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ORIGINAL ARTICLE

Polymorphisms in *NQO1* and *MPO* genes and risk for bladder cancer in Tunisian population

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Abstract

Background: NAD (P) H: quinone oxidoreductase (1) *(NQO1*-**HGNC: 2874)** and myeloperoxidase (*MPO*-**HGNC: 7218**) are two enzymes involved in phase II of the xenobiotic metabolism pathway.

Methods: In this study, a case–control analysis was conducted to investigate the relationship between genetic variations in the *NQO1* **(C609T, rs1800566; IVS1-27 C >G, rs689452)** and *MPO* **(G463A, rs2333227)** genes and the risk for bladder cancer among Tunisian population.

Results: We have found that the *MPO 463GA* genotype was associated with a decreased risk of developing bladder cancer ($p = 0.049$; OR = 0.696; 95% CI 0.484–0.999). In contrast, we have found that the *NQO1 609CT* genotype could increase the risk of bladder cancer patients ($p = 0.0039$; OR = 1.454; 95% CI = 1.017–2.078). Moreover, patients with "NQO1 609 CT/IVS1-27 CG" genotype show a 2.180-fold increasing risk for developing bladder cancer in comparison to the control group with wild genotype. This OR is estimated at 5.6-fold in smokers patients with "NQO1 609 CT/IVS1-27 CG" genotype. Lastly, study findings suggest that the *NQO1 IVS-27 *CG* genotype (rs689452) is associated with a risk of progression to muscle invasive bladder cancer.

Conclusion: Our study suggests that environmental risk factors in association to *NQO1* genotypes (NQO1 609 CT/IVS1-27 CG) play an important role in the development of bladder cancer in Tunisian population.

KEYWORDS

benzene, bladder cancer, *NQO1* and *MPO* polymorphism, RFLP, smoking

1 | INTRODUCTION

Bladder cancer (BC) is the 10th most common form of cancer worldwide, with an estimated 549,000 new cases and 200,000 deaths. It occurs more often in men than in women, with respective incidence and mortality rates of 9.6 and 3.2 per 100,000 in men (Bray et al., 2018). Urothelial cell carcinomas (UCC) represent more than

90% of bladder tumors and are classified as muscle invasive (MIBC) and non-muscle invasive (NMIBC) stages (Chalasani et al., 2009).

According to the Cancer Registry of the north of Tunisia (1999–2003), BCa is the most frequent urological cancer in Tunisia in men and in 2018 it became the second malignant tumor with an age-standardized incidence rates of 17.7 in men and 2.0 per 100 000 in women, respectively. In 2018, the number of new BCa cases was 1323 (Bray et al., 2018). Bladder cancer is considered as a smoking-related cancer (Simonis et al., 2014). Indeed, it has been reported that tobacco was associated with 5.5-fold increased risk of BC (Freedman et al., 2011). This risk was attributed to many compounds of tobacco such as 4-aminobiphenyl, 3-amino-1,4-dimethyl-5*H*-pyrido [4,3-b] indole (Trp-P-1), toluene, benzo[a]pyrene, benzene (Talhout et al., 2011). Moreover, excess risk of bladder cancer has been reported among several occupational groups such as painters, rubber industry workers, hairdressers and barbers, dry cleaners, transportation workers, and printers (Letašiová et al., 2012). These groups may be exposed to aromatic amines, polycyclic aromatic hydrocarbons, diesel exhaust, and chlorinated hydrocarbons. Recently, the large study of Hadkhale et al. (2016) provides evidence of an association between occupational exposure to trichloroethylene, perchloroethylene, aliphatic and aromatic hydrocarbon solvents, benzene and toluene, and bladder cancer risk. Among these solvents, only benzene and trichloroethylene are currently classified as Group 1 carcinogens by International Agency for Research on Cancer (IARC) (International Agency for Research on Cancer and Weltgesundheitsorganisation, 2012). Benzene is an industrial chemical ubiquitous in the environment due to the emission from gasoline and combustion of hydrocarbons. It has been used as a component of inks in printing industries and later on in other chemical and drug industries. In Europe, benzene is primarily used in drugs, dyes, insecticides, and plastics. In fact, it has been reported that concentration of benzene in the breathing air of smokers can be 10–20 times higher than for nonsmokers (Johnson et al., 2007; Wallace, 1996). Two potential mechanisms by which benzene metabolites may damage cellular macromolecules to induce toxicity include the covalent binding of reactive metabolites of benzene and the capacity of benzene metabolites to induce oxidative damage (Carbonari et al., 2016). According to the benzene metabolic detoxification pattern (Carbonari et al., 2016), the NQO1 and MPO enzymes were implicated in the last phase of benzene metabolism. *NQO1* (NAD(P)H:quinone oxidoreductase (1) catalyzes the two-electron reduction and detoxification of quinones and their derivatives, avoiding the formation of free radicals (semiquinones) and ROS, hence protecting cells against the adverse effects of quinones and their derivatives (Ross et al., 2000). It is previously shown to detoxify a number of natural and synthetic compounds and, conversely, to activate certain anticancer agents (Larson et al., 1999). *MPO* (myeloperoxidase) is not directly involved in the benzene metabolic pathways. It is an endogenous oxidant enzyme that generates reactive oxygen species (ROS). In tissue, the *MPO* activity is balanced by the two- or four-electron reduction catalyzed by the *NQO1* (Guo et al., 2010).

