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Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis

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Abstract

Aromatic and heterocyclic amines require metabolic activation to electrophilic intermediates that initiate carcinogenesis. *N*-Acetyltransferase 1 (NAT1) and 2 (NAT2) are important enzymes in the biotransformation of these carcinogens and exhibit genetic polymorphism. Human *NAT1* and *NAT2* alleles are listed at: http://www.louisville.edu/medschool/pharmacology/NAT.html by an international gene nomenclature committee. The high frequency of the NAT1 and NAT2 acetylation polymorphisms in human populations together with ubiquitous exposure to aromatic and heterocyclic amines suggest that *NAT1* and *NAT2* acetylator genotypes are important modifiers of human cancer susceptibility. For cancers in which *N*-acetylation is a detoxification step such as aromatic amine-related urinary bladder cancer, NAT2 slow acetylator phenotype is at higher risk. Multiple studies have shown that the urinary bladder cancer risk is particularly high in the slowest NAT2 acetylator phenotype or genotype (*NAT2*5*). In contrast, for cancers in which *N*-acetylation is negligible and *O*-acetylation is an activation step such as for heterocyclic amine-related colon cancer, NAT2 rapid acetylator phenotype is at higher risk. Although studies have found associations between *NAT1* genotype and various cancers, the findings are less consistent and are not well understood. Since cancer risk requires exposure to aromatic and/or heterocyclic amine carcinogens modified by *NAT1* and/or *NAT2* acetylator genotype, the results from human epidemiology studies are dependent upon the quality and accuracy of the exposure assessment and genotype determination. Conclusions require understanding the relationship between genotype and phenotype, as well as the role of genetic variation in carcinogen metabolism, DNA repair, and host susceptibility. Investigations have been carried out in rapid and slow acetylator rodent models in which both exposure and genetic variability are tightly controlled. Human *NAT1* and *NAT2* alleles have been characterized by recombinant expression to further understand the effects of nucleotide polymorphisms on function and phenotype.

Keywords: *N*-Acetylation polymorphism; Single nucleotide polymorphisms; Aromatic amines; Heterocyclic amines; Urinary bladder cancer; Colon cancer

1. Introduction

Aromatic and heterocyclic amines require metabolic activation to electrophilic intermediates that initiate carcinogenesis. *N*-Acetylation and *O*-acetylation are catalyzed by two *N*-acetyltransferase (E.C.2.3.1.5) isozymes, *N*-acetyltransferase 1 (NAT1) and 2 (NAT2). Over 25 human *NAT1* (Table 1) and *NAT2* (Table 2) alleles have been identified. The listing is updated and published at http://www.louisville.edu/medschool/pharmacology/NAT.html by an international gene nomenclature committee [1].

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Table 1
Human *NAT1* alleles[a]

NAT1 allele	Nucleotide change(s)	Amino acid change(s)
*NAT1*3*	$C^{1095}A$	None
*NAT1*4*	None	None
*NAT1*5*	$G^{350,351}C$, $G^{497--499}C$, $A^{884}G$, Δ^{976}, Δ^{1105}	$Arg^{117} \rightarrow$ Thr, $Arg^{166} \rightarrow$ Thr, $Glu^{167} \rightarrow$ Gln
*NAT1*10*	$T^{1088}A$, $C^{1095}A$	None
*NAT1*11A*	$C^{-344}T$, $A^{-40}T$, $G^{445}A$, $G^{459}A$, $T^{640}G$, $\Delta 9$ between 1065 and 1090, $C^{1095}A$	$Val^{149} \rightarrow$ Ile, $Ser^{214} \rightarrow$ Ala
*NAT1*11B*	$C^{-344}T$, $A^{-40}T$, G^{445} A, $G^{459}A$, $T^{640}G$, $\Delta 9$ between 1065 and 1090	$Val^{149} \rightarrow$ Ile, $Ser^{214} \rightarrow$ Ala
*NAT1*11C*	$C^{-344}T$, $A^{-40}T$, $G^{459}A$, $T^{640}G$, $\Delta 9$ between 1065 and 1090, $C^{1095}A$	$Ser^{214} \rightarrow$ Ala
*NAT1*14A*	$G^{560}A$, $T^{1088}A$, $C^{1095}A$	$Arg^{187} \rightarrow$ Gln
*NAT1*14B*	$G^{560}A$	$Arg^{187} \rightarrow$ Gln
*NAT1*15*	$C^{559}T$	$Arg^{187} \rightarrow$ Stop
*NAT1*16*	[AAA] immediately after 1091, $C^{1095}A$	None
*NAT1*17*	$C^{190}T$	$Arg^{64} \rightarrow$ Trp
*NAT1*18A*	$\Delta 3$ between 1065 and 1087, $T^{1088}A$, $C^{1095}A$	None
*NAT1*18B*	$\Delta 3$ between 1065 and 1090	None
*NAT1*19*	$C^{97}T$	$Arg^{33} \rightarrow$ Stop
*NAT1*20*	$T^{402}C$	None
*NAT1*21*	$A^{613}G$	$Met^{205} \rightarrow$ Val
*NAT1*22*	$A^{752}T$	$Asp^{251} \rightarrow$ Val
*NAT1*23*	$T^{777}C$	None
*NAT1*24*	$G^{781}A$	$Glu^{261} \rightarrow$ Lys
*NAT1*25*	$A^{787}G$	$Ile^{263} \rightarrow$ Val.
*NAT1*26A*	[TAA] insertion between 1065 and 1090 $C^{1095}A$	None
*NAT1*26B*	[TAA] insertion between 1065 and 1090	None
*NAT1*27*	$T^{21}G$, $T^{777}C$	None
*NAT1*28*	[TAATAA] deletion between 1065 and 1090	None
*NAT1*29*	$T^{1088}A$, $C^{1095}A$, Δ^{1025}	None

[a] From http://www.louisville.edu/medschool/pharmacology/NAT.html.

