Toxicogenomics: History and Current Applications

Linking genetic makeup to drug responsiveness could lead to better, safer therapies while enhancing drug discovery and development

Wendell W. Weber

We often assume that therapeutic drugs, particularly those available in the United States that are subject to the stringent Food and Drug Administration (FDA) approval process, carry the seal of safety and effectiveness for everyone. However, some drugs work as expected in only a fraction of patients and thus may lead to complications—some of them serious—for others who take those same drugs.

Thus, effective responses, even for many popular drugs, are far from complete but instead range from 40–75%, according to Brian B. Spear of Abbott Laboratories, North Chicago, Ill. (Table 1). Moreover, adverse drug reactions rank as a leading cause of hospitalization and death in the United States, according to P. N. Corey and Bruce H. Pomeranz of the Departments of Zoology and Physiology, University of Toronto, Toronto, Ontario, Canada. Meanwhile, the costs of dealing with those adverse events can reach higher than $177 billion per year, according to Frank R. Ernst and Amy J. Grizzle of the College of Pharmacy, University of Arizona, Tucson.

With such issues in mind, Ronald Polk of Virginia Commonwealth University, Richmond, organized a symposium, “The Future of Toxicogenomics in Antimicrobial Drug Development and Approval,” that was held in Chicago, Illinois, during the 43rd Annual ICAAC meeting in September 2003. I and other participants described toxicogenomics, drew attention to how genetics can predispose some individuals to clinically important adverse drug reactions, and explained how toxicogenomics could help in designing drugs to deal with some of these challenges.

Toxicogenomics: a Brief History

Toxicogenomics, an outgrowth of the human genome project, is closely allied to pharmacogenetics, which analyzes effects of heredity on responses of humans to drugs. In some circles, individuals mistakenly assume pharmacogenetics to have the same origin as toxicogenomics. However, although pharmacogenetics is benefiting from new technologies, it began at least 100 years ago before pharmacologists began applying genetic principles to dissect human drug responses in earnest (Table 2). The current enthusiasm surrounding these fields reflects optimism over their prospects for contributing to the discovery of drug therapies that can be tailored to the specific needs of individuals.

Pharmacogenetics and toxicogenetics trace to genetic principles uncovered by Gregor Mendel about 1865 that were subsequently lost and rediscovered by about 1900. Key developments in these two closely connected fields fall roughly into four periods.

During the first period, the cellular foundations of heredity were established when chromosomes were identified as the locus of heredity, and Archibald Garrod proposed the concept of chemical individuality to explain inborn errors of metabolism. Garrod, who believed that enzymes were physiological agents responsible for detoxifying exogenous chemicals, noted that this mechanism might fail in some persons who lacked one or more appropriate detoxifying enzymes. Prior to this groundbreaking proposal, physiological chemists demonstrated metabolic mechanisms that enable humans who are exposed to exogenous chemicals to transform and excrete them harmlessly before they accumulate.
toxic levels. Moreover, Charles Langley and Paul Ehrlich had inferred the existence of drug receptors to explain the localized action of drugs on tissues. During the second period, about the middle of the 20th century, scientists identified DNA as the hereditary material, and its double helix structure established the molecular foundations of heredity. During this same period, other scientists determined that human cells carry 23 pairs of chromosomes. Meanwhile, researchers recognized that chromosomal aberrations are associated with at least two specific pathologies, Down syndrome (trisomy 21) and chronic myelogenous leukemia (the Philadelphia chromosome). More subtle heritable variants involving drug-metabolizing enzymes further marked this period as the beginning of pharmacogenetics as an experimental science (Table 2).

The third period, from the 1970s until about 1990, came as biologists developed technologies for cloning and sequencing genes as well as expressing proteins—a period when the informational foundations of heredity were fully established. These concepts and technologies were soon adopted in the laboratories of pharmacogenetic investigators, who used these methods to study and categorize variations in human drug responses attributable to genetic variations of enzymes, receptors, and other proteins.

Subsequently, we entered and remain in the genomic period of pharmaco/toxicogenetic history. By the 1990s, investigators in this field firmly incorporated genetics, making the genomic basis of heredity a critical goal. A central strategy involves assessing the impact of genomics on pharmaceutical product development, safety, and efficacy. Whereas specific genes could only be inferred by classical techniques, molecular biology and other modern technologies enable investigators to observe genes directly and, potentially, to study the structural and functional allelic forms of thousands of genes simultaneously in many individuals and across population groups.

