Pharmacogenomics and its applications

By Robert M. White, Sr., PhD, and Steven H.Y. Wong, PhD

The Great Paracelsus (Philipus Aureolus Theophrastus Bombastus von Hohenheim-Paracelsus, 1493-1541) is remembered by toxicologists for his famous quote: "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy."[1] Indeed, the basic principle from the quotation still applies today. The right dose of morphine that will produce a blood level of approximately 0.07 mg/L to 0.083 mg/L is essential for the relief of pain. An overdose that results in a markedly increased blood level (0.2 mg/L to 2.3 mg/L) can cause death due to central nervous system depression.[2] In general, the same applies to all chemical substances.

As an important exception to Paracelsus' statement, however, there are instances in which a drug should be avoided completely. An example is found in the administration of succinylcholine as a skeletal muscle relaxant in surgery patients. Most patients can convert succinylcholine into inert metabolites through an enzyme called pseudocholinesterase because most patients possess two alleles for the common gene that can produce active enzyme. A certain small percentage of patients, however, carries the genes that are aberrant or silent and, thus, either produce protein (enzyme) that is defective in its function or cannot produce the protein that is capable of inactivating administered succinylcholine at all. Thus, these patients expire or suffer severe sequelae from administration of the drug.[3]

Before embarking upon a cursory journey through pharmacogenomics, a very brief discussion of DNA (deoxyribonucleic acid) and its products is in order.

Along the same line of thinking, the dose of a given drug for certain individuals may differ from that used for the majority of the population due to genetic differences between the individual and the general population (e.g., a reduced dose of 6-mercaptopurine in the presence of reduced thiopurine methyl transferase, or TPMT, activity due to a genetic deficiency in synthesis of the active enzyme).[4,5] Therefore, in the Third Millennium, an expansion of Paracelsus' statement might be that the "right dose" for one individual may not be the "right dose" or even the right drug for another, due to differences in each individual's genetic makeup. The determination of what is the "right dose" and, in some cases, what is even the right drug for a given individual constitutes the reason for the development of the sciences of pharmacogenetics and pharmacogenomics.

Although often used interchangeably, there are subtle differences between pharmacogenetics and pharmacogenomics. Pharmacogenetics focuses on individual traits with respect to one compound or drug. Thus, pharmacogenetics, which historically actually preceded pharmacogenomics, looks at the responses of different individuals to one drug, while pharmacogenomics studies the differences among several compounds with regard to a single genome.[6,7] Pharmacogenomics is concerned with the systematic assessment of how chemical compounds (e.g., drugs) modify the overall expression pattern

Glossary[11,23]

Allele. One or several forms of a gene of a single individual compared with other individuals. An allele is present on a specific site (genetic locus) of a chromosome controlling a particular characteristic and giving rise to noticeable hereditary difference. A single allele is inherited separately from maternal and paternal origin. Thus, with exception to unmatched sex chromosomes, every individual has two alleles for each gene.

Genotype. The precise genetic constitution (i.e., genomes, genes, or alleles at one locus or place) that determines the phenotype (observable characteristics) of an organism.

Heterozygote. An individual that carries a pair of different alleles of a particular gene (inherited from two parents) on each member of a pair of chromosomes.

Homozygote. An individual whose genotype has two identical alleles (each derived from one parent) at a given locus or place on a pair of homologous chromosomes.

Phenotype. The observable characteristics or physical appearance of an organism, resulting from and determined by its expressed genes.

Point mutation. A mutation in which one base in the DNA chain is substituted with another, resulting in a change in the resulting amino acid sequence (protein).

Polymorphism. "Many faces." The difference in genetic sequences among individuals, groups, or populations.

Wild-type. The allele, genotype, or phenotype that naturally occurs in the normal population or, in the case of microbes, the standard laboratory strain of a given organism.

Xenobiotic. A foreign chemical including drugs, industrial chemicals, pollutants, pyrolysis products in food, and toxins produced by plants and animals.

in certain tissues. Pharmacogenomics is not focused on the differences between individuals. Rather, pharmacogenomics focuses on differences among several drugs or compounds with regard to a generic set of expressed or nonexpressed genes. The focus in pharmacogenomics is on compound variability. For the purposes of this brief introduction, the two terms will be used interchangeably unless stated otherwise.