Since aromatic and heterocyclic amines require metabolism to exert their carcinogenic effects, genetic polymorphisms in a number of important phase 1 (e.g. cytochrome P450s) and 2 (e.g. glutathione-*S*-transferases) metabolizing enzymes may modify cancer risk. Our laboratory has investigated the biotransformation of these carcinogens by NAT1 and NAT2. The high frequency of the NAT1 and NAT2 acetylation polymorphisms in human populations together with ubiquitous exposure to aromatic and heterocyclic amines suggest that *NAT1* and *NAT2* acetylator genotypes are important modifiers of human cancer susceptibility. Since human populations encounter many different chemicals and it is very difficult to quantify exposures to aromatic and heterocyclic amines over long periods of time, we have utilized animal models to test the effects of acetylator polymorphisms on carcinogenic risk from these compounds.

2. Investigations in animal models

In order to test the specific role of *NAT2* acetylator genotype independent of genetic polymorphisms in other genes important in the carcinogenesis process such as other carcinogen metabolizing and DNA repair enzymes, we have constructed congenic Syrian hamster lines that differ only at the *NAT2* gene locus and other closely linked loci [2,3] and transgenic mice in which human rapid acetylator NAT2 (*NAT2*4*) was over-expressed specifically in the prostate [4]. We have also characterized and utilized rapid and slow acetylator rat strains [5]. A *NAT2* gene-dose effect on *N*-acetyltransferase activity is expressed in urinary bladder and prostate of the rat [5] and Syrian hamster [6].

Since 3,2′-dimethyl-4-aminobiphenyl (DMABP) is an aromatic amine carcinogen that produces tumors in multiple organs of rats and hamsters, we administered DMABP to rapid and slow acetylator congenic

Table 2
Human *NAT2* alleles[a]

NAT2 allele	Nucleotide change(s)	Amino acid change(s)
*NAT2*4*	None	None
*NAT2*5A*	$T^{341}C$, $C^{481}T$	$Ile^{114} \rightarrow Thr$
*NAT2*5B*	$T^{341}C$, $C^{481}T$, $A^{803}G$	$Ile^{114} \rightarrow Thr$, $Lys^{268} \rightarrow Arg$
*NAT2*5C*	$T^{341}C$, $A^{803}G$	$Ile^{114} \rightarrow Thr$, $Lys^{268} \rightarrow Arg$
*NAT2*5D*	$T^{341}C$	$Ile^{114} \rightarrow Thr$
*NAT2*5E*	$T^{341}C$, $G^{590}A$	$Ile^{114} \rightarrow Thr$, $Arg^{197} \rightarrow Gln$
*NAT2*5F*	$T^{341}C$, $C^{481}T$, $C^{759}T$, $A^{803}G$	$Ile^{114} \rightarrow Thr$, $Lys^{268} \rightarrow Arg$
*NAT2*6A*	$C^{282}T$, $G^{590}A$	$Arg^{197} \rightarrow Gln$
*NAT2*6B*	$G^{590}A$	$Arg^{197} \rightarrow Gln$
*NAT2*6C*	$C^{282}T$, $G^{590}A$, $A^{803}G$	$Arg^{197} \rightarrow Gln$, $Lys^{268} \rightarrow Arg$
*NAT2*6D*	$T^{111}C$, $C^{282}T$, $G^{590}A$	$Arg^{197} \rightarrow Gln$
*NAT2*7A*	$G^{857}A$	$Gly^{286} \rightarrow Glu$
*NAT2*7B*	$C^{282}T$, $G^{857}A$	$Gly^{286} \rightarrow Glu$
*NAT2*10*	$G^{499}A$	$Glu^{167} \rightarrow Lys$
*NAT2*11*	$C^{481}T$	None
*NAT2*12A*	$A^{803}G$	$Lys^{268} \rightarrow Arg$
*NAT2*12B*	$C^{282}T$, $A^{803}G$	$Lys^{268} \rightarrow Arg$
*NAT2*12C*	$C^{481}T$, $A^{803}G$	$Lys^{268} \rightarrow Arg$
*NAT2*13*	$C^{282}T$	None
*NAT2*14A*	$G^{191}A$	$Arg^{64} \rightarrow Gln$
*NAT2*14B*	$G^{191}A$, $C^{282}T$	$Arg^{64} \rightarrow Gln$
*NAT2*14C*	$G^{191}A$, $T^{341}C$, $C^{481}T$, $A^{803}G$	$Arg^{64} \rightarrow Gln$, $Ile^{114} \rightarrow Thr$, $Lys^{268} \rightarrow Arg$
*NAT2*14D*	$G^{191}A$, $C^{282}T$, $G^{590}A$	$Arg^{64} \rightarrow Gln$, $Arg^{197} \rightarrow Gln$
*NAT2*14E*	$G^{191}A$, $A^{803}G$	$Arg^{64} \rightarrow Gln$, $Lys^{268} \rightarrow Arg$
*NAT2*14F*	$G^{191}A$, $T^{341}C$, $A^{803}G$	$Arg^{64} \rightarrow Gln$, $Ile^{114} \rightarrow Thr$, $Lys^{268} \rightarrow Arg$
*NAT2*14G*	$G^{191}A$, $C^{282}T$, $A^{803}G$	$Arg^{64} \rightarrow Gln$, $Lys^{268} \rightarrow Arg$
*NAT2*17*	$A^{434}C$	$Gln^{145} \rightarrow Pro$
*NAT2*18*	$A^{845}C$	$Lys^{282} \rightarrow Thr$
*NAT2*19*	$C^{190}T$	$Arg^{64} \rightarrow Trp$

[a] From http://www.louisville.edu/medschool/pharmacology/NAT.html.

hamsters and rats and measured the amount of DNA adducts formed in various tissues. DMABP-DNA adduct levels in prostate and urinary bladder were dose-dependent in hamsters [7] and rats and significantly higher in slow than rapid acetylator rats (Fig. 1). Heterocyclic amines such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) also induce prostate tumors in the rat [9,10]. Heterocyclic amines do not undergo significant *N*-acetylation, but following *N*-hydroxylation catalyzed by cytochrome P4501A2, undergo *O*-acetylation catalyzed by *N*-acetyltransferase. We administered PhIP to wild-type and transgenic mice specifically over-expressing human *NAT2*4* in the prostate and measured PhIP DNA adduct levels. We found that over-expression of human *NAT2*4* in mouse prostate did not increase the level of PhIP DNA adducts in the prostate [4]. These results suggest that slow NAT2 acetylators are at increased risk of prostate and urinary bladder tumors following exposures to aromatic amine carcinogens such as DMABP, and that rapid NAT2 acetylators are not at increased risk to prostate cancer following exposures to heterocyclic amine carcinogens such as PhIP. Whether or not these results obtained in rodent models extrapolate to humans remains to be investigated.

