

Association of *NAT1* and *NAT2* Polymorphisms to Urinary Bladder Cancer: Significantly Reduced Risk in Subjects with *NAT1*10*¹

Ingolf Cascorbi,² Ivar Roots, and Jürgen Brockmöller

Institute of Clinical Pharmacology, University Medical Center Charité, Humboldt University, D-10098 Berlin [I. C., I. R., J. B.]; Institute of Pharmacology, Ernst Moritz Arndt University, D-17487 Greifswald [I. C.]; and Center of Pharmacology, Georg August University, D-37075 Göttingen [J. B.], Germany

ABSTRACT

The role of hereditary polymorphisms of the arylamine *N*-acetyltransferase 1 (*NAT1*) gene in the etiology of urinary bladder cancer is controversial. *NAT1* is expressed in the urothelium and may *O*-acetylate hydroxyl amines, particularly in subjects with low *NAT2* activity. Thus, *NAT1* polymorphisms may affect the individual bladder cancer risk by interacting with environmental factors (smoking and occupational risks) and by interacting with the *NAT2* gene. We studied the frequencies of the *NAT1* haplotypes *3, *4, *10, *11, *14, *15, *17, and *22 in 425 German bladder cancer patients and 343 controls by PCR-RFLP. *NAT1*10* allelic frequency was lower in bladder cancer patients (15.1%) compared with controls (20.4%; $P = 0.012$). Genotypes that included *NAT1*10* were significantly less frequent among the cases (odds ratio adjusted for age, gender, and smoking, 0.65; 95% confidence interval, 0.46–0.91; $P = 0.013$). Two subtypes of *NAT1*11* were detected: *11A (–344T, –40T, 445A, 459A, 640G, and 1095A) and *11C (–344T, –40T, 459A, 640G, and 1095A). The allele frequency of *NAT1*11* was 4.3% in the cases versus 3.9% in the controls. The rare low-active *NAT1*14A* was overrepresented in the cases ($P = 0.026$). With regard to the *NAT2* genotype, our data showed: (a) a partial linkage of *NAT1*10* to *NAT2*4*; (b) a clear underrepresentation of *NAT1*10* genotypes among rapid *NAT2* genotypes in the cases studied (odds ratio, 0.39; 95% confidence interval, 0.22–0.68; $P = 0.001$), and (c) a gene-gene-environment interaction. *NAT2*slow/NAT1*4* genotype combinations with a history of occupational exposure were 5.96 (2.96–12.0) times more frequent in cancer cases than in controls without risk occupation ($P < 0.0001$). Hence, our data suggest that individuals provided with *NAT2*4* and *NAT1*10* are at a significantly lower risk for bladder cancer, particularly when exposed to environmental risk factors.

INTRODUCTION

In humans, *N*-acetylation and *O*-acetylation of aromatic amines and some heterocyclic amines are mediated by two arylamine *N*-acetyltransferases, *NAT1*³ and *NAT2*, both of which exhibit significant genetic polymorphisms. In early phenotype-based studies, the slow acetylator phenotype turned out to be at a higher risk for cancer of the urinary tract (1–3). Genotyping studies demonstrated that particularly slow acetylators, who were exposed to cigarette smoke or contamination at the workplace, were at increased risk for urinary bladder cancer (4, 5). As *NAT1* but not *NAT2* is expressed in the bladder (6), the question arose whether or not the local *NAT1* activity contributes to the formation of highly reactive acetoxy esters (7) and whether polymorphic *NAT1* genotypes (8) could modulate an individual's risk to get bladder cancer. Some studies indicated that the *NAT1*10* genotype was overrepresented among bladder cancer patients (9, 10)

and was associated with increased activity. Moreover, it led to enhanced DNA adduct levels (11). However, until now, published results have been conflicting (12). Recently we were able to show that *NAT1*10* does not alter enzyme activity toward *ex vivo* formation of *p*-amino benzoic acid, whereas there was a trend toward decreased acetylation rates of the rare *NAT1*11* (13). There is evidence that the amino acid replacements Arg187Gln in *NAT1*14* and Arg64Trp in *NAT1*17* lead to a significant reduction of the enzyme activity, whereas *NAT1*15* does not yield an active protein because of the formation of a premature stop codon by Arg187Stop (13–15). More than 20 human *NAT1* haplotypes had been identified until recently (16). In addition to *NAT1* alleles *3, *4, *10, *11A, *11B, *14, *15, and *17, we have analyzed allele *22 to cover all common haplotypes expected in Middle Europeans. The functional impact of the Asp251Val exchange in *NAT1*22* is not yet clear. The aim of our study was to investigate the frequency of *NAT1* and *NAT2* genotypes in bladder cancer patients in a case-control study and to elucidate the *NAT1/NAT2* as well as the gene-environment interaction.

MATERIALS AND METHODS

Subjects of Investigation. On the occasion of their first surgery for urinary bladder cancer, 425 mostly incident Caucasian patients (277 males and 148 females; median age, 73; 25th–75th percentiles, 63–80 years) were recruited by the departments of urology at the Benjamin Franklin Medical Center, the Free University of Berlin, and the Krankenhaus Neukölln, Berlin. The control group consisted of 343 patients (220 males and 123 females; median age, 65; range, 56–73 years) from the same catchment area and were hospitalized because of a variety of nonmalignant diseases (4). Patients were selected during the time period 1994–1998; they gave their informed consent, and the study was approved by the Ethics Committee of the Charité University Medical Center. To avoid confounding by ethnicity, only subjects of German extraction were included, as defined by their names, and places of birth. Smoking behavior and occupation were recorded. The total cigarette consumption was expressed in PYs (1 PY = consumption of one pack of 20 cigarettes/day for 1 year). Bladder cancer risk occupations were defined on the basis of epidemiological studies (17, 18) and are given elsewhere (4).