Many studies have reported that polymorphisms of NQO1 (+ 125860. NAD(P) H DEHYDROGENASE, QUINONE 1; NQO1) and *MPO* (* 606989. MYELOPERO XIDASE; MPO) may significantly modify the individual susceptibility to benzene toxicity and also have been associated with increasing bladder cancer risk (Basma et al., 2013; Zhang et al., 2015). There have been more than 93 single nucleotide polymorphisms (SNPs) identified in the *NQO1* gene (Guha et al., 2008). The most widely studied SNP of *NQO1* is a C to T change at nucleotide position 609 (rs1800566), also known as *NQO1*2* (Sato et al., 2010). This results in a proline to serine amino acid change at codon 187 that is associated with a loss of enzyme activity due to the instability of the protein product (Traver et al., 1992). Indeed genotype–phenotype studies have demonstrated that the mutated-type TT genotype compared to the wild-type CC genotype was associated with an increased risk of myeloid leukemia and bladder carcinoma (Larson et al., 1999; Wa et al., 1997). The *NQO1* (**rs689452** IVS1-27C>G) is an intronic polymorphism located in a protein binding motif that has the potential to modulate *NQO1* protein activity (Kim & Hong, 2015). This polymorphism is little described in literature. A single G463A base transition in the *MPO* promoter region was identified at the SP1 binding site, which might modify benzene metabolism. Indeed, the *MPO 463*A* allele has been associated with reduced mRNA expression and its transcription activity is approximately 25 times lower than the MPO463*G allele in vitro due to reduced binding of SP1 (Piedrafita et al., 1996). Thus, individuals with one or more copies of the A-allele may be afforded a protection due to the decreased transcriptional activity of MPO and subsequent decreased metabolic activation of procarcinogens. This reduced activity has been reported to be associated with risk for several human cancers such as liver, ovarian, bladder, and lung cancer (Hecht et al., 2003; Schabath et al., 2002).

The present case–control study with 260 bladder cancer cases and 271 controls from Tunisia, was performed to investigate the impacts of SNPs in *NQO1* intron-1 (IVS1-27C>G, **rs689452**), exon 6 (Pro187Ser C > T, **rs1800566**), and in *MPO* promoter region (G463A, **rs2333227**) in association with environmental risk factors in the increasing risk of bladder cancer. Moreover, we correlated molecular results with clinicopathological characteristics of patients.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

This project was approved by a Charles Nicolle ethical committee. Informed consent was obtained from all individual participants included in the study.

2.2 | Subjects

A total of 260 BC patients (89.61% male and 10.38% female with a mean age of 68.71 ± 12.50 years), were included in this study. The number of BC samples included in this study was collected after calculating the estimated samples with relative precision estimated à 25% and CI at 95%. All patients had previously undergone bladder biopsy for detection of BC at the Department of Urology at Charles Nicolle Hospital of Tunis, Tunisia. They were confirmed by the result of the pathology report. Clinical and epidemiological data of BC cases are summarized in Table 1. Patients were interviewed when was signed the consent. Clinical characteristics of bladder cancer were obtained from medical records. The controls were matched to BC patients for age, sex, and geographic origin.

2.3 | Molecular analysis

Peripheral blood samples were collected from all subjects into tubes with ethylene diamine tetra-acetic acid (EDTA) at pH 8. Genomic DNA was extracted by a conventional phenol/chloroform protocol [20]. The quantity and quality of extracted DNA were estimated by a Nanodrop. The *NQO1**2 was genotyped in first step by PCR-RFLP. The PCR for *NQO1* reaction was carried out in a total volume of 25 μl containing 50 ng of genomic DNA, 1 X of DNA polymerase Taq buffer with $MgCl_2$, 0.3 mM of dNTP, 0.4 μM of each primer, and 1 U of Taq DNA polymerase (Invitrogen™). Thermal cycling conditions were: an initial step of 95℃ for 10 min followed by 35 cycles of denaturing step at 95℃ for 45 s, 56℃ for 45 s, and 72℃ for 45 s, and a final elongation step at 72℃ for 10 min. The PCR product was resolved by agarose gel electrophoresis (1%) and visualized by UV radiation after ethidium bromide staining. The *NQO1*2* polymorphisms were genotyped by