DMABP, PhIP, and 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx) induce colon tumors in the rat. We administered DMABP to rapid and slow acetylator congenic hamsters and rats and measured aberrant crypt foci (ACF), the earliest morphologically recognized precursor of colon tumors. Colon ACF were higher in rapid than slow acetylator congenic hamsters administered DMABP (Fig. 2). Similarly,

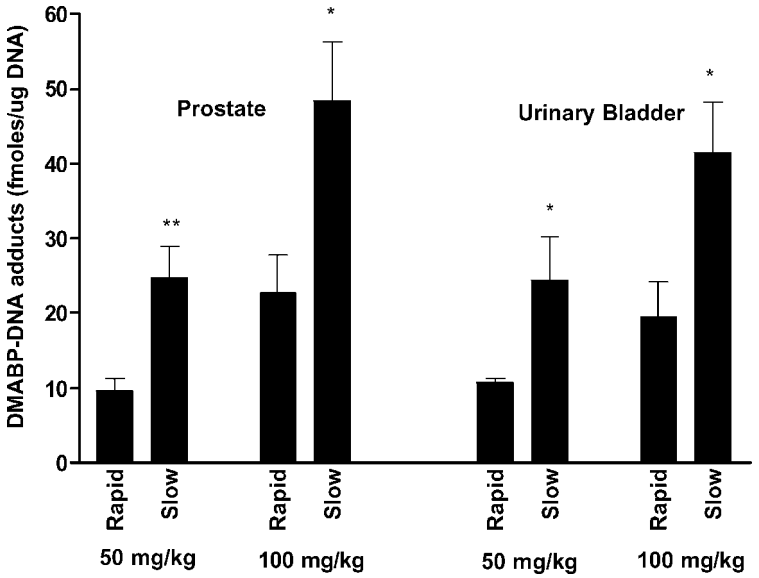

Fig. 1. Total DMABP DNA adducts in prostate and urinary bladder of rapid and slow acetylator rats following administration of 50 or 100 mg/kg DMABP. Adduct levels were significantly higher in slow than rapid acetylators ($^{*}P < 0.05$; $^{**}P < 0.01$). Adapted from Jiang et al. [8].

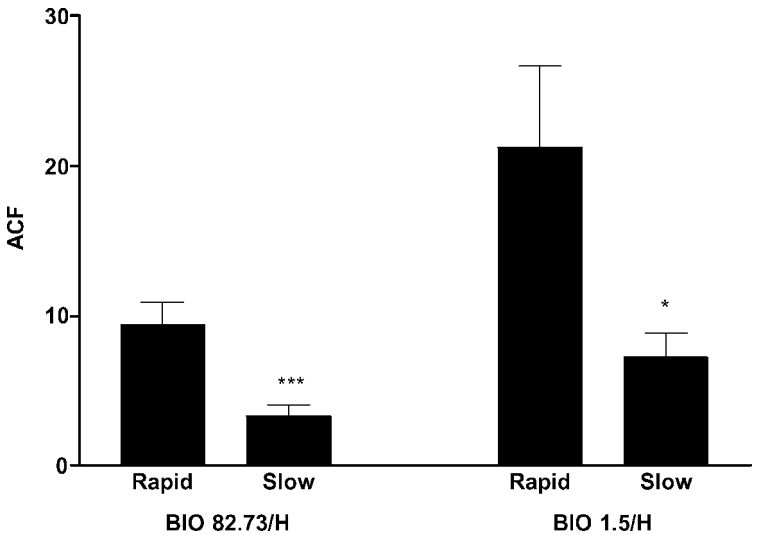

Fig. 2. Aberrant crypt foci (ACF) in colons of rapid and slow acetylator congenic hamsters (strains BIO 82.73/H and BIO 1.5/H) following administration of DMABP. ACF levels were significantly higher in rapid than slow acetylators ($^{*}P < 0.05$; $^{***}P < 0.001$). Adapted from Feng et al. [11]; Paulsen et al. [12].

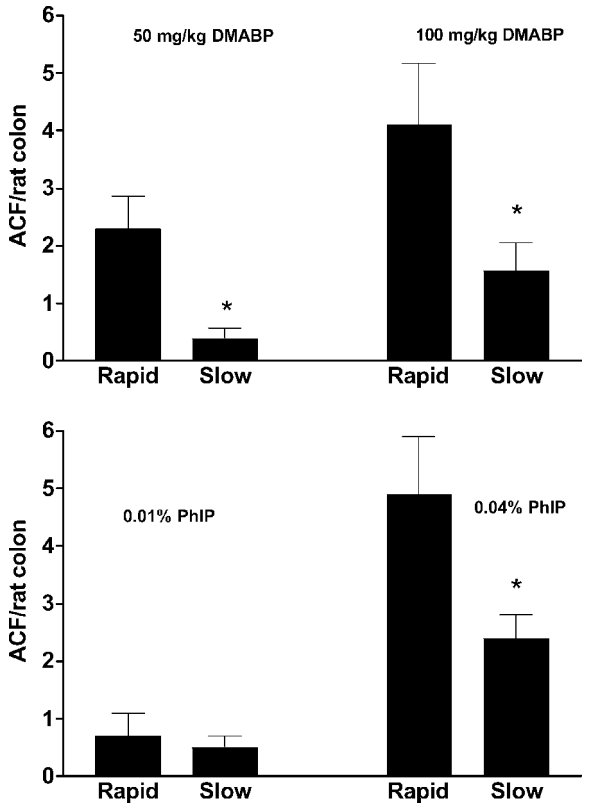

Fig. 3. ACF in colons of rapid and slow acetylator rats following administration of 50 or 100 mg/kg DMABP (upper panel) or 0.01 or 0.04% dietary PhIP (lower panel). ACF levels were significantly ($P < 0.05$) higher in rapid than slow acetylators following both doses of DMABP and the higher dose of PhIP. Adapted from Feng et al. [13] and Purewal et al. [14].

colon ACF were dose-dependent and higher in rapid than slow acetylator rats following administration of DMABP or PhIP (Fig. 3).

