BioTechnologia vol. 100 (2) • pp. 155–168 • 2019
Journal of Biotechnology, Computational Biology and Bionanotechnology RESEARCH PAPERS
http://doi.org/10.5114/bta.2019.85846

Joint effect of N-acetyltransferase 2 gene and smoking status on bladder carcinogenesis in Algerian population

ASMA RIBOUH-ARRAS [1]*, NAOUEL CHAOUI-KHEROUATOU [1], AHMED HIRECHE [1], NOUREDDINE ABADI [2], DALILA SATTA [1]

[1] Department of Animal Biology, Laboratory of Cellular and Molecular Biology, University Constantine 1, Constantine, Algeria

[2] Laboratory of Biology and Molecular Genetic, University Constantine 3, Constantine, Algeria

Abstract

Background. Toxic compounds are detoxified by several xenobiotic metabolizing enzymes such as N-acetyltransferase 2 (NAT2). The role for NAT2 genetic polymorphisms in different malignancies risk has been the subject of numerous studies. In the current study we investigated the association of genetic NAT2 variants or their corresponding acetylator phenotypes with bladder cancer risk. The relationship between NAT2 genotype/phenotype and smoking status was also evaluated as potential risk factor of urinary bladder cancer. *Material and methods.* As few data on the association between genetic polymorphisms of NAT2 and bladder cancer are available in the Algerian population, we performed an extensive identification of NAT2 variants in 175 bladder cancer patients and 189 healthy controls by direct PCR sequencing of the coding region. *Results.* Thirteen previously described SNPs were identified in this study; only T341C and G590A were associated with increased risk of bladder cancer ($P < 0.05$). NAT2 slow acetylator phenotype is at higher risk (OR = 2.45, 95% CI = 1.41–4.35) with the greatest risk noted for the allele NAT2*5. When combined to smoking status, T341C and G590A SNPs were of significant correlation with bladder cancer risk ($P < 0.05$) among non smokers. A correlation that increased among smokers. However, a relationship emerged only when smoking habit was considered between C345T, C481T and A803G SNPs and bladder cancer risk ($P < 0.05$). Our study showed a strong interaction between NAT2 slow acetylator phenotype and smoking ($P = 7.20e^{-6}$). *Conclusions.* These findings provided evidence of an additive effect between smoking status and NAT2 slow acetylation in influencing bladder cancer risk.

Key words: single nucleotide polymorphisms, smoking, phenotype, urinary bladder cancer, N-acetyltransferase 2 (NAT2)

Introduction

Worldwide, bladder cancer is recognized as the tenth most common cancer, with statistical data showing 549 393 newly diagnosed cases and 199 922 cancer-related deaths for both genders in 2018 (Ferlay et al., 2018). In North African men, bladder cancer is the third most frequent cancer after liver and lung cancers, and twelfth in women with an annual incidence rate of 14.3/100 000 in men and 3.2/100 000 in women. In Algerian men, bladder cancer is the first most common genitourinary malignant disease accounting for an incidence rate of 13/100 000 person- years and a mortality rate of 5.8/100 000 person-years (Ferlay et al., 2018).

Tobacco smoking and occupational exposure to aromatic amines are identified to be the most important risk factors for this disease (Freedman et al., 2011; Chu et al., 2013), but other lifestyle, environmental, as well as hereditary factors have also attracted interest, suggesting individual susceptibility to bladder carcinogenesis (Cohen et al., 2000; Chu et al., 2013).

Toxic compounds such as 4-aminobiphenol, acrolein, and oxygen free radicals contained in tobacco and other aromatic amines are also known to be present in several industrial compounds, especially those used in farming, chemical plants, rubber industry, painting, and textiles (Dolin, 1992; Viel et al., 1995; Ward et al., 1996; Car-

* Corresponding author: University Constantine 1, Route Ain El Bey, 25000 Constantine, Algeria; e-mail: asma.ribouh@hotmail.fr

reón et al., 2010; Pira et al., 2010; Tsai et al., 2011). These compounds have been identified as group I carcinogens by the International Agency of Research on Cancer (Sanderson et al., 2007; Tao et al., 2012; Pesch et al., 2013). These substances as well as various polycyclic aromatic hydrocarbons have been reported to be carcinogenic to urinary bladder (Garcia-Closas et al., 2005; Tao et al., 2012).

These compounds act by forming an adduct with human DNA, thus exerting their carcinogenic effect and subsequently leading to the development of bladder cancer (Tao et al., 2012). The bladder urothelium like many other tissues, expresses various enzymes, for example, N-acetyltransferase 2 (*NAT2*), that help in metabolizing xenobiotic substances (Inatomi et al., 1999; Khedhiri et al., 2010; Tao et al., 2012). This detoxifying enzyme catalyzes the N-acetylation reaction of exogenous chemicals present in diet, cigarette smoke, and environment (Sanderson et al., 2007; Song et al., 2009; Rihs et al., 2011; Di Pietro et al., 2012; Guaoua et al., 2014).

Humans present wide inter-individual variability with regard to NAT2 enzyme activity, which is caused by mutations in *NAT2* gene (Di Pietro et al., 2012; Guaoua et al., 2014), a gene mapped to the human chromosome 8p22 having an open reading frame of 870 bp (Di Pietro et al., 2012).

Till date, over 25 polymorphisms have been detected in the *NAT2* coding region among the populations of different ethnic origins (Hein, 2002; Di Pietro et al., 2012). These genetic variations in the *NAT2* gene affect the enzyme activity and result in the formation of three different NAT2 phenotypes: fast, intermediate, and slow acetylators (Sanderson et al., 2007; Song et al., 2009; Guaoua et al., 2014).

Thirty-six allelic variants of *NAT2* gene have been reported, and each allele is classified as fast or slow depending on the combination of different single nucleotide polymorphisms (SNPs) present in its coding region (Khedhiri et al., 2010; Di Pietro et al., 2012). *NAT2* *4 allele is defined as the wild-type allele and has been associated with the fast acetylation phenotype (Hein et al., 2000a; Walker et al., 2009; Toure et al., 2012). Individuals homozygous for rapid *NAT2* acetylator alleles are classified as rapid acetylators, those homozygous for slow *NAT2* acetylator alleles as slow acetylators, and those possessing one rapid and one slow *NAT2* acetylator alleles as intermediate acetylators (Hein, 2006).

Many studies have analyzed the relationship between *NAT2* slow acetylation phenotype and risk of bladder cancer (Vatsis et al., 1995; Hein, 2002; García-Closas et al., 2005; Sanderson et al., 2007). However, results were controversial at the level of individual studies, which may be explained by the differences in exposure to bladder carcinogens and ethnicity, as well as variations in the relative number of cases and statistical power limitations in various studies (Moore et al., 2011; Selinski et al., 2013; Zhu et al., 2015). In the present study, we first identified *NAT2* variants among 364 subjects through complete sequencing of the *NAT2* coding region. Then, we evaluated the association between polymorphisms within the gene and their corresponding acetylator phenotypes and bladder cancer risk. Finally, we assessed the combined effect of *NAT2* genotypes and smoking status on susceptibility to bladder cancer.

Materials and methods

Subjects

The study population comprised 175 patients and 189 controls. Bladder cancer patients diagnosed with the disease during the period 2014–2016 at the Urology Departments of Daksi Renal Clinic and Central Hospital University of Constantine and Tizi-Ouzzou cities, Algeria, were included in the study.

