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*Perspectives in Pharmacology*

# Genetics and Susceptibility to Toxic Chemicals: Do You (or Should You) Know Your Genetic Profile?

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Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP, cytochrome P450 enzyme; P450, cytochrome P450; FMO, flavin-containing monooxygenase; FDA, Food and Drug Administration; GST, glutathione *S*-transferase; mEH, microsomal epoxide hydrolase; NAT, *N*-acetyltransferase; sEH, soluble epoxide hydrolase; UGT, UDP glucuronosyltransferase; TPMT, thiopurine methyltransferase.

**Abstract**

This review is based on a symposium/roundtable session, sponsored by the Division of Toxicology of the American Society for Pharmacology and Experimental Therapeutics, that was held at the 2002 Experimental Biology meeting in New Orleans, Louisiana. The focus is on the role of pharmacogenomics in determining individual susceptibility to chemically induced toxicity. An individual's risk of disease from exposure to toxic chemicals is determined by a complex interplay between genetics, physiology, and concurrent or prior exposures to drugs and other chemicals. The first section of the review defines the basics of pharmacogenetics and pharmacogenomics and assesses the current state of the science. Selected applications to specific enzyme systems are summarized by way of example. New, state-of-the-art approaches to studying genetic determinants of susceptibility, including analytical methods and transgenic technology are then discussed. Finally, ethical and legal concerns with the application of this knowledge and methodology to human health will be discussed.

**Pharmacogenetics and Susceptibility to Toxic Chemicals: Historical Perspective, Current Status and Future Challenges**

The objective of this article is to place the field of pharmacogenetics and pharmacogenomics into historical perspective, to review its current status, and also to examine the promises and challenges of the future. But what is pharmacogenetics and what is the difference between this term and pharmacogenomics – a phrase that has arisen much more recently? In fact, both involve the study of genetically determined variation in xenobiotic response. However, pharmacogenetics has classically focused on one or perhaps two loci to explain intersubject variation. With improvements in analytical technology coupled with the sequencing of the human genome and the technological advances spurred by this endeavor, as well as improvements in data analysis, it has become possible to take a more global approach to underlying causes of variation and thus, the advent of the field of pharmacogenomics.

Several key principles are widely recognized in pharmacogenetics and pharmacogenomics. First, causative polymorphisms will be heritable and will occur at high frequency, but low penetrance (defined as the probability of a genetic trait being expressed). Thus, a genetic variation leading to a pharmacogenetic polymorphism will occur at an allelic frequency greater than 1%. However, the resulting phenotype will only be observed after a suitable environmental exposure. Second, not all pharmacogenetic traits are of high clinical or toxicological significance. Rather, their importance is determined by the frequency of exposure eliciting the phenotype, the narrowness of the therapeutic index or sharpness of the dose-response curve, the limited availability of alternative clearance pathways, and the absence of alternative therapeutics or chemicals. A third important principle has arisen as we have learned more about the temporal-specific expression of xenobiotic metabolizing enzymes. Examples within pediatric pharmacology have been described wherein temporal changes in gene expression result in phenotypes inconsistent with genotype. Importantly, this incongruence resolves with time. These cases emphasize the continued importance of phenotype as individuals who have not reached a critical age may be deficient in a particular enzyme simply because the

onset of expression has yet to occur, not because of a variant allele. Specific examples include transient trimethylaminuria due to the low or absent expression of *FMO3* in the neonate and infant (Mayatepek and Kohlmüller, 1998; Koukouritaki et al., 2002) and the relatively low clearance of theophylline in the neonate due to the late onset of *CYP1A2* expression (Nassif et al., 1981; Sonnier and Cresteil, 1998). Other examples can be found in Leeder (2001). Finally, genetic variability at any single locus has already, or soon will be well defined. However, it is clear that many pharmacogenetic traits result from complex haplotypes wherein variation at multiple loci define a susceptible population. Defining and clinically proving the significance of such complex haplotypes will serve as a major challenge for pharmacogenomics.

The advances that spawned the field of pharmacogenomics have also resulted in a renewed enthusiasm for its promise for human health within both commercial and academic settings. However, before discussing both the current state of the field, as well as future promises and challenges, it would be appropriate to examine some historical highlights.

