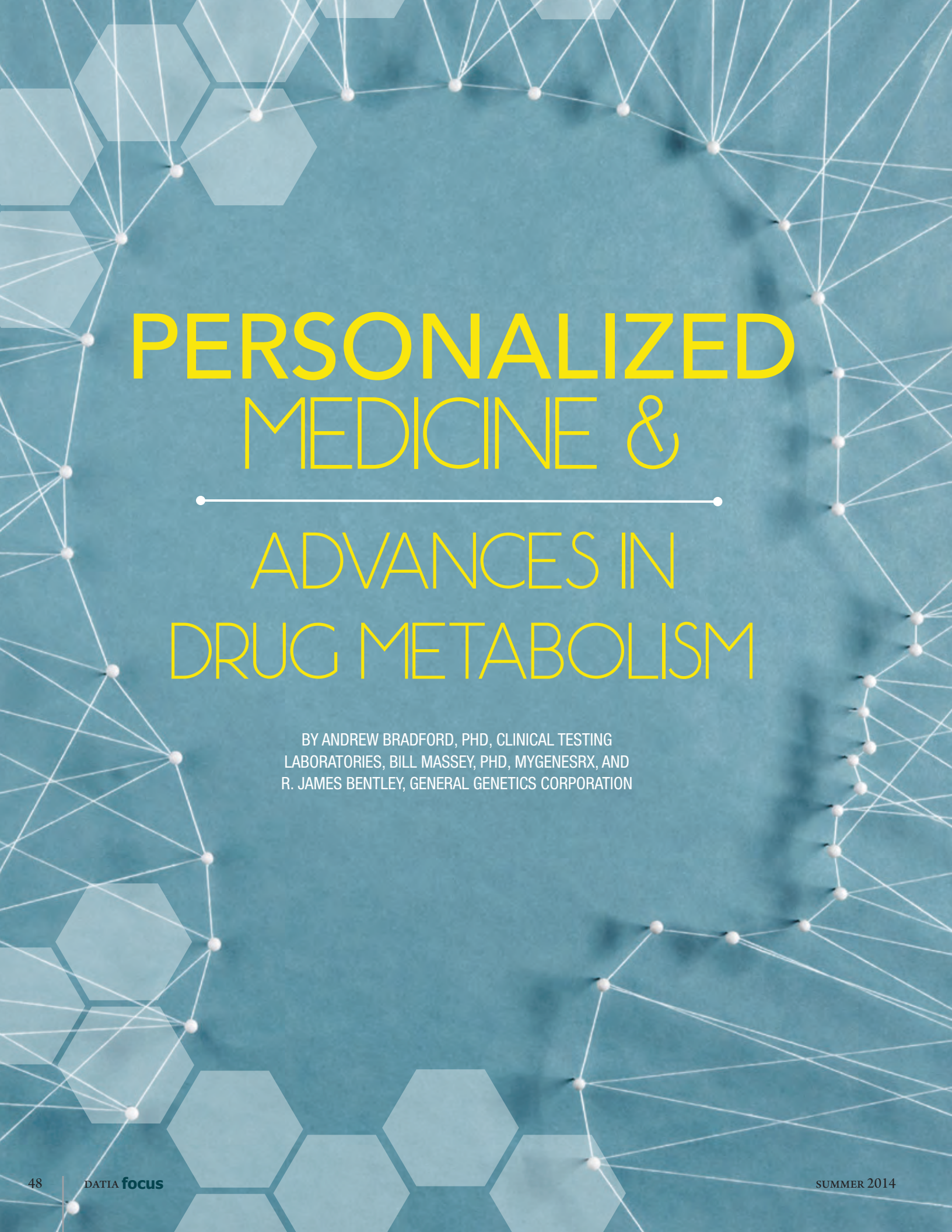


PERSONALIZED MEDICINE & ADVANCES IN DRUG METABOLISM

BY ANDREW BRADFORD, PHD, CLINICAL TESTING LABORATORIES, BILL MASSEY, PHD, MYGENESRX, AND R. JAMES BENTLEY, GENERAL GENETICS CORPORATION

