



Heterocyclic Amines, Cytochrome P4501A2, and N-Acetyltransferase: Issues Involved in Incorporating Putative Genetic Susceptibility Markers into Epidemiological Studies

RASHMI SINHA, PHD, AND NEIL CAPORASO, MD

PURPOSE: Heterocyclic amines (HCAs), which are found mainly in well-cooked meat, require metabolic activation to function as mutagens and animal carcinogens. Enzymes such as cytochrome P4501A2 (CYP1A2) and N-acetyltransferase (NAT2) perform this task and are subject to interindividual variation. The source of this variation may be genetic, as in the case of NAT2, or both genetic and environmental as with CYP1A2. The present study examined the effect of HCAs on the NAT2 and CYP1A2 phenotypes in 33 males and 33 females.

METHODS: The subjects consumed a low HCA-containing diet for 1 week followed by a high HCA diet for the subsequent week. The subjects were phenotyped for CYP1A2 and NAT2 at the time of entry into the study (free-living), 1 week later (end of low-HCA or low-induction diet) and 2 weeks later (end of high-HCA or high-induction diet).

RESULTS: Consistent with genetic sources of variability, NAT2 showed little effect of a high-HCA diet and exhibited high intraindividual correlation. CYP1A2, in contrast, was induced by a high-HCA diet and exhibited a more modest intraindividual correlation.

CONCLUSIONS: Incorporating putative genetic susceptibility makers in population studies requires consideration of issues of induction and inhibition of metabolizing enzymes, and effects of covariates. *Ann Epidemiol* 1997;7:350-356. Published by Elsevier Science Inc.

KEY WORDS: Heterocyclic Amines, Cytochrome P4501A2, N-acetyltransferase MeIQx, Genotype, Phenotype, Inducible Enzymes.

INTRODUCTION

Humans are exposed to heterocyclic amines when they consume diets containing meats cooked at high temperature. These compounds, similar to other xenobiotics, are metabolized by various hepatic and extrahepatic enzymes. Although these reactions can facilitate the attachment of polar groups that increase water solubility and allow elimination, they can also activate heterocyclic amines (HCAs) and make them potent mutagens and carcinogens.

The cancer risk to humans posed by HCAs in the diet may depend upon the extent to which these compounds are activated *in vivo* (1). The initial activation step is thought to be N-oxidation by liver cytochrome P4501A2 (CYP1A2) (2). The N-hydroxy arylamine metabolite is O-acetylated in the liver itself or transported to the appropriate target organ where it is O-acetylated by the polymorphic N-acetyltransferase (NAT2) to form an arylamine-DNA adduct.

Both of these enzymes can be measured by evaluating the excretion of caffeine metabolites in urine after caffeine consumption (3). The measured phenotype can distinguish between slow and rapid O-acetylators and N-oxidizers. The role of both of these enzymes in the metabolism of carcinogens has led to population studies that have implicated the slow acetylator phenotype as a risk factor for bladder cancer (4). There is also evidence that rapid metabolizers for both NAT2 and CYP1A2 have increased susceptibility to colon cancers (5).

There is considerable interindividual variability in the activity of both CYP1A2 and NAT2 (3, 6). The reasons for the variability of these two enzymes are different. For NAT2, the variability is primarily due to genetic variants that determine function (7), with environmental factors playing a minor role in the phenotype. In contrast, both genetic and environmental factors are likely to be responsible for the variability in CYP1A2 (3, 8-10). Various environmental factors, such as smoking, or certain dietary components, e.g., cruciferous vegetables and polycyclic aromatic hydrocarbons (PAHs), as well as HCAs in high-temperature cooked meats are known to induce enzyme activity (11, 12). Despite intense study and the identification of a few polymorphic variants of the CYP1A2 gene, a clear genetic source of the variability remains to be demonstrated (13).

From the Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD 20892.

Address reprint requests to: Dr. Rashmi Sinha, Division of Cancer Epidemiology, National Cancer Institute, Executive Plaza North, Rm. 430, 6130 Executive Blvd., Rockville, MD 20892.

Received March 3, 1997; accepted April 4, 1997.