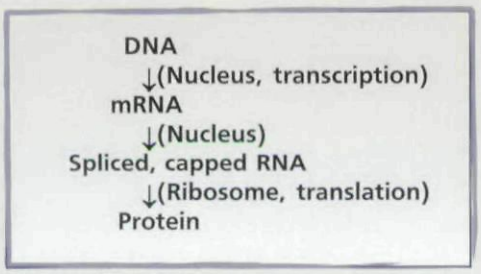

Figure 1. "Central dogma"

Before embarking upon a cursory journey through pharmacogenomics, a very brief discussion of DNA (deoxyribonucleic acid) and its products is in order. DNA is the genetic code that determines all of an individual's characteristics, including the synthesis of the proper proteins essential for life. The so-called "central dogma" of molecular biology is outlined in Figure 1.[8] DNA is a long chain of chemically linked (phosphate bond), single nucleotides. Although there is a great deal more detail to the system due to phenomena such as splice variants,[9] basically DNA is transcribed in the cell nucleus by an enzyme called RNA polymerase to yield messenger RNA, or mRNA. DNA sequences called promoters and enhancers are also usually required to initiate RNA synthesis. Silencers balance the effects of promoters and enhancers when synthesis is not required. A stop codon (a series of the nucleotides that tell the polymerase to stop transcription) also is required so that only the required RNA, not an almost infinitely long RNA, is produced. Regions of the DNA that do not code for amino acids are called introns. The introns are spliced out before the completed mRNA is capped (*vide infra*) and exported from the nucleus for translation. The regions of the DNA that do code for protein are called exons. RNA that has had the introns removed is capped on the 5-prime end with a special nucleotide called 7-methylguanosine and has a poly-adenosine (poly-A) "tail" added to the 3-prime end. The capped mRNA with its poly-A "tail" is then translated into protein in a cellular apparatus called a ribosome.[9]

Perhaps the best-characterized and most extensively studied area in pharmacogenomics is biotransformation, which is commonly referred to as metabolism.

Also required before pharmacogenomics can be discussed is a basic knowledge of the proteins that are the end product of the transcription of DNA and the translation of RNA. Essentially, a protein is a long chain of chemically linked

Continues on page 22

A commentary on pharmacogenomics: What can it do?

By Richard Gabriel

What does pharmacogenomics mean? It triggers, in some minds, thoughts of science-fiction and suspense thrillers where cloning and genetic testing often portray a dark, lurking, evil specter. A lingering public fear is that abuse of genetic testing could create potential harm for individuals afflicted with inherited diseases, as well as perpetuating "racial" issues. While these are rational fears, understanding the origin of a disease rooted within the human genetic code can lead scientists to discover treatments and some day — perhaps — even a cure.

Forty years ago when I was in high school, genetic testing and genetic manipulation generally involved farmers, livestock, and crops. We knew that certain families in Pennsylvania and Minnesota were prone to genetic disorders. We also knew about Woody Guthrie, Lou Gehrig, four- and five-leafed clover, and the genetic defects of people who survived the atomic bombs of Hiroshima and Nagasaki. I never imagined then that I would run a genomics company that is able to determine an individual's genetic ancestry, potentially identify an individual who left his DNA at a crime scene, or differentiate a drug responder from a nonresponder.

Since our company is one of the first to launch a genetic heritage product into the marketplace, ANCESTRYbyDNA for genealogy and DNAWitness for forensics, I ask myself: How can I convince others that even though we do not yet understand all of the ramifications of pharmacogenomic testing, population structure, and genetic heritage, we will make it safer to test and, by understanding test results, we will move forward to face today's challenge of treating and maybe *curing* diseases?

