Potential for Incorporation of Genetic Polymorphism Data in Human Health Risk Assessment

The toxicity of chemicals entering the body is governed by many factors with the host's ability to metabolize the chemical of prime importance. In some cases, this metabolism activates the chemical leading to a more toxic metabolite while in others it leads to detoxification and excretion. Host metabolism of environmental chemicals can be affected by lifestyle, dietary and exposure factors which can induce or inhibit the metabolizing enzymes. Additionally, the genetic makeup of the host can affect chemical metabolism as there are numerous variants (polymorphisms) that can affect the function of metabolizing enzymes. This has been shown to affect the disposition of therapeutic drugs and environmental toxicants. Polymorphisms can also occur in detoxification/ antioxidant defense genes and repair genes. Such gene variations may affect an individual's ability to metabolize, detoxify and repair damage related to exposure to toxic chemicals and thus affect the degree of inter-individual variability in both the internal dose experienced for a given external dose and the risk that results even for the same internal dose.

This project focused upon how genetic polymorphisms can contribute to the variability in enzyme activity in key metabolizing enzymes and defense mechanisms that occur across the human population. Technically defined, polymorphisms are heritable changes in DNA sequence resulting in two or more alleles of a particular gene within a population: the prevalent (or wild- type) allele and the minority (or variant) allele. The minimal frequency for a variant allele to be considered a polymorphism is 1%, with lower frequencies considered to be rare. The influence of polymorphisms was first revealed in drug disposition studies in which the reaction of patients to drugs such as isoniazid (tuberculosis drug) or debrisoquine (antihypertensive) was highly variable across the patient population. This variability was found to be due to polymorphism in the acetylation pathway for isoniazid (aceylator phenotype) or in cytochrome P-450 (CYP) for debrisoquine (poor metabolizer phenotype). These cases have led to an explosion of examples in which the genetic basis for pharmacokinetic variability has been revealed.

Since many of these polymorphic enzymes also process environmental toxicants, it is important to consider the implications of genetic polymorphisms on toxicology and risk assessment. Such an understanding will improve the characterization of uncertainty and variability, an endeavor of great importance to the replacement of default uncertainty factors (*e.g.*, 3.2 fold variability in pharmacokinetics across the population) with actual distributions that support probabilistic assessments. However, until now the application of pharmacogenetics to environmental toxicology has received relatively little attention.

This project evaluated the potential impact of genetic polymorphisms in 10 metabolizing enzymes on the variability in enzyme function across ethnically diverse populations. Typically there are multiple polymorphisms within a single gene. Our analysis identified those polymorphisms most likely to have an impact on enzyme function and in certain cases used Monte Carlo analysis to simulate the population distribution of this function.

For other enzymes, the project assessed the feasibility of creating such distributions for the most influential and common polymorphisms.

Table 1 provides an overview of the enzymes analyzed. They were chosen on the basis of their being: (1) well characterized in terms of where polymorphisms (genotype) occur and how these variants affect enzyme function (phenotype); (2) involved in the toxic mechanism for numerous xenobiotics; (3) implicated in human susceptibility to environmentally-mediated disease; and (4) exemplary of a range of polymorphisms that can affect metabolism. The selected systems are functionally diverse including Phase I metabolizing enzymes (CYP2E1; CYP2D6; and paraoxonase-1, PON1), Phase II conjugating enzymes (glutathione transferases, GSTs; N-acetyltransferases, NATs; UDP-glucronyltransferase, UGTs; and sulfotransferases, SULTs), and enzymes involved in metabolic detoxification although in some cases their action has been associated with chemical activation (epoxide hydrolase, EH; NADPH quinine oxidoreductase 1, NQO1; aldehyde dehydrogenase 2, ALDH-2).

An extensive review was performed for each enzyme to describe: 1) the enzyme's measurement and function; 2) key substrates; 3) role in chemical activation or detoxification; 4) effect of genotype on phenotype, considering both coding region polymorphisms that affect enzyme structure and upstream polymorphisms that affect gene expression; 5) the frequency of key polymorphisms in the population; and 6) the molecular epidemiology evidence that these polymorphisms can modulate disease outcomes. By combining these various inputs, we analyzed whether a given polymorphism was likely to have sufficient effect on enzyme activity and to be of sufficient frequency to affect the distribution of enzyme activity and thus chemical fate across the population. However, it is important to note that there are many other factors that can affect chemical fate and internal dose and so the variability in enzyme activity described in the current analysis needs to be brought within the context of more comprehensive toxicokinetic modeling.

