Pathol. Oncol. Res. (2011) 17:879–886
DOI 10.1007/s12253-011-9398-3

RESEARCH

Smoking and Polymorphisms in Xenobiotic Metabolism and DNA Repair Genes are Additive Risk Factors Affecting Bladder Cancer in Northern Tunisia

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Received: 5 November 2010 / Accepted: 30 March 2011 / Published online: 8 June 2011

Abstract Cancer epidemiology has undergone marked development since the nineteen-fifties. One of the most spectacular and specific contributions was the demonstration of the massive effect of smoking and genetic polymorphisms on the occurrence of bladder cancer. The tobacco carcinogens are metabolized by various xenobiotic metabolizing enzymes, such as the super-families of N-acetyltransferases (NAT) and glutathione S-transferases (GST). DNA repair is essential to an individual's ability to respond to damage caused by tobacco carcinogens. Alterations in DNA repair genes may affect cancer risk by influencing individual susceptibility to this environmental exposure. Polymorphisms in NAT2, GST and DNA repair genes alter the ability of these enzymes to metabolize carcinogens or to repair alterations caused by this process. We have conducted a case-control study to assess the role of smoking, slow NAT2 variants, GSTM1 and GSTT1 null, and XPC, XPD, XPG nucleotide excision-repair (NER) genotypes in bladder cancer development in North Tunisia. Taken alone, each gene unless NAT2 did not appear to be a factor affecting bladder cancer susceptibility. For the NAT2 slow acetylator genotypes, the NAT2*5/*7 diplotype was found to have a 7-fold increased risk to develop bladder cancer (OR=7.14; 95% CI: 1.30–51.41). However, in tobacco consumers, we have shown that Null GSTM1, Wild GSTT1, Slow NAT2, XPC (CC) and XPG (CC) are genetic risk factors for the disease. When combined together in susceptible individuals compared to protected individuals these risk factors give an elevated OR (OR=61). So, we have shown a strong cumulative effect of tobacco and different combinations of studied genetic risk factors which lead to a great susceptibility to bladder cancer.

Keywords Bladder cancer · Xenobiotics · DNA repair · Predisposition

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Introduction

The chain of events from environmental exposures to cancer requires hundreds of polymorphic genes encoding proteins involved in the transport and metabolism of xenobitics, or in DNA repair, or in immune or inflammatory response [1]. Once introduced into organism, these xenobiotics are bio-transformed by several enzymes.

The xenobiotic-metabolizing machinery includes oxidative enzymes (phase I), which generally activate compounds that become carcinogenic and phase II conjugating enzymes, considered mainly protective since they detoxify a number of reactive chemical carcinogens [2]. The conjugating process (phase II) is controlled by the super families of glutathione S-transferases and N-acetyltransferases enzymes.

Genetic polymorphisms affecting these enzymes can modify their activity with an effect on individual susceptibility for cancers which are induced by DNA damage [3].

The absence of GSTM1 activity is due to a homozygous deletion in the GSTM1 gene (GSTM1*0 or GSTM1 null genotype). Subjects lacking GSTM1 are at increased risk of cancer, particularly lung and bladder cancer that are environmentally-related cancers [4, 5].

Lack of GSTT1 enzyme activity has been reported in several studies as associated with increased risk for bladder and lung cancers [6, 7]. However, some studies reported that the risk of cancer was increased only among those with the GSTT1 positive (wild-type) genotype [8, 9].

The N-acetyltransferase (NAT) enzymes catalyze the N-acetylation of aromatic amine, which is considered a detoxifying process, expressed in multiple tissues, such as the bladder urothelium [10, 11]. A number of single nucleotide polymorphisms (SNPs) have been reported in the NAT2 coding region. NAT2*4 is considered the "wild-type" allele or haplotype. The genetic alterations underlying the NAT2 polymorphism have been correlated with decreased NAT2 enzyme activity [12].

Indeed, the presence of one or 2 wild-type alleles results in a rapid or an intermediate acetylator phenotype, whereas carrying two mutant alleles results in a slow acetylator phenotype. NAT2 alleles containing the 191G>A (NAT2*14), 341T>C (NAT2*5), 590G>A (NAT2*6), or 857G>A (NAT2*7) SNPs are the slow acetylator NAT2 variants. The slow acetylator phenotypes were found to have an increased risk of bladder cancer among cigarette smokers [13, 14]. Indeed, smokers who are NAT2 slow acetylators have higher levels of 4-aminobiphenyl (ABP) hemoglobin adducts [15, 16]. Furthermore, ABP-DNA adducts in high grade bladder tumors are found at higher levels in smokers who are slow NAT2 acetylators [17, 18].

