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NAT2 slow acetylation and bladder cancer in workers exposed to benzidine

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This study expands a previous study of *NAT2* polymorphisms and bladder cancer in male subjects occupationally exposed only to benzidine. The combined analysis of 68 cases and 107 controls from a cohort of production workers in China exposed to benzidine included 30 new cases and 67 controls not previously studied. NAT2 enzymatic activity phenotype was characterized by measuring urinary caffeine metabolite ratios. PCR-based methods identified genotypes for *NAT2*, *NAT1* and *GSTM1*. NAT2 phenotype and genotype data were consistent. A protective association was observed for the slow *NAT2* genotype (bladder cancer OR = 0.3; 95% CI = 0.1 = 1.0) after adjustment for cumulative benzidine exposure and lifetime smoking. Individuals carrying *NAT1wt/*10* and *NAT1*10/*10* showed higher relative risks of bladder cancer (OR = 2.8, 95% CI = 0.8–10.1 and OR = 2.2, 95% CI = 0.6–8.3, respectively). No association was found between *GSTM1* null and bladder cancer. A metaanalysis risk estimate of case-control studies of NAT2 acetylation and bladder cancer in Asian populations without occupational arylamine exposures showed an increased risk for slow acetylators. The lower limit of the confidence interval (OR = 1.4; 95% CI = 1.0–2.0) approximated the upper confidence interval for the estimate obtained in our analysis. These results support the earlier finding of a protective association between slow acetylation and bladder cancer in benzidine-exposed workers, in contrast to its established link as a risk factor for bladder cancer in people exposed to 2-naphthylamine and 4-aminobiphenyl. Study findings suggest the existence of key differences in the metabolism of mono- and diarylamines.
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Aromatic amines (arylamines) have been strongly linked to bladder cancer.[1] Arylamines must undergo metabolic activation to become carcinogenic. The metabolism of arylamines involves *N*-acetylation, regulated by the enzymes *N*-acetyltransferases 1 and 2 (NAT1 and NAT2).[2] It has been shown that *NAT2* is polymorphic, and the lack of 2 functional alleles is responsible for decreased enzyme activity, conferring the slow acetylation phenotype.[2] Numerous studies have shown that individuals in the general population classified as slow acetylators are at an increased risk of bladder cancer.[3] In these studies, the only suspected source of arylamine exposure was tobacco smoke, which has been reported to contain 4-aminobiphenyl and 2-naphthylamine.[4] This observation is supported by a case-series metaanalysis in which the association between smoking and bladder cancer was stronger among slow acetylators than among rapid acetylators.[5]

For those exposed to arylamines in the workplace, the relationship between NAT2 acetylation status and bladder cancer has not been as clear. In a number of studies of workers, slow acetylation has been reported as a risk factor for bladder cancer.[6–12] In several of these studies, workers were exposed to arylamine mixtures.[6,13] Other studies did not offer adequate detail of the exposure assessment or analytical methods employed.[11] In 1993, Hayes *et al.*[14] reported a protective but not statistically significant association between NAT2 slow acetylation and bladder cancer in workers in production and dye manufacturing plants in China (OR = 0.5; 95% CI = 0.1–1.8). In contrast to other occupational studies, in the study by Hayes *et al.*[14] workers were exposed exclusively to benzidine, suggesting that the model proposed at the time[15] for deactivation of aromatic amines as bladder carcinogens by *N*-acetylation was not appropriate for benzidine.

This study expands a previous study that evaluated the impact of *NAT2* polymorphisms on bladder cancer in men occupationally exposed to benzidine in the absence of other arylamine exposures. We conducted a nested case-control study in a cohort of 2,612 workers exposed to benzidine for at least one year, between 1945 and 1977, in the cities of Tianjin, Shanghai, Jilin, Henan and Chonquin. Information was available for 2,515 workers, including 1,850 men and 665 women.[16] A separate study of male members of this cohort exposed to benzidine reported a standardized incidence ratio for bladder cancer of 25.0 (95% CI = 16.9–35.7) when compared to an unexposed group.[17] We also investigated the effect of *NAT1* and glutathione *S*-transferase μ1 (*GSTM1*) polymorphisms on bladder cancer in these workers. To increase study power, we pooled our data with the case-control series from the same cohort studied by both Hayes *et al.*[14] and Rothman *et al.*[18] In addition, we conducted a metaanalysis of bladder cancer in nonoccupationally exposed Asian populations who were exposed to monoarylamines in cigarette smoke.