TABLE 1 Clinical and epidemiological characteristics of bladder cancer patients and controls

Clinical and epidemiological parameters	Bladder cancer	Controls
Samples sizes	N = 260	N = 271
Male	233(89.61%)	210(77.49%)
Female	27(10.38%)	61(22.50%)
Mean age at diagnosis (years)	67.63 ± 12.50	63.77 ± 11.43
Smoking status		
Smokers	208(80%)	146(53.87%)
Nonsmokers	29(11.15%)	125(46.13%)
ND	23(8.85%)	0(0.00%)
Number of pack/years		
<20 PY	35(16.83%)	74(50.68%)
≥20 PY	173(83.17%)	72(49.32%)
Exposure to professional risk factors (farmer, painter, building, chemical factory, etc.)		
Not exposed	126(48.46%)	240(88.56%)
Exposed	84(32.30%)	31(11.44%)
ND	50(19.24%)	0(0.00%)
TNM classification		
LG NMIBC	126(48.46%)	-
HG NMIBC	47(18.08%)	—
MIBC	66(25.38%)	—
ND	21(8.08%)	—

Abbreviations: HG, High Grade; LG, Low Grade; MIBC, Muscle Invasive Bladder Cancer; ND, Not Determined; PY, Packet per Year.

PCR-RFLP (restriction fragment length polymorphism) using 10 U of HinfI, for 4 h at 37℃ and analyzed by agarose gel electrophoresis at 1.5% (Ergen et al., 2007). Moreover, the genotyping of *NQO1*2*, rs689452, and rs2333227 was also done by NGS (next generation sequencing) (Li et al., 2016).

2.4 | Statistical analysis

The Hardy–Weinberg equilibrium test was calculated by the software package Arlequin (version 3.01) [21]. The difference of genotypes frequencies between cases and controls was determined by the chi-square test using IBM SPSS software 23. In addition, odds ratios (ORs) and their 95% confidence intervals (95% CI). A p value was considered significant at <0.05.

3 | RESULTS

All samples were found to be in Hardy–Weinberg equilibrium ($p > 0.05$) for rs1800566, rs689452, and rs2333227 in both the case and control groups (Table 2). The frequencies of *NQO1* 609*T (rs1800566*T) were estimated at 0.249 and 0.273 in respective control group and bladder cancer patients. The analysis of genotypic distribution for rs1800566 variation in codominant model showed that the *NQO1* 609CT genotype could increase the risk of bladder cancer patients in comparison with the reference group harboring the *NQO1* 609CC genotype ($p = 0.039$; OR = 1.454; 95% CI = 1.017–2.078) (Table 3). However, the analysis of the genotypic distribution for rs1800566 between patients and controls in the recessive and dominant models does not report a significant association (Table 3). The frequencies of *NQO1* IVS1-27*G (rs689452*G) allele were, respectively, estimated at 0.129 and 0.138 in the control group and bladder cancer patients. The comparison of rs689452 genotypes frequencies between the control group and bladder cancer patients does not report a significant difference in all models (Table 3). The linkage disequilibrium test showed that rs1800566 polymorphism is in linkage disequilibrium with rs689452 variation (Table 4). Moreover, the combined analysis of rs180056 and rs689452 in the *NQO1* gene showed that patients who inherited the *NQO1*609 CT / IVS1-27 CG genotype were associated with 2.180-fold increased risk for bladder cancer development compared to the reference group harboring the wild homozygous genotype (*NQO1* 609 CC / IVS1-27 CC) (Table 5). This additive effect could be explained by the high frequency of *NQO1* T-G haplotype in patients (11.153%) compared to the control group (5.535%). Conversely, when comparing the genotypic distribution for *MPO* G463A variation (rs2333227) between the case and control groups, the *MPO* 463GA genotype was found to be associated with a decreased risk of bladder cancer development in comparison with the reference group harboring the *MPO* wild genotype ($p = 0.049$; OR = 0.696; 95% CI = 0.484–0.999). Besides, no additive effect was found between the *MPO* 463GA genotype and *NQO1* polymorphisms (Table 5).

