Genetic Differences in Drug Disposition

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Genetic polymorphisms of drug metabolizing enzymes are well recognized. This review presents molecular mechanisms, ontogeny and clinical implications of genetically determined intersubject variation in some of these enzymes. Included are the polymorphic enzymes N-acetyl transferase, cytochromes P4502D6 and 2C, which have been well described in humans. Information regarding other Phase I and Phase II polymorphic pathways, such as glutathione and methyl conjugation and alcohol and acetaldehyde oxidation continues to increase and are also discussed. Genetic factors effecting enzyme activity are frequently important determinants of the disposition of drugs and their efficacy and toxicity. In addition, associations between genetic differences in these enzymes and susceptibility to carcinogens and teratogens have been reported. Ultimately, the application of knowledge regarding these genetic factors of enzyme activity may guide medical therapy and minimize xenobiotic-induced disease.

The recognition of the importance of intersubject variation in drug metabolism has increased markedly over the past 20 years. Intersubject variation may be genetically determined, environmentally induced, or more commonly, a combination of both. Pharmacogenetics is the study of the inherited traits that are responsible for unusual responses to drugs or xenobiotics. The inherited traits determining drug disposition may be either genetic polymorphisms, defined as Mendelian traits that exist in the population in at least two phenotypes neither of which is rare, or rare single gene defects. Genetically determined variation in drug disposition leads to responses that may be immediate, subacute, or delayed and may be either positive or adverse. The clinical consequences of genetic polymorphisms includes increased risk of adverse drug reactions, a lack of efficacy, or an association with disease states. The identification of patients with genetic predisposition using history, screening tests, and family studies may allow *a priori* dosage adjustment, alterations in time scheduling of therapeutic monitoring, or the use of drug alternatives. In this review, the Cytochrome P450 oxidative polymorphisms and N-acetylation are detailed as the best studied examples of phase I and phase II genetic polymorphisms. Other genetically polymorphic pathways, including glutathione and methyl conjugation and alcohol and acetaldehyde oxidation, as well as the genetic polymorphism of aryl hydrocarbon hydroxylase induction, are also briefly described. Maturation of the polymorphic pathways is summarized, and examples of resulting kinetic, therapeutic, and toxic differences are presented. Data regarding potential differences in susceptibility to xenobiotic-induced diseases, including carcinogenesis and teratogenesis are outlined.

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THE EVOLUTION OF KNOWLEDGE

Drug metabolism polymorphisms have been typically discovered after observations of marked intersubject differences in drug response during preclinical or clinical trials. Subsequently, definitive pharmacokinetic studies in which individuals were given an oral dose of probe drug and either plasma or urine collected were performed. For urinary sampling, the ratio of the total urinary yield of the parent drug over 8 hours to the total urinary yield of metabolite was calculated as the metabolic ratio. Next, the metabolic ratios of large populations were evaluated using either frequency histograms or normit plots. Bimodal distributions suggested two populations of phenotypes, termed extensive and poor metabolizers. Family studies confirmed the genetic nature of phenotypic bimodality. For some of the genetic polymorphisms, the responsible enzymes have been

isolated, and the antibodies subsequently raised have permitted studies of substrate specificity.

Utilization of multiple molecular biology tools has clarified fine details of the structure of some of the involved genes and the definition of the mutations causing absent or defective proteins, allowing the development of simple tests to detect these mutations. An insertion, deletion, or rearrangement mutation in either the regulatory or structural sequence of the gene for a drug-metabolizing enzyme may decrease intracellular concentration or eliminate the enzyme protein or may structurally alter the enzyme with consequent changes in enzyme function. A defect in transcription, RNA processing or RNA stability may occur. At the protein level, either a decreased intracellular concentration or total absence of the protein may occur secondary to a diminished rate of synthesis or an accelerated degradation. Alternatively, there may be a normal intracellular concentration of a mutant enzyme protein. Finally, an enzyme may be structurally intact, but exhibit decreased affinity or maximal velocity for substrates or a change in stereoselectivity.

TABLE I

Drugs and Other Environmental Chemicals Subject to Polymorphic Acetylation in Humans

- Hydrazines
  - Isoniazid
  - Hydralazine
  - Phenelzine
  - Acetylhydrazine
  - Hydrazine
- Arylamines
  - Procainamide
  - Dapsone
  - Sulfamethazine
  - Sulfapyridine
  - Aminoglutethimide
- Carcinogenic arylamines
  - Benzidine
  - 2-aminofluorene
  - β-naphthylamine
  - 4-aminobiphenyl
- Drugs metabolized to amine
  - Sulfasalazine
  - Nitrazepam
  - Clonazepam
  - Caffeine
  - Acebutolol

N-ACETYLATION (N-ACETYLTRANSFERASE-2)

The earliest described and most widely recognized genetic polymorphism of drug metabolism is that of N-acetylation. This polymorphism affects the disposition of many diverse xenobiotics (Table I). Using one of several probe compounds, including isoniazid, sulfadimidine, procainamide, caffeine, dapsone, sulfamethazine or isoniazid, individuals may be separated into either fast or slow acetylator phenotypes. About half of Caucasian populations are slow acetylators. Compared with fast acetylators, slow acetylators achieve a higher parent drug blood concentration after the usual therapeutic dose of acetylated drugs and are more likely to develop unwanted effects. These toxic effects include sulfasalazine-induced hemolysis and hydrazine or arylamine-induced peripheral neuropathy and lupus erythematosus.[1-4] The relationship between acetylator phenotype and therapeutic response is less clear; however, on the basis of higher cure rates in slow acetylators,[5] it has been advocated that rapid acetylators with pulmonary tuberculosis receive twice weekly isoniazid, while slow acetylators are treated once a week.