What pharmacogenomics means — and what it does not — is a legitimate concern about which members of the pharmaceutical industry and the healthcare market should be aware and be willing to take time to explain. Regulators from Capitol Hill to local communities can have a dramatic impact upon the emerging field of pharmacogenomics. Points of view based upon scientific discovery or knowledge (or not) must be addressed head-on and dealt with in a straightforward manner. Reporters have asked me why National Institutes of Health researchers (some of whom head genetic research groups) think that determining an individual's genetic heritage is a trivial exercise, and that our DNAPrint testing is a sham and should be banned. While many good arguments exist as to why pharmacogenomics should be avoided, these are strong criticisms from eminent scientists in the fields of genetics and population structure — the very fields we believe hold keys to unlocking the origins of a vast majority of diseases afflicting humankind.

The FDA (U.S. Food and Drug Administration) adopted an opinion on pharmacogenomic testing as it relates to new drug approval and published this landmark document in November 2003. Our FDA and similar government bodies across the globe are chartered with one very important mission: to protect patients from harm derived from toxic medicines, poor quality control, or deceptive practices. This first rational position written by FDA regulators demonstrates just how important our government believes improving drug response and personalized medicine is.

Pharmaceutical executives, researchers, physicians, and medical professionals have an opportunity through knowledge of a patient's genetic heritage to change the way drugs are developed, approved, regulated, and used to treat illnesses. Linking a drug, such as Herceptin, to a genetic disorder and having the FDA approve it is a tremendous stride. The pharmaceutical industry has an even more profound opportunity to further develop drugs for genetic ancestors that are often hidden under an individual's physical appearance.

Medicines work differently in different peoples. The most recent example is the discovery of a gene passed down to Russians vis-a-vis the Mongolian invaders. The Mongols apparently liked their drink — fermented mare's milk — which required a particular enzyme for digestion. When Mongols mingled with European tribes — who derived their preferred alcoholic drink from grapes and various fruits, grains, or honey — this inherited Mongol enzyme eventually afflicted millions of Russians. Although they do not look "Mongolian," the Russian

Continues on page 24

Clinical application: Depression

In order to provide therapeutic efficacy, the tricyclic antidepressant nortriptyline must achieve serum levels of 5 ng/mL to 150 ng/mL.[23] Usually, a therapeutic level is achieved by adjustment of a standard dose. Nortriptyline is metabolized to its hydroxy metabolite, 10-hydroxynortriptyline, by CYP2D6. Individuals with different genotypes and multiple copies of CYP2D6 metabolize nortriptyline at markedly different rates. When poor metabolizers of debrisoquin (a drug used to determine an individual's CYP2D6 status) with no functional CYP2D6 gene, intermediate metabolizers with one functional CYP2D6 gene, extensive metabolizers with two functional CYP2D6 genes, ultra-rapid metabolizers with duplicated CYP2D6*2 genes, and one ultra-rapid metabolizer with 13 copies of the CYP2D6*2 gene are compared, the results are quite striking. On one end of the spectrum, the individuals with no functional copy of CYP2D6 have maximal levels of serum nortriptyline of 51 ng/mL to 71 ng/mL. On the other end of the spectrum, an individual with 13 copies of the CYP2D6*2 gene had a maximal serum level of only 13 ng/mL. Needless to say, this represents an outstanding example where a "standard dose" will not necessarily achieve the required serum level of the active drug and, thus, the desired results.[12] A recent study further substantiated the prediction of amitriptyline and nortriptyline concentrations based on CYP2D6*4, *10, and *41 genotyping.[13]

Selective serotonin reuptake inhibitors, such as fluvoxetine, paroxetine and fluoxetine, probably exert their action through the serotonin transporter protein. The serotonin transporter gene (5-HTT) shows several polymorphisms. One polymorphism is in the transcriptional control region upstream of the 5-HTT coding sequence. It is either a 44-base pair insertion (long variant) or deletion (short sequence). One group has reported that individuals homozygous for the long variant and heterozygous individuals respond better to fluvoxamine than do individuals who are homozygous for the short variant.[24] Interestingly, another group reported just the opposite.[25] The disparity between the studies is a reminder that other factors that may never be separated out affect genomic expression and phenotypic response.