Material and methods

Study population

This nested case-control study included surviving members of a 1972–1981 retrospective cohort study of male workers exposed to benzidine in China.[17] All cohort members had been employed in benzidine production and use facilities for at least a year between 1945 and 1977. Both cases and controls were recruited from a bladder cancer screening program conducted in local occupational

Abbreviations: 1X, 1-methyluracyl; AFMU, 5-acetylamino-6-formylamino-methyluracyl; *GSTM1*, glutathione *S*-transferase μ1; HPLC, high-performance liquid chromatography; NAT1 and NAT2, *N*-acetyltransferases 1 and 2; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

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TABLE I – DISTRIBUTION OF SUBJECT CHARACTERISTICS IN BLADDER CANCER CASES AND CONTROLS EXPOSED TO BENZIDINE

Characteristic	Current study		Pooled analysis	
	Cases (n = 30)	Controls (n = 67)	Cases (n = 68)	Controls (n = 107)
Age (years in 1993), mean ± SD	62.2 ± 8.1	61.6 ± 9.3	63.5 ± 8.0	62.1 ± 9.1
Benzidine exposure (years), mean ± SD	12.6 ± 7.8	9.7 ± 6.9	11.5 ± 7.5	9.8 ± 7.4
Benzidine cumulative exposure, mean ± SD (score × years exposure)	36.5 ± 41.9	21.6 ± 28.0	44.9 ± 44.6	22.7 ± 25.5
Lifetime smoking, *n* (%)				
≥ 100 cigarettes	25 (83.3)	42 (62.7)	56 (82.3)	74 (67.9)
< 100 cigarettes	5 (16.7)	24 (35.8)	12 (17.6)	35 (32.1)
Pack-years (to 1993), mean ± SD	26.3 ± 21.1	25.3 ± 16.2	24.2 ± 21.9	18.5 ± 19.8
Cancer type, *n* (%)				
Transitional cell carcinoma (TCC)	23 (76.7)		55 (80.9)	
Squamous carcinoma	1 (3.3)		1 (1.5)	
Adenocarcinoma			1 (1.5)	
Mixed TCC/adenocarcinoma			1 (1.5)	
Not determined	6 (20.0)		10 (14.7)	

hospitals in 5 Chinese cities. Details of the screening program have been described.[16,19] Briefly, those in the screening program were monitored, on a routine basis, by urine cytology for bladder cancer. The identified cases were confirmed pathologically.

Two groups of workers were studied. The first group (referred to here as the ''current study'') included benzidine-exposed incident cases diagnosed with urinary bladder cancer from 1992 through 1997. Sixty-seven benzidine-exposed controls with negative urine cytology were frequency-matched to 30 cases by age (in 10-year age groups). The study purpose was explained and consent was received using procedures approved by participating institutional review boards. Trained Chinese interviewers administered a questionnaire concerning demographics, smoking history, medication use, consumption of alcohol and caffeine, as well as detailed occupational and health histories. Blood and urine specimens (after administration of a caffeine tablet) were collected.

The second study group (the pooled analysis) included 68 cases and 107 controls from the current study pooled with cases from the same cohort diagnosed between 1965 and 1991 and their corresponding controls. Results from this case-control series were published elsewhere.[14,18] As study participants' names were not available for the earlier series, a verification procedure was followed to guarantee that they were not in the current study. This procedure included review of birthdays, work, medical and smoking histories, as well as genotype status. Data from the earlier series on subjects who could also be in the current study were not included in the pooled analysis. We could not rule out the inadvertent inclusion of 2 controls from the 1991 series in the current study; consequently, we excluded data from the earlier series on these 2 workers from the pooled analysis. In addition, one of the controls in the earlier series[14,18] became a case in the current study, so his data as a control were excluded.

Exposure assessment

All participants had been employed in benzidine production and dye manufacture facilities for at least 1 year. Measurements of benzidine concentrations in the air of benzidine production facilities and in contaminated skin and urine of selected workers confirmed persistent exposure to benzidine. Review of work histories, materials and products indicated that benzidine was the only arylamine used in the facilities.[17] Because quantitative levels of benzidine exposure were unknown for each job, we assigned a benzidine exposure level (1 = low, 3 = medium, 9 = high) to each job title held by the workers, based on knowledge of the plants' operations and the available industrial hygiene data as described by Bi *et al*.[17] Cumulative exposure to benzidine in each job was estimated as the product of the benzidine exposure level times duration of exposure.

Phenotyping

Eligible participants were administered a caffeine tablet (100 mg; NoDoz; Bristol-Myers Squibb, Princeton, NJ) by mouth in the morning after voiding. Participants were told not to drink caffeinated beverages from midnight and until 5 hr after drug administration. At 4 and 5 hr after drug administration, participants were asked to void; they collected urine specimens only at the end of the 5-hr period. Specimens were transferred to tubes with ascorbic acid and frozen in a −70°C freezer or in dry ice for transportation.[20]

NAT2 enzymatic activity was characterized by measuring urinary caffeine metabolite ratios reflecting this activity. Secondary caffeine metabolites 5-acetylamino-6-formylamino-methyluracyl (AFMU) and 1-methyluracyl (1X) were measured in urine extracts by HPLC as described by Butler *et al*.[20] The AFMU/1X ratio was used to determine acetylator phenotype.