MORE THAN 60 YEARS **have passed since Watson and Crick deciphered the elegant structure of the DNA double helix from a pattern of spots on X-ray film. Since the discovery of the structure and function of DNA, molecular biology has contributed enormously to our current understanding of virtually every facet of biological sciences.**

Currently, discoveries on how genetic variation affects an individual's response to particular drugs, also known as the field of pharmacogenomics, are spurring a revolution in medical care known as personalized medicine. A growing body of knowledge in pharmacogenomics has demonstrated that even the most subtle of changes in the nucleotide sequence of DNA, so-called single nucleotide polymorphisms (SNPs), can have an enormous impact on the safety and effectiveness of drugs. Other types of genetic variation such as those that affect the extent of gene expression and gene copy number variations (CNVs) are also crucial determinants of patient genotype (their genetic makeup) and can affect an individual patient's response to drugs.

A paradigm shift in medicine is underway, and the idea that one-drug-fits-all is being replaced with a promise of personalized medicine based on the genetic makeup of the individual. It has been clear since the 1950s that individual response to drugs varies greatly and can be due to factors such as environment, gender and ethnicity, to name a few. We have learned from clinical genetics that a patient's genotype is the most important factor underlying an individual's response to a drug. With today's advancements in genomic technology and the precipitously decreasing costs of such diagnostics, there exists a promise of personalized medicine becoming available to everyone in the very near future.

The cost of genotyping a person's entire genetic code, also called whole genome sequencing, is plummeting. Sequencing a patient's entire genome is approaching the $1000 mark. The speed of sequencing has accelerated beyond rates that Moore's law would predict, thanks to new next generation sequencing (NGS) technologies such as the Ion Torrent (Life Technologies, Inc.) and Illumina's machines.

Figure 1. Metabolic Genes.

Variation in the Cytochrome P450 metabolic genes can lead to higher or lower concentrations of drugs. Since recommended dosing assumes normal metabolism, individuals with genetic variants that impact drug metabolism may require dose adjustments or, in some cases, should avoid drugs impacted by genetic variants.

