[CANCER RESEARCH 55, 5226–5229, November 15, 1995]

Advances in Brief

Polymorphism in the *N*-Acetyltransferase 1 (*NAT1*) Polyadenylation Signal: Association of *NAT1*10* Allele with Higher *N*-Acetylation Activity in Bladder and Colon Tissue

Douglas A. Bell,[1] Alaa F. Badawi, Nicholas P. Lang, Kenneth F. Ilett, Fred F. Kadlubar, and Ari Hirvonen[2]

Laboratory of Biochemical Risk Analysis, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709 [D. A. B, A. H.]; Division of Molecular Epidemiology, National Center for Toxicological Research, Jefferson, Arkansas 72079 [A. F. B, F. F. K.]; University of Arkansas Medical Sciences, Arkansas Cancer Research Center, Little Rock, Arkansas 72205 [N. P. L.]; and Department of Pharmacology, University of Western Australia, Perth, Australia 6009 [K. F. I.]

Abstract

Exposures to carcinogens present in the diet, in cigarette smoke, or in the environment have been associated with increased risk of bladder and colorectal cancer. The aromatic amines and their metabolites, a class of carcinogen implicated in these exposures, can be *N*- or *O*-acetylated by the NAT1 and NAT2 enzymes. Acetylation may result in activation to DNA-reactive metabolites or, in some cases, detoxification. Many studies have focused on genetic variation in *NAT2* and its potential as a risk factor in bladder and colorectal cancer; however, NAT1 activity is higher in bladder and colonic mucosa than NAT2, and the NAT1 enzyme also exhibits phenotypic variation among human tissue samples. We hypothesized that specific genetic variants in the polyadenylation signal of the *NAT1* gene would alter tissue levels of NAT1 enzyme activity and used a PCR-based method to distinguish polymorphic *NAT1* alleles in samples obtained from 45 individuals. When the *NAT1* genotype was compared with the NAT1 phenotype in bladder and colon tissue samples (*p*-aminobenzoic acid activity), we observed a ~2-fold higher NAT1 enzyme activity in samples from individuals who inherited a variant polyadenylation signal (*NAT1*10* allele). This is the first observation relating a genetic polymorphism in *NAT1* to a rapid/slow NAT1 phenotype in humans.

Introduction

The human NAT1[3] enzyme can carry out *N*-acetylation of carcinogenic aromatic amines (1, 2). NAT1 also catalyzes the *O*-acetylation of *N*-hydroxyarylamines and an intramolecular *N,O*-acetyltransfer reaction that converts arylhydroxamic acids to mutagenic acetoxy esters (1–3). The NAT1 enzyme is expressed in bladder epithelial tissue and in cell lines derived from bladder epithelium (4, 5). Recent *in vitro* studies have suggested that NAT1 may have an important role in the metabolism of some bladder carcinogens (2, 6); thus, variation in NAT1 activity among human populations might affect risk of carcinogen-induced carcinoma of the bladder as well as that of the colon.

The NAT2 enzyme has long been known to be polymorphic (reviewed in Ref. 7), and recent studies have described the molecular genetic basis for the rapid/slow acetylation phenotype (8–12). In contrast, the NAT1 enzyme has generally been considered to be monomorphic (reviewed in Ref. 13) for two reasons: (*a*) until very recently, only one form of the NAT1 enzyme had been isolated; and (*b*) human population studies of NAT1 phenotypic variation appeared to show a unimodal distribution. However, some recent studies have suggested the potential for high and/or low activity alleles of *NAT1* (14, 15). These studies measured NAT1 enzyme activity in whole blood using PABA or *p*-aminosalicylic acid (14, 15). A preliminary report also has suggested that there are some rare *NAT1* alleles that lack activity (16). In addition, Vatsis *et al.* (17) have demonstrated that sequence polymorphisms in the 3′ untranslated region of *NAT1* occur relatively frequently in human populations. However, as yet, there have been no comparisons between NAT1 phenotype and *NAT1* genotype for the known alleles. Thus, it was unknown if differences in tissue levels of NAT1 among humans are related to specific sequence differences in the *NAT1* structural gene. In this study, we measured the *in vitro* NAT1 enzyme activity of bladder and colon tissue samples using PABA (a NAT1 specific substrate) and found that higher PABA activity is associated with a specific *NAT1* polyadenylation signal sequence variant (*NAT1*10*).