The alterations caused by carcinogens exposure are generally removed by DNA repair enzymes to assure DNA integrity. The nucleotide-excision repair (NER) pathway has been reported to be the most significant modulator of bladder cancer risk, with other pathways playing less prominent roles [19]. Polymorphisms in NER genes may cause variations in DNA repair capacity and increase susceptibility to bladder cancer through complex gene–gene and gene–smoking interactions [20].

Xeroderma pigmentosum complementation group C, D and G polymorphisms (XPC Lys939Gln, XPD Lys751Gln and XPG Asp1104His, respectively) has been hypothesized to have a role in bladder cancer risk, but results from prior molecular epidemiologic studies and genotype-phenotype analyses are conflicting [21–25]. The most important result in Caucasian population that has been reported in association with increased risk of bladder cancer was the mutated homozygous Lys939Gln for XPC [22].

In Tunisia, bladder cancer is the most prevalent cancer of the uro-genital tract and the second most frequent cancer affecting men [26]. This type of disease is typically of multi-factor origin with the additive effect of low penetrance genes and environmental factors. Combination of these factors might change between subjects and from one population to another.

Bladder cancer is a pertinent model to study such genetic and environmental factors interaction. In order to concretely illustrate this concept, we performed a case-control study with the aim of investigating the combined effect on bladder cancer of selected variations in two different pathway genes GSTM1, GSTT1, NAT2, XPC, XPD and XPG according to tobacco smoking in the Northern Tunisian population.

Material and Methods

Subjects

A total of 125 patients with bladder urothelial cell carcinoma (UCC) and 125 healthy controls were included in the present study. Patients were recruited from the Department of Urology at the Charles Nicole Hospital in Tunis. All were from North of Tunisia, 90% of them were men and their mean age at diagnosis was 67.14±9.5. The control group consisted of non-related healthy subjects without history of malignant disease who were matched to those in the case group for gender proportion, geographic origin, and age. Smoking status information was available for all controls and only for 109 patients. Under informed consent, peripheral blood samples were collected into tubes with EDTA (pH 8).

DNA Preparation and Genotyping

Genomic DNA was extracted from leukocytes using a phenole/chloroform procedure [27]. The quality of genomic DNA was controlled by electrophoresis on a 1% agarose gel stained with ethidium bromide. GSTM1 and GSTT1 null alleles were identified using a multiplex-polymerase chain reaction (PCR)-based method as described by Arand et al. [28]. For NAT2, a PCR was carried out as described by Hsieh et al. [29]. The whole intronless NAT2 gene resulted in 1093-base pair amplification. It was then digested with 5 U of KpnI (Promega), 10 U of BamHI (Promega), 5U of TaqI (Promega) and 10U of AluI (Promega), to reveal NAT2*5, NAT2*7, NAT2*6, and NAT2*14 alleles, respectively. Digestion was performed overnight at 37°C for KpnI, BamHI, and AluI and at 65°C for TaqI. The obtained fragments were separated in 2% agarose gel. Individuals with 2 wild alleles (NAT2*4/*4) were classified as rapid

acetylators, whereas the others were classified as intermediate acetylators when they had one mutant allele, and as slow acetylators when they had 2 mutant alleles including NAT2*5, NAT2*6, NAT2*7, or NAT2*14 [30, 31].

Polymorphisms for XPC Lys939Gln, XPD Lys751Gln and XPG Asp1104His are detected by PCR followed by digestion with PvuII, PstI and Hsp92II enzymes (Promega), respectively. Digestion was performed during 4 h at 37°C for all enzymes and the obtained fragments are separated in 2.5% agarose gel. Primers used in this study have been previously described [32, 33].

Statistical Analysis

Relative risks were estimated by calculating the odds ratios (OR) with 95% confidence intervals (CI) at the 0.05 ($\chi 2$ test with Yates' correction, and Fisher's exact test) significance level [34]. Departures from Hardy-Weinberg equilibrium were tested using the software package Arlequin (version 3.01).