Genotyping Procedure. DNA was isolated from leukocytes by standard phenol/chloroform extraction. *NAT1* genotypes were identified according to Henning *et al.* (19) with the addition of mutations C190T (as it occurs in *NAT1*17*), G445A (*11A), C559T (*15), G560A (*14A and *14B), and A752T, which occurs in *NAT1* haplotype *22 (Fig. 1).

Apart from T1088A, which was discriminated by allele-specific PCR, all single nucleotide polymorphisms were determined by PCR-RFLP. For A–40T, G560A, and C1095A, mismatch primers were used, introducing restriction-enzyme cleavage sites in dependence on the respective mutation (Table 1). Primer pairs (TIB Molbiol, Berlin, Germany), restriction endonucleases (New England Biolabs, Schwalbach, Germany), and characteristic DNA fragment patterns are given in Table 2. Fragments were separated with 2–3.5% 3:1 NuSieve agarose gels (Biozym, Hessisch-Oldendorf, Germany).

NAT2 genotypes were analyzed essentially as described (20, 21). PCR analyses identified *NAT2* mutations G191A, C282T, T341C, C481T, G590A, and A803G, which were finally addressed to alleles *4, *5A, *5B, *5C, *6A, *12A, and *14B, respectively.

Statistics. The study was of case-control design, with group matching of cases to controls by age and gender. Expected genotype frequencies were calculated by the Hardy-Weinberg equation from the allele frequencies. Odds ratios appeared with 95% CIs and two-sided P , calculated by the χ^2 test or

Received 12/8/00; accepted 4/20/01.

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 Supported by Grant 01 GG 9845/5 from the German Federal Ministry of Education, Science, Research, and Technology.

² To whom requests for reprints should be addressed, at Institut für Pharmakologie, Ernst-Moritz-Arndt-Universität, Friedrich-Loeffler-Strasse 23D, D-17487 Greifswald, Germany. Phone: 49 3834-865650; Fax: 49 3834-865651; E-mail: cascorbi@uni-greifswald.de.

³ The abbreviations used are: *NAT1*, arylamine *N*-acetyltransferase 1; *NAT2*, arylamine *N*-acetyltransferase 2; PY, pack year; CI, confidence interval.

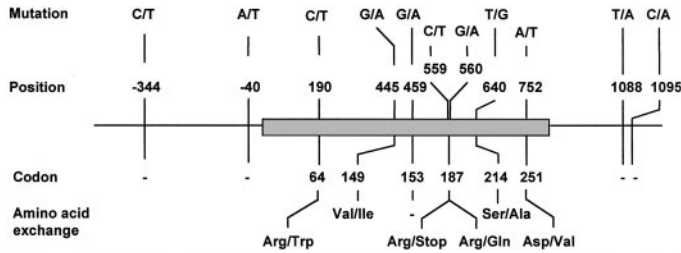


Fig. 1. Common *NAT1* polymorphisms investigated in this case-control study and resulting amino acid changes.

Fisher's exact test when a cell counted <5. Crude odds ratios were calculated from the ratio of specified *versus* wild-type genotypes in cases compared with the ratio in controls or other strata. Relative risks were computed by multiple logistic regression analysis, with adjustment for age, gender, and *NAT* genotypes and with smoking and occupational risk as confounding factors. All tests were analyzed using an SPSS 9.0 program.

RESULTS

***NAT1* Haplotypes.** Nine haplotypes of *NAT1* could be identified (Table 3): Most common was *NAT1**4, with 75.3% in bladder cancer patients and 72.4% in controls. Strikingly, *NAT1**10 was significantly less frequent in the cases than in the controls (15.1% *versus* 20.4%). The odds ratio of the allelic frequencies was 0.71 (95% CI, 0.54–0.93; *P* = 0.012).

*NAT1**11 occurred in 4.3% of the cases and in 3.9% of the controls. However, in 1.2% of the cases and 0.4% of the controls, the G445A transition in *11 was lacking. This novel haplotype was assigned to *NAT1**11C (GenBank accession no. AF308866). The *11A variant, defined by –344T, –40T, 445A, 459A, 640G, and 1095A, was found in 3.1% of the cases and 2.5% of the controls. An *11 allele with 1095C (*11B) was not observed. de Leon *et al.* (22) discussed sterical hindrances as the putative reason why G445A had not been detected in the initial sequencing of the *NAT1**11 (8). However, we have checked this mutation repeatedly by *Bbs*I digest. Only the wild-type 445G resulted in the cleavage of the initial 878-bp fragment into three smaller fragments, and these results could be confirmed by DNA sequencing. The distinction between *NAT1**11A and *11C haplotypes revealed no significant differences regarding bladder cancer risk.

Interestingly enough, the low-active *NAT1* variant *14A occurred three times more frequently in the cases than in the controls (*P* = 0.026), although at rather low frequency. Other haplotypes were too rare for statistical analysis. Therefore, all additional analyses were focused on *NAT1* alleles *4, *10, and *11.

***NAT1* Genotypes.** All genotypes were in Hardy-Weinberg equilibrium; expected frequencies calculated from the allelic frequencies did not differ significantly from observed ones (Table 4). When taking into consideration only the genotypes which included *NAT1**4 or

*NAT1**10, a significant lower frequency of *NAT1**4/*10 and *10/*10 in comparison to *NAT1**4/*4 was disclosed. The crude odds ratio was 0.64 (95% CI, 0.46–0.89; *P* = 0.007), the odds ratio adjusted for age, gender, and extent of smoking was 0.65 (95% CI, 0.46–0.91; *P* = 0.013). There was a 1.53-fold lower risk for carriers of one or two *NAT1**10 alleles compared with the wild-type *NAT1**4/*4. In females, there was a 2.7-fold reduction of risk (odds ratio, 0.37; 95% CI, 0.21–0.65; *P* < 0.001), whereas in males an underrepresentation of *NAT1**10 that was not significant observed.