Genotyping

Participants donated approximately 8.5 ml of venous blood collected by venipuncture; contents of each tube were transferred to centrifuge tubes and frozen at −70°C. Specimens were shipped on dry ice. DNA was isolated from peripheral leukocytes by high-salt precipitation and resuspended in TE buffer (10 mM Tris/1 mM EDTA).

Genotyping was performed using 50 ng of genomic DNA and polymerase chain reaction (PCR)-based methods. *NAT2* genotypes *NAT2*4* (WT), *NAT2*5* (T341C, C481T, A803G), *NAT2*6* (G590A), *NAT2*7* (G857A) and *NAT2*14* (G191A) were determined using the PCR-based restriction fragment length polymorphism (RFLP) methods of Doll *et al*.[21] *NAT1* polymorphisms that were representative of the *NAT1* alleles *NAT1*4* (WT), *NAT1*3* (C1095A), *NAT1*10* (T1088A, C1095A) and *NAT1*11* (del 9) were assessed using the PCR-RFLP methods of Bell *et al*.[22] *NAT1*14* (G560A), *NAT1*15* (C559T) and *NAT1*17* (C190T) alleles were determined using the method of Boissy *et al*.[23] and Katoh *et al*.[24] In Table II and in the text, WT represents genotypes containing *NAT1*4* alleles.

The presence of the *GSTM1* allele was determined using the multiplex PCR method of Chen *et al*.[25] This technique does not distinguish between heterozygote and homozygote *GSTM1*-positive genotype but conclusively identifies null genotypes.

Statistical analysis

Statistical procedures for all variables included exploratory data analysis and descriptive statistics. A time series analysis comparing latency (date first exposed to benzidine to date of diagnosis) was conducted for both current and previous cases.[14] To assess the

TABLE II – ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR BLADDER CANCER IN BENZIDINE-EXPOSED WORKERS ACCORDING TO DIFFERENT CHARACTERISTICS

Characteristic	Current study				Pooled analysis			
	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI
Benzidine cumulative exposure[1] (cumulative level-years)								
Low (< 30)	17	50	1.0		32	78	1.0	
Medium (30–59)	4	9	1.3	0.3–5.0	15	17	2.7	1.1–6.3
High (≥ 60)	5	6	2.5	0.6–9.4	17	10	4.4	1.8–10.8
Lifetime smoking[2]								
No	5	24	1.0		25	44	1.0	
Yes	25	42	2.7	0.8–9.1	34	55	1.9	0.8–4.2
Tobacco (pack-year)[2]								
0	5	24	1.0		12	35	1.0	
< 20	13	22	2.7	0.7–10.3	24	34	2.0	0.8–5.1
≥ 20	8	18	2.2	0.5–8.8	28	38	2.1	0.7–4.1
NAT2 phenotype[3]								
Rapid (> 0.29)	26	47	1.0		59	81	1.0	
Slow (≤ 0.29)	2	15	0.3	0.1–1.6	6	24	0.4	0.1–1.1
NAT2 genotype[3]								
Rapid	29	50	1.0		62	83	1.0	
Slow (No *NAT2*4*)	1	15	0.2	0.0–1.4	6	25	0.3	0.1–1.0
NAT1 genotype[3]								
wt/wt	4	17	1.0		NA[4]	NA	NA	NA
wt/*10	13	20	2.8	0.6–8.3				
*10/*10	11	21	2.2	0.8–7.0				
GSTM1 genotype[3]								
+/+, 0/+	14	33	1.0		29	49	1.0	
0/0 (null)	16	32	1.3	0.5–3.4	39	56	1.1	0.6–2.3

[1]ORs and 95% CIs adjusted for lifetime smoking.–[2]ORs and 95% CIs adjusted for cumulative benzidine exposure.–[3]ORs and 95% CIs adjusted for cumulative benzidine exposure and lifetime smoking.–[4]Not analyzed.

associations of NAT2 phenotype and genotype data and bladder cancer risk before and after controlling for confounding factors, unconditional logistic regression models were used. Also, interactions between cumulative benzidine exposure or cigarette smoking and bladder cancer were analyzed by logistic regression methods. Statistical procedures were repeated, pooling the new cases and controls from this study with previous cases and controls.[18] SAS Version 8.0 was used for these analyses (SAS Institute, Cary, NC). A summary odds ratio (OR) and 95% confidence interval (CI) was calculated by pooling the results of studies conducted in general Asian populations using metaanalytic techniques that weighted the estimated β coefficient for each individual study by a function of its variance.[26] Homogeneity between studies was tested using the Q-statistic at a 0.05 level using Comprehensive Meta Analysis software (Biostat, Englewood, NJ).