Selected Abbreviations and Acronyms

HCA = heterocyclic amines
PAHs = polycyclic aromatic hydrocarbons
CYP1A2 = cytochrome P4501A2
NAT2 = N-acetyltransferase
MelQx = 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline
 r = correlation coefficient

The inducible or noninducible nature of these enzymes needs to be considered before incorporating measures of interindividual activities into epidemiologic studies. The implications of this concept will be discussed in the context of a metabolic study in which subjects consumed a controlled diet and had repeated measures of CYP1A2 and NAT2 activities.

METHODS

Sixty-six subjects (33 men and 33 women) participated in a 2-week metabolic study that has been previously described (11). During the first week, subjects consumed meat cooked at low temperature (low-induction diet), and during the second week they ate meat cooked at high temperature (high-induction diet). A 7-day menu cycle for the two diet periods was used. All foods were the same except for the way the meats were cooked. The study was designed to minimize exposure to known inducers of CYP1A2. The subjects were all nonsmokers. Cruciferous vegetables, estrogens (women), vitamin and mineral supplement use, and alcohol consumption were not permitted during the controlled dietary period (11).

The subjects were phenotyped for CYP1A2 and NAT2 at the time of entry into the study (free-living), 1 week later (end of low-induction diet), and 2 weeks later (end of high-induction diet). The CYP1A2 and NAT2 phenotypes were measured by the ratio of urinary caffeine metabolites (3). At each time point, subjects refrained from consuming caffeine-containing foods and beverages for 24 hours before the sampling and for at least 5 hours after taking the test dose. Subjects consumed 3.6 g of instant coffee (114 mg caffeine) in 9-oz (266 ml) of water. The subjects voided 4 hours after consuming the coffee, and provided a 1-hour urine sample at the end of the 5th hour. Caffeine and its metabolites were extracted and analyzed from the urine by the method described by Butler and colleagues (3). The NAT2 phenotype was determined from the ratio of the concentrations of 5-acetylamino-6-formylamino-3-methyluracil to 1-methylxanthine and the phenotype for CYP1A2 was estimated from the ratio: 1,7-dimethylxanthine + 1,7-dimethyluric acid to 1,3,7-trimethylxanthine. The coefficient of variation for the quality control samples over the time

period of analysis was 8.7% for NAT2 and 9.5% for CYP1A2.

Blood was collected at the three time points (free-living period, end of low-induction diet period, and end of high-induction diet period). DNA was extracted from the buffy coat and used to genotype for the four most common NAT2 mutations and wild type (to determine the acetylator status) (14, 15). By use of genotype information, the wild-type homozygous and heterozygous are classified as rapid acetylators; the homozygous mutants comprise the slow acetylator group.

MelQx was measured in first 12-hour urine sample after the subjects consumed their first meal containing meat cooked at a high temperature containing 9 ng of MelQx per gram of cooked meat (11). The amount of meat consumed by each subject depended on their body weight (3.1-4.0 g of cooked meat/kg body weight). The subjects collected all urine produced between the evening meal and breakfast the next morning (~ 12 hours). Total unconjugated MelQx in the urine was measured by a method using gas chromatography-mass spectrometry (16, 17). A stable isotope-labeled analogue of MelQx was used as an internal standard in the assay, and the mass spectrometer was operated in the electron-capture negative-ion chemical ionization mode with ammonia as the reagent gas. MelQx and its labeled analogue were chromatographed as their di-3,5-bistrifluoromethylbenzyl derivatives. The coefficient of variation for the quality control samples over the time period of analysis was 5.1% for MelQx.

RESULTS

The distribution of the log NAT2 for each of the three time periods: free-living, low induction diet, and high induction diet exhibited a clear bimodal distribution corresponding to the rapid- and slow-acetylator phenotype (Figure 1). The distribution of the phenotype data within each genotype is shown in Figure 2. The bimodal phenotypic distribution could be clearly differentiated by the genotype. The log NAT2 phenotype distribution did not vary over the different dietary regimens.