There were no differences in the frequency of *NAT1* alleles between patients younger or older than the median age. Evaluating gene-environment interactions, a stratification to the extent of smoking and to occupational exposure was performed. The frequencies of *NAT1* genotypes were not different in nonsmokers or in the three groups of smokers. However, patients with *NAT1**4/*10 or *10/*10 having no occupational risk were at a lower risk for bladder cancer as compared with controls with *NAT1* wild-type *4/*4 (odds ratio, 0.60; 95% CI, 0.40–0.89; *P* = 0.012; Fig. 2).

*NAT1**11 genotypes were similarly distributed among the cases and the controls (adjusted odds ratio, 0.88; 95% CI, 0.45–1.73; *P* = 0.71). Stratification to gender, age, smoking, or risk occupation also revealed no significant diversity (Fig. 3).

When *NAT1* *ex vivo* *p*-amino salicylic acid acetylation was measured, *NAT1* alleles *14 and *15 were associated with a significant reduction of the enzyme activity (13). *NAT1**17 and *22 are also assumed to provide low activity (14). In cancer patients, there was a slight overrepresentation of these rare slow *NAT1* variants (4.7% *versus* 2.3%), which, however, did not reach statistical significance (crude odds ratio, 2.07; *P* = 0.081) and which was even smaller when

Table 2 Restriction endonucleases used for recognition of *NAT1* mutations

Single nucleotide polymorphism	Restriction enzyme	Amplification with primers	Fragment length	
			Wild type	Mutation
C-344T	MaeII or Tail	N1-1F /N1-1R	71	338
			409	409
A-40T	SspI	N1-1F /N1-1R	376	33
C190T	Tsp590I	N1-8F /N1-8R	358	139 110 73 43 26
			224	139 134 129 110 73 43 26
G445A	BbsI	N1-8F /N1-8R	455	310 113
G459A	BslI	N1-1F /N1-5R	568	310
			406	324 278 230 160 141 1
C559T	DdeI	N1-4F/N1-IIIIR	554	406 278 160 141 1
			159	88
G560A	Alw26I	N1-4F/N1-IIIIR	127	88 32
			211	36
T640G	AlwNI	N1-1F /N1-5R	247	
			817	723
A752T	BglII	N1-8F /N1-8R	817	482 241
			767	111
C1095A	BbsI	N1-5F /N1-IIR	878	
			396	39
			435	

Table 1 Oligonucleotide primers used for amplification of the entire *NAT1* gene and of smaller fragments, enabling reliable detection of point mutations

Name ^a	Binding positions	Fragment length (bp)	Sequence
N1-1F	–415––394	1540	5'-GAA ATT GAG TGG GTC AGG TAC C
N1-5R	1125–1106		5'-TTC CAA GAT AAC CAC AGG CC
N1-1R	–7––39	409 (with N1-1F)	5'-CTA AGC AAG GAA AAC AAA ACG AAA GCA AAT AAT
N1-5F	691–710	435	5'-GTT CAC TGT TTG GTG GGC TT
N1-IIR	1125–1096		5'-TTC CAA GAT AAC CAC AGG CCA TCT TTA GAA
N1-6R	1108–1088	418 (with N1-5F)	5'-GCC ATC TTT AAA ATA CAT TTA
N1-7R	1108–1088		5'-GCC ATC TTT AAA ATA CAT TTT
N1-4F	344–364	247	5'-ATG GCA GGA ACT ACA TTG TCG
N1-IIIIR	561–590		5'-CGA GGC TTA AGA GTA AAG GAG TAG ATG TCT
N1-8F	–16–4	878	5'-TCC TTG CTT AGG GGA TCA TG
N1-8R	861–840		5'-AAA TCT ATC ACC ATG TTT GGG C

^a F, forward primer; R, reverse primer; roman numerals, designed primers; mismatch base is indicated in italic bold.

Table 3 NAT1 mutations determined in 425 urinary bladder cancer patients compared to 343 controls and designation to NAT1 haplotypes

Allele	Nucleotide at position (nt)											Cases % ^b	Controls % ^b	OR ^a	P
	-344	-40	190	445	459	559	560	640	752	1088	1095				
*3	C	A	C	G	G	C	G	T	A	T	A	3.1	3.1	1.04	NS ^c
*4	C	A	C	G	G	C	G	T	A	T	C	75.3	72.4	1.00	
*10	C	A	C	G	G	C	G	T	A	A	A	15.1	20.4	0.71	0.012
*11A	T	T	C	A	A	C	G	G	A	T	A	3.1	2.5	1.19	NS
*11C	T	T	C	G	A	C	G	G	A	T	A	1.2	0.4	2.59	NS
*14A	C	A	C	G	G	C	A	T	A	A	A	1.9	0.6	3.11	0.034
*15	C	A	C	G	G	T	G	T	A	T	C	0.2	0.0		
*17	C	A	T	G	G	C	G	T	A	T	C	0.2	0.1	1.55	NS
*22	C	A	C	G	G	C	G	T	T	T	C	0.0	0.4		

^a OR, odds ratio. ORs refer to wild-type allele NAT1*4.

^b Percent of alleles (i.e., 850 in cases and 686 in controls).

^c NS, not significant.

Table 4 Frequency distribution of NAT1 genotypes among 425 urinary bladder cancer patients and 343 controls

Expected frequencies are calculated from the allelic frequencies (Table 1) by the Hardy-Weinberg law.

