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Original Article

CYP2E1 and *NQO1* genotypes and bladder cancer risk in a Lebanese population

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Abstract: Urinary bladder cancer incidence in Lebanon ranks among the highest in the world. Cytochrome P450 2E1 (CYP2E1), NAD(P)H quinone oxidoreductase1 (NQO1), and N-Acetyltransferase1 (NAT1), are drug-metabolizing enzymes (DMEs) involved in the metabolism of carcinogens, such as arylamines and heterocyclic amines, implicated in bladder cancer. The present study attempts to investigate the role of these DMEs genetic polymorphism in bladder cancer risk among Lebanese men. 54 cases and 106 controls were recruited from two hospitals in Beirut. An interview-based questionnaire was administered to assess suspected environmental and occupational risk factors. PCR-RFLP was performed on blood-based DNA samples to determine DMEs genotypes. Associations between bladder cancer and putative risk factors were measured using adjusted odds ratios (ORs) and their 95% confidence intervals (CIs). Results showed *CYP2E1* c1/c1, *NAT1*14A*, and smoking, to be risk factors for bladder cancer. No significant differences in frequency distribution of the *NQO1* genotypes were found in cases versus controls. The odds of carrying the *CYP2E1* c1/c1 genotype were 4 times higher in cases compared to controls (OR=3.97, 95% CI: 0.48-32.7). The odds of carrying at least one *NAT1*14A* allele were 14 times higher in cases versus controls (OR= 14.4, 95% CI: 1.016-204.9). Our study suggests *CYP2E1* c1/c1*, NAT1*14A,* and smoking, as potential risk factors for bladder cancer in Lebanese. Further studies with larger samples must be conducted to confirm these findings.

Keywords: Cytochrome P450 CYP2E1, NQO1, N-Acetyltransferase NAT1, bladder cancer, Lebanese

Introduction

Globally, bladder cancer is the 10th most leading cancer and accounts for 3.3% of all malignancies [1]. Highest incidence rates are reported in Europe, North America and Australia, compared to lower rates in developing countries as in Africa and Asia [2, 3]. In Lebanon, the incidence of bladder cancer has been markedly increasing in the past few years [4, 5]. According to the latest Lebanese National Cancer Registry (NCR) Report, bladder cancer incidence is one of the highest in the world, with an age-adjusted incidence rate of ASR=32 per 100,000 [6]. Histologically, most cases of bladder cancer are papillary transitional cell carcinoma [7]. Eighty five percent of cases are men with most of them above 50 years of age.

Genetic factors have been found to modulate the internal dose of carcinogens and affect the risk of developing bladder cancer [8]. Many procarcinogens may require enzymatic bioactivation to become ultimate carcinogens and cause genotoxicity [9]. Therefore, genes coding for Phase I and Phase II drug-metabolizing enzymes (DMEs) may impact toxic outcome, hence, may be important cancer risk factors.

Human *CYP2E1* is constitutively expressed in the liver and to lesser extent in other organs and tissues, including human urothelial cells [10]. It is a key enzyme in the metabolic activation of many low-molecular-weight carcinogens, such as vinyl chloride, benzene, and tobacco-specific nitrosamines [11, 12]. Two *CYP2E1* polymorphisms, *CYP2E1*6* (rs6413432) and *CYP2E1*5B* (rs2031920), have been frequently studied. *CYP2E1*6* is a DraI polymorphism caused by a T to A at 7632 in intron 6. *CYP2E1*6* does not affect gene transcription but may have an effect on the catalytic activity of the enzyme

[13]. *CYP2E1*5B* is a PstI polymorphism caused by a G to C at 1293 in the non-coding region. G and C are named c1 and c2 alleles, respectively. Studies showed that *CYP2E1*5B* modulates transcription of the gene in vitro [14]. Frequency of the c2 allele and reported associations between this polymorphism and risk for many types of cancer, including bladder, have been inconsistent and varied among ethnic groups [15-19].