All subjects were from the northern region of Algeria, with an age range between 24 and 90 years. All the bladder cancer cases, including 165 males and 10 females, were confirmed by clinical histopathology and staged according to the Tumor-Node-Metastasis (TNM) classification system of the Union International Contre le Cancer (UICC, 1997). Tumors were graded according to the World Health Organization 1973 classification. The control group included healthy individuals without a family history of cancer who were approximately matched for gender proportion, geographic origin, and age range to those in the case group.

After obtaining informed consent from the participants, a detailed questionnaire was administered to both cases and controls to obtain study information.

DNA extraction

Blood samples were obtained from the peripheral veins of each participant using a veno jet-system (Terumo™, France) and collected in a vacutainer EDTA

tube (Dutscher, France). Genomic DNA was extracted from leukocytes using standard NaCl extraction method according to the protocol suggested by Miller and coworkers (1988). The quality of DNA obtained by the recommended method was evaluated by determining A260/A280 ratio using a NanoDrop spectrophotometer (Thermo Scientific, France). DNA samples were stored at –20°C until further analyses.

PCR amplification and sequencing analysis

A fragment of length 771 bp spanning the coding region of *NAT2,* was amplified by Polymerase Chain Reaction (PCR) using the following specific primers: F: CA TGGAGTTGGGCTTAGAGG and R: GGGTGATACAT ACACAAGGGTTT. The reference fragment of *NAT2* gene was taken from Ensembl Genome Browser database (ID: ENSG00000 156006).

The amplification reaction (PCR) was carried out in 20 µl of total reaction mixture containing 2 µl (20 ng) of template DNA, 4 µl (0.2 mM) of dNTPs, 1.2 µl (0.3 µM) each of Oligo F and Oligo R, 2 µl of 1 × buffer, 0.2 µl (0.05 U/µl) of Taq Hotstar (QIAGEN, Germany) and 9.4 µl of H_2O.

PCR amplification was carried out using a Mastercycler pro thermocycler (Eppendorf, France) as follows: an initial heat activation step at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 30 s and extension at 72°C for 1 min, and finally an extension cycle was performed at 72°C for 10 min. The quality of the obtained PCR fragments was assessed by analyzing them on a 1.5% agarose gel. Afterward, the PCR products were purified by using ExoSAP-IT reagent (GE HEALTHCARE, France), which involved the addition of 2 µl of ExoSAP to 5 µl of PCR product. This purification procedure was carried out in two steps and performed by using Mastercycler pro (Eppendorf, France): first step at 37°C for 15 min followed by a second step at 80°C for 15 min. The purified PCR products (10–15 ng of DNA) were then utilized to perform double-stranded sequencing reaction in the presence of the aforementioned primers using the Big Dye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, France). The sequencing reactions were carried out using a Mastercycler pro (Eppendorf, France) under the following cycling conditions: initial denaturation at 96°C for 1 min, and 25 cycles at 96°C for 20 s, 50°C for 15 s, and 60°C for 4 min. The amplicons were directly sequenced using an ABI 3130 XL automated DNA sequencer (Applied Biosystems, France). The sequenced products were analyzed using Sequencher v5.0 software.

Statistical analysis

The characterization and designation of the sequenced alleles were performed using the *NAT2* allele nomenclature published in 1995 (Vatsis et al., 1995).

All the statistical analyses were performed using the R software version 3.2.1. The genotypic and allelic frequencies of the *NAT2* polymorphisms within the two populations were estimated by direct counting method. Chi-square test or Fisher's test was used to calculate the odds ratios (ORs) with the corresponding 95% confidence interval (95% CI) to determine the association between *NAT2* gene polymorphisms or corresponding acetylator phenotypes and susceptibility to bladder cancer, as well as to assess the outcomes following their additive interactions with smoking status. Significance was set at *P*-value less than 0.05.

Results

Subject characteristics

The characteristics of the study population are given in Table 1. As expected, cases and controls appeared to be adequately matched for age and gender with 92.57% males in the case group versus 88.89% males in the control group ($P = 0.28$). The mean ages were 59.57 ± 16.56 and 60.83 ± 14.87 years for the cases and controls respectively ($P = 0.60$). Smoking was more widespread among patients with bladder cancer (69.71%) than among controls (40.21%), considering that controls were taken randomly ($P = 2.78e^{-8}$).

According to the TNM staging of bladder cancer, patients were classified into non-muscle-invasive (Ta = 20.57% and T1 = 47.43%) and muscle-invasive (T2 = 22.86% and T3 = 9.14%) groups. We also classified the patients according to their cancer grade into three groups: well differentiated (G1 = 18.86%), moderately differentiated (G2 = 24%) and poorly differentiated (G3 = 57.14%).

Genotypic and allelic frequencies of NAT2 SNPs and their association with bladder cancer risk

A total of 13 different SNPs have been identified during the sequencing analysis of the *NAT2* coding region

Table1. General characteristics of the bladder cancer patients and controls subjects

Characteristics	Patients % (n = 175)	Controls % (n = 189)	OR (CI 95%)	P-value
Gender				
Males	162 (92.57%)	168 (88.89%)		**0.28** [b]
Females	13 (7.43%)	21 (11.11%)		
Age (years)				
< 50	35 (20%)	42 (22.22%)		
≥ 50	140 (80%)	147 (77.78%)		**0.60** [a]
Cigarette smoking				
Never or former smokers	53 (30.29%)	113 (59.79%)		
Current smokers	122 (69.71%)	76 (40.21%)	**3.42 (2.21–5.28)**	**2.78 e^{-8}** [a]
Grade				
G1	33 (18.86%)			
G2	42 (24%)			
G3	100 (57.14%)			
TNM stage				
Ta	36 (20.57%)			
T1	83 (47.43%)			
T2	40 (22.86%)			
T3	16 (9.14%)			

[a] – Chi-square test; [b] – Fisher test

among the bladder cancer patients and controls representing the Algerian population (Table 2). All those SNPs have been previously described in various studies (Boukouvala, 2016) and no new mutation was identified in this study. The allelic and genotypic frequencies and their calculated ORs are presented in Table 2.

Among the 13 SNPs, T341C and G590A polymorphisms have shown statistically significant association with bladder cancer risk in the patients possessing heterozygous genotype (OR = 2.36, 95% CI = 1.44–3.86 and OR = 2.58, 95% CI = 1.52–4.37, respectively) or mutant homozygous genotype (OR = 2.54, 95% CI = 1.47–4.38 and OR = 2.67, 95% CI = 1.62–4.40, respectively). However, no significant association was observed between the risk of bladder cancer and the occurrence of SNPs G191A, C282T, C345T, C481T, and A803G for genotypic and allelic distributions.

The genotypic and the allelic distributions of C403G and G857A SNP variants in the bladder cancer patients were not significantly different from those of the control group (P= 0.16 and P= 0.8, respectively), and the number of individuals carrying these two polymorphisms was too small (six patients and two controls for C403G SNP, seven patients and nine controls for G857A SNP).

Finally, no evidence has been registered in cancer patients and controls for the presence of *NAT2* A434C, C638T, G838A, and A845C SNPs.

Frequencies of predicted phenotypes and their association with bladder cancer risk

Based on the consensus nomenclature of *NAT2* alleles (Vatsis et al., 1995; Hein et al., 2000b; Boukouvala, 2016), a total of eight previously described alleles have been identified among patients and controls (*NAT2**4, *NAT2**5, *NAT2**6, *NAT2**7, *NAT2**12, *NAT2**13, *NAT2**14, and *NAT2**18); moreover, each allele is characterized by specific SNP, for example, the SNPs T341C, G590A, and G191A are characteristic of the alleles *NAT2**5, *NAT2**6, and *NAT2**14, respectively. Combination of different SNPs for *NAT2**4 wild-type allele, and combination of SNPs for other alleles identi-

fied in our study are presented in Table 3. Furthermore, different combinations of these alleles were used to predict the acetylation phenotype for all the individuals of our study population (Table 4).