(amide or peptide bond) amino acids. The sequence in the chain of amino acids is called the primary structure. The chain may form loops and/or helices (secondary structure), and the loops and helices may fold to form the tertiary structure. Further, the protein may exist by itself, associate with other protein chains like itself (e.g., dimerize to form an aggregate of two like chains), or associate with dissimilar protein chains to form the final, active structure, which is known as the quarternary structure. Further, a protein may be chemically and functionally changed (post-translational modification) by the addition of phosphate groups (phosphorylation), glucuronic acid groups (glucuronidation), or other groups, or by the addition or removal of amino acids or short stretches of amino acids called polypeptides. The study of proteins, along with the analysis by 2D gel and mass spectrometry and data analysis by bioinformatics, constitute the emerging field of clinical proteomics.[10,11]

Proteins can act as hormones (e.g., insulin, glucagons, chorionic gonadotrophin); enzymes, which are biological catalysts (e.g., lactate dehydrogenase, alkaline phosphatase, creatine kinase); structural components (e.g., troponin, collagens); receptors (e.g., opiate receptors, cholesterol receptors); and a plethora of other functions essential for life. When

Clinical application: Forensics

A 9-year-old born with probable fetal alcohol syndrome is treated with a combination of methylphenidate, clonidine, and fluoxetine for multiple behavioral problems. Over a period of time, the individual is hospitalized in status epilepticus, followed by cardiac arrest, and expires. Based on the levels of fluoxetine and its metabolite norfluoxetine in the deceased's post-mortem blood, the adoptive parents are suspected of homicide. The remainder of the adopted children are removed from the household. Due to the vociferous claims of the adoptive parents that there was no foul play involved, the deceased individual is tested for genetic polymorphism. Indeed, a polymorphism in the CYP2D6, which resulted in the poor metabolism of fluoxetine, was discovered. Based on the results of the post-mortem genetic testing, the adoptive parents of the deceased were exonerated and reunited with the remainder of the children.[26]

From a recently published study of assessing pharmacogenomics as an adjunct of molecular autopsy for forensic pathology/toxicology, CYP2D6 was genotyped for methadone deaths.[27] One of the three methadone deaths with poor phenotype based on genotyping results was a 41-year-old female who was six months pregnant. She was diagnosed with heart murmur and rheumatoid arthritis and treated with methadone. In addition, tricyclic antidepressants were prescribed for her depression. After celebrating New Year's Eve with her husband, she was found unresponsive in the living room the following morning. Toxicology analysis showed: methadone, 0.7 mg/L, amitriptyline, 1.5 mg/L, and nortriptyline, 2.2 mg/L. Pharmacogenomics tests showed CYP2D6*4 homozgyous, corresponding to poor metabolizer as a result of deficient CYP2D6 enzyme. The lack of enzyme predisposed her to the inability to hydroxylate methadone, amitriptyline, and nortriptyline, resulting in an overall accumulation and drug toxicity. Death certification showed the cause of death to be mixed drug overdose, and manner of death, accident.[27]

a protein is formed from DNA that was in the correct sequence, the DNA is correctly transcribed, and the RNA is correctly translated; a fully functional protein usually results. If the DNA code is incorrect (although exceptions exist here also) or there is a defect in the mechanism that creates the protein from the original DNA code, however, a partially functional protein, a nonfunctional protein, or even a protein that is deleterious to cell function may be produced. Gene duplication, where the gene is functional and codes for the correct protein, can result in the overproduction of protein.[12]

Also before pharmacogenomics can be discussed, it is helpful to review a few pharmacologic fundamentals. First, although it sounds obvious, before a drug can have any effect on an individual, the drug must somehow enter the individual's body. Entry can be accomplished through inhalation (e.g., a bronchodilator used for the treatment of asthma), absorption through the skin (e.g., a topical anesthetic such as cocaine or lidocaine) or mucous membranes (e.g., pilocarpine eye drops), parenterally (any number of drugs that may be delivered intravenously, intramuscularly, or subcutaneously), or, most commonly, orally (a large number of drugs, including such common substances as aspirin and acetaminophen) where the drug is absorbed from the gastrointestinal tract. Once inside an individual, a drug needs to be transported to the site where it will have its effect. A drug may have no action whatsoever (e.g., insulin), may act directly on a receptor to produce the desired effect (e.g., morphine for analgesia), or may require activation (e.g., the production of morphine from codeine for analgesia). Both before and after the desired effect has been produced, drugs may be excreted (multiple routes such as urine and bile) unchanged (e.g., free morphine), excreted as metabolites (e.g., the excretion of morphine glucuronide, which is an inactive metabolite of morphine), or excreted as a combination.[14] Proteins are essential to carry out all of the aforementioned steps in drug metabolism.