NQO1, on the other hand, is a ubiquitous flavoprotein that functions as an antioxidant enzyme and a xenobiotic detoxifier [20, 21]. *NQO1* catalyzes a two-electron reduction of quinones to hydroquinones, hence, prevents formation of free radicals [22]. It also bioactivates environmental carcinogens, such as nitro-aromatic compounds and heterocyclic amines, present in tobacco smoke as well as in processed food [23]. Moreover, overexpression of *NQO1* mRNA in tumor tissue was reported in many types of cancer, including bladder [24-26]. So far, more than 20 single nucleotide polymorphisms (SNPs) in the *NQO1* gene have been reported [27]. *NQO1*1*, designated as the wild-type C allele, encodes for an enzyme with normal activity. *NQO1*2* encodes a nonsynonymous mutation (C609T, P187S), designated as the T allele, that has negligible activity in heterozygotes [28], and total absence of enzymatic activity in homozygous mutant T/T carriers [29]. Consequently, upon exposure to carcinogens, this polymorphism may affect metabolism and influence susceptibility to cancer. Results from previous studies investigating *NQO1* genotype and bladder cancer risk were controversial [30-32].

Accordingly and given the above, the current hospital-based case-control study sought to examine the potential association of *CYP2E1* and *NQO1* genotypes and bladder cancer risk among Lebanese men residing in Lebanon.

Materials and methods

Study design and population

This study employed a case-control design. Two major medical centers in the Capital Beirut participated: St. Georges Hospital University Medical Center and Bahman Hospital. 54 cases and 106 hospital controls were recruited. The sample size was guided by power analysis that revolved around: 80% power; Type 1 error of 5%; estimated odds ratio (OR) of 3; and a proportion of exposure of *NAT1*14A* among cases of 21.4%, based on data observed in earlier studies [33-35]. A ratio of 1:2 of cases to controls was used.

Cases were selected, based on a histological confirmation of bladder cancer, from a pool of 284 identified bladder cancer patients, diagnosed between 2002 and 2008, according to the medical records of both hospitals. They were randomly selected based on the most recent year of diagnosis. Exclusion criteria consisted of the following: females, non-Lebanese, patients less than 50 years of age, first-degree relatives, deceased patients before the beginning of the study, patients with missing contact information, as well as refusals.

Controls were conveniently selected from the same settings. They were either visitors at one of the two hospitals or coming for routine clinical check-up. These were also Lebanese males, 50 years or older, with no present or previous history of cancer, or any other systemic illness or chronic disease. In the control group, all subjects with history of bladder cancer or other types of cancer or urinary infections, or chronic diseases, were excluded.

Data collection and statistical analysis

IRB was obtained prior to conducting the study. All subjects signed an informed consent before the interview. All cases and controls were administered a standardized questionnaire during a face-to-face interview and provided a blood sample. Data collection was conducted by trained graduate students, in native language (Arabic). Information sought included: age, family history, alcohol consumption, dietary habits, smoking habits, occupational info, use of hair dyes, chronic diseases and urinary infections.

Following collection, the data was managed and analyzed using the Statistical Package for Social Sciences (version 16; SPSS, Chicago, IL). Analysis revolved around univariate, bivariate, and multivariate analysis. Univariate analysis employed frequency and percentage distributions for the categorical variables, as well as means, standard deviations, and ranges for the