As summarized in the table and in Ginsberg et al (2009a; 2010) most of the enzyme systems studied had influential polymorphisms that are sufficiently well characterized to develop distributions of enzyme activity. In some cases (*e.g.*, CYP2D6, UGTs, SULTs) there are multiple single nucleotide polymorphisms (SNPs) which are influential and must be accounted for to model inter-individual variability in enzyme activity. In other cases (*e.g.*, GSTs, ALDH2) there is a single inactivating polymorphism creating the null (no activity) phenotype which governs the variability in enzyme activity. Difficulties arise because of the overlap in functional activity between isozymes within the same family of enzymes so that if one family member is highly polymorphic, the polymorphism may be muted as the related enzymes compensate for losses in activity caused by the genetic variant. The net influence on chemical metabolism may be difficult to predict. The GSTs M1, T1 and P1 are a case in point; this project modeled the three enzymes separately as well as in various combinations to simulate chemicals that are substrates specifically for only one, compared with all three, isozymes. This example demonstrated that substrate specificity across isozymes is an important determinant of the influence of the null polymorphism in a single GST. Two enzyme systems, CYP2E1 and

epoxide hydrolase, do not have sufficient evidence of genotype effect on phenotype to perform a polymorphism-based assessment of inter-human variability in activity. In these cases, human liver bank studies assessing enzyme levels and substrate turnover can be used to characterize the likely *in vivo* variability across the population.

Our major finding for enzymes that were subjected to full Monte Carlo analysis (NATs, GSTs, PON1, ALDH2, CYP2D6) is that inter-subject variability in enzyme activity can be large. In these cases, a substantial percentage of the population (≥20%) was estimated to be more than a half log (3.2-fold) different from the median when comparing the 99^{th} percentile to the population median activity. The 3.2-fold differential is used as an evaluation benchmark because of the common assumption in non-cancer risk assessment that toxicokinetics contributes 3.2-fold to the overall 10-fold inter-individual variability uncertainty factor. This assumption has become a convenient default which can be replaced on a case-specific basis by pharmacokinetic modeling that takes into account the types of genetic polymorphisms described in the current project. For example, the percentage of individuals that are more than 10-fold different from the median can also be high in certain cases as shown for GSTM1 and T1 due to the common null polymorphisms in these enzymes. In other cases (ALDH2, NAT2, CYP2D6) genetically distinct subgroups exist which are more than 10-fold different than the median activity, and while they are less than 10% of the population, can still be an important group to consider in developing population estimates of internal dose and risk.

As noted above, it is important to keep in mind that the variation in function for a particular enzyme does not necessarily equate with population variability in internal dose since other pharmacokinetic factors (*e.g.*, overlapping or compensating pathways, blood flow limitations) may modify the influence of the metabolism polymorphism. Therefore, the current results are best used to highlight which polymorphic enzymes would most benefit from refined modeling analysis when evaluating the risks associated with substrates for those pathways. The enzyme variability data developed in this analysis may be used as input distributions for such physiologically-based pharmacokinetic (PBPK) modeling analyses. The population distributions presented for these enzymes are generally consistent with evidence from the drug metabolizing literature for bimodal distributions of enzyme activity in clinical pharmacokinetic studies as seen classically with NAT2 and CYP2D6.

Of the other five enzyme systems analyzed (Table 1), four of the five were determined to be suitable for Monte Carlo analysis and PBPK modeling. Of the multiple SNPs in the SULT superfamily of enzymes, our analysis prioritized SULT1A1*2. Among the UGT enzymes the following SNPs were prioritized: UGT1A1*6, UGT1A1*7, UGT1A1*28, UGT1A7*3, UGT2B15*2 and the UGT2B17 null polymorphism. Regarding NQO1, the null polymorphism (NQO1*2) is highly influential and prevalent and thus is important for follow-up analysis.. These identified SNPs have the potential to affect internal dose and risk and thus merit distributional analysis and variability assessment. Our review of EH failed to find a consistently large enough effect of genotype on phenotype to merit further consideration of variability modeling based upon genotype.

