N-Acetyltransferase Polymorphism and Human Cancer Risk

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Abstract

Because of the important role of *N*-acetyltransferase (NAT) enzymes in both metabolic activation and detoxification of certain precarcinogens, such as homo- and heterocyclic arylamines, extensive research in the past has focused on the relationship between the distribution of different variants of these enzymes and cancer susceptibility. In this context, we examined the relationship between the acetylator type of two NAT isozymes (NAT1 and NAT2) and cancer risk. It was shown that any independent overall association of those diseases with acetylation for either *NAT1* or *NAT2* is likely to be weak at most. Besides individual genetic profile, differences in the degree of exposure to environmental precarcinogens should also be considered. It was suggested that smoking and red meat intake were associated with both *NAT1* and *NAT2* genotype in the carcinogenesis. A gene-gene interaction, even linkage between *NAT1* and *NAT2* may also exist.

Key words: *N*-acetyltransferase, cancer risk, smoking, red meat intake

Introduction

The increased risk of cancer was related to the increased carcinogen exposure in the environment and workplace. In environmental exposures, lifestyle factors such as smoking and diet become the main attributable exposures. Since the majority of carcinogenic chemicals do not produce their biological effect directly, they require metabolic activation before they can react with cellular macromolecules and form covalent adducts¹⁾, which is generally regarded as a critical, initiating event in the multistep process of chemical carcinogenesis. Thus, the enzymes that are responsible for such bioactivation/detoxification should play an important role in the interaction between individuals and xenobiotics²⁾. The relationship of carcinogen-DNA adduct level, acetyltransferase expression and human cancer susceptibility have been reviewed by Badawi³⁾. The allelic variations of *NAT1* and *NAT2* were reviewed by Grant et al.⁴⁾. The purpose of this review is to briefly summarize the current state of knowledge concerning NATs and cancer risk.

General aspects of *N*-acetyltransferases

There are two types of enzyme reactions involved in the metabolism of xenobiotics. Phase-I enzymes are involved in

converting many compounds to reactive electrophilic metabolites, while phase-II enzymes usually act as inactivating enzymes. *N*-acetyltransferases (NAT), which belong to Phase-II enzymes, have two similar genes designated *NAT1* and *NAT2*⁵⁻⁷⁾. Each gene is intronless, located on the short arm of chromosome 8 [8p22] and has an open reading frame of 870 bp^{8,9)}. There are two kinetically distinct isozymes, NAT1 and NAT2. NAT2 is expressed primarily in the liver, with lower activities present in the pancreas and the colon; while NAT1 is found in various extrahepatic tissues, including colon, bladder, lung and breast¹⁰⁻¹³⁾.

The main carcinogens from cigarette smoke and fried red meat are aromatic amines (AA) and heterocyclic aromatic amines (HA) such as 4-aminobiphenyl and 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP)¹⁴⁻¹⁶⁾.

Hepatic NAT2 can compete with cytochrome P4501A2 (CYP1A2) which bio-activates (*N*-oxidation) arylamines by its detoxification (*N*-acetylation) reaction^{17,18)}. The arylamine metabolites can also enter the circulation and then be reabsorbed by the local tissues, where they could be further catalyzed by the NAT enzymes (*O*-acetylation and *N*, *O*-acetylation, predominantly NAT1 activity)¹⁹⁾. The resulting unstable acetoxy esters and *N*-acetyl-acetoxy esters can spontaneously decompose to electrophilic DNA- or protein-binding nitrenium ions²⁰⁾ (Fig. 1.).

To date, nine *NAT1* variant alleles and 15 *NAT2* variant alleles have been described²¹⁻²³⁾. According to the distribution of the NAT enzyme metabolite ratio, two kinds of phenotypes (fast acetylator and slow acetylator) were determined. In *NAT1*, the 'mutant-type' *NAT1**10 allele²⁴⁾ is associated with the fast acetylator; while in *NAT2*, the 'wild-type' *NAT2**4 allele⁵⁾ is associated with the rapid acetylator (Table 1.).

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Fig. 1 Proposed main pathway for *N*-acetyltransferases in the activation of arylamine carcinogens.