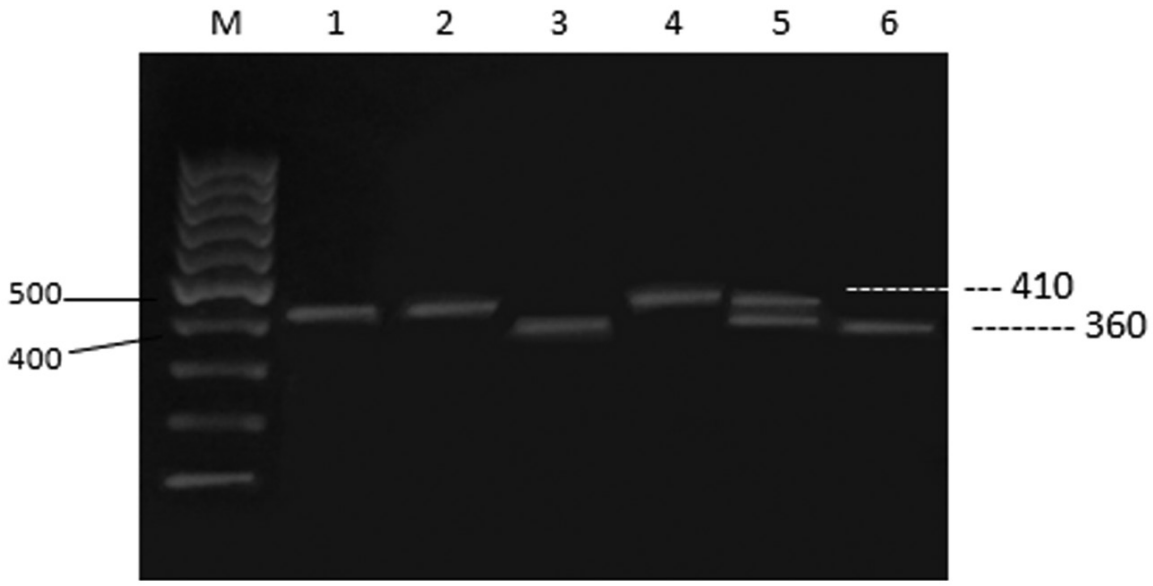

Figure 1. Genotype pattern for *CYP2E1* analyzed by RFLP. Lane M shows a 100 bp DNA ladder representing fragment size in base pair (bp) units; lane 1 represents undigested PCR product (410 bp); lane 2 and 4 show variant *CYP2E1* c2/c2 genotypes (410 bps); lanes 3 and 6 show the wild type *CYP2E1* c1/c1 genotypes (360 and 50 bps); lane 5 shows the heterozygous *CYP2E1* c1/c2 genotypes (410; 360; and 50 bps).

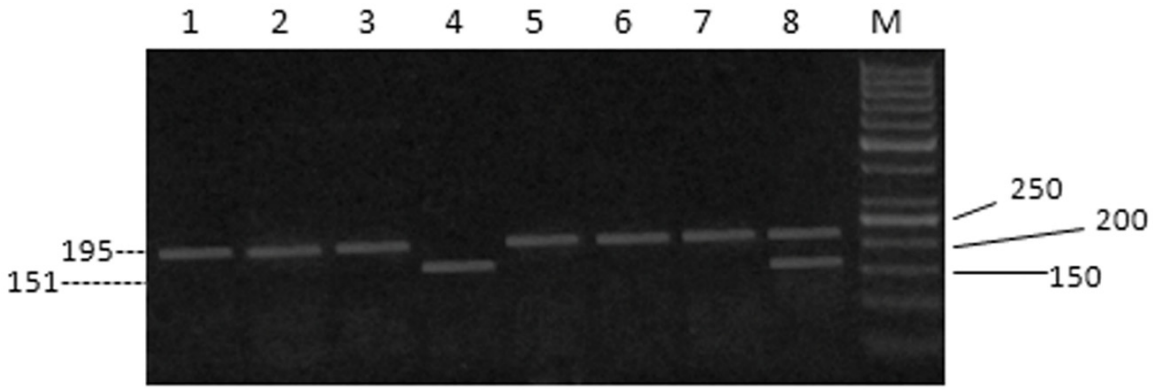

Figure 2. Genotype pattern for *NQO1* analyzed by RFLP. Lane M shows a 50 bp DNA marker representing fragment size in base pair (bp) units; lanes 1-3 and lanes 5-7 show the wild type *NQO1* C/C genotype (195, and 35 bps); lane 4 shows variant *NQO1* T/T genotype (151, 44, and 35 bps); lane 8 shows the heterozygous *NQO1* C/T genotype (195, 151, and 35 bps).

continuous variables. Bivariate analysis employed Chi-squared and Fisher's exact test to check for crude associations between the main outcome variable (urinary bladder) and various exposure and confounding variables. Multivariate analysis consisted of a backward multivariate logistic regression model, focusing on different exposure and confounding variables that showed significant results during the bivariate analysis. The final model incorporated the exposure and confounding variables that displayed the most significant odds ratios.

A detailed description of the above methods for this study is cited elsewhere [36, 37].

DNA sources and extraction

2-ml of blood was collected in EDTA-treated tubes from each participating subject, and 1ml of 100% Ficoll was added. Buffy coats appeared after centrifugation at 2500 g for 10 minutes. DNA extraction was performed using a ready-to-use extraction kit (Qiagen) according to manufacturer's instructions and stored at -20°C until genotypes were analyzed.

