput technologies including genomics, transcriptomics, metabolomics, and proteomics to significantly enhance the ability to generate and test pharmacogenomic hypotheses and to translate those hypotheses into clinical practice. PK, pharmacokinetics; PD, pharmacodynamics.

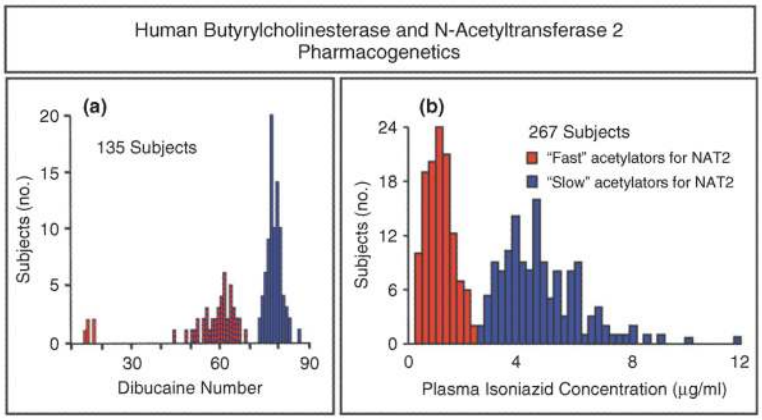

FIGURE 2.

(a) Butyrylcholinesterase (BCHE) genetic variation. The figure shows data for 135 members of 7 unrelated families selected on the basis of a proband with atypical BCHE. These subjects were phenotyped for percentage inhibition of BCHE by dibucaine. Subjects homozygous for the trait of atypical BCHE are shown at the far left. (b) *N*-Acetyltransferase 2 genetic variation. Plasma concentrations of isoniazid in 267 subjects 6 hours after the administration of an identical oral dose are shown. The bimodal frequency distribution results from polymorphisms in the *NAT2* gene. (Modified with permission from Refs 12,14. Copyright 1957, 1960 BMJ Publishing Group).

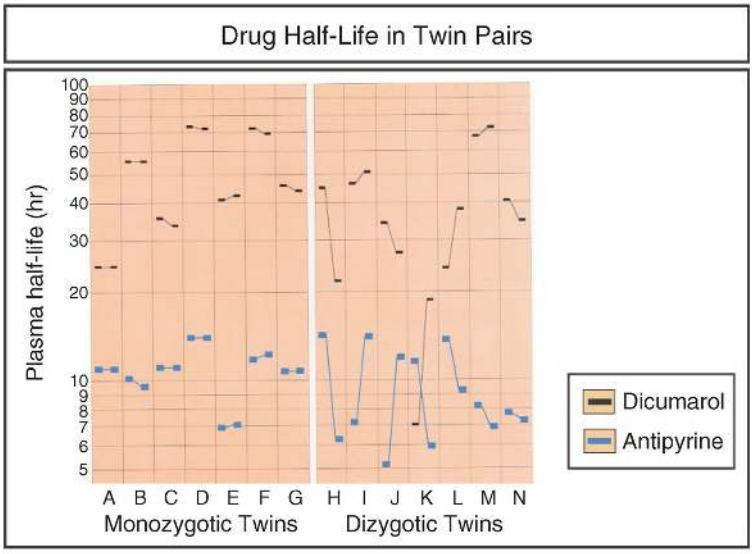

FIGURE 3.
Plasma dicumarol and antipyrine half-life values in monozygotic and dizygotic twin pairs. (Data from Vesell and Page[23] with permission).

