Arylamine N-Acetyltransferase 1 (NAT1) and 2 (NAT2) Polymorphisms in Susceptibility to Bladder Cancer: The Influence of Smoking

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Abstract

Aromatic amines are involved in the etiology of bladder cancer. These compounds are acetylated by N-acetyltransferase 1 (NAT1) and 2 (NAT2), and epidemiological studies have shown that the slow NAT2 acetylator phenotype is associated with increased risk of bladder cancer and may be associated with decreased risk of colorectal cancer. By using PCR-RFLP analyses to identify three known slow acetylator alleles (M1, M2, and M3) and the wild-type, or fast, allele, the NAT2 genotypes were determined. No association between the NAT2 slow acetylator genotype and bladder cancer was found either by crude analyses (odds ratio [OR], 1.32; 95% confidence interval [CI], 0.91-1.92) or by logistic regression analyses adjusted for age, gender, and smoking exposure (OR, 1.22; 95% CI, 0.92-1.62). A similar observation was made when the cases were divided into incident and surviving cases. Dividing the cases by pathological classification (benign or malignant) did not alter this finding. Likewise, analyses of the NAT1 and glutathione S-transferase \( \mu \) 1 (GSTM1) genotypes showed no associations between the NAT1 or GSTM1 genotypes and bladder cancer risk.

However, restricting the analysis to people exposed to potential bladder carcinogens (e.g., smokers) among cases and controls, a small but significant association between the slow acetylator genotype and bladder cancer risk was revealed among all cases with malignant tumors (OR, 1.35; 95% CI, 1.02-1.80) and among incident cases with malignant tumors (OR, 1.50; 95% CI, 1.04-2.16). The allele frequencies in the group consisting of smokers showed an overrepresentation of the NAT2 M1 (NAT2*5) allele in the incident case group. The NAT1 and GSTM1 genotypes were not associated with increased risk of bladder cancer among smokers. Analyses of genetic combinations of NAT1/NAT2 as potential risk factors for bladder cancer seem to indicate that the normal NAT1/fast NAT2 genotype may be a protective genotype compared with the other genotype combinations. Analyses of genetic combinations of NAT2/GSTM1 did not reveal any combination of NAT2 and GSTM1 genotypes associated with increased bladder cancer risk.

Introduction

The initial studies on acetylation phenotyping as a risk factor in bladder cancer were conducted in the early 1980s, and the term molecular epidemiology was introduced (1, 2). In these studies, a significant overrepresentation of the slow acetylator phenotype in bladder patients was reported. The studies strongly suggested that the slow acetylator phenotype predisposes to bladder cancer, especially among individuals exposed to aromatic amine carcinogens either from occupational or environmental sources (3-5).

Arylamines are a group of chemicals used widely in the industry and are formed as a consequence of human activity and lifestyle. The carcinogenic potency of these substances has clearly been demonstrated, and a role in the etiology of bladder cancer seems evident (6).

The carcinogenic forms of arylamines are formed by bioactivation (N-oxidation) in the liver in a cytochrome P4501A2-catalyzed reaction (7). In a competing hepatic detoxifying reaction (N-acetylation), the NAT3 enzymes (predominantly NAT2 activity) inactivate the proximate carcinogens (8). The arylamine metabolites can then enter the circulation, and after reabsorption into the bladder epithelium, further activation steps catalyzed by the NAT enzymes (O-acetylation and N,O-acetylation, predominantly NAT1 activity) may occur (9). The resulting DNA-reactive species are capable of forming DNA adducts.

Arylamines are tobacco smoke constituents (e.g., 2-naphthylamine and 4-aminobiphenyl; Ref. 10), and it has been demonstrated that smokers have higher arylamine DNA adducts than nonsmokers (11). The adduct level in exfoliated bladder cells of smokers was higher in slow acetylators than in fast acetylators (12). Furthermore, one of the major adducts identified in biopsies isolated from bladder patients who were smokers was an arylamine DNA adduct \([N-(\text{deoxyguanosin-8-yl})-4\text{-aminobiphenyl}]\); Ref. 13. Thus, although no strong epidemiological data support a link between NAT2 status and smoking-induced bladder cancer, there are substantial indications that arylamines found in tobacco smoke may play an important role in smoking-induced bladder carcinogenesis.