In total, out of 175 patients and 189 controls, 85.71% of patients and 70.90% of controls were predicted to be slow acetylators, whereas the rapid/intermediate acetylators were identified in 14.29% and 29.10% of patients and controls, respectively (Table 4).

As presented in Table 4, when compared to the reference group (rapid/intermediate acetylators), the slow acetylation phenotype was associated with an increased risk for bladder cancer (OR = 2.45, 95% CI = 1.41–4.35, P = 0.0009).

When studied separately, the slow acetylators *NAT2**5/5 and *NAT2**5/6 were significantly associated with the development of bladder cancer (OR = 2.91, 95% CI = 1.55–5.45, P = 0.0008 and OR = 3.19, 95% CI = 1.55–6.68, P = 0.0008, respectively).

Effect of smoking status and genetic polymorphisms of NAT2 on bladder cancer risk

The stratification of patients and controls according to *NAT2* genotypes and tobacco consumption (reference group: non-smokers harboring the wild-type genotype for each of the studied SNPs) revealed a significant association between *NAT2* T341C and G590A variants and bladder cancer risk, for both non-smokers and smokers. The risk was found to be escalated in smokers (OR = 7.50, 95% CI = 3.37–17.77, $P = 3.49e^{-8}$ and OR = 13.41, 95% CI = 4.91–40.11, $P = 5.06e^{-9}$ for heterozygous and homozygous *NAT2* T341C variant; OR = 5.60, 95% CI = 2.74–11.81, $P = 2.67e^{-7}$ and OR = 7.29, 95% CI = 3.46–15.91, $P = 7.78e^{-9}$ for heterozygous and homozygous *NAT2* G590A variant).

A significant association has been observed between other mutations of *NAT2* (C345T, C481T, and A803G) and bladder cancer risk among smokers possessing non-mutant homozygous genotype (OR = 2.69, 95% CI = 1.49–4.85, P = 0.0009; OR = 2.81, 95% CI = 1.40–5.78, P = 0.002 and OR = 2.25, 95% CI = 0.94–5.47, P = 0.004, respectively), heterozygous genotype (OR = 3.16, 95% CI = 1.67–6.10, P = 0.0001; OR = 4.77, 95% CI = 2.03–11.61, P = 0.0001; and OR = 2.69, 95% CI = 1.30–5.67, P = 0.004, respectively), and mutant homozygous genotype (OR = 4.11, 95% CI = 2.09–8.29, $P = 1.15e^{-5}$; OR = 6.55, 95% CI = 2.83–15.86, $P = 9.72e^{-7}$; and OR = 3.66, 95% CI = 1.54–9.06, P = 0.001, respectively). This association was not observed in the non-smokers group (Table 5).

In contrast, no significant association has been registered between the risk of bladder cancer and the *NAT2* polymorphisms G191A, C282T, C403G, A434C, C638T, G838A, A845C, G857A among smokers and non-smokers (Table 5).

Association and stratification analysis between the predicted acetylation phenotype, smoking status, and bladder cancer risk

The risk of bladder cancer related to the *NAT2* acetylation phenotype was further investigated by stratification of smoking (Table 6). The results showed no significant association between the patients and controls for the rapid/ intermediate acetylation phenotype among smokers group (P = 0.46).

In contrast, the slow acetylation phenotype has been associated with an increased risk of bladder cancer in the smokers group (OR = 6.77, 95% CI = 2.67–18.93, $P = 7.20e^{-6}$); however the association was not significant in the case of non-smokers group (P = 0.52).

Discussion

The prevalence of bladder cancer was found to be high in Algeria. There has been an alarming increase in the number of newly diagnosed year after year; in the recent decades, the incidence of bladder cancer increased from 2.2 to 8.7 per 100 000 persons (Hamdi Cherif et al., 2010; Hamdi Cherif et al., 2014). Various previous epidemiological studies on humans showed the presence of a relationship between the *NAT2* genotype and its acetylation profile with the increased risk of urinary bladder cancer (Garcia-Closas et al., 2005; Song et al., 2009; Pesch et al., 2013). The lack of sufficient knowledge regarding the profile of the acetylator phenotype in the Algerian population is a short coming that may affect the success of treatment. It is well-known that Arylamine N- acetyltransferases play an important role in the detoxification of xenobiotic compounds such as therapeutic drugs. It has been reported that clinical consequences in drugs therapies are applied differently for slow and rapid acetylators (Meisel, 2002).

The sequencing of the *NAT2* coding region, revealed that only T341C and G590A polymorphisms have been significantly associated with an increased risk of bladder cancer in the tested population. This result is partially in

Table 2. Genotypic and allelic frequencies of NAT2 SNPs and their association with bladder cancer risk

	SNP	Genotypes and alleles	N [%]		*P*-value	OR	CI (95%)
			patients	controls			
rs 1801279	**G191A**	GG	125 (71.43)	146 (77.24)		1.00 (reference)	
		GA	21 (12.00)	13 (06.89)	0.10 [b]	1.88 [b]	0.85–4.27
		AA	29 (16.57)	30 (15.87)	0.77 [b]	1.12 [b]	0.61–2.06
		G	271 (77.43)	305 (80.69)			
		A	79 (22.57)	73 (19.31)	0.28 [a]	1.05 [a]	0.78–1.41
rs1041983	**C282T**	CC	110 (62.86)	126 (66.67)		1.00 (reference)	
		CT	35 (20.00)	32 (16.93)	0.41 [a]	1.25 [a]	0.72–2.15
		TT	30 (17.14)	31 (16.40)	0.72 [b]	1.10 [b]	0.63–1.94
		C	255 (72.86)	284 (75.13)			
		T	95 (27.14)	94 (24.87)	0.48 [a]	1.12 [a]	0.80–1.56
rs 1801280	**T341C**	TT	44 (25.14)	85 (44.97)		1.00 (reference)	
		TC	77 (44.00)	63 (33.34)	**0.0006** [a]	**2.36** [a]	**1.44–3.86**
		CC	54 (30.86)	41 (21.69)	**0.0007** [a]	**2.54** [a]	**1.47–4.38**
		T	165 (47.14)	233 (61.64)			
		C	185 (52.86)	145 (38.36)	**$9.17e^{-5}$** [a]	**1.80** [a]	**1.34–2.41**
rs 45532639	**C345T**	CC	87 (49.72)	125 (66.14)		1.00 (reference)	
		CT	45 (25.71)	36 (19.05)	0.02 [a]	1.79 [a]	1.07–3.01
		TT	43 (24.57)	28 (14.81)			
		C	219 (62.57)	286 (75.66)			
		T	131 (37.43)	92 (24.34)	0.18 [a]	0.55 [a]	0.22–1.27
rs12720065	**C403G**	CC	169 (96.57)	187 (98.94)		1.00 (reference)	
		CG	6 (03.43)	2 (01.06)	0.16 [b]	3.30 [b]	0.58–33.95
		GG	0 (00.00)	0 (00.00)			
		C	344 (98.29)	376 (99.47)			
		G	6 (01.71)	2 (00.53)	0.16 [b]	3.27 [b]	0.58–33.37
rs72554616	**A434C**	AA	175 (100.00)	189 (100.00)		1.00 (reference)	
		AC	0 (00.00)	0 (00.00)			
		CC	0 (00.00)	0 (00.00)			
		A	350 (100.00)	378 (100.00)			
		C	0 (00.00)	0 (00.00)			
rs1799929	**C481T**	CC	71 (40.57)	95 (50.26)		1.00 (reference)	
		CT	54 (30.86)	48 (25.40)	0.1 [a]	1.50 [a]	0.91–2.47
		TT	50 (28.57)	46 (24.34)	0.14 [a]	1.45 [a]	0.87–2.40
		C	196 (56.00)	238 (62.96)			
		T	154 (44.00)	140 (37.04)	0.05 [a]	1.33 [a]	0.99–1.79
rs1799930	**G590A**	GG	50 (28.57)	97 (51.32)		1.00 (reference)	
		GA	56 (32.00)	42 (22.22)	**0.0003** [a]	**2.58** [a]	**1.52–4.37**
		AA	69 (39.43)	50 (26.46)	**0.0001** [a]	**2.67** [a]	**1.62–4.40**
		G	156 (44.57)	236 (62.43)			
		A	194 (55.43)	142 (37.57)	**$1.55e^{-6}$** [a]	**2.06** [a]	**1.53–2.77**