Table 1. Socio-Demographic Background of Cases and Controls (n=160)

Variables		Bladder Cancer	
		Cases	Control
Age	Mean (SD)	67.1 (8.1)	65.6 (11.3)
		P-value=0.39	
Years of Education	Mean (SD)	8.26±6.76	7.88±5.34
		P-value=0.69	
Salary (US $)	Mean (SD)	1721 (2435)	1885 (2230)
		P-value=0.003	
		N (%)	N (%)
Salary (US $)			
Less than 500 $		11 (27.5)	36 (52.9)
Between 500 $ and 1000 $		16 (40)	27 (39.7)
Between 1000 $ and 5000 $		12 (30)	5 (7.4)
More than 5000 $		1 (2.5)	0 (0)
		P-value=0.003	
Current Residence			
Beirut		18 (33.3)	79 (79)
Mount Lebanon		25 (46.3)	15 (15)
North		2 (3.7)	4 (4)
South		4 (7.4)	2 (2)
Bekaa		5 (9.3)	0 (0)
		P-Value=0.00	
Histological Type and Grade		Bladder Cancer Cases N (%)	
Papillary Transitional Cell Carcinoma		45 (84.9)	
Transitional Cell Carcinoma (NOS)		5 (9.4)	
Adenocarcinoma (NOS)		1 (1.9)	
Unspecified Bladder Cancer		2 (3.8)	
Low Grade		26 (51)	
High Grade		25 (49)	

PCR-RFLP for CYP2E1 and NQO1

In *CYP2E1* genotyping, PCR primers 5'-CCA GTC GAG TCT ACA TTG TCA-3' and 5'-TTC ATT CTG TCT TCT AAC TGG-3' were used as following: 20 µl reaction mixtures contained 2 µl Taq buffer, 2 µl dNTPs, 1 µM primers, 100-150 ng DNA template, 1.5 units of Taq polymerase and nuclease-free water as described [17]. After an initial denaturation at 94°C for 4 min, 30 cycles of 60 seconds at 94°C, 60 seconds at 58°C, and 60 s at 72°C were performed, followed by a final extension step of 7 min at 72°C. A PCR product of 410 bp was generated. Agarose gel electrophoresis followed PCR amplification to validate the correct product size.

In *NQO1* genotyping, PCR primers 5'-TCC TCA GAG TGG CAT TCT GC-3' and 5'-TCT CCT CAT CCT GTA CCT CT-3' were used [17]. After an initial denaturation at 94°C for 4 min, 36 cycles of 10 seconds at 94°C, 30 seconds at 62°C, and 60 seconds at 72°C were performed, followed by a final extension step of 5 min at 72°C. A PCR product of 230 bp was generated. Agarose gel electrophoresis followed PCR amplification to validate the correct product size.

After PCR, an aliquot of 10 µl of *CYP2E1* PCR product was digested with RsaI (PstI) overnight at 37°C, to detect *CYP2E1*5B* c1 and c2 polymorphism. For *NQO1*, an aliquot of 10 µl of the PCR product was digested with *Hinf*I overnight at 37°C, to detect *NQO1* C and T polymorphism. Agarose gel electrophoresis followed digestion, and DNA fragments examination was performed under UV light (**Figures 1** and **2**).

Results

Our results indicate no significant differences between cases and controls in terms of age and education. The average age in cases was 67.1 (±8.1) years compared to 65.6 (±11.3) years in controls. Most of the subjects in this study completed at least eight years of education. On average, the monthly income was reported around US $ 1800. Both cases and controls resided mainly in Beirut (33.3% versus 79%), and Mount Lebanon (46.5% versus 15%). Most of the bladder cancer cases were diagnosed with papillary transitional cell carcinoma (84.9%). Fifty-one percent had a low cancer grade versus 49% with a high grade (**Table 1**).

At the level of genotypic and allelic frequency distribution, cases were more likely than controls to show clustering of the following: *CYP2E1* c1/c1 (80% vs. 54.1 %, significant), *NQO1* T/T (14.6% vs. 11.1%), *NAT1*14A* (53.7% vs. 11.3%, significant), *NAT1*14B* (13% vs. 4.7%), and *NAT1*15* (48.1% vs. 40.6%). Moreover, cases were less likely than controls to show clustering of the following genotypes and alleles: *NQO1* C/C (66.7% vs. 67.8%), *NQO1* C/T (18.8% vs. 21.1%), *NAT1*10* (20.4% vs. 50.9%,

Table 2. Bivariate Analysis of Genetic Predisposition among Cases and Controls (n=160)