**Historical Perspective.** Perhaps the first recorded observation of individual variation in response to a xenobiotic exposure was that by Pythagoras in 510 B.C., when he noted that some, but not all individuals develop hemolytic anemia in response to fava bean ingestion. It was not until the report by Gorrod and Oxon in 1902 (1902), however, that it was suggested that genetically determined differences in biochemical processes were the cause of adverse drug reactions and interindividual differences in toxicity were due to enzyme deficiencies. Thirty years later, another significant advance was made when Snyder described the first population-based study to identify ethnic variation in a pharmacogenetic trait, i.e., phenylthiocarbamate non-taster phenotype (Snyder, 1932). Such variability across different ethnic groups is now recognized as a common property of most pharmacogenetic traits.

The next two decades saw a remarkable increase in both an awareness and interest in pharmacogenetics due to adverse responses to two commonly used and important therapeutics: Isoniazid-induced neuropathy was observed as a relatively common adverse reaction, which we now know is due to deficiency in the *N*-acetyltransferase 2 (*NAT2*)-encoded arylamine *N*-

acetyltransferase; and prolonged apnea was observed in patients who had been treated with succinylcholine and who had a deficiency in pseudocholinesterase. It was also during this era that Motulsky (1957) expanded and refined the ideas originally proposed by Gorrod and Oxon, suggesting that interindividual differences in drug efficacy, as well as adverse drug reactions were due, in part, to genetic variation and Vogel (1959) first coined the term, "pharmacogenetics." Recognizing this emerging field, Werner Kalow organized a symposium at the 1965 meeting of the Federation of American Societies for Experimental Biology entitled "Experimental and Clinical Aspects of Pharmacogenetics." It would be several years, however, before the first, clinically important polymorphisms would be described in a member of the cytochrome P450 (P450) superfamily, now recognized as playing a prominent role in drug metabolism. In 1977, Smith and colleagues described an idiosyncratic, hypotensive response to debrisoquine that correlated with a deficiency in drug hydroxylation (Mahgoub et al., 1977). Two years later, Eichelbaum and his colleagues reported that poor metabolic clearance of the antiarrhythmic, sparteine, was due to a deficiency in *N*-oxidation (Eichelbaum et al., 1979). It was later shown that both of these responses, as well as several others, are due to allelic variants at the *CYP2D6* locus (Gonzalez et al., 1988). The identification of pharmacogenetic polymorphisms in other enzymes involved in drug metabolism followed quickly, including genetic variation in thiopurine methyltransferase (Weinshilboum and Sladek, 1980) and glutathione *S*-transferase (Seidegård and Pero, 1985). Some examples of enzymes exhibiting polymorphisms and some of the possible consequences of these variations are shown in **Table 1**.

In recognition of the growing interest in pharmacogenetics and its implications for toxicology, as well as pharmacology, Gill Omen and Harry Gelboin organized a Banbury Conference in 1983 entitled “Genetic Variability in Response to Chemical Exposure” (1984) and Richard Weinshilboum and Elliot Vesell organized symposia entitled, "Human Pharmacogenetics" and "New Directions in Pharmacogenetics," respectively, at the 1984 meeting of the Federation of American Societies for Experimental Biology. Finally, 1991 saw the birth of the journal, *Pharmacogenetics*, which focuses on genetic determinants of drug and

other chemical responses in both humans and animal models. This obviously is a cursory overview of the rich history of pharmacogenetics, merely describing highlights of the pioneering studies and events that set the stage for the current interest and activity in this field. For a more complete history, the reader is referred to other recent excellent reviews (Nebert, 1997; McLeod and Evans, 2001; Leeder, 2001).

It is interesting to note that the Human Genome Project was serendipitously conceived, in part, as a result of toxicology. The Alta Summit, sponsored by the Department of Energy and the International Commission for Protection Against Environmental Mutagens and Carcinogens, was held in December of 1984 to determine if new methods could be developed to detect increases in the mutation rates among Hiroshima and Nagasaki bombing survivors and their children, as well as in U.S. populations exposed to mutagenic chemicals, Agent Orange, and radiation from atmospheric testing of nuclear weaponry during the 1950s. The report from this meeting subsequently served as the impetus for the Human Genome Project (DeLisi, 1988; Cook-Deegan, 1989). Although conceived in response to issues related to toxicity, it is ironic that toxicology is now undergoing a revolution as a result of the Human Genome Project that will undoubtedly impact mechanistic research related to toxicology as well as safety and risk assessments.

