RESEARCH ARTICLE

Distribution and Role of N-acetyltransferase 2 Genetic Polymorphisms in Bladder Cancer Risk in a Lebanese Population

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Abstract

Background: In Lebanon, bladder cancer (BC) has an unusually high prevalence. Individuals who are exposed to aromatic amines from smoking or certain occupations and carrying the slow N-acetyl transferase 2 (NAT2) acetylator' phenotype may be at a higher risk. **Methods:** Data and DNA from 115 Lebanese BC cases and 306 controls were examined. Ten NAT2 single nucleotide polymorphisms were genotyped, seven of which were then included in haplotype and phenotype analysis. **Results:** BC patients were more likely to be males (87.8% vs. 54.9%) and current smokers (60.9% vs. 26.5%) when compared to controls. In both groups, most participants had the slow NAT2 acetylator phenotype (66.1% of BC cases vs 62.7% of controls; P=0.302) with the NAT2*5B and *6A haplotypes being the most common. The odds ratio (95%CI) of having BC among slow NAT2 acetylators was 1.157 (0.738-1.815) and remained non-significant after adjustment [1.097 (0.666-1.806)]. Sensitivity analysis with a subgroup of 113 cases and 84 controls for which occupational history was available revealed a statistically significant association between slow NAT2 acetylators and BC in females only. The sample size was however very small and the CI quite wide. **Conclusions:** This is the first study to evaluate the distribution of NAT2 haplotypes and their potential role in BC in a Lebanese population. The absence of any significant association may be due to the relatively small sample size, the unavailability of matching by gender, and the lack of evaluation of genetic interactions with extent of active and passive smoking, exposure to environmental pollutants, diet, and other genes. The potential association limited to females needs further evaluation.

Keywords: NAT2- bladder cancer- Lebanese

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Introduction

According to Globocan data from 2012 (www.globocan.iarc.fr), bladder cancer (BC) is the 9th most common cancer worldwide comprising 3.1% of all cases. In Lebanon, BC appears to have an unusually high prevalence. Data for the period of 2003 to 2008 revealed BC to be the second most common after prostate cancer in males with an incidence rate of 34 per 100,000. Although the rate was much lower in females (9 per 100,000), it is alarming that the calculated annual percent changes were on the rise from 2003 to 2008, and the projected rates for 2018 are even higher in both males (41.2 per 100,000) and females (13.4 per 100,000) (Shamseddine et al., 2014). These trends may be attributed to increased exposure to a variety of environmental and occupational carcinogens including the commensurate increase in tobacco smoking (Dhaini and Kobeissi, 2014; Baris et al., 2009; IARC monograph 100E-6, 2013; Jaafar et al., 2014; Malats and Real, 2015; Sauter G, 2014; Sibai et al., 2016).

It is known that many of the carcinogens require metabolic activation in order to induce their toxic effect; therefore, it is supposed that genetic typing of the relevant drug metabolizing enzymes (DMEs) may explain some of the variability in the cancer process. According to the International Agency for Research on Cancer (IARC), there are at least 62 carcinogens in cigarette smoke with polycyclic aromatic hydrocarbons (PAHs), aromatic amines and N-nitroso compounds being highly toxic to the urothelium (IARC monograph 100E-6, 2013). In the liver, these compounds are activated by cytochrome P450 (CYP450) into reactive metabolites that may be further activated by O-acetylation via the N-acetyltransferase 1 (NAT1) enzyme in the urothelium. Carcinogens and