NAT1 genotype	Bladder cancer		Controls		Expected %
	Observed		Observed		
	n	%	n	%	
*3/*3	1	0.2	1	0.3	0.1
*3/*4	18	4.2	14	4.1	4.4
*3/*10	3	0.7	5	1.5	1.2
*3/*11A	1	0.2	0	0.0	0.2
*3/*11C	1	0.2	0	0.0	0.0
*3/*14A	1	0.2	0	0.0	0.0
*4/*4	241	56.7	175	51.0	52.5
*4/*10	98	23.1	108	31.5	29.6
*4/*11A	22	5.2	16	4.7	3.6
*4/*11C	8	1.9	2	0.6	0.6
*4/*14A	11	2.6	3	0.9	0.8
*4/*17	1	0.2	1	0.3	0.2
*4/*22	0	0.0	3	0.9	0.6
*10/*10	8	1.9	12	3.5	4.2
*10/*11A	3	0.7	1	0.3	1.0
*10/*11C	1	0.2	1	0.3	0.2
*10/*14A	4	0.9	1	0.3	0.2
*10/*15	2	0.5	0	0.0	0.0
*10/*17	1	0.2	0	0.0	0.1
ND					0.4
Total	425	100.0	343	100.0	100.0

^a ND, not detected.

the odds ratio was adjusted for age, gender, and smoking (odds ratio, 1.81; *P* = 0.18). The number, however, was too low to study the gene-gene interaction with NAT2.

Combination with NAT2. As shown in an earlier publication (4), slow NAT2 acetylators were statistically significantly overrepresented when the odds ratio was adjusted for age, gender, smoking habits, and occupational exposure by logistic regression analysis. In this study, their relative risk was 1.36; a 95% CI of 0.99–1.86 and a *P* = 0.058 was of marginal statistical significance, with a crude odds ratio of 1.29 (95% CI, 0.96–1.73; *P* = 0.088). For smokers with more than 20 PYs and for subjects with exposure to occupational risks, relative risk adjusted for age and gender, 95% CI, and *P* were 1.70, 1.09–2.65, and 0.019 and 1.87, 1.02–3.43, and 0.042, respectively.

A cross table of NAT1*4 and *10 genotypes versus NAT2 genotypes disclosed a partial linkage of NAT1*10 with NAT2*4 (*P* < 0.001). The distribution of NAT1*10 among slow and rapid NAT2 acetylators differed drastically. Among slow NAT2 acetylators, an equal distribution of NAT1*4/*10 between the cases (26.2%) and the controls (26.7%) was found. In rapid NAT2 acetylators, 32.2% of the cases and 51.3% of the controls carried NAT1*4/*10. The odds ratio of NAT1*4/*10 compared with NAT1*4/*4 was as low as 0.39 (95% CI, 0.22–0.68; *P* = 0.001; Table 5).

A logistic regression analysis including age, gender, PYs, occupa-

tional risk, as well as presence of NAT1*10 and NAT2 acetylator status, revealed a relative risk for NAT1*10 carriers of 0.71 (95% CI, 0.51–0.99; *P* = 0.047) and of 1.29 (95% CI, 0.93–1.77; *P* = 0.12), for slow NAT2 acetylators, thus indicating the significant impact of NAT1*10 on urinary bladder cancer risk.

Testing for gene-gene-interactions, NAT1 genotypes (NAT1*4/*10* or NAT2*4/*4) combined with NAT2 (*4/slow or NAT2*4/*4) were clearly less frequent than NAT1*4/*4 combined with NAT2*slow/slow (crude odds ratio, 0.50; 95% CI, 0.33–0.76; *P* = 0.001). Adjustment for age and gender revealed an odds ratio of 0.43, a 95% CI of 0.28–0.67, and a *P* = 0.0001. Thus the bladder cancer risk for slow acetylators combined with NAT1*4 was increased 2.3 times compared with rapid acetylators with NAT1*10 genotypes.

In the next step we tested for gene-gene-environment interactions. Multiple logistic regression analysis revealed no additional impact of smoking, but in the group of patients with a history of occupational exposure to hazardous compounds, NAT2*slow/NAT1*4 were 5.96 (95% CI, 2.96–12.0) more frequent than in controls with risk occupation (*P* < 0.0001). With testing extremes (occupational exposure

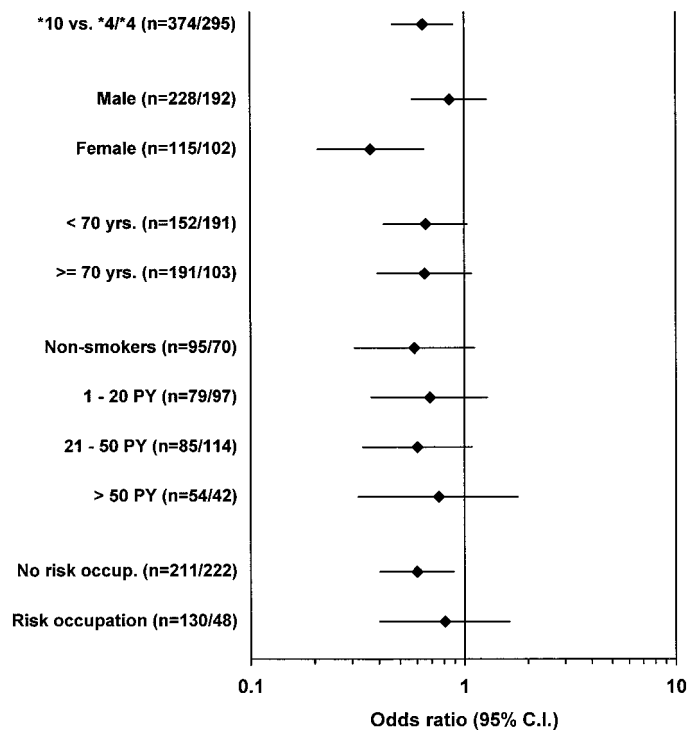


Fig. 2. Association of NAT1*10-consisting NAT1-genotypes to urinary bladder cancer in different subgroups stratified to gender, median age, cigarette consumption, and occupational risk (odds ratios given with 95% CIs). Number of individuals given for cases/controls.

