Genotyping for Polymorphisms in Xenobiotic Metabolism as a Predictor of Disease Susceptibility

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Polymorphisms in many xenobiotic metabolizing enzymes occur leading to variation in the level of enzyme expression in vivo. Enzymes showing such polymorphisms include the cytochrome P450 enzymes CYP1A1, CYP1A2, CYP2A6, CYP2D6, and CYP2E1 and the phase two metabolism enzymes glutathione S-transferase M1 (GSTM1) and arylamine N-acetyltransferase 2 (NAT2). In the past, these polymorphisms have been studied by phenotyping using in vivo administration of probe drugs. However, the mutations which give rise to several of these polymorphisms have now been identified and genotyping assays for polymorphisms in CYP1A1, CYP2A6, CYP2D6, CYP2E1, GSTM1, and NAT2 have been developed. Specific phenotypes for several of the polymorphic enzymes have been associated with increased susceptibility to malignancy, particularly lung and bladder cancer, and Parkinson’s disease. These associations are likely to be due to altered activation or detoxification of chemicals initiating these diseases, including components of tobacco smoke and neurotoxins. The substrate specificity and tissue distribution of polymorphic enzymes implicated in disease causation discussed with particular reference to previously described disease-phenotype associations.—Environ Health Perspect Vol 102(Suppl 9):55–61 (1994)

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Introduction

There is considerable variation in expression of xenobiotic-metabolizing enzymes in the general population. This variability is due, at least in part, to the presence of genetic polymorphisms where mutations in the wild-type gene result in the synthesis of enzymes with impaired activity. Individuals expressing one or more of these mutated enzymes may differ from normal individuals in their susceptibility to certain diseases where exposure to xenobiotics is a possible cause. There is now clear evidence at the metabolic level for polymorphisms in the cytochrome P450 enzymes CYP1A1, CYP1A2, CYP2A6 and CYP2D6 and in N-acetyltransferase 2 (NAT2) and glutathione S-transferase M1 (GSTM1) which carry out phase 2 conjugation reactions. In the case of CYP2D6, NAT2, and GSTM1, the genetic basis of the polymorphism is also well understood. There is also evidence at the genetic level for a polymorphism in the enzyme CYP2E1. As summarized in Table 1, simple genotyping assays have been developed for a number of these polymorphisms. Associations between specific phenotypes for certain polymorphic enzymes and susceptibility to cancer, particularly lung and bladder cancer, Parkinson’s disease, and the autoimmune disease systemic lupus erythematosus have been described. These associations may be due to differences in the ability of the various enzyme phenotypes to activate or detoxicate chemical toxins or, alternatively, to linkage disequilibrium where a particular allele coding for another gene with a direct role in determining disease susceptibility shows genetic linkage with an allelic variant of the xenobiotic-metabolizing enzyme.

This article considers associations between particular metabolic phenotypes and disease susceptibility, with particular emphasis on methods for determining phenotypes. The relevance of the enzyme polymorphism-disease associations from what is known regarding substrates and tissue-specific expression is also considered. However, it should be noted that metabolism at the site of the tumor may not be essential because many carcinogens may migrate to other tissues following metabolism in the liver (1).

CYP1A1

CYP1A1 metabolizes a range of polycyclic aromatic hydrocarbons including benzo[a]pyrene and is inducible by various aromatic hydrocarbons which act by binding to the Ah receptor, a transcriptional activator of CYP1A1. There appears to be little significant constitutive CYP1A1 expression in human liver but the enzyme is detectable in lung tissue from smokers (2). An apparent polymorphism with respect to inducibility of CYP1A1 has been detected by use of an in vitro assay of lymphocytes which can classify individuals as high or low inducers with 3-methylcholanthrene (3). In two studies, the high inducibility phenotype was found to be more common among lung cancer patients than in a group of smoking controls (3,4). However, other workers (5–7) have failed to reproduce these findings.