Two enzymes catalyzing N-acetylation, N-Acetyltransferase 1 and 2 (NAT1 and NAT2), are expressed in human liver, and both show selectivity for arylamine substrates.[6] NAT1 has higher affinity and substrate turnover for monomorphic arylamines, such as p-aminosalicylic acid and p-aminobenzoic acid, while NAT2 isoforms have higher affinity for the polymorphic arylamines substrates, such as sulfamethazine and procainamide. Thus, the NAT2 enzyme is responsible for the genetic polymorphism of N-acetylation. The detailed human tissue distribution of NAT1 and NAT2 remains to be determined, but it seems likely from the rabbit model that NAT2 is present in liver and gut, while NAT1 is present more widely.[7] From the arylamine substrate specificity of human peripheral blood cells, the acetylation enzyme present in blood is almost certainly NAT1.[8,9] The enzyme NAT2 exists as two isoforms, NAT2A and NAT2B. In genetically slow acetylators, the liver content of both NAT2A and NAT2B is markedly reduced.[10]

NAT1 and NAT2 are encoded at two genetic loci on chromosome 8.[11] A lack of correlation of NAT1 and NAT2 enzyme activities in the human liver cytosol suggests that the two genes are independently regulated.[6] Isolation of the DNA and corresponding cDNA for the polymorphic NAT2 locus has been well described.[11-13] Using PCR and NAT2 specific oligonucleotide primers, at least 4 alleles, one fast (F1) and 3 slow (S1, S2, and S3) may be present at the polymorphic NAT2 locus.[14,15] The fast allele in both heterozygotes and homozygotes produces the fast phenotype. In homozygotes, the three slow alleles are associated with slow acetylation *in vivo* without significant difference in the resulting acetylation ac-

tivities among the three alleles. In either autopsied livers or in transfected cells, the mRNA levels determined by the three mutant alleles are not different from mRNA levels from the fast F1 allele, suggesting that the difference in NAT activity is mainly determined by the amount of enzyme protein.[15] In Caucasians, the most common allele at the polymorphic NAT locus in Caucasians is the S1 which has a frequency of 45%.[14] In the Japanese population, the absence of this allele in association with a 68% frequency of the F1 allele explains the markedly lower incidence of slow acetylation (6.6%) in this population.[14,16]

DEBRISOQUINE/SPARTEINE (CYTOCHROME P450 2D6)

The genetic polymorphism associated with debrisoquine and sparteine metabolism is the first and most fully described genetic polymorphism of a Cytochrome P450 enzyme. The observation of unusual subject sensitivity to the hypotensive effects of debrisoquine led to this discovery.[17] Contemporaneously, German investigators identified a polymorphism from observations of unusual neurologic side effects in some patients treated with the oxytocic agent sparteine.[18] From cosegregation of poor metabolizers[19] and from competitive inhibition studies,[20] these polymorphic responses were shown to reflect differences in the activity of the same Cytochrome P450 enzyme, currently labeled Cytochrome P450 2D6 (CYP2D6). Using the metabolic ratio of parent drug to its 4-hydroxymetabolite, investigators separated individuals into extensive and poor metabolizer phenotypes and found 7–10% of Caucasian populations deficient in the activity of this enzyme.[21,22]

Purification and characterization of the responsible enzyme led to studies that confirmed the cosegregation of debrisoquine and sparteine, bufuralol, encainide, and propranolol and allowed the development of specific polyclonal antibodies, which has allowed substrate specificity studies.[23] The large number of drugs that are oxidized by CYP2D6 (Table II) and the 10–20 fold difference in disposition of some of these substrates account for the tremendous clinical interest in this polymorphism. Multiple drugs lack cosegregation with the debrisoquine polymorphism (Table III). Although criteria have not been formed to structurally assess whether a compound is metabolized by this enzyme *a priori*, all CYP2D6 metabolized substrates have a basic nitrogen and are oxidized at a site within 0.5–0.7 nm of this basic nitrogen.[24] Not only does the polymorphism alter the rate of metabolism, the stereoselectivity of CYP2D6 may also be modified. In extensive metabolizers, inactive R-metoprolol is metabolized faster than the active S-enantiomer, whereas metabolism is not stereoselective in poor metabolizers.[25] For some substrates, such as imipramine, the disposition of CYP2D6 substrates may exhibit nonlinear kinetics in extensive metabolizers but linear kinetics in poor metabolizers.[26] Phenotypic differences may be reduced by functional impairment of the enzyme in extensive metabolizers. For example, quinidine is a competitive inhibitor that almost completely abolishes the *in vivo* metabolism of debrisoquine.[27] Hence, a genetic extensive metabolizer taking quinidine would phenotypically appear to be a poor metabolizer. Other compounds that inhibit debrisquine metabolism *in vivo* include thioridazine, levomepromazine, and propafenone.[28,29] Compounds that competitively inhibit enzyme activity *in vitro* which

TABLE II

Drugs That Undergo Oxidation by Cytochrome P450 2D6

- Beta Blockers
  - Propranolol
  - Metroprolol
  - Timolol
  - Bufuralol
- Antiarrhythmics
  - Sparteine
  - N-propylajmaline
  - Propafenone
  - Flecainide
  - Encainide
- Tricyclic antidepressants
  - Nortriptyline
  - Desipramine
  - Climipramine
  - Imipramine
  - Amitriptyline
- Neuroleptics
  - Perphenazine
  - Thioridazine
- Miscellaneous
  - Codeine
  - Debrisoquine
  - 4-Hydroxyamphetamine
  - Phenformin
  - Amiflamine
  - Perhexiline
  - Dextromethorphan
  - Guanoxan
  - Indoramin
  - Methoxyamphetamine
  - Methoxyphenamine
  - Tomoxetine
  - Ethylmorphine