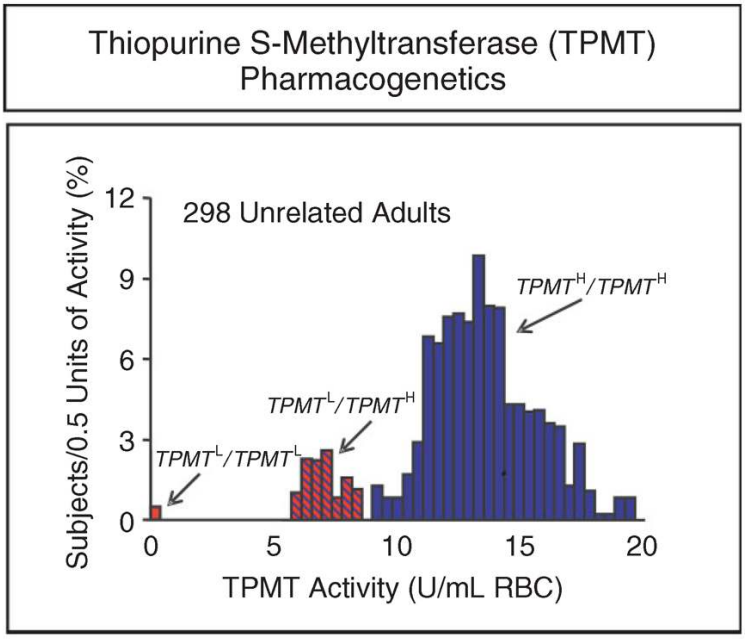

FIGURE 4.

Thiopurine S-methyltransferase (TPMT) pharmacogenetics. The frequency distribution shows the level of red blood cell (RBC) TPMT activity in 298 randomly selected Caucasian blood donors. Presumed genotypes for the TPMT genetic polymorphism are also shown. *TPMT*^{L} and *TPMT*^{H} (low and high, respectively) were allele designations used before the molecular basis for the polymorphism was established. (Modified with permission from Ref 28. Copyright 1980 the American Society of Human Genetics).

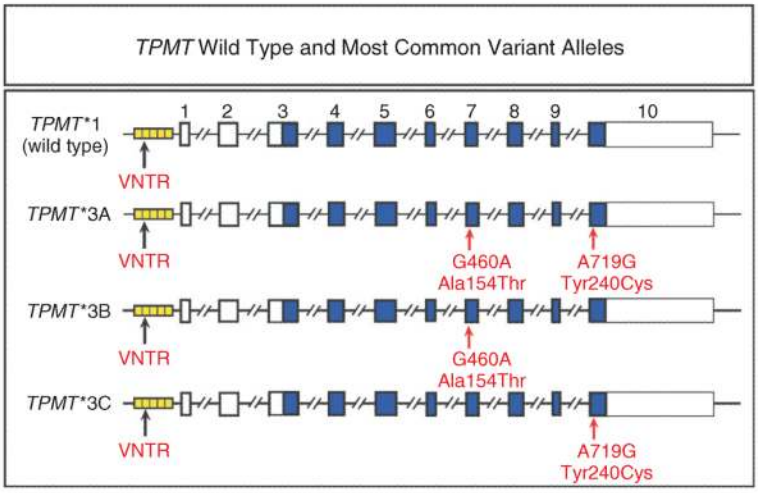

FIGURE 5.

Human thiopurine S-methyltransferase (*TPMT*) alleles. *TPMT* *1 is the most common allele (wild type), *TPMT* *3*A* is the most common variant allele in Caucasians, and T*PMT* *3*C* is the most common variant allele in East Asian subjects. Rectangles represent exons, with blue areas representing the open reading frame. Arrows indicate the locations of two common single nucleotide polymorphisms (SNPs) as well as a 5′-flanking region GC-rich variable number of tandem repeats (VNTR). (Modified with permission from Ref 29. Copyright 2006 Annual Reviews www.annualreviews.org).

