***Review***

# Molecular Genetics and Epidemiology of the *NAT1* and *NAT2* Acetylation Polymorphisms[1]

**David W. Hein,[2] Mark A. Doll, Adrian J. Fretland, Matthew A. Leff, Stephanie J. Webb, Gong H. Xiao, Udaya-Sankar Devanaboyina, Norma A. Nangju, and Yi Feng**

Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky 40292

## Abstract

**The focus of this review is the molecular genetics, including consensus *NAT1* and *NAT2* nomenclature, and cancer epidemiology of the *NAT1* and *NAT2* acetylation polymorphisms. Two *N*-acetyltransferase isozymes, NAT1 and NAT2, are polymorphic and catalyze both *N*-acetylation (usually deactivation) and *O*-acetylation (usually activation) of aromatic and heterocyclic amine carcinogens. Epidemiological studies suggest that the *NAT1* and *NAT2* acetylation polymorphisms modify risk of developing urinary bladder, colorectal, breast, head and neck, lung, and possibly prostate cancers. Associations between slow *NAT2* acetylator genotypes and urinary bladder cancer and between rapid *NAT2* acetylator genotypes and colorectal cancer are the most consistently reported. The individual risks associated with *NAT1* and/or *NAT2* acetylator genotypes are small, but they increase when considered in conjunction with other susceptibility genes and/or aromatic and heterocyclic amine carcinogen exposures. Because of the relatively high frequency of some *NAT1* and *NAT2* genotypes in the population, the attributable cancer risk may be high. The effect of *NAT1* and *NAT2* genotype on cancer risk varies with organ site, probably reflecting tissue-specific expression of NAT1 and NAT2. Ethnic differences exist in *NAT1* and *NAT2* genotype frequencies that may be a factor in cancer incidence. Large-scale molecular epidemiological studies that investigate the role of *NAT1* and *NAT2* genotypes and/or phenotypes together with other genetic susceptibility gene polymorphisms and biomarkers of carcinogen exposure are necessary to expand our current understanding of the role of *NAT1* and *NAT2* acetylation polymorphisms in cancer risk.**

## Introduction

Comprehensive reviews of the acetylation polymorphism (NAT2) have been published previously (1–4). Several shorter reviews including information on the *NAT1* and *NAT2* acetylation polymorphisms have been published within the past few years (*e.g.,* 5–14). This review focuses on the molecular genetics and cancer epidemiology of the *NAT1* and *NAT2* acetylation polymorphisms.

The acetylation polymorphism (NAT2) was discovered over 40 years ago following differences observed in tuberculosis patients to isoniazid toxicity (15). Subsequently, the differences in isoniazid toxicity were attributed to genetic variability in *N*-acetyltransferase (EC 2.3.1.5), a cytosolic phase II conjugation enzyme primarily responsible for deactivation of isoniazid (16, 17). Indeed, the polymorphism was termed the "isoniazid acetylation polymorphism" for many years until the importance of the polymorphism in the metabolism and disposition of other drugs and chemical carcinogens was fully appreciated (1, 2).

## *N*-Acetyltransferase Isozymes

An early paradox was the observation that the *N*-acetylation of isoniazid and other drugs such as sulfamethazine divided human populations into rapid, intermediate, and slow acetylator phenotypes, whereas the *N*-acetylation of other drugs such as *p*-aminosalicylic acid yielded an apparently normal (monomorphic) distribution (18). Based on this observation, drugs such as isoniazid and sulfamethazine were termed "polymorphic" substrates for *N*-acetylation, whereas drugs such as *p*-aminosalicylic acid were termed "monomorphic." The biochemical basis for this observation relates to substrate specificity and molecular genetics of two distinct *N*-acetyltransferase isozymes, NAT1 and NAT2. The concept was suggested by Jenne (18), confirmed initially in the Syrian hamster (19–21), and subsequently confirmed in man (22). The classical isoniazid slow acetylator phenotype(s) is due, at least in part, to reduction(s) in NAT2 protein (23, 24) with a frequency that is approximately 50–60% in Caucasian populations but shows striking ethnic differences (reviewed in Ref. 2). For example, slow acetylator phenotype is much more frequent in Egyptians but is much less frequent in Asians (2). The NAT2 acetylation polymorphism is very important in clinical pharmacology and toxicology because of its primary role in the activation and/or deactivation of a large and diverse number of aromatic amine and hydrazine drugs used in clinical medicine (for reviews, see Refs. 1, 2, and 4). The NAT1 isozyme was initially described as monomorphic because of its selectivity for *p*-aminosalicylic acid and other substrates yielding "monomorphic" distributions in human populations (1, 2). However, subsequent studies showed that *NAT1* is not monomorphic in human populations but rather is subject to a separate polymorphism (21, 22). Endogenous substrates for human NAT1 and NAT2 are not known, although the folic acid catabolite, *p*-aminobenzoylglutamate is *N*-acetylated by NAT1 (27, 28), suggesting a role for NAT1 in folate metabolism.

*N*-Acetyltransferases metabolize a number of aromatic and heterocyclic amine carcinogens that produce tumors in rodents (29). Site-directed mutagenesis studies have established a critical cysteine (amino acid 68) within the catalytic site that participates directly in acetyl transfer between the acetyl-CoA

Received 8/5/99; revised 10/18/99; accepted 10/20/99.
[1] Supported in part by USPHS Grant CA-34627 from the National Cancer Institute.
[2] To whom requests for reprints should be addressed. Phone: (502) 852-5141; Fax: (502) 852-7868; E-mail: d.hein@louisville.edu.

*Table 1* Human *NAT1* alleles[a]

| Allele | Nucleotide change(s) | Amino acid change(s) | References[b] |
|---|---|---|---|
| *NAT1*3* | C1095A | None | 40 |
| *NAT1*4* | None | None | 25 |
| *NAT1*5* | G350,351C, G497–499C, A884G, Δ976, Δ1105 | $Arg^{117}$→Thr, $Arg^{166}$→Thr, $Glu^{167}$→Gln | 49 |
| *NAT1*10* | T1088A, C1095A | None | 25 |
| *NAT1*11* | C−344T, A−40T, G445A, G459A, T640G, Δ9 between 1065–1090, C1095A | $Val^{149}$→Ile, $Ser^{214}$→Ala | 50 |
| ***NAT1*14A*** | G560A, T1088A, C1095A | $Arg^{187}$→Gln | 51, 52 |
| ***NAT1*14B*** | G560A | $Arg^{187}$→Gln | 52, 53 |
| ***NAT1*15*** | C559T | $Arg^{187}$→Stop | 51, 53 |
| *NAT1*16* | [AAA] immediately after 1091, C1095A | None | 54 |
| ***NAT1*17*** | C190T | $Arg^{64}$→Trp | 55, 56 |
| *NAT1*18A* | Δ3 between 1064–1087, T1088A, C1095A | None | 57 |
| *NAT1*18B* | Δ3 between 1064–1091 | None | 58 |
| ***NAT1*19*** | C97T | $Arg^{33}$→Stop | 56 |
| *NAT1*20* | T402C | None | 56 |
| *NAT1*21* | A613G | $Met^{205}$→Val | 56 |
| ***NAT1*22*** | A752T | $Asp^{251}$→Val | 56 |
| *NAT1*23* | T777C | None | 56 |
| *NAT1*24* | G781A | $Glu^{261}$→Lys | 56 |
| *NAT1*25* | A787G | $Ile^{263}$→Val | 56 |
| *NAT1*26A* | [TAA] insertion between 1066 and 1091, C1095A | None | 59 |
| *NAT1*26B* | [TAA] insertion between 1066 and 1091 | None | 60 |
| *NAT1*27*[c] | T21G, T777C | None | |
| *NAT1*28* | [TAATAA] deletion between 1085 and 1090 | None | 61 |
| *NAT1*29* | T1088A, C1095A, Δ1025 | None | 62 |

[a] Updated from Ref. 26. *NAT1* nomenclature is accessible on the internet at a website.[3] Low activity alleles are shown in bold. Allelic frequencies vary with ethnicity (6, 11).
[b] Initial reports as opposed to an exhaustive list.
[c] V. A. Smelt, A. Upton, J. Adjaye, M. A. Payton, N. Johnson, H. J. Mardon, and E. Sim, Expression of arylamine *N*-acetyltransferases in preterm placentas and in human preimplantation embryos, manuscript submitted.

cofactor and acceptor substrates (30). Aromatic amines and hydrazines (*N*-acetylation) and *N*-hydroxy-aromatic and -heterocyclic amines (*O*-acetylation) are both examples of acceptor substrates that, in general, are deactivated (*N*-acetylation) or activated (*O*-acetylation) by NAT1 and/or NAT2 (reviewed in Ref. 3). NAT1 and NAT2 also catalyze the intramolecular *N,O*-acetyltransfer of *N*-hydroxy-*N*-acetyl-aromatic amines (3, 31). It is often suggested that human NAT2 activity is highest in the liver and gastrointestinal tract, whereas human NAT1 activity is expressed in many extrahepatic tissues, but the relative contribution of hepatic *versus* extrahepatic activation and/or deactivation of these carcinogens by NAT1 and NAT2 is not fully understood. In Syrian hamsters (32, 33) and humans (34), *N*- and *O*-acetyltransferase expression in many extrahepatic tissues is comparable to that in the liver and underscores the potential importance of *N*-acetyltransferases in extraheptic activation/deactivation pathways. Because both NAT1 and NAT2 catalyze the metabolic activation (via *O*-acetylation) of aromatic and heterocyclic amine carcinogens (31, 35–37), genetic polymorphism in NAT1 and/or NAT2 may modify the cancer risk related to exposures to these carcinogens. The metabolic activation of many *N*-hydroxy heterocyclic amine carcinogens is catalyzed to a greater extent by human NAT2 than NAT1 (35, 37), but tissue-specific expression is also important for the effect of the *NAT1* and *NAT2* acetylation polymorphisms in cancer risk.

## Molecular Genetics

NAT1 and NAT2 are products of single, intronless protein-coding exons of 870-bp open reading frames encoding 290 amino acids (38, 39). *NAT1*, *NAT2*, and a pseudogene, *NATP*, are located on human chromosome 8p22 (40, 41), a region frequently displaying loss of heterozygosity in human tumors (42). A third *NAT* gene (*NAT3*) has been identified in the mouse (43, 44). *NAT1* and *NAT2* share 87% nucleotide homology in the coding region, yielding 55 amino acid differences. Whereas *NAT1* derives its entire transcript from a single exon, *NAT2* mRNA is derived from both the protein-coding exon and a second noncoding exon of 100 bp located about 8 kb upstream of the translation start site (40, 45).

Seven missense (G191A, T341C, A434C, G590A, A803G, A845C, and G857A) and four silent (T111C, C282T, C481T, and C759T) substitutions have been identified thus far in the *NAT2* coding exon. *NAT2*4* is considered the wild-type allele because of its absence of any of these substitutions. However, *NAT2*4* is not the most common allele in many ethnic groups, including Caucasians and Africans. *NAT2* alleles containing the G191A, T341C, A434C, G590A, and/or G857A missense substitutions are associated with slow acetylator phenotype(s). Striking ethnic differences in the frequencies of these missense substitutions (reviewed in Ref. 6) are responsible for the corresponding ethnic differences in frequency of slow acetylator alleles (reviewed in Ref. 11) and phenotype(s) (reviewed in Ref. 2). For example, the G191A substitution common to the *NAT2*14* gene cluster is present in African Americans and native Africans, but it is virtually absent in Caucasian populations. *NAT1* allelic frequency has been reviewed recently (6, 11). *NAT1*4* is the most common wild-type allele in all populations studied to date. Ethnic differences have been observed. For example, *NAT1*10* is more frequent in Mexican and African Americans (46) than in Caucasians (47) and Asians (48).

As of this date (October 1999), 24 different *NAT1* (Table 1) and 26 different *NAT2* (Table 2) alleles have been identified in human populations. A number of *NAT1* and *NAT2* alleles

*Table 2* Human *NAT2* alleles[a]

| Allele | Nucleotide change(s) | Amino acid change(s) | References[b] |
|---|---|---|---|
| *NAT2*4* | None | None | 24, 38, 39, 45, 49, 63, 64 |
| *NAT2*5A* | T341C, C481T | $Ile^{114}$→Thr | 38, 65, 66, 69 |
| *NAT2*5B* | T341C, C481T, A803G | $Ile^{114}$→Thr, $Lys^{268}$→Arg | 39, 66, 68 |
| *NAT2*5C* | T341C, A803G | $Ile^{114}$→Thr, $Lys^{268}$→Arg | 65, 66, 67, 69 |
| *NAT2*5D* | T341C | $Ile^{114}$→Thr | 70–73 |
| *NAT2*5E* | T341C, G590A | $Ile^{114}$→Thr, $Arg^{197}$→Gln | 70 |
| *NAT2*5F* | T341C, C481T, C759T, A803G | $Ile^{114}$→Thr, $Lys^{268}$→Arg | 74 |
| *NAT2*6A* | C282T, G590A | $Arg^{197}$→Gln | 24, 38, 39, 65, 66 |
| *NAT2*6B* | G590A | $Arg^{197}$→Gln | 66, 71, 72 |
| *NAT2*6C* | C282T, G590A, A803G | $Arg^{197}$→Gln, $Lys^{268}$→Arg | 71, 72 |
| *NAT2*6D* | T111C, C282T, G590A | $Arg^{197}$→Gln | 73 |
| *NAT2*7A* | G857A | $Gly^{286}$→Glu | 38, 49, 66 |
| *NAT2*7B* | C282T, G857A | $Gly^{286}$→Glu | 24, 65 |
| *NAT2*12A* | A803G | $Lys^{268}$→Arg | 66, 76 |
| *NAT2*12B* | C282T, A803G | $Lys^{268}$→Arg | 66, 72 |
| *NAT2*12C* | C481T, A803G | $Lys^{268}$→Arg | 69, 71, 72 |
| *NAT2*13* | C282T | None | 66, 67, 70, 75 |
| *NAT2*14A* | G191A | $Arg^{64}$→Gln | 69, 77 |
| *NAT2*14B* | G191A, C282T | $Arg^{64}$→Gln | 77, 78 |
| *NAT2*14C* | G191A, T341C, C481T, A803G | $Arg^{64}$→Gln, $Ile^{114}$→Thr, $Lys^{268}$→Arg | 70–72 |
| *NAT2*14D* | G191A, C282T, G590A | $Arg^{64}$→Gln, $Arg^{197}$→Gln | 70, 72 |
| *NAT2*14E* | G191A, A803G | $Arg^{64}$→Gln, $Lys^{268}$→Arg | 70 |
| *NAT2*14F* | G191A, T341C, A803G | $Arg^{64}$→Gln, $Ile^{114}$→Thr, $Lys^{268}$→Arg | 72 |
| *NAT2*14G* | G191A, C282T, A803G | $Arg^{64}$→Gln, $Lys^{268}$→Arg | 73 |
| *NAT2*17* | A434C | $Gln^{145}$→Pro | 79 |
| *NAT2*18* | A845C | $Lys^{282}$→Thr | 79 |