The frequencies of *NAT2* slow acetylator alleles vary among ethnic groups (Japanese 0.33; Koreans 0.32; Chinese 0.47; Africans 0.74; and Caucasian 0.76)^{4, 25}, whereas the data on ethnic distribution of *NAT1*10* is still limited (Japanese 0.42-0.45; Asians 0.50; and Caucasian 0.16-0.21)²⁵⁻²⁸

Proposed genetic linkage between *NAT1* and *NAT2* polymorphism

Because *NAT1* and *NAT2* loci are very close in genomic terms (the minimum distance between these two genes is approximately 170 kb and the maximum distance is 360 kb²⁹), a proposed linkage disequilibrium between these two genes has recently been suggested. Palmqvist et al.³⁰ found that the relationship between *NAT1* and *NAT2* polymorphism was non-random in both the control and the colorectal cancer groups. In a haplotype analysis³¹, the combination *NAT1*10/NAT2*4* was found 3.5 times more frequently than would be expected. Moreover, results of an epidemiological study in Germany also indicated that *NAT1*10* frequency was twofold higher in the *NAT2*4/*4* carrier than in the slow *NAT2* coding genotypes³². This linkage may confound studies of *NAT1* and *NAT2* polymorphism with disease susceptibility.

Table 1 Association between NATs phenotypes and main genotype locus^a

NAT1		NAT2	
Fast acetylator alleles	Slow acetylator alleles	Fast acetylator alleles	Slow acetylator alleles
<i>NAT1*10</i> ^b	<i>NAT1*4</i> (WT) ^c	<i>NAT2*4</i> (WT)	<i>NAT2*5A</i> (M1)
	<i>NAT1*3</i>		<i>NAT2*6A</i> (M2)
	<i>NAT1*11</i>		<i>NAT2*7A</i> (M3)
			<i>NAT2*14A</i> (M4)

^a From Vatsis et al.²¹, Grant et al.⁹.

^b In *NAT1*, both homozygote and heterozygote of *NAT1*10* are fast acetylator genotypes, while all others are slow acetylator genotypes; In *NAT2*, both homozygote and heterozygote of *NAT2*4* are fast acetylator genotypes, while all others are slow acetylator genotypes.

^c WT, wild type; M, mutant.

NATs polymorphism, cancer and precancerous diseases

Colorectal adenomatous polyps and colorectal cancer

Although only a small proportion of adenomatous polyps develop into malignancy, adenomas are generally considered precursors of most large-bowel cancers³³⁻³⁵. Therefore, inference can be made from polyps to colorectal cancer. Lang et al.³⁶ reported an elevated but not significant risk in the fast acetylator; however, the association between the risk of polyps and the *NAT2* acetylation has not yet been demonstrated by genotypic method³⁷⁻³⁹ (Table 2).

By phenotypic assay, Lang et al.⁴⁰ and Ilett et al.⁴¹ firstly reported that a significantly higher proportion of fast acetylators in patients with a history of colorectal cancer was found. This relationship was supported by Kadlubar et al.¹⁷. However, the same result could not be obtained in Spain⁴². Because disease status or chemotherapeutic agents may affect acetylation, almost all of the studies were performed by genotypic assay rather than by phenotypic assay, after *NATs* genotypic polymorphism was found⁵⁻⁷. However, most of these genotypic studies failed to detect a significant role for *NAT2* genotype^{26, 43-48}. Only two groups in Australia and Portugal respectively found consistent results with the hypothetical mechanism of food-contained arylamine activation by an *NAT2*-mediated *O*-acetyltransferase in the liver and colon. Accordingly, the slow acetylator phenotype should decrease the generation of critical intracellular concentrations of such ultimate carcinogens and consequent tumourigenesis upon environmental exposure to precursors^{39, 49}.

Comparison of *NAT* activity in cytosolic preparations from the human colon suggests that the *NAT1* but not the *NAT2* gene product is the predominant enzyme activity⁴³. This implies that a previous association between the *NAT2* polymorphism and colon cancer may arise partly as a result of differences in activity encoded by the *NAT1* gene.

Probst-Hensch et al.³⁸ reported that, in incident polyps, though the *NAT2* phenotype had no effect on polyps, subjects with the *NAT1* fast acetylator (homozygous or heterozygous for the *NAT1*10* allele) were at an increased risk for developing colorectal adenomas (OR 2.92, 95% CI 1.23-4.24).