The comparison between bladder cancer patients and controls depending on their tobacco status and analyzed polymorphisms (rs1800566, rs689452, and rs2333227) showed a significant increased risk for bladder cancer development in smokers harboring the *NQO1*609 CT genotype compared to the reference group (Table 6). It was also shown that the risk for bladder cancer development increases more than 5.6-fold times in smokers harboring "*NQO1* 609 CT / IVS1-27 CG" compared to nonsmokers with homozygous genotype ($p = 0.002$; OR = 5.607; 95% CI = 1.847–17.02) (Table 6). This result suggests the presence of additive effect between "*NQO1* 609 CT / IVS1-27 CG" and tobacco smoking. In fact, an additive effect between "*NQO1* 609 CT / IVS1-27 CG" and exposition to professional risk factors was demonstrated ($p = 0.0004$; OR = 4.895; 95% CI = 2.027–11.82) (Table 7). In contrast, the inheritance of *MPO* 463GA was associated with a protective effect in none exposed patients compared to the reference group with wild genotype (Table 7). Eventually, a multinomial logistic regression analysis was used to investigate the predictive role of genetic and epidemiological parameters on tumor stages and grade of bladder cancer. This analysis did not reach a significant association between epidemiological parameters (including exposition to professional risk and tobacco status) and advanced tumor groups (NMIBC HG and MIBC) (Table 8). Furthermore, no significant association between rs233322, rs1800566, and the NMIBC HG or MIBC was found. However, the *NQO1* IVS-27 *CG genotype (rs689452) appeared to be associated with a risk of progression to MIBC ($p = 0.012$; expected OR for multinomial regression = 2.726).

4 | DISCUSSION

In this Tunisian population-based case–control study, the effect of environmental risk factor and polymorphisms in the *NQO1* (**rs689452 and rs1800566)** and *MPO* genes (**rs2333227**) on bladder cancer (BC) development and their association with clinical and epidemiological parameters were investigated.

The alleles frequencies for *NQO1* 609*T (*NQO1*2* **rs1800566)** and IVS1-27*G (**rs689452)** were, respectively, estimated at 0.249 and 0.129 in the control group. These frequencies are slightly different to those reported

TABLE 2 Hardy–Weinberg equilibrium for rs1800566, rs689452, and rs2333227

	Controls (N = 271)				Cases (N = 260)			
Gene / Polymorphisms	**Obs. Het**	**Exp. Het**	***p*-value**	**SD**	**Obs. Het**	**Exp. Het**	***p*-value**	**SD**
NQO1C609T rs1800566	0.35055	0.37477	0.33350	0.00050	0.44615	0.39778	0.06056	0.00023
NQO1 IVS1-27 C >G rs689452	0.22140	0.22536	0.78655	0.00040	0.23077	0.23904	0.60101	0.00050
MPO G463A rs2333227	0.46125	0.44319	0.58131	0.00046	0.37692	0.41770	0.13511	0.00037

Abbreviations: Exp. Het, Expected heterozygous genotype; Obs. Het, Observed heterozygous genotype; SD, Standard Deviation.

in Caucasian populations (Kim & Hong, 2015; Lajin & Alachkar, 2013). The comparison of rs689452 genotype frequencies between the control group and bladder cancer patients does not report a significant difference in all models. This result is in-line with previous reported studies (Figueroa et al., 2008; Guo et al., 2010; Kim & Hong, 2015). However, the analysis of genotypic distribution for rs1800566 variation in codominant model showed that the *NQO1* 609 CT genotype was associated with a 1.454-fold increased risk of bladder cancer patients in comparison with the reference group harboring the *NQO1* 609CC genotype. This result is consistent with recent meta-analysis and reviews (Guha et al., 2008; Lajin & Alachkar, 2013; Zhang et al., 2015). Previous studies have also suggested that *NQO1*rs1800566 is associated with an increased risk of several kinds of cancers, such as lung cancer, colorectal cancer, and esophageal cancer (Kiyohara et al., 2005; Yanling et al., 2013; Zheng et al., 2014). Positive association between this genotype and cancers can be explained by the reduction of detoxifying power for toxic quinone and free radicals and the decreased stability of p53. Indeed, in vitro evidence suggests that decreased *NQO1* activity cannot reduce the formation of benzo(a)pyrene quinone-DNA adducts generated by cytochrome P450 reductase (Joseph & Jaiswal, 1994). It is also observed that *NQO1* knockout mice and *NQO1* polymorphism in humans that result in the loss of its oxidoreductase activity are associated with a lower expression and induction of tumor suppressor p53 and consequently an increased risk of tumor development (Iskander et al., 2004). The linkage disequilibrium test showed that rs1800566 polymorphism is in linkage disequilibrium with rs689452 variation. Besides, the combined analysis of rs180056 and rs689452 in the *NQO1* gene showed that patients who inherited the *NQO1*609 CT/IVS1-27 CG genotype were associated with 2.180-fold increased risk for bladder cancer development compared to the reference group harboring the wild homozygous genotype (*NQO1* 609 CC/IVS1-27 CC). This additive effect could be explained by the high frequency of *NQO1* T-G haplotype in patients compared to controls. This finding confirms the study of Hee Kim and Yun-Chul Hong (Kim & Hong, 2015) who showed that these two loci exist as a haplotype block in Korean population (Kim & Hong, 2015). In fact the risk for bladder cancer development was 4.89- and 5.6-fold times higher for the group of patients "exposed to professional risk factors" and a group of "smokers" harboring "NQO1 609 CT / IVS1-27 CG", respectively, in comparison to the reference groups. These results suggest that haplotype of the *NQO1* gene play an important role in the development of bladder cancer by interaction with smoking (80% of patients were smokers) and professional risk factors (30% of patients were exposed to professional risk factors). This interaction could be essentially attributed to the metabolism of Benzene which is a common component in tobacco and industrial products. In fact, it has been reported that the concentration of benzene in the breathing air of smokers can be 10–20 times higher than in nonsmokers (Johnson et al., 2007; Wallace, 1996). Benzene has also been used as a component of inks in the printing industries and it is primarily used in drugs, dyes, insecticides, and plastics. Moreover, Nebert and al. (2002) in a comprehensive review, demonstrated that a lowered or absent *NQO1* activity can increase the risk of bone marrow toxicity, after environmental exposure to benzene and benzene-like compounds. In cancer patients, the *NQO1*2* allele appears to be associated with increased risk of chemotherapy-related myeloid leukemia (Nebert et al., 2002). In our study, the inheritance of NQO1 T-G haplotype and exposure to benzene is associated with the accumulation of reactive metabolites of benzene which could induce oxidative damage. Interestingly, our study has shown that smoker-patients harboring "*NQO1* 609 CT/IVS1-27 CG" and simultaneously exposed to professional risk factors were at 9.2-fold increased risk for developing bladder cancer compared to the reference group ($p = 0.0003$, OR = 9.204; 95% CI = 2.746–30.843). These findings emphasize this additive effect between smoking, professional risk factors and *NQO1* haplotype.