rs138707146	**C638T**	CC	175 (100.00)	189 (100.00)		1.00 (reference)	
		CT	0 (00.00)	0 (00.00)			
		TT	0 (00.00)	0 (00.00)			
		C	350 (100.00)	378 (100.00)			
		T	0 (00.00)	0 (00.00)			
rs 1208	**A803G**	AA	46 (26.28)	55 (29.10)		1.00 (reference)	
		AG	82 (46.86)	83 (43.92)	0.51[a]	1.18 [a]	0.71–1.94
		GG	47 (26.86)	51 (26.98)	0.73 [a]	1.10 [a]	0.63–1.92
		A	174 (49.71)	193 (51.06)			
		G	176 (50.29)	185 (48.94)	0.71 [a]	1.05 [a]	0.78–1.41
rs 563935	**G838A**	GG	175 (100.00)	188 (99.47)		1.00 (reference)	
		GA	0 (00.00)	1 (00.53)			
		AA	0 (00.00)	0 (00.00)			
		G	350 (100.00)	377 (99.74)			
		A	0 (00.00)	1 (00.26)			
rs 56054745	**A845C**	AA	173 (98.86)	189 (100.00)		1.00 (reference)	
		AC	2 (01.14)	0 (00.00)			
		CC	0 (00.00)	0 (00.00)			
		A	348 (99.43)	378 (100.00)			
		C	2 (00.57)	0 (00.00)			
rs179931	**G857A**	GG	168 (96.00)	180 (95.24)		1.00 (reference)	
		GA	7 (04.00)	9 (04.76)	0.8 [b]	0.83 [b]	0.25–2.57
		AA	0 (00.00)	0 (00.00)			
		G	343 (98.00)	369 (97.62)			
		A	7 (02.00)	9 (02.38)	0.8 [b]	0.83 [b]	0.26–2.55

[a] – Chi-square test; [b] – Fisher test

agreement with the study of Brokmoller et al. (1996) comprising 374 cases and 373 controls from Germany, where T341C but not G590A SNP was associated with bladder cancer risk. It has been reported that mutations at these two positions result in the production of non-synonymous SNPs that subsequently lead to a significant reduction in the NAT2 protein activity due to protein degradation (Hein, 2006; Zang et al., 2007; Selinski et al., 2013; Boukouvala, 2016). Furthermore Zang et al. (2007) and Walraven et al. (2008) demonstrated that both T341C and G590A SNPs are associated with a reduction in N- and O-acetylation capacity in different bacterial and eukaryotic expression systems.

On the other hand, no association has been found between the *NAT2* polymorphisms G191A, C282T, C345T, C481T, and A803G and bladder cancer risk in our study population. Similar results were observed in a meta-analysis conducted by Selinski et al. (2013). This may be explained by differences in the nature of the SNPs and the mechanism by which they affect the NAT2 enzyme activity. Actually, it has been reported that C282T and C481T SNPs are synonymous changes and seem to exert no effect on NAT2 acetylation capacity or protein stability (Zang et al., 2007; Selinski et al., 2013; Boukouvala, 2016). It has been shown that G191A, C345T, and A803G SNPs are missense mutations that cause amino acid changes in a mature protein (Walraven et al., 2008; Toure et al., 2012; Boukouvala, 2016). Polymorphisms C345T and A803G seem to have no functional effect on NAT2 enzyme, in contrast to the G191A SNP which has been associated with reduced catalytic activity (Toure et al., 2012; Selinski et al., 2013; Boukouvala, 2016).

NAT2 G191A SNP variant presents equal allelic and genotypic distributions between the controls and pa-

Table 3. Nature of NAT2 alleles identified in the study population

	G191A	C282T	T341C	C345T	C403G	C481T	G590A	A803G	G838A	A845C	G857A
NAT2*4	**G**	**C**	**T**	**C**	**C**	**C**	**G**	**A**	**G**	**A**	**G**
NAT2*5			**C**								
*5A			**C**			T					
*5B			**C**			T		G			
*5C			**C**					G			
*5D			**C**								
*5E			**C**				A				
*5G		T	**C**			T		G			
*5J		T	**C**				A				
*5K		T	**C**								
*5 (341+590+803)			**C**				A	G			
*5 (341+345)			**C**	T							
NAT2*6							**A**				
*6A		T					**A**				
*6B							**A**				
*6C		T					**A**	G			
*6E						T	**A**				
*6F							**A**	G			
*6I		T					**A**		A		A
*6J		T					**A**				A
*6L		T		T			**A**				
*6N		T				T	**A**				
*6 (282+590+838)		T					**A**		A		
*6 (590+857)							**A**				A
*6 (481+590+857)						T	**A**				A
NAT2*7											**A**
*7A											**A**
*7B		T									**A**
*7C		T						G			**A**
NAT2*12								**G**			
*12A								**G**			
*12B		T						**G**			
*12 (282+481+803)		T				T		**G**			
NAT2*13		**T**									
*13A		**T**									
*13 (282+838)		**T**							A		
NAT2*14	**A**										
*14A	**A**										
*14B	**A**	T									
*14D	**A**	T					A				
*14G	**A**	T						G			
*14 (191+282+838)	**A**	T							A		
NAT2*18										**C**	

Table 4. Nat 2 genotypes and predicted phenotypes frequencies in patients vs controls subjects

