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PHARMACOGENOMICS: Unlocking the Human Genome for Better Drug Therapy

Howard L McLeod[1] and William E Evans[2]
[1]*Washington University Medical School, Department of Medicine, Division of Oncology, St. Louis, Missouri 63110-1093; e-mail: hmcleod@imgate.wustl.edu*
[2]*St. Jude Children's Research Hospital, Department of Pharmaceutical Sciences, and the University of Tennessee, Memphis, Tennessee 38105; e-mail: william.evans@stjude.org*

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■ **Abstract** There is great heterogeneity in the way humans respond to medications, often requiring empirical strategies to find the appropriate drug therapy for each patient (the "art" of medicine). Over the past 50 years, there has been great progress in understanding the molecular basis of drug action and in elucidating genetic determinants of disease pathogenesis and drug response. Pharmacogenomics is the burgeoning field of investigation that aims to further elucidate the inherited nature of interindividual differences in drug disposition and effects, with the ultimate goal of providing a stronger scientific basis for selecting the optimal drug therapy and dosages for each patient. These genetic insights should also lead to mechanism-based approaches to the discovery and development of new medications. This review highlights the current status of work in this field and addresses strategies that hold promise for future advances in pharmacogenomics.

THE PROMISE OF PHARMACOGENOMICS

The past 50 years have seen major improvements in the length and quality of life. Diseases such as polio and smallpox have been virtually eliminated through vaccination, while improved public health initiatives have had major impacts on diseases such as cholera and HIV. In addition, the morbidity and mortality of hypertension, diabetes, and many infectious diseases have been substantially reduced through the availability of medical interventions. However, optimal therapy is relatively elusive for current major killers, such as coronary artery disease, cerebral vascular events, and many cancers. Furthermore, there is clear evidence of significant heterogeneity in the efficacy and toxicity of most therapeutic agents, when viewed across the population. Unfortunately, prospective identification of those patients who are most likely to benefit from a specific therapy is not routinely possible for many diseases and medications. This is particularly important in the current health

care environment, where cost containment and evidence-based initiatives are having a significant influence on patient care. Understanding the molecular basis of drug action and genetic determinants of drug response should enlighten our use of many medications, toward the ultimate goal of giving the right drug at the right dose to the right patient at the right time. It should also lead to mechanism-based approaches to the discovery and development of new medications.

The field of pharmacogenomics encompasses a wide range of efforts designed to elucidate genetic determinants of drug toxicity and efficacy (1). It is also concerned with the effects of therapeutic agents on the patterns of gene expression in complex tissues. Research strategies involve the isolation and evaluation of variants in genetic code amongst individuals and the establishment of definitive relationships between these genetic polymorphisms and drug response. These genetic polymorphisms include nucleotide repeats, deletions, insertions, and mutations that influence gene expression and/or function. This review introduces the current technology for pharmacogenomic analyses and illustrates their application to answer questions important for advancing human pharmacology.

CURRENT PHARMACOGENOMIC TECHNOLOGY

The principles of pharmacogenomics have been around for decades, but the recent rapid development of the field has been the result of new technological advances in high throughput DNA and mRNA analysis and in the processing of these data in an efficient manner. The most dramatic change has been the introduction of arrays for the simultaneous assessment of multiple genes. Initial studies used robotics-based systems to "print" a series of gene clones onto a silicone-coated glass slide (2). By labeling the mRNA of interest with a fluorochrome, a correlation was found between the fluorescence intensity emitting from each gene clone and the measured level of gene expression. This approach has been modified to use large gene clones from the Human Genome Project, small oligonucleotides for specific genes, and cDNA derived from differential expression projects (2). Arrays are currently constructed on nylon filters or glass slides, with slides allowing greater density of genes per experiment and nylon generally being more reproducible. The improvements in robotics and fluid physics is such that up to 64,000 gene clones can be evaluated on a single 1 inch by 1 inch slide. The gene expression arrays have enabled a degree of genomic analysis not feasible in the recent past (detailed below). For example, it is estimated that the quantity of data available from a single array containing 64,000 genes (generated in approximately 48 hours) would have taken a researcher over 20 years to complete by Northern blot analysis.

The ability to obtain information on patient genotype in a rapid manner has also greatly improved in the past few years. Strategies such as fluorescence energy transfer detection (3), fluorescence polarization (4), kinetic PCR (5), mass spectrometry (6, 7), oligonucleotide ligation/flow cytometry (8), HPLC fragment analysis (9), and mini-sequencing (10) have all been used to increase the throughput

of genotype information from genomic DNA. Analysis of 1,000 to 5,000 genotypes per day is routine in many pharmacogenomics laboratories, with automated multiplex assays extending this to 100,000 genotypes per day. The ideal approach for rapid genotyping is not yet clear, but a large amount of effort is currently being expended to test various approaches in the clinical setting.

Computational biology, or bioinformatics, has been instrumental in the development of pharmacogenomics. The gene expression arrays and high throughput genotyping techniques generate a large amount of data in a single experiment, much more than can be evaluated using commonly available spreadsheets or manual approaches. Therefore, software has been developed that not only captures the experimental data, but includes comparison of results with existing genome databases, generation of dendrograms for sequence homology, and pattern recognition to pull together genes with similar patterns of expression, as part of the initial algorithm. This provides the investigator with a powerful and comprehensive output on which rapid interpretation and implementation of data can be made.

Informatics has spawned the field of *in silico* biology, in which mining of computer databases for genomic information is performed without laboratory experimentation. This has been useful for gene discovery, in that existing transcripts in the expressed sequence tag (EST) databases can be constructed to provide the coding sequence of a new gene. In addition, *in silico* approaches have been used to identify new polymorphisms in gene coding regions, using computer algorithms that align different EST clones with overlapping sequences. Nucleotides that appear to differ can be identified, and this approach has revealed nearly 100,000 new putative polymorphisms in the human genome (11). Efforts are under way to complete this approach for all sequences in the EST and human genome databases and to confirm their frequency in world populations. This approach will not identify all polymorphisms in the human genome, because only coding regions are available in the EST databases, prohibiting generation of sequence from the 5′ end, 3′ end, or intronic regions. In addition, the clones available for comparison are not comprehensive representations of diverse populations and so will not contain many important polymorphisms.

TAKING PHARMACOGENOMICS TO PATIENTS

Rational Therapeutics

Interpatient variability in response to drug therapy is the rule, not the exception, for almost all medications. This variation is potentially regulated by a number of processes, including drug transport, drug metabolism, cellular targets and signalling pathways (e.g. G-protein-coupled receptors), and cellular response pathways (e.g. apoptosis, cell cycle control, etc) (1). Currently an empirical treatment approach is typically taken for many diseases, with factors such as clinician familiarity with available medications and knowledge of dosing schedules influencing the choice of medications. This relatively crude method reflects the lack of a single

optimal treatment strategy for most diseases and the large number of medications available for most illnesses. For example, how does one rationally select the best treatment option for hypertension in a 78-year-old Caucasian female when there are nearly 100 different diuretics, β-blockers, calcium channel antagonists, angiotensin converting enzyme inhibitors, α-adrenergic blockers, and angiotensin II receptor antagonists available? Although there are published guidelines for the selection of drug classes in the treatment of hypertension, the final choice of medications remains empirical, if not arbitrary, for the great majority of patients. Variations on this scenario can be made by altering patient age, gender, race, or the therapeutic area under consideration. Over the past 15 years, data have been accumulating that provide a stronger scientific basis for patient-specific selection of medications and their dosages (1). This includes molecular mechanisms for variation in drug efflux and metabolism, cellular targets (e.g. receptor conformation), and heterogeneity in disease pathogenesis and phenotype. Even at a superficial level, the complexity of these different sources of variation is apparent. Heterogeneity in genotype for drug efflux, metabolism, and receptor pathways is now well established for numerous medications, and these can have additive or even synergistic effects on therapeutic success or toxicity (1). Although there is much work to be done before broad use of pharmacogenomics becomes routine in patient care, there are many clear lessons to be learned from current successes. Ultimately, the paradigm for selecting optimal medications and dosages will utilize a panel of genotypes that are disease-specific to identify the subset of patients who are destined to fail therapy with certain medications because they are genetically predisposed to toxicity or lack of efficacy (Figure 1).