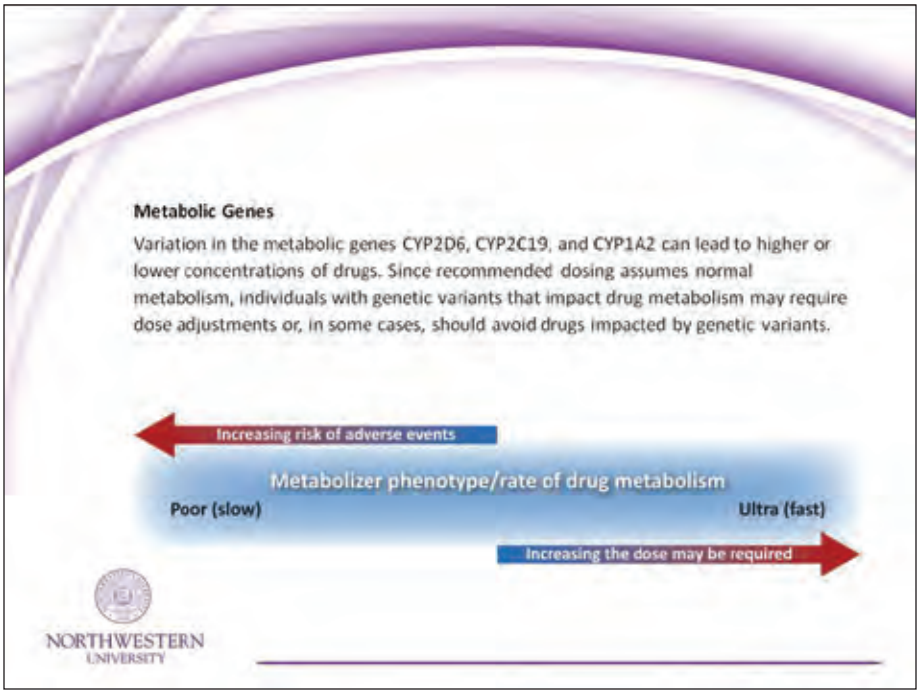
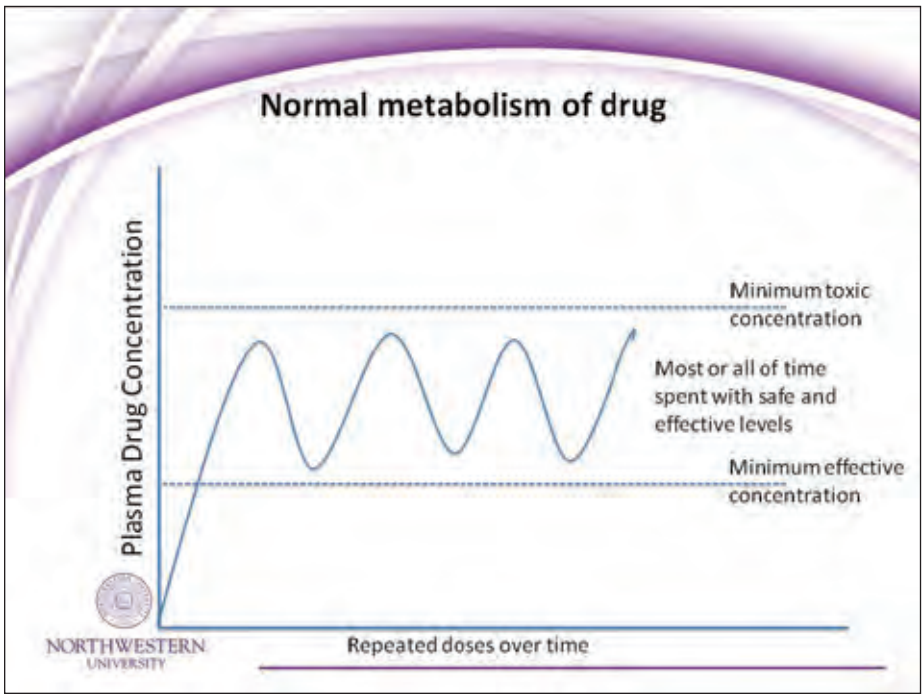


Figure 2. Normal metabolic genes result in effective drug therapy



Thus, these technologies are becoming affordable to not only high profile individuals such as Steve Jobs and author Christopher Hitchens, but to the average citizen. These men notably had their genomes and the genomes of their tumor cells sequenced to look for differences between the two in order to choose the correct treatment. Unfortunately they lost their battles with cancer, but the approach is a very rational one that will likely become the norm. Leukemia researcher Dr. Lukas Wartman is a success story, where RNA sequencing of his own leukemia cancer cells revealed an overabundance of a particular receptor tyrosine kinase (RTK) that was fueling the cancer's growth. Dr. Wartman was able to use this information to choose an available cancer medicine that targeted that RTK, but his insurance would not pay because that drug was for pancreatic cancer, not leukemia. He scraped together the money to pay for the drug and survived, but this points out the clash between the old way of thinking, that is, one-drug-fits-all, and the new way, which is a personalized medicine approach.

Cancer is not the only disease where genotyping is of great benefit. In fact, mainstream genotyping has to the present day mainly focused on drug metabolizing enzymes such as the cytochrome P450 super family of enzymes, which metabolize the vast majority of presently prescribed drugs. SNPs in these CYP enzymes greatly influence the blood levels of the active form of prescribed drugs, and contribute to over- and under-dosing. In the case of Plavix for example, variations in the CYP2C19 gene affect the metabolic conversion of this inactive pro-drug into its active form. Genotyping CYP2C19 for each patient requiring Plavix ensures an optimal dosage is prescribed. Likewise, variations in the CYP2D6 gene affect the efficacy of many different types of drugs, as CYP2D6 acts

on approximately 25 percent of all clinically used medications.