[a] Updated from Ref. 26. *NAT2* nomenclature accessible on the internet.[3] The allelic frequencies vary markedly with ethnicity. For review, see Ref. 11.
[b] Initial reports, as opposed to an exhaustive list.

have been identified recently, and there is a critical need to standardize and widely publicize consensus gene nomenclature (80–82). A consensus *NAT* nomenclature was published in 1995 (26). An international NAT nomenclature committee was formed at the First International Workshop on the Arylamine *N*-Acetyltransferases (81) held in Cairns, Australia in October 1998 to update, possibly revise, and publish an internet-accessible website for *NAT* nomenclature updates (82).[3] Some of the nucleotide substitutions and corresponding *NAT1* and *NAT2* alleles are rare and reflect nucleotide diversity (frequency of 0.0003–0.005) rather than genetic polymorphism (defined as frequency greater than 0.01). Moreover, some of the nucleotide substitutions are silent (do not change primary amino acid sequence) and may therefore be considered insignificant. However, recent studies have shown that silent single-nucleotide polymorphisms in gene coding regions yield allele-specific mRNA variants that differ markedly in structural folds (83) that may alter splicing, processing, translational control, and/or regulation of the mRNA.

Potential genotype and deduced phenotype misclassifications occur using methods designed to detect a small subset (usually described as *WT*, *M1*, *M2*, *M2*, *M3*, and sometimes *M4*) of the alleles (Table 3). The *M1* allele is detected by possession of the C481T substitution. This silent substitution is found in many but not all of the *NAT2*5* alleles and is found in *NAT2*12C*, which is actually a rapid allele. The *M2* allele is detected by possession of the G590A missense substitution, which is found in *NAT2*5*, *NAT*6*, and *NAT2*14* allelic clusters. The *M3* allele is detected by possession of the G857A missense substitution, which thus far has been identified only on *NAT2*7A* and *NAT2*7B*. An *M4* allele was initially discovered in African Americans (69, 77) and is detected by possession of the G191A missense substitution. Thus far, this substitution has only been identified on the *NAT2*14* gene cluster. The *WT* allele was formerly defined as the absence of C481T, G590A, G857A, and sometimes the G191A nucleotide substitutions. Because *NAT1*, *NAT2*, and *NATP* are highly homologous, PCR primers must be specific for *NAT1* or *NAT2*. Because most *NAT1* and *NAT2* alleles have multiple nucleotide substitutions, it is important that the correct phase be determined to identify their location on one or the other of the homologous chromosomes in the diploid genome (78). This and other pitfalls in *NAT2* genotyping have been reviewed recently (84). Several published methods to detect *NAT1*10* do not distinguish it from *NAT1*14A* and perhaps other *NAT1* alleles. Genotype misclassification in epidemiology investigations can produce substantial bias, requiring large expansions in sample sizes, particularly if gene-environmental interactions are considered (85, 86).

Acetylation polymorphisms also have been characterized in animal models (reviewed in Ref. 7). *NAT1* (Table 4) and *NAT2* (Table 5) in nonhuman species are highly homologous to both human *NAT1* and *NAT2* (Table 6). In fact, substrate specificities for Syrian hamster, mouse, and rat NAT2 may resemble human NAT1 more than they do human NAT2 (1). Several different mechanisms are responsible for NAT2 polymorphisms in nonhuman species. The molecular basis for slow acetylation is a *NAT2* gene deletion in the rabbit (87), a nonsense mutation yielding a truncated NAT2 enzyme in the Syrian hamster (88–90), and a missense substitution (91) yielding an unstable NAT2 enzyme (44, 92) in the mouse. In contrast, no *NAT1* polymorphisms have been identified in nonhuman species except for a single silent T60C mutation in Syrian hamster *NAT1* (93).

[3] The website is presently located at www.louisville.edu/medschool/pharmacology/NAT.html.

*Table 3* Examples of *NAT2* genotype/phenotype misclassifications

| *NAT2* genotype[a] (comprehensive) | Deduced phenotype[a] (comprehensive) | *NAT2* genotype[b] (simple) | Deduced phenotype[b] (simple) |
|---|---|---|---|
| **4/*5C* | Intermediate | *Wt/Wt* | Rapid |
| **4/*12C* | Rapid | *Wt/M1* | Intermediate |
| **4/*14A* | Intermediate | *Wt/Wt* | Rapid |
| **4/*14B* | Intermediate | *Wt/Wt* | Rapid |
| **4/*14E* | Intermediate | *Wt/Wt* | Rapid |
| **5B/*5C* | Slow | *Wt/M1* | Intermediate |
| **5B/*14A* | Slow | *Wt/M1* | Intermediate |
| **5B/*14B* | Slow | *Wt/M1* | Intermediate |
| **5C/*6A* | Slow | *Wt/M2* | Intermediate |
| **5C/*7B* | Slow | *Wt/M3* | Intermediate |
| **5C/*12A* | Intermediate | *Wt/Wt* | Rapid |
| **5C/*13* | Intermediate | *Wt/Wt* | Rapid |
| **6A/*12C* | Intermediate | *M1/M2* | Slow |
| **6A/*14A* | Slow | *Wt/M2* | Intermediate |
| **6A/*14B* | Slow | *Wt/M2* | Intermediate |
| **12A/*14B* | Intermediate | *Wt/Wt* | Rapid |
| **13/*14B* | Intermediate | *Wt/Wt* | Rapid |
| **14A/*14A* | Slow | *Wt/Wt* | Rapid |

[a] Examples of *NAT2* genotypes and deduced phenotypes from ongoing studies in our laboratory. The frequency of the genotypes varies with ethnic origin.
[b] The original *NAT2* nomenclature identified a wild type (*Wt*) allele and three mutant *M1*, *M2*, and *M3* alleles. Deduced phenotypes are defined as follows: rapid acetylators, *Wt/Wt*; intermediate acetylators, *Wt*/mutant; slow acetylators, mutant/mutant. A "simple" *NAT2* genotype method distinguishes only these alleles.

*Table 4* *NAT1* alleles in nonhuman species[a]

| Allele | Nucleotide changes[b] | Amino acid changes[b] | Species (phenotype)[c] |
|---|---|---|---|
| *NAT1*1* | | | Rabbit (rapid) |
| *NAT1*2* | None (coding region) | None | Rabbit (slow) |
| *NAT1*6* | | | Mouse (rapid) |
| *NAT1*7* | None (coding region) | None | Mouse (slow) |
| *NAT1*8* | | | Syrian hamster (rapid) |
| *NAT1*9* | T60C | None | Syrian hamster (slow) |
| *NAT1*13* | | | Rat (rapid and slow) |

[a] Adapted from Ref. 7.
[b] Relative to *NAT1* alleles of the species.
[c] No functional differences have been observed between rapid and slow acetylator *NAT1* in these species.

## Genotype/Phenotype Relationships

The effects of *NAT1* and *NAT2* nucleotide substitutions on acetyltransferase catalytic activities have been investigated primarily in recombinant expression systems (30, 31, 50, 52, 55, 69, 73, 94–97). Nucleotide substitutions identified in human *NAT1* and *NAT2* allelic variants yield reductions in substrate affinity, catalytic activity, and/or protein stability of the recombinant *N*-acetyltransferase allozyme. Recombinant human NAT2 5, NAT2 6, NAT2 14, and NAT2 17 clusters yield variable reductions in catalytic activity associated with the slow acetylator phenotype, whereas recombinant human NAT2 12 and NAT2 13 clusters catalyze *N*-,*O*-, and *N*,*O*-acetyltransferase activities at levels comparable to those of the rapid acetylator NAT2 4 (Fig. 1). Although one study suggested that *NAT2*13* was associated with slow acetylator phenotype *in vivo* (67), the observation related to a *NAT2* genotyping artifact (84). A recent study (98) found that *NAT2*13* is associated with rapid acetylator phenotype *in vivo*, consistent with recombinant expression systems (31, 94). Recombinant NAT2 proteins differ in intrinsic stability because the NAT2 6, NAT2 7, NAT2 14, and NAT2 18 clusters are less stable than the others (6, 69, 73, 94). The NAT2 7B allozyme has a higher affinity than other NAT2 allozymes for sulfamethazine and dapsone (96) but not for 2-aminofluorene (94, 96), suggesting that expression of acetylator phenotype is dependent on substrate even if it is selective for NAT2 *versus* NAT1. Some, but not all, of the nucleotide substitutions in human *NAT2* yield reductions in the quantity of recombinant NAT2 protein in eukaryotic expression systems (24, 38, 68, 73, 99). Because most of the structure-function information has been derived from recombinant expression systems, more data from human tissues are needed to investigate tissue-specific factors and other regulatory factors.

Because multiple mechanisms for reductions in *N*-acetyltransferase activity are associated with various nucleotide substitutions present on *NAT2* alleles, the ability to distinguish acetylator phenotypes is complex and is a function of the sensitivity and specificity of the phenotype method. Indeed, some studies report phenotypic differences within the slow acetylator phenotype (67, 84, 100). Phenotype is influenced by a number of factors including diet, disease, and drug therapy. Depending on the probe drug and analytical method used, acetylation phenotypes are often not clearly separate or distinct but rather exhibit continuous and overlapping variability due to numerous genetic and/or environmental factors, including the large number and diversity of *NAT2* genotypes present in human populations. This finding is not unique to acetylation phenotypes (101). The relative specificity of the substrate for NAT2 *versus* NAT1 at the concentrations obtained *in vivo* will also affect acetylator phenotype. Aromatic and hydrazine drugs such as isoniazid, dapsone, procainamide, and sulfamethazine have also been used in patients treated with these drugs (reviewed in Refs. 1 and 2). Caffeine is most commonly used as a probe drug for NAT2 phenotype determinations (102), and excellent *NAT2* genotype/phenotype correlations have been reported (6, 67). Although administration of caffeine is a relatively noninvasive method for determining NAT2 acetylator phenotype, this method requires the quantitation of secondary metabolites with key intermediates that are unknown. Thus, genetic and/or environmental effects on a number of enzyme systems (*e.g.*, cytochrome P450 or xanthine oxidase) may affect the levels of the metabolites used in the phenotype determina-

*Table 5* *NAT2* alleles in nonhuman species[a]

| Allele | Nucleotide changes[b] | Amino acid changes[b] | Species (phenotype) |
|---|---|---|---|
| *NAT2*1* | | | Chicken |
| *NAT2*2* | | | Rabbit (rapid) |
| *NAT2*3* | *NAT2* deleted | No transcript or protein | Rabbit (slow) |
| *NAT2*8* | | | Mouse (rapid) |
| *NAT2*9* | A296T | $Asn^{99}$→Ile | Mouse (slow) |
| *NAT2*15* | | | Syrian hamster (rapid) |
| *NAT2*16A* | T36C, A633G, C727T | $Arg^{243}$→Stop | Syrian hamster (slow) |
| *NAT2*16B* | T36C, C325T, A633G, C727T | $Arg^{243}$→Stop | Syrian hamster (slow) |
| *NAT2*20* | | | Rat (rapid) |
| *NAT2*21A* | G361A, G399A, G522A, G796A | $Val^{121}$→Ile; $Val^{266}$→Ile | Rat (slow) |
| *NAT2*21B* | G361A, G399A, C672T, G796A | $Val^{121}$→Ile; $Val^{266}$→Ile | Rat (slow) |

[a] Adapted from Ref. 7.
[b] Relative to *NAT2* alleles of the species.

*Table 6* Nucleotide and deduced amino acid sequence identity of rodent *NAT1* and *NAT2* with human *NAT1* and *NAT2*[a]

| Species | Gene | Human *NAT1 (NAT1*4)* | | Human *NAT2 (NAT2*4)* | |
|---|---|---|---|---|---|
| | | % Nucleotide identity[b] | % Deduced amino acid identity | % Nucleotide identity[b] | % Deduced amino acid identity |
| Mouse | *NAT1*6* | 80 | 73 | 82 | 72 |
| Rat | *NAT1*13* | 81 | 76 | 80 | 74 |
| Syrian hamster | *NAT1*8* | 78 | 71 | 77 | 69 |
| Mouse | *NAT2*8* | 79 | 80 | 79 | 74 |
| Rat | *NAT2*20* | 84 | 81 | 80 | 74 |
| Syrian hamster | *NAT2*15* | 84 | 82 | 80 | 75 |

[a] Adapted from Ref. 7.
[b] Coding region.

tion. For example, NAT1 phenotype influences the urinary caffeine ratio used as a measure of NAT2 phenotype (103). Other potential artifacts in the use of caffeine to determine acetylation phenotype have also been reported (104). An ELISA method to measure caffeine metabolic ratios is presently under development (105).