Materials and Methods

Human urinary bladder (n = 26) and colorectal (n = 19) mucosa were obtained from the U.S. Cooperative Tissue Network, the Sir Charles Gairdner Hospital (Perth, Western Australia), and the John L. McClellan Memorial Veterans Medical Center (Little Rock, AR). Information on subjects concerning gender, age, medications, tobacco use, alcohol consumption, or life style was not available. Following collection, samples were immediately frozen in liquid nitrogen and stored at −80°C until used. Homogenates of thawed tissue were prepared as described elsewhere (18) to allow preparation of both tissue cytosols and DNA. DNA was isolated from 10,000 × g pellets, using a modified phenol extraction procedure (19). The supernatants of the 100,000 × g centrifugation was used as a source of tissue cytosol. DNA (20) and protein (21) content were determined as described. *In vitro* acetylation of PABA to *N*-acetyl-PABA (a specific indicator of NAT1 activity) was measured under linear conditions (acetyl-CoA, 2 mM) essentially as described (22, 23).

Statistical analyses (Mantel-Haenszel χ^2, Fisher's exact test, t test of log transformed data, Mann-Whitney test, normality tests, and box-and-whisker plots) were carried out using the SigmaStat (Jandel Corp., San Rafael Madera, CA) and Epitest software packages (Finnish Institute of Occupational Health). Probit transformations of the data were carried out by plotting the enzyme activity against the corresponding percentage under the normal probability curve, applying the method of Butler *et al.* (24).

For *NAT1* genotype analysis by PCR, we used a mutant primer PCR-RFLP method to discriminate between three sequence variants in the *NAT1* gene (25). These alleles are distinguished by changes in the 3′ region of the *NAT1* gene in and around the putative mRNA polyadenylation signal at nucleotide 1086 ("AATAAA"; Fig. 1). The alleles identified by this method were: (*a*) *NAT1*4*, the most common allele[4] and presumably, the "wild type" allele that contains a "T" at nucleotide 1088 and a "C" at nucleotide 1095; (*b*) *NAT1*10*, a variant in which nucleotide 1088 = "A" and nucleotide 1095 = "A" (the change at

Received 7/12/95; accepted 10/5/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom correspondence should be addressed at C3–03, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709.

[2] A. H., Visiting Fellow, National Institute of Environmental Health Sciences, was supported by a National Institute of Environmental Health Sciences Intramural Research Award to D. A. B. and had partial support from the Finnish Academy of Sciences, the Finnish Cancer Society, and the Finnish Work Environmental Fund.

[3] The abbreviations used are: NAT1 and NAT2, *N*-acetyltransferase 1 and 2, PABA, *p*-aminobenzoic acid.

[4] See Ref. 26 for an explanation of *NAT1* allele nomenclature.

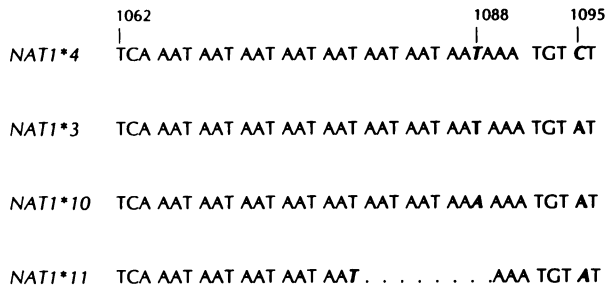

Fig. 1. Sequence of *NAT1* alleles in the 3′ untranslated region. *NAT1*10* differs from *NAT1*4* at two nucleotide positions (nucleotides 1088 and 1095). The nucleotide 1088 change shifts the position of the putative polyadenylation signal. *NAT1*11* differs from *NAT1*4* by a 9-bp deletion in the 24-bp trinucleotide repeat sequence (nucleotides 1065–1088), a C > A change at nucleotide 1095, and a T > G at nucleotide 640. The exact position of the 9-bp deletion cannot be determined, but it is possible that *NAT1*11* is a reversion of *NAT1*10* (they share the nucleotide 1095 C > A change). The position of PCR primer N1536R is shown; it contains a mismatch at nucleotide 1098, which generates a partial *Mbo*II site.

nucleotide 1088 results in a shift in the mRNA polyadenylation signal); (*c*) *NAT1*11*, a variant that contains a 9-bp deletion in the trinucleotide repeat sequence ("AAT") occurring immediately upstream of nucleotide 1088 with a T > G (Ser > Ala) change at nucleotide 640. The sequence variants are illustrated in Fig. 1.