Bell et al.²⁶ first showed a cancer risk associated with the *NAT1* gene (Table 3.). However, in a later prospective study, an independent overall association of colorectal cancer with *NAT1* could not be observed⁴⁸.

Bladder cancer

Studies that associate NATs to bladder carcinogenesis were begun with the observation that subjects with a history of arylamine-related occupational exposure history had higher prevalence of bladder cancer⁵⁰. However, when the exposure condition was excluded, most of the studies failed to show that *NAT* polymorphism had any relation with the bladder cancer by either phenotypic or genotypic assay⁵⁰⁻⁵⁵. Only two groups reported a direct significant association between the *NAT* phenotype and bladder cancer^{56, 57}, however, the sample size of one study⁵⁶ was small (Table 2.). A study in Japan reported that the slow acetylator genotype of *NAT2* was a significant risk factor for urothelial cancer patients⁵⁸, in which 78% of the cases were bladder cancers.

NAT1 is also expressed at high levels in the bladder mucosa, and involvement of the *NAT1*10* allele in the bioactivation of aromatic amines in bladder mucosa has been shown. For

Table 2 A summary of results from studies that evaluated the association of NAT2 polymorphism and cancer risk

Diseases	Risk acetylator	Place of study	Subjects		Results (OR, 95% CI) ^a	Notes	References
			Case	Control			
Colorectal adenomatous polyps	Fast	USA	41	205	1.92 (0.97-3.78) ^{bc}		36)
	Fast	USA	447	487	1.08 (0.83-1.40)		37)
	Fast	USA	441	484	1.10 (0.84-1.44)	Total subjects	38)
	Fast		441	282	0.84 (0.53-1.32)	Incident cases within 23 years	
Colorectal cancer	Fast		441	282	0.74 (0.41-1.33)	Incident cases within 5 years	
	Fast	Australia	89	110	1.1 (0.6-2.1)		39)
	Fast	USA	43	41	2.48 (1.02-6.03) ^{bc}		40)
	Fast	Australia	49	41	3.80 (1.53-9.44) ^{bc}		41)
	Fast	Spain	109	96	1.14 (0.67-1.9) ^{bc}		42)
	Fast	USA	38	100	2.12 (1.00-4.51) ^{bc}	Cases consisted of 20 cancer and 18 polyp patients	17)
	Fast	USA	44	28	0.96 (0.37-2.49) ^c		43)
	Fast	Japan	234	329	0.83 (0.48-1.44) ^c		44)
	Fast	USA	34	205	0.86 (0.41-1.80) ^{bc}		36)
	Fast	UK	202	112	1.1 (0.71-1.80) ^c		26)
Bladder cancer	Fast	UK	96	103	0.82 (0.45-1.49) ^c		45)
	Fast	Australia	110	110	1.8 (1.0-3.3)		39)
	Fast	UK	174	174	0.95 (0.61-1.49)		46)
	Fast	Portugal	114	210	2.04 (1.28-3.24)		49)
	Fast		114	210	3.46 (1.73-6.93)	NAT2*4/*4 only	
	Fast	USA	212	221	0.80 (0.53-1.19)		48)
	Fast	USA	689	803	1.07 (0.94-1.22) ^c		78)
	Slow	Denmark	71	74	1.74 (0.90-3.38) ^{bc}		50)
	Slow	Sweden	115	118	1.13 (0.65-1.98) ^{bc}		
	Slow	UK	111	95	1.52 (0.86-2.68) ^{bc}		51)
	Slow	Poland	67	22	2.82 (1.06-7.47) ^{bc}		56)
	Slow	China	66	15	0.3 (0.1-1.3) ^b		52)
	Slow		66	15	0.5 (0.1-1.8)		
	Slow	UK	189	59	2.60 (1.45-4.66) ^c		57)
	Slow	Germany	374	373	1.32 (0.97-1.80)		53)
	Slow	Denmark	254	242	1.22 (0.92-1.62)		54)
	Slow	USA	230	203	0.96 (0.68-1.36) ^c		55)
	Slow	Japan	102	100	3.04 (1.22-7.60)	Cases were patients with urothelial cancer (Bladder n=78, renal pelvis n=7, ureter n=7, overlap n=10)	58)
Breast cancer	Slow	Spain	160	132	1.10 (0.69-1.75) ^c		59)
	Slow	USA	304	327	1.11 (0.80-1.54) ^c	Total subjects	60)
	Slow		119	114	0.9 (0.7-2.0)	Premenopausal	
	Slow		185	213	1.3 (0.8-1.9)	Postmenopausal	
	Slow	USA	465	466	0.9 (0.7-1.2)		61)
	Slow	USA	488	472	0.88 (0.68-1.14) ^c	Total subjects	62)
	Slow		236	213	1.1 (0.7-1.6)	Premenopausal	
	Slow		252	256	0.7 (0.5-1.0)	Postmenopausal	
	Slow	USA	303	317	0.87 (0.64-1.19) ^c	Total subjects	63)
	Slow		118	114	0.99 (0.58-1.70) ^c	Premenopausal	
Lung cancer	Slow		185	203	0.78 (0.52-1.18) ^c	Postmenopausal	
	Fast	UK	53	31	0.91 (0.37-2.21) ^b		65)
	Fast	UK	126	273	1.20 (0.78-1.84) ^{bc}		66)
	Fast	Spain	108	243	0.98 (0.63-1.52) ^c		67)
	Fast	Germany	389	657	1.05 (0.81-1.36) ^b		71)
	Fast		155	310	1.10 (0.73-1.65)		
	Fast		155	310	2.36 (1.05-5.32)	NAT2*4/*4 only	
	Fast	Japan	124	376	0.75 (0.41-1.37) ^c		68)
	Fast	Sweden	185	164	1.0 (0.6-1.5)		69)