Association of a CYP1A1 genotype detectable by restriction fragment length polymorphism (RFLP) analysis using the enzyme MspI with increased susceptibility to lung cancer, particularly squamous cell carcinoma, among smokers has been reported for a Japanese population (8). However, no such association was detected in a similar study of a Norwegian population (9). The MspI polymorphism has recently been demonstrated to be linked to an amino acid substitution in the heme-
Table 1. Detection of polymorphisms linked to impaired xenobiotic metabolism and/or altered disease susceptibility.

| Enzyme          | Polymorphism or allelic variant | Assay                        | Reference         |
|-----------------|---------------------------------|------------------------------|--------------------|
| CYP1A1          | In 3′-flanking region A to G (Val42 to Ile) | RFLP analysis with MspI | Kawajiri et al. (8) |
| CYP2A6          | CYP2A6V(T1496 to A, G96 to A) | Allele-specific PCR | Yamano et al. (18) |
| CYP2D6          | CYP2D6A (deletion of A_887 in exon 5) | PCR (allele specific or with HpaII digest) | Daly et al. (31); Broly et al. (32); Smith et al. (41) |
| CYP2D6          | CYP2D6B (G1923 to A) | PCR (with BsrNI digest or allele-specific) | Daly et al. (31); Broly et al. (32); Smith et al. (41) |
| CYP2D6          | CYP2D6D (deletion of CYP2D6) | RFLP analysis with XbaI | Daly et al. (31); Broly et al. (32); Skoda et al. (76) |
| CYP2E1          | Polymorphism in intron 2 Several point mutations in 5′-flanking region | RFLP analysis with DraI | Uematsu (49) |
| GSTM1 Null allele (deletion of GSTM1) | PCR with Rsal or PstI digest | Hayashi et al. (50) |
| NAT2            | M1 (T_341 to C, C_481 to T, A_903 to G) | PCR (with KpnI digest or allele-specific) | Hickman and Sim (66) |
| M2 (C_34 to T, G_396 to A) | PCR (with TaqI digest or allele-specific) | Hickman and Sim (66) |
| M3 (G_973 to A) | PCR (with BamHI digest or allele-specific) | Hickman and Sim (66) |

binding region of the enzyme (10). The base change which gives rise to this substitution can be readily detected by an allele-specific polymerase chain reaction (PCR) assay. The MspI genotype apparently associated with increased lung cancer susceptibility also appears to cosegregate with the high inducibility-phenotype for CYP1A1 (11). In view of the expression of CYP1A1 in lung tissue and the nature of its substates, further studies on the role of polymorphisms in this enzyme in relation to lung cancer susceptibility appear appropriate.

CYP1A2

CYP1A2 is known to activate arylamine procarcinogens (12). There is evidence that it activates tobacco smoke condensate to a genotoxic product (13) and, in lymphoblastoid cells stably expressing this gene, a considerably increased mutation rate and decreased cell survival compared with the wild-type has been observed on exposure to the tobacco smoke-specific nitrosamine 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (14). Although expressed constitutively in liver, CYP1A2 has not been detected in lung (15) and it is unclear whether CYP1A2 expression is a risk factor for lung cancer development. However, tobacco-derived arylamines may undergo activation by CYP1A2 in the liver and play a role in the initiation of bladder carcinogenesis (16). Using caffeine as a probe drug, a polymorphism for CYP1A2 has been detected in three separate populations. Within each of these populations caffeine metabolism in nonsmokers showed a trimodal distribution with 12 to 13% of subjects classified as slow metabolizers of caffeine (17). Among smokers considerable variability in levels of metabolism was also seen but the distinction between phenotypes was blurred, presumably due to induction of CYP1A2 (17). The genetic basis of this apparent polymorphism is not yet understood.