To differentiate the *NAT1*10* allele from the *NAT1*4* allele, the *NAT1* reverse primer contained a mismatched base (T > G) at nucleotide 1098. Subsequent PCR amplification of the *NAT1*4* allele with this primer resulted in a PCR product containing a new *Mbo*II restriction site. PCR conditions for the *NAT1* PCR-RFLP method were the same as in (25). Briefly, *NAT1*-specific primers N1306F (5′ cta ttt aga ata agg agt aa) and N1536R (5′ aca ggc cat ctt tag aa) (15 pmol each), 2.0 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate, and 1 unit Taq DNA polymerase were added to 100 ng of genomic DNA in a volume of 30 μl; and the PCR was heated to 94°C for 4 min and then subjected to 35 cycles of 94°C for 30 s, 42°C for 30 s, and 72°C for 45 s. A final 72°C extension for 5 min was performed.[5] Following *Mbo*II digestion and electrophoresis on a high resolution agarose gel (4% Metaphor; FMC Bioproducts, Rockland, ME), genotypes containing the *NAT1*4* and *NAT1*11* alleles produced distinct band patterns (Fig. 2). The *Mbo*II digestion of the *NAT1*4* allele cut the PCR product into fragments of 105, 71, 45, and 26 (Fig. 2, *Lane 1, NAT1*4* homozygote). Digest of a *NAT1*10* allele results in fragments of 131, 75, 45, and 26 bp (Fig. 2, *Lane 3, NAT1*10* homozygote). The *NAT1*11* allele can be distinguished by observation of a 9-bp mobility shift of the 131-bp band to a 122-bp band (Fig. 2, *Lanes 4, 5,* and *6,* heterozygote and homozygote). PCR fragment digestion patterns, along with the genotypes, are shown in Fig. 2. We have confirmed these genotypes using a second allele-specific PCR method for nucleotide 1088 (data not shown; method described in Ref. 25). For ~80 individuals participating in other studies, we have confirmed PCR-RFLP genotypes by direct sequencing of the polymorphic region (data not shown). More information with regard to *NAT1* alleles and genotyping can be found in Vatsis *et al.* (26) and Bell *et al.* (25).

Results and Discussion

Probit plots of NAT1 activity in bladder and colon tissue samples are shown in Fig. 3 and demonstrate that there is a relatively large sample-to-sample variation within each tissue type. We observed an ~8-fold variation among bladder tissue samples and a 53-fold variation among colon tissue samples. Average NAT1 activity in colon tissue was about 8-fold higher than in bladder tissue (Table 1). The sample-to-sample variation observed in Figs. 3 and 4 (particularly the very low activity for some samples) may be caused, in part, by degradation of the NAT1 enzyme. Instability of NAT1 has been observed in other studies (1, 2); however, the relationship between activity and genotype strongly suggests that there might be a molecular genetic determinant to the variation in NAT1 activity.

[5] Recently, we have also used a new forward primer N1208F (5′ gac tct gag tga ggt aga aat a; annealing at 55°C), which has increased specificity for *NAT1*.

In both tissue types, *NAT1*10* alleles were significantly overrepresented among individuals with high NAT1 activity relative to low NAT1 activity. Within the bladder tissue samples (Fig. 3*A*), the frequency of *NAT1*10* alleles (7 of 12) among the 12 individuals with "high" NAT1 activity (>2.2 nmol/min/mg protein) was significantly greater relative to the frequency of *NAT1*10* alleles (1 of 13) among the 13 lowest activity individuals (Mantel-Haenszel $\chi^2 = 7.1$; $P = 0.008$). One individual was heterozygous for the *NAT1*11* allele (9-nucleotide deletion and nucleotide 640 Ser > Ala) and was excluded from the statistical analysis. In colon tissue (Fig. 3*B*), the frequency of *NAT1*10* alleles among the 8 individuals with "high" NAT1 activity (7 of 8; >20 nmol/min/mg protein) was significantly higher relative to the frequency of *NAT1*10* alleles among the 11 lowest activity individuals (4 of 11; Mantel-Haenszel $\chi^2 = 4.7$; $P = 0.037$). Among all samples, only one individual was homozygous for the *NAT1*10* allele (in the colon tissue samples). Thus, in sample sets from both tissues, there were significant associations between the *NAT1*10* allele and higher NAT1 activity. Although the high/low grouping used in this analysis is somewhat arbitrary and the size of these sample sets is small, the differences we observed are highly suggestive of a phenotypic effect for the *NAT1*10* allele.