2.1. Characterization of human NAT1 and NAT2 alleles though recombinant expression in yeast and bacteria

If cancer risk from aromatic and/or heterocyclic amine carcinogens is modified by *NAT1* and/or *NAT2* acetylator genotype, the results from human epidemiology studies depend upon the quality and accuracy of the genotype determination. Furthermore, conclusions from acetylator genotyping studies require understanding the relationship between genotype and phenotype. To better understand this relationship, we expressed human *NAT1* and *NAT2* alleles in yeast and bacteria to characterize and compare the various human NAT1 and NAT2 allozymes. Human *NAT1*4* is defined as the reference human *NAT1* allele [1] because it is associated with high activity and is the most frequent allele in the original populations studied (Caucasians). Variant human *NAT1* alleles have been identified that possess a number of nucleotide polymorphisms in the coding and 3′-untranslated region (Table 1). The relationship between *NAT1* genotype and phenotype remains unclear. We expressed human *NAT1*4* and 12 variant *NAT1* alleles possessing nucleotide polymorphisms in the coding region in yeast (*Schizosaccharomyces pombe*). We found that five of the recombinant human NAT1 allozymes (NAT1 14B, 15, 17, 19, and 22) catalyzed both *N*-acetylation and *O*-acetylation at rates substantially below that of NAT1 4 and the other NAT1 allozymes (Fig. 4). Substantially lower levels of NAT1 protein were expressed by these same variant *NAT1* alleles and the reduced level of NAT1 17 and NAT1 22 protein and catalytic activities appear to be related to decreased stability (Fig. 5). Although these studies shed light on the identity and possible mechanism for slow acetylator *NAT1* alleles, they do not address the phenotype of alleles such as *NAT1*10* that have nucleotide polymorphisms only in the 3′-untranslated region. The *NAT1*10* allele has been associated with a rapid acetylator phenotype both in vitro [17] and in vivo [18]. However, recombinant expression studies have failed to confirm this conclusion [19,20]. The matter is further complicated by the fact that *NAT1*10* and *NAT2*4* appear to be in linkage disequilibrium [19] and that human NAT1 catalytic activity may be regulated by substrate concentration [21]. It is clear that further studies are needed to understand the role of substrate regulation and nucleotide polymorphisms in the 3′-untranslated region of *NAT1* on mRNA and protein expression and stability.

The human *NAT2* acetylator polymorphism was identified much earlier (reviewed in [22]) and many studies have investigated the relationship between genotype and phenotype. Human *NAT2*4* is defined as the reference human *NAT2* allele [1] because it is associated with high activity and is the most frequent allele in the original population studied (Japanese). All the nucleotide polymorphisms identified to date

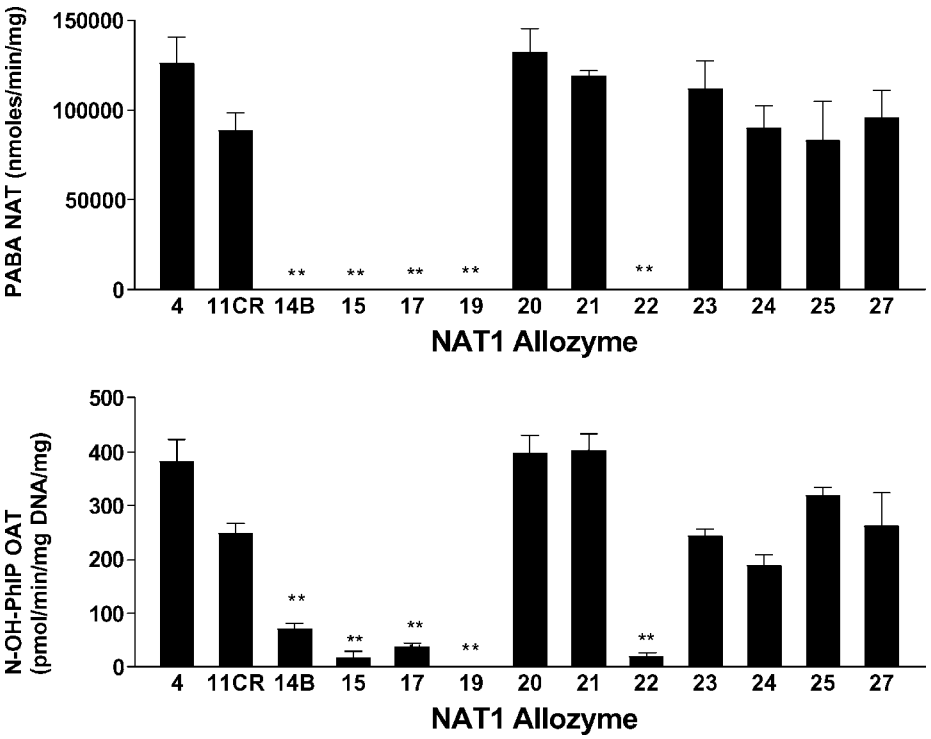

Fig. 4. *p*-Aminobenzoic acid (PABA) *N*-acetyltransferase (upper panel) and *N*-hydroxy-PhIP *O*-acetyltransferase activities (lower panel) of recombinant human NAT1 allozymes expressed in yeast. 11CR refers to *NAT1*11* coding region only. ** Significantly lower than reference NAT1 4 ($P < 0.01$). Adapted from [15].