Polymorphic Drug Metabolism

One of the most developed examples of clinical pharmacogenomics, in the context of therapeutics, involves the genetic polymorphism of thiopurine methyltransferase (TPMT). TPMT catalyzes the S-methylation of the thiopurine agents azathioprine, mercaptopurine, and thioguanine (12, 13). These agents are commonly used for a diverse range of medical indications, including childhood leukemia, rheumatoid arthritis, inflammatory bowel disease, dermatologic disorders, and solid organ transplantation. The principal cytotoxic mechanism of these agents is generally considered to be mediated via the incorporation of thioguanine nucleotides (TGN) into DNA. Thus, thiopurines are inactive prodrugs that require metabolism to TGN to exert cytotoxicity; this activation is catalysed by multiple enzymes, the first of which is hypoxanthine phosphoribosyl transferase. Alternatively, these agents can be inactivated via oxidation by xanthine oxidase or methylation by TPMT. In hematopoietic tissues, xanthine oxidase is negligible, leaving TPMT as the only inactivation pathway. TPMT activity is highly variable and polymorphic in all large populations studied to date; approximately 90% of individuals have high activity, 10% have intermediate activity, and 0.3% have low or no detectable enzyme activity (14, 15). Family studies have shown that TPMT activity is inherited as

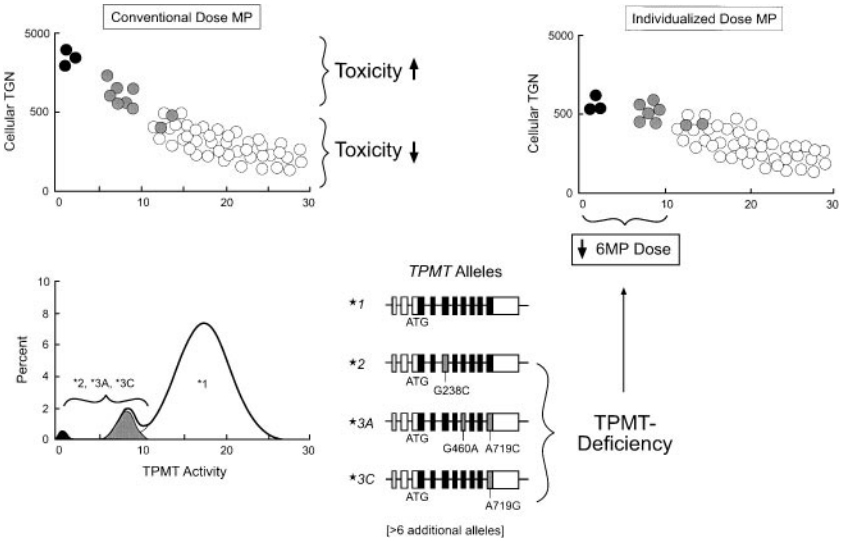

Figure 2 Patients who inherit TPMT deficiency or heterozygocity accumulate excessive cellular concentrations of active thioguanine nucleotides (TGN), predisposing them to severe hematopoietic toxicity. However, reducing the dosage of thiopurines (i.e. mercaptopurine, azathioprine, thioquanine) in TPMT-deficient or -heterozygous patients permits thiopurine therapy without acute toxicity. Molecular diagnostics, based on TPMT genotype, can now be used to prospectively identify TPMT-deficient patients, minimizing the risk of dose-limiting toxicity.

an autosomal codominant trait. Patients who inherit TPMT deficiency accumulate excessive cellular concentrations of TGN, predisposing them to hematopoietic toxicity (Figure 2), which can be fatal (16).

The molecular basis for polymorphic TPMT activity has now been defined for the majority of patients. Whereas 8 TPMT alleles have been identified, 3 alleles (*TPMT***2, TPMT***3A, TPMT***3C*) account for about 95% of intermediate or low enzyme activity cases (Figure 2) (12, 13). The mutant allele *TPMT***2* is defined by a single nucleotide transversion (G238C) in the open reading frame, leading to an amino acid substitution at codon 18 (Ala>Pro) (17). *TPMT***3A* contains two nucleotide transition mutations (G460A and A719G) in the open reading frame, leading to amino acid substitutions at codon 154 (Ala>Thr) and codon 240 (Tyr>Cys) (18), whereas *TPMT***3C* contains only the A719G transition mutation (18, 19). All three alleles are associated with lower enzyme activity, owing to enhanced rates of proteolysis of the mutant proteins (20). By using allele-specific PCR or PCR-RFLP to detect the three signature mutations in these alleles, a rapid and relatively inexpensive assay is available to identify >90% of all mutant alleles (21). In Caucasian populations, *TPMT***3A* is the most common mutant *TPMT* allele (3.2–5.7% of *TPMT* alleles), whereas *TPMT***3C* has an allele frequency of 0.2–0.8% and *TPMT***2* represents 0.2–0.5% of *TPMT* alleles (13, 21). Studies in

Caucasian, African, and Asian populations have demonstrated the broad utility of this approach (22–24) while revealing that the frequency of these mutant *TPMT* alleles differs among various ethnic populations. For example, East and West African populations have a frequency of mutant alleles similar to that of Caucasians, but all mutant alleles in the African populations are *TPMT*3C* (22). Among African Americans, *TPMT*3C* is the most prevalent allele, but *TPMT*2* and *TPMT*3A* are also found, reflecting the integration of Caucasian and African American genes in the US population (23). In Asian populations, *TPMT*3C* is the predominant mutant allele (100% of mutant alleles in published studies to date).

The presence of *TPMT*2, TPMT*3A, or TPMT*3C* is predictive of phenotype; patients heterozygous for these alleles all have intermediate activity, and subjects homozygous for these alleles are TPMT deficient (21, 23). In addition, compound heterozygotes (*TPMT*2/3A, TPMT*3A/3C*) are also TPMT deficient, as would be expected (21). Whereas most studies have used erythrocytes as a surrogate tissue for measuring TPMT activity, studies have also shown that TPMT genotype determines TPMT activity in leukemia cells (15, 25), as would be expected for germline mutations.