	Predicted phenotype	Patients [%]	Controls [%]	*P*-value	OR (CI 95%)
NAT2 *5/*5	slow	53 (30.29)	40 (21.16)	**0.0008** [a]	**2.91 (1.55–5.45)**
NAT2 *5/*6	slow	41 (23.43)	28 (14.81)	**0.0008** [b]	**3.19 (1.55–6.68)**
NAT2 *5/*7	slow	03 (01.71)	04 (2.12)		
NAT2 *5/*14	slow	02 (01.14)	00 (00.00)		
NAT2 *6/*6	slow	15 (08.57)	30 (15.87)		
NAT2 *6/*7	slow	00 (00.00)	02 (1.06)		
NAT2 *7/*14	slow	00 (00.00)	02 (1.06)		
NAT2 *6/*14	slow	17 (09.71)	03 (1.59)		
NAT2 *14/*14	slow	19 (10.86)	25 (13.23)		
Total slow		**150 (85.71)**	**134 (70.90)**	**0.0009** [a]	**2.45 (1.41–4.35)**
NAT2 *4/*6	intermediate	02 (01.14)	10 (5.29)		
NAT2 *4/*7	intermediate	01 (00.57)	00 (00.00)		
NAT2 *4/*14	intermediate	02 (01.14)	09(4.76)		
NAT2 *5/*12	intermediate	04 (02.29)	12 (6.35)		
NAT2 *5/*13	intermediate	00 (00.00)	02 (1.06)		
NAT2 *6/*12	intermediate	00 (00.00)	03 (1.59)		
NAT2 *6/*13	intermediate	01 (00.57)	03 (1.59)		
NAT2 *4/*5	intermediate	12 (06.86)	09 (4.76)		
NAT2 *4/*4	rapid	00 (00.00)	02 (1.06)		
NAT2 *4/*12	rapid	01 (00.57)	01 (0.52)		
NAT2 *12/*13	rapid	00 (00.00)	02 (1.06)		
NAT2 *13/*14	rapid	01 (00.57)	02 (1.06)		
NAT2 *14/*18	rapid	01 (00.57)	00 (00.00)		
Total rapid/intermediate		**25 (14.29)**	**55 (29.10)**		1 (Reference)

[a] – Chi-square test; [b] – Fisher test

tients and does not show an association with urinary bladder cancer in our population study. This non-association can be explained by the very low number of patients harboring the change compared with those carrying the wild-type *NAT2* genotype (Table 2), despite the fact that G191A variant is more frequent in Africans (Hein, 2006; Sabbagh et al., 2008).

Our results also showed no association with bladder cancer risk for C403G and G857A SNPs. This may be related to the less number of subjects carrying these SNPs in our population study (Table 2). Patin et al. (2006) and Teixeira et al. (2007) suggested that the *NAT2*C403G SNP may or may not alter the enzyme activity depending on the population it is expressed in. On the other hand, the G857A polymorphism has been identified by Toure et al. (2012) as a substrate-dependent inactivating mutation and has been found to be more frequent in South India and Korea than in the other tasted populations (Hein, 2006).

The remaining SNPs (A434C, C638T, G838A, and A845C) were absent in both patients and controls. This can be interpreted by the fact that the nature and frequency of the *NAT2* polymorphisms vary remarkably between the populations of different ethnic origins. In this context, it has been demonstrated by Patin et al. (2006) and Teixeira et al. (2007) that these four SNPs occur at a low frequency in some populations like Brazilian and Sub-Saharan African populations and may or may not alter the *NAT2* acetylation activity.

Table 5. Nat2 polymorphisms and bladder cancer risk stratified by smoking

	Non-smokers				Smokers			
	Patients [%]	Controls [%]	*P*-value	OR (CI 95%)	Patients [%]	Controls [%]	*P*-value	OR (CI 95%)
G191A								
GG	50 (94.34)	70 (61.95)		1 (reference)	75 (61.48)	76 (100.00)	0.18 [a]	1.38 (0.85–2.23)
GA	3 (05.66)	13 (11.50)	0.10 [b]	0.32 (0.05–1.27)	18 (14.75)	0 (00.00)		
AA	0 (00.00)	30 (26.55)			29 (23.77)	0 (00.00)		
C282T								
CC	30 (56.60)	50 (44.25)		1 (reference)	80 (65.57)	76 (100.00)	0.05 [b]	1.75 (0.97–3.17)
CT	10 (18.87)	32 (28.32)	0.15 [b]	0.52 (0.19–1.28)	25 (20.49)	0 (00.00)		
TT	13 (24.53)	31 (27.43)	0.43 [b]	0.70 (0.28–1.63)	17 (13.94)	0 (00.00)		
T341C								
TT	12 (22.64)	52 (46.02)		1 (reference)	32 (26.23)	33 (43.42)	**0.0003** [b]	**4.15 (1.78–10.19)**
TC	19 (35.85)	30 (26.55)	**0.02** [b]	**2.71 (1.08–7.08)**	58 (47.54)	33 (43.42)	**3.49e^{-8}** [b]	**7.50 (3.37–17.77)**
CC	22 (41.51)	31 (27.43)	**0.008** [b]	**3.04 (1.24–7.77)**	32 (26.23)	10 (13.16)	**5.06e^{-9}** [b]	**13.41 (4.91–40.11)**
C345T								
CC	47 (88.68)	95 (84.07)		1 (reference)	40 (32.78)	30 (39.47)	**0.0009** [b]	**2.69 (1.49–4.85)**
CT	4 (07.55)	10 (08.85)	1 [b]	0.80 (0.17–2.99)	41 (33.61)	26 (34.21)	**0.0001** [b]	**3.16 (1.67–6.10)**
TT	2 (03.77)	8 (07.08)	0.5 [b]	0.50 (0.05–2.68)	41 (33.61)	20 (26.32)	**1.15e^{-5}** [b]	**4.11 (2.09–8.29)**
C403G								
CC	52 (98.11)	113 (100.00)		1 (reference)	117 (95.90)	74 (97.37)	**3.54e^{-8}** [a]	**3.43 (2.21–5.32)**
CG	1 (01.89)	0 (00.00)			5 (04.10)	2 (02.63)	0.04 [b]	5.37 (0.84–58.11)
GG	0 (00.00)	0 (00.00)			0 (00.00)	0 (00.00)		
A434C								
AA	53 (100.00)	113 (100.00)		1 (reference)	122 (100.00)	76 (100.00)	**2.78e^{-8}** [a]	**3.42 (2.21–5.28)**
AC	0 (00.00)	0 (00.00)			0 (00.00)	0 (00.00)		
CC	0 (00.00)	0 (00.00)			0 (00.00)	0 (00.00)		
C481T								
CC	20 (37.73)	50 (44.25)		1 (reference)	51 (41.80)	45 (59.21)	**0.002** [b]	**2.81 (1.40–5.78)**
CT	23 (43.40)	32 (28.32)	0.1 [b]	1.78 (0.79–4.05)	31 (25.41)	16 (21.05)	**0.0001** [b]	**4.77 (2.03–11.61)**
TT	10 (18.87)	31 (27.43)	0.6 [b]	0.80 (0.29–2.09)	40 (32.79)	15 (19.74)	**9.72e^{-7}** [b]	**6.55 (2.83–15.86**
G590A								
GG	20 (37.73)	74 (65.49)		1 (reference)	30 (24.60)	23 (30.26)	**2.37e^{-5}** [b]	**4.76 (2.17–10.74)**
GA	10 (18.87)	12 (10.62)	**0.02** [b]	**3.04 (1.02–9.03)**	46 (37.70)	30 (39.48)	**2.67e^{-7}** [b]	**5.60 (2.74–11.81)**
AA	23 (43.40)	27 (23.89)	**0.003** [b]	**3.12 (1.40–7.08)**	46 (37.70)	23 (30.26)	**7.78e^{-9}** [b]	**7.29 (3.46–15.91)**
C638T								
CC	53 (100.00)	113 (100.00)		1 (reference)	122 (100.00)	76 (100.00)	**2.78e^{-8}** [a]	**3.42 (2.21–5.28)**
CT	0 (00.00)	0 (00.00)			0 (00.00)	0 (00.00)		
TT	0 (00.00)	0 (00.00)			0 (00.00)	0 (00.00)		
A803G								
AA	20 (37.74)	35 (30.97)		1 (reference)	26 (21.31)	20 (26.32)	**0.004** [b]	**2.25 (0.94–5.47)**
AG	20 (37.74)	43 (38.06)	0.69 [b]	0.81 (0.35–1.87)	62 (50.82)	40 (52.63)	**0.004** [b]	**2.69 (1.30–5.67)**
GG	13 (24.52)	35 (30.97)	0.39 [b]	0.65 (0.25–1.62)	34 (27.87)	16 (21.05)	**0.001** [b]	**3.66 (1.54–9.06)**