CYP2A6

CYP2A6 is a constitutive cytochrome P450 which is expressed in human liver at variable levels (18). This enzyme may also be expressed in other tissues, including nasal tissue (19). CYP2A6 carries out 7-hydroxylation of coumarin but also appears to activate certain procarcinogens, including hexamethylphosphoramide (19), N-nitrosodimethylamine (20), NNK (14) and aflatoxin B1 (21). Using coumarin as a probe drug, the formation of 7-hydroxy-coumarin has been investigated in a group of volunteers. Considerable variation in the percentage of drug excreted as 7-hydroxy metabolites has been observed with levels of conversion varying from 10 to 120% of the dose (22). A variant cDNA (CYP2A6v) which lacks coumarin 7-hydroxylase activity and has only three nucleotide differences compared with the wild-type CYP2A6, one of which gives rise to the amino acid substitution Leu_160 to His, has been isolated (18). DNA samples from the volunteer group previously investigated by coumarin phenotyping have been analyzed for the presence of the mutation which gives rise to the amino acid substitution using an allele-specific PCR assay (Daly AK, Vaz A, Cholerton S, and Idle JR, unpublished data). The variant allele was detected at a frequency of 0.02 and was not present in four subjects who showed recovery of coumarin metabolites of less than 40%. However, three subjects were heterozygous for the variant allele and showed recoveries of metabolites of 59.7%, 61.8% and 79.5% (mean 67%). These values were significantly different from the mean recoveries of the remaining population (90.1%, n = 104) on the basis of unpaired t-testing (p < 0.02). This suggests that the CYP2A6/CYP2A6v genotype leads to some impairment of coumarin metabolism. It is likely that other mutations in CYP2A6 associated with poor metabolism of coumarin remain undetected.

CYP2D6

CYP2D6 metabolizes a variety of drugs used therapeutically, including antiarrhythmics, antidepressants, and neuroleptics (23). There is also evidence that CYP2D6 can metabolize the neurotoxins 1,2,3,4-tetrahydroisoquinoline (TIQ) (24) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (25). Studies of lymphoblastoid cells stably transfected with CYP2D6 indicate that the tobacco-specific nitrosamine NNK is a CYP2D6 substrate (14), although in inhibition studies NNK showed little effect on metoprolol metabolism (26). CYP2D6 is expressed constitutively in human liver (27) and no inducers of this enzyme have been identified. Studies on the expression of CYP2D forms
which lung; probe CYP2D6 cDNA to hybridize to a CYP2D6 cDNA probe in both tissues (Figure 1). However, the major band approximately 8S in liver is at least 10-fold more intense than the corresponding band in lung. Two other higher molecular weight transcripts are also detected in liver but the larger and stronger of these is not detected in lung. It is not yet known whether CYP2D6 transcripts are detectable in human lung. No CYP2D6 transcripts were detected in a human lymphoblastoid cell line (Figure 1).

CYP2D6 is polymorphic and 3.2 to 11.5% of individuals in various European populations lack this enzyme activity (30). These individuals, termed poor metabolizers (PMs) have been identified in the past by administration of the drug debrisoquine, sparteine, or dextromethorphan followed by analysis of urinary metabolites. A number of inactivating mutations in CYP2D6 have now been identified and are summarized in Table 1. The CYP2D6A and CYP2D6B alleles can be detected by PCR-based assays using either restriction digests or allele-specific primers. The CYP2D6D allele represents complete deletion of the coding gene and, at present, can only be detected by RFLP analysis with the enzyme XbaI. In addition to the CYP2D6D allele which is represented by a 13-kb band on RFLP analysis with XbaI, a separate band of 11 kb, which is also associated with the PM phenotype (31) and probably represents another type of large deletion, can be detected in some individuals.

By combination of PCR and RFLP assays, 95% of European phenotypic PMs can be identified as genotypic PMs (31–33). The remaining 5% of PMs are likely to have rare mutations in one or both alleles. In a sequencing study on DNA from a subject classified phenotypically as a PM but with an apparent heterozygous genotype, we have detected a single base substitution (G206C to A) in exon 4 of CYP2D6 which results in substitution of Glu for Gly. Using allele-specific PCR, approximately 100 subjects have been screened for the presence of this mutation; but in addition to the original subject studied, the mutation has only been detected in one other individual (also a PM), giving an allele frequency of 0.01.

In the past, phenotyping studies using debrisoquine or another CYP2D6-specific probe drug have suggested associations between particular phenotypes and disease susceptibility. As discussed above, there is evidence that CYP2D6 may activate the procarcinogen NNK while detoxicating certain neurotoxins. These findings as well as increasing evidence for extrahepatic expression of CYP2D6 may account for these phenotype-disease associations. Two independent studies of lung cancer patients have demonstrated a reduced frequency of PMs and a generally increased level of metabolism of debrisoquine compared with several types of control group (34,35). Similarly, among cases of aggressive bladder cancer, a reduced frequency of the PM phenotype was observed (36). A study of Parkinson’s disease patients observed an increased frequency of PMs compared with controls (37), although some of these findings were later ascribed to interference by other drugs in the debrisoquine phenotyping procedure (38). An increased frequency of the autoimmune disease systemic lupus erythematosus among poor metabolizers has also been reported (39). With the advent of genotyping techniques, studies on disease susceptibility can be carried out with increased accuracy, since there is no possibility of interference by other drugs in the procedure and the majority of subjects can be accurately categorized as homozygous or heterozygous extensive metabolizers or PMs.