TABLE III

Drugs That Are Not Eliminated by Cytochrome P450 2D6

Alfentanil
Acetanilide
Amobarbital
Antipyrine
Carbamazepine
Carboxymethylcysteine
Cholesterol
Cyclosporine
Desmethyldiazepam
Diazepam
Ethinyloestradiol
Ethosuximide
Haloperidol
Maprotiline
Mephenytoin
Methaqualone
Midazolam
Nifedipine
Phenytoin
Pinacidil
Prazosin
Propranolol
Quinidine
Theophylline
Tolbutamide

may be *in vivo* inhibitors include important drugs such as haloperidol, fluphenazine and trifluperidol, metoclopramide, apomorphine, phenylcyclopropylamine, diphenhydramine and nicardipine and the estrogen norethindrone acetate.[30] These compounds likely bind to CYP2D6 and might convert a genetically extensive metabolizer to a phenotypic poor metabolizer. Inducers of CYP2D6, such as antipyrine and rifampicin, will exaggerate the differences observed in metabolic ratios between extensive and poor metabolizers, but have minimal effect on drug elimination.[31]

The debrisoquine/sparteine polymorphism is caused by mutations of the CYP2D6 gene, which is part of a gene cluster on chromosome 22 that includes several related pseudogenes.[32] Analysis of genotype may be performed using allele-specific probing of PCR amplified DNA. Restriction fragment length polymorphism (RFLP) analysis after digestion with the endonuclease XbaI usually results in fragments of 29, 44, and 11.5 kb length,[33] or in a smaller number of individuals, 16+9 kb.[34] Unfortunately, RFLP analysis does not distinguish between extensive and poor metabolizers because a 44 kb and a 29 kb fragment may occur in both. A 29 kb fragment may represent either the wild type (normal) allele or may represent mutant alleles, called 2D6(A) and 2D6(B). The 2D6(A) mutant allele features a deletion in exon 5, while the 2D6(B) mutant allele contains multiple mutations leading to a splicing defect.[35] Some individuals with the 2D6(B) mutation and splicing defect, have an additional gene in the cluster that renders a 44 kb fragment instead of a 29 kb fragment.[36] In Chinese populations, a large segment of the population has a 44 kb fragment associated with the gene insertion, but without the splicing defect.[37] These individuals are extensive metabolizers in contrast to Caucasians with a 44 kb fragment who are poor metabolizers. The 2D6(C) mutant allele involves deletion of 3 base pairs in exon 5, resulting in a single amino acid deletion in the protein. The protein produced appears to function, but at markedly reduced levels.[38] In individuals with the 2D6(D) mutation, the entire gene is deleted, resulting in an 11.5 kb fragment.[39] Using PCR and RFLP analysis, these four mutant alleles have been shown to account for over 95% of deficient metabolizers of debrisoquine with approximately 43, 31, 14 and 9% of the mutant alleles in poor metabolizers associated with the 29(B), 44 kb, 11.5 and 29(A) fragments, respectively.[40] Using a nested primer strategy, allele specific oligonucleotide probing of PCR amplified DNA has correctly predicted 100% of extensive metabolizers and 86%–97% of poor metabolizers in two large independent population studies.[41,42]

The frequency of the poor metabolizer debrisoquine phenotype appears to be markedly lower in non-Caucasian populations. Conflicting studies have reported 8.6[43] and 0% of black Nigerians to be poor metabolizers.[44] In 80 Ghanaians, no individuals were poor metabolizers of sparteine while 6% were poor metabolizers of debrisoquine.[45] Similarly, black South Africans exhibited no evidence of poor metabolism for sparteine.[46] African American children had a lower incidence of the poor metabolizer phenotype for debrisoquine (1.9%) compared with Caucasian children (7.7%).[47] The incidence of poor metabolizers in Orientals, including Japanese, Thai, Malaysian, and Chinese populations, appears to be 0–2%.[48-52] A low incidence of the debrisoquine polymorphism has been reported in other ethnic groups of Mongolian origin with 3.2% and 0% of Greenlanders and Amerindians phenotyped as poor metabolizers, respectively.[53,54] The regulation of sparteine and debrisoquine oxidation may be different in different ethnic groups.[45,55]

PCR techniques to determine CYP2D6 genotypes are highly accurate[41,42] and genetic influences account for 0.79 of variation in the activity of the debrisoquine enzyme, with non-genetic factors such as age, smoking, and alcohol use having little or no impact on enzyme activity.[48,56] Thus, PCR determina-

tions of genotype may replace debrisoquine phenotype studies for some purposes. Genotyping may be useful when subjects are already taking inducers or inhibitors which alter phenotype or when giving a probe compound or collecting total urine may be medically or ethically difficult. Pregnant, pediatric, and geriatric patients may be examples of the latter; however, information regarding hormonally or developmentally induced alterations in enzyme activity must be considered. For instance, during pregnancy, the hepatic clearance of metoprolol, an antihypertensive metabolized by CYP2D6, increases with resulting plasma concentrations being only 12% to 55% of those seen in the nonpregnant state.[57] Thus, although genotyping may be easier to perform than phenotyping, the application to specific clinical questions may be less direct.

MEPHENYTOIN HYDROXYLATION (CYTOCHROME P4502C)

The observation of unusual clinical sensitivity to a low dose of the anticonvulsant drug mephenytoin led to the discovery of this genetic polymorphism.[58,59] Mephenytoin is a chiral compound that displays stereoselective metabolism *in vivo*. In normal or extensive metabolizers, S-mephenytoin is oxidized to 4'-hydroxymephenytoin, which is then excreted as the glucuronide conjugate over about 4 days, while the R-enantiomer is preferentially N-demethylated and slowly excreted with only about 10% excreted in 14 days.[60] Thus, in the normal individuals, the clearance of the S-enantiomer is about 200-fold greater than the R-enantiomer.[60] In genetically deficient subjects, this stereoselectivity is virtually absent and both enantiomers undergo demethylation and slow excretion. The mephenytoin polymorphism and this change in stereoselective metabolism is generally described using 8-hour urinary R/S mephenytoin ratios with extensive and poor metabolizers having very low ratios and ratios of 1 or more, respectively. Poor metabolizers of mephenytoin are characterized by a high Km and a low Vmax for S-mephenytoin hydroxylation.[24] This is consistent with results of *in vitro* studies using human autoantibodies suggesting that the mephenytoin hydroxylation deficiency is caused by a structural change leading to a functionally altered enzyme.[61] Family studies suggest the defect in S-mephenytoin hydroxylation is consistent with autosomal recessive inheritance.[62,63] The enzyme responsible for mephenytoin hydroxylation has not been identified; however, it is believed to belong to the Cytochrome P4502C family. Although 4-hydroxylation of R-mephenytoin is catalyzed by 2C9, no activity has been observed against S-mephenytoin.[64]