Results

Among the bladder cancer patients, 16% (20/125) were non-smokers and 71.2% (89/125) used 20 packs a year or more. Among the control group, 53.6% (67/125) were non-smokers versus 46.4% (58/125) smokers (Table 1). The comparison of patients and control group according to tobacco status shows that smoking is a risk factor for bladder cancer development (p=9.5 10^{-6}; OR=3.99; CI 95% 2.08–7.69).

Genotype distributions for GSTM1, GSTT1, NAT2, XPC, XPD and XPG in 125 bladder cancer cases and 125 controls are summarized in Table 1. The distribution of GSTT1, GSTM1 null genotypes (GSTM1*0 and GSTT1*0), SNPs of the NAT2 gene and XPC, XPD and XPG variations among both groups were in agreement with the Hardy-Weinberg equilibrium.

Frequencies of the GSTM1 null genotype in the control group and bladder cancer cases were 44.8% and 50.4%, respectively (Table 1). The comparison of GSTM1*0 frequencies in the groups of patients and controls did not show a significant statistic difference (p=0.44). Study of the combined effect of smoking and GSTM1 genotypes in bladder cancer cases and controls suggests that smokers with the GSTM1 null genotype were at 6.40-fold increased risk for developing bladder cancer when compared to non-smokers carrying the wild-type GSTM1 genotype (Table 2). Frequencies of the GSTT1 null genotype in the control group and bladder cancer cases were 30.4% and 24%, respectively (Table 1). As the GSTM1*0 variant, the GSTT1*0 polymorphism did not appear to be a factor for bladder cancer susceptibility (p=0.31). Studied separately, XPC, XPD and XPG mutated genotypes did not appear to affect bladder cancer susceptibility when compared to wild genotypes as a reference group (p value=0.56, 0.21 and 0.91, respectively).

The frequencies of NAT2*4, NAT2*5, NAT2*6, NAT2*7, and NAT2*14 variants in the control group as compared with bladder cancer cases were 51.6% versus 43.2%; 43.6% versus 48.4%; 2% versus 1.2%; 2.8% versus 5.2%; and 0% versus 2%, respectively (data not shown). NAT2 genotypes were categorized as homozygous mutant (slow), heterozygous wild-type/mutant (intermediate), and homozygous wild-type (rapid). The NAT2 genotype frequencies of the case group (22.4% rapid, 41.6% intermediate and 36% slow) were not significantly different from the control group (32% rapid, 39.2% intermediate and 28.8% slow), with p values estimated at 0.24 and 0.11 (Table 1). In contrast, a significant difference in genotypic frequencies between cases and controls was detected for the NAT2*5/*7 diplotype (p=0.01). This genotype was found to be over-represented in patients and presented a 7-fold increased risk of developing bladder cancer as compared to the control group (OR=7.14; 95% CI: 1.30–51.41).

When patients genotypes were considered according to tobacco status (Table 2), null GSTM1, wild GSTT1, slow NAT2, XPC (CC), XPD (AA + AC) and XPG (CC) were respectively revealed as risk genotypes in smoker patients. Indeed, the risk value given by smoker status considered alone is OR=4.

While, smokers harboring separately slow NAT2 or mutated homozygous genotype for XPC were at 11 to 12-fold increased risk for developing bladder cancer when compared to non-smokers with respectively rapid NAT2 or wild type XPC (Table 2).

To investigate and evaluate the additive effects of these genetic risk factors, we have shown in Table 3 the association, according to the smoker status, of multiple factors simultaneously and with different possible combinations on bladder cancer susceptibility.

Non-smokers, harboring respectively GSTM1 wild, GSTT1 null, rapid and intermediate NAT2, XPC (AA + AC), XPD (CC) and XPG (GG + GC) were supposed to be protected.

Since our studied cancer is a multifactorial pathology which depends on additive effects of multiple risk factors, a single factor by itself should be without any effect on bladder cancer susceptibility. Hence, we supposed that protected individuals are non-smokers carrying zero to one (0–1) genetic risk factor. Considered as references, frequencies of these protected individuals are compared with those of persons at risk for bladder cancer which are smokers carrying at least one, two, three, four or five genetic risk factors.