		Cases	Controls
		N (%)	N (%)
Allele Clustering			
NAT1*14A		29 (53.7)	12 (11.3)
		P-value=0.00	
NAT1*14B		7 (13)	5 (4.7)
		P-value=0.06	
NAT1*10		11 (20.4)	54 (50.9)
		P-value=0.00	
NAT1*15		26 (48.1)	43 (40.6)
		P-value=0.36	
NAT1*3		7 (13)	14 (13.2)
		P-value=0.96	
NAT1*4		14 (25.9)	49 (46.2)
		P-value=0.013	
CYP2E1 (Genotype)			
c1/c1		36 (80.0)	46 (54.1)
c1/c2		2 (4.4)	12 (14.1)
c2/c2		7 (15.6)	27 (31.8)
		P-value=0.013	
NQO1 (Genotype)			
C/C		32 (66.7)	61 (67.8)
C/T		9 (18.8)	19 (21.1)
T/T		7 (14.6)	10 (11.1)
		P-value=0.82	
Familial History of Cancer			
Yes		18 (33.3)	8 (7.6)
No		36 (66.7)	97 (92.4)
		P-Value=0.00	
Family members			
Father	(Yes)	6 (11.1)	2 (1.9)
		P-Value=0.012	
Mother	(Yes)	3 (5.6)	3 (2.9)
		P-Value=0.40	
Sister	(Yes)	4 (7.4)	1 (1)
		P-Value=0.027	
Brother	(Yes)	8 (14.8)	1 (1)
		P-Value=0.00	
Grandfather-father side	(Yes)	0	0
Grandfather-mother side	(Yes)	0	0
Grandmother-father side	(Yes)	0	0
Grandmother-mother side	(Yes)	1 (1.9)	0
Other relatives	(Yes)	0	1 (1)
		P-Value=0.48	

significant), *NAT1*3* (13% vs. 13.2%), and *NAT1*4* (25.9% vs. 46.2%, significant), *NAT1*5* and *NAT1*16* were not detected in any of the cases or controls. Besides, cases were significantly more likely to report a familiar history of cancer than controls (33.3% versus 7.6%) (**Table 2**).

In order to explore potential independent effects with bladder cancer, two multivariate models were constructed. **Table 3** illustrates the crude results of the multivariate analysis. **Table 4** shows results of the best-fit model, constructed at *p*-values of 0.1 or lower. Smoking, occupational exposure to combustion fumes, and *NAT1*14A* allele were found to be significantly independent risk factors for bladder cancer. Prostate related symptoms and *CY2E1 c1/c1* genotype were found to be important risk factors, but not statistically significant. When comparing odds ratios, the odds of smoking were found to be 1.03 times higher in cases compared to controls. The odds of *NAT1*14A* allele clustering were 14.4 times higher in cases compared to controls. The odds of occupational exposure to combustion fumes were 7.7 times higher in cases compared to controls. The odds of *CYP2E1* c1/c1 genotype clustering were 3.97 times higher in cases compared to controls, while, the odds of prostate-related symptoms were 6.05 times higher in cases compared to controls (**Table 4**).

When testing for gene-environment interaction (*NAT1* and *CYP2E1* with smoking), the interplay of the presence and absence of both *NAT1*14A* and *CYP2E1* c1/c1 with smoking did not yield a different OR for smoking (**Table 5**).

Discussion

An adequate comprehension of the underlying risk factors for bladder cancer in the context of Lebanon is becoming an urgent priority [4, 7, 38-40]. In this study we focused on the effects of *DMEs* genetic polymorphism on bladder cancer risk. We also investigated possible gene-environment interactions. In summary, we found *CYP2E1* c1/c1 genotype to be significantly clustered among cases compared to controls, while, no major genotypic frequency distribution differences were observed for *NQO1*. In the multivariate analysis, we observed a 4-fold and a 14-fold increased bladder cancer risk for carriers of the *CYP2E1* c1/c1 genotype and the *NAT1*14A* allele, respectively. We also found that smokers, subjects with pros-

Table 3. Multivariate Logistic Regression Analysis (Crude Model)