Results

The current nested case-control study included 30 cases and 67 controls. Twenty-three cases were diagnosed with transitional cell carcinoma, 1 had squamous carcinoma and 6 had an unspecified bladder cancer diagnosis (Table I). Since cases were identified through a screening program, most of them did not present invasive disease. A listing of most cases and their WHO grade and TNM stage is provided in Hemstreet *et al.*[19] Cases and controls did not differ in age and smoking (mean cigarette pack-years), but 35.8% of controls, compared to 16.7% of cases, had smoked < 100 cigarettes in their lifetime. Cases were exposed to benzidine longer than were controls (Table I), and the mean cumulative (log-transformed) benzidine exposure was higher among cases ($p < 0.0001$).

A time series analysis was conducted comparing latency for both previous and current cases (date first exposed to benzidine to date of diagnosis). A statistically significant difference was not observed between the 2 sets of data (mean latency = 26.7 years for previous cases *vs.* 31.6 years for current cases; $p = 0.12$). A difference in mean year of first exposure was observed (1955 for previous cases *vs.* 1959 for current cases; $p < 0.02$).

Risk analyses were conducted separately for the current case-control study and for the pooled study by adding eligible cases and controls from the previous series.[14,18] From the 1991 case-control study, we added 38 bladder cancer cases diagnosed through 1991 and 40 controls, all occupationally exposed to benzidine only. Available information for this latter group included questionnaire and pathology data, NAT2 phenotype and *NAT2* genotype information, as well as *GSTM1* genotype data. The mean ages at diagnosis were 63.3 ± 6.8 and 63.2 ± 7.0 years, respectively. Thirty-two cases were diagnosed with transitional cell carcinomas, one with an adenocarcinoma, one with a mixed transitional cell and adenocarcinoma; 4 diagnoses were unspecified.

Table II shows associations between several characteristics and the development of bladder cancer after adjustment for confounding factors. We observed a higher but nonsignificant relative risk of bladder cancer among smokers in the current study (OR = 2.7; 95% CI = 0.8–9.1) and in the pooled analysis (OR = 1.9; 95% CI = 0.8–4.2). No dose-response effect was observed when data were stratified by tobacco consumption in either the current study or the pooled analysis (Table II).

An increased relative risk of bladder cancer was observed (OR = 1.7; 95% CI = 1.4–2.6) when cumulative benzidine exposure was expressed as a continuous variable in the current study, after adjustment for lifetime smoking. We observed a slightly stronger association for the pooled data (OR = 2.0; 95% CI = 1.5–2.9), also adjusted for lifetime smoking. When cumulative exposure was divided in tertiles, subjects with medium and high benzidine exposures showed an exposure-dependent increase in bladder cancer risk in both analysis groups, relative to lower-exposed subjects (Table II). Unadjusted ORs in the current study changed minimally after adjustment for age, tobacco use and alcohol or tea consumption. Adjustment for age and tobacco use did not alter the results in the pooled analysis. Nonetheless, the results are presented adjusted for lifetime smoking.

In tests for NAT2 phenotype, a histogram of the logarithmically transformed caffeine metabolites AFMU/1X ratio showed a distinct bimodal distribution (Fig. 1). We placed the cutoff for slow

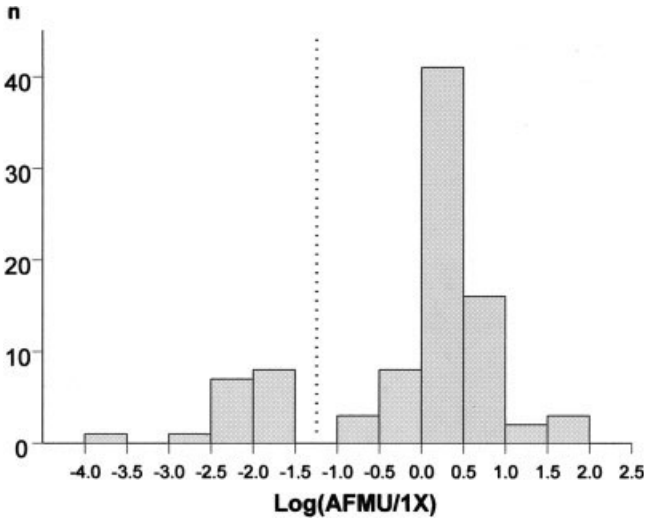


FIGURE 1 – Histogram of NAT2 phenotypic activities as obtained by the caffeine test in Chinese workers exposed to benzidine. From the distinct bimodal distribution, an antimode of log(0.29) = −1.25 was obtained (dotted line).

and rapid acetylators at log(0.29) = −1.25. Among 90 individuals tested for NAT2 phenotype, 17 (19%) had an AFMU/1X ratio ≤ 0.29. Ninety-five subjects had genotype testing. Of those, 16 (17%) did not have the *NAT2*4* allele but had other alleles and were therefore classified as slow acetylators. The remaining 79 (83%) had the *NAT2*4* allele on one or both chromosomes and were considered rapid acetylators. A high concordance (96%) was found between *NAT2* genotype and NAT2 phenotype measured by caffeine metabolites.