Proteins play an active role in the disposition of drugs and their metabolites (*vide infra*). Proteins in the intestinal enterocytes, such as breast cancer resistance protein (BCRP), multidrug resistance-associated protein (MRP2), and multidrug resistance protein (MDR1), are involved in the transport of xenobiotics into the intestinal lumen.[15] A member of the peptide transporter family such as PepT1 facilitates absorption from the gut lumen and tubular reabsorption in the kidney. Drugs such as valaciclovir, valganciclovir, and captopril are known to be transported by PepT1. OCTN2, which is part of the organic cation transporter family, is involved in both the efflux and influx of drugs such as

Continues on page 24

inheritors of that particular Mongolian gene are unable to process ethanol derived from fruit or potatoes, thus proving more susceptible to the disease of alcoholism. On the other hand, many Mongolian people express the phenotypic descriptors of the Mongolian race, yet, if given a drink of Russian vodka, are very capable of drinking it and not turning into an alcoholic. Population structure and genetic inheritance has absolutely nothing to do with the Russian or the Mongolian "races" but everything to do with genetic ancestry.

When we conduct clinical trials focusing on African-Americans or Hispanics, what are we testing? Genetic heritage is a component of race, but not all of the descriptors we use for race are inherent in our genes. We have genes for eye color, hair color, height, weight, skin shade, and other phenotypic descriptors. We have a series of identifiers that we call ancestry informative markers (or AIMs) that span the entire human genome. These markers can be linked to the four major subcontinental groups from which all humans evolved (based on our current understanding of human evolution): subSaharan African, East Asian, European, and Native American. We have been able to link these markers to such fundamental information as drug metabolism.

Although they do not look "Mongolian," the individuals in Russia who have inherited that gene are unable to process ethanol derived from fruit or potatoes.

We know that phenotypic descriptors, such as high blood pressure, are linked to inherited genetic traits, not an individual's race. Appearance is as deceptive and elusive as the mystery of life itself. We know that prostate cancer among African-Americans is not linked to an individual who *says* he is African-American and *appears* to be an African-American but *is* linked to the gene he inherited from subSaharan Africans. We know that statins and the negative or positive response to statin treatment is linked to genetic ancestry. And we certainly know that among Russians, alcoholism can most likely be linked to an invading East-Asian army's genetic make-up. Identifying these inherited descriptors — along with learning whether we can or cannot absorb or metabolize certain fundamental compounds — can be used to alleviate pain and suffering.

We are working on our first pharmacogenomic product (our first target is Taxol), in conjunction with the H. Lee Moffitt Cancer Center and Research Institute in Tampa, Florida, and should be able to predict, with 95% accuracy, whether or not a cancer patient responds to a particular medication. Although our company's research in pharmaceutical prediction has not yet met our company's standards for a product launch nor our internal FDA standards for discussions with the U.S. government, we are highly encouraged by our discoveries, as are the doctors and scientists at Moffitt and their patients with whom we have talked.

Our company code of ethics, like many others, speaks not only to our corporate behavior but also to our determination to root out and end any abuse of our science, products, or technology. We are mindful of our responsibility and very much aware that our research and products can be misused. It is similar to the discovery of any compound for treatment of a disease: There are always associated side effects, lethal to some patients. Although we hope to minimize or reduce side effects, their elimination — while probably unlikely — remains a worthy aspiration.