**Current Status.** The push to complete the human genome led to rapid advances in high-throughput technologies. Today, these same technologies are being use to catalogue genetic variants in molecules relevant to pharmacology and toxicology, including receptors, signaling molecules, transporters, and metabolic enzymes (http://www.genome.utah.edu/genesnps/ and http://www.ncbi.nlm.nih.gov/SNP/). These same technologies are also allowing for high-throughput genotyping, enabling large population studies to identify multiple loci that contribute to complex phenotypes. There have also been rapid advances in assessing global responses. Thus, gene expression and proteomic microarrays are being used to map changes in mRNA and protein expression, while metabonomics is being used to assess changes in metabolite profiles. Of course, the most powerful approach is when all three of these complementary technologies are used to assess phenotype. The advent of such high-throughput technologies has resulted in

massive data sets that in turn, have spurred major advances in bioinformatics, as well as unprecedented cross-discipline collaboration. Finally, there have also been major successes in identifying and validating *in vivo* probes for assessing phenotype in relatively non-invasive manners.

**Promises and Challenges.** The above advances in pharmacogenetics and pharmacogenomics have provided renewed excitement in the potential to attain some of the field's long-term objectives in human health. Challenges, however, still exist. The promise of rational versus empirical therapeutics was touted early on as a rationale for studies in pharmacogenetics. Thus, based on genotype and/or phenotype, individualized therapeutic entities and dosing regimens would be selected to maximize benefits to the patient, while minimizing risk. Obstacles still exist, however, before such therapeutic strategies can be implemented. The cost of routine genotyping/phenotyping in a clinical setting is a challenge that likely can be addressed with relative ease. More difficult will be the education of health care providers and third-party payers with regards to the benefits and cost-effectiveness of such a strategy. Finally, several ethical issues remain to be resolved, particularly with regards to confidentiality.

High-throughput technologies offer the promise of high density and robust pharmacogenomics. Investigators will be able to perform global searches for genetic variants associated with a disease or responsive phenotype, as well as use global changes in gene expression as a phenotypic measure. Indeed, many of these technologies currently are being applied with intriguing results. Yet, several significant obstacles also face the field. Although the high-throughput methods offer tremendous promise, the ability to show significant association of multiple variants (i.e., a complex haplotype) to either a complex or even simple phenotype will require large population studies that are costly and technically difficult. Further, bioinformatics is still struggling with the most appropriate means to analyze such large data sets, which is compounded by the lack of standardization across platforms. With respect to analyzing changes in gene expression as a measure of response in the human, it will be critical to obtain early-stage tissue. However, this usually is an extremely difficult, if not impossible task. The alternative, i.e.,

the use of animal models, offers its own challenges, particularly with respect to extrapolating resulting data to the human.

In the area of drug discovery and development, pharmacogenomics and pharmacogenetics offer the ability to identify novel drug targets, as well as the tools to predict both efficacy and toxicity. Knowledge of genetic variability that impacts response can also be used to increase the efficiency of clinical trials by the rational selection of patients. Finally, this same knowledge can be used in the design of specific pharmacophores for responder versus non-responder populations. To achieve this promise, there is a need for improved functional genomics, some of which is discussed in the previous paragraph. Further, although there has been tremendous effort expended to implement this approach, no examples have emerged that offer proof of the underlying principles. Such an achievement would provide an important impetus for the field.

Pharmacogenomics and pharmacogenetics offer the ability to identify susceptible populations for environmental risk assessment, as well as the ability to improve our understanding of environmental toxicant mechanism(s) of action. However, once again, challenges are apparent. To date, there have been numerous inconsistencies in reports associating specific variants with a particular outcome. Some of these have undoubtedly arisen because of inadequate study design, which in turn, relate to cost and feasibility. Nevertheless, such inconsistencies mislead both the scientific and lay communities. The application of pharmacogenomics and pharmacogenetics to environmental health offers its own ethical challenges, again, particularly in the area of confidentiality. Finally, it is well recognized that significant inter-ethnic differences exist in the frequencies of pharmacogenetic variants. Although important to recognize, these differences also become problematic in attempting to apply this knowledge in risk assessment. It is rare to identify isolated populations that would strictly adhere to these rules. The substitution of specific haplotypes for ethnicity in such association studies would allow for more rigorous results and conclusions.