The enthusiasm for TPMT pharmacogenetics has been further stimulated by the finding that TPMT genotype identifies patients who are at risk of toxicity from mercaptopurine or azathioprine. Numerous studies have shown that TPMT-deficient patients are at very high risk of developing severe hematopoietic toxicity when treated with conventional doses of thiopurines (26, 27). More recent studies have shown that patients who are heterozygous at the TPMT gene locus are at intermediate risk of dose-limiting toxicity (28–30). In a study of 67 patients treated with azathioprine for rheumatic disease, six patients (9%) were heterozygous for mutant TPMT alleles (28), and therapy was discontinued in five of the six patients because of low leukocyte count within one month of starting treatment. The sixth patient had documented noncompliance with azathioprine therapy. Patients with wild-type TPMT received therapy for a median of 39 weeks without complications compared with a median of 2 weeks in patients heterozygous for mutant TPMT alleles (28). A second study in patients with Japanese rheumatic disease receiving azathioprine recently confirmed the importance of a heterozygous TPMT genotype for predicting systemic toxicity (29). Futhermore, Relling et al (30) showed that TPMT-deficient patients tolerated full doses of mercaptopurine for only 7% of scheduled weeks of therapy, whereas heterozygous and homozygous wild-type patients tolerated full doses for 65% and 84% of scheduled weeks of therapy, respectively, over the 2.5 years of treatment. The percentage of weeks in which mercaptopurine dosage had to be decreased to prevent toxicity was 2%, 16%, and 76% in wild-type, heterozygous, and homozygous mutant individuals, respectively (30). Collectively, these studies demonstrate that the influence of TPMT genotype on hematopoietic toxicity is most dramatic for homozygous mutant patients, but is also of clinical relevance for heterozygous individuals, who represent about 10% of patients treated with these medications. TPMT deficiency has also been linked to a higher risk of second malignancies among patients with acute lymphoblastic

leukemia, including topoisomerase-inhibitor-induced acute myeloid leukemia (31, 32) and radiation-induced brain tumors (33). Therefore, prospective knowledge of a patient's TPMT status permits patient-specific dosages that reduce the risk of acute toxicity from thiopurine medications (Figure 2) and may identify those at higher risk of second malignancies.

Polymorphic Drug Targets

Genetic polymorphism of the β2-adrenoreceptor exemplifies a clinically relevant polymorphism in a drug target (34). The β2-adrenoreceptor is a G protein–coupled receptor that interacts with endogenous catecholamines and various medications. These receptors are widely distributed and play an important role in regulating cardiac, vascular, pulmonary, and metabolic functions (34). Studies of such physiologic functions of β2-adrenoreceptor in humans have revealed substantial interpatient variation in receptor function and responsiveness to stimulation. In the heart, activation of β2-adrenoreceptor results in an increased rate and force of cardiac muscle, whereas β2-adrenoreceptor stimulation in the lungs acts to relax airway smooth muscle. Influences on lipolysis in subcutaneous fat have also been described, possibly through regulation of lipid mobilization, energy expenditure, and glycogen breakdown. Understanding the molecular basis for variability in the β2-adrenoreceptor has recently been assisted by the identification of five distinct single nucleotide polymorphisms, each associated with altered expression, down regulation, or coupling of the receptor (34). Alteration at amino acid 16 (Arg>Gly) appears to have relevance in pulmonary disease, with patients homozygous for Arg exhibiting a greater response to β2 agonist medications (35, 36). For example, the FEV_1 response to oral albuterol was 6.5-fold higher in patients with an Arg/Arg genotype at codon 16 compared with Gly/Gly patients, even though similar plasma drug concentrations were achieved (35). In contrast, the alteration at codon 27 (Gln>Glu) does not appear to influence lung function, but there is an association between the Gln/Gln genotype and an increased incidence of obesity (37, 38). This relationship appeared to be more prominent in men and could be overcome with exercise (38). The mutant allele for codon 16 (frequency 0.61) and codon 27 (frequency 0.43) are relatively common and are therefore under intensive investigation for their clinical relevance. A less common allele contains a mutation at Codon 164 (Thr>Ile), with a mutant allele frequency of 0.05. The clinical significance of this polymorphism was identified in patients with heart failure: A 42% one-year survival was observed in patients with the Thr/Ile genotype compared with 76% in patients with Thr/Thr (39). This finding led to the suggestion that patients with the Ile164 polymorphism and heart failure should be considered as candidates for early aggressive intervention or cardiac transplantation. More recent findings indicate that β2 receptor haplotype is more informative than individual SNPs in predicting response to beta agonists in asthmatics (39a).

Considerable variation in patient response to therapy can also be observed in clinical trials. Understanding the mechanistic basis for differences in drug

response can be used to identify disease phenotypes to which specific therapy should be directed. An example of this approach was seen in the treatment of Alzheimer's disease, where 83% of patients without an apoE4 genotype had an improvement in total response and cognitive response to tacrine therapy, compared with 40% in patients with apoE4 (40). The specific interaction between the apolipoprotein genotype and tacrine therapy has not been elucidated, but this association suggests that apoE4 plays a role in cholinergic dysfunction in Alzheimer's disease, in a way that cannot be overcome by therapy with acetylcholinesterase inhibitors such as tacrine. Although this relationship between apolipoprotein genotype and improvement in Alzheimer's disease needs to be confirmed, it provides a putative genetic approach for selecting therapy for this disorder.

Although the above examples are illustrative of clinically relevant single nucleotide polymorphism (SNP), many genes with a putative role in the regulation of drug activity do not have clearly defined genetic polymorphisms associated with drug response or disease phenotypes. Therefore, considerable time and money are currently being invested in the production of large libraries of single nucleotide polymorphisms (41) that can be further investigated for an association with drug response. This includes nonprofit ventures (e.g. The SNP Consortium) that release all information to the public free of charge and private SNP efforts from a number of biotech companies (e.g. Genset, Celera Genomics, Incyte). SNPs are the most abundant type of DNA sequence variation in the human genome, with an estimated frequency of 1 in 1000 bases (11, 42). A SNP is a site on the DNA in which a single base pair varies among individuals in a population. If a SNP is found within a small, unique segment of DNA, it serves as both a physical landmark and as a genetic marker whose transmission can be followed from parent to child. According to theoretical models, if the genotype of a group of individuals with a common disease and a group without the disease are studied, certain genotypes may be consistently associated with those individuals who have the disease (41). Owing to linkage disequilibrium, alleles of genetic markers in close proximity to a disease-modifying mutation are often found to be associated with the disease, even though they themselves are not involved in disease pathogenesis or drug response (41). Once localized, these specific chromosomal regions can be analyzed further to identify disease-associated genes and mutations. This molecular/population genetic approach also provides a strategy to identify genes associated with other phenotypes, such as drug toxicity or therapeutic benefit. This approach can be used for genome-wide mapping in which no a priori genes or genomic regions are assumed to be associated with the drug effect under investigation. The number of subjects and the numbers of markers needed for such a study depend on the level of contribution of the specific locus to the complex trait (e.g. a single causative mutation is easier to find than an alteration that is one of several contributors to a phenotype). It is estimated that 60,000 markers, at 50-kb spacing, will be needed to blanket the genome in an association study with 1,000 individuals (e.g. 500 patients with toxicity and 500 patients tolerating therapy). If 1,000 individuals are

to be genotyped with 60,000 markers, 60,000,000 genotyping assays will have to be completed for each study. This requires a dramatic advance in high throughput genotyping techniques for this approach is to be used in a timely and cost-efficient manner. An alternative approach (Figure 3) uses an educated guess as to which of the 100,000 genes in the human genome are likely to be important contributors to the clinical phenotype (43), then a search for informative polymorphisms in these genes. This is especially useful for classes of agents with clearly defined biochemistry, allowing candidate gene selection, as exemplified by the recent preliminary studies of clozapine response in schizophrenia (43a). This candidate gene approach substantially reduces the number of loci under evaluation, but will miss genes with no anticipated role in the drug's in vivo activity. It is through efforts such as these that the next wave of pharmacogenetic predictive tools will emerge, requiring extensive in vitro and in vivo functional analyses to determine the role of each specific SNP in selecting optimal drug therapy.