G838A								
GG	53 (100.00)	113 (100.00)		1 (reference)	122 (100.00)	75 (98.68)	**2.09e^{-8}** [a]	**3.46 (2.24–5.35)**
GA	0 (00.00)	0 (00.00)			0 (00.00)	1 (01.32)		
AA	0 (00.00)	0 (00.00)			0 (00.00)	0 (00.00)		
A845C								
AA	53 (100.00)	113 (100.00)		1 (reference)	120 (98.36)	76 (100.00)	**4.45e^{-8}** [a]	**3.36 (2.17–5.19)**
AC	0 (00.00)	0 (00.00)			2 (01.64)	0 (00.00)		
CC	0 (00.00)	0 (00.00)			0 (00.00)	0 (00.00)		
G857A								
GG	51 (96.23)	108 (95.58)		1 (reference)	117 (95.90)	72 (94.74)	**4.86e^{-8}** [a]	**3.44 (2.20–5.36)**
GA	2 (03.77)	5 (04.42)	1 [b]	0.84 (0.07–5.39)	5 (04.10)	4 (05.26)	0.4 [b]	0.63 (0.17–2.27)
AA	0 (00.00)	0 (00.00)			0 (00.00)	0 (00.00)		

[a] – Chi-square test; [b] – Fisher test

With regard to phenotype, the results show that slow acetylators are most frequent in the Algerian population (85.71% for patients vs 70.90% for controls) and that NAT2*5 is the most represented allele (Table 4). These findings are in accordance with those reported by Guaoua et al (2014) for Moroccan population. Furthermore, we registered a strikingly significant association between slow acetylator phenotypes and bladder cancer risk, consistent with several previous studies (Garcia-Closas et al., 2005; Gu et al., 2005; Rothman et al., 2007; Song et al., 2009; Moore et al., 2011; Pesch et al., 2013). Additionally, it has been reported that the balance between activation and detoxification of carcinogens affects the extent of DNA damage that occurs in the cells (Safarinejad et al., 2013). Individuals possessing a NAT2 slow acetylation phenotype are less efficient in detoxifying the arylamines and other environmental carcinogens. The metabolites of arylamines are transported through the circulatory system and are absorbed into the bladder epithelium. The metabolic activation results in the acquisition of carcinogenic properties through the formation of DNA adducts that may eventually lead to the development of bladder cancer (Zang et al., 2004).

Interestingly, some slow acetylator phenotypes showed a significant association with bladder cancer risk, such as those corresponding to *NAT2**5/5 and *NAT2**5/6 genotypes (Table 4). This finding has been confirmed by several earlier studies which showed that urinary bladder cancer risk was higher in the individuals possessing *NAT2**5 allele, and proposed enhanced protein degradation as the mechanism for the reduced amounts of NAT2 protein and activity (Brockmoller et al., 1996; Okkels et al., 1997; Zang et al., 2004; El Desoky et al., 2005).

The classification of patients and controls according to the *NAT2* polymorphisms and smoking status showed that *NAT2* T341C and G590A SNPs displayed a strong correlation with bladder cancer risk, an association that exhibited an increasing trend with cigarette consumption (Table 5).

C403G, A434C, C638T, G838A, A845C, and G857A SNP variants of *NAT2* have shown an association with the homozygous wild-type genotype for each variant only in tobacco smokers (Table 5). Our findings confirm that cigarette smoking is the predominant risk factor for bladder cancer (OR = 3.42, 95% CI = 2.21–5.28), as shown previously in Table 1 and as observed in other published data (Brennan et al., 2000; Zeegers et al., 2004). Moreover, for a long time, carcinogens found in tobacco have been implicated in bladder cancer etiology among smokers (Probst-Hensch et al., 2000). On the other hand, besides the large number of *NAT2* polymorphisms reported, polymorphisms in other genes such as N-acetyltransferase 1 (*NAT1*) and glutathione S-transferases M1 and T1 (*GSTM1* and *GSTT1*) can also alter the ability of xenobiotic enzymes to metabolize carcinogens (Khedhiri et al., 2010).

No association was registered for the G191A and C282T SNPs with bladder cancer risk in non-smokers (Table 5). However, association of these SNPs with the bladder cancer risk in smokers could not be estimated because of their rare occurrence within the control group.

Table 6. Association of the predicted phenotype, smoking status and the bladder cancer risk

Predicted phenotypes	No-smokers				Smokers			
	patients [%]	controls [%]	P-value	OR (CI 95%)	patients [%]	controls [%]	P-value	OR (CI 95%)
Rapid/intermediate	8	23		1 (reference)	17	32	0.46	1.51 (0.51–4.80)
Slow	45	90	0.52	1.43 (0.56–4.01)	105	44	**$7.20e^{-6}$**	**6.77 (2.67–18.93)**

Also, no association has been noted between the *NAT2*-C345T, C481T, and A803G SNPs and bladder cancer susceptibility in non-smokers; however, in the smoker category, these polymorphisms have shown a remarkable association with the development of bladder cancer (Table 5). This could be explained by the fact that the joint effects of NAT2 polymorphisms and smoking status exhibit a strong biological effect and increase the risk of bladder cancer (Garcia-Closas et al., 2005).

In this study, we also showed that the NAT2 slow-acetylator phenotype was significantly associated with bladder carcinogenesis among smokers (Table 6). This gene-environment interaction has strong biological effect on NAT2 enzyme activity because slow acetylators are unable to detoxify the aromatic amines rapidly by N-acetylation. Moreover, tobacco is considered to be the first source of exposure to aromatic amines, which are suspected to be the primary carcinogens for bladder cancer in cigarette (Tao et al., 2012). Our findings are in agreement with several previous studies (Brockmoller et al., 1996; Hein, 2006; Moore et al., 2011). Furthermore, many other studies provided evidence for the fact that the NAT2 slow acetylation phenotype interacts with tobacco smoking as a function of exposure intensity rather than exposure duration (Lubin et al., 2007; Yuan et al., 2008; Moore et al., 2011). It has been suggested that among the slow acetylators, detoxification of arylamines present in cigarette smoke may became saturated at higher smoking intensity, thus minimizing the effect of NAT2 detoxification to a greater extent in slow acetylators (Lubin et al., 2007; Moore et al., 2011).

Conclusions

NAT2 T341C and G590A SNPs are found to be risk factors for bladder cancer. NAT2 slow acetylator phenotype is associated with higher risk of bladder cancer, with the greatest risk observed for the allele NAT2*5 harboring the *NAT2* T341C SNP.

We also found that tobacco smoking interacts additively with *NAT2* T341C and G590A SNPs in influencing the bladder cancer risk. Moreover, *NAT2* C345T, C481T, and A803G SNPs were associated with bladder cancer risk only in patients of the smokers group.

Furthermore, our analysis provides strong evidence for the fact that smoking increases the risk of bladder cancer in NAT2 slow acetylators.

Our findings support the need for the conductance of future studies on the potential gene–gene and gene–environment interactions among larger sample sizes.