Figure 3 presents the distribution of log CYP1A2 for each of the time periods: free-living, low-induction diet, and high-induction diet. The CYP1A2 had a Gaussian distribution during each of the three periods. For the 1 week period corresponding to the low-induction diet, there was some apparent contraction of the variability (Figure 3), as compared with the free-living period. During the high-induction diet, there was an increase in mean/median-log CYP1A2 activity, indicated by the shift of the distribution to the right. This observation is consistent with induction of CYP1A2 by dietary intake.

The within-person correlation for the NAT2 phenotype

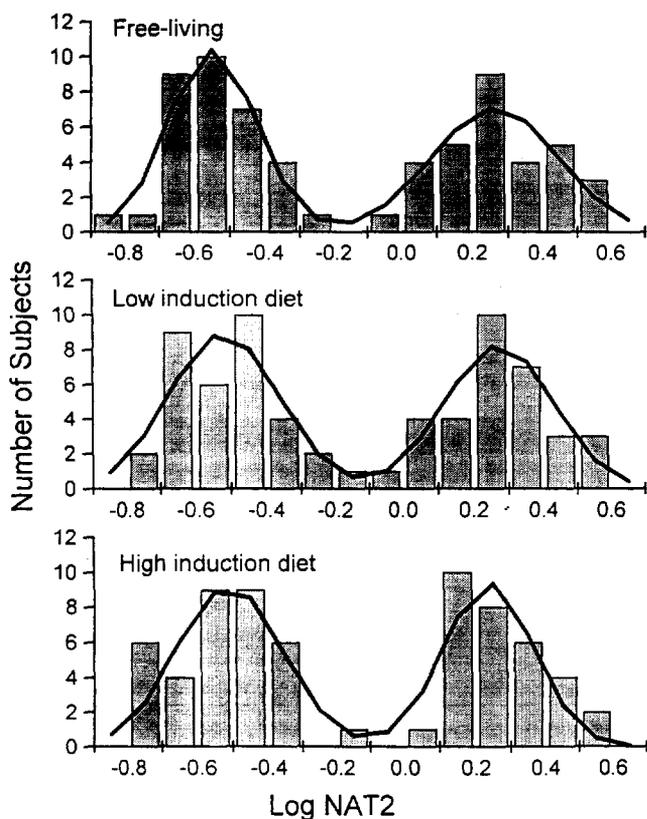


FIGURE 1. NAT2 (log transformed) distribution at three points in a metabolic study: entry, end of low-induction dietary period, and end of high-induction dietary period.

between the three different time periods of the study exceeded 0.96 (Table 1), while the correlations (Pearson) for CYP1A2 were much lower (0.33–0.54). The highest correlation was found between the low-induction diet period and the high-induction diet period ($r = 0.54$, Pearson). For both enzymes, the within-person correlations were highly significant.

At present, urinary unconjugated MelQx is the best available biomarker of dietary intake of MelQx, with an 0.52 correlation (Pearson) between the amount of MelQx consumed and the quantity excreted in the urine. We calculated the percentage of unconjugated MelQx in the urine as: total MelQx in a 12-hour urine specimen/total MelQx consumed $\times 100$. There was no association between percentage unconjugated urinary MelQx and NAT2 activity. In contrast, Figure 4 demonstrates the relationship between the percentage unconjugated MelQx in the 12-hour urine sample and the log CYP1A2 activity. The slope of the regression line shows the negative relationship ($r = -0.27$; $P = 0.03$) between percentage unconjugated MelQx in the urine and CYP1A2 activity. The subjects with higher CYP1A2 activity had less total unconjugated MelQx in the urine than