Table 3
Summary effects of SNPs on *N*-acetyltransferase catalytic activity, levels of expressed protein, and intrinsic protein stability[a]

SNP	Amino acid change	Sulfamethazine *N*-acetyltransferase activity[b]	2-Aminofluorene *N*-acetyltransferase activity[b]	NAT2 protein expression[b]	NAT2 protein stability[b]
T111C	None	+++	+++	+++	+++
C190T	R64W	+	+	+	+
G191A	R64Q	+	+	++	+
C282T	None	+++	+++	+++	+++
T341C	I114T	+	+	+	+++
A434C	E145P	+	+	+	+++
C481T	None	+++	+++	+++	+++
G590A	R197Q	++	++	+	+
G759T	None	+++	+++	+++	+++
A803G	K268R	+++	+++	+++	+++
A845C	K282T	+++	++	+++	+
G857A	G286E	+++	++	+++	+

+, 0–35%; ++, 36–70%; +++, >70%.

[a] Recombinant human NAT2 allozymes expressed in yeast. Adapted from [23,24].

[b] Relative to NAT2 4.

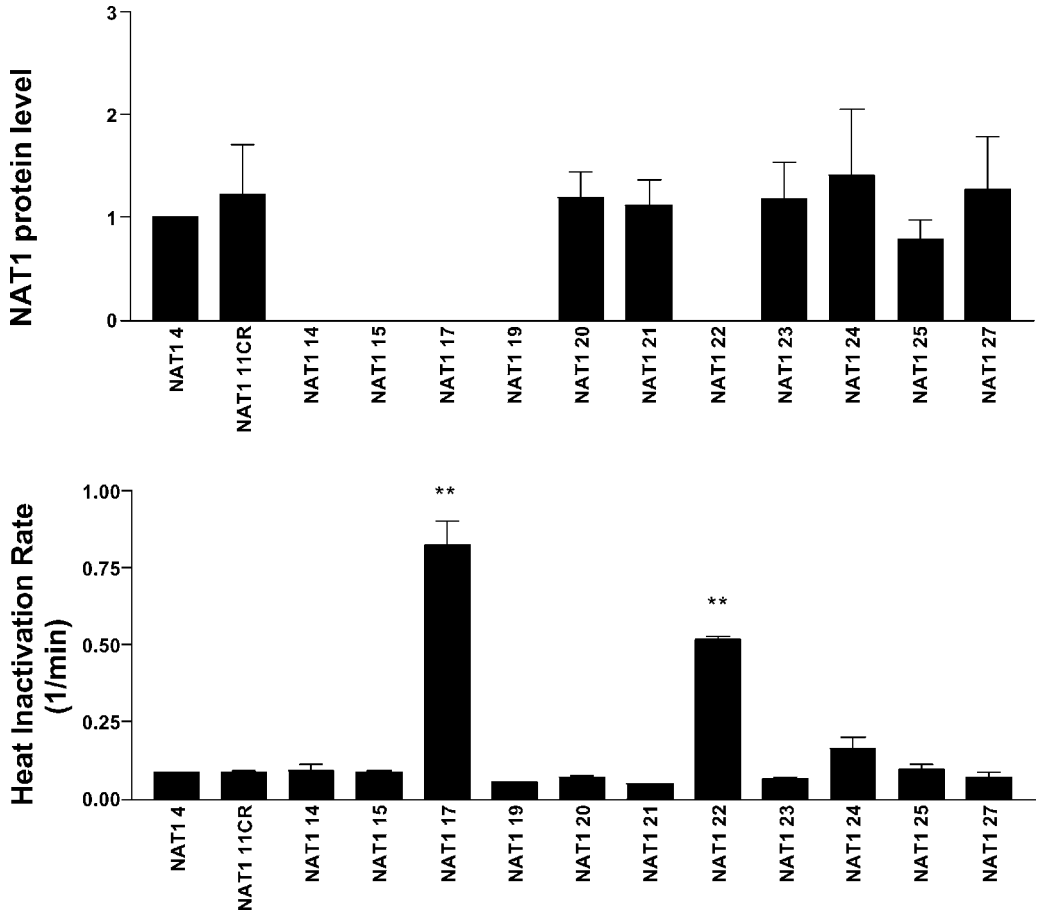

Fig. 5. NAT1 protein expression levels (upper panel) and protein stability (lower panel) of human NAT1 4 (reference) and variant NAT1 allozymes recombinantly expressed in yeast. Protein levels for NAT1 14, 15, 17, 19, and 22 were below the level of detection following Western blot analysis. NAT1*11CR refers to *NAT1*11* coding region only. ** Significantly different than NAT1 4 ($P < 0.01$). Adapted from [15,16].

in *NAT2* are present in the coding region and most occur in combination (Table 2). We expressed human *NAT2*4* and 12 variant *NAT2* alleles possessing single nucleotide polymorphisms (SNPs) in the coding region in yeast (*S. pombe*). We found that several of the SNPs were associated with reduced catalytic activity (Fig. 6). The effects of each SNP on *N*-acetylation and *O*-acetylation were highly correlated for human NAT1 and NAT2 (Fig. 7). However, there was considerable difference among the SNPs in the extent of the reduction as C190T, G191A, T341C, and A434C reduced these activities to less than 35% of reference NAT2 4 (Table 3). Substantially lower levels of NAT2 protein were associated with C190T, T341C, A434C, and G590A (Fig. 8). C190T, G191A, A845C and G857A SNPs encoded NAT2 proteins that were significantly less stable (Fig. 8). These results suggest multiple mechanisms for human slow *NAT2* acetylator phenotype as C190T, T341C, A434C, and G590A reduce protein expression whereas C190T, G191A, A845C, and G857A reduce NAT2 protein stability. We have expressed human *NAT2*4* reference and 14 other variant *NAT2* alleles possessing combinations of SNPs in *Escherichia coli*. The *NAT2*5* cluster (all possessing T341C) showed the greatest reduction in *N*-acetylation, *O*-acetylation and *N*,*O*-acetylation