Always teetering on the edge of the abyss (where we could plunge into a quagmire of despair and misuse), science, discovery, and invention can also help us leap across the abyss to a new horizon. The same drive that carries us today helped our ancestors cross the frozen Bering Sea or migrate up from Africa and settle the continents. Every day scientists, doctors, and healthcare workers stride toward a greater knowledge of disease over the "frozen tundra" of genetic testing. □

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quinidine and verapamil. Although numerous polymorphisms exist among the transporter proteins, their influence on pharmacogenomics is unclear and still in its infancy.[16]

A clear example of how a protein and a mutation of that protein can affect a drug's absorption, however, is found with the cardiac glycoside digoxin. P-glycoprotein, which is a membrane protein that functions as an exporter of xenobiotics from cells, is a product of the MDR1 gene. Although several models have been proposed for P-glycoprotein's action, basically P-glycoprotein acts to move xenobiotics from epithelial cells into the adjacent lumen. P-glycoprotein is found in numerous cells associated with excretory function. In the case of digoxin (and certain other drugs), reduced intestinal absorption of the drug can be associated with induction (increased amounts) of the enzyme or the C3435T (where the deoxyribonucleotide cytidine replaces the deoxyribonucleotide thymidine at position 3435 in the DNA sequence that codes P-glycoprotein) mutation of P-glycoprotein. Thus, the mutant form of the protein causes a lowered overall intestinal absorption of digoxin by excreting more back into the intestinal lumen than the wild-type protein.[17]

Once a drug has entered the bloodstream, it is transported to various parts of the body where the drug may be activated or inactivated by certain enzymes (*vide infra*) by a process commonly referred to as biotransformation — or metabolism; be excreted unchanged; interact with a receptor or other location where the desired (and, sometimes, undesired or side-effect) action(s) may take place; or be stored (e.g., the retention of Δ^9-tetrahydrocannabinol or THC in body fat or lead in bone) for future uses such as those previously described. Many drugs and other xenobiotics express their pharmacodynamic action by interacting with a specific protein receptor. As an example, morphine acts at what are called μ receptors. Indeed, polymorphism is exhibited by the various opiate receptors.[18]

Perhaps the best-characterized and most extensively studied area in pharmacogenomics is biotransformation. Fundamentally, biotransformation can be divided into two areas — Phase I and Phase II. Both Phase I and Phase II are designed to make xenobiotics more polar and, thus, more water-soluble. By being more polar and more water-soluble, metabolites are more easily excreted into excretory fluids such as urine. Phase I reactions include hydrolysis, reduction, and oxidation. Phase I biotransformation may activate a drug (known in this case as a prodrug) into a biologically active form or may inactivate an active drug. An example of activation is seen with the conversion of Tegafur into the active anticancer agent 5-fluorouracil (5-FU). An example of deactivation is seen with the oxidation of ethanol into acetaldehyde by alcohol dehydrogenase and the further oxidation of acetaldehyde into acetate by aldehyde dehydrogenase. Phase II biotransformation may or may not be preceded by Phase I biotransformation. Phase II biotransformation reactions involve glucuronidation; sulfation; acetylation; methylation; conjugation with glutathione; and conjugation with amino acids such as glycine, taurine, and glutamic acid.[19]

Hydrolysis as a Phase I chemical breakdown pathway has been mentioned above in the case of pseudocholinesterase

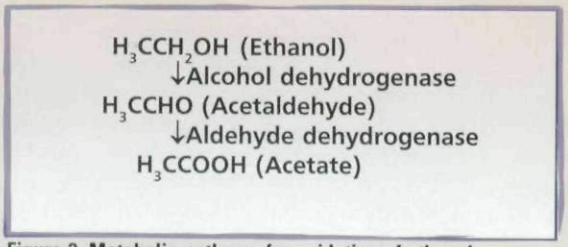

Figure 2. Metabolic pathway for oxidation of ethanol

and its variants. Reduction of the drug 5-fluorouracil, which was discussed above under activation of Tegafur, shows polymorphism in the 5-FU reduction when 5-FU is metabolized (deactivated) to its reduction product, 5-fluorodihydrouracil, in rare individuals who are deficient in the enzyme dihydropyrimidine dehydrogenase (DPD). Individuals who are deficient in DPD show toxicity, which may be fatal, to bone marrow and intestines due to increased levels of 5-FU.[19]

Since a more polar (and, thus, more water-soluble) product usually results, oxidation is one of the most common metabolic pathways in mammals. Due to genetic polymorphism, the metabolism of the most routinely observed analyte in toxicology, ethanol or ethyl alcohol, actually is more complex than usually visualized by the simple pathway depicted in Figure 2.