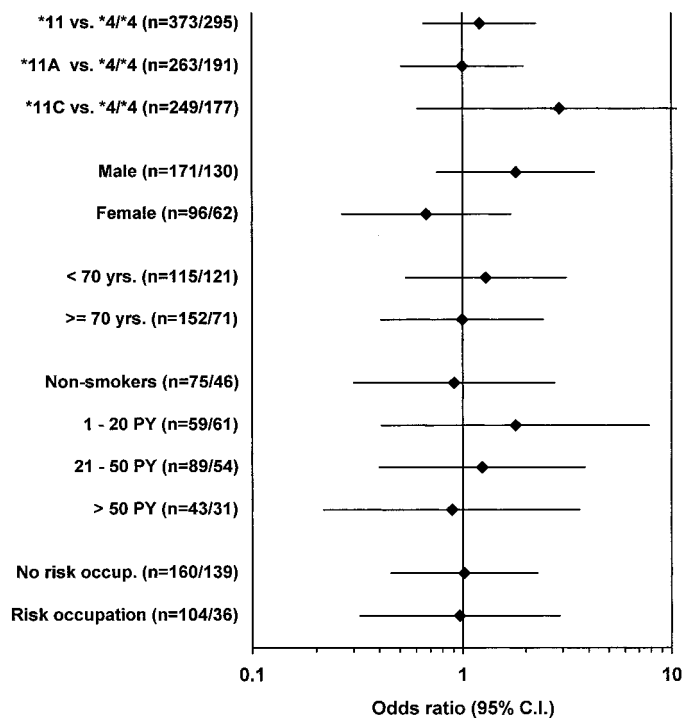


Fig. 3. Association of NAT1*11-consisting NAT1-genotypes to urinary bladder cancer in different subgroups stratified to gender, median age, cigarette consumption, and occupational risk (odds ratios given with 95% CIs). Number of individuals given for cases/controls.

and smoking versus no environmental risk factors), an adjusted odds ratio of 4.15 (95% CI, 1.95–8.83; *P* = 0.0002) was calculated (Table 6).

DISCUSSION

NAT2 and Bladder Cancer. Low NAT2 activity is doubtless a risk factor for bladder cancer, particularly for those individuals who smoke or who are exposed to specific occupational hazards (23). A meta-analysis of studies totaling 2000 European cases and 2500 controls showed a slight, but statistically significant, overrepresentation of NAT2 slow acetylators (odds ratio, 1.4; 95% CI, 1.2–1.6; Ref. 24).

According to the current theory of the role of *N*-acetyltransferases in bladder cancer etiology, a decrease in arylamine *N*-acetylation rates in the liver enforces *N*-hydroxylation mediated by cytochrome P4501A2, which in turn leads to increased concentrations of hydroxyl amines in the urinary bladder (25). Local *O*-acetylation by the *N*-acetyltransferases, expressed in the urothelium (6) would subsequently produce aromatic acetoxy esters, which may disintegrate into highly reactive nitrenium ions (7, 8, 26). These intermediates can react

nonspecifically with the urinary bladder epithelium by the formation of adducts (27).

Role of NAT1*10 in Bladder Cancer. When the polymorphic character of NAT1 was disclosed (28), the question arose whether highly active NAT1 isoforms could modulate the risk of bladder cancer or could modify the consequence of slow NAT2-mediated acetylation. NAT1*10 was suspected of leading to higher enzymatic activity than NAT1*4 in the colon, the bladder (9), and the liver (29), thus possibly causing increased adduct rates in these tissues (11). We, therefore, based the present study on the working hypothesis that NAT1*10-mediated enzyme activity affects the individual’s risk for urinary bladder cancer.

As reviewed by Hirvonen (30), the results of other studies on the role of NAT1 are conflicting, thus making it difficult to understand the functional significance of different NAT1 alleles in certain types of cancer. In contrast to positive findings (10), some studies showed no evidence of an association of NAT1 genotypes with cancer of the bladder (12), colon (31, 32), or larynx (19). Moreover, phenotypic *ex vivo* experiments using *p*-amino salicylic acid as a substrate did not prove an increased acetylation activity in carriers of NAT1*10 compared with wild-type NAT1*4 carriers (13, 15).

Role of Other NAT1 Alleles. NAT1*14, as well as *17 and *22 are associated with low enzyme activity in *in vitro* experiments (33), and NAT1*15 carriers lack enzyme activity at all. One-third of all NAT1*11 alleles among bladder cancer patients and 20% of controls lacked the G455A single nucleotide polymorphism, designated as the novel allele *11C. It is still unclear how NAT1*11C affects enzyme activity. G445A, absent in haplotypes *11A and *11B, codes for a replacement of Val/Ile at codon 149. *In vitro* experiments showed increased activity of the isolated 149 Ile variant (12), whereas *ex vivo* acetylation of *p*-amino benzoic acid was slightly decreased by NAT1*11 (13).

Linkage of NAT1 to NAT2. The linkage disequilibrium of the NAT1*10 to the rapid NAT2*4 haplotype, which we observed in our sample, confirms the results of at least two other studies (19, 33). Because of the short distance of 170–360 kb on chromosome 8p21.3–23.1 (34), such a cosegregation of defined NAT1/NAT2 traits is not unlikely. The frequency of NAT1*10 alleles did not differ in the cases and in the controls with slow NAT2 genotypes (25.0% versus 27.6%), however, NAT1*10 alleles were considerably less frequent in the patient group with rapid NAT2 genotypes (33.8 versus 50.3%; *P* = 0.003). A disequilibrium of the distribution of NAT1*10 was also detected in a sample of 240 young healthy volunteers genotyped for NAT1 (26.4% in slow acetylators and 51.9% in rapid NAT2 genotypes; data not shown).