A small number of other drugs cosegregates with mephenytoin. Mephenytoin hydroxylase catalyzes the 3'-hydroxylation of hexobarbital.[65,66] The oxidation of mephobarbital cosegregates with mephenytoin hydroxylation,[67,68] and the incidence of adverse reactions to mephobarbital is similar to the incidence of slow metabolizers.[49,69] Because tolbutamide hydroxylase copurified with mephenytoin hydroxylase, tolbutamide oxidation was believed to cosegregate with mephenytoin hydroxylation. However, no cosegregation of poor metabolizers occurs,[70] and tolbutamide is oxidized by both CYP2C8 and C9, neither of which metabolize S-mephenytoin. Mephenytoin 4'hydroxylation is inhibited *in vitro* by cortisone and ethinyloestradiol and weakly by cortisol, estradiol, adrenosterone, and testosterone.[71] Competitive inhibition occurs with ethotoin, mephobarbital, methsuximide, phensuximide, and high doses of warfarin.[72] Other commonly used anticonvulsants such as ethosuximide, phenobarbital, phenytoin, diazepam, and primidone do not competitively inhibit.[72] An aryl residue alpha to the carbonyl carbon of an N-alkyl lactam in a 5 or 6 membered ring is a minimal requirement for interaction with the 4'hydroxylase.

The defect in S-mephenytoin metabolism is found in about 2–5% of Caucasian populations[73,74] and 18–23% of Japanese populations.[49,50] In Chinese populations, studies report 5–17.4% poor metabolizers.[50,74] In contrast, no poor metabolizers were identified in 90 Panamanian Amerindians.[75] The relative importance of genetic and non-genetic influences on mephenytoin hydroxylase is unknown; however, among extensive metabolizers, clearance of S-mephenytoin varies by more than an order of magnitude, suggesting non-genetic influences are important. Age may have an influence on mephobarbital hydroxylation, which cosegregates with mephenytoin oxidation.[76] The suggestion of an age specific effect of gender overlooked likely confounding from oral contraceptive use.[76,77]

OTHER CONJUGATION POLYMORPHISMS: GLUTATHIONE AND METHYL TRANSFERASES

Glutathione S-transferases (GSTs) comprise a family of proteins that conjugate electrophilic molecules with glutathione to render them less toxic. The multiples forms of GST are divided into three classes, acidic, neutral and basic, based on their pH in isoelectric focusing. Each class shares significant physical and immunologic properties and substrate specifici-

ties. Three genetic loci, GST1, GST2, and GST3, encoding human liver GST isoenzymes have been characterized.[78–80] Of these, the GST1 locus, which encodes for the neutral mu form, appears to be polymorphic with three possible alleles, GST1-0 (null), GST1-1 and GST1-2.[81] Although mu GST is a relatively small contributor to total GST activity, this polymorphism is of interest because of the relatively high specific activity of the mu class for epoxides.[82] The null variant is found in every ethnic group tested thus far with a gene frequency ranging from 43 to 82%.[78,83] Individuals who are homozygous for the null allele have significantly lower total GST activity[79] and lack any immunologically detectable GST1 isozyme.[84] The other possible alleles at the GST1 locus, GST1-1 and GST1-2, have been cloned and sequenced; however, the localization of the human mu class genes has not been resolved.[85,86]

Methylation, an important pathway for many drugs, neurotransmitters and xenobiotics, is catalyzed by a number of enzymes, two of which exhibit genetic polymorphism. Thiopurine methyl transferase (TPMT) and thiomethyl transferase (TMT) have been identified in the human red blood cell and act on thiol groups.[87,88] TPMT is present in the red blood cell lysate and catalyzes methylation of 6-thioguanine, azathioprine and 6-mercaptopurine.[89] Segregation analysis of family studies suggests TPMT enzyme activity is controlled by a single locus with two alleles.[90] Eighty nine percent of Caucasians have high TPMT activity and represent the homozygous high activity genotype, while 11% have intermediate activity and are heterozygotes.[90] Rare individuals lack TPMT activity and are homozygous for the low activity allele. This phenotype distribution yields low and high activity allele frequencies of 6% and 94%, respectively. The activity of TPMT in the red blood cell correlates well with the relative activity in other sites, including the lymphocyte, kidney and liver.[91–93] Low levels of activity reflect quantitatively low enzyme concentrations.[92] The biochemical and physical properties of TPMT are similar in animals with low and high activity, suggesting a non-structural polymorphism.[94] The other polymorphic methyl transferase, thiol methyltransferase (TMT), is membrane bound and catalyzes the methylation of a number of nonpurine molecules, such as 2-mercaptoethanol, captopril, and N-acetylcysteine.[95] The biochemical properties of human liver TMT are very similar to those of human red blood cell TMT.[96] TMT activity in red blood cells is genetically regulated with about 98% heritability.[97] Fifty-eight percent of individuals have high activity, whereas 42% have low activity.[98] Whether genetic variation in red blood cell membrane TMT parallels the variation of TMT in other tissues is unknown.