Our results presented in Table 3 show that the population number decreases with the increase of risk factors analyzed simultaneously. We have also found a progressive increase

Table 1 Tobacco status and genotype distribution of *NAT2, GSTT1, GSTM1, XPC, XPD* and *XPG* in bladder cancer cases and controls from north Tunisia

	Genotypes	Controls (%) (n=125)	Cases (%) (n=125)	p [a] value	OR (95%CI)
Smoking status					
Non-smoker	–	67	20	–	1*
Smoker [c]	–	58	89	$9.5\ 10^{-6}$	3.99 (2.08–7.69)
GSTM1	Wild	69 (55.2)	62 (49.6)	–	1*
	Null	56 (44.8)	63 (50.4)	0.44	–
GSTT1	Wild	87 (69.6)	95 (76)	–	1*
	Null	38 (30.4)	30 (24)	0.31	–
NAT2					
Rapid	NAT2*4/*4	40 (32)	28 (22.4)	–	1*
Intermediate	NAT2*4/*5	42 (33.6)	47 (37.6)	0.19	–
	NAT2*4/*6	3 (2.4)	1 (0.8)	0.52	–
	NAT2*4/*7	4 (3.2)	3 (2.4)	0.75	–
	NAT2*4/*14	0	1 (0.8)	0.87	–
	IA Subtotal	49 (39.2)	52 (41.6)	0.24	–
Slow	NAT2*5/*5	32 (25.6)	30 (24)	0.51	–
	NAT2*5/*6	1 (0.8)	1 (0.8)	0.63	–
	NAT2*5/*7	2 (1.6)	10 (8)	0.01	7.14 (1.30–51.41)
	NAT2*5/*14	0	3 (2.4)	0.15	–
	NAT2*6/*7	1 (0.8)	0	0.84	–
	NAT2*6/*14	0	1 (0.8)	0.87	–
	SA Subtotal	36 (28.8)	45 (36)	0.11	–
XPC	AA	57 (45.6)	53 (42.4)	–	1*
	AC	52 (41.6)	52 (41.6)	0.89	–
	CC	16 (12.8)	20 (16)	0.56	–
	Total	125	125	–	–
XPD	AA	62 (49.6)	65 (52)	–	1*
	AC	52 (41.6)	55 (44)	0.92	–
	CC	11(8.8)	5 (4)	0.21	–
XPG	GG	46 (36.8)	48 (38.4)	–	1*
	GC	61 (48.8)	56 (44.8)	0.74	–
	CC	18 (14.4)	21 (16.8)	0.91	–

RA rapid scetylator, *IA* intermediate acetylator, *SA* slow acetylator

[a] Yates correction

[*] Reference group

[c] ≥20 packet years

in OR, according to the number of analyzed genetic risk factors. These values of OR were increased from 7.46 to a maximal value of 61 (p=3 10^{-5}; OR=61.75; CI 95% 5.25–1711.89) when 4 risk factors were studied simultaneously in smoker patients.

Discussion

We have undertaken this case-control study to investigate the combined effect of smoking, slow NAT2, GSTM1 and GSTT1 null genotypes, XPC, XPD and XPG on susceptibility to bladder cancer in a Tunisian population. In the control group, our results confirmed the frequency of the GSTM1 null genotype (GSTM1*0) previously reported for healthy Tunisians and for the Caucasian population [35, 36]. However, the GSTT1*0 frequency was lower than that reported for healthy Tunisians and was higher than that observed in the Caucasian population [35, 36]. Frequencies of the GSTM1 and GSTT1 null genotypes in the control group were compared to those found in bladder cancer cases. Our results indicated that the GSTM1 null genotype was not associated with bladder cancer susceptibility. When we stratified patients and controls according to their smoking status, our data suggested an additive effect of tobacco and GSTM1 null genotype but the smoking effect was predominant. This result was in agreement with a meta-analyses performed by Garcia-Closas et al. [21], which showed that the GSTM1 null genotype increases the overall risk of bladder cancer in smokers.