In a second step, we analyzed only samples with NAT1*4/*4, *4/*10, and *10/*10 (Table 5). Among rapid NAT2 acetylators, only 36.4% of the cases but 58.1% of the controls carried NAT1*4/*10 or *10/*10 (odds ratio, 0.41; 95% CI, 0.24–0.70; *P* = 0.001). This underrepresentation of NAT1*10/*10 and NAT1*4/*10 in rapidly

Table 5 Distribution of NAT1*4 and *10 genotypes among slow and rapid NAT2 acetylator genotypes

NAT2 genotype	NAT1 genotype	Cases %	Controls %	Odds ratio ^a	95% CI	<i>P</i>
All	NAT1*4/*4	69.5	59.2	1.00		
<i>n</i> = 347 cases	NAT1*4/*10	28.2	36.7	0.66	0.47–0.94	0.02
<i>n</i> = 289 controls	NAT1*10/*10	2.3	4.2	0.53	0.20–1.40	0.20
Slow	NAT1*4/*4	72.5	70.9	1.00		
<i>n</i> = 229 cases	NAT1*4/*10	26.2	26.7	1.04	0.65–1.66	0.86
<i>n</i> = 172 controls	NAT1*10/*10	1.3	2.3	0.75	0.15–3.87	0.73
Rapid	NAT1*4/*4	63.6	41.9	1.00		
<i>n</i> = 118 cases	NAT1*4/*10	32.2	51.3	0.39	0.22–0.68	0.001
<i>n</i> = 117 controls	NAT1*10/*10	4.2	6.8	0.42	0.12–1.46	0.17

^a Adjusted for age, gender, and extent of smoking.

Table 6 Gene-gene interaction and gene-gene-environment interaction of NAT1 and NAT2 genotypes

Environmental risk (no. of cases/controls) ^a	NAT2 genotype	NAT1 genotype ^b	Cases %	Controls %	Odds ratio ^c	95% CI	P
(425/337)	Slow	All	63.2	56.7	1.35	1.00–1.84	0.051
	Rapid	All	36.8	43.3	1.00 ^d		
	Slow	NAT1*4	47.3	41.2	2.09	1.36–3.22	
(425/337) Smoking (297/254)	Rapid	NAT1*10	12.5	21.7	1.00 ^d		0.004
	Slow	NAT1*4	50.5	40.9	2.06	1.26–3.36	
	Rapid	NAT1*10	13.1	21.7	1.00 ^d		
Nonsmoking (121/82)	Slow	NAT1*4	37.2	42.7	2.37	0.92–6.06	0.07
	Rapid	NAT1*10	10.7	22.0	1.00 ^d		
					1.81	1.22–2.69	
Smoking vs. nonsmoking Occupational exposure (156/63)	Slow	NAT1*4	46.8	36.5	2.42	0.99–5.56	0.054
	Rapid	NAT1*10	10.9	19.9	1.00 ^d		
	Slow	NAT1*4	46.5	42.5	1.90	1.14–3.17	
No occupational exposure (260/252)	Rapid	NAT1*10	13.5	23.0	1.00 ^d		0.014
					5.96	2.96–12.0	
					2.58	0.93–7.09	
Occupational exposure vs. no occupational exposure Occupational exposure + smoking (126/51)	Slow	NAT1*4	50.8	33.3	2.58	0.93–7.09	0.067
	Rapid	NAT1*10	11.9	19.6	1.00 ^d		
	Slow	NAT1*4	39.6	41.9	2.14	0.78–5.86	
No occupational exposure + nonsmoking (91/62)	Rapid	NAT1*10	12.1	24.2	1.00 ^d		0.14
					4.15	1.95–8.83	
Occupational exposure + smoking vs. no occupational exposure + nonsmoking							0.0002

^a History of occupational exposure or smoking was lacking in some subjects.

^b NAT1*4 genotypes summarize all genotypes, but *10, NAT1*10 comprises hetero- and homozygotes.

^c Adjusted for age and gender.

^d Reference.

acetyating patients suggests a linkage between NAT2*4 and NAT1*10 in bladder cancer etiology.

In an American study (10), the distribution of NAT1*10 among slow and rapid NAT2 acetylators in 191 white controls was similar to the distribution in our study (21.2% versus 48.3%), whereas in the 205 bladder cancer patients, NAT1*10 carriers were overrepresented among the slow NAT2 genotypes (34.7% versus 50.5%).

The theory of the implication of NAT1/NAT2 combinations in the etiology of cancer is supported by studies of colon cancer patients. Rapid acetylation seems to augment the risk of colon cancer (35), particularly in individuals who have NAT2*4 and NAT1*10 and who consume considerable amounts of red meat (36).

In our study, carriers of NAT2*4 and NAT1*10 were protected from bladder cancer, thus contradicting initial observations (9, 10). Particularly subjects with occupational hazards were at increased risk (adjusted odds ratio, 5.96; $P < 0.0001$). The data suggest that earlier findings on the association of slow NAT2 acetylators with bladder cancer risk should be reconsidered with respect to the role of NAT1*10. It is possible that our results are also partly attributable to our consideration of haplotypes other than *3, *4, *10, and *11, as determined by Taylor *et al.* (10), although it is not very likely that additional undiscovered alleles may exist. In fact, carriers of low-active NAT1 variants such as *14, *15, *17, and *22 were slightly (although not significantly) overrepresented among bladder cancer patients.