The NATs are encoded by two highly similar genes (14, 15). Both enzymes are expressed in the bladder urothelium (9, 16)....
16, 17). The NAT1 enzyme has only recently been shown to be polymorphically distributed in humans (9, 18–20), whereas the NAT2 polymorphism was demonstrated years ago (reviewed in Ref. 21). The genetic alterations underlying the NAT2 polymorphism have been determined and correlated with decreased NAT2 enzyme activities (15, 22–25). The four major NAT2 alleles are represented by the wild-type allele (NAT2*4) and three mutant (M1–M3) alleles (NAT2*5, NAT2*6A, and NAT2*7A; the new nomenclature reviewed in Ref. 26). The presence of at least one wild-type allele results in a fast acetyl phenotype, whereas carrying two mutant alleles results in an intermediate phenotype. Other acetylator alleles exist (27), however, the exact result of these mutations remains unclear. One other mutant allele (M4, or NAT2*14) seems to be restricted to individuals of African ancestry (28). Sequencing of variant NAT1 alleles has identified a polymorphism at the polyadenylation signal of the NAT1 gene (19). Subsequently, a correlation between the structural heterogeneity and the phenotypic expression indicated that low and higher acetylation variants exist (20). Homozygous NAT1*4 individuals were associated with normal NAT1 acetylation activity, and hetero- and homozygous NAT1*10 individuals with higher acetylation activity, here referred to as normals and fast acetylators, respectively. Recently, an increased aromatic amine DNA adduct level was demonstrated in individuals carrying at least one variant NAT1*10 allele (9). Furthermore, the allele has been associated with an increased risk of colorectal cancer (29). The frequencies of the NAT1 acetylator alleles in different populations are presently unknown. However, in a small American study (n = 26), the NAT1 allele frequencies were in the order NAT1*4 > NAT1*10 > NAT1*11 (20).

In several epidemiological studies, molecular biology methods (the PCR-RFLP technique) have been used to determine the NAT2 genotypes (30–35). A few studies have focused on bladder cancer (16, 36–38), and of these only the latter two assessed the risk of bladder cancer by NAT2 slow acetylator status and smoking (37, 38). To verify the hypothesis that inherited slow acetylators are more susceptible to bladder cancer than fast acetylators, and to investigate the importance of the NAT1 genetic polymorphism as a risk factor in bladder cancer, the NAT1 and NAT2 genotype frequencies were determined in a case-control study. In addition, genetic combinations of the NAT1, NAT2, and GSTM1 genotypes were evaluated as potential risk factors of bladder cancer.

Materials and Methods

Study Design

The study was designed as a case-control study. Approximately 90% of the patients attending the outpatient clinics at the Department of Urology for control of bladder cancers in the period between January 1, 1993 and October 1, 1995 were recruited. Eligibility criteria included: alive at time of diagnosis, histologically verified bladder tumor, and Danish ethnic background (i.e., because both the GSTM1 and NAT2 gene frequencies show variations in different populations; Refs. 39 and 40).

The control group consisted of patients with other non-cancerous diseases of the urinary tract: prostatic hyperplasia (n = 63), hematuria (n = 7), urethral stricture (n = 7), stone disease (n = 32), nephropathy (n = 15), urinary incontinence (n = 64), cystitis (n = 16), and other diseases (n = 38). Eligibility criteria for controls included Caucasian race, Danish ethnic background, and diagnosis of diseases not associated with smoking or with risk of bladder cancer. Cases and controls were unmatched. Demographic data of both case and control groups are presented in Table 1. A total of 254 cases and 242 controls were included in the study.

The project was approved by the Regional Ethical Committee, and all participants signed an informed consent.