For the GSTT1 gene, our results suggested that the GSTT1 null genotype was not associated with bladder cancer risk. Of the published studies, some suggested an increased risk with the GSTT1 null genotype [13, 37] and

Table 2 Combined effect of smoking, xenobiotic and gene repair enzyme genotypes in bladder cancer cases and controls

Tobacco status	Genotypes	Controls	Cases	p [a] value	OR (95%CI)
GSTM1					
Non-smoker	Wild	38	11	–	1[b]
	Null	29	9	0.90	–
Smoker [c]	Wild	31	39	0.0006	4.35 (1.78–10.77)
	Null	27	50	$7.8\ 10^{-6}$	6.40 (2.63–15.83)
GSTT1					
Non-smoker	Wild	49	13	–	1[b]
	Null	18	7	0.67	–
Smoker [c]	Wild	38	71	10^{-7}	7.04 (3.22–15.62)
	Null	20	18	0.01	3.39 (1.29–9.05)
NAT2					
Non-smoker	Rapid	21	4	–	1[b]
	Intermediate	26	10	0.44	–
	Slow	20	6	0.77	–
Smoker [c]	Rapid	19	19	0.01	5.25 (1.34–22.30)
	Intermediate	23	34	0.0006	7.76 (2.12–30.97)
	Slow	16	36	$3\ 10^{-5}$	11.81 (3.11–48.98)
XPC					
Non-smoker	AA	31	8	–	1[b]
	AC	25	10	0.59	–
	CC	11	2	1.00	–
Smoker [c]	AA	26	39	0.0002	5.81 (2.13–16.33)
	AC	27	35	0.0008	5.02 (1.83–14.16)
	CC	5	15	0.0001	11.63 (2.80–52.30)
XPD					
Non-smoker	AA	38	10	–	1[b]
	AC	23	9	0.62	–
	CC	6	1	0.91	–
Smoker [c]	AA	24	44	$7\ 10^{-6}$	6.97 (2.75–18.06)
	AC	29	41	0.0001	5.37 (2.15–13.70)
	CC	5	4	0.27	–
XPG					
Non-smoker	GG	24	7	–	1[b]
	GC	32	9	0.82	–
	CC	11	4	0.94	–
Smoker [c]	GG	22	35	0.001	5.45 (1.83–16.81)
	GC	29	38	0.003	4.49 (1.56–13.38)
	CC	7	16	0.001	7.84 (1.99–32.85)

[a] Yates correction

[b] Reference group

[c] ≥20 packs/year

Table 3 Combined effect of smoking and the 5 genetic risk factors of *NAT2, GSTM1, GSTT1, XPC* and *XPG* in bladder cancer patients and controls

	Controls	Cases	p [a] value	OR
Protected				
Non smoker with 0–1 Genetic risk factor	19	4	–	1[b]
Smokers [c] with minimum 1 Genetic risk factor	56	88	0.0002	7.46 (2.23–27.49)
Smokers [c] with minimum 2 Genetic risk factors	39	73	0.00006	8.89 (2.6–33.44)
Smokers [c] with minimum 3 Genetic risk factors	18	39	0.00009	10.29 (2.73–42.24)
Smokers [c] with minimum 4 Genetic risk factors	1	13	0.00003	61.75 (5.25–1711.89)
Smokers [c] with minimum 5 Genetic risk factors	0	1	–	–

[a] Fisher test

[b] Reference group

[c] ≥20 packs/year

others reported no association [38]. In our study, the comparison of patients and controls according to the GSTT1 genotype and tobacco status suggested an aggravating effect of wild-type GSTT1 genotype in smokers, which confirmed the study of Kim et al. [39].

For the NAT2 gene, we have shown that the frequencies of alleles encoding slow acetylator variants NAT2*5, NAT2*6, NAT2*7, and NAT2*14 were similar to that reported for the Caucasians populations [40]. Globally, the NAT2 slow acetylator in our control group, collected in the North of Tunisia, was 28.8%. This value is lower than that reported in other studies on the middle coast of Tunisia (49.7%) [41] and also lower than that observed among American Caucasians [42]. The comparison of the slow NAT2 frequency in controls to that reported for patients without stratification did not show a significant statistic difference (p=0.19). A significant difference in genotypic frequencies between cases and controls was only detected for the NAT2*5/*7 diplotype (p=0.01). This genotype was found to be over-represented in patients and presented a 7-fold increased risk of developing bladder cancer compared to the control group. This association could be explained by the presence of the NAT2*5 haplotype. Indeed, some studies found that bladder cancer risk was high in individuals possessing NAT2*5 haplotypes [13, 43]. The 341T>C (I114T) SNP associated with NAT2*5 alleles or haplotypes yields a very large reduction in NAT2 activity [44] resulting from protein degradation [45]. However, we have reported that the homozygous genotype of NAT2 (NAT2*5/NAT2*5) was not associated with an increased risk of bladder cancer, which suggested that the NAT2*5 haplotype would act preferentially in association with the NAT2*7 haplotype. N-acetylation is considered as a major detoxification step for carcinogenic aromatic arylamines. The comparison of patients and controls according to the NAT2 genotype and tobacco status has shown a significant statistical difference which was in agreement with several other results [13, 14, 46]. These studies reported that in smokers, slow NAT2 increases the quantity of electrophilic-active products which induces local somatic mutations in oncogenes and/or anti-oncogenes, initiating tumoral progression.