**Current Applications of Genomics to Pharmacotoxicology**

Experience indicates that removing an offending substance from the immediate environment or restricting access to it is the best way to manage individuals who suffer toxic reactions to specific drugs or other substances to which they may be exposed. Moreover, researchers continue to identify polymorphic enzymes, receptors, and other proteins that can play a part in such drug responses and thus can also serve as markers to predict how patients are likely to react when exposed to particular drugs (Table 3). When profiles for such markers are used to guide physicians, they can substantially reduce or avert adverse drug responses when treating susceptible individuals.

For example, CYP2D6 is one such genetic marker. This highly variable gene (more than 80 variants have already been identified) can be used to predict both the efficacy and the toxicity of 20–25% of all prescribed drugs. Investigators identified three distinct CYP2D6 genotypes—extensive metabolizers (EMs), poor metabolizers (PMs), and ultrarapid metabolizers (URMs). Indeed, the six most common CYP2D6 functional variants account for up to 99% of CYP2D6 of all EMs, PMs, and URMs across worldwide populations. Single-nucleotide polymorphism (SNP) profiling for these variants can be performed rapidly and inexpensively.

Consider the effect of CYP2D6 polymorphism on responses to the analgesic drug codeine. Codeine achieves its therapeutic effect after endogenous enzymes transform it into morphine via CYP2D6-encoded metabolism. Among Caucasians ingesting the drug, clinical evidence indicates that codeine is effective in EMs analgesia, ineffective in PMs, and induces morphine toxicity (euphoria, fuzzy vision, abdominal pain) in URMs. With this information in hand, physicians would know to administer conventional doses of codeine to EMs, to select an alternative drug to treat PMs, and to administer a lower dose of codeine to treat URMs (Fig. 2).

Similarly, deafness attributable to treatment with aminoglycoside antibiotics and the life-

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**Table 1. Response rates for various drug types**

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Efficacy rate</th>
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<tbody>
<tr>
<td>Oncology</td>
<td>25–40%</td>
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<tr>
<td>Alzheimer’s Incontinence</td>
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<tr>
<td>Asthma</td>
<td>40–60%</td>
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<tr>
<td>Cardiac arrhythmias</td>
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<td>Diabetes</td>
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<td>Migraine</td>
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<td>Osteoporosis</td>
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<tr>
<td>Rheumatoid arthritis</td>
<td></td>
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<tr>
<td>Schizophrenia</td>
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<tr>
<td>Depression (SSRI)</td>
<td>60–80%</td>
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<tr>
<td>Analgesic (Cox-2)</td>
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</tbody>
</table>

**For all types:**

- Annual prescriptions: 3.2 billion
- Adverse responses: 2.1 million
- Hospitalizations: 1 million
- Deaths: 100,000
- Annual cost: $177.4 billion
Recognizing Drug as Jaundice Source Led to Career in Pharmacogenetics

In the early 1960s, when Wendell Weber was a pediatric resident in San Francisco, he became fascinated by a case involving a six-year-old African American boy admitted for jaundice. Although the child’s eyes were yellow and other doctors thought he had hepatitis B, Weber disagreed with their diagnosis. “He was bouncing around and wasn’t otherwise acting sick,” he recalls. “I thought there must be something else going on.”

The young resident surmised correctly—that the child was reacting to an antibiotic he had received earlier. Indeed, the boy was deficient in glucose-6-phosphate-dehydrogenase, an enzyme that ordinarily protects red blood cells from effects of the drug but whose absence renders those cells fragile. Thus, after the drug treatment, many of his red cells broke down, converting hemoglobin to the bile pigment, bilirubin, inducing jaundice.

“Blacks are particularly disposed to this condition,” Weber says. “It’s evolutionary. In areas of malaria, individuals had a better chance of survival when their blood cells broke down, because the malaria-carrying parasite could not survive.”

The experience was a turning point for Weber, now 78, luring him into a research career in pharmacogenetics. “It really led me to think about how little we knew about the reactions people experienced to drugs,” he says.

Since 1998, Weber has been professor emeritus in pharmacology at the University of Michigan, Ann Arbor, where he spent 30 years studying the genetic susceptibility of humans to the potentially dangerous effects of drugs, foods, and other substances. Before that, he taught and conducted research for a decade, starting in 1963, at New York University School of Medicine, in the department of pharmacology.