Table I  Demographic characteristics of the study population

<table>
<thead>
<tr>
<th>Gender</th>
<th>Cases</th>
<th>Malignant*</th>
<th>Benign*</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>133 (52)</td>
<td>84 (62)</td>
<td>49 (42)</td>
<td>118 (49)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>121 (48)</td>
<td>52 (38)</td>
<td>69 (58)</td>
<td>124 (51)</td>
</tr>
<tr>
<td>Age Mean (±SD)</td>
<td>69 (±10)</td>
<td>69 (±9)</td>
<td>70 (±11)</td>
<td>64 (±12)</td>
</tr>
<tr>
<td>Smoking status*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers (%)</td>
<td>30 (12)</td>
<td>16 (9)</td>
<td>14 (12)</td>
<td>55 (24)</td>
</tr>
<tr>
<td>Ex-smokers (%)</td>
<td>75 (30)</td>
<td>37 (29)</td>
<td>38 (32)</td>
<td>73 (32)</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>148 (58)</td>
<td>83 (62)</td>
<td>65 (56)</td>
<td>98 (44)</td>
</tr>
</tbody>
</table>

* Pathological classification: Malignant, bladder tumors, stage T1–T4; Benign, bladder tumors, stage Ta and Tis.

Information not available for 16 control patients and 1 case patient.

Questionnaire

The questionnaire included information on age, gender, smoking habits, occupation, and family history of chronic diseases. The information on occupational exposure included known risk factors for bladder cancer (i.e., exposure to chemicals, motor exhaust, and metals).

Tobacco Smoking

Smoking status (non-, ex-, and current) and duration of time since quitting smoking were recorded. Detailed information on smoking history was used to calculate lifetime smoking exposure. One PY corresponded to the smoking of 20 cigarettes/day in 1 year.

Classification of Bladder Tumors

The bladder tumors were classified based on tumor type, T category, and grade (Union International Contre Cancer classification). The tumors were classified as benign (Ta, Tis) or malignant (T1–T4). A total of 237 (93.3%) of the cases was diagnosed with TCC, 3 (1.2%) with SCC, 8 (3.1%) with CIS, 4 (1.6%) with papilloma, 1 (0.4%) with adenocarcinoma, and 1 (0.4%) with an unidentified tumor. Among all cases, incident cases, and surviving cases, 118 (46.6%), 30 (31.6%), and 88 (55.3%) were benign tumors, respectively. The corresponding numbers of malignant tumors were 136 (53.4%), 65 (68.4%), and 71 (44.7), respectively.

Blood Samples

Blood samples from the bladder cancer group and the control group were collected and stored at -20°C until DNA extraction.

DNA Extraction and Genotyping by PCR Analyses

DNA was extracted from blood using the method described by Spurr et al. (41). NAT2 Genotype. Approximately 100 ng of DNA was used in a nested PCR assay with two sets of primers to amplify DNA from cases and controls. The first set of primers were: 5'-
AATTAGTCACACGGAGA-3’ (position -74 to -57) and 5’-GAGAGTGATCATGCTAGA-3’ (position 1117–1137).

The PCR conditions for this set of primers were: (a) 1 cycle; denaturation at 94°C for 4 min; (b) 25 cycles; annealing at 59°C for 30 s, extension at 72°C for 45 s, and denaturation at 94°C for 30 s; (c) 1 cycle; annealing at 60°C for 1 min, extension at 72°C for 10 min, and cooling to 30°C for 10 s. These primers amplify a 1211-bp product.

A total of 10 μl (250 ng of DNA) of this product was used in a second amplification using the primers: 5’-GCT-GGGTTGTGACCTCCT-3’ (position 366–386) and 5’-TT-GGGTGATGATACAAAAAGGG-3’ (position 891–913). The conditions for the second set of primers were identical to the first set, except 35 cycles were used. This set of primers produced a 547-bp product.