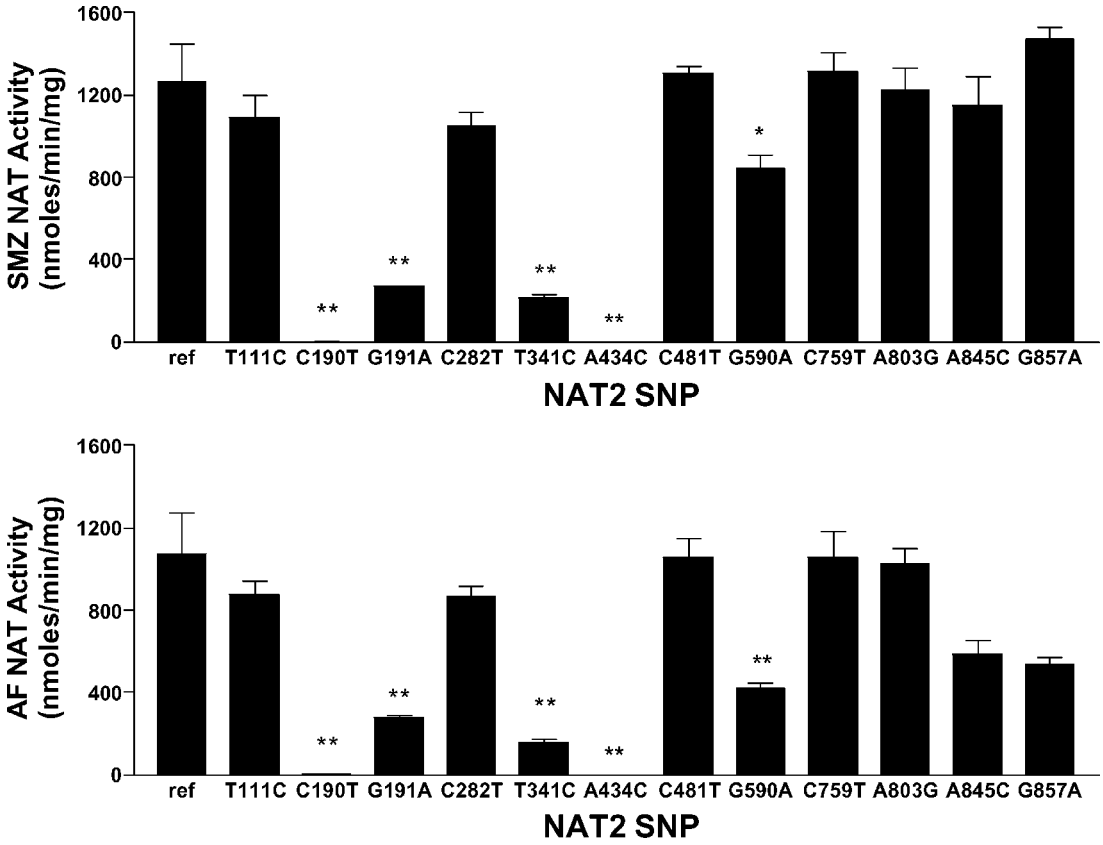

Fig. 6. Sulfamethazine (SMZ; upper panel), and 2-aminofluorene (AF; lower panel) *N*-acetyltransferase activities of human NAT2 4 (reference) and variant NAT2 allozymes expressed in yeast. A434C activities were non-detectable. ** Significantly lower than NAT2 4 ($P < 0.01$). Adapted from [23,24].

activities, followed by the *NAT2*14* cluster (all possessing G191A), followed by the *NAT2*6* cluster (all possessing G590A) followed by the *NAT2*7* cluster (all possessing G857A). The other variant *NAT2* alleles did not differ significantly from reference *NAT2*4* (Fig. 9). These results suggest that human slow acetylator phenotype is not homogeneous but rather consists of multiple phenotypes dependent upon the inheritance of specific SNPs and alleles. Further human genotype/phenotype studies are needed to test this hypothesis.

3. Human epidemiological investigations

A number of studies have shown associations between slow *NAT2* acetylator genotype and urinary bladder cancer (reviewed in [19]) and this finding has been confirmed in meta-analyses (Rothman et al., this volume). Three studies found that urinary bladder cancer risk was highest in individuals possessing *NAT2*5* genotypes [26–28]. The T341C SNP associated with *NAT2*5* alleles yielded very large reductions in NAT2 protein and activity (Table 3). One of the original studies by Cartwright et al. [29] is of particular interest for several reasons. First, the population studied had documented exposures to aromatic amine dyes. Second, NAT2 acetylator phenotype was assessed by measurement of monoacetyl-dapsone to dapsone metabolic ratios in plasma. Dapsone is an NAT2 selective substrate (more so that caffeine which is commonly used today because of its safety) and measurement of monoacetyl-dapsone to dapsone metabolic ratios in plasma is a more direct assessment

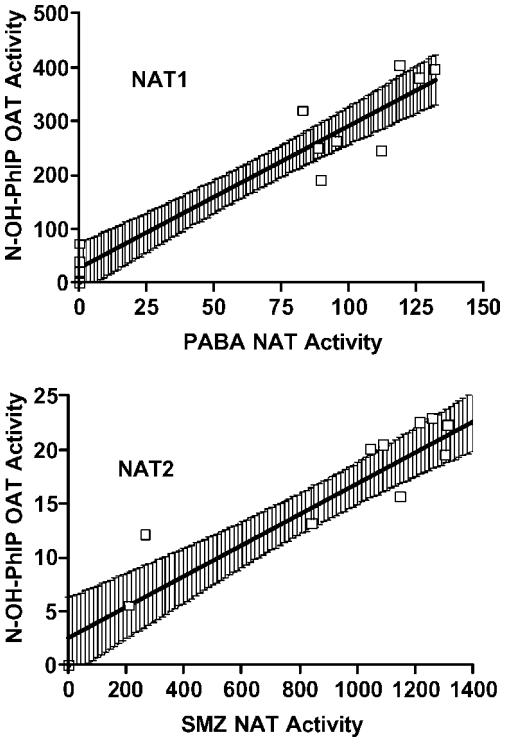

Fig. 7. Correlation of *N*-acetyltransferase and *O*-acetyltransferase activities catalyzed by recombinant human NAT1 (top panel) and NAT2 (bottom panel) allozymes expressed in yeast. Shading represents 95% confidence interval for best fit correlation. Each square represents activities of a recombinant NAT1 or NAT2 allozyme.