NAT1 phenotype has been determined *in vivo* using *p*-aminosalicylic acid as a probe drug (51). Measurement of the urinary or plasma metabolite ratio after administration of low doses of *p*-aminosalicylic acid allowed for the identification of individuals with marked impairments of NAT1, but less than 50% of phenotypic variation *in vivo* appears to be related to variation in NAT1 activity (51). Measurement of NAT1 activity in blood lysates *in vitro* has been used for phenotype determinations (51, 55), but tissue-specific regulation of NAT1 is unknown. One study in a Caucasian Australian population reported a bimodal distribution of NAT1 activity in human peripheral blood mononuclear cells, with approximately 8% exhibiting a slow NAT1 acetylator phenotype (55). Kinetic studies showed that slow acetylator NAT1 individuals exhibited $V_{max}$ levels approximately 50% of rapid acetylators, whereas the $K_m$ was similar for the two phenotypes (55). Because the acetylation activity of the variant NAT1 is minimal, these findings suggest that *NAT1* alleles are codominant (55). This is analogous to *NAT2* alleles in the Syrian hamster, which exhibit a striking Mendelian intermediary inheritance, yielding doubled expression of both *N*- and *O*-acetylation in the presence of two functional (*i.e.*, homozygous rapid) alleles (Refs. 19 and 106–108; Table 7). Preliminary studies suggest that NAT1 is subject to regulatory control (55, 109, 110). Recently, a hormone response element was identified in the *NAT2* promoter region that mediates androgenic regulation of *N*-acetyltransferase activity in mouse kidney (111).

Several *NAT1* alleles (Table 1) have been associated with reduced function *in vivo* or via recombinant expression in bacteria (51, 55, 56). Recombinant expression of *NAT1*21*, *NAT1*24*, and *NAT1*25* in bacterial systems resulted in allozymes with *N*-acetyltransferase activities 2–3-fold higher than NAT1 4 (56). Some studies suggest that *NAT1*10* may be a rapid acetylator allele because it has been associated with slight increases in *N*-acetyltransferase activity in bladder and colon (112), liver (113), and erythrocytes (52) and increased carcinogen-DNA adduct binding in the urinary bladder (114). Each of these studies reported that individuals possessing the *NAT1*10* allele had slightly higher levels of *N*-acetyltransferase activity. However, in each study, there was considerable overlap, and the increase was either nonsignificant or of marginal significance. The instability of human NAT1 (22, 36) is also a problem for investigations in human tissues. *NAT1*10* has no nucleotide substitutions in the coding region, but the substitution (T1088A) in the 3′-untranslated region alters a polyadenylation signal (TAATAA→TAAAAA) that may enhance mRNA stability (112, 115). However, other studies did not find that NAT1 10 was a high activity allozyme (51, 55, 116). Thus, the relationship between *NAT1* genotype and phenotype remains poorly understood.

## Molecular Epidemiology

Human epidemiological studies have investigated the role of *NAT1* and/or *NAT2* polymorphisms in a number of cancers. Aromatic amines such as 4-aminobiphenyl and heterocyclic amines such as PhIP[4] are present in cigarette smoke (117, 118).

[4] The abbreviation used is: PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine.

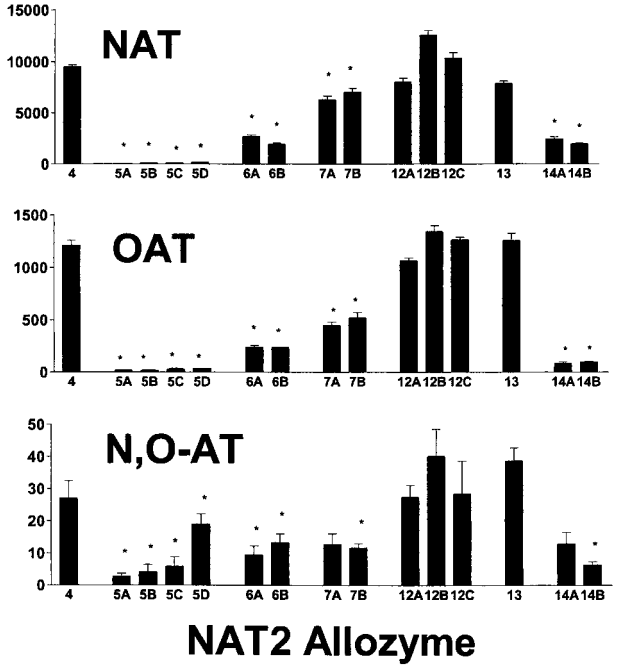


*Fig. 1.* Relative capacity of recombinant human *N*-acetyltransferase allozymes to catalyze the *N*-acetylation of 2-aminofluorene (*top*), the *O*-acetylation of *N*-hydroxy-2-aminofluorene (*center*) and the *N*,*O*-acetylation of *N*-hydroxy-*N*-acetyl-2-aminofluorene (*bottom*). Levels of acetyltransferase for each allozyme were *N*-acetylation > *O*-acetylation > *N*,*O*-acetylation. *, significantly lower than NAT2 4 ($P < 0.05$). NAT activities are expressed in pmol/min/unit protein; OAT and *N*,*O*-AT activities are expressed in pmol/min/mg DNA/unit protein. Adapted from Ref. 31.

Heterocyclic amines are also present in the diet as protein pyrolysis products formed when meat is cooked well done (29). Aromatic and heterocyclic amine carcinogens produce tumors at a number of sites in rodents but require metabolic activation to mutate DNA and initiate carcinogenesis. After *N*-oxidation, the *N*-hydroxy-aromatic and *N*-hydroxy-heterocyclic amines are further activated (via *O*-acetylation) by *N*-acetyltransferases to acetoxy intermediates, which react spontaneously with DNA to form DNA adducts (3, 31–37). Thus, biological plausibility for relationships between the *NAT1* and/or *NAT2* acetylation polymorphisms are strongest for cancers related to aromatic or heterocyclic amine exposures.

The role of NAT1 *versus* NAT2 and/or the role of rapid *versus* slow acetylator genotype in cancer predisposition differs between organ sites, as might be expected with tissue-specific expression of the NAT1 and NAT2 enzymes. Earlier studies primarily investigated relationships between acetylator phenotype (NAT2) and cancer incidence. Since the identification and cloning of human *NAT1* and *NAT2* about 10 years ago, most studies have investigated *NAT2* genotype and cancer incidence, either alone or in combination with phenotype. Recent studies have investigated *NAT1* genotype, usually in combination with *NAT2* genotype. Because multiple carcinogen metabolism enzymes are involved in the activation and deactivation of chemical carcinogens, many studies test interactions among multiple carcinogen metabolizing genes. A discussion of these gene-gene interactions is beyond the scope of this review. Similarly, many studies attempt to estimate exposure to chemical carcinogens and test for gene-environmental interactions. Investigations of gene-gene and/or gene-environmental interactions require larger sample sizes to ensure sufficient statistical power. New genotype technologies (*e.g.*, Refs. 78 and 119–122) are facilitating these studies, and many large epidemiological studies are ongoing. As such, this review represents a status of the field, which is expected to expand exponentially in the near future. Present information on the role of *NAT1* and/or *NAT2* acetylation polymorphism(s) in predisposition to urinary bladder, colorectal, breast, lung, head and neck, and prostate cancers is summarized below.

## Urinary Bladder Cancer

The first association between slow acetylator phenotype and urinary bladder cancer was reported 20 years ago (123). The mechanism for this association suggests that slow NAT2 acetylation of aromatic amine carcinogens competes poorly with metabolic activation via cytochrome P450(s) and/or prostaglandin H-synthases, thus accounting for higher risk in the slow NAT2 phenotype(s). In a subsequent study (100), English chemical dye workers with documented exposure to aromatic amine carcinogens showed a striking association (odds ratio = 16.7; $P = 0.00005$) between urinary bladder cancer and slow acetylator phenotypes. The association was strongest in the slowest acetylator phenotypes. As previously reviewed (3), a number of studies confirmed these observations, and, as expected, the associations between slow acetylator phenotype(s) and urinary bladder cancer are strongest in studies in which there are documented exposures to aromatic amine carcinogens. One prominent exception is a study of a cohort of workers in China who had been employed in benzidine production and use. A case-control study of surviving bladder cancer cases from this cohort showed that neither slow NAT2 acetylator phenotype nor genotype was associated with increased risk of urinary bladder cancer (124). However, these results are explained by the observation that benzidine is a much better substrate for NAT1 than NAT2 (113). Recent *NAT2* genotype studies (125–128) show associations with urinary bladder cancer that are highest for particular *NAT2* alleles associated with slow acetylator phenotype(s), consistent with the earlier phenotypic studies (100). Among smokers, NAT2 slow acetylators have higher levels of 4-aminobiphenyl hemoglobin adducts than rapid acetylators (129, 130).

An association between urinary bladder cancer and smokers possessing the *NAT1*10* allele was reported in two studies (131, 132) but not in two others (116, 127). In the two former studies (131, 132), the association was highest among smokers who possessed both the *NAT1*10* allele and were slow *NAT2* acetylators, suggesting that higher levels of NAT1-catalyzed activation (*O*-acetylation) within the urinary bladder increase risk. Immunochemical detection of NAT1 has been reported in human urinary bladder, whereas NAT2 was below the level of detection with the antibody used (133). However, both *NAT1* and *NAT2* mRNA are readily detectable (134). High levels of metabolic activation of *N*-hydroxy-aromatic amines have been reported in human urinary bladder cytosol (135) and human uroepithelial cells (136). Levels of activation were slightly higher in individuals possessing the *NAT1*10* allele (112). Administration of aromatic amines to mice (137) and rats (138), but not to congenic Syrian hamsters (139, 140), resulted in higher DNA adducts in the urinary bladder of slow acetylators than rapid acetylators. NAT2 activity expressed in urinary bladder is comparable to that observed in other extrahepatic tissues in Syrian hamster (141), rat (142), and mouse (143). In mouse and human, immunohistochemistry has revealed that *N*-acetyltransferase expression is limited to the transitional ep-

*Table 7* Acetylator genotype-dependent *N*-acetyltransferase activity in colon cytosol of congenic Syrian hamster[a]

| Substrate | *NAT2* genotype | Phenotype | *N*-Acetyltransferase activity (nmol/min/mg) | | |
|---|---|---|---|---|---|
| | | | Cytosol | *NAT1* | *NAT2* |
| 2-Aminofluorene | *NAT2*15/*15* | Rapid | 6.16 ± 1.51 | 1.39 ± 0.19 | 24.8 ± 3.2 |
| | *NAT2*15/*16A* | Intermediate | 2.69 ± 0.30 | 0.97 ± 0.19 | 11.7 ± 1.6 |
| | *NAT2*16A/*16A* | Slow | 0.53 ± 0.04 | 0.96 ± 0.26 | 0.74 ± 0.29 |
| | | | ($P < 0.0001$) | | ($P < 0.0001$) |
| 3,2′-Dimethyl-4-aminobiphenyl | *NAT2*15/*15* | Rapid | 0.97 ± 0.24 | 1.06 ± 0.41 | 2.98 ± 0.36 |
| | *NAT2*15/*16A* | Intermediate | 0.59 ± 0.07 | 0.68 ± 0.18 | 1.31 ± 0.15 |
| | *NAT2*16A/*16A* | Slow | 0.31 ± 0.04 | 1.00 ± 0.35 | 0.05 ± 0.16 |
| | | | ($P < 0.005$) | | ($P < 0.0005$) |

[a] Adapted from Refs. 107 and 108.

ithelium and the linings of minor blood vessels (133, 144). *N*-Acetyltransferase expression in the kidney has been detected in the cuboidal epithelium of the proximal convoluted tubules, with more intense staining in the cortex than in the medulla (133). Expression in the proximal convoluted tubules is consistent with a role for *N*-acetyltransferase(s) in the activation and/or deactivation of carcinogens excreted into the urinary bladder. Moreover, a very recent study reported an association between slow acetylator *NAT2* genotype and renal cell carcinoma (145).

### Colorectal Cancer

Heterocyclic amines are associated with colorectal cancer in rodents (29) but are poor substrates for *N*-acetylation (33, 34, 36, 146, 147). Thus, a biologically plausible mechanistic hypothesis suggests that rapid *NAT1* and/or *NAT2* acetylators should more readily activate *N*-hydroxy-heterocyclic amine carcinogens within the colon to their ultimate carcinogenic forms, thereby predisposing them to colorectal cancer. Human colon cytosols activate *N*-hydroxy heterocyclic amine carcinogens to DNA adducts catalyzed by *N*-acetyltransferases (34, 112, 148). Because human populations are genetically heterogeneous, and exposures to heterocyclic amines are difficult to estimate, it is not surprising that the results are inconsistent. Several studies (149–151) found an association between rapid *NAT2* acetylator phenotype and colorectal cancer, whereas other studies (152–157) did not. Five studies (158–162) reported an association between rapid *NAT2* acetylator phenotype and colorectal cancer for individuals consuming well-done meat and, presumably, higher levels of heterocyclic amine carcinogens (163). Consistent with this hypothesis, rapid NAT2 acetylators who consumed pan-fried meats had higher levels of urinary mutagenicity than slow acetylators (164). One study found the association to colorectal cancer was limited to homozygous rapid (*NAT2*4/*4*) acetylators (151), a finding that was also observed for lung (165) and laryngeal (166) cancers.

Bell *et al.* (115) found an association between the *NAT1*10* allele and colorectal cancer, and the risk was highest among *NAT2* rapid acetylators. Another study also showed a higher risk for colorectal cancer in individuals who consumed well-done meat and possessed both the *NAT1*10* allele and rapid acetylator *NAT2* genotype (161). *NAT1*10* and *NAT2*4* are in linkage disequilibrium (166, 167), which may be a factor in the association of the *NAT1*10* allele with colorectal cancer. Two studies reported a lack of association between the *NAT1*10* allele and colorectal cancer (48, 56). The disparity in results may relate to misclassification of *NAT1*10* alleles because a common test to detect the *NAT1*10* allele does not distinguish between *NAT1*10* and *NAT1*14A* (55) or other *NAT1* alleles.