**NAT2 RFLP Analysis.** The NAT2 alleles (wild-type or NAT2*4) M1 [NAT2*5 = NAT2*5B (S1a), NAT2*5A (S1b), and NAT2*5C (S1c)], M2 (with no differentiation between NAT2*6A or B), and M3 (with no differentiation between NAT2*7A or B) were identified by restriction enzyme digestion (KpnI, TaqI, DdeI, and BamHI) at the mutation sites 481 C-T, 590 G-A, 803 A-G, and 857 G-A of the NAT2 gene. The M1, M2, and M3 alleles lack the KpnI, TaqI, and BamHI sites, resulting in incomplete digestion of the DNA. Complete digestion identifies the wild-type allele. Combinations of band patterns identify the NAT2 genotypes (Table 3).

The mutation 803 A-G is almost always associated with the 481 C-T mutation (42), and individuals carrying the 803 A-G mutation without the 481 C-T mutation were considered carriers of one wild-type allele. Approximately 95% of the slow acetylator alleles are identified by this method. The restriction enzyme fragments were run on a 3% agarose gel and visualized by staining the gel with ethidium bromide, followed by UV transillumination.

**NAT1 Genotype.** To detect the NAT1 genotypes, the method described by Bell *et al.* (20) was used with some modifications. The PCR was altered from a single-step PCR to a nested PCR to increase the specificity and the quantity of PCR products. Briefly, two sets of primers specific for the NAT1 gene were constructed. Set 1: 5’-GATCAAGTFGTGAGAAGAAAT-3’ (position 1096–1115) and 5’-GCT-GGTCCTGGAAGCTCCT-3’ (position 169–193) and 5’-CTAGCATAAATCAC-GG-3’ (position 891–913). This set of primers produced a band of 547 bp. Set 2: 5’-GACTCTGAGTGGTAGAAAT-3’ (position 768–789) and 5’-CCACAGGCCATCTCTAGAA-3’ (position 1096–1115). This set of primers produced a band of 347 bp.

To differentiate the NAT1*4 and NAT1*10 alleles, the reverse primer of the second set of primers contains a mismatched base at nucleotide 1098 (underlined), which creates a new MboII restriction enzyme site when amplifying the NAT1*4 allele. The two sets of primers were used in sequence (5 μl of PCR product 1 was used in the second PCR), followed by digestion of the second PCR product with the MboII restriction enzyme (Life Technologies). The PCR conditions were as described for the NAT2 PCR. The digests were electrophoresed on 6% acrylamide gels, stained by ethidium bromide, and detected by UV transillumination (Fig. 1).

**GSTM1 Genotype.** The GSTM1 genotypes were determined as described previously (43). The procedure has previously been validated with a specificity of >99% (44, 45). All primers were obtained from DNA Technology (Science Park, Aarhus, Denmark).

**Statistics**

Statistical analyses were performed using the Statistical Package for the Social Sciences program (SSPS Inc., Chicago, IL). Initially, the variables were analyzed with χ² test or Mantel Haenszel’s test for stratified data (46).

In a second step, logistic regression (Epidemiological Graphics, Estimation, and Testing package, Cytel Software Corp., Seattle, WA) was performed with additionally explanatory variables in the model to control for confounding. If the variables did not contribute significantly to the model and were not considered to be an obligatory confounder, they were left out of the final model. The logistic regression included as obligatory confounders age (<65 and ≥65), gender, and smoking. Smoking was categorized according to smoking status (nonsmokers, smokers, and ex-smokers). In a second analysis, the cases were categorized according to lifetime history of smoking calculated in PYs (nonsmokers, smokers, and ex-smokers). To test the possible influence of time since quitting smoking, the ex-smokers were subdivided into four categories in a third analysis (those who quit smoking <1 year ago, 1–4 years ago, 5–9 years ago, and ≥10 years ago). To test the possible influence of time since quitting smoking, the ex-smokers were subdivided into four categories in a third analysis (those who quit smoking <1 year ago, 1–4 years ago, 5–9 years ago, and ≥10 years ago). Ex-smokers who quit smoking <1 year ago were coded as smokers. Only first-order interactions were studied in the analyses. The results are reported as ORs and 95% CIs. OR was defined as the odds of case patients having a susceptible genotype divided by the odds of controls having a susceptible genotype.