Most of Weber’s work involved studying acetylation, a process in which natural chemicals help the body excrete waste. In recent years, the results of the Human Genome Project have provided many additional insights. “Once recombinant DNA techniques were invented in the 1970s, and widely applied in labs in the 1980s, you could measure genes directly,” he says. “You could prove what was only an inference before. It opened the way to all kinds of investigations. . . .”

Weber grew up in a small town about 50 miles west of St. Louis, Mo. His interest in science began early, he says, noting that he received a chemistry set at age 5 and that it contained a recipe and materials to make a red “wine”—with the ominous warning against drinking it. “It was made with phenolphthalein, a dye that turns red, which is also used as a laxative,” he recalls. “Little did I know I would run into all of this again learning about pharmacology.”

He received a B.A. in chemistry in 1945 from Central College in Fayette, Mo., and a Ph.D. in physical chemistry in 1950 from Northwestern University in Evanston, Ill. Almost a decade later, he graduated from medical school at the University of Chicago. Before entering medical school, he served as a civilian research analyst for the U.S. Army, studying chemical, biological, and radiological warfare. “We were steeped in this 40 years ago,” he says. “We hear an awful lot about this today, but the same sort of stuff was going on then. It just seems new to many people— because it’s now another generation of people.”

Weber and his wife, a retired sociologist, have two grown children and two grandchildren. His daughter is an artist and financial advisor; his son is a professional cellist.

An affinity for the arts runs in the family. Weber likes to paint and, drawing on 10 years of classical piano training, he also plays jazz piano. Many of his oil paintings—landsape, portraits, and still-lifes—hang in his home. “It’s a very good way to relax and get away from what I am doing all the rest of the time,” he says. “When you paint, you can’t do anything else. It really gets your mind away from everything around you.”

Marlene Cimons
Marlene Cimons is a freelance writer in Bethesda, Md.
main toxic complication. For instance, about 14 years ago in one district of Shanghai, 22% of deaf-mutes had been treated with an aminoglycoside and, among these, more than one-fourth had relatives with ototoxic deafness. Similarly, in Japan streptomycin-induced deafness was identified among members of 28 families.

In both instances, the pattern of deafness among family members was consistent with the susceptibility being maternally transmitted, thus suggesting that variants carried on the mitochondrial genome could be contributing to this adverse drug effect. Subsequent analysis of three of the Chinese families with multiple cases of streptomycin-induced deafness revealed that a point mutation in the mitochondrial ribosomal RNA gene at nucleotide position 1555 is associated with this disorder, according to Nathan Fischel-Ghodsian and colleagues at the Cedars of Lebanon Medical Center and the University of California Los Angeles School of Medicine. They recommend that aminoglycoside use be better monitored—and avoided for maternal relatives in those families with maternally inherited deafness.

Meanwhile, life-threatening hypersensitivity is the main toxic effect associated with use of abacavir, a potent HIV-1 reverse transcriptase inhibitor, in about 5% of HIV-infected individuals. Recently, in a Western Australia cohort of 200 such individuals who were treated for at least 6 weeks with this drug, Simon Mallal and collaborators at the Centre for Clinical Immunology and Biomedical Statistics, Royal Perth Hospital and Murdoch University, Perth, Western Australia, identified 18 (9%) cases of abacavir hypersensitivity. These investigators suspect that genetic susceptibility to this adverse reaction lies within the major histocompatibility complex (MHC). Within the cohort studied, mapping of candidate MHC susceptibility loci revealed a particular ancestral haplotype, HLA-B*5701, HLA-DR7, HLA-DQ-3, among this group of abacavir-sensitive cases as well as in 4 (2%) of 167 abacavir-tolerant cases. Withholding abacavir from individuals who possess this haplotype should significantly reduce the prevalence of abacavir hypersensitivity.