The role of the *NAT2* acetylator polymorphism in colorectal cancer has been investigated in animal models. These models are particularly useful in that age, carcinogen exposure, and diet can be carefully matched, and genetic variability in genes other than *NAT2* is virtually eliminated in the congenic models. Furthermore, the presence of a single mechanism for slow acetylator genotype in these models, resulting from homozygosity for a single slow acetylator *NAT2* allele (Table 5), results in clear and unambiguous genotype/phenotype correlations, as illustrated by the levels of *N*-acetyltransferase activity expressed in the colon (Table 7). This is particularly true when the NAT1 and NAT2 isozymes in colon mucosa are isolated, separated from each other, and tested separately (Table 7). NAT1 and NAT2 mRNA are widely distributed in humans (168), but comprehensive data on NAT1 and NAT2 activities are lacking for human tissues, reflecting the availability of fresh human tissue and the instability of human NAT1. NAT1 and NAT2 are distributed differently in rabbit, Syrian hamster, mouse, and rat small intestine (169, 170). *N*-Acetyltransferase expression at the cellular level was restricted to epithelial cells exposed to the lumen of both the small intestine and colon. Immunohistochemical staining of mouse tissues with anti-NAT2 antibody demonstrated intense staining in the columnar epithelial cells of the villus tip of small intestine, with stain intensity decreasing along the crypt/villus axis, and little to no staining in the crypts of Lieberkuhn (144). Similarly, in rat small intestine, *N*-acetyltransferase expression was greatest in epithelial cells isolated from mid-villus and villus tip (171). *N*-Acetyltransferase expression in humans has been observed in epithelial cells of all types in the small intestine villus, but not in the crypts of Lieberkuhn (172). Staining was restricted to epithelial cells at the luminal surface, with no staining in the crypts. Under controlled exposure and genotype, a higher frequency of aberrant crypt foci (a preneoplastic lesion for colorectal cancer) is found in rapid acetylators than in slow acetylators using two different congenic hamster models and an inbred rat model (Table 8). Recently, higher levels of PhIP-DNA adducts were detected in the colons of rapid *versus* slow acetylator rats given PhIP (176). These studies support the role of the *NAT2* acetylation polymorphism in genetic predisposition to colorectal cancer, suggesting that homozygous rapid acetylators exposed to high levels of heterocyclic amines through consumption of consistently well-done meat are at a higher risk.

*Table 8* Frequency of aberrant crypt foci in homozgous rapid and slow acetylator congenic hamsters and inbred rats administered 3,2-dimethyl-4-aminobiphenyl[a]

| Species (strain) | *NAT2* genotype | Phenotype | Aberrant crypt foci |
|---|---|---|---|
| Syrian hamster (Bio.82.73/H) | *NAT2*15/*15* | Rapid | 9.38 ± 1.55 |
| | *NAT2*16A/*16A* | Slow | 3.30 ± 0.73 |
| | | | $P < 0.001$ |
| Syrian hamster (Bio 1.5/H) | *NAT2*15/*15* | Rapid | 21.2 ± 5.5 |
| | *NAT2*16A/*16A* | Slow | 7.20 ± 1.66 |
| | | | $P < 0.05$ |
| Rat (F344 and WKY) | *NAT2*20/*20* | Rapid | 4.11 ± 1.06 |
| | *NAT2*21A/*21A* | Slow | 1.57 ± 0.48 |
| | | | $P < 0.05$ |

[a] Adapted from Refs. 173–175.

## Breast Cancer

Several studies have investigated an association between NAT2 acetylator phenotype or genotype and breast cancer, but the findings have been very inconsistent. NAT2 acetylator phenotype was not associated with breast cancer in three (177–179), studies. However, rapid acetylator phenotype was associated with breast cancer risk (180, 181) or advanced disease at first presentation (182) in three other studies. Recently, the association between *NAT2* acetylator genotype and breast cancer has been investigated in relation to smoking and diet. Two studies found that red meat consumption and *NAT2* genotype were not associated with breast cancer risk (183, 184). However, in a preliminary report, rapid/intermediate *NAT2* genotypes were associated with breast cancer risk in women who consistently consume very well-done meat (185). The relationship between *NAT2* genotype and breast cancer among smoking women has varied. Two studies reported a higher risk among slow acetylators (186, 187), one found no difference between rapid and slow acetylators (188) and one reported a higher risk among rapid acetylators (189). *NAT1* and *NAT2* mRNA has been detected in human mammary cells (190). Human mammary cells from rapid acetylators activated heterocyclic amines to DNA adducts to a greater extent than cells derived from slow acetylators (191). However, higher levels of DNA adducts were reported in mammary tissue from slow acetylators than in mammary tissue from rapid acetylators (192).

Recent investigations have explored the relationship between *NAT1* acetylation polymorphism and breast cancer risk. NAT1, but not NAT2, enzymatic activity was detected in human mammary epithelial cells (190). One study (189) observed little modification of smoking effects for breast cancer according to *NAT1* genotype, except among postmenopausal women with the *NAT1*10* allele. A more recent study reported an elevated risk of breast cancer in smokers who consistently consumed well-done red meat and possessed the *NAT1*11* allele (47). The acetylator phenotype of individuals possessing the *NAT1*11* allele is not yet fully understood. The G445A; $Val^{129} \rightarrow Ile$ substitution present in *NAT1*11* (50) was not included in previous descriptions of this allele (26). A subsequent study found that this missense substitution yielded recombinant NAT1 protein that catalyzed the metabolic activation of *N*-hydroxy-aromatic amines at rates up to 2-fold higher than that of wild-type recombinant NAT1 (50). However, another study (51) reported that recombinant expression of *NAT1*11* did not produce a higher activity allozyme.

## Lung Cancer

Early studies investigating the role of the NAT2 phenotype in susceptibility to lung cancer were either negative (193) or showed a slight overrepresentation of rapid acetylators (194, 195). A subsequent *NAT2* genotype study (165) basically confirmed the latter finding but showed more clearly that the highest risk was found in smokers with the homozygous rapid acetylator (*NAT2*4/*4*) genotype. Similarly, Nyberg *et al.* (196) reported an increased risk for lung cancer for rapid acetylators who were smokers. In contrast, two studies (70, 197) did not find an association between *NAT2* acetylator genotype and lung cancer. Increased risks of asbestos-associated malignant mesothelioma were observed in slow acetylators in Finnish studies, especially when exposed to high levels of asbestos (198, 199). The role of *NAT1* genotype in lung cancer risk has also been investigated. One study (197) reported an association between low activity *NAT1* alleles (*NAT1*14* and *NAT1*15*) with lung cancer. A second study (46) did not find an association between *NAT1* genotype and lung cancer, but the genotype assay did not distinguish the *NAT1*14* or *NAT1*15* alleles.

## Head and Neck Cancer

Head and neck cancers are strongly associated with smoking, and several studies have explored the role of *NAT1* and *NAT2* polymorphisms in the incidence of head and neck cancer in smokers. The slow NAT2 acetylator phenotype was associated with the development of head and neck cancer in Caucasians (200, 201) and with the development of esophageal (202) and laryngeal (203) cancers in Japan. The homozygous slow acetylator NAT2 genotype was associated with an increased risk of oral/pharyngeal cancer, but not laryngeal cancer (204). The homozygous rapid acetylator (*NAT2*4/NAT2*4*) genotype was strongly associated with laryngeal cancer in a German study (167). *NAT1*10* allele, but not NAT2 phenotype, was associated with oral squamous cell carcinoma in a Japanese study (205). *NAT1*10* was associated with head and neck cancers in one study (206) but with neither oral/pharyngeal nor laryngeal cancer in another study (204).

## Prostate Cancer

Three studies have explored associations between acetylator genotypes and prostate cancer. No relationship between *NAT2* genotype and prostate cancer was observed in two studies (207, 208), and aromatic amine *N*-acetyltransferase activity levels in human prostates were independent of *NAT2* genotype (207). An association between *NAT1*10* and prostate cancer was reported recently (209). Higher levels of DNA adducts in the prostate were reported in slow *versus* rapid acetylator rats given 3,2′dimethyl-4-aminobiphenyl (138), but the opposite was observed in rapid and slow acetylator rats given PhIP (176). Transgenic mice in which human NAT2 was overexpressed specifically in the prostate did not increase levels of DNA adducts in the prostate of mice given PhIP (210).

## Summary

Polymorphisms exist for both NAT1 and NAT2. Because they catalyze the *N*-acetylation (usually deactivation) and *O*-acetylation (usually activation) of aromatic and heterocyclic amine carcinogens, genetic polymorphisms in NAT1 and/or NAT2 may modify risk associated with carcinogen exposures. Although our understanding is still incomplete, particularly for NAT1, information on the molecular genetics and structure-

function of NAT1 and NAT2 has increased substantially over the past decade, facilitating the development of molecular epidemiological studies exploring the relationship between the acetylation polymorphisms and cancer. Animal models have well-defined molecular mechanisms for the slow NAT2 acetylator phenotype. In contrast, molecular mechanisms for slow NAT2 acetylator phenotypes in humans appear to be much more complex and are not well understood. Human epidemiological studies suggest that the NAT1 and NAT2 acetylation polymorphisms modify predisposition to urinary bladder, colorectal, breast, head and neck, lung, and possibly prostate cancers, but there is inconsistency in the results. The inconsistencies may relate, in part, to differences in carcinogen exposures, genotype and/or phenotype methods, insufficient sample sizes, and/or other susceptibility genes and factors. Associations between slow *NAT2* acetylator genotypes and urinary bladder cancer and between rapid *NAT2* acetylator genotypes and colorectal cancer are the most consistently reported associations. Individual risks associated with *NAT1* and/or *NAT2* genotypes are small, but they increase when considered in conjunction with other susceptibility genes and/or aromatic and heterocyclic amine carcinogen exposures. Because of the relatively high frequency of some *NAT1* and *NAT2* genotypes, the attributable risk of cancer in the population may be high. Because most aromatic and heterocyclic amine carcinogens are metabolized by NAT1 and NAT2, both genotypes should be determined using methods that minimize or eliminate allele misclassification. Data on carcinogen exposure should also be assessed when possible. The effect of *NAT1* and *NAT2* genotype on cancer risk varies with organ site, probably reflecting tissue-specific expression of NAT1 and NAT2. Ethnic differences in *NAT1* and *NAT2* genotype frequencies may be a factor in cancer incidence. Large-scale molecular epidemiological studies that investigate the role of *NAT1* and *NAT2* genotypes and/or phenotypes together with other genetic susceptibility gene polymorphisms and biomarkers of carcinogen exposure are necessary to improve our understanding of the role of the *NAT1* and *NAT2* acetylation polymorphisms in cancer risk.

## References


1. Weber, W. W., and Hein, D. W. *N*-Acetylation pharmacogenetics. Pharmacol. Rev., *37:* 25–79, 1985.

2. Weber, W. W. The Acetylator Genes and Drug Response. New York: Oxford University Press, 1987.

3. Hein, D. W. Acetylator genotype and arylamine-induced carcinogenesis. Biochim. Biophys. Acta, *948:* 37–66, 1988.

4. Evans, D. A. P. *N*-Acetyltransferase. Pharmacol. Ther., *42:* 157–234, 1989.

5. Evans, D. A. P. Genetic Factors in Drug Therapy: Clinical and Molecular Pharmacogenetics. Cambridge, UK: Cambridge University Press, 1993.

6. Grant, D. M., Hughes, N. C., Janezic, S. A., Goodfellow, G. H., Chen, H. J., Gaedigk, A., Yu, V. L., and Grewal, R. Human acetyltransferase polymorphisms. Mutat. Res., *376:* 61–70, 1997.

7. Hein, D. W., Doll, M. A., Fretland, A. J., Gray, K., Deitz, A. C., Feng, Y., Jiang, W., Rustan, T. D., Satran, S., and Wilkie, T. R. Rodent models of the human acetylation polymorphism: comparisons of recombinant acetyltransferases. Mutat. Res., *376:* 101–106, 1997.

8. Lang, N. P. Acetylation as an indicator of risk. Environ. Health Perspect., *105:* 763–766, 1997.

9. Meyer, U. A., and Zanger, U. M. Molecular mechanisms of genetic polymorphisms of drug metabolism. Annu. Rev. Pharmacol. Toxicol., *37:* 269–296, 1997.

10. Weber, W. W. Pharmacogenetics. New York: Oxford University Press, 1997.

11. Cascorbi, I., Brockmoller, J., Mrozikiewicz, P. M., Muller, A., and Roots, I. Arylamine *N*-acetyltransferase activity in man. Drug Metab. Rev., *31:* 489–502, 1999.

12. Hulla, J. E., Miller, M. S., Taylor, J. A., Hein, D. W., Furlong, C. E., Omiecinski, C. J., and Kunkel, T. A. Symposium overview: the role of genetic polymorphisms and repair deficiencies in environmental disease. Toxicol. Sci., *47:* 135–143, 1999.

13. Wormhoudt, L. W., Commandeur, J. N. M., and Vermeulen, N. P. E. Genetic polymorphisms of human *N*-acetyltransferase, cytochrome P450, glutathione-*S*-transferase and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. Crit. Rev. Toxicol., *29:* 59–124, 1999.

14. Hein, D. W. *N*-Acetyltransferase genetics and their role in predisposition to aromatic and heterocyclic amine-induced carcinogenesis. Toxicol Lett., in press.

15. Hughes, H. B., Biehl, J. P., Jones, A. P., and Schmidt, L. H. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. Am. Rev. Res. Dis., *70:* 266–273, 1954.

16. Evans, D. A. P., and White, T. A. Human acetylation polymorphism. J. Lab. Clin. Med., *63:* 394–403, 1964.

17. Weber, W. W., and Hein, D. W. Clinical pharmacokinetics of isoniazid. Clin. Pharmacokinet., *4:* 401–422, 1979.

18. Jenne, J. W. Partial purification and properties of the isoniazid transacetylase in human liver: its relationship to the acetylation of *p*-aminosalicylic acid. J. Clin. Investig., *44:* 1992–2002, 1965.

19. Hein, D. W., Kirlin, W. G., Ferguson, R. J., and Weber, W. W. Biochemical investigation of the basis for the genetic *N*-acetylation polymorphism in the inbred hamster. J. Pharmacol. Exp. Ther., *234:* 358–364, 1985.

20. Smith, T. J., and Hanna, P. E. *N*-Acetyltransferase multiplicity and bioactivation of *N*-arylhydroxamic acids by hamster hepatic and intestinal enzymes. Carcinogenesis (Lond.), *7:* 697–702, 1986.