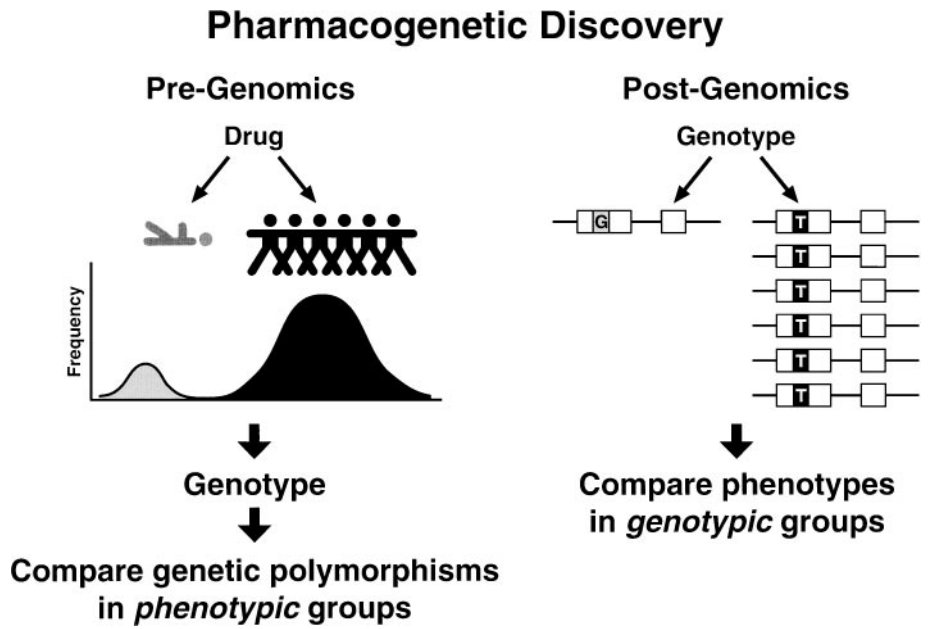

Figure 3 The evolving paradigm for discovery of genetic polymorphisms associated with aberrant drug disposition or effects. In the pregenomics era (e.g. 1950–2000) discoveries were most often made after an unusual phenotype was observed and family studies established an inherited basis, eventually leading to discovery of the genetic basis of the inherited phenotype. In the postgenomics era many more discoveries will begin with elucidation of genetic polymorphisms in candidate genes (e.g. those known to be involved in the metabolism, transport, or targets of the candidate medication), and then large population studies will identify links between these gene polymorphisms and drug effects in patients. (Adapted from MV Relling, personal communication.)

High-Density Pharmacogenomics

Although a candidate gene approach is applicable to agents with clearly defined mechanisms of action, metabolism, and/or toxicity, the pathways influencing a new agent are often unknown. In that circumstance, a genome-wide approach with no a priori assumptions for loci of interest may be the only option. An alternative to low-density genomic approaches, such as SNP analysis, is comparative genomic hybridization (CGH). CGH involves a competitive in situ hybridization of fluorescently labelled test (e.g. tumor) and control (e.g. normal tissue) DNA onto normal metaphase chromosomes from an unrelated healthy donor (44). Computer-assisted fluorescence microscopy is then used to assess the intensity of signal across each chromosome. The differences in test and control fluorescence intensity reflect the change in DNA amount for specific regions of the human genome. If chromosomes or chromosomal subregions are present in identical quantity within both test and control DNA, an equal contribution from each fluorochrome is seen. However, a change in the fluorescent signal is seen if certain chromosomal subregions are gained or lost in the test DNA. CGH is applicable to DNA only and has primarily been used in the context of tumor biology, identifying novel alterations associated with the acquisition of cancer (45). With current technology, CGH has a theoretical limit of detection for gain and loss of genetic material of 5–10 Mb. However, gain of DNA in regions as small as 50 kb have been described in situations in which high-level amplification has occurred (44).

CGH has been used to evaluate a genomic basis for resistance to anticancer chemotherapy. The genomes of cell lines resistant to raltitrexed and 5-fluorouracil, both antimetabolite anticancer agents that inhibit the thymidylate synthase enzyme, were compared with that found in the corresponding parent, sensitive cell line (45). The genome of cell lines resistant to raltritrexed differed from the sensitive cell lines by only a gain of part of the small arm of chromosome 18 (45). This region was subsequently shown to be an amplification of the thymidylate synthase gene on chromosome 18p11.32 (45). The 5-fluorouracil-resistant cell line also had chromosome 18p gain (45). However, several additional regions were associated with drug resistance, including gain of chromosomes 7p11.1-22 and 6p23-25 and loss of chromosomes 2p11.2 and 9p22-q31 (45). These data are especially interesting in the context of our current understanding of raltitrexed and 5-fluorouracil mechanisms of action. Raltitrexed is a thymidylate synthase–specific agent, with no known alternative mechanisms of action. 5-fluorouracil inhibits thymidylate synthase, but also affects RNA and DNA synthesis through false base incorporation (46). This study has generated chromsome loci that will be used to identify specific genes influencing drug resistance to 5-fluorouracil. Similar findings have been generated for cell lines resistant to the anticancer agent cisplatin (47). Although the above studies demonstrate proof of principle for use of CGH in pharmacogenomics, this technique now needs to be applied in the context of agents with unknown mechanisms of action.

Genome-wide analysis of copy number has recently been applied to array-based assays to allow a more automated and rapid CGH approach. The published studies confirm the viability of such an approach, with high resolution mapping of regions of loss and gain in human breast cancer (48). The CGH array approach is limited in the extent of the genome that is evaluable on a single chip and has only been applied to specific chromosomal regions (e.g. chromosome 20) or with superficial coverage of the genome (48, 49). CGH arrays have not yet been used in the context of pharmacogenomics, and CGH's ability to detect only large changes in chromosomal structure represents a substantial limitation of the methodology.

Gene Expression

The development of glass and nylon membrane microarrays has revolutionized the way gene expression is evaluated in all areas of medicine, including pharmacology. Initial studies have focused on gene expression along biologic pathways and have provided an increased understanding of the regulation of cellular proliferation and the cell's response to nutrient stimulation (50). More recently, gene expression arrays have been used in the molecular classification of disease and have highlighted the great genetic heterogeneity among cells with histologically similar appearance (51, 52). For example, Alizadeh et al (51) set out to characterize gene expression in diffuse large B-cell lymphoma (DLBCL), selected because of clinical heterogeneity: 40% of patients respond well to current therapy, whereas the remainder die of disease. By using a "lymphochip" containing 17,856 genes that are preferentially expressed in lymphoid cells, the investigators demonstrated the presence of two molecularly distinct forms of the disease: germinal center B-like DLBCL and activated B-like DLBCL. More important, patients with germinal center B-like DLBCL had a superior overall survival than those with activated B-like DLBCL (5-year survival of 76% versus 16%, respectively) (51). This study provides substance to the proposed use of gene expression arrays as a prospective tool for individualizing patient therapy. Based on the results of Alizadeh, patients with activated B-like DLBCL will not benefit from standard therapy, and experimental treatment approaches should be considered. Alternatively, patients with germinal center B-like DLBCL may be currently "over treated" because of their "good risk" status, and treatment strategies with more manageable side-effect profiles may need to be considered. However, not all evaluations of gene expression array technology have demonstrated usefulness for therapeutics. A molecular evaluation of acute leukemia demonstrated the feasibility of cancer classification based solely on gene expression monitoring rather than the traditional histopathological evaluation (52). The molecular approach is less subjective and not as cumbersome as pathologist-based approaches. This approach was able to differentiate between acute myeloid and acute lymphoid leukemia, and between B-lineage and T-lineage acute lymphoid leukemia within the latter group. However, no strong gene expression signature was evident in this study for those patients achieving

disease remission after induction chemotherapy. This should not be surprising, in that the activity of chemotherapy is a dynamic process in which a series of events must occur down specific molecular pathways. The inability of static measures of a "snapshot" of gene expression to satisfactorily predict therapeutic response is not unreasonable. Indeed, evaluation of the patterns of gene expression during serum starvation was only informative when considered in the context of cluster evaluation of data over a time course (53).