### Meeting Important Challenges Could Provide Clinical Dividends

A major challenge facing investigators is to devise large-scale, high-throughput procedures for genotyping and phenotyping polymorphisms of pharmacogenetic interest as well as better ways to use this knowledge to develop risk profiles describing individual susceptibility to drugs (and other chemicals). Based on almost 50 years of accumulated biochemical, pharmacological, and genetic evidence, a strong case can be made for analyzing genetic polymorphisms and creating a catalog of human genetic diversity in terms

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**TABLE 2. Historical background of toxicogenomics**

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<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Mendel’s laws discovered and rediscovered</td>
<td>DNA found to be hereditary material</td>
<td>Mechanisms by which cells read genes worked out</td>
<td>Large-scale, high-throughput techniques for analysis of DNA and RNA developed</td>
</tr>
<tr>
<td>Human biotransformation of chemicals discovered</td>
<td>DNA double helix described</td>
<td>Recombinant DNA technologies invented and adopted by many laboratorians</td>
<td>Microarray technologies for SNP and gene expression developed</td>
</tr>
<tr>
<td>Drug receptors inferred</td>
<td>Protein polymorphism established</td>
<td>PCR for cloning &amp; sequencing genes invented</td>
<td>Toxicogenomics evolves as an outgrowth of microarray technologies applied to toxicology</td>
</tr>
<tr>
<td>Chromosomes defined as locus of heredity</td>
<td>Human chromosomes enumerated</td>
<td>DNA polymorphisms of numerous enzymes, receptors and other proteins observed</td>
<td>Construction of risk profiles for drug susceptibility and haplotype maps (<a href="http://www.hapmap.org">www.hapmap.org</a>) initiated</td>
</tr>
<tr>
<td>Chemical individuality of humans defined</td>
<td>Down syndrome &amp; CML associated with chromosomal aberrations</td>
<td>Beginning of experimental pharmacogenetics marked by discovery of human drug metabolizing enzyme variants</td>
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of SNPs and gene insertions, deletions, repeats, and rearrangements. This catalogue will help researchers to better determine how the genetic makeup of an individual affects his or her susceptibility to disease and responsiveness to specific drugs, foods, and other substances. It will eventually enable researchers to discover better therapies and improve prospects for personalized medicine.

A salient property of monogenic traits associated with adverse drug responses (Table 1) is their ability to predict how individuals will respond to particular drugs. However, in any particular family, only one gene locus is thought to be defective. Hence, as more and more pharmacogenetic knowledge accumulates, it seems likely that only a small proportion of phenotypic outcomes can be predicted from genetic analysis at single loci. Principal among the problems encountered in dissecting the molecular basis of even the simplest single-gene disorders are the modifying effects of other genes. As these monogenic traits are analyzed in greater depth, we expect to incorporate polygenic effects of modifier genes into schemes for reliably predicting the risks and effectiveness of specific drug treatments.

By combining SNP and gene expression profiling with microarray technologies, researchers are developing powerful new tools for analyzing responses from hundreds of genes in a single experiment. For instance, Ann-Christine Syvanen of the Department of Medical Sciences, Molecular Medicine, Uppsala University Hospital, Uppsala, Sweden, and collaborators adapted profiling on microarrays to screen a panel of 74 SNPs of 25 genes encoding proteins involved in blood pressure regulation for their effect on response to antihypertensive drug treatment. They found that only a few haplotypes (combinations of a few SNPs) are needed to predict approximately 50% of the variation in individual responses to treatment. This pilot study highlights the potential of microarray-based technology for SNP profiling of predictive pharmacogenomic markers.

Gene expression profiling with microarrays has resonated strongly among clinical investigators who see this approach to analyzing genetically defined molecular markers providing a general means for understanding the pathogenesis of many diseases. For example, the potential value of gene expression monitoring is being brought into sharper focus as researchers use it to identify new classes of cancer and to assign cancers to known subclasses. Gene expression pattern analysis is being used to classify leukemias and to identify molecular variants that underlie melanoma, lymphoma, and breast cancer, according to Todd R. Golub of the Dana-Farber Cancer Institute and Harvard Medical School, Boston, Mass.

For example, analyzing a panel of 50 genes enabled clinicians to distinguish cases of acute myelogenous leukemia (AML) from acute myeloblastic leukemia (ALL). Because treatment of ALL generally relies on corticosteroids, vincristine, methotrexate, and L-asparaginase, whereas most AML regimens rely on daunorubicin and...
cytarabine, this observation has immediate therapeutic implications.