For XPC, XPD and XPG mutated alleles; frequencies were reported for the first time in Tunisia and estimated at 33%, 29%, and 38%, respectively (results are submitted). These frequencies were higher than those reported in Caucasian and American population [22, 24]. The comparison of patients and controls according to XPC, XPD and XPG genotypes without any stratification did not show any significant statistical differences. However, according to tobacco status, important significant difference in genotypic frequencies between cases and controls was detected for smokers carrying mutated homozygous genotype for XPC compared to the reference group (p=0.0001, OR=11.63; CI 95%: 2.80–52.30).

Xeroderma pigmentosum type C (XPC) encodes an important DNA damage recognition protein that binds to damaged DNA at a very early stage during DNA repair [47]. Our result confirmed other studies previously reported on XPC polymorphism association with bladder cancer [22, 48].

Bladder cancer is a multifactorial pathology; it seems that several genetic risk factors are involved in the development of the disease. Hence, one single factor alone should be without influence and should not affect bladder cancer susceptibility. In our case-control study, we have compared genotypic frequencies of the different studied genes between protected individuals (non-smokers carrying zero to one genetic risk factor) and individuals at risk (smokers with at least one, two, three, four or five genetic risk factors). We have concluded that more a subject presents risk factor more the susceptibility to the disease increases as shown by OR increasing value. The simultaneous association of 5 risk factors along with cigarette smoker status gives a very high probability to develop bladder cancer. This result can be explained by the fact that persons who are exposed to high intensity of tobacco carcinogens are unable to detoxify these components especially if they carry altered xenobiotic enzymes, and are unable to eliminate mutations caused by DNA adducts if they present in addition an altered DNA repair enzymatic pathway.

According to our results, smoker status, NAT2 slow and intermediate genotypes, the GSTT1 wild-type allele, GSTM1 null genotype, XPC and XPG mutated genotypes are additive factors leading to bladder cancer. Mainly most of our patients present at least one of the studied risk factors, but the risk for bladder cancer increases with the number of accumulated risk factors.

Smoker status is the main environmental risk factor, however, in our study some patients with bladder cancer were non smokers or low smokers, among whom, four non-smoker persons were without or with only one genetic risk factor (Table 3) indicating that some other factors, that we have not investigated yet, could be involved in the bladder cancer.

Indeed, according to previous results, we have shown that polymorphism in genes encoding enzymes implicated in folate metabolism are also risk factor for bladder cancer [49].

In conclusion, to our knowledge, this is the first study achieved in Tunisia and particularly in North Tunisia presenting evidence that the risk of bladder cancer was very high for smokers having the association of 4 genetic risk factors involving different enzymatic pathways.