^a The risk acetylator Vs the other acetylator; OR, odds ratio; CI, confidential interval^b b, phenotypic assay; all others were genotypic assay.^c Modified from the original paper

Table 3 A summary of results from studies that evaluated the association of NAT1 fast acetylator and cancer risk

Diseases	Place of study	Subjects		Results (OR, 95% CI) ^a	Notes	References
		Case	Control			
Colorectal adenomatous polyps	USA	441	484	1.04 (0.79-1.36)	Total subjects	38)
		441	104	1.81 (1.12-2.92)	Incident cases within 23 years	
		441	59	2.29 (1.23-4.24)	Incident cases within 5 years	
Colorectal cancer	England	202	112	1.9 (1.2-3.1)		26)
	USA	212	221	0.93 (0.61-1.49)		48)
Bladder cancer	Denmark	254	242	1.11 (0.78-1.60)		54)
	USA	230	203	1.67 (1.14-2.45) ^b		55)
Breast cancer	USA	492	468	0.91 (0.71-1.17) ^b		62)
Lung cancer				Unknown		

^a The fast acetylator *V*s the slow acetylator; OR, odds ratio; CI, confidential interval

^b Modified from the original paper

example, DNA adduct levels of 4-aminobiphenyl were found to be 2-fold higher in individuals with the *NAT1*10* allele ¹⁹. Preliminary epidemiologic data suggest that the *NAT1*10* allele is a risk factor in smoking-related bladder cancer ⁵⁵ (Table 3.).

Breast cancer

The relationship of the risk of breast cancer with the *NAT* genotype has been studied in a series of studies. However, no current results indicate a direct association between them, no matter whether a premenopausal or a postmenopausal condition was considered ⁵⁹⁻⁶³ (Table 2.).

The expression of the human *NAT1* in mammalian and bacterial cells showed that *NAT1* activates a broader class of aromatic amines to compounds that can adduct to DNA, often more efficiently than does *NAT2* ^{13, 64}. However, association could not be found between the *NAT1* genotype and breast cancer risk when the environmental exposure was not considered ⁶² (Table 3.).

Lung cancer

Though the *NAT* fast acetylator phenotype was considered as a risk factor for lung cancer susceptibility, most studies failed to demonstrate it ⁶⁵⁻⁶⁹. Only Cascorbi et al. reported that the relatively small subgroup of genetically homozygous fast acetylators (*NAT2*4/*4*), which provide significantly higher acetylation rates than heterozygotes ⁷⁰, were at significantly increased risk for lung cancer ⁷¹ (Table 2.).