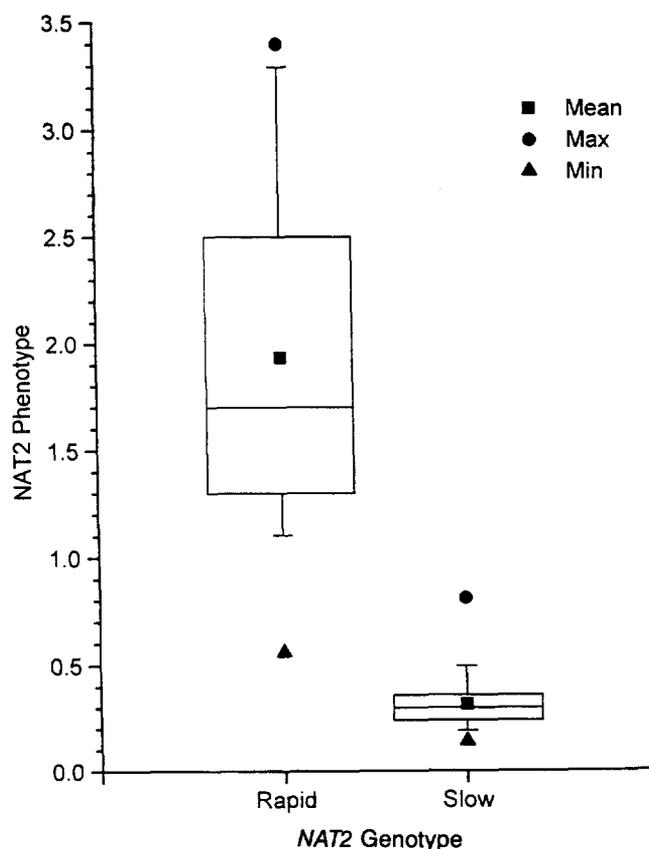


FIGURE 2. NAT2 enzyme activity categorized into slow and fast acetylator status by genotype and phenotype.

did the subjects with lower enzyme activity. Thus, interindividual variation in CYP1A2 activity has an effect on MelQx metabolism.

DISCUSSION

Both NAT2 and CYP1A2 are thought to be involved in the activation of HCAs (1–3, 11). When both these enzymes were measured at three different times in a metabolic study, we found that CYP1A2 was induced by well-done meat while NAT2 was not (11). Furthermore, the inverse relationship observed between CYP1A2 activity and urinary unmetabolized MelQx indicates that interindividual variation in CYP1A2 activity affects MelQx metabolism (18).

Metabolism of HCA by these enzymes provides a good example of the interaction between putative genetic susceptibility factors and environmental exposure to potential carcinogens. The results raise some general implications for epidemiologic studies that incorporate such measures (5).

Our results show that NAT2 is not induced, while CYP1A2 is induced by various compounds. In particular, PAHs, cruciferous vegetables, tobacco smoke, and well-done

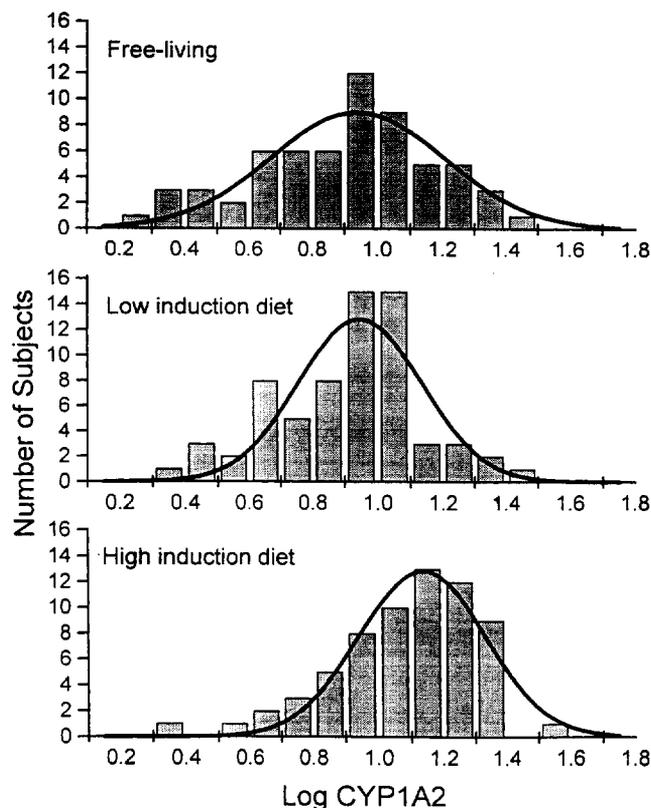


FIGURE 3. CYP1A2 (log transformed) distribution at three points in a metabolic study: entry, end of low-induction dietary period, and end of high-induction dietary period.

cooked meat containing HCAs may induce CYP1A2 while oral contraceptive use results in inhibition (11, 19-21). Given the very high intraperson correlation between phenotypes performed under different dietary regimens, it would appear that for NAT2, and most probably for other noninducible enzymes whose activity is under heritable genetic control, a single measurement in time would be adequate to classify an individual with regard to their phenotype.