of hepatic NAT2 phenotype than is urinary ratios of caffeine metabolites, because the latter are influenced by other factors (including *NAT1* genotype). Third, the NAT2 phenotype data was not separated into two phenotypes (rapid and slow), but rather into eight ranges of metabolic ratios. Five of these ratios (0.3 and greater) correspond to rapid acetylators, and the other three (0.01–0.09; 0.1–0.19; and 0.2–0.29) correspond to different levels of slow acetylator phenotype. As shown in Table 4, urinary bladder cancer risk increased as NAT2 phenotype decreased ($P_{trend} = 0.0006$). The risk was markedly increased in the slowest NAT2 phenotype (OR, 20.8; 95% CI, 2.63–164). These results, together with the findings of studies showing the highest urinary bladder cancer risk in *NAT2*5* genotypes [26–28] are consistent with the recombinant expression studies described above. They suggest that NAT2 slow acetylator phenotype is not homogeneous, but rather that multiple slow acetylator phenotypes exist resulting from different SNPs and mechanisms. In a recent study, we have observed a similar effect on prostate cancer susceptibility, particularly when in combination with glutathione-*S*-transferase null genotypes [30] or the *NAT1*10* allele (Table 5). Further studies are needed to confirm these pilot observations. Interestingly the effect of *NAT1*10* on urinary bladder cancer risk is unclear, as studies have reported that *NAT1*10* either increases [32], decreases [33], or has no effect [27] on risk of urinary bladder cancer.

High red meat intake is associated with an increased risk of colorectal cancer and with adenomatous polyps. Carcinogens generated during high temperature cooking of red meats include heterocyclic amines such as MeIQx and PhIP. NAT1 and NAT2 are expressed at high levels in human colon, and it would be expected that an effect of rapid *NAT1* or *NAT2* acetylator genotype would be most prominent in colon cancer, particularly with documented exposure to heterocyclic amine carcinogens. The role of *NAT1* and *NAT2* acetylator genotypes in colorectal cancer incidence have recently been reviewed [19,34,35]. Some studies have shown increased risk in rapid *NAT1*

Table 4
NAT2 phenotype and urinary bladder cancer[a]

Metabolite ratio[b]	Controls (%)	Cases (%)	Crude OR (95% CI)[c]
0.3+ (rapid)	48 (42.9)	37 (33.3)	1.0 (reference)
0.20–0.29	32 (28.6)	17 (15.3)	0.69 (0.33–1.42)
0.10–0.19	31 (27.7)	41 (42.3)	1.72 (0.91–3.23)
0.01–0.09	1 (0.9)	16 (14.4)	20.8 (2.63–164)

[a] Adapted from [29].
[b] Monoacetyl-dapsone/dapsone in plasma 2–6 h after administration of 50 mg dapsone.
[c] Crude odds ratios determined by Fisher's exact test ($P_{trend} = 0.0006$).

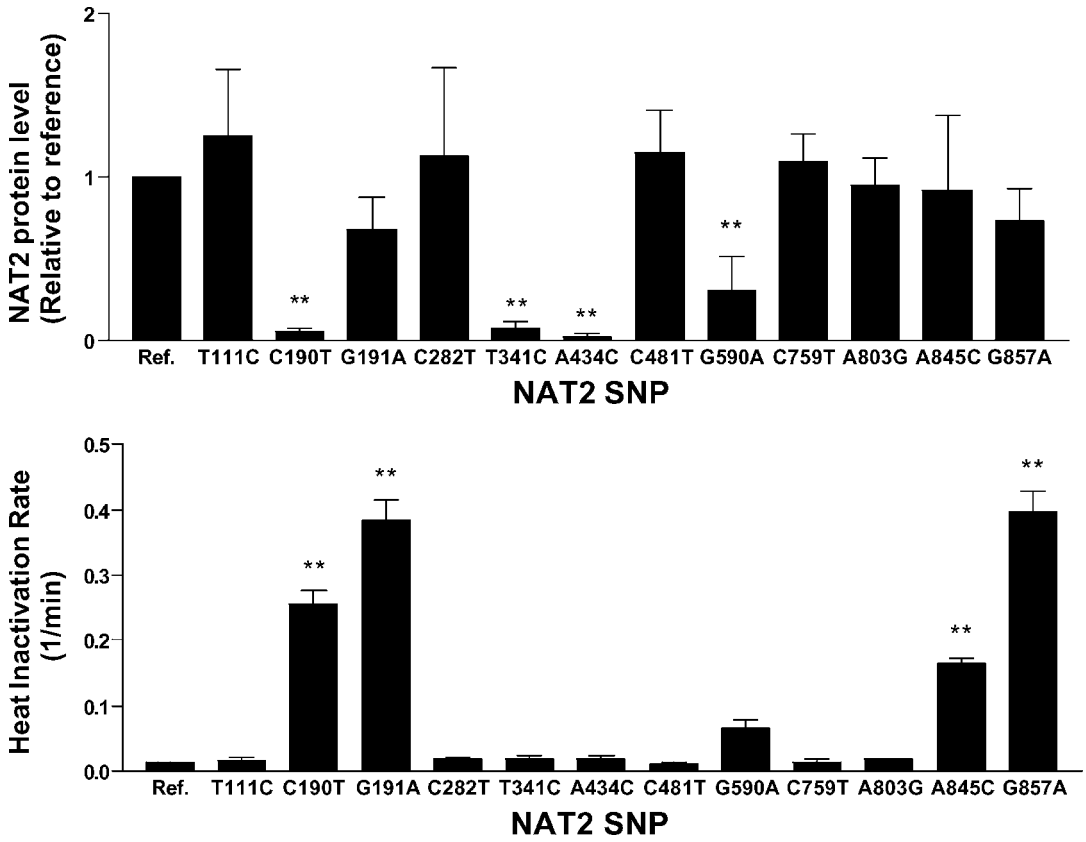

Fig. 8. NAT2 protein expression levels (top panel) and heat inactivation rate constants (bottom panel) of human NAT2 4 (reference) and variant NAT2 allozymes recombinantly expressed in yeast. ** Significantly lower than NAT2 4 ($P < 0.01$). Adapted from [23,24].