**Results**

**NAT1 and NAT2 Genotype Frequencies.** The NAT2 and NAT1 genotype frequencies are shown in Table 2, and statistical analysis revealed that there was no significant difference between the genotype distribution among cases and controls. The NAT2 genotypes were categorized as homozygous mutant (slow), heterozygous wild-type mutant (intermediate), and ho-
mozygous wild type (fast). The NAT2 genotype frequencies of the case group (60.5% slow, 35.2% intermediate, and 4.3% fast) were not significantly different from the control group (60.5% slow, 37.6% intermediate, and 6.6% fast) in this analysis (\( \chi^2 = 1.91, P = 0.39 \)).

NAT1 and NAT2 Allele Frequencies. The NAT2 and NAT1 allele frequencies were not significantly different (Table 3).

Risk of Bladder Cancer by Smoking. Table 4 outlines the risk of bladder cancer by smoking habits and smoking exposure (PYs). The age- and gender-adjusted ORs of smoking, i.e., as smoking habits, were between 2 and 4 in all groups presented in the table. The OR increased in the medium (21–50 PYs) and heavy (>51 PYs) smoking strata. Among incident cases, the OR in these two strata increased, whereas among surviving cases the OR decreased. There was a tendency of decreasing risk with time since quitting smoking.

Risk of Bladder Cancer by NAT1, NAT2, and GSTM1 Genotypes. The crude and adjusted ORs are presented in Table 5. Logistic regression analysis of the incident cases showed that there was a weak, but nonsignificant, association between the slow acetylator genotype and bladder cancer risk (OR, 1.35; 95% CI, 1.02-1.75). The association between the slow acetylator genotype and bladder cancer risk (OR, 1.50; 95% CI, 1.04-2.16) in the control group (45.4 and 28.1%, respectively). Among nonsmok-
NAT2 genotypes were not risk factors of bladder cancer. The NAT2/GSTM genotype as reference category. The OR fluctuated from 0.98 to 1.10 with a 95% CI range of 0.65–1.80, and the corresponding OR among ex-smokers was between 0.87 and 1.40 with 95% CI ranging from 0.61 to 2.16. The NAT1 and GSTM1 genotypes were not associated with bladder cancer risk among smokers (data not shown). In all analyses, there was no effect of occupational exposure on the risk estimates.

**Risk of Bladder Cancer by NAT2/GSTM1 and NAT1/NAT2 Genetic Combinations.** Combinations of the GSTM1 and NAT2 genotypes were not risk factors of bladder cancer. The OR of carrying the combined slow NAT2/GSTM1*5/*0 genotypes was 1.51, and the 95% CI was 0.89–2.55 with the fast NAT2/GSTM1 genotype as reference category. The NAT1 and NAT2 genotype combinations were not risk factors for bladder cancer except among incident cases who are smokers. As presented in Table 7, the reference group (normal NAT1/fast NAT2) seems to be a protective genotype combination compared with all other NAT1/NAT2 genotype combinations.

**Discussion**

The early observation by Lower et al. (1) in a Danish urban population showed that the slow acetylator phenotype was clearly overrepresented among bladder cancer patients. Additional studies conducted in Denmark demonstrated that the same applied to a rural (low-risk) population (47), and subsequently, an overrepresentation of the slow acetylator phenotype was found among bladder cancer patients with the most aggressive form of bladder carcinoma (T3–T4; Ref. 5). In the present study, these findings were not confirmed; however, it is shown that the NAT2 slow acetylator genotype is a risk factor for bladder cancer among smokers, and the risk was highest among patients with malignant bladder cancer (T1–T4).