The poor predictive power of "static" array analysis in some studies (52) could also reflect the limited number of genes tested, rather than a deficiency of the approach. Indeed, this conclusion is supported by the recent evaluation of the expression of 8000 genes in the National Cancer Institute panel of 60 human cancer cell lines, which has been used over the past 10+ years to test the cytotoxicity of over 70,000 putative anticancer agents (54). In this analysis, gene expression patterns were used to evaluate the relationship between drug-activity patterns and mechanism of action and to assess gene-drug activity correlations for predictive purposes. For example, the antimetabolite chemotherapy agent 5-fluorouracil is known to be degraded by dihydropyrimidine dehydrogenase (DPD) (55). High DPD would be expected to decrease exposure of cells to the active form of 5-fluorouracil. Consistent with this hypothesis, a significant negative correlation between DPD mRNA expression and 5-fluorouracil potency was observed among the NCI cell line panel ($r = -0.53$) (54). Most cell lines with low DPD mRNA were sensitive to 5-fluorouracil (14/18), and all seven of the colon cancer cell lines were in this category. This observation is consistent with the clinical use of 5-fluorouracil as an active agent in colorectal cancer (55). The utility of this pharmacogenomic approach for selection of therapy for specific tumor subtypes was further illustrated with the amino acid depletion agent L-asparaginase. This agent takes advantage of the lack of asparagine synthetase in some malignant cells, making them dependent on exogenous L-asparagine (54). Overall, a correlation between asparagine synthetase mRNA expression and L-asparaginase cytotoxicity was observed ($r = -0.44$). However, further examination noted a much stronger relationship between asparagine synthetase mRNA expression and L-asparaginase cytotoxicity in the 6 leukemia cell lines ($r = -0.98$) than in the other cell lines ($r = -0.32$) (54). These findings are consistent with the activity of L-asparaginase in acute leukemia and support the use of asparagine synthetase expression as a predictive marker for guiding use of this agent. These data can be explored further by individual investigators using the NCI Drug Discovery Website (http://dtp.nci.nih.gov/). Many additional publicly and privately funded studies of gene expression and drug sensitivity are being performed and will provide the basis for prospective studies of prognostic prediction and characterization studies of drug-activity relationships.

Gene expression array analysis may allow investigators to begin to qualitatively define the elusive "therapeutic index" for specific agents. Both static and dynamic approaches of analyzing gene expression in normal and disease tissues will allow mechanism of action and mechanism of toxicity to be clarified. This will enable

enlightened strategies for modulation of therapy, new agent design, or tissue targeting to be developed, based on direct in vivo observations, rather than theory alone.

Drug Development

Growth in the field of pharmacogenomics has been heavily influenced by the pharmaceutical industry and its desire for a "smarter" drug development process. The potential applications of pharmacogenomics extends from identification of novel targets against which new therapies are designed to tools for predicting efficacy or toxicity during clinical development (1). Pharmacogenomics also has the potential to make the drug development process more efficient, by decreasing the number of patients required to show efficacy in early clinical trials (56). Human and mouse SNP projects are being utilized in an attempt to find specific genes or genomic loci that are associated with the disease of interest. Similar approaches are being conducted using gene expression arrays. Disease tissue is used to produce mRNA for comparison with normal reference tissue. The goal of this approach is gene hunting, and arrays covering the broadest range of known and unknown genes are desired. One goal of the SNP and gene array hunting exercise is the identification of novel targets for therapy. These can be putative modulators of the disease phenotype or new mechanisms of disease. After identification of the target, a great deal of effort must be expended to confirm the viability of the target, in terms of normal-disease tissue expression, pattern of normal tissue expression for toxicity prediction, and frequency of expression in the disease tissue. There are not yet any published examples detailing the efficiency and success rate of such an approach in the early drug development process.

Gene expression arrays are also being applied to define the mechanism of action for new compounds or to screen for direct influence of an agent on a specific pathway. Even agents developed in the most mechanistically based program can display surprises during in vivo evaluation. For example, inhibitors of HMG-CoA reductase, used to control cholesterol levels, were subsequently found to inhibit farnysyl transferase activity in the cell signalling pathway of the oncogenetic ras (57). By using expression arrays, a profile of the genes altered after drug exposure can be generated and may thereby yield a greater understanding of mechanisms of action. Gene expression arrays can also be used during screening of candidate compounds. By constructing arrays for genes involved in a pathway of interest, in vitro or even in vivo gene dynamics can be used as a functional readout for drug activity.

There is now a rapidly growing effort to identify SNPs that will be useful for identifying patients who are likely to benefit from a specific agent or those likely to experience unacceptable toxicity. Examples of TPMT or $\beta 2$ adrenoreceptor SNPs predicting risk of toxicity or outcome are detailed above. In addition, a SNP in the *ALOX5* gene promoter has been found to be associated with the antiasthma efficacy of inhibitors of 5-lipoxygenase, thereby altering leukotriene production (58). There are numerous other genetic polymorphisms in drug-metabolizing enzymes, transporters, and targets, a compilation of which

can be found at www.science.org/features/data/1044449.shl. Most, if not all, pivotal phase II/III studies now include collection of blood for genomic DNA, in order to subsequently evaluate whether SNPs can provide a more intelligent use of the medication under evaluation.

Pharmacogenomics as a Public Health Tool

Although the promise of pharmacogenomics is enormous, it is likely to have the greatest initial benefit for patients in developed countries, owing to expense, availability of technology and the focus of initial research. However, pharmacogenomics should ultimately be useful to world populations. There is clear evidence of ethnic variation in disease risk, disease incidence, and response to therapy (59). In addition, many polymorphic drug metabolizing enzymes have qualitative and quantitative differences among racial groups (59). For example, the COMT low activity allele is less frequent in African and East Asian populations (60). Because COMT inactivates methyldopa, one of the most commonly prescribed antihypertensive medications in those regions of the world, this has important potential implications (61). In addition, COMT influences the activity of levodopa for Parkinson's disease and the production of estrogen metabolites associated with breast cancer.

One approach to applying pharmacogenomics to public health is through SNP allele frequency analysis in defined populations. For example, TPMT genotype in world populations suggests that TPMT-mediated toxicity from azathioprine or mercaptopurine would be lower in Japanese or Chinese populations than Caucasians (13). In contrast, a higher mutant allele frequency was found in the Ghanaian and Kenyan populations (13). In addition, further analysis of the major tribes of Ghana found distinct differences in TPMT allele frequency, ranging from 9.9% heterozygotes in the Ewe population to 13.8% in Fanti individuals (MM Ameyaw & HL McLeod, submitted). Even greater ethnic differences have been established for other polymorphic drug-metabolizing enzymes (e.g. NAT2, CYP2D6, CYP2C19), and this will likely be the case for most pharmacogenomic traits, including drug transporters and targets. This general approach needs to be more extensively evaluated, but does offer the potential for generating information that will have broad application to the development of clinical practice guidelines and national formularies in developing countries.