In summary, our data suggest that individuals provided with NAT2*4 and NAT1*10 are at a significantly lower risk for bladder cancer, particularly when exposed to environmental risk factors. However, the etiology of bladder cancer remains difficult to understand with respect to polymorphisms of xenobiotic metabolizing enzymes (37).

ACKNOWLEDGMENTS

We are grateful for the skillful technical assistance of Hannelove Maszynski, Petra Pietsch, and Petra Lohse.

REFERENCES

- Cartwright, R. A., Glashan, R. W., Rogers, H. J., Ahmad, R. A., Barham-Hall, D., Higgins, E., and Kahn, M. A. Role of *N*-acetyltransferase phenotypes in bladder

carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. *Lancet*, 2: 842–845, 1982.

- Lower, G. M., Jr., Nilsson, T., Nelson, C. E., Wolf, H., Gamsky, T. E., and Bryan, G. T. *N*-acetyltransferase phenotype and risk in urinary bladder cancer: approaches in molecular epidemiology. Preliminary results in Sweden and Denmark. *Environ. Health Perspect.*, 29: 71–79, 1979.
- Vineis, P., Bartsch, H., Caporaso, N., Harrington, A. M., Kadlubar, F. F., and Tannenbaum, S. R. Genetically based *N*-acetyltransferase metabolic polymorphism and low-level environmental exposure to carcinogens. *Nature (Lond.)*, 369: 154–156, 1994.
- Brockmüller, J., Cascorbi, I., Kerb, R., and Roots, I. Combined analysis of inherited polymorphisms in arylamine *N*-acetyltransferase 2, glutathione *S*-transferases M1 and T1, microsomal epoxide hydrolase, and cytochrome P450 enzymes as modulators of bladder cancer risk. *Cancer Res.*, 56: 3915–3925, 1996.
- Risch, A., Wallace, D. M. A., Bathes, S., and Sim, E. Slow *N*-acetylation genotype is a susceptibility factor in occupational and smoking related bladder cancer. *Hum. Mol. Genet.*, 4: 231–236, 1995.
- Kloth, M. T., Gee, R. L., Messing, E. M., and Swaminathan, S. Expression of *N*-acetyltransferase (NAT) in cultured human uroepithelial cells. *Carcinogenesis (Lond.)*, 15: 2781–2787, 1994.
- Hein, D. W., Doll, M. A., Rustan, T. D., and Ferguson, R. J. Metabolic activation of *N*-hydroxyarylamines and *N*-hydroxyarylamides by 16 recombinant human NAT2 allozymes: effects of 7 specific NAT2 nucleic acid substitutions. *Cancer Res.*, 55: 3531–3536, 1995.
- Vatsis, K. P., and Weber, W. W. Structural heterogeneity of Caucasian *N*-acetyltransferase at the NAT1 gene locus. *Arch. Biochem. Biophys.*, 301: 71–76, 1993.
- Bell, D. A., Badawi, A. F., Lang, N. P., Ilett, K. F., Kadlubar, F. F., and Hirvonen, A. Polymorphism in the *N*-acetyltransferase 1 (NAT1) polyadenylation signal: association of NAT1*10 allele with higher *N*-acetylation activity in bladder and colon tissue. *Cancer Res.*, 55: 5226–5229, 1995.
- Taylor, J., Umbach, D., Stephens, E., Castranio, T., Paulson, D., Robertson, C., Mohler, J., and Bell, D. The role of *N*-acetylation polymorphism in smoking-associated bladder cancer: evidence of a gene-gene-environment three-way interaction. *Cancer Res.*, 58: 3603–3610, 1998.
- Badawi, A. F., Hirvonen, A., Bell, D. A., Lang, N. P., 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, 1997.
- Okkels, H., Sigsgaard, T., Wolf, H., and Autrup, H. Arylamine *N*-acetyltransferase 1 (NAT1) and 2 (NAT2) polymorphisms in susceptibility to bladder cancer: the influence of smoking. *Cancer Epidemiol. Biomark. Prev.*, 6: 225–231, 1997.
- Bruhn, C., Brockmüller, J., Cascorbi, I., Roots, I., and Borchert, H. Correlation between genotype and phenotype of the human arylamine *N*-acetyltransferase type 1 (NAT1). *Biochem. Pharmacol.*, 58: 1759–1764, 1999.
- Butcher, N. J., Ilett, K. F., and Minchin, R. F. Functional polymorphism of the human arylamine *N*-acetyltransferase type 1 gene caused by C190T and G560A mutations. *Pharmacogenetics*, 8: 67–72, 1998.
- Hughes, N. C., Janezic, S. A., McQueen, K. L., Jewett, M. A. S., Castranio, T., Bell, D. A., and Grant, D. M. Identification and characterization of variant alleles of human acetyltransferase NAT1 with defective function using *p*-aminosalicylate as an *in-vivo* and *in-vitro* probe. *Pharmacogenetics*, 8: 55–66, 1998.
- Hein, D. W., Grant, D. M., and Sim, E. Update on consensus arylamine *N*-acetyltransferase gene nomenclature. *Pharmacogenetics*, 10: 291–292, 2000.
- Vineis, P., and Simonato, L. Proportion of lung and bladder cancers in males resulting from occupation: a systematic approach. *Arch. Environ. Health*, 46: 6–15, 1991.