When comparing genotypic distribution for *MPO* G463A variation (rs2333227) between the case and control groups, it was found that the *MPO* 463GA genotype was associated with a decreased risk of bladder cancer

TABLE 3 Comparison of genotypes distribution of NQO1 and MPO in controls and bladder cancer (BC) from Tunisian population

Variant	Genotypes	Controls N = 271	Bladder cancer N = 260	Controls group vs Bladder cancer patient		
				p value	OR	95% CI
NQO1 Exon 6C609T- (rs1800566)	**Codominant model**					
	CC	156(57.60%)	131(50.38%)	—	1*	—
	CT	95(35.00%)	116(44.61%)	**0.039**	**1.454**	1.017–2.078
	TT	20(7.40%)	13(05.00%)	0.495	0.774	0.370–1.615
	Dominant model					
	CC	156(57.60%)	131(50.38%)	—	1*	—
	CT+TT	115(42.44%)	129(49.61%)	0.097	1.335	0.948–1.881
	Recessive model					
	TT	20(7.40%)	13(05.00%)	—	1*	—
	CC+CT	251(92.60%)	247(95.00%)	0.258	1.513	0.736–3.110
	Alleles					
	(Wild) C	407	378	—	1*	—
	(Mutated) T	135	142	0.373	1.132	0.861–1.489
NQO1 IVS1-27 C >G	**Codominant model**					
(rs689452)	CC	206(76.01%)	194(74.61%)	—	1*	—
	CG	60(22.14%)	60(23.077%)	0.773	1.061	0.706–1.596
	GG	5(01.85%)	6(02.30%)	0.692	1.274	0.382–4.243
	Dominant model					
	CC	206(76.01%)	194(74.61%)	—	1*	—
	CG+GG	65(23.99%)	67(25.77%)	0.652	1.094	0.738–1.622
	Recessive model					
	GG	55(01.85%)	6(02.30%)	—	1*	—
	CC+CG	266(98.15%)	254(97.70%)	0.708	0.795	0.239–2.639
	Alleles					
	(Wild) C	472	448	—	1*	—
	(Mutated) G	70	72	0.655	1.083	0.761–1.543
MPO G463A(rs2333227)	**Codominant model**					
	GG	119(43.91%)	134(51.54%)	—	1*	—
	GA	125(46.12%)	98(37.70%	**0.049**	**0.696**	0.484–0.999
	AA	27(09.96%)	28(10.77%)	0.782	0.921	0.513–1.650
	Dominant model					
	GG	119(43.91%)	134(51.54%)	—	1*	—
	GA+AA	152(56.09%)	126(48.46%)	0.078	0.736	0.591–1.171
	Recessive model					
	AA	27(09.96%)	28(10.77%)	—	1*	—
	GG+GA	244(90.04%)	232(89.23%)	0.760	0.916	0.52–1.035
	Alleles					
	(Wild) G	367	366	—	1*	—
	(Mutated) A	175	154	0.346	0.882	0.680–1.154

Bold values indicate a statistical significant association $p < 0.05$.

Abbreviations: 1*, Reference group; 95% CI, confidence interval; OR, odds ratio.