Variable	Testing for Independent Effect
	Adjusted OR (CI), *P*-value
Income (Reference<500 $/month)	
500-1000	2.86 (0.28-28.4), 0.56
>1000	3.58 (0.19-88.13), 0.396
Smoking	1.034* (1.007-1.06), 0.01
Occupational Exposure to Diesel (Reference No)	7.0 (0.74-65.8), 0.089
Prostate (Reference: None)	5.96* (0.48-74.4), 0.16
Family History of Cancer (Reference: None)	2.64 (0.05-136.6), 0.63
Allele (Reference: None)	
*NAT1*14A*	17.8 (0.87-362), 0.061
*NAT1*10*	2.01 (0.1-41.3), 0.63
CYP2E1 C1/C1 (Reference: C1/C2 or C2/C2)	3.27 (0.24-45.4), 0.37
NQO1 C/C (Reference: C/T or T/T)	0.72 (0.08-6.6), 0.77
Nagelkerke R2	0.69

*Significant at or less than 0.05.

tate-related symptoms, and subjects with cancer family history, have statistically significant higher odds of bladder cancer. Findings on *NAT1*, environmental factors, and occupational factors, are discussed elsewhere [36, 37].

Although, our findings are supported by some studies, the literature on *CYP2E1* remains controversial. A study conducted in 2003 among Korean men reported a statistically significant higher frequency of *CYP2E1* c1/c1 genotype among bladder cancer patients compared to controls (57.9% vs. 47.9%; *p*-value<0.01) [17]. However, Anwar *et al.* failed to find any association between *CYP2E1* alleles and bladder cancer risk in Egyptians [41]. Similarly, studies conducted among Indians and German Caucasians showed no association between *CYP2E1* polymorphism and bladder cancer risk [42-44].

Although, our results suggest c1/c1 as a risk factor, the low power of the interaction analysis precludes us from making definitive conclusions. Phenotypes may be dependent upon various endogenous and environmental factors. These inconsistent results may be partially explained by inter-ethnic differences in *CYP2E1* allelic and genotypic frequencies. In Lebanon, only one study explored the allelic frequency distribution of *CYP2E1* among a group of cancer-free subjects. Allelic frequencies of *CYP2E-1*5B* and *CYP2E1*6* were found to be 0.7% and 6.3%, respectively [45]. These findings are remarkably different from those assessed in other studies of different ethnicities [46, 47]. For example, *CYP2E1*5B* frequencies were lower than those observed in Caucasians, and in Iranians [48, 49]. Interestingly, the frequency of *CYP2E1* c2 allele in the total sample in our present study (25.6%) is similar to that reported by Choi *et al.* and other Asian populations (24-30%) [17, 50], but much higher than that reported among Caucasians (2-3%) [49, 51]. On the other hand, the frequency of *NQO1*-T allele in our study (19.3%) is similar to that observed in Caucasians and African-Americans (19-22%) [52-56], however, less than that reported for Asians (38-42%) [17, 56].

The hypothesis of *CYP2E1*, and other DMEs, contributing to bladder cancer risk is not particularly novel. However, our study reenforces this hypothesis by further investigating this association among a population characterized by a remarkably high bladder cancer incidence as well as a distinct toxicogenetic profile [34, 45, 57]. The potential molecular basis for the association between *CYP2E1* c1/c1 genotype and bladder cancer risk may be related to the level of expression of the gene. *CYP2E1* c2/c2 genotype shows a 10 times higher transcriptional activity compared to *CYP2E1* c1/c1 genotype using a CAT assay [14]. Therefore, upon exposure, detoxification of bladder carcinogens in c1/c1 subjects may compete poorly with different DME bioactivation pathways, leading to increased cancer susceptibility. This hypothesis requires further investigation prior to drawing conclusions. Our observed results could also be related, in part, to differences in chemical exposure, and other genetic factors related to metabolism.

When testing for gene-environment interactions, the interplay of the presence and absence of the *NAT1*14A* allele and the *CYP2E1* c1/c1 genotype with smoking did not yield any significant changes in odds ratios. This has led us to

Table 4. Multivariate Logistic Regression Analysis (Best Fit)

Variable	Testing for Independent Effect
	Adjusted OR (CI), *P*-value
Smoking	1.031* (1.006-1.057), 0.015
Occupational Exposure to Diesel fumes (Reference No)	7.7 (0.93-63.9), 0.059
Prostate-related symptoms (Reference: None)	6.05 (0.74-49.6), 0.09
Allele (Reference: None)	
*NAT1*14A*	14.4* (1.016-204.9), 0.049
*NAT1*10*	1.56 (0.09-25.66), 0.75
CYP2E1 C1/C1 (Reference: C1/C2 or C2/C2)	3.97 (0.48-32.7), 0.19
Nagelkerke R2	0.66

*Significant at or less than 0.05. - This is the best-fit model chosen based on the crude model (Table 3): smoking, occupational exposure to diesel fumes, prostate-related symptoms, NAT1*14A, NAT1*10, and CYP2E1 C1/C1.

conclude that it is unlikely that the impact of smoking is exacerbated in carriers of both *NAT1*14A* and *CYP2E1* c1/c1.