SNP and gene expression profiling are also being used by the pharmaceutical industry to gain insights into the toxic effects of chemicals on biological systems, to predict risks associated with chemical toxicity, and to identify human molecular fingerprints consistent with known toxic mechanisms in experimental models. Initially, however, industry viewed the role of pharmaco/toxicogenetics in drug discovery and development with considerable skepticism. Then, the belief that knowing genotype is sufficient to determine and predict the responses of susceptible people somehow prevailed. But this view seriously underestimated the breadth and complexity of human drug response because it did not adequately account for extrinsic factors and intrinsic factors (other than heredity) known to affect this process, and opinions have gradually swung back toward a more balanced position which sees elucidation of genetic diversity as a more rational strategy to diminish costly drug failures and develop better drugs with improved safety and efficacy.

**Applying Principles to Drug Development**

Typically, drug discovery begins with researchers selecting promising candidate chemical entities having biological activities that correlate with relevant treatment targets and proceeds through preclinical and clinical phases to validate safety and efficacy of the better candidates. Until recently, studying functional changes of single genes in appropriate animals and in human cells were the primary approaches to validate the treatment target. Although such approaches are time-tested, they are also slow, labor-intensive, and expensive.

On the other hand, by knowing the full complement of human genes, drug development researchers have at their disposal a much broader range of targets at which to aim potential therapeutic interventions. Moreover, they may also take advantage of high-throughput technologies, global gene expression analysis, and genome-wide functional analyses. With the aid of gene expression monitoring, investigators can readily analyze the effects of hundreds or thousands of genes on toxicity and efficacy of drug candidates, conducting thousands of tests in parallel instead of sequentially, thus streamlining the drug discovery process while enhancing prospects for better therapies.

An individual’s unique genetic makeup is an important predictor of his or her responsiveness to specific drugs, foodborne toxins, and other chemicals. Toxicogenomics is a comparatively new field of biological inquiry now providing insights into the toxic effects of chemicals on biological systems and helping investigators to predict risks associated with exposure to these agents. Historically, toxicogenomics is closely allied to its older partner, pharmacogenetics, now often termed pharmacogenomics.

Both fields are primarily concerned with the influence of heredity on person-to-person differences in response to therapeutic agents, and

<table>
<thead>
<tr>
<th>Genetic Marker</th>
<th>Drug</th>
<th>Adverse Drug Reaction</th>
<th>Comment</th>
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</thead>
<tbody>
<tr>
<td>CYP2C9</td>
<td>Warfarin</td>
<td>Bleeding</td>
<td>Reduced dose associated with variant CYP2C9 alleles</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Triple therapy for <em>Helicobacter pylori</em> GI infection (omeprazole or lansoprazole plus amoxicillin or clarithromycin)</td>
<td><em>H. pylori</em> eradication rate reduced in 2C19 extensive metabolizers</td>
<td>Reduced eradication rate attributed to homozygous (72.7%) &amp; heterozygous (92.1%) extensive metabolizers, compared to poor metabolizers (97.8%)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Codeine</td>
<td>Morphin toxicity</td>
<td>Toxicity attributed to an ultrarapid CYP2D6 genotype</td>
</tr>
<tr>
<td>Dihydouracil dehydrogenase</td>
<td>5-fluorouracil</td>
<td>Stomatitis, diarrhea and pancytopenia</td>
<td>Toxicity attributed to a nonfunctional enzyme due to point mutation in splice site</td>
</tr>
<tr>
<td>UDPGT1A1</td>
<td>Irinotecan</td>
<td>Neutropenia and diarrhea</td>
<td>Severe toxicity attributed to a mutant promoter UDPGT1A1*28</td>
</tr>
<tr>
<td>Thiopurine methyltransferase</td>
<td>6-Mercaptopurine, 6-thioguanine, azathioprine</td>
<td>Severe neutropenia</td>
<td>Neutropenia and CNS leukemia associated with TPMT deficiency. Leukemic relapse associated with elevated TPMT activities</td>
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</tbody>
</table>
researchers are working to understand how these differences relate to almost 50 years of accumulated pharmacological and genetic evidence that helps to predict how humans respond to particular drugs. By establishing associations between the unique genetic makeup of individuals and their responsiveness to specific drugs, we expect to discover better therapies and improve prospects for providing individuals with personalized medicine. And by combining this knowledge with technology for high-throughput screening of candidate drugs early on, we also expect to streamline and enhance the process of drug discovery.

SUGGESTED READING