The first enzyme in the oxidation of ethanol — alcohol dehydrogenase — is a zinc-containing dimer, which means that the functional enzyme consists of two protein chains and the element zinc. The subunits are designated α, β, γ, π, χ, or σ. The subunits are encoded by six different genetic loci (ADH1A, ADH1B*1, ADH1C*1, and ADH4-ADH7 — formerly ADH1 through ADH7, respectively). To further add to the complexity of the system, there are three allelic variants of the beta subunit designated β_1, β_2, and β_3 and two allelic variants of the gamma chain designated γ_1 and γ_2, giving rise to, respectively, ADH1B*1, ADH1B*2, ADH1B*3, ADH1C*1, and ADH1C*2. The nine subunits of ADH can combine to form homodimers (i.e., both chains are identical). Further, the α, β, and γ chains and their allelic variants can form heterodimers (i.e., the two chains are different) with each other, but not with the other types of chains.

The different molecular forms of ADH are divided into four classes. Class 1 contains ADH1A, ADH1B*1, and ADH1C*1, which can be considered isozymes. ADH1A contains either two alpha subunits or one alpha plus one beta or gamma subunit. ADH1B*1 contains either two beta subunits, which could be β_1, β_2, or β_3 or a beta subunit plus a gamma subunit, which could be γ_1 or γ_2. ADH1C*1 contains two gamma subunits that could be γ_1 or γ_2. ADH1B enzymes that differ in the type of β subunit are known as allelozymes, as are ADH1C enzymes that differ in the type of γ subunit. Accordingly, ADH1B*1 is an allelozyme composed of β_1 subunits, ADH1B*2 is an allelozyme composed of β_2 subunits, and ADH1B*3 is an allelozyme composed of β_3 subunits. Class II contains ADH4, which is made up of two π subunits. Class III contains ADH5, which is made up of two χ subunits. Class IV contains ADH7, which is made up of two σ subunits.

It is the Class I ADH isozymes that are of the most interest to the practicing toxicologist, as it is these isozymes that are involved in the oxidation of ethanol and methanol. ADH1B*2 is an atypical isozyme that is responsible for an unusually rapid conversion of ethanol into acetaldehyde in 90% of the Pacific Rim Asian population, but is expressed to a lesser degree in Caucasians, Native Americans, and Asian Indians. Aldehyde dehydrogenase (ALDH) oxidizes aldehydes (like acetaldehyde) to the corresponding carboxylic acid. Twelve ALDH genes (ALDH1 through ALDH10,

Continues on page 26

SSDH, and MMSDH) have been identified in humans. ALDH2 is primarily responsible for oxidizing simple aldehydes like acetaldehyde. A genetic polymorphism for ALDH2 has been identified in humans. A high percentage of Japanese, Chinese, Koreans, Taiwanese, and Vietnamese populations are deficient in ALDH2 due to a point mutation ($Glu_{487} \rightarrow Lys_{487}$). This inactive allelic variant of ALDH2, known as ALDH2*2, is found in the same population that has a high incidence of the atypical form of ADH — ADH2*2, which means that these individuals rapidly convert ethanol to acetaldehyde, but only slowly convert acetaldehyde to acetic acid. As a result, many Asians experience a flushing syndrome after consuming alcohol. Thus, what is considered to be very simple by most toxicologists — especially those who deal with driving-under-the-influence forensic cases — actually can be quite rich in detail for certain individuals within a diverse array of ethnic groups.[20,21]