TABLE 4 Linkage disequilibrium (LD) between rs1800566 and rs689452 in the *NQO1* gene

Group	LnLHood LD	LnLHood LE	Chi-square test value	*p* (LD)
Cases	−387.03391	−392.00328	9.93874	0.00162
Controls	−394.01887	−405.34272	22.64771	0.0000

Abbreviations: LnLHood LD, likelihood of linkage disequilibrium; LnLHood LE, likelihood of linkage equilibrium; *p*(LD), probabilities from likelihood ratio test.

TABLE 5 Combined effect of rs1800566, rs689452, and rs2333227 in bladder cancer susceptibility

Variant	Genotypes	Controls N = 271	Bladder cancer N = 260	Controls group vs Bladder cancer patient		
				p value	OR	95% CI
rs1800566 and rs689452 (NQO1 C609T and NQO1 IVS1-27C>G)						
	CC/CC	106	94	—	1*	—
	CC/CG	45	31	0.355	0.776	0.454–1.326
	CC/GG	5	6	0.626	1.353	0.399–4.57
	CT/CC	80	87	0.331	1.226	0.812–1.850
	CT/CG	**15**	**29**	**0.025**	**2.180**	**1.101–4.313**
	CT/GG	0	0	0.952	1.127	0.022–57.358
	TT/CC	20	13	0.417	0.733	0.345–1.554
	TT/CG	0	0	0.952	1.127	0.022–57.358
	TT/GG	0	0	0.952	1.127	0.022–57.358
rs1800566 and rs2333227 (NQO1 C609T and MPO G463A)						
	CC/GG	70	67	—	1*	—
	CC/GA	71	45	0.107	0.662	0.401–1.093
	CC/AA	15	19	0.467	1.323	0.621–2.816
	CT/GG	43	60	0.151	1.457	0.870–2.440
	CT/GA	41	47	0.509	1.1977	0.700–2.047
	CT/AA	11	9	0.744	0.854	0.333–2.193
	TT/GG	6	7	0.733	1.218	0.389–3.814
	TT/GA	13	6	0.162	0.482	0.173–1.342
	TT/AA	1	0	0.520	0.348	0.013–8.696
rs689452 and rs2333227 (IVS1-27C>G and MPO G463A)						
	CC/GG	93	94	—	1*	—
	CC/GA	89	79	0.541	0.878	0.578–1.332
	CC/AA	24	21	0.664	0.865	0.451–1.661
	CG/GG	23	36	0.150	1.548	0.852–2.812
	CG/GA	34	18	**0.047**	**0.523**	0.276–0.992
	CG/AA	3	6	0.344	1.978	0.480–8.147
	GG/GG	3	4	0.721	0.319	0.287–6.056
	GG/GA	2	1	0.568	0.494	0.044–5.549
	GG/AA	0	1	0.506	2.968	0.119–73.801

Bold values indicate a statistical significant association $p < 0.05$.

1*, Reference group; 95% CI, confidence interval; OR, odds ratio.

development in comparison with the reference group harboring the *MPO* wild genotype ($p = 0.049$; OR = 0.696; 95% CI = 0.484–0.999). This result is consistent with the findings of Chikako Kiyohara et al. (2005). This protective effect was also observed in nonexposed patients to professional risk factors. This data supports the idea that

TABLE 6 Comparison of genotypes distribution for rs1800566, rs689452, and rs2333227 in bladder cancer patients according to tobacco status

Gene/ Variation	Genotypes	Bladder cancer				Controls				**Bladder cancer** (NS) **Vs Controls** (NS)		**Bladder cancer** (S) **Vs Controls** (S)		**Bladder cancer** (S) **Vs Controls** (NS)		**Bladder cancer** (≥ 20PY) **Vs Controls** (≥ 20PY)		**Bladder cancer** (≥ 20PY) **Vs Controls** (< 20PY)	
		NS	<20PY	≥20PY	S	Nonsmokers (NS)	<20PY	≥20PY	Smokers (S)	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (CI 95%)**
NQO1 (rs1800566)																			
	CC	19	20	76	96	76	39	41	80	—	1*	—	1*	—	1*	—	1*	—	1*
	CT	10	15	86	101	41	29	25	54	0.954	0.975(0.415– 2.293)	0.050	1.558(0.999–2.430)	**0.005**	1.950(1.217–3.124)	**0.0384**	1.855(1.033– 3.332)	0.149	1.521(0.859–2.694)
	TT	0	0	11	11	8	6	6	12	0.320	0.230(0.012–4.174)	0.544	0.763(0.319–1.823)	0.862	1.088(0.417–2.840)	0.983	0.989(0.341– 2.868)	0.910	0.940(0.323–2.734)
NQO1 (rs689452)																			
	CC	24	20	130	150	99	58	49	107	—	1*			—	1*	—	1*	—	1*
	CG	5	14	38	52	25	13	22	35	0.721	0.825(0.286– 2.378)	0.818	1.059(0.645–1.738	0.250	1.372(0.799–2.356)	0.174	0.651(0.350–1.209)	0.458	1.304(0.646–2.630)
	GG	0	0	6	6	1	3	1	4	0.854	1.353(0.0535–34.25)	0.918	1.070(0.294–3.884)	0.205	3.960(0.469–33.39)	0.455	2.261(0.265–19.267	0.875	0.892(0.215– 3.691)
MPO (rs233322)																			
	GG	13	16	94	110	52	35	32	67	—	1*	—	1*	—	1*	—	1*	—	1*
	GA	12	17	62	79	58	34	33	67	0.669	0.827(0.346–1.974)	0.145	0.718(0.460–1.121)	0.068	0.643(0.401–1.033)	0.132	0.639(0.357–1.145)	0.183	0.679(0.383–1.201)
	AA	4	2	17	19	15	5	7	12	0.920	1.066(0.302–3.757)	0.927	0.964(0.440–2.112)	0.181	0.598(0.282–1.271)	0.699	0.826(0.314–2.175)	0.665	1.266(0.434–3.690)
rs1800566 and rs689452																			
	CC/CC	16	11	54	65	54	28	24	52	—	1*	—	1*	—	1*	—	1*	—	1*
	CT/CG	2	6	21	27	4	5	6	11	0.566	1.687(0.282–10.074)	0.094	1.96 (0.891–4.327)	**0.002**	5.607(1.847–17.02)	0.399	1.555(0.557–4.343)	0.156	2.177(0.741–6.392)