Overall, these analyses provide examples of how one can analyze the likelihood that a polymorphism will make a difference in chemical fate and show how the genetics information for the key polymorphisms may, in turn, be translated into metabolic variability distributions for input into pharmacokinetic models. While there are many polymorphic enzymes worthy of consideration, the list of enzymes analyzed in the current set is relevant to environmental toxicology and should be immediately useful to risk assessors and modelers.

Options for incorporating this information into human health risk assessments include:

1) Establishing priorities for further analysis: analysis of genotype influence on enzyme activity can help to prioritize pathways that may most contribute to pharmacokinetic variability for a given xenobiotic. PBPK modeling is resource intensive and contains its own array of uncertainties. Therefore, it should be used selectively. The current analysis of variability in enzyme activity is an important first step when considering whether to describe variability in internal dose via PBPK modeling. In fact, the current analysis provides the variability distributions needed for PBPK/Monte Carlo analyses.

2) Developing a screening level pathway-specific pharmacokinetic adjustment factor: analysis of variability for an individual enzyme does not necessarily predict variability of the intact pharmacokinetic system. However, it may be possible to develop a pathway-specific adjustment factor without PBPK modeling. This would necessitate having the in vitro genotype/phenotype data (*e.g.*, gene expression systems) supported by in vivo pharmacokinetic data, typically with therapeutic drug substrates, or with other types of biomarker data (*e.g.*, DNA adducts). ALDH2, NAT2, CYP2D6 datasets are such examples and thus may be most amenable to development of a pathway-specific pharmacokinetic adjustment of the enzymes thus far assessed. However, this is a screening approach that does not represent the level of analysis and exploration of variability and uncertainty that is possible in PBPK modeling.

3) PBPK modeling: as described above, polymorphism-based changes in enzyme function may be incorporated into a PBPK model so that the influence of variability in a single pathway may be assessed in a framework that takes into account other pharmacokinetic factors (blood flows, organ size, compensating pathways). Enzyme activity would thus be represented by a population distribution rather than a single point estimate rate constant. The output would be a PBPK/Monte Carlo analysis that depicts the full distribution of internal doses and risks possible across the population. The distributions developed in this project may be incorporated into such modeling efforts.

Ultimately these analyses will lead to refinement of estimates of internal dose and risk that capture the variability caused by genetic polymorphisms that influence xenobiotic metabolism and the function of detoxification and repair systems.

Table 1
Overview of Enzymes Evaluated for Potential Impact of Genetic Polymorphisms on Human Health Risk Assessment

Enzyme	Major Polymorphisms	Distributional Analysis	Reference/Link
Phase I			
CYP2D6	7 different SNPs contribute to poor metabolizer phenotype	Yes	Neafsey et al., 2009a, 196808
CYP2E1	5 different SNPs in regulatory sequences may alter gene expression	Not feasible	Neafsey et al., 2009b, 196814
Phase II			
GSTM1/T1/P1	Null polymorphisms in M1 and T1; 3 coding region SNPs in P1 alter activity in substrate-dependent manner	Yes	Ginsberg et al., 2009b, 196816
NAT1/NAT2	5 different SNPs in NAT2 contribute to slow acetylator phenotype	Yes	Walker et al., 2009, 194764
SULT (6 isozymes)	SULT1A1*2 associated with 2 to 10 fold less activity	Feasible	Ginsberg et al. 2010, 633899
UGT	4 enzymes with influential SNPs	Feasible	Ginsberg et al. 2010, 633899
Detoxification			
ALDH2	*2: null activity	Yes	Ginsberg et al., 2002, 195866
EPHX1	Tyr113His – small effect His139Arg – small effect	Not feasible	Ginsberg et al. 2010, 633899
NQO1	*2: null activity	Feasible	Ginsberg et al. 2010, 633899
PON1	1 coding region SNP alters activity in substrate-dependent manner; 2 regulatory sequence SNPs decrease gene expression	Yes	Ginsberg et al., 2009c, 196823

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