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**Use of Gene Knock-Out and Transgenic Mice to Study the Roles of Xenobiotic-Metabolizing Enzymes in Toxicology**

Numerous enzymes exist that metabolize foreign chemicals. Commonly referred to as xenobiotic-metabolizing enzymes, they consist of P450, flavin-containing monooxygenases (FMOs), and epoxide hydrolases that metabolize oxygen and a series of conjugating enzymes. These enzymes are responsible for the metabolism of drugs and dietary chemicals as well as environmental and dietary toxins and carcinogens. Although many toxins and carcinogens are direct-acting, many others are inert compounds that require metabolic activation to electrophiles to exert their damaging effects. Based of this, one can predict that the cellular levels of these enzymes can determine the extent of metabolism, either inactivation and activation, and possible damage produced by a particular chemical. This is especially germane to the known existence of polymorphisms in xenobiotic-metabolizing enzymes in humans and to the search for associations between levels of expression of these enzymes or genetic variants/mutations and cancer incidence in humans.

P450s are a superfamily of hemoproteins; over 2500 (including 977 in animals) have been identified (http://drnelson.utmem.edu/CytochromeP450.html). Only a limited number of mammalian P450s, however, metabolize toxins and carcinogens, and these forms are conserved in different mammalian species, including the widely studied rat and mouse experimental models, and humans. These include CYP1A1, CYP1A2, CYP1B1 and CYP2E1. This is in contrast to the marked species differences in expression and catalytic activities of the P450s within the other major CYP2 subfamilies. Thus, it is assumed that data obtained from studying carcinogenesis in rodents with focus on CYP1A1, CYP1A2, CYP1B1 and CYP2E1 can be extrapolated to humans. Standard biochemical analysis using microsomes, purified enzymes, and recombinant P450s has yielded considerable information about the catalytic activities of these P450s toward a number of known drugs and other chemicals of relevance to human health. It had never been established with certainty, however, that P450s are required for toxicity and chemical carcinogenesis in an intact animal model. To investigate this using a genetic system, P450 null

mice were produced and subjected to analysis. This approach is an example of how transgenic technology can be applied to the study of pharmacogenetics as it can be used to model different genotypes.

The CYP1A1 (Dalton et al., 2000), CYP1A2 (Buters et al., 1996; Pineau et al., 1995) CYP1B1 (Buters et al., 1999) and CYP2E1 (Lee et al., 1996) genes were subjected to targeted gene disruption. All mice were phenotypically normal; they reproduced, developed without problems and exhibited no traits that would indicate that these enzymes were critical for development and physiological homeostasis. Thus, at least in laboratory mice kept under controlled conditions and with standard rodent chows, some P450s have no important function other than to metabolize xenobiotics. The microsomal (Miyata et al., 1999) and soluble (Sinal et al., 2000) epoxide hydrolase null mice (designated mEH and sEH, respectively) also had no deleterious phenotypes except that the sEH-nulls had a gender-specific blood pressure phenotype. Since the mice lacking expression of P450s and epoxide hydrolases have no adverse phenotypes, they are ideally suited for use in toxicity and cancer bioassays.

The following is an example of how these mouse models can be used in the study of chemical toxicity. Acetaminophen is a widely used, over-the counter analgesic and generally considered a safe drug. However, there are infrequent reports of lethal hepatic necrosis in humans and this can be reproduced in experimental animals (Rumack, 2002). Acetaminophen metabolism by P450 results in production of a highly reactive electrophilic metabolite, *N*-acetyl-*p*-benzoquinoneimine, that is rapidly conjugated and inactivated by glutathione. Under conditions of low cellular levels of glutathione, however, the quinone metabolite can cause cell death by binding to critical macromolecules. The P450 forms that metabolize acetaminophen have been investigated using native and recombinant enzymes and the results indicated an involvement of CYP1A2, CYP2E1 and CYP3A4 (Patten et al., 1993).