Abbreviations: 1*, Reference group; 95% CI, confidence interval; NS, Nonsmoker; OR, odds ratio; S, Smoker.

TABLE 7 Comparison of genotypes distribution for rs1800566, rs689452, and rs2333227 in bladder cancer patients according to professional risk factors

Gene/ Variation	Genotypes	Bladder cancer		Controls		Bladder cancer (NE) Vs Controls (NE)		Bladder cancer (E) Vs Controls (E)		Bladder cancer (E) Vs Controls (NE)	
		Nonexposed (NE)	Exposed (E)	Nonexposed (NE)	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)
		NQO1 (rs1800566)									
	CC	70	37	136	20	—	1*	—	1*	—	1*
	CT	50	44	84	11	0.529	1.156(0.734–1.820)	0.077	2.162(0.918-5.088)	**0.012**	**1.925**(1.150–3.222)
	TT	6	3	20	0	0.268	0.582(0.223–1.517)	0.382	3.826(0.188-77.76)	0.356	0.551(0.155–1.956)
		NQO1 (rs689452)									
	CC	98	56	186	20	—	1*	—	1*	—	1*
	CG	28	25	49	11	0.761	1.084(0.641–1.833)	0.639	0.811(0.338-1.944)	0.068	1.694(0.961–2.987)
	GG	3	3	5	0	0.860	1.138(0.266–4.865)	0.543	2.539(0.125-51.32)	0.355	1.992(0.461–8.600)
		MPO (rs233322)									
	GG	66	42	102	17	—	1*	—	1*	—	1*
	GA	45	34	113	12	**0.040**	**0.615**(0.387–0.978)	0.756	1.146(0.482-2.727)	0.241	0.730(0.432–1.235)
	AA	15	8	25	2	0.835	0.927(0.455–1.888)	0.566	1.619(0.311-8.42)	0.571	0.777(0.324–1.861)
		rs1800566 and rs689452									
	CC/CC	49	24	94	12	—	1*	—	1*	—	1*
	CT/CG	10	15	12	3	0.310	1.598(0.645–3.961)	0.206	2.500(0.604-10.34)	**0.0004**	**4.895**(2.027–11.82)

Bold values indicate a statistical significant association $p < 0.05$.

Abbreviations: 1*, Reference group; E, Exposed to Professional risk factors; NE, Nonexposed to Professional risk factor.

TABLE 8 Logistic regression: effect of bladder cancer risk factors on tumors groups