With regard to inducible enzymes, the situation becomes more complex. One measure of phenotype may reflect only a recent exposure to inducers or inhibitors. In contrast to metabolic studies where small numbers of healthy subjects can undergo multiple phenotypings under controlled conditions, population studies require large numbers of subjects who must undergo phenotyping under less controlled circumstances. Case-control differences may extend to factors that may influence the phenotype determinations as well. Furthermore, the compounds that are metabolized by the enzymes are often inducers themselves, e.g., alcohol and P4502E1 (22); HCAs in high temperature meat and CYP1A2 (11). In view of the generally deleterious effect of misclassification on epidemiological inference, the representativeness of a single measure under these circumstances

TABLE 1. Within-person correlation at different times of the study for NAT2 and CYP1A2 enzyme activity

Enzyme	Pearson correlation coefficient
NAT2	
Baseline and end of low-induction period	0.96 (n = 64; p = 0.0001)
Baseline and end of high-induction period	0.96 (n = 64; p = 0.0001)
End of low-induction period and end of high-induction period	0.97 (n = 66; p = 0.0001)
CYP1A2	
Baseline and end of low-induction period	0.39 (n = 61; p = 0.002)
Baseline and end of high-induction period	0.33 (n = 60; p = 0.01)
End of low-induction period and end of high-induction period	0.54 (n = 65; p = 0.001)

becomes an issue. However, even for inducible enzymes, there may still be an underlying "fixed" (potentially genetic) component, as seen in the correlation of 0.54 for the CYP1A2 phenotype between the two time periods when the subjects were on the controlled diet. It would be worth investigating the intraindividual variability of inducible enzymes both in a free-living population and in controlled study to get an estimate of how many measures are needed to get the "usual" phenotype.

Depending on the focus of the study, different strategies to deal with variability in the phenotype may be appropriate. In a methodologic study such as this one, which was designed to identify and characterize sources of variability, maximal control of all known and suspected inducers and inhibitors is necessary. For epidemiological studies that investigate the inducible enzyme as a risk factor (exposure) for disease, major inducers or inhibitors generally need to be restricted. Matching or adjustment may be difficult because the time course of the induction may be unknown, the threshold for the exposure to trigger induction may vary, or the dose-response relationship may be poorly understood. Furthermore, information on other risk factors needs to be carefully collected in order to control for residual confounding. When the enzyme is considered as an effect modifier (i.e., a factor that determines how other exposures influence risk), it is necessary to group the continuous values into meaningful categories. To the extent that the enzyme subsumes risk from other exposures (i.e., smoking induces CYP1A2 and is also a risk factor for bladder cancer), it may be difficult to separate these effects and establish such a classification.

The question of when to use genotype as compared to phenotype is quite pertinent when considering epidemiologic designs (23). It is generally more feasible to collect DNA from blood or buccal swabs than to perform an elaborate procedure to phenotype a particular enzyme. For most