To investigate the role of individual P450s in acetaminophen toxicity *in vivo*, the CYP1A2-null, CYP2E1-null and CYP1A2/CYP2E1 double-null mice were compared (Zaher et al., 1998). All three null mouse lines were more resistant to acetaminophen toxicity than wild-

type mice with the rank order of sensitivity wild-type > CYP1A2-null >> CYP2E1-null >>> double-null (**Fig. 1**). These studies established that the principal P450 responsible for acetaminophen toxicity is CYP2E1, in agreement with the *in vitro* data and studies with ethanol treatment (Prasad et al., 1990). Interestingly, the double-null mice were highly resistant to toxicity, indicating the involvement of both CYP1A2 and CYP2E1. These studies show the utility of P450-null mice in assessing the function of P450s in chemical-induced toxicity and in modeling a genetic polymorphism in which the variant phenotype is null.

**Gene Expression Profiling in Mechanistic and Predictive Toxicology**

Toxicogenomics, an emerging subdiscipline, incorporates bioinformatics, genomics, proteomics and metabonomics into toxicology. The inclusion of toxicogenomics into predictive and mechanistic toxicology and preclinical safety assessments of new chemical entities in research and drug development has resulted in the accumulation of vast quantities of data that must be accurately and efficiently indexed and archived to facilitate data analysis and the extraction of decision-supportive information. This section provides an overview of the incorporation of microarray technology into mechanistic and predictive toxicology and discusses the infrastructure required to fully utilize this technology. More specifically, issues related to the development of dbZach (http://dbzach.fst.msu.edu, a toxicogenomic supportive relational database), the construction of model specific cDNA/EST arrays, study design, and data analysis are presented.

Many consider microarrays to be an enabling technology that can provide unprecedented volumes of information regarding mechanisms of action of known toxicants as well as potential toxicities of chemical substances. Microarray assays can simultaneously interrogate the expression of thousands of genes within a model of interest under several conditions. The ability to capture the expression of each gene in response to changes in cellular state (e.g., differentiation, disease) or environment (e.g., exposure to drug or chemical) is commonly referred to as expression profiling. The relative abundance of transcripts within a cell or tissue is

assumed to be indicative of specific cellular reactions in response to treatment or to other changes in cellular state. Consequently, mechanisms or modes of action of the response can be inferred from the observed transcriptional profile. Expression profiles, therefore, constitute a detailed molecular phenotype that can be reverse engineered to classify the perturbation or treatment and support investigation into the mechanisms of action of the compound (Nuwaysir et al., 1999).

To date, microarray assays have been used to demonstrate the potential of global gene expression profiling to accurately diagnose tumor status based on gene expression alone (Alon et al., 1999; Perou et al., 1999; Alizadeh et al., 2000). It is expected that similar profiling strategies can be used to predict the toxicity of drug candidates and other chemicals and to characterize their potential toxicity based on the similarity of their expression profiles compared to profiles obtained from known toxicants with defined mechanisms of action. Proof of this principle has been demonstrated in a several model systems, including yeast (Marton et al., 1998; Hughes et al., 2000), mammalian cells in culture (Burczynski et al., 2000; Waring et al., 2001b) and mouse liver (Waring et al., 2001a; Hamadeh et al., 2002a, 2002b).

Measuring changes in gene expression over time and across dose provides critical information regarding the kinetics and coordination of gene expression that contribute to the dynamic processes of cellular homeostasis and toxicity. Analyzing gene expression data across multiple treatments or responsive species also reveals underlying similarities among different conditions, thus producing correlates of gene behavior that can be used to predict and diagnose cellular responses to exogenous chemicals. A number of multivariate methods have been used to extract ordered subsets of information from disordered sets of multi-conditional gene expression data, including hierarchical clustering and principal component analysis, as well as partitioning methods such as K-means clustering and self-organizing maps. In general, these methods attempt to find order among disordered data sets by grouping similar objects together. Grouping based on similarity of expression has been used to identify genes that have similar function and/or are co-regulated (Marton et al., 1998; Burczynski et al., 2000; Hughes et al., 2000). Therefore, it is