In Table 1, the mean NAT1 activities in bladder and colon tissue are shown for samples from individuals with the *NAT1*10* allele compared with those homozygous for *NAT1*4*, and these data are displayed graphically in box-and-whisker plots. Bladder tissue samples from subjects with the *NAT1*10* allele (all heterozygotes) had a mean NAT1 activity of 4.6 ± 2.6 nmol/min/mg protein ($n = 8$); while those homozygous for *NAT1*4* ($n = 17$) had mean NAT1 activity of 2.3 ± 1.6 pmol/min/mg protein. This 2-fold difference was significant (*t*-test of log transformed data, $t = -2.74$, $df = 23$, $P = 0.012$). Colon mucosa samples from subjects with the *NAT1*10* allele also had higher mean NAT1 activity (28.6 ± 14.1 nmol/min/mg protein *versus* 16.6 ± 13.1 nmol/min/mg protein), but this difference was of borderline significance (*t*-test on log transformed data, $t = -2.04$, $df = 17$, $P = 0.057$). The limited number of samples in both bladder and colon tissue data sets and the high degree of variation within genotype groups indicate that these results should be interpreted cautiously. As can be seen in the plots in Figs. 3 and 4, there are a few samples with *NAT1*10* alleles that have low activity and a few *NAT1*4* alleles among the high activity samples.

The degree of variation within groups and across tissue types suggests that there are other determinants of bladder and colon NAT1 activity in addition to the effect of the altered polyadenylation signal that is present in the *NAT1*10* allele. Deacetylase may have an important role in benzidine metabolism in human liver (27) and expression of deacetylase varies among humans (28). However, it is unlikely that there is enough deacetylase activity in cytosol preparations from bladder and colon tissues to impact the levels of acetylated PABA. Transcriptional effects, particularly tissue-specific factors, may also be important in determining variability in NAT1 activity among individuals and between tissue types.

The recognition of the polyadenylation signal by RNA polymerase during transcription initiates cleavage of the growing mRNA chain followed by the subsequent addition of the poly(A) sequence to the transcript. Polyadenylation state [length of the poly(A) tail] can influence the degradation or stability of a mRNA molecule (29), and there are numerous examples where turnover of protein and mRNA are coordinately regulated (29–31). The putative polyadenylation signal of the *NAT1* gene is altered in the *NAT1*10* allele (T>A change at nucleotide 1088; Fig. 1). This change creates a new consensus polyadenylation signal that is shifted three nucleotides in the 5′ direction and is flanked on the 3′ side by an adenine triplet (AATAAA AAA). We speculate that the new polyadenylation signal present in