References

1. Cohen SM, Johansson SL (1992) Epidemiology and etiology of bladder cancer. Urol Clin North Am 19:421–428
2. Raunio H, Husgafvel-Pursiainen K, Anttila S et al (1995) Diagnosis of polymorphisms in carcinogen-activating and inactivating enzymes and cancer susceptibility: a review. Gene 159:113–121
3. Kaprio J (2000) Science, medicine, and the future. Genetic epidemiology. B Med J 320:1257–1259
4. Ford JG, Li Y, O'Sullivan MM et al (2000) Glutathione S-transferase M1 polymorphism and lung cancer risk in African-Americans. Carcinogenesis 21:1971–1975
5. Karagas MR, Park S, Warren A et al (2005) Gender, smoking, glutathione- S-transferase variants and bladder cancer incidence: a population-based study. Cancer Lett 219:63–69
6. Abdel-Rahman SZ, Anwar WA, Abdel-Ala WE et al (1998) GSTM1 and GSTT1 genes are potential risk modifiers for bladder cancer. Cancer Detect Prev 22:129–138
7. Salagovic J, Kalina I, Stubna J et al (1998) Genetic polymorphism of glutathione S-transferase M1 and T1 as a risk factor in lung and bladder cancers. Neoplasma 45:312–317
8. Hengstler JG, Kett A, Arand M et al (1998) Glutathione S-transferase T1 and M1 gene defects in ovarian carcinoma. Cancer Lett 130:43–48
9. Katoh T, Inatomi H, Kim H et al (1998) Effects of glutathione S-transferase (GST) M1 and GSTT1 genotypes on urothelial cancer risk. Cancer Lett 132:147–152
10. Badawi AF, Hirvonen A, Bell DA et al (1995) Role of aromatic amine acetyltransferases, NAT1 and NAT2, in carcinogen-DNA adducts formation in human urinary bladder. Cancer Res 55:5230–5237
11. Kloth MT, Gee RL, Messing EM et al (1994) Expression of N acetyltransferase (NAT) in cultured human uroepithelial cells. Carcinogenesis 15:2781–2787
12. Blum M, Grant DM, McBride W et al (1990) Human arylamine N-acetyltransferase genes: isolation, chromosomal localization, and functional expression. DNA Cell Biol 9:193–203
13. Brockmoller J, Cascorbi I, Kerb R et al (1996) Combined analysis of inherited polymorphisms in arylamine N-acetyltransferase 2, glutathione S-transferases M1 and T1, microsomal epoxide hydrolase and cytochrome P450 enzymes as modulators of bladder cancer risk. Cancer Res 56:3915–3925
14. Risch A, Wallace DMA, Bathers S et al (1995) Slow N-acetylation genotype is a susceptibility factor in occupational and smoking related bladder cancer. Hum Mol Genet 4:231–236
15. Probst-Hensch NM, Bell DA, Watson MA et al (2000) N-acetyltransferase 2 phenotype but not NAT1*10 genotype affects aminobiphenylhemoglobin adduct levels. Cancer Epidemiol Biomarkers Prev 9:619–623
16. Yu MC, Skipper PL, Taghizadeh K et al (1994) Acetylator phenotype, aminobiphenylhemoglobin adduct levels, and bladder cancer risk in white, black, and Asian men in Los Angeles, California. J Nat Cancer Inst 86:712–716
17. Airoldi L, Orsi F, Magagnotti C et al (2002) Determinants of 4-aminobiphenyl-DNA adducts in bladder cancer biopsies. Carcinogenesis 23:861–866
18. Hao GY, Zhang WD, Chen YH et al (2004) Relationship between genetic polymorphism of NAT2 and susceptibility to urinary bladder cancer. Zhonghua Zhong Liu Za Zhi 26:283–286
19. Wu XF, Dzenis YA (2006) Guided self-assembly diblock copolymer thin films on chemically patterned substrartes. J Chem Phys 125:174–1777
20. Chen CH, Shun CT, Huang KH et al (2007) Stopping smoking might reduce tumour recurrence in non-muscle invasive bladder cancer. BJU Int 100:281–286
21. Garcıa-Closas M, Malats N, Real FX et al (2006) Genetic variation in the nucleotide-excision repair pathway and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 15:536–542
22. Sanyal S, De Verdier PJ, Steineck G et al (2007) Polymorphisms in XPD, XPC and the risk of death in patients with urinary bladder neoplasms. Acta Oncol 46:31–41
23. Zhu Y, Yang H, Chen Q et al (2008) Modulation of DNA damage/DNA repair capacity by XPC polymorphisms. DNA Repair 7:141–148
24. Dworaczek H, Xiao W (2007) Xeroderma pigmentosum: a glimpse into nucleotide-excision repair, genetic instability, and cancer. Crit Rev Oncog 13:159–177
25. Shen M, Hung RJ, Brennan P et al (2003) Polymorphisms of the DNA repair genes XRCC1, XRCC3, XPD, interaction with environmental exposures, and bladder cancer risk in a case-control study in northern Italy. Cancer Epidemiol Biomarkers Prev 12:1234–1240
26. Sellami A, Jlidi R, Hsaıri M et al (2000) Registre du cancer du Sud Tunisie 1997. Hôpital Habib Bourguiba 2:32–35
27. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor
28. Arand M, Muhlbaur R, Hengstler J et al (1996) A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione S transferase GSTM1 and GSTT1 polymorphisms. Annal Biochem 236:384–386
29. Hsieh FI, Pu YS, Chern HD et al (1999) Genetic polymorphisms of N-acetyltransferase 1 and 2 and risk of cigarette smoking-related bladder cancer. Br J Cancer 81:537–541
30. Fukino K, Sasaki Y, Hirai S et al (2008) Effect of N-acetyltransferase 2(NAT2), CYP2E1 and Glutathione-S-transferase (GST) genotypes on the serum Concentrations of isoniazid and metabolites in tuberculosis patients. J Toxicol Sci 33:187–195
31. Vastis KP, Weber WW, Bell DA et al (1995) Nomenclature for N-acetyltransferases. Pharmacogenetics 5:1–17
32. Sanyal S, Festa F, Sakano S et al (2004) Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 25:729–734
33. De Ruyck K, Szaumkessel M, De Rudder I et al (2007) Polymorphisms in base-excision repair and nucleotide-excision repair genes in relation to lung cancer risk. Mutat Res 631:101–110
34. O'Gorman TW, Woolson RF (1993) The effect of category choice on the odds ratio and several measures of association in case-control studies. Commun Stat 22:1157–1171
35. Ouerhani S, Tebourski F, Slama MR et al (2006) The role of glutathione transferases M1 and T1 in individual susceptibility to bladder cancer in a Tunisian population. Ann Hum Biol 33:529–535
36. Garte S, Gaspari L, Alexandrie AK et al (2001) Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev 10:1239–1248
37. Kempkes M, Golka K, Reich S et al (1996) Glutathione transferase GSTM1 and GSTT1 null genotypes as potential risk factors for urothelial cancer of the bladder. Arch Toxicol 71:123–126
38. Lee SJ, Cho SH, Park SK et al (2002) Combined effect of glutathione S-transferase M1 and T1 genotypes on bladder cancer risk. Cancer Lett 77:173–179
39. Kim WJ, Kim H, Kim CH et al (2002) GSTT1-null genotype is a protective factor against bladder cancer. Urology 60:913–918
40. Hein DW (2006) N-acetyltransferase 2 genetic polymorphism: effects of carcinogen and haplotype on urinary bladder cancer risk. Oncogene 25:1649–1658
41. Khedhaier A, Hassen E, Bouaouina N et al (2008) Implication of xenobiotic metabolizing enzyme gene (CYP2E1, CYP2C19, CYP2D6, mEH and NAT2) polymorphisms in breast carcinoma. BMC Cancer 28:109–121

