

Review

Molecular Genetics and Epidemiology of the *NAT1* and *NAT2* Acetylation Polymorphisms¹

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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.

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Table 1 Human NAT1 alleles^a

Allele	Nucleotide change(s)	Amino acid change(s)	References ^b
<i>NAT1*3</i>	C1095A	None	40
<i>NAT1*4</i>	None	None	25
<i>NAT1*5</i>	G350,351C, G497-499C, A884G, Δ976, Δ1105	Arg ¹¹⁷ →Thr, Arg ¹⁶⁶ →Thr, Glu ¹⁶⁷ →Gln	49
<i>NAT1*10</i>	T1088A, C1095A	None	25
<i>NAT1*11</i>	C-344T, A-40T, G445A, G459A, T640G, Δ9 between 1065-1090, C1095A	Val ¹⁴⁹ →Ile, Ser ²¹⁴ →Ala	50
<i>NAT1*14A</i>	G560A, T1088A, C1095A	Arg ¹⁸⁷ →Gln	51, 52
<i>NAT1*14B</i>	G560A	Arg ¹⁸⁷ →Gln	52, 53
<i>NAT1*15</i>	C559T	Arg ¹⁸⁷ →Stop	51, 53
<i>NAT1*16</i>	[AAA] immediately after 1091, C1095A	None	54
<i>NAT1*17</i>	C190T	Arg ⁶⁴ →Trp	55, 56
<i>NAT1*18A</i>	Δ3 between 1064-1087, T1088A, C1095A	None	57
<i>NAT1*18B</i>	Δ3 between 1064-1091	None	58
<i>NAT1*19</i>	C97T	Arg ³³ →Stop	56
<i>NAT1*20</i>	T402C	None	56
<i>NAT1*21</i>	A613G	Met ²⁰⁵ →Val	56
<i>NAT1*22</i>	A752T	Asp ²⁵¹ →Val	56
<i>NAT1*23</i>	T777C	None	56
<i>NAT1*24</i>	G781A	Glu ²⁶¹ →Lys	56
<i>NAT1*25</i>	A787G	Ile ²⁶³ →Val	56
<i>NAT1*26A</i>	[TAA] insertion between 1066 and 1091, C1095A	None	59
<i>NAT1*26B</i>	[TAA] insertion between 1066 and 1091	None	60
<i>NAT1*27</i>	T21G, T777C	None	
<i>NAT1*28</i>	[TAATAA] deletion between 1085 and 1090	None	61
<i>NAT1*29</i>	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 extrahepatic 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, 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	W1/W1	Rapid
*4/*12C	Rapid	W1/M1	Intermediate
*4/*14A	Intermediate	W1/W1	Rapid
*4/*14B	Intermediate	W1/W1	Rapid
*4/*14E	Intermediate	W1/W1	Rapid
*5B/*5C	Slow	W1/M1	Intermediate
*5B/*14A	Slow	W1/M1	Intermediate
*5B/*14B	Slow	W1/M1	Intermediate
*5C/*6A	Slow	W1/M2	Intermediate
*5C/*7B	Slow	W1/M3	Intermediate
*5C/*12A	Intermediate	W1/W1	Rapid
*5C/*13	Intermediate	W1/W1	Rapid
*6A/*12C	Intermediate	M1/M2	Slow
*6A/*14A	Slow	W1/M2	Intermediate
*6A/*14B	Slow	W1/M2	Intermediate
*12A/*14B	Intermediate	W1/W1	Rapid
*13/*14B	Intermediate	W1/W1	Rapid
*14A/*14A	Slow	W1/W1	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 (W1) allele and three mutant M1, M2, and M3 alleles. Deduced phenotypes are defined as follows: rapid acetylators, W1/W1; intermediate acetylators, W1/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 non-significant 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 (TAAATAA→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 PhIP⁴ are present in cigarette smoke (117, 118).

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

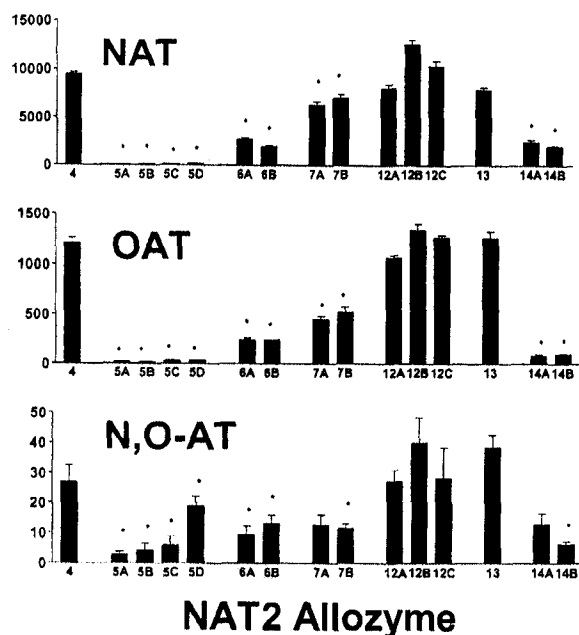


Fig. 1. Relative capacity of recombinant human *N*-acetyltransferase allozymes to catalyze the *N*-acetylation of 2-aminofluorene (top), the *O*-acetylation of *N*-hydroxy-2-aminofluorene (center) and the *N,O*-acetylation of *N*-hydroxy-*N*-acetyl-2-aminofluorene (bottom). Levels of acetyltransferase 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 re-

quire 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	<i>NAT2</i> genotype	Phenotype	<i>N</i> -Acetyltransferase activity (nmol/min/mg)		
			Cytosol	<i>NAT1</i>	<i>NAT2</i>
2-Aminofluorene	<i>NAT2*15/*15</i>	Rapid	6.16 ± 1.51	1.39 ± 0.19	24.8 ± 3.2
	<i>NAT2*15/*16A</i>	Intermediate	2.69 ± 0.30	0.97 ± 0.19	11.7 ± 1.6
	<i>NAT2*16A/*16A</i>	Slow	0.53 ± 0.04 (<i>P</i> < 0.0001)	0.96 ± 0.26	0.74 ± 0.29 (<i>P</i> < 0.0001)
3,2'-Dimethyl-4-aminobiphenyl	<i>NAT2*15/*15</i>	Rapid	0.97 ± 0.24	1.06 ± 0.41	2.98 ± 0.36
	<i>NAT2*15/*16A</i>	Intermediate	0.59 ± 0.07	0.68 ± 0.18	1.31 ± 0.15
	<i>NAT2*16A/*16A</i>	Slow	0.31 ± 0.04 (<i>P</i> < 0.005)	1.00 ± 0.35	0.05 ± 0.16 (<i>P</i> < 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 homozygous 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
Syrian hamster (Bio 1.5/H)	NAT2*15/*15	Rapid	21.2 ± 5.5
	NAT2*16A/*16A	Slow	7.20 ± 1.66
Rat (F344 and WKY)	NAT2*20/*20	Rapid	4.11 ± 1.06
	NAT2*21A/*21A	Slow	1.57 ± 0.48
			<i>P</i> < 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; Val¹²⁹→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.

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