21. Ozawa, S., Abu-Zeid, M., Kawakubo, Y., Toyama, S., Yamazoe, Y., and Kato, R. Monomorphic and polymorphic isozymes of arylamine *N*-acetyltransferases in hamster liver: purification of the isozymes and genetic basis of *N*-acetylation polymorphism. Carcinogenesis (Lond.), *11:* 2137–2144, 1990.

22. Grant, D. M., Beer, M., Blum, M., and Meyer, U. A. Monomorphic and polymorphic human arylamine *N*-acetyltransferases: a comparison of liver isozymes and expressed products of two cloned genes. Mol. Pharmacol., *39:* 184–191, 1991.

23. Grant, D. M., Morike, K., Eichelbaum, M., and Meyer, U. A. Acetylation pharmacogenetics: the slow acetylator phenotype is caused by decreased or absent arylamine *N*-acetyltransferase in human liver. J. Clin. Investig., *85:* 968–972, 1990.

24. Deguchi, T. Sequences and expression of alleles of polymorphic arylamine *N*-acetyltransferase of human liver. J. Biol. Chem., *267:* 18140–18147, 1992.

25. Vatsis, K. P., and Weber, W. W. Structural heterogeneity of Caucasian *N*-acetyltransferase at the NAT1 gene locus. Arch. Biochem. Biophys., *301:* 71–76, 1993.

26. Vatsis, K. P., Weber, W. W., Bell, D. A., Dupret, J-M., Price-Evans, D. A., Grant, D. M., Hein, D. W., Lin, H. J., Meyer, U. A., Relling, M. V., Sim, E., Suzuki, T., and Yamazoe, Y. Nomenclature for *N*-acetyltransferases. Pharmacogenetics, *5:* 1–17, 1995.

27. Minchin, R. F. Acetylation of *p*-aminobenzoylglutamate, a folic acid catabolite, by recombinant human arylamine *N*-acetyltransferase and U937 cells. Biochem. J., *307:* 1–3, 1995.

28. Ward, A., Summer, M. J., and Sim, E. Purification of recombinant human *N*-acetyltransferase type 1 (NAT1) expressed in *E. coli* and characterization of its potential role in folate metabolism. Biochem. Pharmacol., *49:* 1759–1767, 1995.

29. Layton, D. W., Bogen, K. T., Knize, M. G., Hatch, F. T., Johnson, V. M., and Felton, J. S. Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. Carcinogenesis (Lond.), *16:* 39–52, 1995.

30. Dupret, J-M., and Grant, D. M. Site-directed mutagenesis of recombinant human arylamine *N*-acetyltransferase expressed in *E. coli*: evidence for direct involvement of $Cys^{68}$ in the catalytic mechanism of polymorphic human NAT2. J. Biol. Chem. *267:* 7381–7385, 1992.

31. Hein, D. W., Doll, M. A., Rustan, T. D., and Ferguson, R. J. Metabolic activation of *N*-hydroxyarylamines and *N*-hydroxyarylamides by 16 recombinant human NAT2 allozymes: effects of 7 specific NAT2 nucleic acid substitutions. Cancer Res., *55:* 3531–3536, 1995.

32. Hein, D. W., Flammang, T. J., Kirlin, W. G., Trinidad, A., and Ogolla, F. Acetylator genotype dependent metabolic activation of carcinogenic *N*-hydroxyarylamines by S-acetyl coenzyme A-dependent enzymes of inbred hamster tissue cytosols: relationship to arylamine *N*-acetyltransferase. Carcinogenesis (Lond.), *8:* 1767–1774, 1987.

33. Ogolla, F., Ferguson, R. J., Kirlin, W. G., Trinidad, A., Andrews, A. F., Mpezo, M., and Hein, D. W. Acetylator genotype-dependent expression of arylamine *N*-acetyltransferase and *N*-hydroxy-arylamine *O*-acetyltransferase in Syrian inbred hamster intestine and colon: identity with the hepatic acetylation polymorphism. Drug Metab. Dispos., *18:* 680–685, 1990.

34. Turesky, R. J., Lang, N. P., Butler, M. A., Teitel, C. H., and Kadlubar, F. F. Metabolic activation of carcinogenic heterocyclic aromatic amines by human liver and colon. Carcinogenesis (Lond.), *12:* 1839–1845, 1991.

35. Minchin, R. F., Reeves, P. T., Teitel, C. H., McManus, M. E., Mojarrabi, B., Ilett, K. F., and Kadlubar, F. F. *N*- and *O*-acetylation of aromatic and heterocyclic amine carcinogens by human monomorphic and polymorphic acetyltransferases expressed in COS-1 cells. Biochem. Biophys. Res. Commun., *185:* 839–844, 1992.

36. Hein, D. W., Doll, M. A., Rustan, T. D., Gray, K., Feng, Y., Ferguson, R. J., and Grant, D. M. Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. Carcinogenesis (Lond.), *14:* 1633–1638, 1993.

37. Hein, D. W., Rustan, T. D., Ferguson, R. J., Doll, M. A., and Gray, K. Metabolic activation of aromatic and heterocyclic *N*-hydroxyarylamines by wild-type and mutant recombinant human NAT1 and NAT2 acetyltransferases. Arch. Toxicol., *68:* 129–133, 1994.

38. Blum, M., Demierre, A., Grant, D. M., Heim, M., and Meyer, U. A. Molecular mechanism of slow acetylation of drugs and carcinogens in humans. Proc. Natl. Acad. Sci. USA, *88:* 5237–5241, 1991.

39. Vatsis, K. P., Martell, K. J., and Weber, W. W. Diverse point mutations in the human gene for polymorphic *N*-acetyltransferase. Proc. Natl. Acad. Sci. USA, *88:* 6333–6337, 1991.

40. Blum, M., Grant, D. M., McBride, W., Heim, M., and Meyer, U. A. Human arylamine *N*-acetyltransferase genes: isolation, chromosomal localization, and functional expression. DNA Cell Biol., *9:* 193–203, 1990.

41. Hickman, D., Risch, A., Buckle, V., Spurr, N., Jeremiah. S. J., McCarthy, A., and Sim, E. Chromosomal localization of human genes for arylamine *N*-acetyltransferase. Biochem. J., *297:* 441–445, 1994.

42. Matsus, N., Thygesen, P., Stacey, M., Risch, A., and Sim, E. Mapping AAC1, AAC2, and AACP, the genes for arylamine *N*-acetyltransferases, carcinogen metabolising enzymes on human chromosome 8p22, a region frequently deleted in tumours. Cytogenet. Cell Genet., *77:* 290–295, 1997.

43. Kelly, S. L., and Sim, E. Arylamine *N*-acetyltransferase in Balb/c mice: identification of a novel mouse isoenzyme by cloning and expression *in vitro*. Biochem. J., *302:* 347–353, 1994.

44. Fretland, A. J., Doll, M. A., Gray, K., Feng, Y., and Hein, D. W. Cloning, sequencing, and functional expression of NAT1, NAT2, and NAT3 derived from C3H/HeJ (rapid) and A/HeJ (slow) acetylator inbred mouse: functional characterization of the activation and deactivation of aromatic amine carcinogens. Toxicol. Appl. Pharmacol., *142:* 360–366, 1997.

45. Ebisawa, T., and Deguchi, T. Structure and restriction fragment length polymorphism of genes for human liver arylamine *N*-acetyltransferases. Biochem. Biophys. Res. Commun., *177:* 1252–1257, 1991.

46. Ishibe, N., Wiencke, J. K., Zuo, Z-F., McMillan, A., Spitz, M., and Kelsey, K. T. Polymorphisms in the *N*-acetyltransferase 1 (NAT1) gene and lung cancer risk in a minority population. Biomarkers, *3:* 219–226, 1998.

47. Zheng, W., Deitz, A. C., Campbell, D. R., Wen, W-Q., Cerhan, J. R., Sellers, T. A., Folsom, A. R., and Hein, D. W. *N*-Acetyltransferase 1 genetic polymorphism, cigarette smoking, well-done meat intake, and breast cancer risk. Cancer Epidemiol. Biomark. Prev., *8:* 233–239, 1999.

48. Probst-Hensch, N. M., Haile, R. W., Li, D. W., Sakamota, G. T., Louie, A. D., Lin, B. K., Frankl, H. D., Lee, E. R., and Lin, H. J. Lack of association between the polyadenylation polymorphism in the NAT1 (acetyltransferase 1) gene and colorectal adenomas. Carcinogenesis (Lond.), *17:* 2125–2129, 1996.

49. Ohsako, S., and Deguchi, T. Cloning and expression of cDNAs for polymorphic and monomorphic arylamine *N*-acetyltransferases from human liver. J. Biol. Chem., *265:* 4630–4634, 1990.

50. Doll, M. A., Jiang, W., Deitz, A. C., Rustan, T. D., and Hein, D. W. Identification of a novel allele at the human NAT1 acetyltransferase locus. Biochem. Biophys. Res. Commun., *233:* 584–591, 1997.

51. Hughes, N. C., Janezic. S. A., McQueen, K. L., Jewett, M. A. S., Castranio, T., Bell, D. A., and Grant, D. M. Identification and characterization of variant alleles of human acetyltransferase NAT1 with defective function using *p*-aminosalicylate as an *in-vivo* and *in-vitro* probe. Pharmacogenetics, *8:* 55–66, 1998.

52. Payton, M. A., and Sim, E. Genotyping human arylamine *N*-acetyltransferase type 1 (NAT1): the identification of two novel allelic variants. Biochem. Pharmacol., *55:* 361–366, 1998.

53. Hubbard, A., Moyes, C., Wyllie, A. H., Smith, C. A. D., and Harrison, D. J. *N*-Acetyltransferase 1: two polymorphisms in coding sequence identified in colorectal cancer patients. Br. J. Cancer, *77:* 913–916, 1998.

54. de Leon, J. H., Vatsis, K. P., and Weber, W. W. Catalytic activities of human *N*-acetyltransferase (NAT1) variants expressed in COS-1 cells. FASEB J., *10:* A456, 1996.

55. Butcher, N. J., Ilett, K. F., and Minchin, R. F. Functional polymorphism of the human arylamine *N*-acetyltransferase type 1 gene caused by C190T and G560A mutations. Pharmacogenetics, *8:* 67–72, 1998.

56. Lin, H. J., Probst-Hensch, N. M., Hughes, N. C., Sakamoto, G. T., Louie, A. D., Kau, I. H., Lin, B. K., Lee, D. B., Lin, J. Frankl, H. D., Lee, E. R., Hardy, S., Grant, D. M., and Haile, R. W. Variants of *N*-acetyltransferase NAT1 and a case-control study of colorectal adenomas. Pharmacogenetics, *8:* 269–281, 1998.

57. Deitz, A. C., Doll, M. A., Fretland, A. J., and Hein, D. W. *Homo sapiens N*-acetyltransferase NAT1 (allele *NAT1*18A*) gene, complete cds. GenBank AF032677, 1997.

58. Deitz, A. C., Doll, M. A., Fretland, A. J., and Hein, D. W. *Homo sapiens N*-acetyltransferase NAT1 (allele *NAT1*18B*) gene, complete cds. GenBank AF032678, 1997.

59. Deitz, A. C., Fretland, A. J., Leff, M. A., and Hein, D. W. *Homo sapiens N*-acetyltransferase-1 (*NAT1*) gene, *NAT1*26A* allele, complete cds. GenBank AF071552, 1998.

60. Deitz, A. C., Fretland, A. J., Leff, M. A., Doll, M. A., and Hein, D. W. *Homo sapiens N*-acetyltransferase-1 (*NAT1*) gene, *NAT1*26B* allele, complete cds. GenBank AF067408, 1998.

61. Lo-Guidice, J-M., Marez, D., Barat, F., Spire, C., Chevalier, D., and Broly, F. Human *N*-acetyltransferase 1 (*NAT1*) gene, *NAT1*28* allele. GenBank AF082904, 1999.

62. Lo-Guidice, J-M., Marez, D., Barat, F., Spire, C., Chevalier, D., and Broly, F. Human *N*-acetyltransferase 1 (*NAT1*) gene, *NAT1*29* allele. GenBank AF082903, 1999.

63. Grant, D. M., Blum, M., Demierre, A., and Meyer, U. A. Nucleotide sequence of an intronless gene for a human arylamine *N*-acetyltransferase related to polymorphic drug acetylation. Nucleic Acids Res., *17:* 3978, 1989.

64. Hickman, D., and Sim, E. *N*-Acetyltransferase polymorphism: comparison of phenotype and genotype in humans. Biochem. Pharmacol., *42:* 1007–1014, 1992.

65. Hickman, D., Risch, A., Camilleri, J. P., and Sim, E. Genotyping human polymorphic arylamine *N*-acetyltransferase: identification of new slow allotypic variants. Pharmacogenetics, *2:* 217–226, 1992.

66. Lin, J. J., Han, C-Y., Link B. K., and Hardy, S. Slow acetylator mutations in the human polymorphic *N*-acetyltransferase gene in 786 Asians, blacks, Hispanics, and whites: application to metabolic epidemiology. Am. J. Hum. Genet., *52:* 827–834, 1993.

67. Cascorbi, I., Drakoulis, N., Brockmoller, J., Mauer, A. Sperling, K., and Roots, I. Arylamine *N*-acetyltransferase (NAT2) mutations and their allelic linkage in unrelated Caucasian individuals: correlation with phenotypic activity. Am. J. Hum. Genet., *57:* 581–592, 1995.

68. Abe, M., Deguchi, T., and Suzuki, T. The structure and characteristics of a fourth allele of polymorphic *N*-acetyltransferase gene found in the Japanese population. Biochem. Biophys. Res. Commun., *191:* 811–816, 1993.

69. Ferguson, R. J., Doll, M. A., Rustan, T. D., Gray, K., and Hein, D. W. Cloning, expression, and functional characterization of two mutant (NAT2 191 and NAT2 341/803) and wild-type human polymorphic *N*-acetyltransferase (NAT2) alleles. Drug Metab. Dispos., *22:* 371–376, 1994.

70. Martinez, C., Agundez, J. A. G., Olivera, M., Martin, R., Ladero, J. M., and Benitez, J. Lung cancer and mutations at the polymorphic NAT2 gene locus. Pharmacogenetics, *5:* 207–214, 1995.