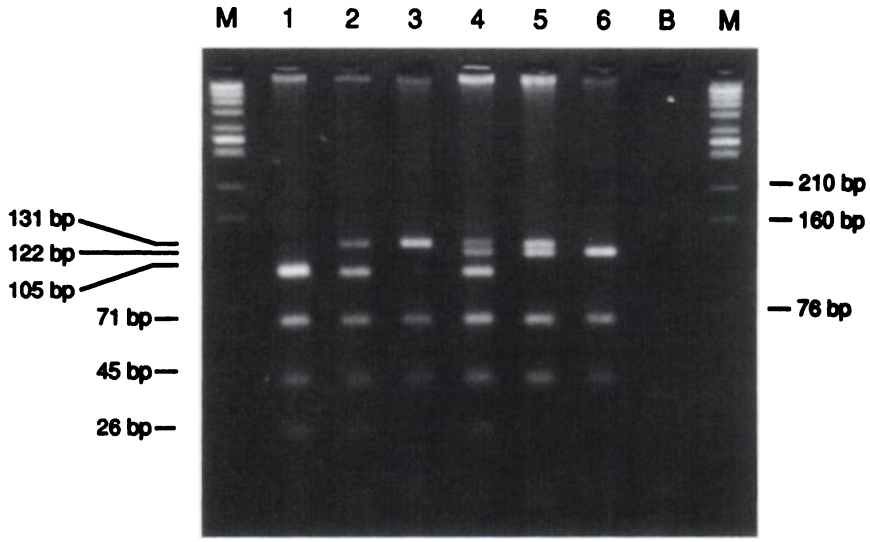

Fig. 2. Gel electrophoresis PCR products from the possible *NAT1* genotypes. *M*, marker ($\phi X174$ *Hin*dIII); *Lane 1*, *NAT1*4/NAT1*4*; *Lane 2*, *NAT1*4/NAT1*10*; *Lane 3*, *NAT1*10/NAT1*10*; *Lane 4*, *NAT1*4/NAT1*11*; *Lane 5*, *NAT1*10/NAT1*11*; *Lane 6*, *NAT1*11/NAT1*11*; *B*, blank, no DNA negative control; *M*, marker ($\phi X174$ *Hin*dIII). In *Lane 4*, the 131-bp band is caused by poor *Mbo*II digestion of the *NAT1*4/NAT1*11* heteroduplex PCR product.

the *NAT1*10* allele produces a more stable mRNA, which in turn impacts NAT1 enzyme levels. Alternative mechanisms may also be possible. For example, the *NAT1*10* allele might be in linkage disequilibrium with other mutations in the coding or regulatory region of *NAT1*. However, preliminary sequencing studies of the coding regions of the *NAT1*10* allele do not support this possibility (18). Moreover, the possibility of linkage between *NAT1*10* and upstream regulatory mutations has not been investigated. We have initiated studies that may reveal the relationship between specific *NAT1* alleles and mRNA levels, mRNA stability, polyadenylation status, and enzyme activity.

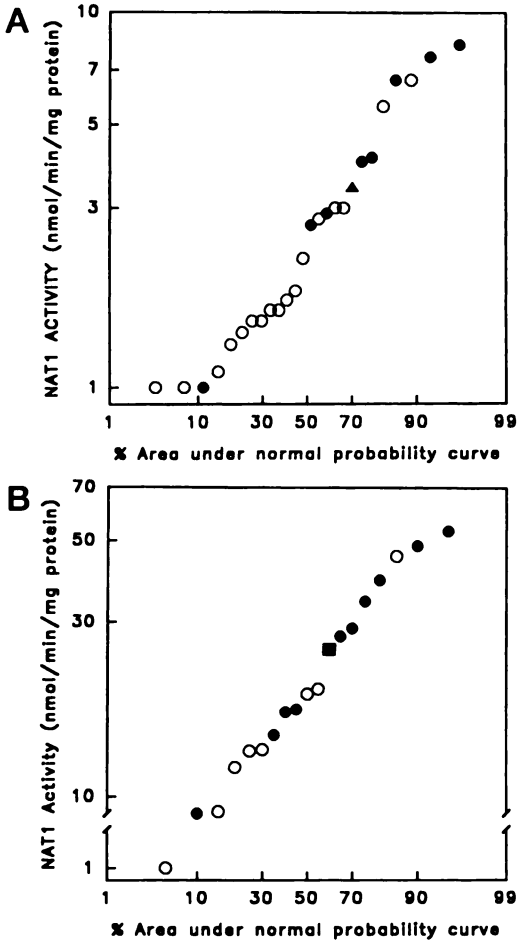

Fig. 3. Probit plots of NAT1 activity in bladder (*A*) and colon (*B*) tissue as measured by *N*-acetyl-PABA. ○, *NAT1*4/NAT1*4* homozygotes. ●, the subjects who were heterozygous for the *NAT1*10* allele. ■, the single *NAT1*10/NAT1*10* homozygote. ▲, a *NAT1*4/NAT1*11* heterozygote. *NAT1*10* alleles were overrepresented among individuals with high NAT1 activity.

Table 1 *Mean NAT1 enzyme activity among* NAT1 *genotypes as measured by PABA acetylation*

	Bladder		Colon	
	NAT1 activity[a]	*n*	NAT1 activity	*n*
NAT1 (all genotypes)	2.9 ± 2.3	26	23.6 ± 14.6	19
*NAT1*10*[b]	4.6 ± 1.6[c]	8	28.6 ± 14.1[d]	11
*NAT1*4*	2.3 ± 2.6	17	16.6 ± 13.1	8
*NAT1*11*	3.3	1		

[a] Values represent mean ± SD pmoles *N*-acetyl-PABA/mg protein/min.
[b] Heterozygous *NAT1*10/NAT1*4* genotypes (except one colon sample was a *NAT1*10/NAT1*10* homozygote).
[c] *t* test on log transformed data, t = −2.74, *P* = 0.012.
[d] *t* test on log transformed data, t = −2.04, *P* = 0.057.