Acknowledgements

The authors acknowledge Prof. Abderrezak Dahdouh, Prof. Taoufik Djaghri (Department of Urology and Renal Transplantation, Daksi Renal Clinic of Constantine) and Prof. Nacer Balasla (Department of Urology, University Hospital of Tizi-Ouzou) for their support. They also extend their thanks to Prof. François Radvanyi and all the staff of the Laboratory of Molecular Oncology, Institut Curie, Paris, France for their help and assistance.

References

Boukouvala S. (2016) Human NAT2 alleles (haplotypes). http://nat.mbg.duth.gr/Human%20NAT2%20alleles_2013.htm. Accessed 03 Dec 2017.

Brennan P., Bogillot O., Cordier S., Greiser E., Schill W., Vineis P., Lopez-Abente G., Tzonou A., Chang-Claude J., Bolm-Audorff U. et al. (2000) *Cigarette smoking and bladder cancer in men: a pooled analysis of 11 case-control studies*. Int. J. Cancer. 86(2): 289–294.

Brokmoller J., Cascorbi I., Kerb R., Roots I. (1996) *Combined analysis of inherited polymorphisms in arylamine N-acetyltransferase 2, glutathione S-transferases Ml and Ti, microsomal epoxide hydrolase, and cytochrome P450 enzymes as modulators of bladder cancer risk*. Cancer Res. 56(17): 3915–3925.

Carreón T., Hein M.J., Viet S.M., Hanley M.W., Ruder A.M., Ward E.M. (2010) *Increased bladder cancer risk among workers exposed to O-toluidine and aniline: a reanalysis*. Occup. Environ. Med. 67(5): 348–350.

Chu H., Wang M., Zhang Z. (2013) *Bladder cancer epidemiology and genetic susceptibility*. J. Biomed. Res. 27: 170–178.

Cohen S.M., Shirai T., Steineck G. (2000) *Epidemiology and etiology of premalignant and malignant urothelial changes*. Scand. J. Urol. Nephrol Suppl. 205: 105–115.

Di Pietro G., Gadelha S.R., Sousa S.M.B., Santana de Melo P.R., Rios Santos F. (2012) *N-acetyltransferase 2: slow, intermediate or fast? A booming question of the molecular epidemiology in cancer research*. Open J. Genet. 2: 221–235.

Dolin P.J. (1992) *A descriptive study of occupation and bladder cancr in England and Wales*. Br. J. Cancer. 65(3): 467–468.

El Desoky E.S., AbdelSalam Y.M., Salama R.H., El Akkad M.A., Atanasova S., von Ahsen N., Armstrong V.W., Oellerich M. (2005) *NAT2*5/*5 genotype (341T > C) is a potential risk factor for schistosomiasis-associated bladder cancer in Egyptians*. Ther. Drug Monit. 27(3): 297–304.

Ferlay J., Ervik M., Lam F., Colombet M., Mery L., Piñeros M., Znaor A., Soerjomataram I., Bray F. (2018) *Global cancer observatory: cancer today*. International Agency for Research on Cancer. https://gco.iarc.fr/today. accessed 1 Feb 2019.

Freedman N.D., Silverman D.T., Hollenbeck A.R., Schatzkin A., Abnet C.C. (2011) *Association between smoking and risk of bladder cabcer among men and women*. JAMA. 306: 737–745.

García-Closas M., Malats N., Silverman D., Dosemeci M., Kogevinas M., Hein D.W., Tardón A., Serra C., Carrato A., García-Closas R. 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.

Gu J., Liang D., Wang Y., Lu C., Wu X. (2005) *Effects of N-acetyltransferase 1 and 2 polymorphisms on bladder cancer risk in Caucasians*. Mutat. Res.-Genet. Toxicol. Environ. Mutagen. 581: 97–104.

Guaoua S., Ratbi I., Laarabi F.Z., Chafai Elalaoui S., Charkaoui Jaouad I., Barkat A., Sefiani A. (2014) *Distribution of allelic and genotypic frequencies of NAT2 and CYP2E1 variants in Moroccan population*. BMC Genet. 15: 156–161.

Hamdi Cherif M., Zaidi Z., Abdellouche D., Hamdi S., Lakhdari N., Djema Benjazia A., Laouamri S., Mahnane A., Moussaoui H., Kadri L., et al. (2010) *Cancer registry of Setif (Algeria): cancer incidence, trend and survival, 1986- 2005*. Afr. J. Cancer 2: 245–258.

Hamdi Cherif M., Serraino D., Mahnane A., Laouamri S., Zaidi Z., Boukharouba H., Cherka D., Rakeb M., Kara L., Ayat A., et al. (2014) *Time trends of cancer incidence in Setif, Algeria, 1986- 2010: an observasional study*. BMC Cancer. 14: 637–644.

Hein D.W., Doll M.A., Fretland A.J., Leff M.A., Webb S.J., Xiao G.H., Devanaboyina U.S., Nangju N.A., Feng Y. (2000a) *Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms*. Cancer Epidemiol. Biomarkers Prev. 9(1): 29–42.

Hein D.W., Grant D.M., Sim E. (2000b) *Update on consensus arylamine N-acetyltransferase gene nomenclature*. Pharmacogenetics 10(4): 291–292.

Hein D.W. (2002) *Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis*. Mutat. Res. 506–507: 65–77.

Hein D.W. (2006) *N-acetyltransferase 2 genetic polymorphism: effects of carcinogen and haplotype on urinary bladder cancer risk*. Oncogene 25(11): 1649–1658.

Inatomi H., Katoh T., Kawamoto T., Matsumoto T. (1999) *NAT2 gene polymorphism as a possible marker for susceptibility to bladder cancer in Japanese*. Int. J. Urol. 6: 446–454.

Khedhiri S., Stambouli N., Ouerhani S., Rouissi K., Marrakchi R., Gaaied A.B., Slama M.B. (2010) *The impact of smoking and polymorphic enzymes of xenobiotic metabolism on the stage of bladder tumors: a generalized orderedlogistic regression analysis*. J. Cancer Res. Clin. Oncol. 136(7): 1111–1116.

Lubin J.H., Kogevinas M., Silverman D., Malats N., García-Closas M., Tardón A., Hein D.W., García-Closas R., Serra C., Dosemeci M. et al. (2007) *Evidence for an intensity-dependent interaction of NAT2 acetylation genotype and cigarette smoking in the Spanish Bladder cancer study*. Int. J. Epidemiol. 36: 236–241.

Meisel P. (2002) *Arylamine N-acetyltransferases and drug response*. Pharmacogenomics 3(3): 349–366.

Miller S.A., Dykes D.D., Polesky H.F. (1988) *A simple salting out procedure for extracting DNA from human nucleated cells*. Nucl. Acids Res. 16(3): 1215.

Moore L.E., Baris1 D.R., Figueroa J.D., García-Closas M., Karagas M.R., Schwenn M.R., Johnson A.T., Lubin J.H., Hein D.W., Dagnall C.L., et al. (2010) *GSTM1 null and NAT2 slow acetylation genotypes, smoking intensity and bladder cancer risk: results from the New England bladder cancer study and NAT2 meta-analysis*. Carcinogenesis 32(2): 182–189.

Okkels H., Sigsgaard T., Wolf H., Autrup H. (1997) *Arylamine N-Acetyltransferase 1 (NA Ti) and 2 (NAT2) polymorphisms in susceptibility to bladder cancer: the influence of smoking*. Cancer Epidemiol. Biomarkers Prev. 6(4): 225–231.