OTHER OXIDATIVE POLYMORPHISMS: ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE

Twin studies suggest that genetic factors play a role in the threefold intersubject variation observed in ethanol metabolism.[99,100] Both alcohol dehydrogenase, which oxidizes ethanol to acetaldehyde, and aldehyde dehydrogenase, which oxidizes acetaldehyde to acetate, exhibit genetic polymorphism. Alcohol dehydrogenase is a dimeric enzyme whose subunit chains are determined by at least six genetic loci, of which at least two, *ADH2* and *ADH3* are polymorphic.[101] The three possible alleles at the polymorphic *ADH2* locus determine subunit chains called $\beta 1$, $\beta 2$, and $\beta 3$, and the two possible *ADH3* locus alleles result in $\tau 1$ and $\tau 2$ subunit chains (Table IV). These subunit chains hybridize to form homo or heterodimers which vary markedly in their kinetic properties. Allele frequencies vary across ethnic groups (Table V).[102] Isolation, purification and sequencing of the isoenzymes led to the development of specific oligonucleotides to detect complementary sequences in cDNA libraries. cDNA clones were then used to detect complimentary sequences in the libraries of human genomic DNA, leading to the isolation of genomic clones for each chain. Both polymorphic ADH loci have been localized to the long arm of chromosome 4.[103] Substrate specificity studies of human ADH have largely addressed the metabolism of various alcohols, however, the oxidation of the sedative drug chloral hydrate appears to involve ADH.[104,105]

There are at least four forms of aldehyde dehydrogenase (ALDH) whose characteristics and activity vary substantially.[106] The major isoenzyme form responsible for acetaldehyde oxidation is the mitochondrial, low Km form, *ALDH2*, encoded by the ALDH2 gene. In Asians and South American Indians,

TABLE IV

Disparity in Alcohol Dehydrogenase Isoenzyme Kinetic Constants Secondary to ADH Genetic Polymorphism

Allele	Enzyme	Km(mM) ETOH	V_{max} Min^{-1}
ADH2*1	$\beta_1\beta_1$	0.05	9
ADH2*2	$\beta_2\beta_2$	0.9	400
ADH2*3	$\beta_3\beta_3$	34	300
ADH3*1	$\gamma_1\gamma_1$	1.0	87
ADH3*2	$\gamma_2\gamma_2$	0.63	35

(Data from Bosron and Li, 1987).

TABLE V

Frequency of Polymorphic ADH Alleles Across Ethnic Groups

	ADH2 Alleles			ADH3 Alleles	
	1	2	3	1	2
Caucasians	>95	<5	<5	50	50
Japanese	35	65	<5	95	5
African Americans	85	<5	15	85	15

a null allele at this locus causes impaired elimination of acetaldehyde and the flushing reaction following ingestion of ethanol.[107] The null allele is dominant, and heterozygotes exhibit ALDH deficiency.[108] The *ALDH2* gene has been localized to chromosome 12,[109] and the null allele encodes a single substitution of lysine for glutamic acid.[110]

POLYMORPHISM OF ENZYME INDUCTION: THE Ah RECEPTOR

Cytochrome P450 1A1 (CYP1A1), or aryl hydrocarbon hydroxylase, catalyzes the conversion of many procarcinogens into active carcinogens and is induced by various polycyclic aromatic hydrocarbons. CYP1A1 induction is a multistep process which has been elucidated using murine models.[111-114] The first step requires the presence of a cytosolic receptor protein, the Ah receptor, whose presence is determined by a genetic polymorphism at the Ah locus. The inducing agent binds to this Ah cytosolic receptor; subsequently, the inducer-receptor complex gains chromatin binding properties. The inducer-receptor complex binds to regulatory elements upstream from the CYP1A1 coding site. The Ah receptor has been refractory to purification; however, it is thought that at least two proteins are involved.[115] The molecular mechanisms and the epidemiology of Ah induction in humans is currently under investigation. Consistent with animal models demonstrating the genetic basis for Ah inducibility,[115,116] induction in human lymphocytes appears trimoidal with segregation analysis strongly suggestive of a single diallelic locus determining the response.[117]

DEVELOPMENTAL ASPECTS OF GENETICALLY POLYMORPHIC PATHWAYS

Although characterization of the ontogeny of the genetically polymorphic pathways is incomplete, several *in vitro* studies of human fetal liver tissue and a limited number of *in vivo* clinical studies have been reported. Acetylation of the polymorphic NAT2 substrate 7-clonazepam was absent in human fetal liver at 11 and 14 weeks, with some activity starting at 16 weeks.[118] However, at gestational ages up to 22 weeks, NAT2 activity is less than 1% of adult activity and bimodality is not detectable. CYP2D6 is not active early in gestation, as codeine and dextromethorphan oxidation also do not occur in human fetal liver at 14–24 weeks gestation.[119] An increase in immunochemically detected protein occurs during the first postnatal week, irrespective of gestational age.[120,121] The increase in protein is associated with the onset of dextromethorphan O-demethylation and is preceded by a rise in CYP2D6 mRNA, suggesting that regulation early is life is primarily at the transcriptional level. *In vitro* evaluation of a single human fetal liver suggests that hexobarbital hydroxylation, which cosegregates with mephenytoin hydroxylation, does not occur during mid-gestation.[122] This finding is consistent with the lack of hexobarbital oxidation observed in sheep fetuses between 12 and 20 weeks.[122]

Glutathione transferase is present in the fetus as early as 11 weeks.[123] Fetal hepatic GST activity is about two-thirds of adult activity, and non-hepatic fetal activity may actually exceed adult activity.[124,125] The polymorphic mu form of glutathione transferase has not been easy to observe in the fetus before 30 weeks gestation, but does increase steadily after that time with normal adult values reached late in infancy.[126] Other GST isoforms, alpha and pi glutathione transferase, are similar to the corresponding adult forms.[127,128] TPMT and TMT activities are present at in human fetal liver at mid-gestation, but the activities are about one third and one sixth that of adult activity, respectively.[129,130] Interestingly, fetal renal TPMT is about twice that of adult hepatic TPMT. For TMT activity, no correlation is observed between fetal hepatic activity and gestational age, suggesting that late antenatal or postnatal ontogeny is important.[129]