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their metabolites are inactivated by hepatic Phase II enzymes such as glutathione S-transferases (GST) and N-acetyltransferase 2 (NAT2) (Antonova et al., 2015; Tsukino et al., 2004). Accordingly, it has been postulated that subjects who carry CYP450 and NAT1 genetic polymorphisms that lead to an increased enzymatic activity, and those with GSTT1 and GSTM1 null genotypes or NAT2 haplotypes that confer the slow acetylator phenotype are at a higher risk of developing BC (Cascorbi et al., 2001; Fontana et al., 2009; McGrathet al., 2006; Song et al., 2009). Results have shown that the interaction between smoking and genetic polymorphisms of DMEs is strongest with GSTM1 null and slow NAT2 acetylators (Antonova et al., 2015; Garcia-Closas et al., 2005; McGrath et al., 2006; Ovsiannikov et al., 2012; Rouissi et al., 2009; Yuan et al., 2008), with the NAT2 association being the most consistent (Moore et al., 2011; Tsukino et al., 2004) as has been shown in 3 recent meta-analyses asserting its role in BC (An et al., 2015; Wu et al., 2016; Zhu et al., 2015). Additional sources of the carcinogenic aromatic amines include a number of occupations many of which have been linked to an increased risk of bladder cancer (Dhaini and Kobeissi, 2014; Malats and Real, 2015; Sauter G, 2014), especially among slow NAT2 acetylators (Covolo et al., 2008; Ovsiannikov et al., 2012; Yuan et al., 2008).

To date, a large number of NAT2 single nucleotide polymorphisms (SNPs) have been reported with 107 documented haplotypes (Boukouvala, 2016; Walker et al., 2009). Studies in Middle Eastern Arabs showed a high prevalence of NAT2*5 and NAT2*6 alleles and a higher frequency of slow acetylators in comparison to other ethnicities (Bu et al., 2004; Hamdy et al., 2003; Sabbagh et al., 2008; Tanira et al., 2003). To our knowledge, no data are available on the potential association of NAT2 genetic polymorphisms with BC in Arabs including the Lebanese except for one study by El-Desoky et al., (2005) who evaluated 3 common NAT2 SNPs and showed that the NAT2*5/*5 genotype is associated with schistosomiasis-related BC in Egyptians. More recently in Lebanon, Basma et al., (2013) showed NAT1*14A to be potentially associated with a higher risk of BC in a small sample of Lebanese people. The current study measured the frequencies of NAT2 genetic polymorphisms in a sample of Lebanese people with and without BC and evaluated whether smokers with slow NAT2 acetylator phenotype are at a higher risk of having BC.

Materials and Methods

Study population

This is a case-control association study. It includes 115 Lebanese subjects who were diagnosed with transitional cell carcinoma (TCC) of the bladder -of which 41.1% were invasive- and who were admitted for treatment at the American University of Beirut Medical Center (AUBMC) between 2012 and 2016. Controls included 84 patients who presented during the same recruitment period to the urology clinics or service for other complaints in addition to 222 historic controls who did not have BC but were previously recruited for a separate study (Esmerian et al., 2011).

Peripheral whole blood was drawn and stored at -80°C. A questionnaire was administered. Due to insufficient numbers or missing data for most of the risk factors, only current smoking (defined as being a cigarette smoker at the time of diagnosis for BC cases, and at the time of recruitment for controls) and alcohol intake were analyzed. Occupational history was available for 113 of the 115 cases and the 84 controls. No such data were available for the 222 historic controls. Medical chart review was performed for TCC histologic diagnosis.

The study was approved by the Institutional Review Board (IRB), and all subjects signed an informed consent.

NAT2 analysis

DNA was isolated from 300 microliters of peripheral blood using the Flexigene DNA isolation kit (Qiagen, Ca, USA) as per manufacturer's guidelines and stored at -20°C until analysis.