71. Agundez, J. A. G., Olivera, M., Ladero, J. M., Lescure-Rodriguez, A., Ledesma, M. C., Diaz-Rubio, M., Meyer, U. A., and Benitez, J. Increased risk for hepatocellular carcinoma in NAT2-slow acetylators and CYP2D6-rapid metabolizers. Pharmacogenetics, *6:* 501–512, 1996.

72. Agundez, J. A. G., Olivera, M., Martinez, C., Ladero, J. M., and Benitez, J. Identification and prevalence study of 17 allelic variants of the human *NAT2* gene in a white population. Pharmacogenetics, *6:* 423–428, 1996.

73. Leff, M. A., Fretland, A. J., Doll, M. A., and Hein, D. W. Novel human *N*-acetyltransferase 2 alleles that differ in mechanism for slow acetylator phenotype. J. Biol. Chem., *274:* 34519–34522, 1999.

74. Woolhouse, N. M., Qureshi, M. M., and Bayoumi, R. A. A new mutation C759T in the polymorphic *N*-acetyltransferase (NAT2) gene. Pharmacogenetics, *7:* 83–84, 1997.

75. Agundez, J. A., Martinez, C., Olivera, M., Ledesma, M. C., Ladero, J. M., and Benitez, J. Molecular analysis of the arylamine *N*-acetyltransferase polymorphism in a Spanish population. Clin. Pharmacol. Ther., *56:* 202–209, 1994.

76. Cascorbi, I., Brockmoller, J., Bauer, S., Reum, T., and Roots, I. NAT2*12A (803A→G) codes for rapid arylamine *N*-acetylation in humans. Pharmacogenetics, *6:* 257–259, 1996.

77. Bell, D. A., Taylor, J. A., Butler, M. A., Stephens, E. A., Wiest, J., Brubaker, L. H., Kadlubar, F. F., and Lucier, G. W. Genotype/phenotype discordance for human arylamine *N*-acetyltransferase (NAT2) reveals a new slow-acetylator allele common in African-Americans. Carcinogenesis (Lond.), *14:* 1689–1692, 1993.

78. Delomenie, C., Sica, L., Grant, D. M., Krishnamoorthy, R., and Dupret, J-M. Genotyping of the polymorphic *N*-acetyltransferase (NAT2*) gene locus in two native African populations. Pharmacogenetics, *6:* 177–185, 1996.

79. Lin, H. J., Han, C-Y., Lin, B. K., and Hardy, S. Ethnic distribution of slow acetylator mutations in the polymorphic *N*-acetyltransferase (NAT2) gene. Pharmacogenetics, *4:* 125–134, 1994.

80. Nebert, D. W., Ingelman-Sundberg, M., and Daly, A. K. Genetic epidemiology of environmental toxicity and cancer susceptibility: human allelic polymorphisms in drug metabolizing enzyme genes, their functional importance, and nomenclature issues. Drug Metab. Rev., *31:* 467–487, 1999.

81. Ilett, K. F., Kadlubar, F. F., and Minchin, R. F. 1998 Meeting on arylamine *N*-acetyltransferases: synopsis of the workshop on nomenclature, biochemistry, molecular biology, interspecies comparisons, and role in human disease risk. Drug Metab. Dispos., *27:* 957–959, 1999.

82. Hein, D. W., Grant, D. M., and Sim, E. Update on consensus arylamine *N*-acetyltransferase gene nomenclature. Pharmacogenetics, in press, 2000.

83. Shen, L. X., Basilion, J. P., and Stanton, V. P., Jr. Single-nucleotide polymorphisms can cause different structural folds of mRNA. Proc. Natl. Acad. Sci. USA, *96:* 7871–7876, 1999.

84. Cascorbi, I., and Roots, I. Pitfalls in *N*-acetyltransferase 2 genotyping. Pharmacogenetics, *9:* 123–127, 1999.

85. Rothman, N., Stewart, W. F., Caporaso, N. E., and Hayes, R. B. Misclassification of genetic susceptibility biomarkers: implications for case-control studies and cross-population comparisons. Cancer Epidemiol. Biomark. Prev., *2:* 299–303, 1993.

86. Rothman, N., Caporaso. N. E., Wacholder, S., Garcia-Closas, M., Lubin, J. H., Marcus, P., Hoover, R. N., and Fraumeni, J. F., Jr. Evaluation of interactions between environmental exposures and common genetic polymorphisms: a population-based epidemiologic perspective. Proc. Am. Assoc. Cancer Res., *40:* 762–763, 1999.

87. Blum, M., Grant, D. M., Demierre, A., and Meyer, U. A. *N*-Acetylation pharmacogenetics: a gene deletion causes absence of arylamine *N*-acetyltransferase in liver of slow acetylator rabbits. Proc. Natl. Acad. Sci. USA, *86:* 9554–9557, 1989.

88. Ferguson, R. J., Doll, M. A., Rustan, T. D., Baumstark, B. R., and Hein, D. W. Polymorphic arylamine *N*-acetyltransferase encoding gene (NAT2) from homozygous rapid and slow acetylator congenic Syrian hamsters. Gene (Amst.), *140:* 247–249, 1994.

89. Nagata, K., Ozawa, S., Miyata, M., Shimada, M., Yamazoe, Y., and Kato, R. Primary structure and molecular basis of polymorphic appearance of an acetyltransferase (AT-II) in hamsters. Pharmacogenetics, *4:* 91–100, 1994.

90. Ferguson, R. J., Doll, M. A., Rustan, T. D., and Hein, D. W. Cloning, expression, and functional characterization of rapid and slow acetylator polymorphic *N*-acetyltransferase encoding genes of the Syrian hamster. Pharmacogenetics, *6:* 55–66, 1996.

91. Martell, J. J., Vatsis, K. P., and Weber, W. W. Molecular genetic basis of rapid and slow acetylator phenotype in mice. Mol. Pharmacol., *40:* 218–227, 1991.

92. de Leon, J. H., Martell, K. J., Vatsis, K. P., and Weber, W. W. Slow acetylation in mice is caused by a labile and catalytically impaired mutant *N*-acetyltransferase (NAT2 9). Drug Metab. Dispos., *23:* 1354–1361, 1995.

93. Ferguson, R. J., Doll, M. A., Rustan, T. D., Baumstark, B. R., and Hein, D. W. Syrian hamster monomorphic *N*-acetyltransferase (*NAT1*) alleles: amplification, cloning, sequencing, and expression in *E. coli*. Pharmacogenetics, *4:* 82–90, 1994.

94. Hein, D. W., Ferguson, R. J., Doll, M. A., Rustan, T. D., and Gray, K. Molecular genetics of human polymorphic *N*-acetyltransferase: enzymatic analysis of 15 recombinant human wild-type, mutant, and chimeric NAT2 allozymes. Hum. Mol. Genet., *3:* 729–734, 1994.

95. Dupret, J-M., Goodfellow, G. H., Janezic, S. A., and Grant, D. M. Structure-function studies of human arylamine *N*-acetyltransferases NAT1 and NAT2: functional analysis of NAT1/NAT2 protein chimeras expressed in *E. coli*. J. Biol. Chem., *269:* 26830–26835, 1994.

96. Hickman, D., Palamanda, J. R., Unadkat, J. D., and Sim, E. Enzyme kinetic properties of human recombinant arylamine *N*-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*. Biochem. Pharmacol., *50:* 697–703, 1995.

97. Delomenie, C., Goodfellow, G. H., Krishnamoorthy, R., Grant, D. M., and Dupret, J-M. Study of the role of the highly conserved residues $Arg^{9}$ and $Arg^{64}$ in the catalytic function of the human *N*-acetyltransferases NAT1 and NAT2 by site-directed mutagenesis. Biochem. J., *323:* 207–215, 1997.

98. O'Neil, W. M., Drobitch, R. K., MacArthur, R. D., Farrough, M. J., Doll, M. A., Fretland, A. J., Hein, D. W., Crane, L. R., and Svensson, C. R. Acetylator phenotype and genotype in patients infected with HIV: discordance between methods for phenotype determination and genotype. Pharmacogenetics, in press, 2000.

99. Fretland, A. J., Leff, M. A., Doll, M. A., and Hein, D. W. Functional analysis of single nucleotide substitutions in the human *NAT2* gene in a yeast expression system. Proc. Am. Assoc. Cancer Res., *40:* 469, 1999.

100. Cartwright, R. A., Glashan, R. W., Rogers, H. J., Ahmad, R. A., Barham-Hall, D., Higgins, E., and Kahn, M. A role of *N*-acetyltransferase phenotypes in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. Lancet, *2:* 842–846, 1982.

101. Vesell, E., and Gaylor, D. Limitations of probit plots in pharmacogenetics: requirement of genetic analyses to test hypotheses based on graphical methods. Pharmacogenetics, *5:* 18–23, 1995.

102. Grant, D. M., Tang, B. K., and Kalow, W. A simple test for acetylator phenotype using caffeine. Br. J. Clin. Pharmacol., *17:* 43–50, 1984.

103. Cribb, A. E., Isbrucker, R., Levatte, T., Tsui, B., Gillespie, C. T., and Renton, K. W. Acetylator phenotyping: the urinary caffeine metabolite ratio in slow acetylators correlated with a marker of systemic NAT1 activity. Pharmacogenetics, *4:* 166–170, 1994.

104. Lorenzo, B., and Reidenberg, M. M. Potential artifacts in the use of caffeine to determine acetylation phenotype. Br. J. Clin. Pharmacol., *28:* 207–208, 1989.

105. Leyland-Jones, B., Banerjee, K., Lang, N., and Wong, P. A reliable method for NAT2 phenotyping by ELISA. Proc. Am. Assoc. Cancer Res., *40:* 53, 1999.

106. Hein, D. W., Doll, M. A., Rustan, T. D., Gray, K., Ferguson, R. J., and Feng, Y. Construction of Syrian hamster lines congenic at the polymorphic acetyltransferase locus (NAT2): acetylator genotype-dependent *N*- and *O*-acetylation of arylamine carcinogens. Toxicol. Appl. Pharmacol., *124:* 16–24, 1994.

107. Hein, D. W., Rustan, T. D., Bucher, K. D., and Miller, L. S. Polymorphic and monomorphic expression of arylamine carcinogen *N*-acetyltransferase isozymes in tumor target organ cytosols of Syrian hamsters congenic at the polymorphic acetyltransferase locus. J. Pharmacol. Exp. Ther., *259:* 699–704, 1991.

108. Hein, D. W., Doll, M. A., Gray, K., Rustan, T. D., and Ferguson, R. J. Metabolic activation of *N*-hydroxy-2-aminofluorene and *N*-hydroxy-2-acetylaminofluorene by monomorphic *N*-acetyltransferase (NAT1) and polymorphic *N*-acetyltransferase (NAT2) in colon cytosols of Syrian hamsters congenic at the NAT2 locus. Cancer Res., *53:* 509–514, 1993.

109. Minchin, R. F., Butcher, N. J., and Pope, C. Characterization of the human *N*-acetyltransferase type I gene promotor: identification of ATCATTT repeats flanking an AP-1 like motif in the minimum promotor sequence. *In:* Proceedings of the First International Workshop on Arylamine *N*-Acetyltransferases, p. 4. Cairns, Australia: , 1998.

110. Butcher, N. J., Ilett, K., and Minchin, R. F. Substrate regulation of human arylamine *N*-acetyltransferase type-1. *In:* Proceedings of the First International Workshop on Arylamine *N*-Acetyltransferases, p. 22. Cairns, Australia: , 1998.

111. Estrada-Rodgers, L., Levy, G. N., and Weber, W. W. Characterization of a hormone response element in the mouse *N*-acetyltransferase 2 (Nat2*) promoter. Gene Expr., *7:* 13–24, 1998.

112. Bell, D. A., Badawi, A. F., Lang, N. P., Ilett, K. F., Kadlubar, F., and Hirvonen, A. Polymorphism in the NAT1 polyadenylation signal: association of *NAT1*10* allele with higher *N*-acetylation activity in bladder and colon tissue samples. Cancer Res., *55:* 5226–5229, 1995.

113. Zenser, R. B., Lakshmi, V. M., Rustan, T. R., Doll, M. A., Deitz, A. C., Davis, B. B., and Hein, D. W. Human *N*-acetylation of benzidine: role of NAT1 and NAT2. Cancer Res., *56:* 3941–3947, 1996.

114. Badawi, A. F., Bell, D. A., Hirvonen, A., and Kadlubar, F. F. Role of aromatic amine acetyltransferases, NAT1 and NAT2, in carcinogen-DNA adduct formation in the human urinary bladder. Cancer Res., *55:* 5230–5237, 1995.

115. Bell, D. A., Stephens, E., Castranio, T., Umbach, D. M., Watson, M., Deakin, M., Elder, J., Duncan, H., Hendrickse, C., and Strange, R. C. Polyadenylation polymorphism in the *N*-acetyltransferase gene 1 (*NAT1*) increases risk of colorectal cancer. Cancer Res., *55:* 3537–3542, 1995.

116. Grant, D. M., Hughes, N. C., Janezic, S. A., Sampson, H., Darlington, G., and Jewett, M. A. Acetylation polymorphisms and risk for bladder cancer: a case-control study. *In:* Proceedings of the First International Workshop on Arylamine *N*-Acetyltransferases, p. 8. Cairns, Australia: , 1998.

117. Patrianakos, C., and Hoffmann, D. Chemical studies on tobacco smoke. LXIV. On the analysis of aromatic amines in cigarette smoke. J. Anal. Toxicol., *3:* 150–154, 1979.

118. Manabe, S., Tohyama, K., Wada, O., and Aramaki, T. Detection of a carcinogen, 2-amino-1-methyl-6-phenylimidazo [4:5-b]pyridine (PhIP) in cigarette smoke condensate. Carcinogenesis (Lond.), *12:* 1945–1947, 1991.

119. Doll, M. A., Fretland, A. J., Deitz, A. C., and Hein, D. W. Determination of human NAT2 acetylator genotype by restriction fragment-length polymorphism and allele-specific amplification. Anal. Biochem., *231:* 413–420, 1995.