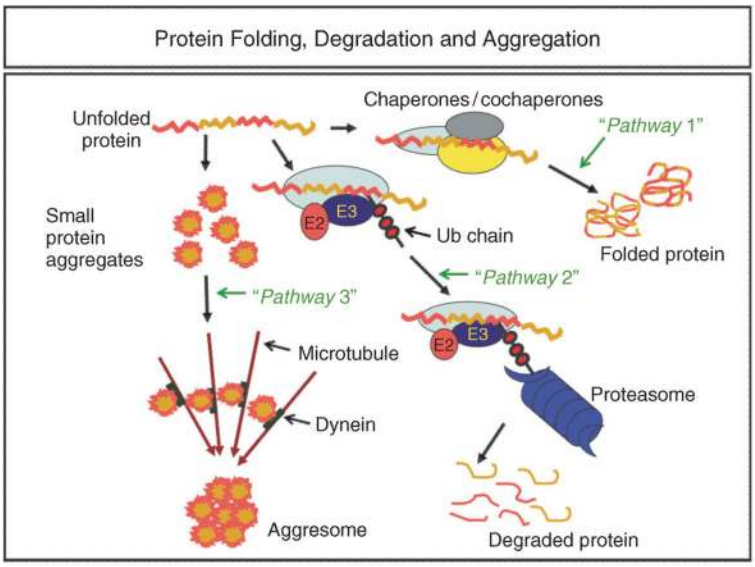

FIGURE 6.

The dynamic balance among protein folding, proteasome-mediated degradation, and aggresome formation. The figure depicts various fates for a protein, including proper folding (*Pathway 1*), misfolding followed by ubiquitination and proteasome-mediated degradation (*Pathway 2*), or aggresome formation (*Pathway 3*). (Modified with permission from Ref 38. Copyright 2006 Nature Publishing Group).

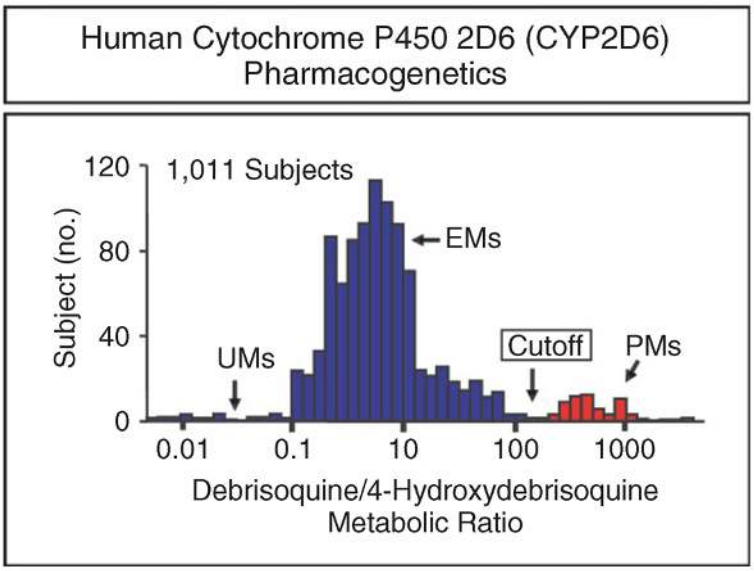

FIGURE 7.

Cytochrome P450 2D6 (CYP2D6) pharmacogenetics. The frequency distribution of the ratio of debrisoquine to its metabolite 4-hydroxydebrisoquine in 1011 Swedish subjects is shown. `Cutoff' marks the demarcation between PMs and EMs. PM, poor metabolizer; EM, extensive metabolizer; UM, ultrarapid metabolizer. (Modified with permission from Ref 42. Copyright 1992 Nature Publishing Group).

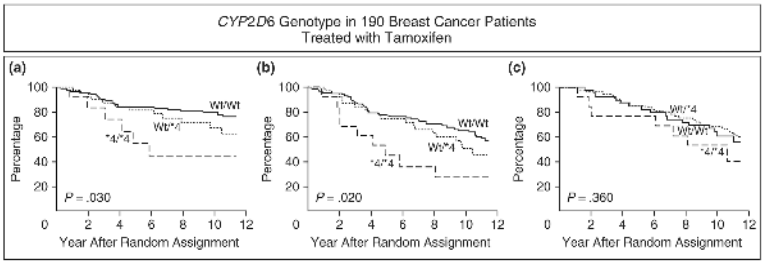

FIGURE 8.