Genotyping for ten SNPs in an 866 bp fragment spanning the coding region of NAT2 was performed using restriction fragment length polymorphism (RFLP) technique as described by Deitz et al. (2000). The 3 primer pairs (5'-GGCTATAAGAACTCTAGGAAC-3' with 5'-AAGGGTTTATTTGTTCCTTATTCTAAT-3' for the initial PCR, 5'-CACCTTCTCCTGCAGGTGACCG-3' with 5'-TGTCAAGCAGAAAATGCAAGGC-3' for the nested T341C PCR, and 5'-TGAGGAGAGGTTGAAGAAGTGCT-3' with 5'-AAGGGTTTATTTTGTTCCTTATTCTAAAT-3' for the nested A803G PCR) were purchased from TibMolBiol (Germany) and the 1X RedTaq ready mix per reaction mix containing Taq polymerase and dNTPs was purchased from Sigma-Aldrich (MO, USA). FastDigest restriction enzymes (Thermo Scientific, MA, USA) were used for SNP detection as such: FokI for C282T; MspI and KpnI combined for G191A, A434C, and C481T; TaqI and BamHI combined for T111C, G590A, C759T, and G857A; and AciI and DdeI for T341C and A803G on nested PCR respectively. Digested products were run on 3% agarose gel (Peqlab, Germany) containing 0.025% ethidium bromide (Amresco, Ohio, USA) for 1 hour and visualized using a Gel Doc instrument (Biorad, Ca, USA).

NAT2 haplotypes and their corresponding phenotypes were assigned based on the NAT2 haplotype nomenclature (Boukouvala S, 2016; Walker et al., 2009) using only seven SNPs as the other 3 were all wild type. NAT2*4 is the reference haplotype and corresponds to rapid NAT2 activity (Table 1). Subjects who had a combination of 2 haplotypes corresponding to rapid NAT2 acetylation activity were categorized as fast acetylators, those who had a combination of 2 haplotypes corresponding to slow NAT2 acetylation activity were categorized as slow acetylators, and those who had one of each were categorized as intermediate acetylators (Boukouvala S, 2016; Walker et al., 2009; Cascorbi et al., 1995; Cascorbi et al., 2001) (Table 2).

Statistical analysis

Data were entered into SPSS v.23.0 (IBM, USA). Minor allele frequencies (MAF) of the NAT2 SNPs were

calculated and tested for Hardy Weinberg Equilibrium (HWE) using chi-square test. Baseline demographic data of cases and controls including age, sex, current smoking and alcohol intake were computed and compared using Student t-test, chi-square test and logistic regression as applicable. Comparisons of NAT2 phenotypes between cases and controls were performed using chi-square test and logistic regression. Sensitivity analysis with the subgroup for which occupational history is available was attempted. Occupational exposure to aromatic amines included having any the following occupations: hairdressers, paint industry, printing ink industry, rubber and cable manufacture, textile and leather works, truck or bus drivers, roofers, chimney sweeps, truck drivers, tar and asphalt workers, brickyard workers, aluminum industry, gas industries and blacksmiths. Univariate and multivariate logistic regressions were also performed to include statistically significant confounders as applicable. Despite the small sample size, analyses were run on the whole sample and after stratification for sex, current smoking and occupational exposure to aromatic amines as these are established risk factors for BC. P values of 0.05 or less and odds ratios (ORs) with 95% confidence intervals (CIs) that did not include the value of 1 were considered statistically significant.

Results

The tested NAT2 alleles were all in HWE. The major haplotypes in controls were NAT2*5B (40.68%), *6A (30.89%),*4 (20.27%) and *7B (3.43%) (Table 1), and genotypes were NAT2*5B/*6A (23.9%), *5B/*5B (19.0%) *4/*5B (14.7%) and *6A/*6A (11.4%) in this order (Table 2). In both groups, the majority of participants had the slow NAT2 acetylator phenotype (66.1% in BC cases vs 62.7% in controls; P=0.302). BC patients were more likely to be males (87.8% vs. 54.9%), current smokers (60.9% vs. 26.5%) and alcohol drinkers (46.1% vs. 28.8%). There was no difference in mean age between cases and controls (64.89± 10.57 vs. 66.92 ± 15.14) (Table 3). The odds ratio of having BC among slow NAT2 acetylators was 1.157 (95%CI: 0.738-1.815) and remained non-significant even after adjusting for sex, current smoking and alcohol intake [1.097 (95%CI: 0.666-1.806)]. Stratification analysis revealed higher odds of having BC