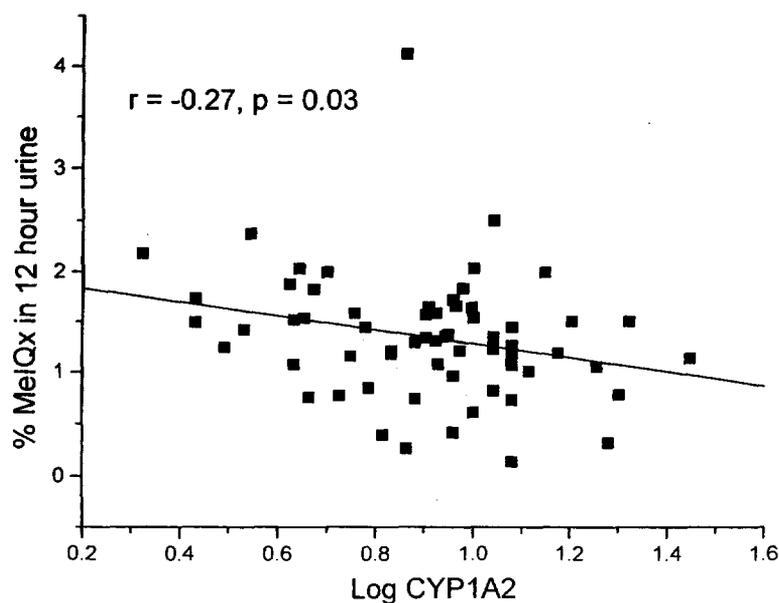


FIGURE 4. Relationship of percentage unconjugated MelQx (total MelQx in 12-h urine/total MelQx consumed \times 100) to CYP1A2 activity (log transformed).

phenotype determinations, subjects are required to take a probe drug and then donate timed blood or urine samples. This procedure is not feasible in many situations due to various restraints: large number of subjects in a cohort study, logistics of sample collection, or metabolic changes in the subject due to disease. In these situations, genotyping would be the best approach. For NAT2, genotyping is preferred because this enzyme is not inducible and the genetic information can categorize subjects quite accurately into slow and fast acetylators. Genotyping would be appropriate for an inducible enzyme if it can be shown that specific mutations account for some component of variation. Genotype assays are subject to limitations as well. The effect of a particular mutation may be complex, or all mutations relevant to

the phenotype may not be known. Furthermore, mutations typically reduce activity or are inactivating, but mutations that enhance activity are also being described (24).

A potential advantage of phenotype assays for inducible enzymes is that they combine all influences of genetic and environmental factors and therefore reflect the physiologic state of the organism. This can also be a disadvantage since the physiologic status of the subject may be distorted by a wide variety of nonspecific factors. These may be associated with the disease state and therefore can distort the assay and bias the findings even though they are not in the causal pathway. For example, phenotype determination using urine samples may be affected by a depleted nutritional state (catabolism), altered absorption, dehydration (with reduced

TABLE 2. Inducers, substrates, and inhibitors for selected P450 enzymes

P450 enzyme	Inducers	Substrates	Inhibitors
CYP1A2	Tobacco smoke, well-done meat, cruciferous vegetables, omeprazole	Aromatic and heterocyclic amides Theophylline Estradiol, caffeine, phenacetin	Furafylline, oral contraceptives
CYP2A6	Barbiturates	Coumarin, nicotine	Diethyldithiocarbamate
CYP2C9	Rifampin	Phenytoin, tolbutamide, piroxicam	Sulfinpyrazone
CYP2D6	None known	Dextromethorphan, hydrocodone, Perphenazine, timolol, Flecainide, many others	Quinidine Fluoxetine Yohimbine
CYP2E1	Ethanol, isoniazid	Ethanol, caffeine, benzene, dapsone, chlorzoxazone, acetaminophen	Disulfiram, phenethyl isothiocyanate
CYP3A4	Dexamethasone, rifampin	Digitoxin, diazepam, erythromycin, flutamide, steroids, tamoxifen, tetrahydrocannabinol, verapamil	Ethinylestradiol, ketoconazole

urine flow), or medication (diuretics with increased urine flow); all of these factors are likely to occur in patients with cancer. These considerations would be relevant to both inducible and noninducible enzymes. Beyond the general consideration of the above factors, even if, as is the case for NAT2, a phenotype is strictly dependent upon a known gene, there must be specific consideration of potential for induction, inhibition, or interference with the assay itself. It is worthwhile to think of all assays as potentially subject to distortion by inducers, inhibitors, and competing substrates, or by interaction with other factors due to physiologic changes. These effects should be routinely anticipated because, as shown in Table 2, inhibitors and inducers exist for virtually all polymorphic P450 enzymes.