Two studies on CYP2D6 genotypes among Parkinson’s disease patients have recently emerged (40,41). In the first, CYP2D6 genotypes were analyzed for 53 Parkinson’s disease patients and 72 controls. A small but not statistically significant increase was observed in the percentage of heterozygous extensive metabolizers but when allele frequencies between the two groups were compared, the CYP2D6B allele frequency was increased from 10.4% in the control group to 21.65% among cases, representing a relative risk of 2.7 (40). These findings could be explained by linkage disequilibrium with an association between CYP2D6B and an allele from another gene with a role in determining susceptibility to this disease. In a study of a larger population (41), an increase in frequency of the PM genotype from 5.0 to 11.8% was observed with a relative risk of 2.54. There is a need for further studies of this type, particularly to investigate the possibility that it may be early-onset Parkinson’s disease in particular that is associated with the PM phenotype due to the role of CYP2D6 in the metabolism of neurotoxins.

A study on the relationship between CYP2D6 genotype and susceptibility to a number of different types of cancer has recently been reported (42). In this study, CYP2D6B allele distributions for 1635 cancer patients and an unmatched control group of 720 random volunteers were examined. No significant difference in the frequency of the CYP2D6B allele between lung cancer patients and controls was observed. Some statistically significant differences in genotype frequencies between patients and controls were obtained for other tumors, including an increase in the percentage of PMs among leukemia patients. Although interesting, this study is very preliminary and there is a need to carry out well-designed case-control studies using genotyping methods which detect a higher percentage of PMs and heterozygotes and are validated by preliminary metabolic phenotyping of the study population. Definitive answers on the role of polymorphism in CYP2D6 in determining disease susceptibility should soon be available.

CYP2E1

CYP2E1 metabolizes nitrosamines, including the procarcinogens N-nitrosodimethylamine and N-nitrosopyrrolidine (43), the tobacco smoke component 3-hydroxypyridine (44) and many common organic solvents including benzene (43). This enzyme is inducible by ethanol, and thus alcohol consumption may influence carcinogenesis involving CYP2E1. The majority of expression in humans is in liver where interindividual variability in levels of expression may
occur (45). Expression has also been detected in rat lung (46), rabbit nasal tissue and kidney (47), and human leucocyte (48).

A polymorphism in intron 2 of the human CYP2E1 gene is detectable by RFLP analysis with Dral. Genotyping analysis of a lung cancer patient group showed a significantly reduced incidence of a rare genotype among cases compared with a control group (49). Two linked polymorphisms in the 5'-flanking region of CYP2E1 affecting levels of transcription have also been identified (50). PCR assays for analysis of these polymorphisms have been described but have not yet been applied to studies on disease susceptibility.

**Glutathione S-Transferase μ**

Glutathione S-transferases are a multigene family of enzymes which conjugate xenobiotics with glutathione (51). In the case of the near-neutral family, a polymorphism has been detected at the human GSTI locus which codes for glutathione S-transferase (GSTMI). Three different allelic variants have been detected, with two of these, GSTMI*A and GSTMI*B, coding for enzymes of similar catalytic activity (52) whereas GSTMI 0 (the null allele) produces no catalytically active enzyme and may represent complete deletion of the coding gene (53). In most racial groups, 40 to 50% of subjects are homozygous for the null allele and lack GSTMI (54). GSTMI is expressed in a variety of human tissues including liver, leucocyte, kidney, and stomach (55). Expression has also been detected in lung but this enzyme appeared to have a faster mobility than the liver form in Western immunoblot experiments and did not appear polymorphic (56).

Substrates for GSTMI include trans-stilbene oxide and benzo[α]pyrene-4,5-oxide (51). GSTMI phenotyping can be carried out in leucocytes by assay for enzyme activity towards trans-stilbene oxide (57) or by immunoblot (58). More recently, genotyping assays have been developed using either RFLP analysis with EcoRI or BamHI (53) or PCR assays (59) where absence of GSTMI amplification indicates that the subject is homozygous for the null allele.