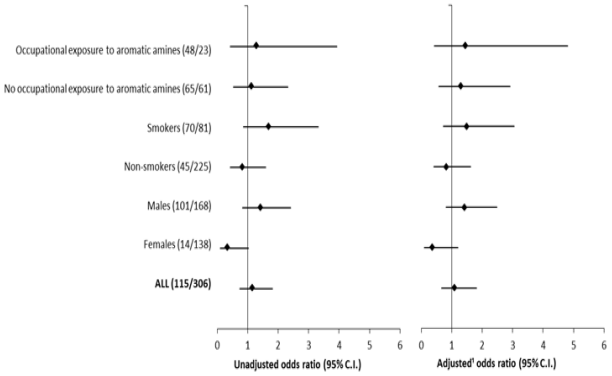

Figure 1. Association between NAT2 Phenotype (Slow vs. Fast and Intermediate) and Bladder Cancer Risk in the Whole Sample and Stratified by Sex, Smoking and Occupational Exposure to Aromatic Amines[2]. Sex and/or smoking, and alcohol intake as applicable. [2]Data were available for a subgroup of 113 cases and 84 controls. Occupational exposure included those who had any of the following occupations: Hairdressers, paint industry, printing ink industry, rubber and cable manufacture, textile and leather works, truck or bus drivers, roofers, chimney sweeps, truck drivers, tar and asphalt workers, brickyard workers, aluminum industry, gas industries and blacksmiths.

Table 1. *NAT2* Genetic Polymorphisms in Bladder Cancer Cases and Controls with Designation of Haplotypes

		Nucleotide position and restriction enzyme							Haplotype frequencies	
		G191A	C282T	T341C	C481T	G590A	A803G	G857A	Cases	Controls
Allele	Phenotype	*MspI*	*Fok*	*Aci*	*Kpn*	*TaqI*	*Dde*	*Bam*	N (%)	N (%)
*4[1]	Rapid	G	C	T	C	G	A	G	42 (18.26)	124 (20.27)
*5A	Slow	.	.	C	T	.	.	.	0	5 (0.83)
*5B	Slow	.	.	C	T	.	G	.	89 (38.69)	249 (40.68)
*5C	Slow	.	.	C	.	.	G	.	6 (2.61)	16 (2.61)
*6A	Slow	.	T	.	.	A	.	.	77 (33.48)	189 (30.89)
*6B	Slow	.	.	.	.	A	.	.	1 (0.44)	0
*6C	Slow	.	T	.	.	A	G	.	0	1 (0.16)
*7B	Slow	.	T	.	.	.	.	A	11 (4.78)	21 (3.43)
*12A	Rapid	.	.	.	.	.	G	.	0	1 (0.16)
*12C	Rapid	.	.	.	T	.	G	.	0	2 (0.32)
*13	Rapid	.	T	.	.	.	.	.	3 (1.30)	3 (0.48)
*14B	Slow	A	T	.	.	.	.	.	0	1 (0.16)
*14D	Slow	A	T	.	.	A	.	.	1 (0.44)	0
Cases	MAF[2] (%)	0.43	40	41.3	39.13	49.67	41.3	4.78		
Controls	MAF[2] (%)	0.16	34.97	44.28	42.16	31.05	44.12	3.43		

[1] Wild type; [2] Minor Allele Frequency

Table 2. Distribution of NAT2 Genotypes and Phenotypes Among Bladder Cancer Cases and Controls