Kaplan-Meier curves for 190 women with breast cancer who were treated with tamoxifen and were genotyped for *CYP2D6*4*. Wt, wild type. (a) Relapse-free time, (b) disease-free survival, and (c) overall survival for patients with the *CYP2D6* genotypes indicated. (Modified with permission from Ref 56. Copyright 2005 American Society of Clinical Oncology).

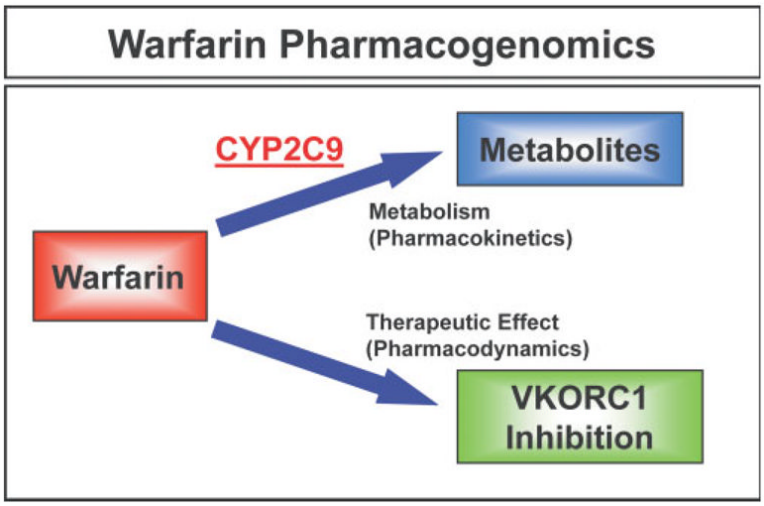

FIGURE 9.

Warfarin pharmacogenomics. The figure shows a schematic representation of pharmacokinetic (CYP2C9-dependent) and pharmacodynamic (VKORC1-dependent) pharmacogenomic factors that influence the final dose of warfarin. (Modified with permission from Ref 29. Copyright 2006 Annual Reviews www.annualreviews.org).

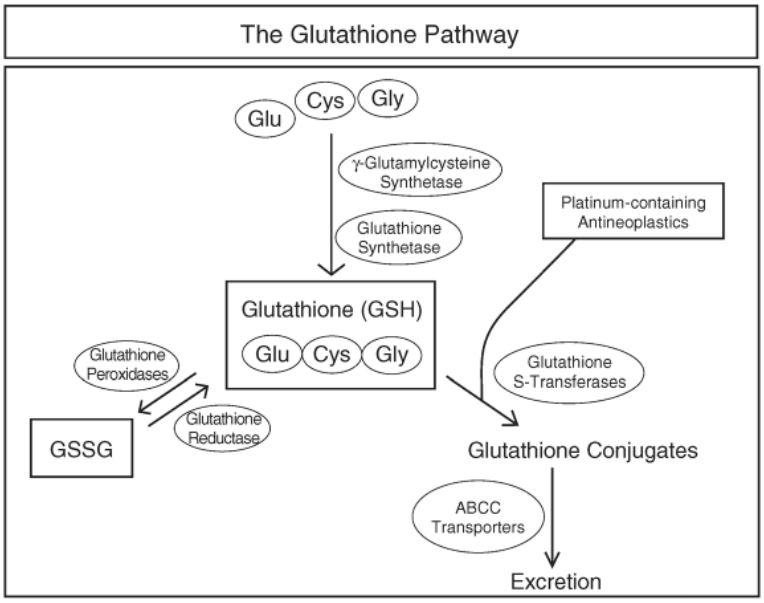

FIGURE 10.

The figure shows a schematic representation of the `glutathione pathway.' Glutathione is synthesized from glutamate (Glu), cysteine (Cys), and glycine (Gly) by γ-glutamylcysteine synthetase and glutathione synthetase. Glutathione redox state is regulated, in part, by glutathione peroxidases, forming oxidized glutathione (GSSG), and by a reaction catalyzed by glutathione reductase. Glutathione is conjugated to substrates both through the action of the glutathione S-transferases and through nonenzymatic reactions. Glutathione conjugates can be excreted from cells by members of the ABCC transporter family.