Cytochrome P450s (CYPs) comprise one of the largest multi-gene families and are widely known for the diversity of chemical reactions they catalyze as well as the variety of substrates upon which they act. Of the 57 known CYPs, a mere six metabolize approximately 90 percent of clinically prescribed drugs. This diversity arose through gene duplication of the original cytochrome P450 gene, and different pressures allowed the generation of metabolic diversity in the gene family. It is presumed that many of the chemical reactions catalyzed by these cytochrome P450 enzymes are the result of animal life having to deal with various biologically active compounds encountered primarily from ingesting plants. The original cytochrome P450 gene coded for an enzyme whose key role was detoxification of oxygen, which was new to Earth's atmosphere at that time. Indeed, molecular oxygen is required for the function of CYPs, and is consumed in the reaction.

Genetic variation within the DNA sequence (i.e., genetic polymorphisms and copy number variations) of cytochrome P450s can affect the structure, function and/or expression of the cytochrome P450 enzymes. Multiple polymorphisms affect the function of CYPs 2D6, 2C19 and 2C9. By performing a simple and low-cost panel of 50 or so SNPs, patients are deemed poor (PM), intermediate (IM), normal (NM), Extensive (EM) or ultra-rapid (UM) metabolizers.

Adverse drug reactions (ADRs) are the third leading cause of death in the U.S. Many ADRs are avoidable when the genotype of the patient is known, as more than 75% of the population has known genetic variations that increase their propensity for ADRs. In addition, genetic variants that affect drug metabolism can exacerbate drug-drug interactions (adverse drug reactions that occur when the patient is taking multiple drugs at the same time) a frequent

Figure 3. In those patients where a gene mutation results in poor metabolism of a certain family of drugs, the body is unable to metabolize or eliminate the target drug from the system. Each succeeding dose will add to the circulating drug level, where a toxic effect from that drug will be displayed.