possible to identify genes that contribute to toxicity and to predict putative mechanisms of action based on correlated global gene expression patterns, which are obtained by comparing responses to uncharacterized drug candidates or chemicals to those of toxicants with well-defined mechanisms. The genes and responses to experimental conditions can both be clustered so that treatments that induce similar expression profiles across all genes on the microarray are in close proximity, inferring mechanistic relationships, while genes that are similar in expression profile across all conditions are closer together in the second dimension, and perhaps functionally related. This approach can be used to generate a molecular phenotype to identify potential mechanisms of toxicity and to illustrate relationships between multiple conditions and gene expression patterns, so that subsets of genes, rather than a single biomarker, can be used as more accurate predictors of toxicity.

Mining of the Human Genome Project for targets for therapeutic agents in combination with high-throughput screening, combinatorial chemistry and improved structure activity predictions has yielded an overwhelming number of new drug candidates. Consequently, microarray technology is being incorporated into preclinical assessment programs to prioritize drug candidates that warrant further development. Moreover, microarrays are proving to be an invaluable tool for elucidating potential mechanisms of toxicity and biomarker discovery. Although the technology has tremendous potential, there are a number of limitations and challenges that must be overcome (Fielden and Zacharewski, 2001), such as extrapolation between platforms (e.g., GeneChips vs. cDNA microarrays) and the fidelity of cDNA/EST identities within distributed clone sets (Halgren et al., 2001; Taylor et al., 2001). Additionally, rigorous statistically-based analysis strategies for large gene expression data sets (Pan, 2002), study designs that consider replicate data sets (Kerr and Churchill, 2001), and integrative data management strategies (Brazma et al., 2000), must be developed that facilitate information extraction and integration of disparate data amassed from chemical, toxicological, pathological and toxicogenomic studies. Unfortunately, currently available databases are not designed to handle issues specific to *in vitro* and *in vivo* toxicogenomic studies, such as different dose levels

and durations of exposure, replicate data sets, orthologous gene representation on arrays and their annotation, extrapolation between species, and sample annotation. Continued development of methods and experimental approaches involving microarrays should help them become much more useful for risk assessment and for documenting genetic polymorphisms.

**Ethical and Legal Implications of Pharmacogenetics**

Much of the excitement surrounding pharmacogenomics stems from the possibility of improving the safety and efficacy of drug interventions. Additionally, by conducting clinical studies in genetically homogeneous populations, it should be possible to use smaller, faster, and cheaper clinical trials. There is even the possibility that certain drugs that have failed clinical trials in broad populations could be "rescued" and demonstrated to be safe and effective when used only by individuals with certain genotypes.

Along with the mixture of hype and hope, the reality is that pharmacogenomics presents a number of challenges from an ethical, legal, and policy standpoint. Among these challenges are (1) the ethical, economic, and policy implications of market segmentation; (2) the ethical and social issues surrounding research in pharmacogenomics, including the generation of sensitive genetic information; and (3) the political challenge of ensuring equal access to beneficial pharmaceutical products developed through pharmacogenomics.

Most of the pharmacogenomic research is currently at the pre-clinical stage. Both at this stage and the later clinical research stage an important, but generally unexplored, issue is whether the target population is supportive of the research. In particular, it is important to consider (1) whether individuals are willing to participate in research by donating biological samples and sharing medical records with investigators; (2) whether individuals are willing to undergo genetic testing as part of the research process; (3) whether individuals have suspicions about the medical research establishment; (4) whether concerns about privacy and confidentiality will cause individuals to decline to participate in research; and (5) whether individuals are concerned about the morality of research into human genetic variation. Although these concerns

are certainly common to all clinical trials, they warrant mention here because of the unique concerns centering around genetics in general.

As the research proceeds to the clinical stage, it will be important to develop inclusion and exclusion criteria based on genotype. One important issue is the ethics of including random or "non-matched" controls in the studies (i.e., individuals whose genotypes do not suggest favorable responses). Informed consent will also be a major concern, including how researchers inform potential participants about the possible economic and social consequences of the research, including possible group-based harms. In this regard, the idea of community consultation before performing research in discrete ethnic groups has been debated in the literature.