18. Silverman, D. T., Levin, L. I., Hoover, R. N., and Hartge, P. Occupational risks of bladder cancer in the United States: I. White men. *J. Natl. Cancer. Inst.*, *81*: 1472–1480, 1989.
19. Henning, S., Cascorbi, I., Münchow, B., Jahnke, V., and Roots, I. Association of arylamine *N*-acetyltransferases *NAT1* and *NAT2* genotypes to laryngeal cancer risk. *Pharmacogenetics*, *9*: 103–111, 1999.
20. Cascorbi, I., Drakoulis, N., Brockmöller, J., Maurer, A., Sperling, K., and Roots, I. Arylamine *N*-acetyltransferase (*NAT2*) mutations and their allelic linkage in unrelated Caucasian individuals: correlation with phenotypic activity. *Am. J. Hum. Genet.*, *57*: 581–592, 1995.
21. Cascorbi, I., Brockmöller, J., Mrozikiewicz, P. M., Bauer, S., Loddenkemper, R., and Roots, I. Homozygous rapid arylamine *N*-acetyltransferase *NAT2* genotype as susceptibility factor for lung cancer. *Cancer Res.*, *56*: 3961–3966, 1996.
22. de Leon, J. H., Vatsis, K. P., and Weber, W. W. Characterization of naturally occurring and recombinant human *N*-acetyltransferase variants encoded by *NAT1*. *Mol. Pharmacol.*, *58*: 288–299, 2000.
23. Marcus, P. M., Hayes, R. B., Vineis, P., Garcia-Closas, M., Caporaso, N. E., Atrup, H., Branch, R. A., Brockmöller, J., Ishizaki, T., Karakaya, A. E., Ladero, J. M., Mommsen, S., Okkels, H., Romkes, M., Roots, I., and Rothman, N. Cigarette smoking, *N*-acetyltransferase 2 acetylation status, and bladder cancer risk: a case-series meta-analysis of a gene-environment interaction. *Cancer Epidemiol. Biomarkers Prev.*, *9*: 461–467, 2000.
24. Marcus, P. M., Vineis, P., and Rothman, N. *NAT2* slow acetylation and bladder cancer risk: a meta-analysis of 22 case-control studies conducted in the general population. *Pharmacogenetics*, *10*: 115–122, 2000.
25. Lang, N. P., and Kadlubar, F. F. Aromatic and heterocyclic amine metabolism and phenotyping in humans. *Prog. Clin. Biol. Res.*, *372*: 33–47, 1991.
26. Wild, D., Feser, W., Michel, S., Lord, H. L., and Josephy, P. D. Metabolic activation of heterocyclic aromatic amines catalyzed by human arylamine *N*-acetyltransferase isozymes (*NAT1* and *NAT2*) expressed in *Salmonella typhimurium*. *Carcinogenesis (Lond.)*, *16*: 643–648, 1995.
27. Nerurkar, P. V., Schut, H. A., Anderson, L. M., Riggs, C. W., Snyderwine, E. G., Thorgeirsson, S. S., Weber, W. W., Rice, J. M., and Levy, G. N. DNA adducts of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in colon, bladder, and kidney of congenic mice differing in *Ah* responsiveness and *N*-acetyltransferase genotype. *Cancer Res.*, *55*: 3043–3049, 1995.
28. Vatsis, K. P., Martell, K. J., and Weber, W. W. Diverse point mutations in the human gene for polymorphic *N*-acetyltransferase. *Proc. Natl. Acad. Sci. USA*, *88*: 6333–6337, 1991.
29. Zenser, T. V., Lakshmi, V. M., Rustan, T. D., Doll, M. A., Deitz, A. C., Davis, B. B., and Hein, D. W. Human *N*-acetylation of benzidine: role of *NAT1* and *NAT2*. *Cancer Res.*, *56*: 3941–3947, 1996.
30. Hirvonen, A. Chapter 20. Polymorphic NATs and cancer predisposition. *IARC Sci. Publ.* *148*: 251–270, 1999.
31. Lin, H. J., Probst-Hensch, N. M., Hughes, N. C., Sakamoto, G. T., Louie, A. D., Kau, I. H., Lin, B. K., Lee, D. B., Lin, J., Frankl, H. D., Lee, E. R., Hardy, S., Grant, D. M., and Haile, R. W. Variants of *N*-acetyltransferase *NAT1* and a case-control study of colorectal adenomas. *Pharmacogenetics*, *8*: 269–281, 1998.
32. Probst-Hensch, N. M., Haile, R. W., Li, D. S., Sakamoto, G. T., Louie, A. D., Lin, B. K., Frankl, H. D., Lee, E. R., and Lin, H. J. Lack of association between the polyadenylation polymorphism in the *NAT1* (*acetyltransferase 1*) gene and colorectal carcinomas. *Carcinogenesis (Lond.)*, *17*: 2125–2129, 1996.
33. Smelt, V., Mardon, H., and Sim, E. Placental expression of arylamine *N*-acetyltransferases: evidence for linkage disequilibrium between *NAT1*10* and *NAT2*4* alleles of the two human arylamine *N*-acetyltransferase loci *NAT1* and *NAT2*. *Pharmacol. Toxicol.*, *83*: 149–157, 1998.
34. Matas, N., Thygesen, P., Stacey, M., Risch, A., and Sim, E. Mapping *AAC1*, *AAC2*, and *AACP*, the genes for arylamine *N*-acetyltransferases, carcinogen metabolising enzymes on human chromosome 8p22, a region frequently deleted in tumours. *Cytogenet. Cell Genet.*, *77*: 290–295, 1997.
35. Roberts-Thomson, I. C., Ryan, P., Khoo, K. K., Hart, W. J., McMichael, A. J., and Butler, R. N. Diet, acetylator phenotype, and risk of colorectal neoplasia. *Lancet*, *347*: 1372–1374, 1996.
36. Chen, J., Stampfer, M. J., Hough, H. L., Garcia-Closas, M., Willett, W. C., Hennekens, C. H., Kelsey, K. T., and Hunter, D. J. A prospective study of *N*-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. *Cancer Res.*, *58*: 3307–3311, 1998.
37. Brockmöller, J., Cascorbi, I., Henning, S., Meisel, C., and Roots, I. Molecular genetics of cancer susceptibility. *Pharmacology (Basel)*, *61*: 212–227, 2000.