Although using knowledge of ethnic differences may be relevant to much of the world's populations, it is significantly limited in places with extensive genetic mixing. For example, it is well known that the African American population has a great degree of geographic and social mixing that provide a basis for genetic heterogeneity. This is illustrated in evaluation of TPMT mutations between African American and West African populations. Although the *TPMT*3C* allele was the most frequently observed variant in both populations, it represented 100% of West African mutant alleles and 52% of African American mutant alleles (22, 23). The remaining African American mutant alleles were *TPMT*2* and *TPMT*3A* (23),

alleles that are common in Caucasians. Therefore, great care must be made when applying pharmacogenomics to public health issues, and testing at the genetic level in each patient will remain the most definitive approach.

NONSCIENTIFIC CHALLENGES FOR PHARMACOGENOMICS

There are a number of issues influencing the development of pharmacogenomics, including many that are of a practical or nonscientific nature. An important limitation to the wide application of pharmacogenomics is the availability of gene expression arrays, high throughput genotyping, and informatics. Currently, there is considerable growth in the number of companies offering both genomics analysis on a fee-for-service basis and the equipment for user-maintained instruments. As technology and competition bring down the high initial capital costs of array and genotype systems, the potential for general application of these approaches will be further enhanced.

A related, and unanswered, question is how much can pharmacogenomics analysis cost and still be a viable adjunct to current medical practice? Currently, the technology for gene expression and genotype assessment is only affordable in the research and development setting or in the context of funded research. Thoughtful pharmacoeconomic analysis is needed to justify and direct the further development of pharmacogenomics for rational therapeutics. On the positive side, once a panel of genotypes has been correctly determined for a given individual, they need not be repeated. It is anticipated that a secured, patient-specific database will be established for each person, into which additional results will be deposited as additional genotypes are determined. This potentially web-based compilation of an individual's established genotypes would then be available to authorized healthcare providers for the selection of optimal therapy for the treatment or prevention of diseases.

Finally, the ethics of genetic analysis is currently under avid discussion and debate. Previously, a system of trust and internal control was utilized to prevent inappropriate use of genetic information. This approach has been very successful, with breach of trust being a rare event. However, the field of bioethics is now focusing on prevention of potential or theoretical abuses of genetic information against individuals. This has led to questions about what information is needed, who should have access to the data, and how they should be used. Issues such as these are deeply challenging, as the insurance carrier paying for genetic testing is the same entity that could use the information to identify disease or therapy risks that could be used to restrict future coverage. However, the great potential gains from pharmacogenomics, in terms of both patient well-being and cost of healthcare, heavily outweigh the risks. Putting such powerful information in the hands of knowledgeable healthcare providers and those involved in the discovery of new approaches to disease treatment or prevention offers so much promise that society

TABLE 1 Examples of pharmacogenomic influences on drug activity[a]

Gene	Drug	Effect	Reference
$\beta 2$ adrenoreceptor	Albuterol	Response (FEV_1) in asthmatics	34, 35, 39a 62–64
5-lipozygenase promoter	ABT-761 (zileuton)	Response in asthmatics	58
Angiotensin-converting enzyme (ACE)	Enalapril, lisinopril, captopril	Renoprotective effects, cardiac indices, blood pressure, IgA nephropathy	65–69 70–72
Potassium channels			
HERG	Quinidine	Drug-induced long QT syndrome	73
	Cisapride	Drug-induced *torsade de pointes*	
KvLQT1	Terfenadine, Disopyramide, meflaquine	Drug-induced long QT syndrome	74
hKCNE2	Clarithromycin	Drug-induced arrhythmia	75
ApoE4	Tacrine	Response in Alzheimer's disease	40
TPMT	Azathioprine, mercaptopurine, thioguanine	Hematopoietic toxicity	12, 28, 30
CYP2C9	Warfarin	Anticoagulant effects	76

[a]Additional examples can be found at www.science.org/feature/data/1044449.shl

must find a way to ensure that inappropriate exploitation does not preclude the vast public good that will emerge from the burgeoning field of pharmacogenomics.

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LITERATURE CITED

1. Evans WE, Relling MV. 1999. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 286:487–91
2. Eisen MB, Brown PO. 1999. DNA arrays for analysis of gene expression. *Methods Enzymol.* 303:179–205
3. So CW, Caldas C, Liu MM, Chen SJ, Huang QH, et al. 1997. EEN encodes for a member of a new family of proteins

containing an Src homology 3 domain and is the third gene located on chromosome 19p13 that fuses to MLL in human leukemia. *Proc. Natl. Acad. Sci. USA* 94:2563–68
4. Chen XN, Levine L, Kwok PY. 1999. Fluorescence polarization in homogeneous nucleic acid analysis. *Genome Res.* 9:492–98
5. Germer S, Holland MJ, Higuchi R. 2000. High-throughput SNP allele-frequency determination in pooled DNA samples by kinetic PCR. *Genome Res.* 10:258–66
6. Griffin TJ, Smith LM. 2000. Single-nucleotide polymorphism analysis by MALDI-TOF mass spectrometry. *Trends Biotechnol.* 18:77–84
7. Kwok PY. 1998. Genotyping by mass spectrometry takes flight. *Nat. Biotech* 16:1314–15
8. Iannone MA, Taylor JD, Chen JW, Li MS, Rivers P, et al. 2000. Multiplexed single nucleotide polymorphism genotyping by oligonucleotide ligation and flow cytometry. *Cytometry* 39:131–40
9. Kuklin A. 1997. Detection of single-nucleotide polymorphisms with the WAVE(TM) DNA fragment analysis system. *Genet. Test.* 1:201–6
10. Syvanen AC. 1999. From gels to chips: "minisequencing" primer extension for analysis of point mutations and single nucleotide polymorphisms. *Hum. Mutat.* 13:1–10
11. Marth GT, Korf I, Yandell MD, Yeh RT, Gu Z, et al. 1999. A general approach to single-nucleotide polymorphism discovery. *Nat. Genet.* 23:452–56
12. Krynetski EY, Evans WE. 1998. Pharmacogenetics of cancer therapy: getting personal. *Am. J. Hum. Genet.* 63:11–16
13. McLeod H, Krynetski E, Relling MV, Evans WE. 2000. Genetic polymorphism of thiopurine methyltransferase and its clinical relevance for childhood acute lymphoblastic leukemia. *Leukemia* 14:567–72
14. Weinshilboum RM, Sladek SL. 1980. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am. J. Hum. Genet.* 32:651–62
15. McLeod HL, Relling MV, Liu Q, Pui C-H, Evans WE. 1995. Polymorphic thiopurine methyltransferase in erythrocytes is indicative of activity in leukemic blasts from children with acute lymphoblastic leukemia. *Blood* 85:1897–902
16. Schutz E, Gummert J, Mohr F, Ollerich M. 1993. Azathioprine-induced myelosuppression in thiopurine methyltransferase deficient heart transplant recipient. *Lancet* 341:436
17. Krynetski EY, Schuetz JD, Galpin AJ, Pui C-H, Relling MV, Evans WE. 1995. A single point mutation leading to loss of catalytic activity in human thiopurine S-methyltransferase. *Proc. Natl. Acad. Sci. USA* 92:949–53
18. Tai HL, Krynetski EY, Yates CR, Loennechen T, Fessing MY, et al. 1996. Thiopurine S-methyltransferase deficiency: two nucleotide transitions define the most prevalent mutant allele associated with loss of catalytic activity in Caucasians. *Am. J . Hum. Genet.* 58:694–702
19. Loennechen T, Yates CR, Fessing MY, Relling MV, Krynetski EY, Evans WE. 1998. Isolation of a human thiopurine S-methyltransferase (TPMT) complementary DNA with a single nucleotide transition A719G (TPMT*3C) and its association with loss of TPMT protein and catalytic activity in humans. *Clin. Pharmacol. Ther.* 64:46–51
20. Tai HL, Krynetski EY, Schuetz EG, Yanishevski Y, Evans WE. 1997. Enhanced proteolysis of thiopurine S-methyltransferase (TPMT) encoded by mutant alleles in humans (TPMT*3A, TPMT*2): mechanisms for the genetic polymorphism of TPMT activity. *Proc. Natl. Acad. Sci. USA* 94:6444–49
21. Yates CR, Krynetski EY, Loennechen T, Fessing MY, Tai HL, et al. 1997.

Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. *Ann. Intern. Med.* 126:608–14
22. Ameyaw MM, Collie-Duguid ES, Powrie RH, Ofori-Adjei D, McLeod HL. 1999. Thiopurine methyltransferase alleles in British and Ghanaian populations. *Hum. Mol. Genet.* 8:367–70
23. Hon YY, Fessing MY, Pui C-H, Relling MV, Krynetski EY, Evans WE. 1999. Polymorphism of the thiopurine S-methyltransferase gene in African Americans. *Hum. Mol. Genet.* 8:371–76
24. Collie-Duguid ES, Pritchard SC, Powrie RH, Sludden J, Li T, McLeod HL. 1999. The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations. *Pharmacogenetics* 9:37–42
25. Coulthard SA, Howell C, Robson J, Hall AG. 1998. The relationship between thiopurine methyltransferase activity and genotype in blasts from patients with acute leukemia. *Blood* 92:2856–62
26. Lennard L, Van Loon JA, Weinshilboum RM. 1989. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. *Clin. Pharmacol. Ther.* 46:149–54
27. Evans WE, Horner M, Chu YQ, Kalwinsky D, Roberts WM. 1991. Altered mercaptopurine metabolism, toxic effects, and dosage requirement in a thiopurine methyltransferase-deficient child with acute lymphocytic leukemia. *J. Pediatr.* 119:985–89
28. Black AJ, McLeod HL, Capell HA, Powrie RH, Matowe LK, et al. 1998. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. *Ann. Intern. Med.* 129:716–18
29. Ishioka S. 1999. Thiopurine methyltransferase genotype and the toxicity of azathioprine in Japanese. *Intern. Med.* 38:944–47
30. Relling MV, Hancock ML, Rivera GK, Sandlund JT, Ribeiro RC, et al. 1999. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *J. Natl. Cancer Inst.* 91:2001–8
31. Relling MV, Yanishevski Y, Nemec J, Evans WE, Boyett JM, et al. 1998. Etoposide and antimetabolite pharmacology in patients who develop secondary acute myeloid leukemia. *Leukemia* 12:346–52
32. Bo J, Schroder H, Kristinsson J, Madsen B, Szumlanski C, et al. 1999. Possible carcinogenic effect of 6-mercaptopurine on bone marrow stem cells: relation to thiopurine metabolism. *Cancer* 86:1080–86
33. Relling MV, Rubnitz JE, Rivera GK, Boyett JM, Hancock ML, et al. 1999. High incidence of secondary brain tumours after radiotherapy and antimetabolites. *Lancet* 354:34–39
34. Liggett SB, Wagoner LE, Craaft LL, Hornung RW, Hoit BD, et al. 2000. beta(2)-Adrenergic receptor pharmacogenetics. *Am. J. Resp.* 161:S197–201
35. Lima JJ, Thomason DB, Mohamed MHN, Fberle LV, Shelf TH, Johnson JA. 1999. Impact of genetic polymorphisms of the beta(2)-adrenergic receptor on albuterol bronchodilator pharmacodynamics. *Clin. Pharmacol. Ther.* 65:519–25
36. Tan S, Hall IP, Dewar J, Dow E, Lipworth B. 1997. Association between beta 2-adrenoceptor polymorphism and susceptibility to bronchodilator desensitisation in moderately severe stable asthmatics. *Lancet* 350:995–99
37. Large V, Hellstrom L, Reynisdottir S, Lonnqvist F, Eriksson P, et al. 1997. Human beta-2-adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. *J. Clin. Invest.* 100:3005–13
38. Meirheaghe A, Halbecque N, Cottel D, Amouyel P. 1999. Beta 2-adrenoceptor gene polymorphism, body weight, and physical activity. *Lancet* 353:869

39. Liggett SB. 1998. Pharmacogenetics of relevant targets in asthma. *Clin. Exp. Allergy* 28(Suppl. 1):77–79

39a. Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, et al. 2000. Complex promoter and coding region beta(2)-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc. Natl. Acad. Sci. USA* 97:10483–88

40. Poirier J, Delisle MC, Quirion R, Aubert I, Farlow M. 1995. Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 92:12260–64

41. Kwok PY, Gu ZJ. 1999. Single nucleotide polymorphism libraries: Why and how are we building them? *Mol. Med. Today* 5:538–43

42. Brookes AJ. 1999. The essence of SNPs. *Gene* 234:177–86

43. Emahazion T, Jobs M, Howell WM, Siegfried M, Wyoni PI. 1999. Identification of 167 polymorphisms in 88 genes from candidate neurodegeneration pathways. *Gene* 238(2):315–24

43a. Arranz MJ, Munro J, Birkett J, Bolona A, et al. 2000. Pharmacogetetic predictions of clozapine response. *Lancet* 355:1615–16

44. Rooney PH, Murray GI, Stevenson DAJ, Haites NE, Cassidy J, McLeod HL. 1999. Comparative genomic hybridization and chromosomal instability in solid tumors. *Br. J. Cancer* 80:862–73

45. Rooney PH, Stevenson DA, Marsh S, Johnston PG, Haites N, et al. 1998. Comparative genomic hybridization analysis of chromosomal alterations induced by the development of resistance to thymidylate synthase inhibitors. *Cancer Res.* 58:5042–45

46. Rustum YM, Harstrick A, Cao S, Vanhoefer U, Yin MB, et al. 1997. Thymidylate synthase inhibitors in cancer therapy: direct and indirector inhibitors. *J. Clin. Oncol.* 15:389–400

47. Leyland Jones B, Kelland LR, Harrap KR, Hiorns LR. 1999. Genomic imbalances associated with acquired resistance to platinum analogues. *Am. J. Pathol.* 155:77–84

48. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, et al. 1998. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat. Genet.* 20:207–11

49. Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, et al. 1999. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nat. Genet.* 23:41–46

50. Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, et al. 2000. Systematic variation in gene expression patterns in human cancer cell lines. *Nat. Genet.* 24:227–35

51. Alizadeh AA, Eisen MB, Davis E, Ma C, Lossos IS, et al. 2000. Distinct types of diffuse large B-cells lymphoma identified by gene expression profiling. *Nature* 403:503–11

52. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, et al. 1999. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 286:531–37

53. Iyer VR, Eisen MB, Ross DT, Schuler G, Moore T, et al. 1999. The transcriptional program in the response of human fibroblasts to serum. *Science* 283:83–87