Patin E., Harmant C., Kidd K., Kidd J., Kidd J., Froment A., Mehdi S.Q., Sica L., Heyer E., Quintana-Murci L. (2006) *Sub-Saharan African coding sequence variation and haplotype diversity at the NAT2 gene*. Hum. Mutat. 27(7): 720.

Pesch B., Gawrych K., Rabstein S., Weiss T., Casjens S., Rihs H.P., Ding H., Angerer J., Illig T., Klopp N., et al. (2013) *N-acetyltransferase 2 phenotype, occupation, and bladder cancer risk: results from the EPIC Cohort*. Cancer Epidemiol. Biomarkers Prev. 22(11): 2055–2065.

Pira E., Piolatto G., Negri E., Romano C., Boffetta P., Lipworth L., McLaughlin J.K., La Vecchia C. (2010) *Bladder cancer mortality of workers exposed to aromatic amines: a 58-year follow-up*. J. Natl. Cancer Inst. 102(14): 1096–1099.

Probst-Hensch N.M., Bell D.A., Watson M.A., Skipper P.L., Tannenbaum S.R., Chan K.K., Ross R.K., Yu M.C. (2000) *N-acetyltransferase 2 phenotype but not Nat1*10 genotype affects aminobiphenyl-hemoglobin adduct levels*. Cancer Epidemiol. Biomarkers Prev. 9: 619–623.

Rihs H.P., Spickenheuer A., Heinze E., Pesch B., Raulf-Heimsoth M., Angerer J., Brüning T. (2011) *Modulation of urinary polycyclic aromatic hydrocarbon metabolites by enzyme polymorphisms in workers of the German Human Bitumen Study*. Arch. Toxicol. 85(suppl. 1): S73–S79.

Rothman N., García-Closas M., Hein D.W. (2007) *Commentary: reflections on G. M. Lower and colleagues' 1979 study associating slow acetylator phenotype with urinary bladder cancer: meta-analysis, historical refinements of the hypothesis, and lessons learned*. Int. J. Epidemiol. 36: 23–28.

Sabbagh A., Langaney A., Darlu P., Gerard N., Krishnamoorthy R., Poloni E.S. (2008) *Worldwide distribution of NAT2 diversity: implications for NAT2 evolutionary history*. BMC Genet. 9: 21–34.

Safarinejad M.R., Safarinejad S., Shafiei N., Safarinejad S. (2013) *Association of genetic polymorphism of glutathione S-transferase (GSTM1, GSTT1, GSTP1) with bladder cancer susceptibility*. Urol. Oncol. 31(7): 1193–1203.

Sanderson S., Salanti G., Higgins J. (2007) *Joint effects of the N-Acetyltransferase 1 and 2 (NAT1 and NAT2) genes and smoking on bladder carcinogenesis: a literature-based systematic HuGE review and evidence synthesis*. Am. J. Epidemiol. 166: 741–751.

Selinski S., Blaszkewicz M., Ickstadt K., Hengstler J.G., Golka K. (2013) *Refinement of the prediction of N-acetyltransferase 2 (NAT2) phenotypes with respect to enzyme activity and urinary bladder cancer risk*. Arch. Toxicol. 87: 2129–2139.

Song D.K., Xing D.L., Zhang L.R., Li Z.X., Liu J., Qiao B.P. (2009) *Association of NAT2, GSTM1, GSTT1, CYP2A6, and CYP2A13 gene polymorphisms with susceptibility and clinicopathologic characteristics of bladder cancer in Central China*. Cancer Detect. Prev. 32: 416–423.

Tao L., Xiang Y.B., Chan K.K., Wang R., Gao Y.T., Yu M.C., Yuan J.M. (2012) *Cytochrome P4501A2 phenotype and bladder cancer risk: the Shanghai bladder cancer study*. Int. J. Cancer. 130(5): 1174–1183.

Teixeira R.L.F., Miranda A.B., Pacheco A.G., Lopes M.Q.P., Fonseca Costa J., Rabahi M.F., Melo H.M., Kritski A.L., Mello F.C.Q., Suffys P.N., et al. (2007) *Genetic pro le of the arylamine N-acetyltransferase 2 coding gene among individuals from two different regions of Brazil*. Mutat. Res. 624: 31–40.

Toure A., Diop C., Cabral M., Fall M., Lhermitte M., Diouf A., Broly F., Allorge D. (2012) *Study of NAT2 genetic polymorphism in West African subjects: example of an healthy non-smoker Senegalese population*. Mol. Biol. Rep. 39: 10489–10496.

Tsai C.W., Hsu C.F., Tsai M.H., Tsou Y.A., Hua C.H., Chang W.S., Lin C.C., Bau D.T. (2011) *Methylenetetrahydrofolate reductase (MTHFR) genotype, smoking habit, metastasis and oral cancer in Taiwan*. Anticancer Res. 31(6): 2395–2399.

Vatsis K.P., Weber W.W., Bell D.A., Dupret J.M., Evans D.A., Grant D.M., Hein D.W., Lin H.J., Meyer U.A., Relling M.V. (1995) *Nomenclature for N-acetyltransferases*. Pharmacogenetics 5(1): 1–17.

Viel J.F., Challier B. (1995) *Bladder cancer among French farmers: does exposure to pesticides in vineyards play a part?* Occup. Environ. Med. 52(9): 587–592.

Walker K., Ginsberg G., Hattis D., Johns D.O., Guyton K.Z., Sonawane B. (2009) *Genetic polymorphism in N-acetyltransferase (NAT): population distribution of NAT1 and NAT2 activity*. J. Toxicol. Environ. Health B Crit. Rev. 12: 440–472.

Walraven J.M., Zang Y., Trent J.O., Hein D.W. (2008) *Structure/function evaluations of single nucleotide polymorphisms in human N-acetyltransferase 2*. Curr. Drug Metab. 9: 471–486.

Ward E.M., Sabbioni G., DeBord D.G., Teass A.W., Brown K.K., Talaska G.G., Roberts D.R., Ruder A.M., Streicher R.P. (1996) *Monitoring of aromatic amines exposures in workers at a chemical plant with a known bladder cancer excess*. J. Natl. Cancer Inst. 88(15): 1046–1052.

Yuan J.M., Chan K.K., Coetzee G.A., Castelao J.E., Watson M.A., Bell D.A., Wang R., Yu M.C. (2008) *Genetic determinants in the metabolism of bladder carcinogens in relation to risk of bladder cancer*. Carcinogenesis 29(7): 1386–1393.

Zang Y., Zhao S., Doll M.A., States J.C., Hein D.W. (2004) *The T341C (Ile114Thr) polymorphism of N-acetyltransferase 2 yields slow acetylator phenotype by enhanced protein degradation*. Pharmacogenetics 14(11): 717–723.

Zang Y., Doll M.A., Zhao S., States J.C., Hein D.W. (2007) *Functional characterization of single-nucleotide polymorphisms and haplotypes of human N-acetyltransferase 2*. Carcinogenesis 28(8): 1665–1671.

Zeegers M.P., Kellen E., Buntinx F., Van den Brant P.A. (2004) *The association between smoking, beverage consumption, diet and bladder cancer: a systematic literature review*. World J. Urol. 21(6): 392–401.

Zhu Z., Zhang J., Jiang W., Zhang W., Li Y., Xu X. (2015) *Risks on N-acetyltransferase 2 and bladder cancer: a meta-analysis*. Onco. Targets Ther. 8: 3715–3720.