Though *NAT1* expression was also detected in all bronchial and lung samples ¹², data concerning its genotypic polymorphism and lung cancer risk are not available.

One explanation for the lack of relationship between the acetylator polymorphism and the risk of the above malignancies would be due to a lack of adequate carcinogens instead of the absence of the enzymatic machinery that converts these carcinogens into their active end products.

NATs polymorphism, smoking and cancer risk

One of the main routes of environmental exposure is smoking, since smokers are exposed to numerous potent carcinogens, including polycyclic aromatic hydrocarbons (PAHs), tobacco-specific nitrosamines and arylamines. A well-recognized heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo [4,5-b]

pyridine (PhIP), typically found in fried beef ¹⁴, was also detected in the urine of smokers ¹⁵ and in the cigarette smoke condensate ¹⁶. The high concentration of PhIP in fried meats has been suggested to explain the increased risk of certain cancers that will be discussed later. The metabolism of PAHs was suggested to be affected by the cytochrome P4501A1 (CYP1A1) and the glutathione S-transferase M1 (GSTM1) pathway ⁷². However, the arylamines, such as 2-naphthylamine and 4-aminobiphenyl, could be either activated (*N*-oxidation) by the cytochrome P4501A2 (CYP1A2) or competitively detoxified (*N*-acetylation) by the *NAT*s enzymes (predominantly *NAT2* activity) in the liver ¹⁸. The arylamine metabolites can then enter the circulation, and be reabsorbed into the target tissues. Further activation steps catalyzed by the *NAT* enzymes (*O*-acetylation and *N*, *O*-acetylation) may occur ¹⁹. *NAT1* was suggested to be the major pathway for acetylation here because *NAT1* has a higher relative activity for most of these compounds than *NAT2* ⁷³.

The *NAT1**NAT2** interaction is supported by a possible metabolic mechanism. If an individual has a *NAT2* fast allele, arylamines may be rapidly detoxified in the liver so that little hydroxylated arylamine may ever reach the bladder epithelium, where *NAT1* could act upon it. Consequently, if a person has a *NAT2* fast allele, his *NAT1* genotype may have little effect on the risk of bladder cancer. Conversely, if an individual has a *NAT2* slow allele, detoxification in the liver may be incomplete, so that excess hydroxylated arylamine reaches the bladder epithelium, making the *NAT1* genotype an important determinant of bladder cancer risk. There are limited experimental data supporting such a pathway; Badawi et al. ¹⁹ found significantly higher arylamine adduct levels in bladder epithelium among individuals with the *NAT2* slow and *NAT1* rapid genotypes compared to other genotypes tested. Okkels et al. ⁵⁴ indicated that the slow *NAT1* and fast *NAT2* genotype may be a protective genotype compared with other genotype combinations. The existence of interaction involving *NAT1*, *NAT2* and smoking was further demonstrated by Taylor et al. ⁵⁵ (Table 4).

The *NAT* * smoking interaction has been well studied. However, its results are still controversial (Table 5). Probst-Hensch et al. ³⁷ observed that *NAT2* Fast acetylator current smokers had 2.25 times higher risk for polyps than those slow acetylator never smokers. This result was consistent with other studies that reported a 1.5-3 times relative risk between cigarette smoking and colorectal adenomas ⁷⁴⁻⁷⁷. As to colorectal cancer, smoking increased the risk of *NAT2* slow acetylators in one study ⁴⁶.

Table 4 The *NAT1* and *NAT2* gene-gene interaction in carcinogenesis

Diseases	Interaction found?	References
Colorectal adenomatous polyps	No	38)
Colorectal carcinoma	Yes	26)
	Yes	48)
Bladder cancer	Yes	54)
	Yes	55)
Breast cancer	Unknown	
Lung cancer	Unknown	

Whereas, in a later research, a relatively higher risk was observed in past smokers who had intermediate/fast acetylators ⁷⁸⁾. The discrepancy between these studies may be partly due to the complexity of the whole metabolic system, which includes other polymorphic enzymes such as CYP1A1, CYP1A2 and GSTM1. Secondly, usually information on diet factors, such as red meat intake, which also have an important effect on carcinogenesis, was unavailable. Thirdly, the misclassification of outcomes in colon cancer research might exist in that the controls used in studies of cancer may have colonic polyps, whereas in studies of adenomas, they do not. This is a consequence of adenoma studies including people who have had a colonoscopy, whereas carcinoma studies do not have that requirement. This type of misclassification bias would result in attenuated association seen in studies of cancer ⁷⁹⁾.