In the current study, slow acetylators had a reduced risk of bladder cancer when compared with rapid acetylators (Table II). We observed a protective association for both NAT2 phenotype and *NAT2* genotype after adjustment for cumulative benzidine exposure. As expected, the associations for NAT2 phenotype and *NAT2* genotype remained in the pooled analysis (Table II).

In the current analysis, individuals carrying *NAT1wt/*10* and *NAT1*10/*10* showed higher relative risks of bladder cancer (OR = 2.8, 95% CI = 0.8–10.1 and OR = 2.2, 95% CI = 0.6–8.3, respectively) compared to individuals with the *NAT1wt/wt* genotype (Table II). Similar risk estimates were obtained when data from individuals carrying the *NAT1wt/wt* genotype were grouped with data from study participants carrying *NAT1wt/*3* (results not shown). We could not conduct a pooled analysis for *NAT1*, as no data were available for the 1991 series. The relative risk of bladder cancer for the *GSTM1*-null genotype was 1.3 (95% CI = 0.5–3.4; Table II). Results remained unchanged after controlling for age, tobacco use, benzidine exposure, or *NAT2* genotype. The pooled analysis also showed no association between *GSTM1* and bladder cancer (OR = 1.1; 95% CI = 0.6–2.3).

There was insufficient power to evaluate the interaction between *NAT2* polymorphisms and benzidine exposure. Therefore, results of the pooled analysis were compared with the results of a metaanalysis of 8 case-control studies of NAT2 slow acetylation and bladder cancer conducted in Asian populations (5 from Japan, 1 from Korea and 2 from Taiwan) not exposed occupationally to arylamines.[34] Table III presents a summary of the results of each study and the result obtained when the data from these studies were pooled. Only results of the fixed model are presented as the results for fixed and random-effects models were practically identical and the hypothesis of homogeneity was not rejected (Q-statistic, $p = 0.36$). The lower limit of the 95% CI of the risk estimate in Asian general populations (OR = 1.4; 95% CI = 1.0–2.0) approximates the upper 95% CI for the estimate obtained in our pooled analysis (OR = 0.3; 95% CI = 0.1–1.0).

Discussion

Benzidine has been associated causally with an increase of bladder cancer in humans and induces malignant neoplasms in experimental animals. The International Agency for Research in Cancer has classified benzidine as a group 1 carcinogen (''sufficient'' evidence of carcinogenicity in humans).[35] In China, benzidine manufacture began in 1956 and halted in 1977.[36] In a cohort of 1,972 benzidine-exposed subjects in China, Bi *et al.*[17] reported an overall bladder cancer SIR of 25, with relative risks ranging from 4.8 to 158.4 for low to high exposures. Benzidine-exposed workers who also smoked cigarettes had a 31-fold risk of bladder cancer, compared to an 11-fold risk observed in nonsmoking workers, suggesting a synergistic effect. Wu[36] reported a similar synergistic effect in a retrospective cohort study of 2,525 workers at benzidine manufacturing plants in China. The results of this study confirm that workers who reportedly ended their exposure in 1977 were still at risk of bladder cancer in 1993. Given that the average latency for chemically induced bladder cancer ranges from 4 to 40 years,[37] it is reasonable to expect that other members of the cohort may yet develop bladder cancer. In our study populations, no differences in latency were observed, and even though a difference was observed in mean year of first exposure, there is no reason to believe that there are substantive differences between both populations.

Tobacco use is a known cause of bladder cancer, with 2- to 3-fold increased risk among individuals who have ever smoked.[5] In this study, lifetime cigarette smoking was a moderate risk factor for bladder cancer, and an exposure-response relationship between cigarette pack-years and bladder cancer was not observed. Reasons for such a finding could be limited information on use of tobacco products other than cigarettes and misreport of cigarette smoking history. Data from the 1996 National Prevalence Survey of Smoking Pattern in China[38] indicate that filtered cigarettes dominate the market in China, particularly among the young. Among older men (age 60+), 20% or more use Chinese pipe tobacco and other tobacco products. These usage patterns, which are also affected by educational level and geographical distribution, may in part explain these findings in a group whose mean age is above 60 years.