In addition to the above example of the oxidation of ethanol, numerous other oxidative pathways for xenobiotics exist in humans and other animals. Many instances of drug oxidation are the result of a group of enzymes known as CYPs (from *CY*tochrome *P*450, the 450 being derived from the cytochrome's maximal absorbance of light at 450 nm). CYPs are categorized according to amino acid sequence homology. CYPs that have less than 40% homology are placed in a different family (e.g., 1, 2, 3, and so on). CYPs that are 40% to 55% identical are assigned to different subfamilies (e.g., 1A, 1B, 1C, and so on). P450 enzymes that are more than 55% identical are classified as members of the same subfamily (e.g., 2B1, 2B2, 2B3). The P450 enzymes are expressed in numerous tissues, but are especially prevalent in liver. CYPs, which exist in mammalian physiology, are so numerous that their complete description is beyond the scope of this basic introduction. According to a recent survey regarding the top 10 pharmacogenomics tests important to human drug metabolism and of interest for test development, five CYPs are CYP2D6, 2C9, 2C19, 3A5, and 2B6.[22]

The final step in drug metabolism is elimination. As stated earlier, elimination can occur with the unchanged drug, a drug that has been subjected to Phase I metabolism, a drug that has been subjected to Phase II metabolism, or a combination. Also

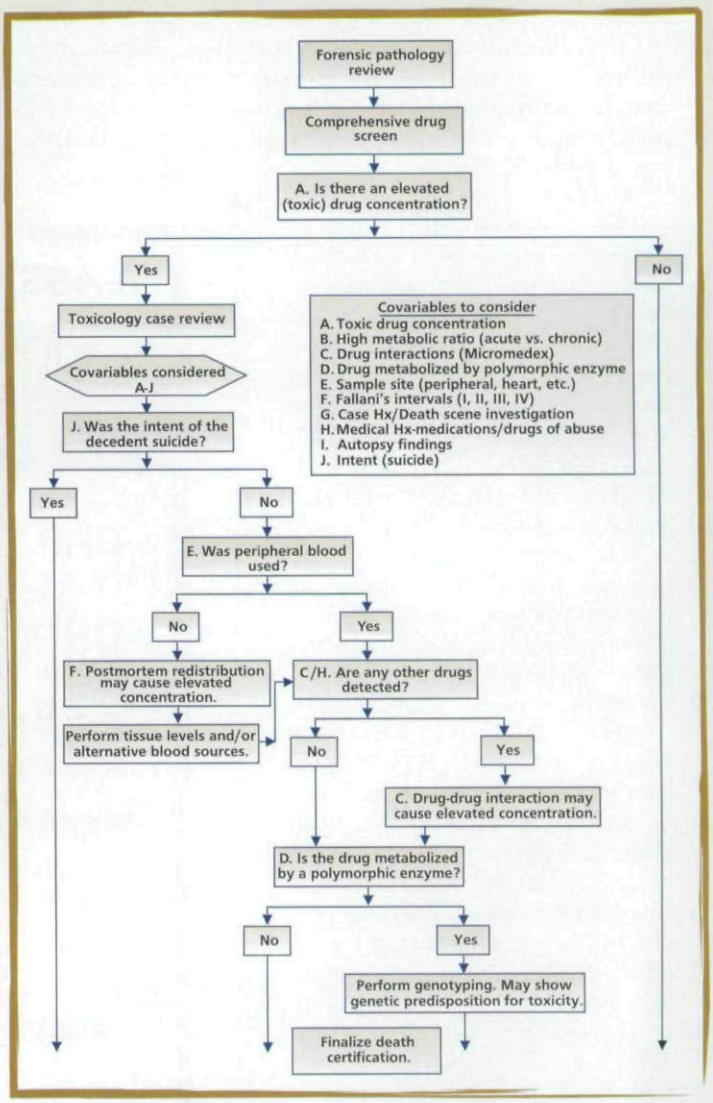

Figure 3. The proposed Milwaukee pharmacogenomic algorithm for forensic toxiology. (Reprinted with permission from the *Journal of Analytical Toxicology* — see Reference 29.)

as briefly discussed above, the proteins involved in elimination can be subject to polymorphism and, thus, are involved in pharmacogenomics. □

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