Once the research has been completed, submission to the Food and Drug Administration (FDA) raises a new set of regulatory issues. The model of greater data from fewer, more homogeneous research participants is new to the FDA and will require additional regulatory analysis. Because of the possibility of smaller research studies, post-approval, Phase IV clinical studies may be of greater importance. Additionally, with drugs likely to be given a narrower range of approval, what will be the effect on "off-label" uses of the drugs?

From a policy perspective, as pharmaceutical companies segment the market, it may become economically infeasible to pursue drug development for individuals with rare genotypes. Consequently, some governmental subsidies – akin to those under the Orphan Drug Act – may be necessary to encourage the development of "small market" drugs.

Over the next several years, as pharmacogenomic-based medications become available, will managed care plans include them in their formularies? Most companies can be expected to undertake a detailed cost-benefit analysis to determine whether the incremental benefits are worth the incremental costs. Even if they are cost-effective, it remains to be seen whether the costs will be borne by consumers or third-party payers and how increased pharmaceutical costs will affect access to health care in general.

Finally, whenever the standard of care in medicine changes there is an increased possibility of liability for those providers who fail to meet the new standard of care. For physicians, the range of possible liability issues includes the failure to order the appropriate genetic tests or to interpret them and explain them to patients properly, the duty to warn patients of possible genotype-specific side effects of medications, and the possible issue of failure to warn at-risk relatives. To meet this heightened standard of care it will be necessary to include instruction in pharmacogenomics in schools of medicine, nursing, pharmacy, and other health care fields, as well as to include new developments in continuing education courses. Pharmacogenomics is a very promising avenue of research, but we must be careful to make sure that there are no unintended social consequences from introducing this technology.

## Conclusions

Building upon the historical approaches taken in the field of pharmacogenetics, the information resource gained from the completion of the Human Genome Project, coupled with the development of high-throughput technologies, the development of sophisticated analytical tools, and the identification of relatively specific *in vivo* phenotypic markers, provides the potential for this field to make rapid advances. Although challenges exist, the promise of pharmacogenomics for human health benefit is exciting and clearly attainable. A number of animal models, including transgenic species, can be used to assess the role of genetic variation in disease susceptibility. However, extrapolation of results from such studies to humans remains a difficult task. There are ethical and legal concerns involved in the application of pharmacogenetics to drug design and clinical practice that must be considered in health care policies for the twenty-first century.

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**Footnote**

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**Figure Legend**

**Fig. 1.** Acetaminophen toxicity in wild-type, CYP1A2-null, CYP2E1-null and CYP1A2/CYP2E1 double null mice. Mice were administered acetaminophen and the number of mice surviving at 48 hours later determined. Data are re-drawn and combined from Zaher et al. (1998).

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TABLE 1

Selected examples of human enzymes exhibiting genetic polymorphisms and potential clinical consequences.

| **Enzyme** | **Polymorphism** | **Examples of Consequences** |
|---|---|---|
| GSTM1 | Null variant | Increased susceptibility to colon cancer |
| NAT2 | Low activity | Increased side effects from antitubercular drug isoniazid (peripheral neuropathy); increased susceptibility to human bladder carcinogen 4-aminobiphenyl |
| UGT1A | Low activity | Hyperbilirubinemia (Crigler-Najjar and Gilbert's syndromes) |
| CYP2C9 | Low activity | Low-dose requirement for warfarin and increased risk for bleeding |
| CYP2C19 | Low activity | Increased risks for side effects from mephenytoin anticonvulsant therapy |
| CYP2D6 | High or low activity | Lack of therapeutic efficacy or increased side effects from antihypertensive debrisoquine (hypotension) |
| ADH1 | Altered affinity and activity | Increased risks for cardiovascular effects; protection against alcohol teratogenicity |
| ALDH2 | Null variant | Facial flushing and reduced risk for alcoholism |
| FMO3 | Null variants | Trimethylaminuria |
| TPMT | High or low activity | Lack of therapeutic efficacy or increased risk for drug-induced myelosuppression |

**Abbreviations:** ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP, cytochrome P450; FMO, flavin-containing monooxygenase; GST, glutathione *S*-transferase; NAT, *N*-acetyltransferase; UGT, UDP glucuronosyltransferase; TPMT, thiopurine methyltransferase.