NAT2	Cases N=115	Controls N=306
Rapid acetylators	6 (5.2)	16 (5.2)
*4/*4	4 (3.5)	14 (4.6)
*4/*12C	0	1 (0.3)
*4/*13	2 (1.7)	1 (0.3)
Intermediate acetylators	33 (28.7)	98 (32.1)
*4/*5A	0	1 (0.3)
*4/*5B	17 (14.8)	45 (14.7)
*4/*5C	4 (3.5)	5 (1.6)
*4/*6A	7 (6.1)	37 (12.1)
*4/*6C	0	1 (0.3)
*4/*7B	3 (2.6)	5 (1.6)
*4/*14D	1 (0.9)	0
*5B/*12A	0	1 (0.3)
*5B/*13	0	1 (0.3)
*5C/*12C	0	1 (0.3)
*6A/*13	0	1 (0.3)
*7B/*13	1 (0.9)	0
Slow acetylators	76 (66.1)	192 (62.7)
*5A/*5B	0	2 (0.7)
*5A/*5C	0	1 (0.3)
*5A/*6A	0	1 (0.3)
*5B/*5B	14 (12.2)	58 (19.0)
*5B/*5C	2 (1.7)	4 (1.3)
*5B/*6A	37 (32.2)	73 (23.9)
*5B/*6B	1 (0.9)	0
*5B/*7B	4 (3.5)	8 (2.6)
*5C/*5C	0	1 (0.3)
*5C/*6A	0	1 (0.3)
*5C/*7B	0	1 (0.3)
*6A/*6A	15 (13.0)	35 (11.4)
*6A/*7B	3 (2.6)	5 (1.6)
*6A/*14B	0	1 (0.3)
*7B/*7B	0	1 (0.3)

within males and current smokers with ORs (95% CI) of 1.420 (0.837-2.410) and 1.688 (0.859-3.316) respectively; however, these did not reach statistical significance even after adjustment (Figure 1 and Supplementary Table 1). When comparing slow, intermediate and fast NAT2 acetylators, the odds ratio remained non-significant (Supplementary Table 2). Analysis within the BC cases showed no statistically significant differences in NAT2 acetylation phenotype and the tumor invasion status even after adjustment for age, sex, current smoking and alcohol intake (data not shown).

As for the subgroup for which occupational history is available, there was a statistically significantly higher percentage of history of occupational exposure to aromatic amines among those with BC when compared to controls: 42.5% vs. 27.4% (P=0.029) (Table 3). The characteristics of the subgroup were relatively similar to the larger group except that cases and controls were similar concerning alcohol intake (Supplementary Table 3). Interestingly, there were no statistically significant associations between NAT2 acetylator status and BC risk except in females, though the sample size was very small (N=14 cases and 35 controls) and the CI quite wide. For instance, the ORs (95%CI) of having BC among NAT2 slow acetylators were 3.927(1.064-14.490) and 5.180 (2.269-22.947) without and with adjustment respectively (Supplementary Table 4). No statistically significant results appeared when stratifying for occupational exposure to aromatic amines (Figure 1 and Supplementary Table 4). Similar results were shown when comparing slow, intermediate and fast NAT2 acetylators (Supplementary Table 5), and when excluding alcohol intake from the adjustment (data not shown).

Discussion

This is the first study to evaluate the distribution of NAT2 haplotypes and their potential role in BC in the Lebanese population. The MAFs and frequencies of the corresponding NAT2 haplotypes, genotypes and phenotypes were similar to Europeans (Walker et al., 2009; Cascorbi et al., 1995; Sabbagh et al., 2008) and Arabs (Bu et al., 2004; Hamdy et al., 2003; Tanira et al., 2003; Woolhouse et al., 1997) with the NAT2*5B and *6A haplotypes that correlate with slow NAT2 enzyme activity being the most common. As expected, results revealed that males, current smokers, alcohol drinkers and those who have occupational history of exposure to aromatic amines were at a higher risk for having BC (Covolo et al., 2008; Cui et al., 2013; Hosen et al., 2015; Klimcakova et al., 2011; Kobeissi, Yassine, Jabbour, Moussa, and Dhaini, 2013; Lubin et al., 2007; Lu, Chung, Huang, and Ko, 2005; Marcus et al., 2000; Ouerhani et al., 2009; Tao et al., 2010). Nevertheless, the risk was the same irrespective of the NAT2 acetylator status. Of note is that the potential association between slow NAT2 acetylator status and BC in females only may be due to mere chance and needs further evaluation, as this was shown in the sensitivity analysis only with a much smaller sample size and a very wide CI. In addition, knowing that smoking habits and occupational risks differ by gender, it would have been preferable to match cases and controls for gender especially that application of logistic regression cannot fully correct this bias.