In the present study, an increased risk of bladder cancer of 2–4 fold was observed among smokers, confirming many larger epidemiological studies [e.g., Augustine et al. (48)]. Furthermore, NAT2 slow acetylator genotype was a risk factor for bladder cancer among smokers. In a recent German study correlating bladder cancer and the NAT2 polymorphism, the smokers were stratified by PYs (1–20 PYs, 21–50 PYs, and >51 PYs), and the highest risk was found among heavy smokers (>51 PYs; Ref. 38). In the present study, however, we find that the OR was increased due to a higher number of slow acetylators in the 21–50 PY strata. This may indicate, contrary to the German study (38), that at high exposure (>51 PYs) acetylator status may be irrelevant, as suggested by Vineis et al. (12). Thus, it is still unclear how (or if) the association between slow acetylator status and bladder cancer risk is dependent on smoking dose.

By restricting the analysis to the exposed cases (the smokers), an increased risk of bladder cancer among the slow acetylator genotype was found. The implication of this is that in investigating a genetic polymorphism, an influence of the NAT2 genotype would be revealed only in analyses comparing exposed individuals (49). The effect of the NAT2 polymorphism would be masked by negative confounding of the nonexposed individuals. Other studies, using phenotyping, have focused on occupationally exposed groups and have found a higher number of slow acetylators among bladder cancer patients (3–5). However, the results of the present study suggest that individuals carrying the slow acetylator genotype have an increased risk of bladder cancer only if they are smokers, and this result is supported by a recent study in Germany (38). However, contrary to the latter study, there was no influence of occupational exposure in the present study. This may be because of the way in which data regarding occupational exposure were collected (only 41% answered the questionnaire on occupational exposure in detail).

It is interesting that the frequency of the NAT2 M1 allele was higher in the exposed (smokers) case group compared with the exposed control group. Although it is not clear whether individuals carrying the NAT2 M1M1 genotype have lower acetylation capacity (in vivo) than other slow acetylator genotype combinations, it suggests that the mutation identifying the NAT2 M1 allele has been associated with the most substantial reduction in N-, O-, and N.O-acetyltransferase activities of all of the NAT2 mutations (50). In any case, it is interesting that among smokers, 55.6% was homozygous M1 compared with 7.1% among controls.

The allele frequencies found in this study match allele frequencies published previously by other investigators (30, 31, 38, 51, 52). In Caucasian populations, the NAT2 M1 allele (NAT2*5) is the most frequent allele (40–50%), in contrast to Asian populations in which the frequency is very low (0–5%; Refs. 14, 42, 53, and 54). Such population differences may have important implications for the incidence of bladder cancer in different ethnic populations.

In a previous study, the GSTM1 genotypes of the same cases and controls were determined. The result of this first study suggested that the cases should be divided into incident and surviving groups to prevent bias by incorporating prevalent cases into a case-control study (43). Hence, the cases were classified into incident and surviving case groups. Furthermore, the study showed that the GSTM1*0/*0 genotype was not a risk factor for bladder cancer, and this finding was confirmed here. However, because the GST and NAT2 enzymes are involved in the metabolism of many smoke-derived compounds (e.g., heterocyclic amines and aromatic nitroso compounds), in theory combinations of these genes may be expected to be risk factors in a smoking-induced disease such as bladder cancer. Moreover, we and others have found higher aromatic DNA adducts in individuals exposed to air pollution carrying the combined GSTM1*0/*0/NAT2 slow acetylator genotype (47, 55). How-

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**Table 6** ORs for the association of the NAT2 slow acetylator genotype to bladder cancer risk according to pathological classification among smokers only.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Benign*</th>
<th>Malignant*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)*</td>
<td>OR (95% CI)*</td>
</tr>
<tr>
<td>All cancer cases</td>
<td>1.04 (0.78–1.37)</td>
<td>1.35 (1.02–1.80)</td>
</tr>
<tr>
<td>Incident†</td>
<td>1.34 (0.81–2.20)</td>
<td>1.50 (1.04–2.16)</td>
</tr>
<tr>
<td>Survivors‡</td>
<td>0.95 (0.70–1.30)</td>
<td>1.25 (0.87–1.80)</td>
</tr>
</tbody>
</table>

* See legend to Table 1.
† OR and 95% CI of NAT2 slow acetylators to NAT2 fast acetylators in cases versus the control group. The OR was adjusted for age and gender.
‡ See legend to Table 4.