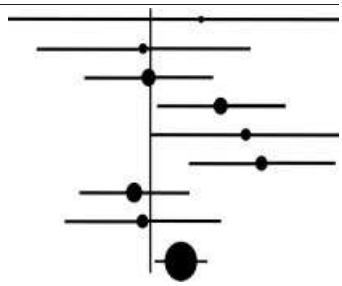
We evaluated the effect that genetic polymorphisms of metabolic enzymes had on the risk of bladder cancer. The results indicated that being a slow acetylator reduces the risk of developing benzidine-induced bladder cancer (OR = 0.2; 95% CI = 0.0–1.4). These findings are consistent with earlier results in subjects from the same cohort (OR = 0.5; 95% CI = 0.1–1.8).[14] Pooling data from both studies increased statistical power and strengthened the results (OR = 0.3; 95% CI = 0.1–1.0).

Even though the data from 2 case-control series were pooled, the sample size was still relatively small ($n = 175$). Nonetheless, there was sufficient statistical power to detect a protective association between slow acetylation and bladder cancer risk from benzidine. The pooled data, however, did not provide sufficient power to evaluate the interaction between slow acetylation and benzidine exposure. Accordingly, we compared the result of the association obtained in this study with the result of pooled studies in Asian populations not exposed occupationally to arylamines. Evidence existed of a gene-environment interaction between NAT2 acetylation and benzidine exposure, as the upper confidence limit of the risk estimate for NAT2 acetylation and bladder cancer in subjects exposed to benzidine adjoins with the lower limit in people not exposed.

This study has several strengths. Combining the data from 2 case-control series makes it the largest study to date conducted in workers exposed to arylamines. It is also the largest study of ben-

TABLE III – ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR NAT2 SLOW ACETYLATION AND BLADDER CANCER IN CASE-CONTROL STUDIES IN ASIAN POPULATIONS NOT EXPOSED OCCUPATIONALLY TO ARYLAMINES. CIRCLES ARE PROPORTIONAL TO STUDY SAMPLE SIZE

Study	Year	Site	Phenotype (P) or genotype (G) (drug used or allele tested)	Slow/rapid acetylators		Odds ratio (95% CI)
				Cases	Controls	
Sone[27]	1986	Japan	P (sulfamethazine)	3/4	39/63	1.8 (0.2–18.8)
Horai *et al*.[28]	1989	Japan	P (dapsone)	3/16	48/238	0.9 (0.3–3.3)
Lee *et al*.[29]	1994	Korea	P (isoniazid)	16/30	82/152	1.0 (0.4–2.1)
Ishizu *et al*.[30]	1995	Japan	P (isoniazid)	20/33	51/129	2.4 (1.1–5.1)
Su *et al*.[1, 31]	1998	Taiwan	G (*4, *5, *6, *7)	8/15	19/72	3.2 (1.0–10.0)
Katoh *et al*.[2, 32]	1999	Japan	G (*4, *5, *6, *7)	22/29	94/209	3.8 (1.6–9.4)
Hsieh *et al*.[33]	1999	Taiwan	G (*4, *5, *6, *7, *14)	15/73	44/183	0.8 (0.4–1.6)
Kontani *et al*.[3, 34]	2001	Japan	G (*4, *5, *6, *7)	8/18	128/273	0.9 (0.3–2.4)
Combined (8)				95/204	519/1333	1.4 (1.0–2.0)[4]



[1]Excludes subjects exposed to arsenic.–[2]Urothelial cancer patients (bladder 96, ureter 13, renal pelvis 7).–[3]Excludes workers employed in the manufacture of dyestuffs, rubber and rubber products, gas and coke ovens workers.–[4]In benzidine-exposed workers in China (this study), the risk for bladder cancer among NAT2 slow acetylators was 0.3 (0.1–1.0).

zidine-exposed workers. In previous studies, workers may have been exposed to mixtures of arylamines, but this group has the unique attribute of having been exposed exclusively to benzidine. Future studies in larger cohorts are unlikely, given that the production and use of benzidine have been banned in many countries. In addition, this study has detailed information on confounding factors and used reliable laboratory methods.

Previous studies in subjects exposed to arylamines mixtures have not shown consistent results for the association between NAT2 slow acetylation and bladder cancer risk. Specifically, the study of Cartwright *et al*.[6] in Great Britain showed a strong association in the analysis of a subgroup of chemical workers in the dye manufacturing industry (OR = 16.7; 95% CI = 2.2–129.1). Results of this magnitude, however, have not been replicated. Cartwright *et al*.[6] indicated that workers were exposed to benzidine, but that other exposures, such as 2-naphthylamine, were likely and that acetylation metabolism is only relevant for some but not all *N*-substituted arylamines. Furthermore, Cartwright *et al*.[6] acknowledged that the bias toward slow acetylation seen in bladder cancer cases could be explained by the preponderance of patients with invasive disease and of the group exposed to arylamines; if these 2 groups were removed from the analysis, the acetylation status among cases and controls would not be different. A German study in workers employed in a benzidine production plant showed an association between slow acetylation and increased bladder cancer relative risk (OR = 4.8; 95% CI = 2.7–8.4).[11] This study, however, did not allow the reader to determine if workers were also exposed to other compounds. Furthermore, analytical procedures were not fully described and precluded further evaluation of the reliability of the methods employed. Hanke and Krajewska[10] also found a positive association between slow acetylation and bladder cancer risk among people reporting exposure to arylamines (type not specified) in Poland (OR = 8.4; 95% CI = 1.9–36.6). In contrast, other studies have included subanalyses in groups reporting occupational exposure to arylamines and have presented evidence of moderate or no associations between NAT2 slow acetylation and bladder cancer risk (with odds ratios ranging from 0.3 to 3.7).[7–9,12,39]