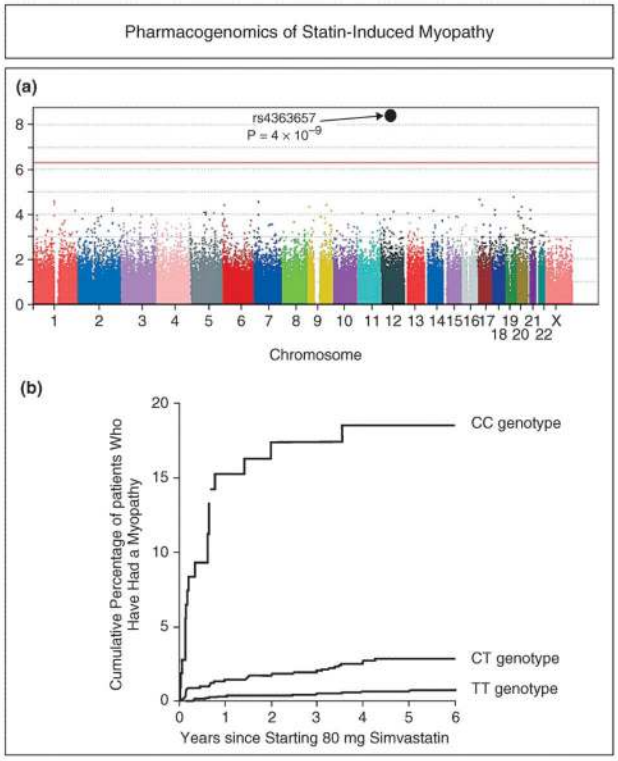

FIGURE 11.

Genome-wide association study (GWAS) of statin-induced myopathy. (a) Association between statin-induced myopathy and each SNP assayed in the GWAS. The x-axis shows the location of SNPs in the genome by chromosome, while the y-axis shows the—$\log_{10}$ of the *p* value for each SNP. (b) Estimated cumulative incidence of myopathy associated with 80 mg of simvastatin daily by genotype at SNP rs4363657. (Modified with permission from Ref 90. Copyright 2008 Massachusetts Medical Society).

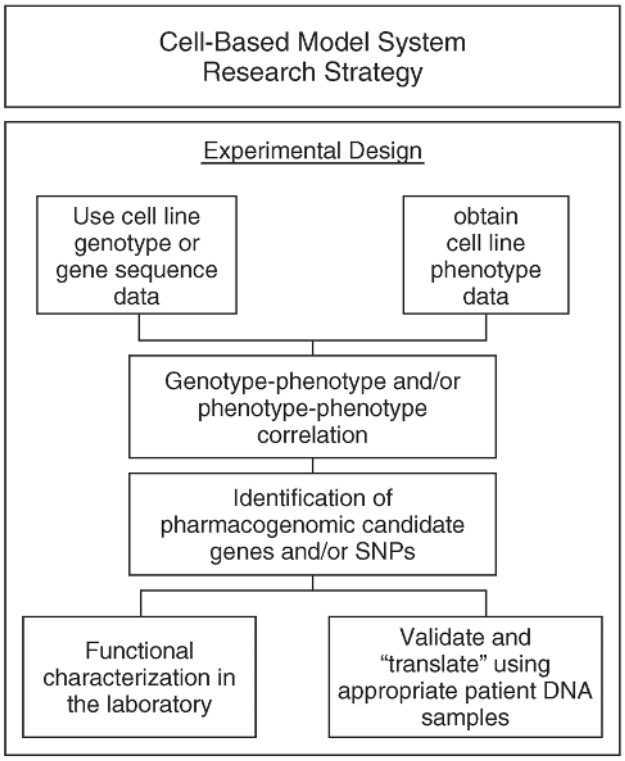

FIGURE 12.
Diagrammatic outline of the use of a cell line-based model system to identify and both functionally and clinically validate pharmacogenomic candidate genes. DNA, deoxyribonucleic acid. (Modified with permission from Ref 92. Copyright 2008 Oxford University Press).