It is interesting that the association between *NAT1*10* and high PABA activity appeared to be stronger in urinary bladder than in colon tissue. This is consistent with our epidemiological studies comparing *NAT1*10* genotypes and risk of colorectal and bladder cancer (25, 32). We reported recently on preliminary evidence showing that the *NAT1*10* allele was associated with a 2.9- to 26-fold increased risk among bladder cancer patients who were also smokers (32),[6] whereas *NAT1*10* was associated with only a 1.9-fold risk among colorectal cancer patients (25). Considering that mean NAT1 activity was about 8-fold lower in bladder relative to colon (Table 1), the *NAT1*10* allele (which presumably effects mRNA stability) may have more impact in tissues where *NAT1* mRNA levels are low. Furthermore, we also have data suggesting that NAT1 activity in bladder is correlated with DNA adduct levels in this tissue (18).

This work is the first demonstration that a DNA sequence polymorphism in the 3′ untranslated region of the *NAT1* gene is associated with differences in NAT1 enzyme activity in human tissues. Tissue samples from individuals carrying the *NAT1*10* allele had about 2-fold more NAT1 enzyme activity than those homozygous for the

[6] J. A. Taylor, D. Umbach, E. Stephens, T. Castranio, D. Paulson, C. Robertson, J. L. Mohler, and D. A. Bell. Role of *N*-acetylation polymorphism at *NAT1* and *NAT2* in smoking-associated bladder cancer, manuscript in preparation.

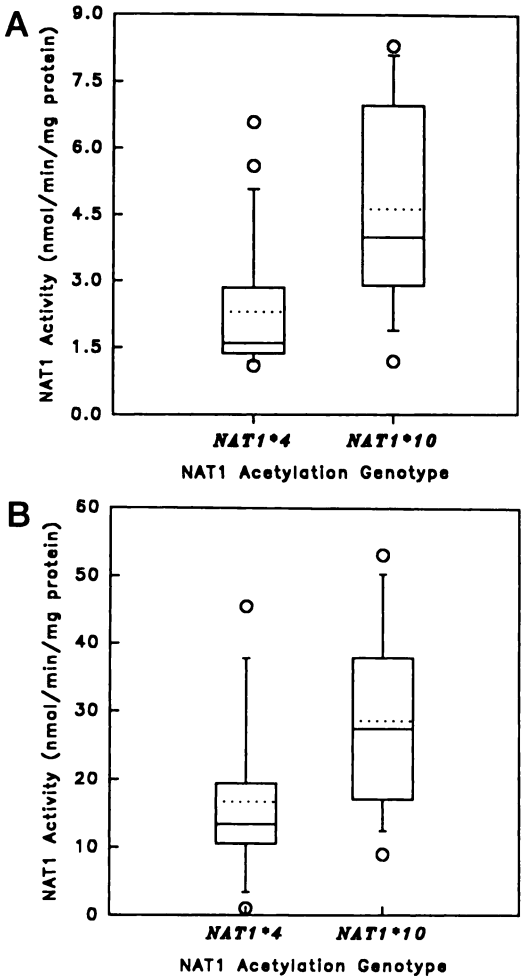

Fig. 4. *A*, bladder tissue cytosol NAT1 activity for *NAT1*4* genotypes *versus NAT1*10* genotypes. *B*, colon mucosa tissue cytosol NAT1 activity for *NAT1*4* genotypes *versus NAT1*10* genotypes. Box-and-whisker plots of bladder and colon tissue NAT1 activity. ···, mean value; ——, median; the *box*, 25th and 75th percentile; *capped line*, 10th and 90th percentile; ○, data values beyond the 10th and 90th percentile.

more common *NAT1*4* allele. Given that the NAT1 enzyme can mediate activation and detoxification pathways for numerous carcinogens, this gene may have an important role in modulating cancer risk. If these preliminary observations can be confirmed by further studies, then the NAT1 genotype will need to be considered as a genetic risk factor in cancer epidemiology.

Acknowledgments

Many thanks to Dr. Denis Grant, Hospital for Sick Children, Toronto, Canada, for many helpful discussions and especially for encouraging us to carry out this study. We thank Drs. Ron Ferguson and John Meadus, National Institute of Environmental Health Sciences, and Dr. Fred Beland, National Center for Toxicological Research, for helpful comments on the manuscript.