NQO1, on the other hand, is also involved in metabolism of carcinogens. However, studies on *NQO1* and cancer risk report contradictory results. While, we did not find any association between *NQO1* genetic variants and bladder cancer risk, Choi *et al.* reported *NQO1* C/C genotypes to be significantly more prevalent in bladder cancer patients compared to controls [17]. In contrast, a multi-ethnic study conducted at the University of Texas in 2003 suggested that *NQO1*-T allele is a risk factor for bladder cancer, especially in men and in ever-smokers [31]. This is further supported by a recent study in a group of Kashmiri Indians [58]. Kashmiri carriers of the *NQO1*-T allele had a higher bladder cancer risk compared to controls. Moreover, a recent meta-analysis, based on 11 case-control studies, also reports a higher bladder cancer risk for *NQO1*-T allele carriers [59]. These contradictory results may also be explained by inter-ethnic differences. In parallel, our findings on *NQO1* may be potentially due to the relatively small sample size, which is one of the limitations in this study.

The statistical power to analyze associations between genotypes and bladder cancer was limited by the modest sample size and the hospital-based nature of recruitment of controls. Although our sample size was guided by power analysis, 65% of targeted patients could not be recruited due to the high proportion of refusals, deceased, and subjects with absent contact information. Ideally, a larger sample size of newly diagnosed cases in the same population would strengthen the assertions of the conclusions drawn in this study. Another limitation of the study is the possibility of other genetic variants and DMEs genetic polymorphism modifying cancer risk, such as N-acetyltransferase2 (*NAT2*) and Glutathione-S-transferases *(GSTs)*. Both enzymes were reported to contribute to bladder cancer in other ethnic groups [60-62]. Hence, further studies should be conducted in the future to investigate both DMEs and their association to bladder cancer risk in this population.

On the other hand, several strengths may be highlighted in this case-control study, which are likely to strengthen the internal validity of the observed findings. The high face validity of the exposure measurements may be attributed to collected data that combines environmental exposures and genetic factors. In addition, different multivariate models were considered before adopting the best-fit model presented herein. All the different models assessed have actually yielded similar patterns and magnitude of association.

In conclusion, our results suggest that *CYP2E1 c1/c1* and *NAT1*14A* may play an important role in influencing bladder cancer risk among Lebanese men. In addition, smoking is confirmed as an independent risk factor for bladder cancer in Lebanon. Although, our findings are based on a small studied group, and the sample cannot be considered representative of the general population, yet, they represent the first evidence in relation to the observed incidence of bladder cancer in the country. The observed effects may be moderate, however, the suggested association between incidence and genetic variation may account for a substantial proportion of cases in the Lebanese context. Our results call for further investigation, preferably using larger prospective cohorts in order to increase internal validity of the sug-

Table 5. Logistic Regression Analysis: Testing for Gene-Environment Interaction (*NAT1/CYP2E1* - Smoking)

Variable		Testing for Gene-Environment Interaction			
Testing for Independent Effect		*NAT1*14A* (Yes) *CYP2E1* c1/c1	*NAT1*14A* (No) *CYP2E1* c1/c1	*NAT1*14A* (YES) *CYP2E1* c1/c2 OR c2/c2	*NAT1*14A* (NO) *CYP2E1* c1/c2 OR c2/c2
	Adjusted OR	OR	OR	OR	OR
Smoking (Years)	1.046*	0.945	1.044*	1.044	1.098

*Significant at or less than 0.05.

gested conclusions. We recommend building upon these observations by carrying out larger multi-center studies incorporating other DME genes, susceptibility genes, and chemical exposures. When confirmed, findings should be followed-up by formation of national-based guidelines for prevention and early detection of bladder cancer in the country.

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Disclosure of conflict of interest

None declared.

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