ADH activity is present in human fetal liver at about 2 months, but at about 3% of adult activity.[131] Human hepatic ADH activity continues to increase during gestation and early childhood with adult activity reached by age 5. The ontogeny of hepatic ADH involves a sequential initiation of expression of the genetically regulated ADH isoenzymes with α chain production beginning in the first fetal trimester, β chains in the second trimester and τ chains after birth.[132] The temporal expression correlates with sequential promoter activation of the corresponding ADH alleles by hepatic nuclear factor 1 (HNF-1), CCAAT/enhancer-binding protein (C/EBPα) liver activator protein (LAP) and D-element binding protein (DBP).[133]

In vivo phenotype studies of the genetically polymorphic pathways in children have been delayed compared with adult studies, partially related to the potential toxicity of the probe substrates classically used in adult studies. Ethically, a probe for use in pediatric patients should be an innocuous, easily administered compound likely to be taken by a large number of children with a phenotype determination that is noninvasive, either using urine or salivary sampling. Dextromethorphan, a common ingredient in nonprescription cough medicine, is oxidized by CYP2D6 to dextrophan, and the urinary metabolic ratio of the parent drug to its metabolite yields phenotypes that totally cosegregate with phenotypes determined with debrisoquine.[134,135] Caffeine, commonly consumed by children in soft drinks, undergoes complex metabolism; however, the urinary ratio of two metabolites, 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (1X) cosegregates with acetylation phenotypes that were determined using sulfamethazine.[136] Evans and colleagues determined CYP2D6 oxidation and acetylation phenotypes in 26 children (ages 3–21, median 10 yr) using dextromethorphan cough syrup and caffeine-containing cola.[137] Unfortunately, in the urine samples of many children, dextromethorphan could not be detected. Although the extensive debrisoquine oxidation phenotype could be assigned, quantitative metabolic ratios could not be determined. The distribution of N-acetylation and debrisoquine ratios in children were similar to those of adult populations. Fifty-eight percent of children were fast acetylators and 8% (2/26) children were poor metabolizers of dextromethorphan. A comparable incidence (6.4%) of dextromethorphan poor metabolizers was reported in 31 healthy children, ages 5–16.[138]

In a study of the maturation of caffeine metabolism in 14 infants, the oldest infant (age 588 days) was a fast acetylator, while the remaining infants (ages 19–434 days) were slow acetylators.[139] The phenotype of a single patient studied longitudinally changed from slow to fast between 54 and 196 days of age.[139] These results are consistent with the fetal liver data and the increased incidence of slow acetylator phenotype in neonates (83%).[140] The mechanism underlying slow acetylation at very early ages is unknown and may involve insufficient Coenzyme-A synthesis in addition to NAT2 maturation.

SIGNIFICANCE OF DRUG METABOLISM POLYMORPHISMS: PHARMACOKINETICS, TOXICITY, AND EFFICACY

Those authors questioning the clinical significance of genetic polymorphisms[141] should remember that genetic polymorphisms of oxidative drugs were discovered because astute investigators wondered about the mechanisms underlying unusual adverse side effects. Genetic polymorphism may result in accumulation of the parent drug, reduced formation of an active metabolite, accumulation of an active metabolite or accumulation of the parent drug and the active metabolite. The determinants of the extent of pharmacokinetic consequences include the amount of hepatic clearance and the relative importance of the defective pathway. In addition to the magnitude of genetically-induced differences in drug disposition, the quantity of clinical use, pharmacodynamic properties of a drug and the size of its therapeutic index are important determinants of clinical significance. The presence of clinical or paraclinical drug effects which may be used for titrating the drug dose decreases the need for *a priori* knowledge regarding genetic differences. Thus, theoretically, therapeutic decisions regarding drugs subject to genetic polymorphism which have widespread clinical use, a low therapeutic index and no means to titrate the dose on the basis of immediate clinical measures of effect would be facilitated by genotype knowledge. Generally, adverse reactions are more frequent in poor metabolizers, with extensive metabolizers at greater risk for decreased efficacy. However, if drug effect is dependent on an active metabolite, the opposite may occur, with poor metabolizers at risk for decreased efficacy and extensive metabolizers at risk for increased toxicity.

The antiarrhythmic drug encainide undergoes Cytochrome P4502D(6) oxidation to several metabolites, *O*-desmethylencainide (ODE), *N*-desmethylencainide (NDE), 3-methoxy-*O*-desmethylencainide (MODE), and *N*-*O*-didesmethylencainide.[142] ODE is a more potent antiarrhythmic agent than the parent drug, while MODE and NDE appear about equipotent to encainide.[143–145] The characteristic electrocardiographic effects associated with antiarrhythmic action, namely an increase in QRS duration, are best correlated with plasma ODE.[146] With either oral or intravenous administration, poor metabolizers have plasma encainide concentrations that are 10–20-fold higher than extensive metabolizers, and the half life is prolonged by three–fourfold with a significant accumulation of unchanged drug.[142] Equally importantly, poor metabolizers have tenfold lower concentrations of ODE associated with an absence of QRS changes.[142]

Flecainide is an effective antiarrhythmic agent whose major metabolic pathway to meta-O-dealkylated flecainide cosegregates with that of sparteine/debrisoquine oxidation, resulting in a tenfold difference in metabolic clearance.[147] A number of patient deaths in association with high serum flecainide concentrations have been reported.[148] In poor meta-

bolizers, increased renal elimination of unchanged drug occurs. However, in renal failure, disposition of flecainide proceeds almost entirely by hepatic oxidation.[149] Thus, therapy in poor metabolizers with renal failure requires close monitoring and reductions greater than that suggested with renal failure alone.