References

1. Grant, D. M., Blum, M., Beer, M., and Meyer, U. A. Monomorphic and polymorphic human arylamine *N*-acetyltransferases: a comparison of liver isozymes and expressed products of two cloned genes. Mol. Pharmacol., *38:* 184–191, 1991.
2. Hein, D. W., Doll, M. A., Rustan, T. D., Gray, K., Feng, Y., Ferguson, R. J., and Grant, D. M. Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and NAT2 acetyltransferases. Carcinogenesis (Lond.), *14:* 1633–1638, 1993.
3. Minchin, R., Reeves, P. T., Teitel, C. H., McManus, M. E., Mojarrabi, B., Ilett, K. F., and Kadlubar, F. F. *N*- and *O*-acetylation of aromatic and heterocyclic amine carcinogens by human monomorphic and polymorphic acetyltransferases expressed in *COS*-1 cells. Biochem. Biophys. Res. Commun., *185:* 839–844, 1992.
4. Kirlin, W. G., Trinidad, A., Yerokun, T., Ogolla, F., Ferguson, R. J., Andrews, A. F., Brady, P. K., and Hein, D. W. Polymorphic expression of acetyl coenzyme A-dependent *O*-acetyltransferase-mediated activation of *N*-hydroxyarylamines by human bladder cytosols. Cancer Res., *49:* 2448–2454, 1989.
5. Frederickson, S. M., Messing, E. M., Resnikoff, C. A., and Swaminathan, S. Relationship between *in vivo* acetylator phenotypes and cytosolic *N*-acetyltransferase and *O*-acetyltransferase activities in human uroepithelial cells. Cancer Epidemiol., Biomarkers & Prev., *3:* 25–32, 1994.
6. Probst, M., Blum, M., Fasshauer, I., D'Orazio, D., Meyer, U., and Wild, D. The role of the human acetylation polymorphism in the metabolic activation of the food carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ). Carcinogenesis (Lond.), *13:* 1713–1717, 1992.
7. Evans, D. P. *N*-acetyltransferase. Pharmacol. & Ther., *42:* 157–234, 1989.
8. Deguchi, T., Mashimo, M., and Suzuki, T. Correlation between acetylator phenotypes and genotypes of polymorphic arylamine *N*-acetyltransferase in human liver. J. Biol. Chem., *267:* 18140–18147, 1990.
9. Blum, M., Demierre, A., Grant, D. M., Heim, M., and Meyer, U. A. Molecular mechanism of slow acetylation of drugs and carcinogens in humans. Proc. Natl. Acad. Sci. USA, *88:* 5237–5241, 1991.
10. Vatsis, K. P., Martell, K. J., and Weber, W. Diverse point mutations in the human gene for polymorphic *N*-acetyltransferase. Proc. Natl. Acad. Sci. USA, *88:* 6333–6337, 1991.
11. Hickman, D., Risch, A., Camilleri, J., and Sim, E. Genotyping human polymorphic arylamine *N*-acetyltransferase: identification of new slow allotypic variants. Pharmacogenetics, *2:* 217–226, 1992.
12. Bell, D. A., Taylor, J. A., Butler, M. A., Stephens, E., Wiest, J., Brubaker, L., Kadlubar, F., and Lucier, G. W. Genotype/phenotype discordance for human arylamine *N*-acetyl transferase (*NAT2*) reveals a new slow-acetylator allele common in African-Americans. Carcinogenesis (Lond.), *14:* 1689–1692, 1993.
13. Vatsis, K. P., and Weber, W. W. Human *N*-acetyltransferases. *In:* F. C. Kauffman (ed.), Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity. Handbook of Experimental Pharmacology, V1. 112, pp. 109–130. Heidelberg: Springer-Verlag, 1994.
14. Grant, D. M., Vohra, P., Avis, Y., and Ima, A. Detection of a new polymorphism of human arylamine *N*-acetyltransferase (NAT1) using *p*-aminosalicylic acid as an *in vivo* probe. J. Basic Clin. Physiol. Pharmacol., *3* (Suppl.): 244, 1992.
15. Weber, W. W., and Vatsis, K. P. Individual variability in *p*-aminobenzoic acid *N*-acetylation by human *N*-acetyltransferase (NAT1) of peripheral blood. Pharmacogenetics, *3:* 209–212, 1993.