120. Bigler, J., Chen, C., and Potter, J. D. Determination of human NAT2 acetylator genotype by oligonucleotide ligation assay. Biotechniques, *22:* 682–690, 1997.

121. Deitz, A. C., Doll, M. A., and Hein, D. W. A restriction fragment length polymorphism assay that differentiates human *N*-acetyltransferase-1 (NAT1) alleles. Anal. Biochem., *253:* 219–224, 1997.

122. Mrozikiewicz, P. M., Cascorbi, I., Brockmoller, J., and Roots, I. Determination and allelic allocation of seven nucleotide transitions within the arylamine *N*-acetyltransferase (NAT2) gene in the Polish population. Clin. Pharmacol. Ther., *59:* 376–382, 1996.

123. Lower, G. M., Jr., Nilsson, T., Nelson, C. E., Wolf H., Gamsky, T. E., and Bryan, G. T. *N*-Acetyltransferase phenotype and risk in urinary bladder cancer: approaches in molecular epidemiology. Preliminary results in Sweden and Denmark. Environ. Health Perspect., *29:* 71–79, 1979.

124. Hayes, R. B., Bi, W., Rothman, N., Broly, F., Caporaso, N., Feng, P., You, X., Yin, S., Woosley, R. S., and Meyer, U. A. *N*-Acetylation phenotype and genotype and risk of bladder cancer in benzidine-exposed workers. Carcinogenesis (Lond.), *14:* 675–678, 1993.

125. Risch, A., Wallace, D. M., Bathers, S., and Sim, E. Slow N-acetylation genotype is a susceptibility factor in occupational and smoking related bladder cancer. Hum. Mol. Genet., *4:* 231–236, 1995.

126. Brockmoller, J., Cascorbi, I., Kerb, R., and Roots, I. Combined analysis of inherited polymorphisms in arylamine *N*-acetyltransferase 2, glutathione *S*-transferases M1 and T1, microsomal epoxide hydrolase, and cytochrome P450 enzymes as modulators of bladder cancer risk. Cancer Res., *56:* 3915–3925, 1996.

127. Okkels, H., Sigsgaard, T., Wolf, A., and Autrup, H. Arylamine *N*-acetyltransferase 1 (NAT1) and 2 (NAT2) polymorphisms in susceptibility to bladder cancer: the influence of smoking. Cancer Epidemiol. Biomark. Prev., *6:* 225–231, 1997.

128. Filiadis, I. F., Georgiou, I., Alamanos, Y., Kranas, V., Giannakopoulos, X., and Lolis, D. Genotypes of *N*-acetyltransferase-2 and risk of bladder cancer: a case-control study. J. Urol., *161:* 1672–1675, 1999.

129. Vineis, P., Bartsch, H., Caporaso, N., Harrington, A. M., Kadlubar, F. F., Landi, M. T., Malaveille, C., Shields, P. G., Skipper, P., Talaska, G., and Tannenbaum, S. R. Genetically based *N*-acetyltransferase polymorphism and low-level environmental exposure to carcinogens. Nature (Lond.), *369:* 154–156, 1994.

130. Yu, M. C., Skipper, P. L., Taghizadeh, K., Tannenbaum, S. R., Chan, K. K., Henderson, B., and Ross, R. K. Acetylator phenotype, aminobiphenyl-hemoglobin adduct levels, and bladder cancer risk in white, black, and Asian men in Los Angeles, California. J. Natl. Cancer Inst., *86:* 712–716, 1994.

131. Taylor, J. A., Umbach, D. M., Castranio, T., Paulson, D., Robertson, C. Mohler, J. L., and Bell, D. A. The role of *N*-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene-exposure three-way interaction. Cancer Res., *58:* 3603–3610, 1998.

132. Katoh, T., Inatomi, H., Yang, M., Kawamato, T., Matsumota, T., and Bell, D. A. Arylamine *N*-acetyltransferase 1 (NAT1) and 2 (NAT2) genes and risk of urothelial transitional cell carcinoma among Japanese. Pharmacogenetics, *9:* 401–404, 1999.

133. Stanley, L. A., Coroneos, E., Cuff, R., Hickman, D., Ward, A., and Sim, E. Immunochemical detection of arylamine *N*-acetyltransferase in normal and neoplastic bladder. J. Histochem. Cytochem., *44:* 1059–1067, 1996.

134. Kloth, M. T., Gee, R. L., Messing, E. M., and Swaminathan, S. Expression of *N*-acetyltransferase (NAT) in cultured human uroepithelial cells. Carcinogenesis (Lond.), *15:* 2781–2878, 1994.

135. Kirlin, W. G., Trinidad, A., Yerokun, T., Ogolla, F., Ferguson, R. J., Andrews, A. F., Brady, P. K., and Hein, D. W. Polymorphic expression of AcCoA-dependent arylamine *N*-acetyltransferase and AcCoA-dependent *O*-acetyltransferase activation of *N*-hydroxyarylamines by human bladder cytosol. Cancer Res., *49:* 2448–2454, 1989.

136. Frederickson, S. M., Hatcher, J. F., Reznikoff, C. A., and Swaminathan, S. Acetyltransferase-mediated metabolic activation of *N*-hydroxy-4-aminobiphenyl by human urothelial cells. Carcinogenesis (Lond.), *13:* 955–961, 1992.

137. Levy, G. N., and Weber, W. W. 2-Aminofluorene-DNA adducts in mouse urinary bladder: effect of age, sex and acetylator phenotype. Carcinogenesis (Lond.), *13:* 159–164, 1992.

138. Jiang, W., Feng, Y., and Hein, D. W. Higher DNA adduct levels in urinary bladder and prostate of slow acetylator inbred rats administered 3,2′-dimethyl-4-aminobiphenyl. Toxicol. Appl. Pharmacol., *156:* 187–194, 1999.

139. Feng, Y., Jiang, W., and Hein, D. W. 2-Aminofluorene-DNA adduct levels in tumor-target and nontarget organs of rapid and slow acetylator Syrian hamsters congenic at the NAT2 locus. Toxicol. Appl. Pharmacol., *141:* 248–255, 1996.

140. Feng, Y., Jiang, W., Deitz, A. C., and Hein, D. W. 3,2′-Dimethyl-4-aminobiphenyl-DNA adduct formation in tumor target and nontarget organs of rapid and slow acetylator Syrian hamsters congenic at the NAT2 locus. Toxicol. Appl. Pharmacol., *140:* 315–321, 1996.

141. Hein, D. W., Kirlin, W. G., Yerokun, T., Trinidad, A., and Ogolla, F. Inheritance of acetylator genotype-dependent arylamine *N*-acetyltransferase in hamster bladder cytosol. Carcinogenesis (Lond.), *8:* 647–652, 1987.

142. Hein, D. W., Rustan, T. D., Bucher, K. D., Furman E. J., and Martin, W. J. Extrahepatic expression of the *N*-acetylation polymorphism towards arylamine carcinogens in tumor target organs of an inbred rat model. J. Pharmacol. Exp. Ther., *258:* 232–236, 1991.

143. Chung, J. G., Levy, G. N., and Weber, W. W. Distribution of 2-aminofluorene and *p*-aminobenzoic acid *N*-acetyltransferase activity in tissues of C57BL/6J rapid and B6.A-Nat$^s$ slow acetylator congenic mice. Drug Metab. Dispos., *21:* 1057–1063, 1993.

144. Stanley, L. A., Mills, I. G., and Sim, E. Localization of polymorphic *N*-acetyltransferase (NAT2) in tissues of inbred mice. Pharmacogenetics, *7:* 121–130, 1997.

145. Longuemaux, S., Delomenie, C., Gallou, C., Mejean, A., Vincent-Viry, M. N., Bouvier, R., Droz, D., Krishnamoorthy, R., Galteau, M. M., Junien, C., Beroud, C., and Dupret, J. M. Candidate genetic modifiers of individual susceptibility to renal cell carcinoma: a study of polymorphic human xenobiotic-metabolizing enzymes. Cancer Res., *59:* 2903–2908, 1999.

146. Trinidad, A., Hein, D. W., Rustan, T. D., Ferguson, R. J., Miller, L. S., Bucher, K. D., Kirlin, W. G., Ogolla, F., and Andrews, A. F. Purification of hepatic polymorphic arylamine *N*-acetyltransferase from homozygous rapid acetylator inbred hamster: identity with polymorphic *N*-hydroxyarylamine *O*-acetyltransferase. Cancer Res., *50:* 7942–7949, 1990.

147. Stillwell, W. G., Kidd, L. C., Wishnok, J. S., Tannenbaum, S. R., and Sinha, R. Urinary excretion of unmetabolized and Phase II conjugates of 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine and 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline in humans: relationship to cytochrome P4501A2 and *N*-acetyltransferase activity. Cancer Res., *15:* 3457–3464, 1997.

148. Kirlin, W. G., Ogolla, F., Andrews, A. F., Trinidad, A., Ferguson, R. J., Yerokun, T., Mpezo, M., and Hein, D. W. Acetylator genotype-dependent expression of arylamine *N*-acetyltransferase in human colon cytosol from non-cancer and colorectal cancer patients. Cancer Res., *51:* 549–555, 1991.

149. Lang, N. P., Chu, D. Z., Hunter, C. F., Kendall, D. C., Flammang, T. J., and Kadlubar, F. F. Role of aromatic amine acetyltransferase in human colorectal cancer. Arch. Surg., *121:* 1259–1261, 1986.

150. Ilett, K. F., David, B. M., Dethon, P., Castleden, W. M., and Kwa, R. Acetylator phenotype in colorectal carcinoma. Cancer Res., *47:* 1466–1469, 1987.

151. Gil, J. P., and Lechner, M. C. Increased frequency of wild-type arylamine-*N*-acetyltransferase allele NAT2*4 homozygotes in Portuguese patients with colorectal cancer. Carcinogenesis (Lond.), *19:* 37–41, 1998.

152. Ladero, J. M., Gonzalez, J. F., Benitez, J., Vargas, E., Fernandez, M. J., Baki, W., and Diaz-Rubio, M. Acetylator polymorphism in human colorectal carcinoma. Cancer Res., *51:* 2098–2100, 1991.

153. Rodriguez, J. W., Kirlin, W. G., Ferguson, R. J., Doll, M. A., Gray, K., Rustan, T. D., Lee, M. E., Kemp, K., Urso, P., and Hein, D. W. Human acetylator genotype: relationship to colorectal cancer incidence and arylamine *N*-acetyltransferase expression in colon cytosol. Arch. Toxicol., *67:* 445–452, 1993.

154. Shibuta, K., Nakashima, T., Abe, M., Mashimo, M., Mori, M., Ueo, H., Akiyoshi, T., Sugimachi, K., and Suzuki, T. Molecular genotyping for *N*-acetylation polymorphism in Japanese patients with colorectal cancer. Cancer (Phila.), *74:* 3108–3112, 1994.

155. Probst-Hensch, N. M., Haile, R. W., Ingles, S. A., Longnecker, M. P., Han, C. Y., Lin, B. K., Lee, D. B., Sakamota, G. T., Franskl, H. D., Lee, E. R., and Lin, H. J. Acetylation polymorphism and prevalence of colorectal adenomas. Cancer Res., *55:* 2017–2020, 1995.

156. Spurr, N. K., Gough, A. C., Chinegwundoh, F. I., and Smith, C. A. D. Polymorphisms in drug-metabolizing enzymes as modifiers of cancer risk. Clin. Chem., *41:* 1864–1869, 1995.

157. Hubbard, A. L., Harrison, D. J., Moyes, C., Wyllie, A. H., Cunningham, C., Mannion, E., and Smith, C. A. D. *N*-Acetyltransferase 2 genotype in colorectal cancer and selective gene retention in cancers with chromosome 8p deletions. Gut, *41:* 229–234, 1997.

158. Lang, N. P., Butler, M. A., Massengell, J., Lawson, M., Stotts, R. C., Hauer-Jensen, M., and Kadlubar, F. F. 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. Biomark. Prev., *3:* 675–682, 1994.

159. Roberts-Thomson, I. C., Ryan, P., Khoo, K. K., Hart, W. J., McMichael, A. J., and Butler, R. N. Diet, acetylator phenotype, and risk of colorectal neoplasia. Lancet, *347:* 1372–1374, 1996.

160. Welfare, M. R., Cooper, J., Bassendine, M. F., and Daly, A. K. Relationship between acetylator status, smoking, diet and colorectal cancer risk in the north-east of England. Carcinogenesis (Lond.), *18:* 1351–1354, 1997.

161. Chen, J., Stampfer, M. J., Hough, H. L., Garcia-Closas, M., Willett, W. C., Hennekens, C. H., Kelsey, K. T., and Hunter, D. J. A prospective study of *N*-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. Cancer Res., *58:* 3307–3311, 1998.

162. Kampman, E., Slattery, M. L., Bigler, J., Leppert, M., Samowitz, W., Caan, B. J., and Potter, J. D. Meat consumption genetic susceptibility and colon cancer

risk: a United States multicenter case-control study. Cancer Epidemiol. Biomark. Prev., *8:* 15–24, 1999.

163. Skog, K., Steineck, G., Augustsson, K., and Jugerstad, M. Effect of cooking temperature on the formation of heterocyclic amines in fried meat products and pan residues. Carcinogenesis (Lond.), *16:* 861–867, 1995.

164. DeMarini, D. M., Hastings, S. B., Brooks, L. R., Eischen, B. T., Bell, D. A., Watson, M. A., Felton, J. S., Sandler, R., and Kohlmeiter, L. Pilot study of free and conjugated urinary mutagenicity during consumption of pan-fried meats: possible modulation by cruciferous vegetables, glutathione *S*-transferase M1, and *N*-acetyltransferase-2. Mutat. Res., *19:* 83–96, 1997.

165. Cascorbi, I., Brockmoller, J., Mrozikiewicz, P. M., Bauer, S., Loddenkemper, R., and Roots, I. Homozygous rapid arylamine *N*-acetyltransferase (NAT2) genotype as a susceptibility factor for lung cancer. Cancer Res., *56:* 3961–3966, 1996.