Another issue in the discussion of incorporating polymorphic enzymes into epidemiologic studies is the debate involving the phenotypic distribution of an enzyme. For example, NAT2 is demonstratively bimodal, as seen by the data on both genotype and phenotype, and in general either measure can accurately classify subjects as fast or slow acetylators. However, the true phenotype distribution of CYP1A2 is under active debate, in part because the optimal methodology to establish CYP1A2 activity is controversial (25), and various studies have reported CYP1A2 distribution to be unimodal, bimodal, or trimodal (3, 26). In the absence of clear genotypes to guide categorization, statistical approaches (i.e., probits, fitting normal distributions, receiver operator approaches) and arbitrary methods (i.e., quartiles) can be used to classify subjects (27-30). Alternatively, when it is not clear how to classify individuals, analyses could use the data as a continuous variable. At present, no genotypic mutations have been identified that can explain the phenotypic or inducibility differences in CYP1A2 in individuals, but active study of this and related genes (i.e., Ah receptor) may yet reveal a genetic component to this enzyme activity. In conclusion, there is increased understanding that the interaction between genetic susceptibility and environmental exposure can impact the risk of cancer. Thus, it is crucial that optimal study design and feasible methodology be developed for such interdisciplinary studies.

REFERENCES

1. Degawa M, Tanimura S, Agatsuma T, Hashimoto Y. Hepatocarcinogenic heterocyclic aromatic amines that induce cytochrome P-448 isozymes, mainly cytochrome P-448H (P-4501A2), responsible for mutagenic activation of the carcinogenesis in rat liver. *Carcinogenesis*. 1989;10:1119-1122.
2. Turesky RJ, Lang NP, Butler MA, Teitel CH, Kadlubar FF. Metabolic activation of carcinogenic heterocyclic aromatic amines by human liver and colon. *Carcinogenesis*. 1991;12:1839-1845.
3. Butler MA, Lang NP, Young JF, Caporaso NE, Vineis P, Hayes RB, Teitel CH, Massengill JP, Lawson MF, Kadlubar FF. Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. *Pharmacogenetics*. 1992;2:116-127.
4. D'Errico A, Taioli E, Chenx X, Vineis P. Genetic polymorphisms and the risk of cancer: A review of the literature. *Biomarkers*. 1996;1:149-173.
5. Lang NP, Butler MA, Massengill J, Lawson M, Stotts RC, Hauser-Jensen M, and Kadlubar FF. Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increases the risk for colorectal cancer or polyps. *Cancer Epidemiol Biomarkers Prev*. 1994;3:675-682.
6. Daly AK, Cholerton S, Gregory W, Idle JR. Metabolic polymorphisms. *Pharmacol Ther*. 1993;57:129-160.
7. Caporaso N, Landi MT, Vineis P. Relevance of metabolic polymorphisms to human carcinogenesis: Evaluation of epidemiologic evidence. *Pharmacogenetics*. 1991;1:4-19.
8. Guengerich FP. Cytochrome P450 enzymes. *American Scientist*. 1993;81:440-447.
9. Guengerich FP. Human cytochrome P-450 enzymes. *Life Sci*. 1992; 50:1471-1478.
10. Gonzalez FJ. Human cytochromes P450: Problems and prospects. *Trends Pharmacol Sci*. 1992;13:346-351.
11. Sinha R, Rothman N, Brown E, Mark S, Hoover R, Caporaso N, Levander O, Knize M, Lang N, Kadlubar F. Pan-fried meat containing high levels of heterocyclic aromatic amines but low levels of polycyclic aromatic hydrocarbons induces cytochrome P4501A2 activity in humans. *Cancer Res*. 1994;54:6154-6159.
12. Nerurkar PV, Anderson LM, Snyderwine EG, Park SS, Thorgerisson SS, Rice JM. Specific induction of hepatic cytochrome P4501a-2 in C57BL/6 and DBA/2 mice treated with 2-amino-3-methylimidazo[4, 5-f]quinolin (IQ). *J Biochem Toxicol*. 1993;8:175-186.
13. Nakajima MT, Yokoi M, Mizutani SS, Kadlubar FF, Kamataki T. Phenotyping of CYP1A2 in Japanese population by analysis of caffeine urinary metabolites: Absence of mutation prescribing the phenotype in the CYP1A2 gene. *Cancer Epidemiol Biomarkers Prev*. 1994;3: 413-421.
14. Vatsis KP, Weber WW, Bell DA, Dupret J, Price Evans DA, Grant DM, Hein DW, Lin HJ, Meyer UA, Relling MV, Sim E, Suzuki T, Yamazoe Y. Nomenclature for N-acetyltransferases. *Pharmacogenetics*. 1995;5:1-17.
15. Weber WW, Hein DW. N-acetylation pharmacogenetics. *Physiol Rev*. 1985;37:25-79.
16. Lynch AM, Knize MG, Boobis AR, Gooderham NJ, Davies DS, Murray S. Intra- and interindividual variability in systemic exposure in humans to 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, carcinogens present in cooked beef. *Cancer Nurs*. 1992;52:6216-6223.
17. Boobis AR, Lynch AM, Murray S, et al. CYP1A2-catalyzed conversion of dietary heterocyclic amines to their proximate carcinogens is their major route of metabolism in humans. *Cancer Res*. 1994;54:89-94.
18. Sinha R, Rothman N, Brown ED, et al. High concentrations of carcinogen PhIP occurs in chicken but are dependent on the cooking method. *Cancer Res*. 1995;55:4516-4519.
19. Conney AH, Pantuck EJ, Hsiao KC, Garland WA, Anderson KE, Alvares AP, Kappas A. Enhanced phenacetin metabolism in human subjects fed charcoal-broiled beef. *Clin Pharmacol Ther*. 1976;20: 633-642.
20. Conney AH. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes Memorial Lecture. *Cancer Res*. 1982;42:4875-4917.
21. Horn EP, Tucker MA, Lambert G, Silverman D, Zametkin D, Sinha R, Hartge T, Landi MT, Caporaso NE. A study of gender-based cytochrome P4501A2 variability: a possible mechanism for the male excess of bladder cancer. *Cancer Epidemiol Biomarkers Prev*. 1995;4:529-533.
22. Girre C, Lucas D, Hispard E, Menez C, Dally S, Menez JF. Assessment