A review of NAT2 slow acetylation and bladder cancer in arylamine-exposed subjects suggested that metabolic differences between Chinese and Caucasian populations might explain these discrepancies.[40] The authors did not provide evidence for their assumption; moreover, a number of arguments preclude us from supporting such an assertion. First, tobacco smoke increases the risk of bladder cancer in both Asian and Caucasian populations.[41–44] Second, exposure to benzidine and other arylamines has been associated with increased risks of bladder cancer in both Asian and Caucasian workers.[17,45–50] Third, as shown in the metaanalysis presented in Table III of Asian populations not exposed to arylamines in the workplace, studies have shown that slow acetylation is a risk factor for bladder cancer comparable to the risk found in Caucasian populations, and the magnitude of the relative risk is the same.[3] Fourth, even though racial differences in the allele distribution among different ethnic groups have been reported, almost all of the alleles found in Caucasian groups have been described in Asian populations.[51,52] Finally, the correlation between *NAT2* genotype and NAT2 phenotype measured by different probes is the same among Chinese and Caucasian populations.[53–55] Therefore, it is unlikely that an ethnic difference could explain these results, as there are no equivalent, well-designed studies conducted in Caucasian populations exposed exclusively to benzidine.

Alternatively, benzidine metabolism studies suggest that the lack of consistency in results may be explained by variations in the metabolism of different aromatic amines (Fig. 2). For aromatic monoarylamines, such as 2-naphthylamine and 4-aminobiphenyl, also found in tobacco smoke, *N*-acetylation appears to be a detoxification pathway; the acetylated metabolite is excreted into the urine before it can be *N*-oxidized to a reactive form.[56] Primary amines are considered easier to oxidize than their acetylated amide products, and slow acetylators should generate higher levels of *N*-hydroxyarylamines than rapid acetylators.[57]

Benzidine, on the other hand, is a diarylamine. In the liver, benzidine appears to be acetylated to *N*-acetylbenzidine, which in turn can be acetylated to *N*,*N*′-diacetylbenzidine by *N*-acetyltransferase enzymes (NAT).[58] In contrast to monoarylamines, *N*-acetylbenzidine has another free amine group that is susceptible to *N*′-oxidation or *N*′-glucuronidation.[59] Consequently, *N*-acetylation is not a detoxifying mechanism for benzidine, but more likely an important activation step. Both benzidine and *N*-acetylbenzidine are detoxified by hepatic glucuronidation and excreted into the bladder lumen as glucuronides. Studies using human, dog and rat liver slices have demonstrated that both *N*-acetylbenzidine and benzidine are released from their glucuronides by acidic urine.[60] It has been hypothesized that the released primary aromatic amines could be further activated within the bladder to initiate carcinogenesis.[57] *In vitro* experiments have demonstrated that benzidine and *N*-acetylbenzidine are both preferred substrates for NAT1, but not NAT2.[61] Therefore, low NAT2 activity (slow acetylation) should not be expected to affect benzidine acetylation or the incidence of bladder cancer in benzidine-exposed populations. Support for this model of benzidine metabolism is provided in a cross-sectional study in Indian workers exposed to benzidine, where the predominant