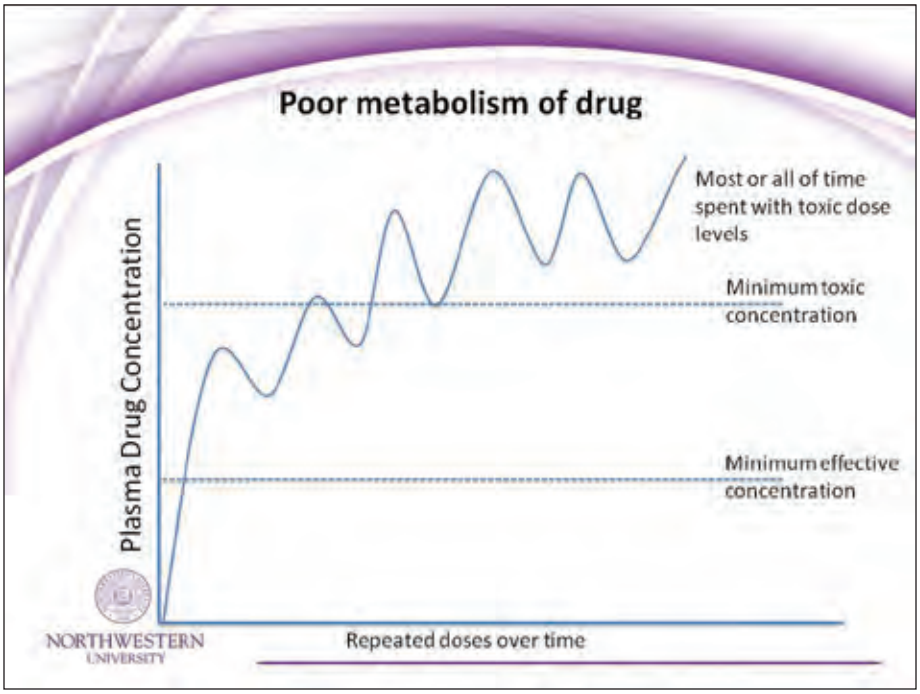
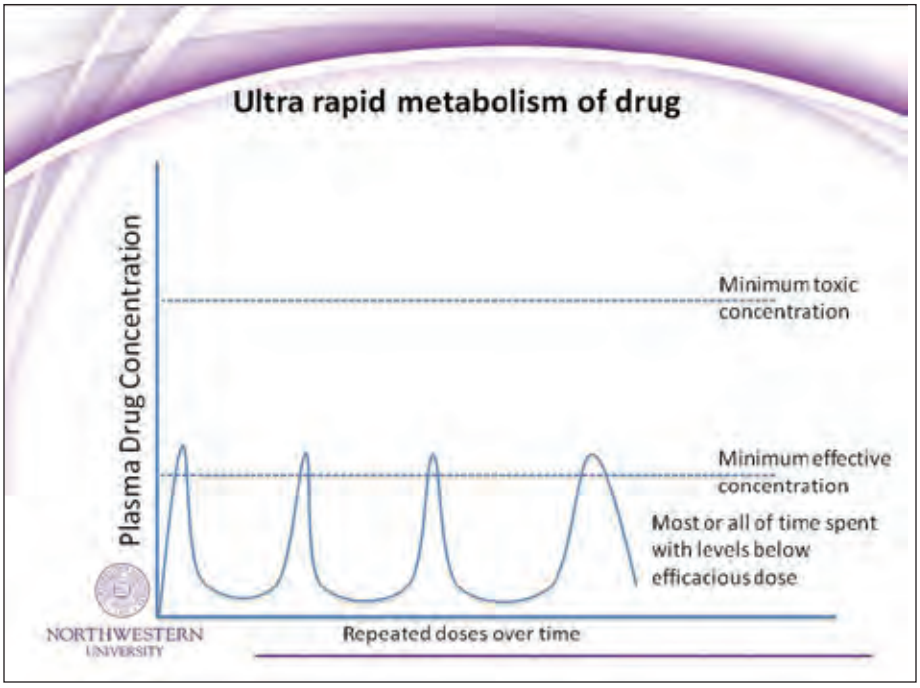


Figure 4. Some genetic variations result in a metabolic enzyme that functionally metabolizes a drug much more rapidly than normal, or in some cases extra copies of a gene will cause to metabolic system to rapidly remove the drug from your bloodstream prior to achieving the intended therapeutic benefits.



We have learned from clinical genetics that a patient's genotype is the most important factor underlying an individual's response to a drug.

occurrence in patients over the age of 65. Patient genotyping allows for more appropriate prescribing and also shifts us further from the trial-and-error, human guinea pig approach that has prevailed to date. Thus, personalized medicine can greatly reduce adverse side effects and increase the effectiveness of drugs, leading to improved safety and better outcomes.

Genotyping the polymorphisms of the cytochrome P450 genes forms the basis for a collection of complimentary tests, or panels, used together to guide the choice of the drugs prescribed for a variety of diseases or conditions. Genotyping the CYPs tells us how a patient metabolizes different classes of available drugs, and at the same time genotyping a patient's genetic variation within a few genes relevant to a particular disease state, can significantly improve the selection of medication prescribed. For instance, if a patient is a normal opiate responder based on his or her OPRM1 genotype (the gene that codes for the opiate receptor), but a poor metabolizer of the pro-drug forms of opiate drugs based on CYP2D6 genotype, then an increase in dosage of pain medication may be indicated in that circumstance.