Propafenone, a Class I antiarrhythmic agent, is metabolized by both 5-hydroxylation and N-dealkylation. The hydroxylation reaction cosegregates with the debrisoquine polymorphism and poor metabolizers exhibit impaired 5-hydroxylation resulting in very low or absent levels of this active metabolite.[28,150] The disposition of the parent drug in poor metabolizers is also altered with largely missing first pass metabolism, greater bioavailability, dramatically reduced apparent oral clearance and much higher steady state concentrations. These higher parent drug concentrations are associated with a greater frequency of CNS side effects in poor metabolizers (67%) compared with extensive metabolizers (14%).[28] Propafenone is delivered as a racemate with both enantiomers exhibiting similar activity on sodium channels. However the S-enantiomer is 100-fold more potent at β receptor blockade.[150] The relationship between the R and S enantiomers is similar in extensive metabolizers and poor metabolizers, but greater concentrations of the S-enantiomer in poor metabolizers may place the poor metabolizer at greater risk of β blockade related side effects.[151] Furthermore, propafenone therapy is frequently combined with a β blocking agent. When given with metoprolol, the oral clearance of metoprolol decreases two fold, while no difference is seen in the metabolism of propafenone. This suggests the dose of metoprolol should be reduced when propafenone is given.[152]

Codeine O-demethylation to its active moiety, morphine, correlates with debrisoquine hydroxylation ability.[153-155] In poor metabolizers and in quinidine-transformed extensive metabolizers, significant morphine production is absent and is associated with a lack of analgesia.[156] In contrast, extensive metabolizer subjects demonstrate measurable morphine and both subjective and objective increases in pain thresholds.

Tricyclic antidepressants undergo 2-hydroxylation which is CYP2D6 mediated and 10-hydroxylation and demethylation which is not.[157] The demethylated metabolites also undergo hydroxylation which is CYP2D6 dependent.[158] The first pass metabolism of imipramine in poor metabolizers is less than in extensive metabolizers; however, the difference is less dramatic than expected because the process is saturated in extensive metabolizers.[159] As a result of impaired elimination in poor metabolizers, accumulation of the secondary amine metabolites which are effective antidepressants may occur with an increased risk of side effect.[160] However, in a small study, the incidence of imipramine related side effects in 5 poor metabolizers was not different than the incidence in 103 extensive metabolizers.[161] Therapeutic management of tricyclic therapy is complicated because symptoms of excessive drug concentrations are similar to those of the disease process under treatment. The metabolic ratio for debrisoquine has been shown to correlate well with plasma nortriptyline and desipramine concentrations, suggesting knowledge of CYP2D6 activity might be helpful in therapeutic decision making.[159] A second complicating factor in tricyclic therapy is the frequent use of combination therapy with neuroleptic drugs. Several reports indicate that neuroleptics inhibit the *in vivo* metabolism of tricyclic antidepressants.[162] This inhibition is at least partially CYP2D6 dependent as the neuroleptics are competitive inhibitors of human liver microsomal metabolism of sparteine[163] and debrisoquine,[164] and neuroleptic therapy has been shown to increase the metabolic ratio of sparteine and debrisoquine *in vivo*.[29,165] In addition to inhibition of CYP2D6, some of the neuroleptics, such as perphenazine are substrates for CYP2D6,[166] while others such as haloperidol are not.[165]

TPMT genotype is an important risk factor for the development of thiopurine-induced myelosuppression. Both 6-mercaptopurine and azathioprine are converted into 6-thioguanine.[167] Red blood cell 6-thioguanine concentrations are directly correlated with adverse effects and inversely correlated with TPMT concentrations.[168,169] Leukemic children with lower TPMT activity are at greater risk of 6-MP induced myelosuppression. In a retrospective study of 21 patients, all five subjects developing azathioprine-induced myelosuppression had low or absent TPMT levels.[170] The mechanism for the inverse relationship between TPMT and 6-thioguanine is unknown.

Recent work suggests a correlation between the genetic polymorphisms for the enzymes involved in alcohol metabolism and *in vivo* ethanol metabolism. In the Chinese, both the *ALDH2* and *ADH2* genotypes influence the alcohol metabolic rate, the alcohol-flush reaction and susceptibility to alcoholism.[171] In the African American population, controlling for the impact of alcohol intake, the presence of the *ADH2*3* allele has been shown to impact ethanol disposition.[172] Caucasians and native Americans do not exhibit variation at these *ADH2* and *ALDH2* loci, and genetic explanations for the intersubject differences in ethanol metabolism in these populations have not been reported. The mechanism of alcohol

related flushing occasionally reported in Caucasians and American Indians is unknown but does not appear related to an *ALDH2*2* allele.

SIGNIFICANCE OF DRUG METABOLISM POLYMORPHISMS: DISEASE SUSCEPTIBILITY

The activation of chemical toxicants and carcinogens by drug metabolizing enzymes is believed important in the pathophysiology of carcinogenesis, mutagenesis, and teratogenesis, as well as other environmentally-induced diseases. Host susceptibility may be partially explained by variation in the genetic control of the activity and inducibility of these enzymes. The association between cancer risk and genetic polymorphisms has been evaluated for N-acetylation, the debrisoquine oxidation, mephenytoin oxidation, and glutathione transferase. Several carcinogenic arylamines, including 2-aminofluorene, methylene-bis-orthochloroaniline, benzidine and α and β naphthylamine are detoxified by acetylation by NAT2.[173] In contrast, the alternate pathway, N-hyroxylation, is the first step in the formation of toxic metabolites. N-hydroxylation, followed by O-acetylation, leads to the formation of acetoxyl arylamines. These derivatives break down spontaneously to form highly reactive arylnitrenium ions, the ultimate metabolite responsible for carcinogenesis and mutagenesis.[174] NAT activity has been reported in human bladder,[175] an interesting finding in view of the 39% increase in the incidence of slow acetylation in bladder cancer patients compared to matched controls.[176,177] Colonic cancer is associated with the fast acetylator phenotype.[178] The incongruity between increased risk of bladder cancer in slow acetylators and increased risk of colon cancer in fast acetylators is unexplained; however, organ specific expression of N- and O-acetyltransferase may be a significant factor. In contrast to bladder cancer and colon cancer, acetylator status and lung cancer risk appear unrelated.[179]