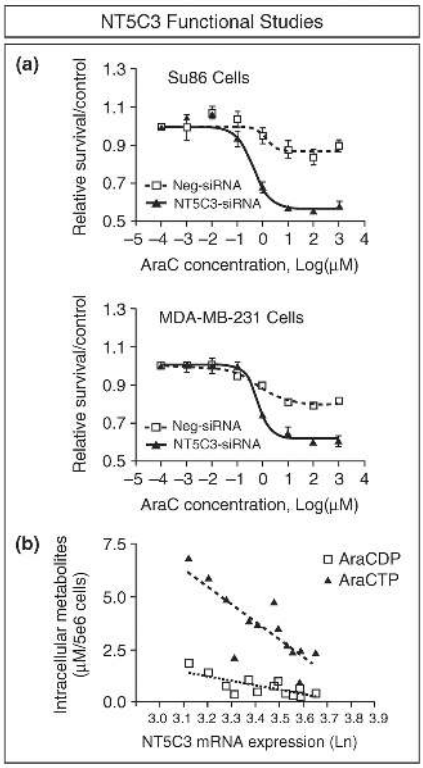

FIGURE 13.

Functional validation of a candidate gene identified using the lymphoblastoid cell line model system. (a) siRNA knockdown of NT5C3 in cancer cell lines shifts the dose–response curves for AraC to the left, as anticipated. (b) Inverse correlation between NT5C3 mRNA levels and levels of AraC phosphorylated active metabolites in lymphoblastoid cells. (Modified with permission from Ref 107. Copyright 2008 AACR).

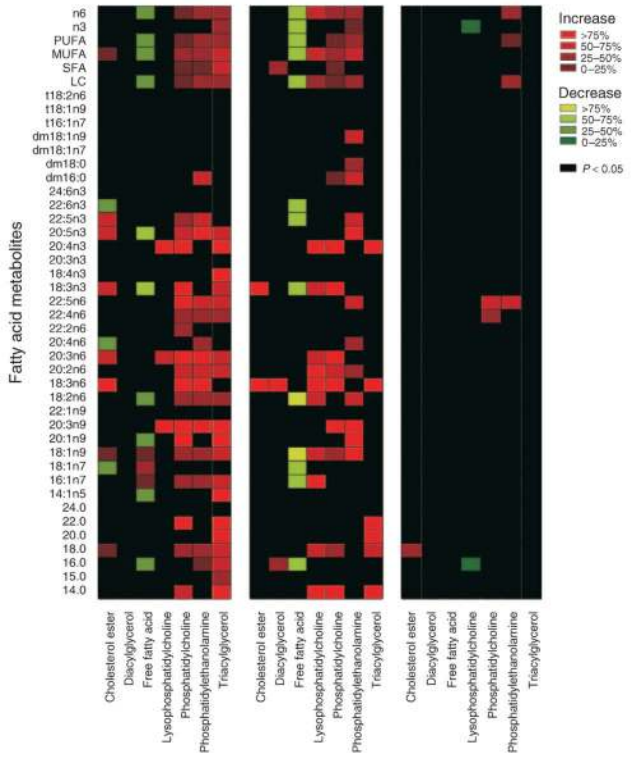

FIGURE 14.

Heat map showing differences in individual lipid metabolites in the plasma of patients with schizophrenia posttreatment as compared with pretreatment using olanzapine (*top panel*), risperidone (*middle panel*), and aripiprazole (*bottom panel*). Fatty acid metabolites are shown as they appear in each distinct lipid class. The percent increase in any lipid after drug treatment is shown in red squares and decreases in green squares.[131] The brightness of each color corresponds to the magnitude of the difference in quartiles. The brighter the square the larger the difference. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; LC, long chain. (Modified with permission from Ref 130. Copyright 2007 Annual Reviews www.annualreviews.org).