- of cytochrome P4502E1 induction in alcoholic patients by chorozone pharmacokinetics. *Biochem Pharmacol.* 1994;47:1503-1508.
23. Le Marchand L, Sivaraman L, Franke AA, et al. Predictors of N-acetyltransferase activity: Should caffeine phenotyping and NAT2 genotyping be used interchangeably in epidemiological studies? *Cancer Epidemiol Biomarkers Prev.* 1996;5:449-455.
 24. Johansson I, Lundqvist E, Bertilsson L, Dahl ML, Sjoqvist F, Ingelman-Sundberg M. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine [see comments]. *Proc Natl Acad Sci USA.* 1993;15;90:11825-11829.
 25. Kadlubar FF, Young JF, Lang NP, Caporaso NE, Sinha R, Landi MT. Correspondence re: Letter to the editor by B.K. Tang and W. Kalow on CYP1A2 phenotyping using caffeine. *Cancer Epidemiology Biomarkers & Prevention.* 1996;5:231-232.
 26. Fuhr U, Rost KL. Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva. *Pharmacogenetics.* 1994;4:109-116.
 27. Jackson PR, Tucker GT, Woods HF. Testing for bimodality in frequency distributions of data suggesting polymorphisms of drug metabolism—hypothesis testing. *Br J Clin Pharmacol.* 1989;28:655-662.
 28. Evans DAP, Mahgoub A, Sloan TP, Idle JR, Smith RL. A family and population study of genetics polymorphism of debrisoquin hydroxylase in a white British population. *J Med Genet.* 1980;17:102-105.
 29. Jackson PR, Tucker GT, Woods HF. Testing for bimodality in frequency distributions of data suggesting polymorphisms of drug metabolism—histograms and probit plots. *Br J Clin Pharmacol.* 1989;28:647-663.
 30. Deleo JM. Receiver operating characteristics laboratory (ROCLAB): Software for developing decision strategies that account for uncertainty. Los Alamitos, CA: IEEE Computer Society, 1993.