The lack of association between NAT2 genetic polymorphisms and BC risk may be mostly attributed to the relatively small sample size knowing that, from 2 recent meta-analyses, the NAT2 slow acetylation status only modestly increases the risk of BC [OR(95%CI): 1.31 (1.11-1.55)] (An et al., 2015; Zhu et al., 2015). It is interesting however that although few of the "negative" association studies were based on small numbers of cases and controls (Wu et al., 2016; Zupa et al., 2009), several did not show any associations despite evaluating a very large population. This is shown by the study of

Table 3. Comparison between Bladder Cancer Cases and Control

		Mean ± SD	Cases (N=115)	Controls (N=306)	P[1]	OR (95%CI)
Age (years)			64.89 ± 10.57	66.92 ± 15.14	0.186	0.990 (0.975-1.005)
Sex	Female	N (%)	14 (12.2)	138 (45.1)		1
	Male	N (%)	101 (87.8)	168 (54.9)	<0.001	5.926 (3.244-10.826)
Current smoking	No	N (%)	45 (39.1)	225 (73.5)		1
	Yes	N (%)	70 (60.9)	81 (26.5)	<0.001	4.32 (2.748-6.794)
Current alcohol intake	No	N (%)	62 (53.9)	218 (71.2)		1
	Yes	N (%)	53 (46.1)	88 (28.8)	0.001	2.118 (1.361-3.296)
Occupational exposure to aromatic amines[2]	No	N (%)	65 (57.5)	61 (72.6)		1
	Yes	N (%)	48 (42.5)	23 (27.4)	0.029	1.959 (1.067-3.596)
NAT2 phenotype 1	Fast	N (%)	6 (5.2)	16 (5.2)		1
	Intermediate	N (%)	33 (28.7)	98 (32.0)		0.898 (0.325-2.485)
	Slow	N (%)	76 (66.1)	192 (62.7)	0.786	1.056 (0.398-2.799)
NAT2 phenotype 2	Fast & Intermediate	N (%)	39 (33.9)	114 (37.3)		1
	Slow	N (%)	76 (66.1)	192 (62.7)	0.302	1.157 (0.738-1.815)

[1] Student-t test or chi-square as applicable; [2] Data were available for a subgroup of 113 cases and 84 controls. The Yes category included those who had any of the following occupations: hairdressers, paint industry, printing ink industry, rubber and cable manufacture, textile and leather works, truck or bus drivers, roofers, chimney sweeps, truck drivers, tar and asphalt workers, brickyard workers, aluminum industry, gas industries and blacksmiths.

Garcia-Glosas et al., (2005) where the odd (95%CI) of BC was 1.37 (0.94-1.99) in a population of 783 cases and 703 controls. It appears that the gene-environment interaction between NAT2 and smoking plays a major role in the increased risk, especially the cumulative exposure to cigarette smoke over time. For instance, Moore et al., (2011) reported that NAT2 slow acetylation is not associated with BC risk (OR; 95%CI) among never (1.04; 0.71-1.51), former (0.95; 0.75-1.20), or current smokers (1.33, 0.91-1.95). However a relationship emerged when smoking intensity was evaluated. Among slow acetylators who ever smoked at least 40 cigarettes/day, risk was elevated among ever (1.82; 1.14-2.91, P=0.07) and current heavy smokers (3.16; 1.22-8.19, P=0.03) compared to rapid acetylators in each category. Similar positive associations were found with various categorizations of smoking intensity such as among those who smoke at least 20 pack-years (Ouerhani et al., 2009), 25 pack-years (Tsukino et al., 2004), 28 pack-years (Gu et al., 2005), and 37.4 pack-years (Cui et al., 2013). Interestingly, environmental tobacco smoke coming from smoking by household members, co-workers and parents during childhood was found to be associated with an increased risk of BC among slow NAT2 acetylators (Tao et al., 2010). In our study, we were unfortunately not able to evaluate the extent of active smoking in pack-years, and did not collect data on passive smoking. In addition, although we specifically inquired about narghile (waterpipe) smoking and categorized it as smoking, it is possible that some participants considered it as a mild and non-harmful habit and hence did not report it (Nakkash and Khalil, 2010; Nakkash, 2011). To our knowledge, no study specifically evaluated the role of NAT2 polymorphisms in narghile smoking, an area where we believe further research is warranted. Another confounding factor is the potentially protective role of NAT2 slow acetylators with the Mediterranean diet that is especially rich in cruciferous vegetables (Lin et al., 2009). Other emerging factors of potential research interest are the exposure to increasing levels of environmental air pollutants from vehicular and generator emission (Farah et al., 2014). Finally, it may be relevant to genotype for additional genes of interest and evaluate not only gene-environment interactions, but also gene-gene interactions such as that between NAT2 and GST (Brockmöller et al., 1996; Rouissi et al., 2009; Yuan et al., 2008).