Extensive metabolism of debrisoquine may be a genetic risk factor for bronchogenic carcinoma; however, this is controversial. Ayesh and coworkers reported that among smokers, very rapid metabolizers of debrisoquine were overrepresented among patients with lung cancer.[180] In a second study controlling for age, gender and smoking, non-occupationally exposed extensive metabolizers of debrisoquine were found to be at a 4 fold increased risk for lung cancer.[181] When combined with asbestos exposure, the relative risk of lung cancer in extensive metabolizers increased 18 fold. In a conflicting report, Speirs and colleagues found no difference in the frequency distribution of debrisoquine metabolism in lung cancer patients compared to controls.[182] Similar debrisoquine metabolic ability was reported in bladder cancer patients and controls.[183] However, in a study in which patients were differentiated by histological criteria, aggressive bladder cancer was strongly associated with efficient debrisoquine metabolism.[184] Cytochrome P450 2D6 is not known to activate any known carcinogens; therefore, the relationship between extensive debrisoquine genotype and carcinogenesis likely reflects genetic linkage.

Presence of the GST1 null allele may predispose to cancer. Using the mu-specific substrate, trans-stilbene oxide, presence of mu GST activity correlates with inhibition of aflatoxin1 DNA adduct formation,[185,186] suggesting an important role for this enzyme in detoxification reactions that relate to cancer risk. The high substrate selectivity of GST1 for epoxides suggests that individuals deficient in GST1 may be at increased risk for cellular and genetic damage following epoxide exposure. The frequency of GST1 deficiency is greater in heavy smokers with lung cancer compared with heavy smokers without lung cancer.[187] The number of GST1 alleles correlates with mu GST activity in lung tissue[188] and high levels of lung mu GST are associated with a decreased risk of lung cancer in smokers.[188] Similarly, individuals who lack mu GST activity are at a threefold greater risk of adenocarcinoma of the stomach and colon.[189] The association of AH inducibility with susceptibility to lung cancer was first suggested by Kellerman[190] and affirmed by the association between high aryl hydrocarbon hydroxylase activity in lymphocytes and lung cancer.[191]

Increased risk for xenobiotic-induced nonmalignant diseases may also be related to genetic differences in metabolic ability. Acetylator status is a risk factor for hydralazine or procainamide-induced SLE.[2,192] In contrast, idiopathic SLE does not appear related to acetylator status.[193] The distribution of debrisoquine phenotypes is altered in patients with systemic lupus erythematosus with an increase in poor metabolizers (21%) relative to the incidence in healthy volunteers (8%).[194] Although previous reports using urinary ratios are conflicting,[195-197] individuals with the mutant forms of CYP2D6 alleles exhibit a two and a half fold increase in risk for Parkinson's disease.[198] Support for the involvement of CYP2D6 in Parkinson's disease includes observations that a substrate of CYP2D6, 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) induces a form of Parkinsonism.[199] Also, within the brain, CYP2D6 is localized to the substantia nigra, the area involved in Parkinsonism,[200] and CYP2D6 has been shown to metabolize several neurotransmitter antagonists.[201,30]

SIGNIFICANCE OF DRUG METABOLISM POLYMORPHISMS: BIRTH DEFECTS

Explanations for the variation in fetal susceptibility to teratogen exposure include differences in the timing and extent of exposure. Analogous to carcinogenic risk, teratogenic risk may involve intersubject variation in the conversion of a proteratogen to either a non-toxic metabolite or to the proximate teratogen. Genetic control of the rates of formation and metabolism of reactive metabolites could determine the availability of these toxic metabolites to bind covalently with either cell macromolecules or DNA, leading to either cell death or mutations. Because maternal metabolic ability is generally orders of magnitude greater than fetal ability, for many exposures it is likely a dominant factor. A report of disparate outcome in heteropaternal twins following intrauterine exposure to phenytoin[202] suggested that genetic differences in the fetus also can influence the likelihood of drug- or xenobiotic-induced teratogenicity. Inherited decreased phenytoin detoxification, documented by cytotoxicity, was observed in 14 of 24 children exposed to phenytoin throughout pregnancy.[203] Twelve of the 14 children had major birth defects, compared with 2 of the 10 children with normal cytotoxic responses to phenytoin. Studies in an animal model suggest genetically controlled inducibility of maternal and fetal hepatic aryl hydrocarbon hydrolase is a factor in environmentally induced birth defects.[204] In an animal model, exposure to benzyopyrene in offspring who were highly Ah inducible resulted in an increase in binding of benzyopyrene, more fetal resorptions, more birth defects and poorer growth only if the mother was noninducible. If the mother had high activity, presumably maternal metabolism of benzopyrene protected the fetus. Similar studies are currently underway to evaluate the alcohol dehydrogenase genotype of mothers and offspring to test whether these factors help explain the variation in alcohol related birth defects.

THE FUTURE

Continued advances in molecular biology will lead to more information regarding the mechanisms and consequences of genetically-determined intersubject variation in drug metabolism. Over the next several years, increasing availability of genetic data regarding an individual's inherited ability to metabolize drugs will likely guide therapeutic decisions, including drug choice, drug dose, and optimum therapeutic monitoring. The ability to determine genotype reliably using PCR technology will facilitate large-scale multinational epidemiologic studies of cancer, birth defects, and other environmentally induced diseases that may be associated with genetic polymorphisms. Multiple genetic polymorphisms of xenobiotic metabolism may be synergistic as risk factors. For example, for some diseases, extensive metabolism for phase I reactions, which generally generate toxic metabolites or intermediates, combined with poor metabolic ability for phase II reactions, which generally detoxify compounds, will represent the greatest risk. Ultimately, the results of these large-scale studies of multiple pathways may lead to the prevention of exposure in at-risk individuals, the detection of xenobiotic-induced disease earlier, or improved treatment using either conventional or genetic therapy.

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