**Table 7** OR of bladder cancer for NAT2 and NAT1 genotype combinations among incident cases, smokers only.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Benign*</th>
<th>Malignant*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)*</td>
<td>OR (95% CI)*</td>
</tr>
<tr>
<td>NAT2 Slow*</td>
<td>3.95 (1.32–11.77)</td>
<td>3.76 (1.07–13.31)</td>
</tr>
<tr>
<td>NAT2 Fast*</td>
<td>1.00 (7/15)</td>
<td>3.60 (1.14–11.41)</td>
</tr>
</tbody>
</table>

* See legend to Table 2.
† OR adjusted for age and gender. Carriers of the NAT1*11 allele were excluded from this analysis.
‡ Number of cases/number of controls.
§ Reference category.

See legend to Table 4.
ever, none of the combinations of the GSTM1 and NAT2 genes was a risk factor for bladder cancer in the present study, suggesting that the two enzymes, in relation to smoke-derived arylamine carcinogens, may have differing substrate specificities.

It might be expected that the NAT enzymes, which are known to be involved in the metabolism of arylamines, could interact in modulating the risk of bladder cancer. However, there are two problems concerning misclassification that need to be addressed when determining the NAT genotypes:

(a) It has been reported that the concordance between NAT1 and NAT2 phenotype and genotype are 70% (9) and 93–98% (32, 36), respectively. Because phenotype-genotype misclassification can introduce serious biases, as shown by Rothman et al. (57), and preliminary studies have shown that other NAT1 alleles exist (19), the altered enzymatic activity (if any) of these has not been determined. Likewise, the frequency of these alleles is unknown, although the NAT1*3 allele frequency has been estimated to be approximately 3% in the American population (30). In any case, the existence of additional alleles may offer one explanation for the lack of matching between NAT phenotype and genotype. Thus, additional work must be carried out to optimize the NAT phenotype/genotype assays, and especially the NAT1 phenotype and/or genotype assays.

(b) As appears from the study of Bell et al. (20), individuals carrying the NAT1*/11 allele apparently have an enzymatic activity intermediate to individuals carrying the NAT1*/4 and individuals carrying the NAT1*/10 allele [i.e., it is unclear to which acetylator group (normal or fast) individuals carrying the NAT1*/11 allele belong; therefore, these individuals are not included in the statistical analyses]. Thus, elucidation of the enzymatic activities of the NAT1*/11 allele or other NAT1 alleles may potentially reveal a trimodal distribution. Consequently, the interpretation of the results of the NAT1 genotype analyses in the present study should be regarded as preliminary.

Badawi et al. (9) have indirectly suggested that the fast NAT1/slow NAT2 genotype combination may increase the risk of bladder cancer (9). The present study was not able to substantiate such a genotype combination as a risk factor; rather, the study indicates that one genotype combination (normal NAT1/fast NAT2) may be protected from bladder cancer compared with other genotype combinations. Alternatively, because of the problems with the NAT1 phenotype/genotype assays, the present studies may not be sensitive enough to distinguish between genotype combinations that increase the risk of bladder cancer.

Recent studies indicate that the NAT1 polymorphism may be a risk factor in bladder cancer (9, 21). These preliminary findings indicate that a certain combination of the two polymorphisms might increase the susceptibility to bladder cancer. It is clear that other enzymes may play equally important roles. The cytochrome P4501A2 may be one of these, because it is induced by and primarily responsible for N-oxidation of carcinogenic arylamines (58). However, at present genetic polymorphisms relating to the CYP1A2 gene have not been identified.

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NAT2341”\(\textsuperscript{3}\))

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