42. Bell DA, Taylor JA, Butler MA et al (1993) Genotype/phenotype discordance for human arylamine N-acetyltransferase (NAT2) reveals a new slow-acetylator allele common in African-Americans. Carcinogenesis 14:1689–1692
43. El Desoky ES, Abdel Salam YM, Salama RH et al (2005) NAT2*5/*5 genotype (341T>C) is a potential risk factor for schistosomiasis- associated bladder cancer in Egyptians. Ther Drug Monit 27:297–304
44. Fretland AJ, Doll MA, Leff MA et al (2001) Functional characterization of nucleotide polymorphisms in the coding region of N-acetyltransferase 1. Pharmacogenetics 11:511–520
45. Zang Y, Zhao S, Doll MA et al (2004) The T341C (Ile114Thr) polymorphism of N-acetyltransferase 2 yields slow acetylator phenotype by enhanced protein degradation. Pharmacogenetics 14:717–723
46. Garcia-Closas M, Malats N, Silverman D et al (2005) NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet 366:649–659
47. Lin J, Swan GE, Shields PG et al (2007) Mutagen sensitivity and genetic variants in nucleotide-excision repair pathway: genotype-phenotype correlation. Cancer Epidemiol Biomarkers Prev 16:2065–2071
48. Fontana L, Bosviel R, Delort L (2008) DNA repair gene ERCC2, XPC, XRCC1, XRCC3 polymorphisms and associations with bladder cancer risk in a French cohort. Anticancer Res 28:1853–1856
49. Rouissi K, Ouerhani S, Oliveira E et al (2009) Polymorphisms in one-carbon metabolism pathway genes and risk for bladder cancer in a Tunisian population. Cancer Genet Cytogenet 195:43–53