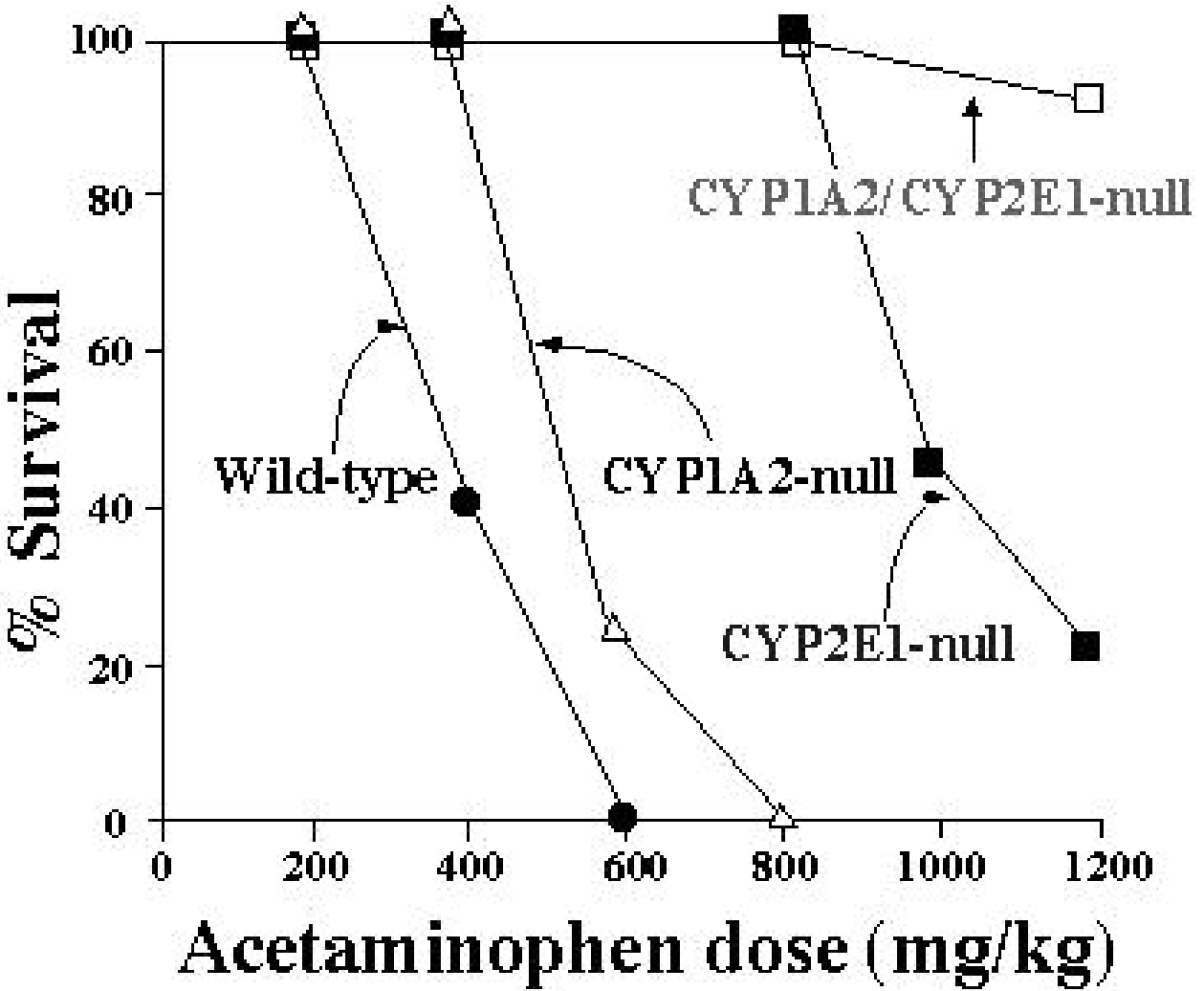
100
80
60
40
20
0
% Survival
0
200
400
600
800
1000
1200
Acetaminophen dose (mg/kg)
CYP1A2/CYP2E1-null
Wild-type
CYP1A2-null
CYP2E1-null

Figure 1.