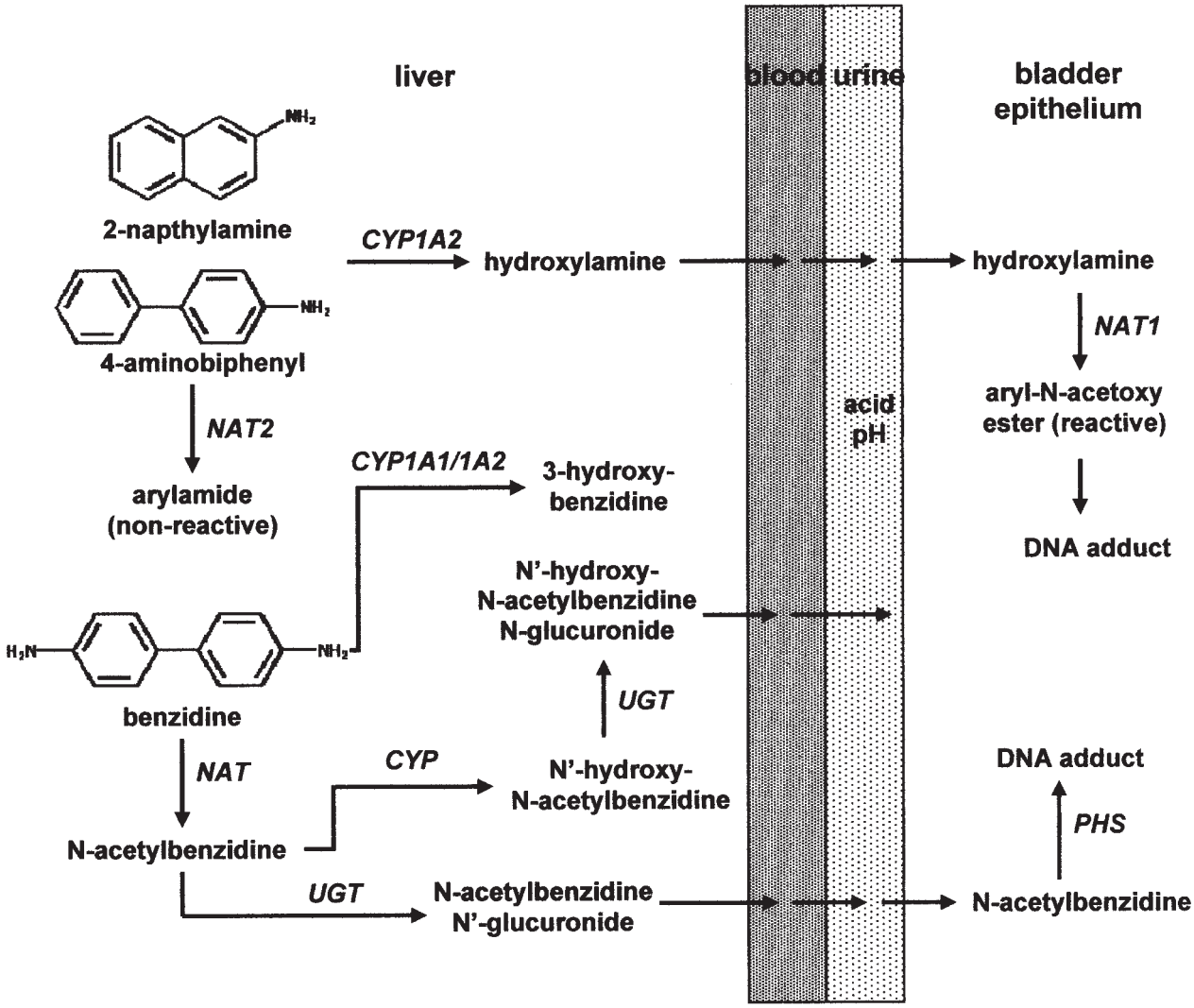


FIGURE 2 – Comparison of key pathways in the metabolism of monoarylamines and diarylamines. CYP1A1/CYP1A2, cytochrome P-450 1A1/1A2; UGT, UDP-glucuronosyltransferase; PHS, prostaglandin H-synthase.

urothelial adduct was *N*-acetylated, but it was not associated with NAT2 activity, NAT2 phenotype, or *NAT2* genotype.[13]

Higher risks of bladder cancer were observed among individuals homozygous and heterozygous for *NAT1*10*. These results were nonstatistically significant, possibly due to inadequate power. Regrettably, no NAT1 data were available for the earlier study, and the quality and quantity of DNA specimens available did not allow genotyping with the methods used for the subjects in the later series. Epidemiologic studies that evaluate this association are required, as human recombinant NAT and liver slice experiments have shown that both benzidine and *N*-acetylbenzidine are preferred substrates for NAT1.[61]

The results of this investigation indicate that GSTM1 deficiency does not increase the risk of developing bladder cancer among workers exposed only to benzidine and are consistent with the initial finding in workers from the same cohort.[18] These results are consistent with a study in Chinese workers exposed to benzidine, where the *GSTM1* 0/0 genotype was not associated with a high cytologic grading in a Papanicolaou test.[62] The results also agree with a study in workers exposed to benzidine in India, in which the *GSTM1* genotype did not affect urine mutagenicity or DNA adduct levels.[18] Results also concur with benzidine metabolism data obtained in studies *in vitro*, where GSTM1 does not appear to catalyze the conjugation of benzidine or its metabolites with glutathione.[18,63]

In summary, these data show that slow *N*-acetylation by NAT2 confers decreased risk for benzidine-induced bladder cancer. These results suggest the existence of key differences in the metabolism of mono- and diarylamines that combine with genotype to affect the individual susceptibility to bladder cancer.

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