Genetic test panels targeting genes related to specific conditions are currently available in the marketplace, and can provide insight into treatment options. For example, there are genotyping panels for pain management, estrogen metabolism, color blindness, cardiovascular disease and mental illness. Many of these panels test for a combination of cytochrome P450 genes

as well as the condition-specific genes. These condition-specific genes are usually discovered as such through large-scale clinical association studies, which show a statistical correlation between certain SNPs and the condition and in response to particular drugs.

Newly-Developed Panels Allow Safe And Effective Treatment Options

Pain and cardio panels look into clinically relevant variations in opiate receptor and statin metabolizing genes, for instance, in conjunction with the cytochrome P450 metabolizer status of the patient to suggest an appropriate dosage of medication and dosage. For patients suffering from neuropsychiatric conditions such as schizophrenia or psychosis, several genes whose functions are brain-specific are evaluated alongside the typical cytochrome P450 drug metabolism genes. Determining the individualized treatment for these patients is critical early on to avoid hospitalization. Genes involved in the biosynthesis and metabolism of dopamine, or the transport of serotonin, are examined for risk variants that can include insertion/deletions and/or SNPs depending on the gene in question. There is significant interplay amongst the genes involved in these neurological conditions, and variants in one gene in combination with those in another may spell trouble (gene-to-gene interactions). It has been clinically shown that choosing the correct initial treatment for these patients is tantamount to a sustained drug effect and

Today, patient genotyping is central to the success of personalized medicine.

for avoiding hospitalization, and that there is a clear association with genotype, drug choice, and these outcomes.

Another panel examines several key genes involved in estrogen metabolism as a breast cancer prevention tool for physicians and consumers. This panel gauges the risk of developing breast cancer from exogenous estrogen in women who may be taking, or considering taking, hormone replacement therapy, or attempting in vitro fertilization, etc. The intent is to lessen the complexity of relieving a patient's menopausal or peri-menopausal symptoms and improving fertility, while avoiding risk of adverse side effects and decreasing cancer risk by dosing according to their estrogen metabolism function. The panel looks at several estrogen metabolizing genes, in which a combination of variants have been shown to increase the risk of breast cancer.

Color vision deficiency or color blindness is a condition that is present from birth but can go unnoticed for years. Genotyping can tell us the exact type of color vision deficiency and the precise level of severity, an advantage over time-consuming and subjective visual tests. Knowing whether a child is color vision deficient can be a major advantage for scholastic achievement, and later on when choosing a career path. There is hope that color vision deficiency can be corrected in the future through gene therapy. Such treatment has been proven effective in primates at the University of Washington.

Today, patient genotyping is central to the success of personalized medicine. Due to the decreasing costs of next generation sequencing (NGS), it appears that this technology will be widely available to patients in the very near future. One particular use for NGS would be in the diagnosis of cancer. Cancer is often an individualistic disease as many cancers are unique to an individual patient. NGS can yield information particular to that cancer and allow an informed decision by the provider as to the optimal cancer drug to be prescribed. These are exciting times as we shift from population-based methods of the past to the individualized medicine of the future. Genetic technology is enabling us to better identify the cause of disease and the correct treatment options.

Andrew Bradford, Ph.D., is Senior Research Scientist at The Clinical Testing Labs (CTL), a division of General Genetics Corporation (GGC). Andrew earned his doctorate in Chemistry from New Mexico State University studying regulatory mechanisms of Grb7, a protein that is overabundant in some of the worst forms of breast cancer. Previously, he studied quantitative gene expression after cadmium exposure in mycelia of the mycorrhizal fungus *glomus intraradices*. He continues to be curious about how environment and genetics impact personal health.

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R. James Bentley. Since graduating from Arizona State University with a degree in chemistry, Jim Bentley has spent 35 years, creating, developing and managing laboratories in the clinical, toxicological, environmental and genetic fields. Today, he is Chief Operating Officer of General Genetics Corporation (GGC). GGC maintains divisions in relationship testing (GTL), forensics (FTL) and clinical genetics (CTL). The main laboratory is in Las Cruces, New Mexico.