16. Hughes, N. C., and Grant, D. M. Cloning and expression of new mutant forms of human acetyltransferase 1 with defective function. *In:* G. Bellwood (ed.), Proceedings of the 10th International Symposium on Microsomes and Drug Oxidations, p. 278. Toronto, Canada, 1994.
17. Vatsis, K. P., and Weber, W. W. Structural heterogeneity of caucasian *N*-acetyltransferase at the *NAT1* locus. Arch. Biochem. Biophys., *301:* 71–76, 1993.
18. Badawi, A. F., Bell, D. A., Hirvonen, A., and Kadlubar, F. F. Role of aromatic amine acetyltransferases, NAT1 and NAT2, in carcinogen-DNA adduct formation in the human urinary bladder. Cancer Res., *55:* 5230–5237, 1995.
19. Kirby, K. S., and Cook, E. A. A new method for the isolation of deoxyribonucleic acid. Biochem. J., *66:* 459–504, 1957.
20. Labarca, C., and Paigen, K. A simple and sensitive DNA assay procedure. Anal. Biochem., *102:* 344–352, 1980.
21. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., *72:* 248–254, 1976.
22. Ilett, K. F., Reeves, P. T., Minchin, R. F., Kinnear, R. E., Watson, H. F., and Kadlubar, F. F. Distribution of acetyltransferase activities in the intestines of rapid and slow acetylator rabbits. Carcinogenesis (Lond.), *12:* 1465–1469, 1991.
23. Ilett, K. F., Ingram, D. M., Carpenter, D. S., Teitel, C. H., Lang, N. P., Kadlubar, F. F., and Minchin, R. F. Expression of monomorphic and polymorphic *N*-acetyltransferases in human colon. Biochem. Pharmacol., *47:* 914–917, 1994.
24. Butler, M. A., Lang, N. P., Yong, J. F., Caporaso, N. E., Vineis, P., Hayes, R. B., Teitel, C. H., Massengill, J. P., Lawsen, M. F., and Kadlubar, F. F. Determination of CYP1A2 and NAT2 phenotypes in human population by analysis of caffeine urinary metabolites. Pharmacogenetics, *2:* 116–127, 1992.
25. Bell, D. A., Stephens, E. A., Castranio, T., Umbach, D. M., Watson, M. A., Deakin, M., Elder, J., Hendrickse, C., Duncan, H., and Strange, R. C. Polyadenylation polymorphism in the acetyltransferase 1 gene (*NAT1*) increases risk of colorectal cancer. Cancer Res., *55:* 3537–3542, 1995.
26. Vatsis, K. P., Weber, W., Bell, D. A., Dupret, J-M., Evans, D. A. P., Grant, D. M., Hein, D. W., Lin, H. J., Meyer, U. A., Relling, M. V., Sim, E., Suzuki, T., and Yamazoe, Y. Nomenclature for *N*-acetyltransferases. Pharmacogenetics, *5:* 1–9, 1995.
27. Lakshmi, V. M., Bell, D. A., Watson, M. A., Zenser, T. V., and Davis, B. B. *N*-Acetylbenzidine and *N,N'*-diacetylbenzidine formation by rat and human liver slices exposed to benzidine. Carcinogenesis (Lond.), *16:* 1565–1571, 1995.
28. Probst, M. R., Beer, M., Beer, D., Jenos, P., Meyer, U., and Gasser, R. Human liver arylacetamide deacetylase. J. Biol. Chem., *269:* 21650–21656, 1994.
29. Surdej, P., Riedl, A., and Jacobs-Lorena, M. Regulation of mRNA stability in development. Annu. Rev. Genet., *28:* 263–282, 1994.
30. Hargrove, J. L., and Schmidt, F. H. The role of mRNA and protein stability in gene expression. FASEB J., *3:* 2360–2370, 1989.
31. Jackson, R., and Standart, N. Do the poly(A) tail and 3' untranslated region control mRNA translation? Cell, *62:* 15–24, 1990.
32. Taylor, J. A., Umbach, D., Stephens, E., Paulson, D., Robertson, C., Mohler, J. L., and Bell, D. A. Role of *N*-acetylation polymorphism at *NAT1* and *NAT2* in smoking-associated bladder cancer. Proc. Am. Assoc. Cancer Res., *36:* 282, 1995.

Polymorphism in the *N*-Acetyltransferase 1 (*NAT1*) Polyadenylation Signal: Association of *NAT1*10* Allele with Higher *N*-Acetylation Activity in Bladder and Colon Tissue

Douglas A. Bell, Alaa F. Badawi, Nicholas P. Lang, et al.

Cancer Res 1995;55:5226-5229.