In conclusion, this is the first study to evaluate the distribution of NAT2 haplotypes and their potential role in BC in a Lebanese population. The absence of association between NAT2 acetylation status and BC risk may be due to the relatively small sample size and the unavailability of matching of cases and controls by gender, as well as the lack of evaluation of genetic interactions with extent of active and passive smoking, environmental pollutants, diet, and other genes. Further studies are needed to elucidate the factors behind the increasing prevalence of BC in Lebanon.

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Supplementary Table 1. Association between NAT2 Phenotype (Slow vs. Fast and Intermediate) and Bladder Cancer Risk

			Unadjusted		Adjusted[1]	
			OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
	Cases	Controls	Fast and Intermediate	Slow	Fast and Intermediate	Slow
Total sample	115	306	1	1.157 (0.738-1.815)	1	1.097 (0.666-1.806)
Stratified by sex						
Female	14	138	1	0.326 (0.103-1.025)	1	0.356 (0.106-1.198)
Male	101	168	1	1.420 (0.837-2.410)	1	1.416 (0.810-2.476)
Stratified by current smoking						
No	45	225	1	0.828 (0.429-1.595)	1	0.821 (0.414-1.626)
Yes	70	81	1	1.688 (0.859-3.316)	1	1.491 (0.728-3.053)

[1]Sex and/or smoking and alcohol intake as applicable

Supplementary Table 2. Association between NAT2 Phenotype (Slow vs. Fast vs. Intermediate) and Bladder Cancer Risk

			Unadjusted			Adjusted[1]		
			OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
	Cases	Controls	Fast	Intermediate	Slow	Fast	Intermediate	Slow
Total sample	115	306	1	0.898 (0.325-2.485)	1.056 (0.398-2.799)	1	0.894 (0.297-2.687)	0.997 (0.348-2.858)
Stratified by sex								
Female	14	138	1	0.870 (0.089-8.455)	0.287 (0.028-2.949)	1	0.887 (0.084-9.358)	0.320 (0.028-3.615)
Male	101	168	1	1.058 (0.332-3.373)	1.488 (0.496-4.465)	1	0.831 (0.245-2.818)	1.214 (0.382-3.855)
Stratified by current smoking								
No	45	225	1	0.414 (0.123-1.393)	0.410 (0.132-1.273)	1	0.525 (0.148-1.867)	0.495 (0.151-1.622)
Yes	70	81	1	3.448 (0.374-31.792)	5.213 (0.587-46.299)	1	3.917 (0.399-38.404)	5.140 (0.547-48.289)

[1]Sex and/or smoking and alcohol intake as applicable

Supplementary Table 3. Comparison between Bladder Cancer Cases and Controls in the Subgroup with Available Occupational History of Exposure to Aromatic Amines