Phenotyping studies on lung cancer patients have indicated that lack of GSTMI activity is associated with an increased risk of lung cancer—particularly adenocarcinoma—development among smokers (57). More recently using both genotyping by RFLP analysis and phenotyping by radioimmunoassay, no significant difference in GSTMI expression was detected between a lung cancer group and a control group but a small but statistically significant increase in lack of GSTMI expression was seen among a subgroup of patients with squamous carcinoma (60). In a recent case-control study on smoking-related cancers (mainly lung cancers) using phenotyping by enzyme activity measurements, there was no overall difference in the percentage of subjects with low levels of enzyme activity (GSTMI negative) between cancer patients and controls but a small increase in the percentage of GSTMI-negative subjects was detected in a subgroup of heavy smokers with smoking-related cancers (61). At present, therefore the relationship between GSTMI expression and lung cancer susceptibility remains unclear.

** Arylamine N-Acetyltransferase 2**

Two forms of N-acetyltransferase (NAT1 and NAT2) carry out N-acetylation of arylamines and certain other reactions such as O-acetylation (62). Expression of NAT2 is polymorphic and about 50% of Caucasians lack this enzyme activity and are termed slow acetylators (63). In addition to metabolizing a group of therapeutically important drugs, NAT2 also acetylates arylamines including benzidine, 2-aminofluorene and β-naphthylamine (16). NAT has been detected in human liver (62) and colon (64). In rabbits, NAT is expressed in a variety of tissues including duodenum, lung, kidney, and bladder mucosa (63).

Slow acetylators are detectable by phenotyping with a number of probe drugs including caffeine and sulphamethazine (63). Recently, the NAT2 gene has been cloned and sequenced and a number of point mutations associated with the slow acetylator phenotype identified. Three variant alleles (Table 1) which lack NAT activity *in vitro* have been identified in several independent studies (65-68). Each variant contains one or more base substitutions with at least one of these introducing an amino acid change. The presence of the variant alleles can be readily detected by PCR assays using either digestion of product with a restriction enzyme or allele-specific methods (66,67), allowing positive identification of greater than 90% of all slow acetylators.

The association between acetylation phenotype and disease susceptibility has received considerable attention. Slow acetylators appear to be at increased risk for development of systemic lupus erythematosus if given certain NAT2 substrates therapeutically (63). In addition, susceptibility to certain types of cancer involving chemical carcinogenesis may be related to acetylator phenotype. The majority of bladder cancer is likely to be related to either occupational exposure or cigarette smoking (69). In a case-control study of occupationally induced bladder cancer, a large excess of slow acetylators was detected in the occupationally exposed group and an odds ratio of 16.7 calculated for risk of disease development in slow acetylators (70).

However, this study found no relationship between acetylator phenotype and development of smoking-related bladder cancer in the absence of confirmed occupational exposure to arylamines and subsequent independent studies have generally confirmed these findings (71-73).

Trace amounts of arylamines may be formed during normal cooking of food. Since these compounds are likely NAT substrates, the possibility that acetylator phenotype might relate to susceptibility to colon cancer was investigated (74,75). An increased frequency of the rapid acetylator phenotype was observed among a group of colorectal cancer patients. The difference in relationship between acetylator phenotype and susceptibility to bladder and colon cancer may be due to NAT primarily acting as a detoxicating enzyme in bladder while in the colon, N-oxidized arylamines may undergo activation by N- and O-acetylation reactions catalyzed by NAT (16). Although the majority of slow acetylators can now be identified by genotyping, studies on genotype-disease susceptibility relationships have not yet been reported.

**Conclusion**

A number of independent studies have now demonstrated the importance of polymorphisms in xenobiotic metabolism as risk factors in the development of diseases associated with chemical exposure. In parallel with these studies, a clearer understanding of the genetic basis of the polymorphisms has emerged, together with more accurate and less invasive methods for screening of populations. Many of the previous studies can now be more accurately replicated and extended and, in addition, the influence of multiple polymorphisms on disease susceptibility examined.
GENOTYPING FOR POLYMORPHISMS IN XENOBOTIC METABOLISM

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