Bladder cancer has been more consistently associated with cigarette smoking than colon cancer. It was shown that genetically susceptible (*NAT2* slow acetylators) individuals may have a higher risk by smoking cigarettes ^{53, 54, 57, 58)}. Though Taylor et al. ⁵⁵⁾ found no association between the *NAT2* genotype and the risk of bladder cancer whether the genotype was considered alone or in combination with smoking, an increased risk for individuals carrying the *NAT1*10* allele among smokers was demonstrated.

Most epidemiologic studies have not yet found a clear association between smoking and breast cancer risk ⁸⁰⁾, and the association between the *NATs* genotype, smoking and breast cancer is also not clear. Ambrosone et al. ⁶⁰⁾ demonstrated that, among postmenopausal women, slow acetylator and smoking increase breast cancer risk in a dose-dependent manner. Another study ⁶¹⁾, which observed that the elevated risks for current smokers who had slow acetylators compared with never smokers

who had fast acetylators were slightly stronger but not statistically significant among postmenopausal women, weakly supported that conclusion. However, The observation of Millikan et al. ⁶²⁾ was different from the former two studies. It indicated that, among postmenopausal women, ORs for smoking within the past 3 years were greater for women with the *NAT2* rapid genotype (OR, 7.4; 95% CI, 1.6-32.6) than the *NAT2*-slow (OR, 2.8; 95% CI, 0.4-8.0) and greater for the *NAT1*10* genotype (OR, 9.0; 95% CI, 1.9-41.8) than the *NAT1-non*10* (OR, 2.5; 95% CI, 0.9-7.2). This result was supported by the observation that the *NAT1* enzyme exhibits higher activity than *NAT2* in the breast ¹³⁾. Since the etiology of breast cancer is largely unknown, it is impossible to give a clear explanation now. Anyway, the available data suggests that cigarette smoking might be a risk factor for post-, but not premenopausal breast cancer. In light of these evidences, it may be assumed that pre-menopausal and post-menopausal breast cancer have different etiologies ⁸⁰⁾.

Though no interaction between the *NAT2* genotype, smoking and lung cancer risk was observed by Martinez et al. ⁶⁷⁾, study of Cascorbi et al. ⁷¹⁾ found that *NAT2*4*4* was statistically significantly overrepresented in patients who had smoked heavily (OR, 3.22; 95% CI, 1.27-7.92). Moreover, Nyberg et al. ⁶⁹⁾ suggested that the *NAT2* slow acetylator genotype may confer an increased risk among never-smokers, and that the fast acetylator genotype interacts with pack-year dose to produce a steeper risk gradient among smokers.

NATs phenotype, red meat intake and cancer risk

Another main route of environmental exposure comes from eating red meat. This is because during the cooking of meats, the meat juices pyrolyze and form heterocyclic aromatic amines (HAA) ¹⁴⁾. HAA concentrations in meat increase with longer cooking times and higher temperatures ⁸¹⁻⁸³⁾. These compounds are carcinogenic in animal tests ^{84, 85)}. In some studies weak positive associations between colon cancer and consumption of "well-done" ⁸⁶⁾, "browned" ⁸⁷⁾, "barbecued" ⁸⁸⁾, or "red meat" ⁸⁹⁾ have been demonstrated. The cancer risk to humans exposed by HAAs in the diet may depend upon the extent to which the compounds are activated in vivo. HAAs need to be metabolically activated in order to act as mutagens or carcinogens ⁹⁰⁾. The initial activation step is thought to be *N*-oxidation by CYP1A2 ⁹¹⁾,

Table 5 Interaction of *NATs* polymorphism and cigarette smoking in carcinogenesis