			Cases (N=113)	Controls (N=84)	P[1]
Age (years)		Mean ± SD	64.63 ± 10.48	66.89 ± 19.12	0.289
Sex	Male	N (%)	99 (87.6)	49 (58.3)	
	Female	N (%)	14 (12.4)	35 (41.7)	<0.001
Current smoking	Yes	N (%)	70 (61.9)	33 (39.3)	
	No	N (%)	43 (38.1)	51 (60.7)	0.002
Current alcohol intake	Yes	N (%)	53 (46.9	30 (35.7)	
	No	N (%)	60 (53.1)	54 (64.3)	0.145
Occupational exposure to aromatic amines[2]	Yes	N (%)	48 (42.5)	23 (27.4)	
	No	N (%)	65 (57.5)	61 (72.6)	0.029
NAT2 phenotype 1	Fast	N (%)	6 (5.3)	4 (4.8)	
	Intermediate	N (%)	33 (29.2)	23 (27.4)	
	Slow	N (%)	74 (65.5)	57 (67.9)	0.939
NAT2 phenotype 2	Fast & Intermediate	N (%)	39 (34.5)	27 (32.1)	
	Slow	N (%)	74 (65.5)	57 (67.9)	0.727

[1] Student-t test or chi-square as applicable; [2] The Yes category included those who had any of the following occupations: hairdressers, paint industry, printing ink industry, rubber and cable manufacture, textile and leather works, truck or bus drivers, roofers, chimney sweeps, truck drivers, tar and asphalt workers, brickyard workers, aluminum industry, gas industries and blacksmiths.

Supplementary Table 4. Association between NAT2 Phenotype (Slow vs. Fast and Intermediate) and Bladder Cancer Risk in the Subgroup with Available Occupational History of Exposure to Aromatic Amines

			Unadjusted		Adjusted[1]	
			OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
	Cases	Controls	Fast and Intermediate	Slow	Fast and Intermediate	Slow
Total sample	113	84	1	1.113 (0.611-2.027)	1	1.381 (0.713-2.676)
Stratified by sex						
Female	14	35	1	3.927 (1.064-14.490)	1	5.180 (1.169-22.947)
Male	99	49	1	0.897 (0.430-1.870)	1	0.961 (0.453-2.040)
Stratified by current smoking						
No	43	51	1	1.575 (0.675-3.673)	1	1.881 (0.740-4.784)
Yes	70	33	1	0.857 (0.353-2.079)	1	0.982 (0.385-2.507)
Stratified by occupational exposure						
No	65	61	1	1.115 (0.537-2.313)	1	1.305 (0.585-2.913)
Yes	48	23	1	1.288 (0.423-3.920)	1	1.447 (0.436-4.799)

[1]Sex and/or smoking and/or occupational exposure and alcohol intake as applicable

Supplementary Table 5. Association between NAT2 Phenotype (Slow vs. Fast vs. Intermediate) and Bladder Cancer Risk in the Subgroup with Available Occupational History of Exposure to Aromatic Amines

			Unadjusted			Adjusted[1]		
			OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
	Cases	Controls	Fast	Intermediate	Slow	Fast	Intermediate	Slow
Total sample	113	84	1	1.155 (0.311-4.288)	1.105 (0.586-2.085)	1	1.573 (0.367-6.728)	1.350 (0.671-2.714)
Stratified by sex								
Female	14	35	1	2.400 (0.181-31.883)	4.267 (1.101-16.537)	1	2.729 (0.183-40.793)	6.195 (1.221-31.423)
Male	99	49	1	1.196 (0.220-6.489)	0.854 (0.394-1.853)	1	1.373 (0.238-7.917)	0.901 (0.405-2.005)
Stratified by current smoking								
No	43	51	1	3.500 (0.628-19.512)	1.300 (0.522-3.239)	1	4.338 (0.644-29.210)	1.540 (0.562-4.219)
Yes	70	33	1	0.224 (0.019-2.608)	0.998 (0.392-2.538)	1	0.198 (0.014-2.872)	1.155 (0.431-3.098)
Stratified by occupational exposure								
No	65	61	1	1.463 (0.232-9.228)	1.078 (0.505-2.302)	1	3.209 (0.425-24.225)	1.170 (0.509-2.692)
Yes	48	23	1	0.773 (0.118-5.076)	1.545 (0.432-5.525)	1	0.648 (0.082-5.152)	1.952 (0.470-8.015)

[1]Sex and/or smoking and/or occupational exposure and alcohol intake as applicable