and/or *NAT2* acetylators, while other studies have not shown these associations. We tested the effect of rapid *NAT2* and *NAT1* (*NAT1*10*) genotypes on colorectal cancer incidence in subjects with high dietary exposures to heterocyclic amine carcinogens [36]. Human dietary exposure to PhIP and MeIQx calculated from a validated food frequency questionnaire, were associated with higher incidence of colorectal adenomas, particularly in individuals with *NAT1*10* (Table 6). These results together with the animal model studies described above, suggest that dietary exposures to heterocyclic amine carcinogens increase risk for human

Table 5
*NAT2/NAT1*10* genotypes in prostate cancer[a]

*NAT2/NAT1*10* Genotypes[b]	Controls (%)	Cases (%)	Crude OR (95% CI)[c]
Rapid/absent	36 (31.3)	6 (13.3)	1.0 (reference)
Rapid/present or slow/present	66 (57.4)	28 (62.2)	2.55 (0.96–6.72)
Slow/absent	13 (11.3)	11 (24.4)	5.08 (1.56–16.5)
*NAT2*5/*5*/present	4 (3.5)	5 (11.1)	7.50 (1.55–15.4)

[a] Adapted from [31].
[b] At risk genotypes defined as *NAT2* slow in combination with *NAT1*10*.
[c] Crude odds ratios determined by Fisher's exact test ($P_{trend} = 0.001$).

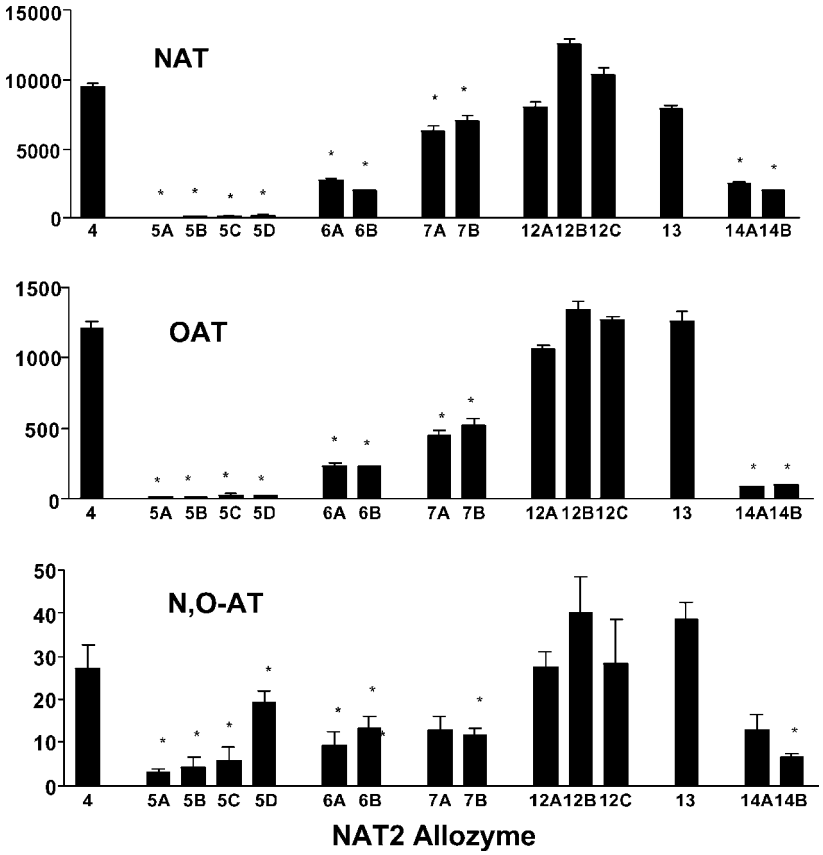

Fig. 9. Relative capacity of recombinant human NAT2 allozymes expressed in *Escherichia coli* to catalyze the *N*-acetylation of 2-aminofluorene (top panel), the *O*-acetylation of *N*-hydroxy-4-aminobiphenyl (center panel) and the *N*,*O*-acetylation of *N*-hydroxy-*N*-acetyl-2 aminofluorene (bottom panel). 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 pmol/min per unit protein; OAT and *N*,*O*-AT activities are pmol/(min mg) DNA per unit protein. Adapted from [25].

Table 6
NAT1 genotype and colorectal adenoma[a]

NAT1 genotype[b]	MeIQx intake (ng)[c]	Cases	Controls	OR (95% CI)[d]
	27.00	78	160	1.0 (reference)
	>27.00	54	32	2.68 (1.58–4.55)
No *NAT1*10*	≤27.00	45	101	1.0 (reference)
No *NAT1*10*	>27.00	32	25	2.44 (1.20–4.99)
*NAT1*10*	≤27.00	33	59	1.23 (0.67–2.24)
*NAT1*10*	>27.00	22	7	7.67 (2.77–21.3) $P = 0.14$[e]

[a] Adapted from [36].

[b] Individuals were classified as rapid acetylators if they possessed at least one *NAT1*10* allele. Due to the low frequency of slow acetylators, all *NAT1* genotypes other than those possessing the *NAT1*10* allele were combined to form the reference group.

[c] Defined by the 80th percentile.

[d] All odds ratios adjusted for age, gender, total caloric intake, fiber intake, reason for screening, physical activity, pack-years of cigarette smoking, and use of non-steroidal anti-inflammatory drugs.

[e] Likelihood ratio test for the interaction between genotype and MeIQx consumption.

colorectal adenomas, and that rapid *NAT1* acetylators are predisposed to the carcinogenic action because of higher rates of metabolic activation and DNA damage.

The role of *NAT1* and *NAT2* acetylator polymorphisms in cancer risk from aromatic and heterocyclic amine carcinogens will become clearer with more precise determinations of both exposures and genotypes. A better understanding of the relationship between NAT1 and NAT2 acetylator genotype and phenotype is also needed. The crystallographic structure of a prokaryotic *N*-acetyltransferase was recently resolved [37]. Molecular modeling of human NAT1 [38] and NAT2 [39] has revealed a cysteine protease-like catalytic triad (Cys^{68}–His^{107}–Asp^{122}). As of this date, crystallization of human or other mammalian *N*-acetyltransferase isozymes have not been reported but is expected in the near future.

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