Diseases	NAT type	Risk acetylator	Interaction found?	References
Colorectal adenomatous polyps	<i>NAT2</i>	Fast	Yes	37)
Colorectal carcinoma	<i>NAT2</i>	Slow	Yes	46)
	<i>NAT2</i>	Fast	Weak ^a	78)
Bladder cancer	<i>NAT2</i>	Slow	Yes	57)
	<i>NAT2</i>	Slow	Yes	53)
	<i>NAT2</i>	Slow	Yes	54)
	<i>NAT2</i>	Slow	No	55)
	<i>NAT1</i>	Fast	Yes	
	<i>NAT2</i>	Slow	Yes	58)
Breast cancer	<i>NAT2</i>	Slow	Yes	60)
	<i>NAT2</i>	Slow	Weak	61)
	<i>NAT2</i>	Fast	Weak	62)
	<i>NAT1</i>	Fast	Weak	
Lung cancer	<i>NAT2</i>	Slow	No	67)
	<i>NAT2</i>	Fast	Yes	71)
	<i>NAT2</i>	Fast	Weak	69)

^a Weak, only weak correlation was found.

Table 6 Interaction of NATs polymorphism and red meat intake in carcinogenesis

Diseases	NAT type	Risk acetylator	Interaction found?	References
Colorectal adenomatous polyps	NAT2	Fast	Yes	36)
	NAT2	Fast	Yes	39)
Colorectal cancer	NAT2	Fast	Yes	39)
	NAT2	Fast	Yes	46)
	NAT1	Fast	Yes	48)
	NAT2	Fast	Yes	
Bladder cancer			Unknown	
Breast cancer	NAT2	Fast	No	63)
Lung cancer			Unknown	

which also varies considerably among individuals ⁹²⁾, and which itself could be induced by HAAs ⁹³⁾. The *N*-hydroxy arylamine can be further *O*-acetylated by NATs to form an arylamine-DNA adduct in either the liver or the appropriate target organ where it is transported. Both NAT1 and NAT2 catalyze the *O*-acetylation of the *N*-hydroxy arylamine. However, NAT2 rather than NAT1 catalyzes the *O*-acetylation of HAAs such as *N*-hydroxy-Glu-P-1, *N*-hydroxy-PhIP and *N*-hydroxy-MeIQx almost exclusively ⁹³⁾. Since NAT2 is expressed primarily in the liver and NAT1 is expressed in extrahepatic tissues, it is thought that some *N*-hydroxy HAAs are *O*-acetylated by NAT2 mainly in the liver. However, other HAAs, such as PhIP which produces low DNA-adduct levels in the liver ⁹⁴⁾, are mainly transported in the blood to the various extrahepatic target tissues, where they are further *O*-acetylated by both NAT1 and NAT2 ^{73, 95)}.

Moreover, concerning red meat intake, a *NAT1***NAT2* interaction might also exist (Table 4.). Bell et al. ²⁶⁾ also observed evidence of interaction between the variant allele of *NAT1* and *NAT2*, such that a significantly increased risk of colorectal cancer among subjects with the *NAT1**10 allele was even higher among *NAT2* fast acetylators. In a later prospective study, it was found that the association of red meat intake with colorectal cancer was stronger among fast acetylators at *NAT1* and *NAT2* loci both

separately and combined ⁴⁸⁾. However, this interaction was not observed by Probst-Hensch et al. ³⁸⁾ for colorectal adenomas. One reason for these inconsistencies is that data on cooking method preference were not or were imperfectly collected. Another reason is that the functional activity of the various *NAT1* alleles has not been completely defined and the degree of acetylation activity of the *NAT1**10 allele is still controversial.

The elevated risk of *NAT2* rapid and red meat intake in carcinogenesis has also been demonstrated in colorectal adenomatous polyps and colorectal cancer ^{36, 39, 46, 48)} (Table 6.). Though in a study of breast cancer the same interaction could neither be found in premenopausal nor postmenopausal subjects, some likely measurement error in evaluation of sources of HAAs and the lack of data on CYP1A2 in that study might account for it ⁶³⁾.

In summary, *NAT*s polymorphism only has a secondary effect on the carcinogenesis of cancers. Its relevance to carcinogenesis could be modified either by the dosage and variety of environmental exposure, or by other polymorphic enzymes. A gene-gene interaction, even linkage between *NAT1* and *NAT2* may also exist.

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