54. Scherf U, Ross DT, Waltham M, Smith LH, Lee JK, et al. 2000. A gene expression database for the molecular pharmacology of cancer. *Nat. Genet.* 24:236–44

55. Milano G, McLeod HL. 2000. Can dihydropyrimidine dehydrogenase impact 5-fluorouracil-based treatment. *Eur. J. Cancer* 36:37–42

56. Fijal BA, Hall JM, Witte JS. 2000. Clinical trials in the genomic era: effects of protective genotypes on sample size and duration of trial. *Control. Clin. Trials* 21:7–20

57. Rowinsky EK, Windle JJ, VonHoff DD.

2000. Ras protein farnesyltransferase: a strategic target for anticancer therapeutic development. *J. Clin. Oncol.* 17:3631–52
58. Drazen JM, Yandava CN, Dube L, Szczerback N, Hippensteel R, et al. 1999. Pharmacogenetic association between *ALOX5* promoter genotype and the response to anti-asthma treatment. *Nat. Genet.* 22(2):168–70
59. Wood AJ. 1998. Ethnic differences in drug disposition and response. *Ther. Drug Monit.* 20:525–26
60. McLeod HL, Syvanen AC, Githang'a J, Indalo A, Ismail D, et al. 1998. Ethnic differences in catechol O-methyltransferase pharmacogenetics: Frequency of the codon 108/158 low activity allele is lower in Kenyan than Caucasian or South-West Asian individuals. *Pharmacogenetics* 8:195–99
61. Weinshilboum RM. 1999. Methylation pharmacogenetics: catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. *Annu. Rev. Pharmacol. Toxicol.* 39:19–52
62. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson. 1997. Association between genetic polymorphisms of the beta2-adrenoceptor and\par response to albuterol in children with and without a history of wheezing. *J. Clin. Invest.* 100:3184–88
63. Hancox RJ, Aldridge RE, Cowan JO, Flannery EM, Herbison GP, et al. 1999. Tolerance to beta-agonists during acute bronchoconstriction. *Eur. Resp. J.* 14:283–87
64. Lipworth BJ, Hall IP, Tan S, Aziz I, Coutie W. 1999. Effects of genetic polymorphism on ex vivo and in vivo function of beta2-adraenoceptors in asthmatic patients. *Chest* 115(2):324–28
65. van der Kleij FG, Navis GJ, Gansevoort RT, Heeg JE. 1997. ACE polymorphism does not determine short-term renal response to ACE-inhibition in proteinuric patients. *Nephrol. Dial. Transplant.* 12(Suppl 2): 42–46
66. Haas M, Yilmaz N, Schmidt A, Neyer U, Arneitz K, et al. 1998. Angiotensin-converting enzyme gene polymorphism determines the antiproteinuric and systemic hemodynamic effect of enalarpril in patients with proteinuric renal disease. *Kidney Blood Press. Res.* 21:66–69
67. Nakano Y, Oshima T, Watanabe M, Matsuura H, Kajiyama G, Kambe M. 1997. Angiotensin I-converting enzyme gene polymorphism and acute response to captopril in essential hypertension. *Am. J. Hypertens.* 10:1064–68
68. O'Toole L, Stewart M, Padfield P, Channer K. 1998. Effect of the insertion/deletion polymorphism of the angiotensin-converting enzyme gene on response to angiotensin-converting enzyme inhibitors in patients with heart failure. *J. Cardiol. Pharmacol.* 32:988–94
69. Sasaki M, Oki T, Iuchi A, Tabata T, Yamada H, et al. 1996. Relationship between the angio tensin converting enzyme gene polymorphism and the effects of enalapril on left ventricular hypertrophy and impaired diastolic filling in essential hypertension: M-mode and pulsed doppler echocardiographic studies. *J. Hypertens.* 14:1403–8
70. Mizuiri S, Hemmi H, Inoue A, Takano M, Kadomatsu S, et al. 1997. Renal hemodynamic changes induced by captopril and\par angiotensin-converting enzyme gene polymorphism. *Nephron* 75:310–14
71. Yoshida H, Mitarai T, Kawamura T, Kitajima T, Miyazaki, et al. 1995. Role of the deletion of polymorphism of the angiotensin converting enzyme gene in the progression and therapeutic responsiveness of IgA nephropathy. *J. Clin. Invest.* 96:2162–69
72. Essen GGV, Rensma PL, Zeeuw DD, Sluiter WJ, Scheffer H, Apperloo AJ. 1996. Association between angiotensin-converting-enzyme gene polymorphism and failure of renoprotective therapy. *Lancet* 347:94–95

73. Priori SG, Barhanin J, Hauer RN, Haverkamp W, Jongsma HJ, et al. 1999. Genetic and molecular basis of cardiac arrhythmias: impact on clinical management part III. *Circulation* 99:674–81
74. Donger C, Denjoy I, Berthet M, Neyroud N, Cruaud C, et al. 1997. KVLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome. *Circulation* 96:2778–81
75. Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, et al. 1999. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 97:175–87
76. Aithal GP, Day CP, Kesteven PJ, Daly AK. 1999. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 353:717–19

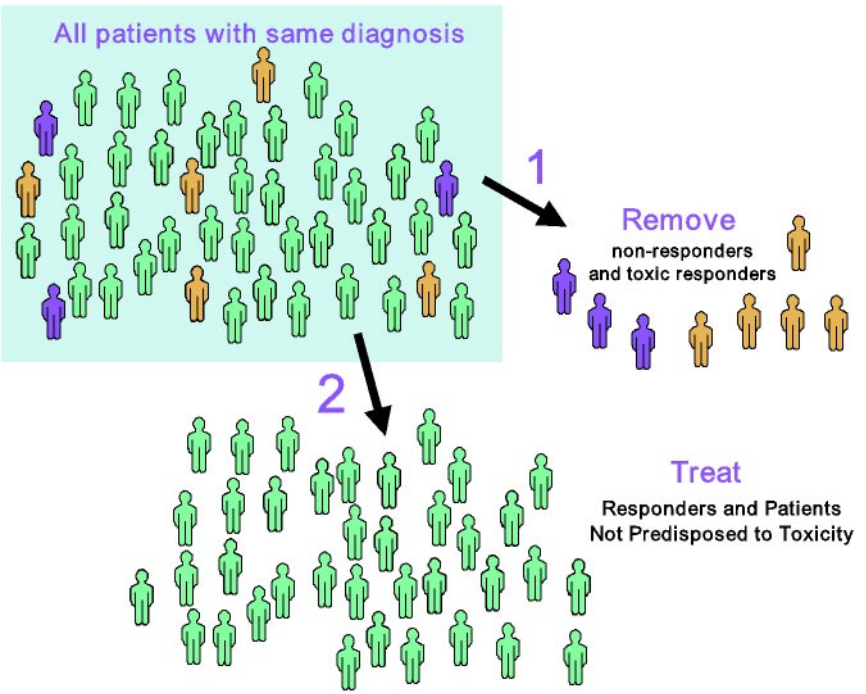

Figure 1 Pharmacogenomics has the potential to identify the subsets of patients who are genetically predisposed to toxicity from specific medications and those who are likely not to respond. Predisposition to toxicity can occur because of an inherited deficiency in drug metabolism, while mutations in drug receptors can alter a patient's response to medications. The subset of patients who are identified as toxic responders or nonresponders would be treated with different dosages or alternative medications.