Groups[a]		B	SE	Wald	df	Sig.	Exp(B)	95% Confidence Interval for Exp(B)	
								Lower bound	Upper BOUND
MIBC	Intercept	−1.716	.763	5.053	1	.025			
	[Tobacco status=<20PY]	.243	.702	.120	1	.729	1.275	.322	5.044
	[Tobacco status=≥20PY]	.353	.602	.343	1	.558	1.423	.437	4.630
	[Tobacco status = NS]	0[b]	.	.	0	.	.	.	.
	[Professional risk factors = E]	−.079	.378	.044	1	.834	.924	.440	1.939
	[Professional risk factors = NE]	0[b]	.	.	0	.	.	.	.
	[rs2333227 = A A]	−.238	.888	.072	1	.788	.788	.138	4.488
	[rs2333227 = G A]	.059	.372	.025	1	.873	1.061	.512	2.201
	[rs2333227 = G G]	0[b]	.	.	0	.	.	.	.
	[rs689452 = C C]	−.044	1.202	.001	1	.971	.957	.091	10.100
	[rs689452 = C G]	1.003	.400	6.283	1	**.012**	2.726	1.244	5.970
	[rs689452 = G G]	0[b]	.	.	0	.	.	.	.
	[rs1800566 = C C]	.399	.634	.396	1	.529	1.490	.430	5.156
	[rs1800566 = C T]	.656	.663	.978	1	.323	1.926	.525	7.064
	[rs1800566 = T T]	0[b]	.	.	0	.	.	.	.
NMIBC HG	Intercept	−1.787	.845	4.472	1	.034			
	[Tobacco status=<20PY]	−.288	.925	.097	1	.755	.750	.122	4.595
	[Tobacco status=≥20PY]	.829	.713	1.353	1	.245	2.292	.567	9.268
	[Tobacco status = NS]	0[b]	.	.	0	.	.	.	.
	[Professional risk factors = E]	.058	.414	.020	1	.888	1.060	.471	2.384
	[Professional risk factors = NE]	0[b]	.	.	0	.	.	.	.
	[rs2333227 = A A]	−20.030	.000	.	1	.	2.001E−9	2.001E−9	2.001E−9
	[rs2333227 = G A]	−.631	.410	2.372	1	.124	.532	.238	1.188
	[rs2333227 = G G]	0[b]	.	.	0	.	.	.	.
	[rs689452 = C C]	.489	.993	.243	1	.622	1.631	.233	11.412
	[rs689452 = C G]	.523	.472	1.229	1	.268	1.688	.669	4.257
	[rs689452 = G G]	0[b]	.	.	0	.	.	.	.
	[rs1800566 = C C]	.031	.655	.002	1	.962	1.032	.286	3.726
	[rs1800566 = C T]	.904	.668	1.830	1	.176	2.469	.667	9.145
	[rs1800566 = T T]	0[b]	.	.	0	.	.	.	.

Logistic regression: Number of observation = 193, Chi-square: 22.644; $p = 0.205$, −2 Log likelihood = 181.637.

Abbreviations: NMIBC LG, non-muscle invasive bladder cancer Low grade; NMIBC HG, non-muscle invasive bladder cancer high grade; PY: Packet Year; NS, Nonsmoker; E, Exposed to Professional risk factors; NE, Nonexposed to Professional risk factor.

[a]The reference category is NMIBC LG.

[b]This parameter is set to zero because it is redundant.

in absence in environmental risk factor, the inheritance of *MPO* 463GA could be protective against bladder cancer. However, the exposure to professional risk factors can induce bladder cancer development independently from the *MPO* genotype. The combined study of genotypic distribution for the *NQO1* and *MPO* genotype between the case and control groups does not report any significant difference. This result is explained by the fact that genetic variations in *NQO1*, which was associated with an increased risk for bladder cancer, is upset by the effect of *MPO* 463GA genotype which was associated with protective effect.

When it comes to multinomial logistic regression analysis, it has been demonstrated that the *NQO1* IVS-27 *CG genotype (rs689452) was associated with a risk of progression to MIBC ($p = 0.012$; expected OR for multinomial regression = 2.726). The positive association between this genotype and advanced forms can be explained by the decreasing stability of p53 (Iskander et al., 2004). Indeed, *TP53* is a common target for carcinogenic agents and an excellent candidate for molecular epidemiology studies (Soussi et al., 2000). It has been also possible to link the mutational pattern of the *TP53* locus with environmental exposures like tobacco smoke, aflatoxin B1, and UV radiation (Hainaut & Pfeifer, 2001). In bladder cancer, which is a tobacco-related cancer, the *TP53* mutation frequency varies from 14 to 61%, with the highest proportion usually associated with tumors of higher stage and grade (Bakkar et al., 2003; Hernández et al., 2005). Eventually, although this work was carried out for the first time in a Tunisian population sample, this study has some limitations. The main limitation of this study is the small sample size and, thus, low statistical power to detect some associations. The second limitation is that environmental exposure data (such as smoking history, alcohol consumption, and environmental exposure.) were not available in all patients, and patients with missing data were excluded from this study.

5 | CONCLUSION

The present study suggests that diplotypes of the *NQO1* gene play an important role in the development of bladder cancer. There is also an additive interaction between smoking, exposition to professional risk factors and polymorphisms in the *NQO1* gene for the risk development of bladder cancer in Tunisian population.

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CONFLICT OF INTEREST

There is no conflict to be declared.

AUTHOR CONTRIBUTIONS

IH: Collecting clinical samples and data, molecular analysis, drafted the manuscript, and analyzes the data. HA: Clinical characterization of the studied population. ZN, KD, MA, SK, and SZ, ET: help in collecting samples. MC: revised the manuscript. SO: designed the study, performed the statistical analysis and drafted, and revised the manuscript.

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