166. Henning, S., Cascorbi, I., Munchow, B., Jahnke, V., and Roots, I. Association of arylamine *N*-acetyltransferases NAT1 and NAT2 genotypes to laryngeal cancer risk. Pharmacogenetics, *9:* 103–111, 1999.

167. Smelt, V. A., Mardon, H. J., and Sim, E. Placental expression of arylamine *N*-acetyltransferases: evidence for linkage disequilibrium between *NAT1*10* and *NAT2*4* alleles of the two human *N*-acetyltransferase loci NAT1 and NAT2. Pharmacol. Toxicol., *83:* 149–157, 1998.

168. Windmill, K. F., McKinnon, R. A., Zhu, X., Gaedigk, A., Grant, D. M., and McManus, M. E. The role of xenobiotic metabolizing enzymes in arylamine toxicity and carcinogenesis: functional and localization studies. Mutat. Res., *376:* 153–160, 1997.

169. Ilett, K. F., Reeves, P. T., Minchin, R. F., Kinnear, B. F., Watson, H. F., and Kadlubar, F. F. Distribution of acetyltransferase activities in the intestines of rapid and slow acetylator rabbits. Carcinogenesis (Lond.), *12:* 1465–1469, 1991.

170. Ware, J. A., and Svensson, C. K. Longitudinal distribution of arylamine *N*-acetyltransferases in the intestine of the hamster, mouse, and rat. Evidence for multiplicity of *N*-acetyltransferases in the intestine. Biochem. Pharmacol., *52:* 1613–1620, 1996.

171. Ware, J. A., Reilly, T. P., and Svensson, C. K. Cellular distribution of *N*-acetyltransferase activity in the rat small intestine. Biochem. Pharmacol., *55:* 1475–1479, 1998.

172. Hickman, D., Pope, J., Patil, S. D., Gakis, G., Smelt, V., Stanley, L. A., Payton, M., Unadkat, J. D., and Sim, E. Expression of arylamine *N*-acetyltransferase in human intestine. Gut, *42:* 402–409, 1998.

173. Feng, Y., Wagner, R. J., Fretland, A. J., Becker, W. K., Cooley, A. M., Pretlow, T. P., Lee, K. J., and Hein, D. W. Acetylator genotype (NAT2)-dependent formation of aberrant crypts in congenic Syrian hamsters administered 3,2′-dimethyl-4-aminobiphenyl. Cancer Res., *56:* 527–531, 1996.

174. Paulsen, J. E., Steffensen, I. L., Namourk, E., Hein, D. W., and Alexander, J. Effect of acetylator genotype on 3,2-dimethyl-4-aminobiphenyl induced aberrant crypt foci in the colon of hamsters. Carcinogenesis (Lond.), *17:* 459–465, 1996.

175. Feng, Y., Fretland, A. J., Rustan, T. D., Jiang, W., Becker, W. K., and Hein, D. W. Higher frequency of aberrant crypt foci in rapid than slow acetylator inbred rats administered the colon carcinogen 3,2′-dimethyl-4-aminobiphenyl. Toxicol. Appl. Pharmacol., *147:* 56–62, 1997.

176. Purewal, M., Fretland, A. J., Schut, H. A. J., Hein, D. W., and Wargovich, M. J. Association between acetylator genotype and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) DNA adduct formation in colon and prostate of inbred Fischer 344 and Wistar Kyoto rats. Cancer Lett., in press.

177. Ladero, J. M., Fernandez, M. J., Palmeiro, R., Munoz, J. J., Jara, C., Lazaro, C., and Periz-Manga, G. Hepatic acetylator polymorphism in breast cancer patients. Oncology (Basel), *44:* 341–344, 1987.

178. Webster, D. J., Flook, D., Jenkins, J., Hutchings, A., and Routledge, P. A. Drug acetylation in breast cancer. Br. J. Cancer, *60:* 236–237, 1989.

179. Ilett, K. F., Detchon, P., Ingram D. M., and Castelden, W. M. Acetylation phenotype is not associated with breast cancer. Cancer Res., *50:* 6649–6651, 1990.

180. Bulovskaya, L. N., Krupkin, R. G., Bochina, T. A., Shipkova, A. A., and Pavlova, M. V. Acetylator phenotype in patients with breast cancer. Oncology (Basel), *35:* 185–188, 1978.

181. Sardas, S., Cok, I., Sardas, O. S., Ilhan, O., and Karakaya, A. E. Polymorphic *N*-acetylation capacity in breast cancer patients. Int. J. Cancer, *46:* 1138–1139, 1990.

182. Philip, P. A., Rogers, H. J., Millis, R. R., Rubens R. D., and Cartwright, R. A. Acetylator status and its relationship to breast cancer and other diseases of the breast. Eur. J. Cancer Clin. Oncol., *23:* 1701–1706, 1987.

183. Ambrosone, C. B., Freudenheim, J. L., Sinha, R., Graham, S., Marshall, J. R., Vena, J. E., Laughlin, R., Nemota, T., and Shields, P. G. Breast cancer risk, meat consumption and *N*-acetyltransferase (NAT2) genetic polymorphisms. Int. J. Cancer, *75:* 825–830, 1998.

184. Gertig, D. M., Hankinson, S. E., Hough, H., Spiegelman, D., Colditz, G. A., Willett, W. C., Kelsey K. T., and Hunter, D. J. *N*-Acetyltransferase 2 genotypes, meat intake and breast cancer risk. Int. J. Cancer, *80:* 13–17, 1999.

185. Deitz, A. C., Zheng, W., Leff, M. A., Gross, M., Xiao, G-h., Doll, M. A., Wen, W-Q., Folsom, A. R., and Hein, D. W. *N*-Acetyltransferase-2 (NAT2) acetylation polymorphism, well-done meat intake and breast cancer risk among post-menopausal women. Proc. Am. Assoc. Cancer Res., *40:* 148, 1999.

186. Ambrosone, C. B., Freudenheim, J. L., Graham, S., Marshall, J. R., Vena, J. E., Brasure, J. R., Michalek, A. M., Laughlin, R., Nemota, T., Gillenwater, K. A., and Shields, P. G. Cigarette smoking, *N*-acetyltransferase 2 genetic polymorphisms, and breast cancer risk. J. Am. Med. Assoc., *276:* 1494–1501, 1996.

187. Huang, C. S., Chern, H. D., Shen, C. Y., and Chang, K. J. Association between *N*-acetyltransferase 2 (NAT2) genetic polymorphism and development of breast cancer in post-menopausal Chinese women in Taiwan, an area of great increase in breast cancer incidence. Int. J. Cancer, *82:* 175–179, 1999.

188. Hunter, D. J., Hankinson, S. E., Hough, H., Gertig, D. A., Garcia-Closas, M., Spiegelman, D., Manson, J. E., Colditz, G. A., Willet, W. C., Speizer, F. E., and Kelsey, K. A prospective study of NAT2 acetylation genotype, cigarette smoking and risk of breast cancer. Carcinogenesis (Lond.), *18:* 2127–2132, 1997.

189. Millikan, R. C., Pittman, G. S., Newman, B., Tse, C-K. J., Selmin, O., Rockhill, B., Savitz, D., Moorman, P. G., and Bell, D. A. Cigarette smoking, *N*-acetyltransferases 1 and 2, and breast cancer risk. Cancer Epidemiol. Biomark. Prev., *7:* 371–378, 1998.

190. Sadrieh, N., Davis, C. D., and Snyderwine, E. G. *N*-Acetyltransferase expression and metabolic activation of the food-derived heterocyclic amines in the human mammary gland. Cancer Res., *56:* 2683–2687, 1996.

191. Stone, E. M., Williams, J. A., Grover, P. L., Gusterson, B. A., and Phillips, D. H. Interindividiaul variation in the metabolic activation of heterocyclic amines and their *N*-hydroxy derivatives in primary cultures of human mammary epithelial cells. Carcinogenesis (Lond.), *19:* 873–879, 1998.

192. Pfau, W., Stone, E. M., Brockstedt, U., Carmicheal, P. L., Marquardt, H., and Phillips, D. H. DNA adducts in human breast tissue: association with *N*-acetyltransferase-2 (NAT2) and NAT1 genotypes. Cancer Epidemiol. Biomark. Prev., *7:* 1019–1025, 1998.

193. Burgess, E. J., and Trafford, J. A. Acetylator phenotype in patients with lung carcinoma: a negative report. Eur. J. Respir. Dis., *67:* 17–19, 1985.

194. Roots, I., Drakoulis, N., Ploch, M., Heinemeyer, G., Loddenkemper, R., Minks, T., Nitz, M., Otte, F., and Koch, M. Debrisoquine hydroxylation phenotype, acetylation phenotypes, and ABO blood groups as genetic host factors of lung cancer risk. Klin. Wochenshr., *66* (Suppl XI)*:* 87–97, 1988.

195. Philip, P. A., Fitzgerald, D. L., Cartwright, R. A., Peake, M. D., and Rogers, H. J. Polymorphic *N*-acetylation capacity in lung cancer. Carcinogenesis (Lond.), *9:* 491–493, 1988.

196. Nyberg, F., Hou, S-M., Hemminki, K., Lambert, B., and Pershagen, G. Glutathione *S*-transferase M1 and *N*-acetyltransferase 2 genetic polymorphisms and exposure to tobacco smoke in nonsmoking and smoking lung cancer patients and population controls. Cancer Epidemiol. Biomark. Prev., *7:* 875–883, 1998.

197. Bouchardy, C., Mitrunen, K., Wikman, H., Husgafvel-Pursiainen, K., Dayer, P., Benhamou, S., and Hirvonen, A. *N*-Acetyltransferase NAT1 and NAT2 genotypes and lung cancer risk. Pharmacogenetics, *8:* 291–298, 1998.

198. Hirvonen, A., Pelin, K., Tammilehto, L., Karjalainen, A., Mattson, K., and Linnainmaa, K. Inherited GSTM1 and NAT2 defects as concurrent risk modifiers in asbestos-related human malignant mesothelioma. Cancer Res., *55:* 2981–2983, 1995.

199. Hirvonen, A., Saarikoski, S. T., Linnainmaa, K., Loskinen, K., Husgafvel-Puriainen, K., Matttson, K., and Vainio, H. Glutathione *S*-transferase and *N*-acetyltransferase genotypes and asbestos-associated pulmonary disorders. J. Natl. Cancer Inst., *88:* 1853–1856, 1996.

200. Drozdz, M., Gierek, T., Jesdryczko, A., Pilch, J., and Piekarska, J. *N*-Acetyltransferase phenotype of patients with cancer of the larynx. Neoplasma (Bratisl.), *34:* 481–484, 1987.

201. Gonzalez, M. V., Alvarez, V., Pello, M. F., Menendez, M. J., Suarex, C., and Cota, E. Genetic polymorphism of *N*-acetyltransferase-2, glutathione *S*-transferase-M1, and cytochromes P450IIE1 and P450IIB in the susceptibility to head and neck cancer. J. Clin. Pathol., *51:* 294–298, 1998.

202. Morita, S., Yano, M., Tasujinaka, T., Ogawa, A., Taniguchi, M., Kaneko, K., Shiozaki, H., Doki, Y., Inoue, M., and Monden, M. Association between genetic polymorphisms of glutathione *S*-transferase P1 and *N*-acetyltransferase 2 and susceptibility to squamous-cell carcinoma of the esophagus. Int. J. Cancer, *79:* 517–520, 1998.

203. Morita, S., Yano, M., Tsujinaka, T., Akiyama, Y., Taniguchi, M., Kaneko, K., Miki, H., Fujii, T., Yoshino, K., Kusuoka, H., and Monden, M. Genetic polymorphisms of drug-metabolizing enzymes and susceptibility to head and neck squamous-cell carcinoma. Int. J. Cancer, *80:* 685–688, 1999.

204. Jourenkova-Mironova, N., Wikman, H., Brouchardy, C., Mitrunen, K. Dayer, P., Benhamou, S., and Hirvonen, A. Role of arylamine *N*-acetyltransferase 1 and 2 (*NAT1* and *NAT2*) genotypes in susceptibility to oral/pharyngeal and laryngeal cancers. Pharmacogenetics, *9:* 533–537, 1999.

205. Katoh, T., Kaneko, S., Boissy, R., Watson, M., Ikemura, K., and Bell, D. A. A pilot study testing the association between *N*-acetyltransferases 1 and 2 and risk of oral squamous cell carcinoma in Japanese people. Carcinogenesis (Lond.), *19:* 1803–1807, 1998.

206. Olshan, A., Watson, M. A., Smith, J., Wiessler, M., and Bell, D. A. Association of *N*-acetyltransferase 1 (NAT1) genotype and smoking with risk of head and neck cancers. Proc. Am. Assoc. Cancer Res., *38:* 331, 1997.

207. Agundez, J. A. G., Martinez, C., Olivera, M., Gallardo, L., Ladero, J. M., Rosado, C., Prados, J., Rodriguez-Molina, J., Resel, L., and Benitez, J. Expression in human prostate of drug-and carcinogen-metabolizing enzymes: association with prostate cancer risk. Br. J. Cancer, *78:* 1361–1367, 1998.

208. Wadelius, M., Autrup, J. L., Stubbins, M. J., Andersson, S-O., Johansson, J-E., Wadelius, C., Wolf, C. R., Autrup, H., and Rane, A. Polymorphisms in *NAT2*, *CYP2D6*, *CYP2C19*, and *GSTP1* and their association with prostate cancer. Pharmacogenetics, *9:* 333–340, 1999.

209. Fukutome, K., Watanaba, M., Shiraishi, T., Murata, M., Uemura, H., Kubota, Y., Kawamura, J., Ito, H., and Yatani, R. *N*-Acetyltransferase 1 genetic polymorphism influences the risk of prostate cancer development. Cancer Lett., *136:* 83–87, 1999.

210. Leff, M. A., Epstein, P. N., Doll, M. A., Fretland, A. J., Devanaboyina, U-S., Rustan, R. D., and Hein, D. W. Prostate-specific human *N*-acetyltransferase 2 (NAT2) expression in the mouse. J. Pharmacol. Exp. Ther., *290:* 182–187, 1999.