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CYP1A2 polymorphisms, occupational and environmental exposures and risk of bladder cancer

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Abstract Cytochrome P4501A2 (*CYP1A2*) is a key enzyme for activation of bladder carcinogens. Polymorphisms in the 5′-noncoding promoter region of *CYP1A2* gene [mainly *-2467T/delT*(rs35694136) and *-163C/A*(rs762551)], are crucial in modifying *CYP1A2* activity in smokers. Within the framework of a hospital-based case/control study, we investigated the relationship between *CYP1A2* polymorphisms, occupational/environmental exposures and bladder cancer (BC) risk. The study population included 185 BC cases and 180 non-cancer controls, all Caucasian males. Data were collected on lifetime smoking, coffee drinking, dietary habits and lifetime occupation, with particular reference to exposure to aromatic amines (AAs) and polycyclic aromatic hydrocarbons (PAHs). A case-only design was applied to study the interaction between *CYP1A2 -2467T/delT* (or *-163C/A*) and occupational and environmental factors. Multiple logistic regression showed a significantly increased risk among heavy smokers (≥ 50 packyears; OR 5.6, 95% CI: 2.5–12.5) and heavy coffee drinkers (> 5 cups/day; OR 3.1, 95% CI: 1.2–7.9). Exposure to AAs showed a significant trend of BC risk with increasing

cumulative exposure (CE) ($P = 0.04$), with heavy smoking as possible confounder. A decreased risk was noted for large leaf vegetable consumption, with significant trend from < 1 /month to > 3 times/week ($P = 0.008$). The case-only analysis showed an interaction between *-2467T/delT* and tobacco smoking > 25 packyears ($P = 0.04$); no interaction was detected between such polymorphisms and coffee consumption, dietary habits and occupational exposure to AAs. No effects were shown with *-163C/A* genotype as well as no overall effect of *CYP1A2* by itself on BC risk. This is the first study suggesting that *CYP1A2 -2467T/delT* modifies the effect of cigarette smoking on BC risk.

Keywords Bladder cancer · *CYP1A2* genotype · Occupational/environmental exposures

Introduction

Cytochrome P4501A2 (*CYP1A2*) is a key phase I enzyme necessary to activate the major recognized bladder carcinogens, i.e., aromatic amines (AAs) and polycyclic aromatic hydrocarbons (PAHs), which require metabolic activation before exerting their damaging effects [1]. The formation of reactive metabolites of AAs and PAHs and their binding to DNA to give stable adducts are considered to be critical steps in their carcinogenic process. High *CYP1A2* activity was therefore suggested as a susceptibility factor for bladder cancer (BC) as well as lung and colon cancer, when exposure to AAs and PAHs which are present in the main stream of tobacco smoke has been implicated in the etiology of such diseases [2]. Increased *CYP1A2* activity was associated to the inducibility of *CYP1A2* [1, 3, 4] by a number of environmental factors, such as AAs, PAHs, heterocyclic amines, nitrosamines,

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whose exposure occurs via tobacco smoking, occupational exposure, diet [5–7]. More recently, inducibility of CYP1A2 was also associated to the occurrence of specific polymorphisms in the CYP1A2 gene. In our previous works, we showed that polymorphisms, all located in the 5′-noncoding promoter region (–2467T/delT(rs35694136) and –163C/A(rs762551) having the main effect on CYP1A2 activity) are crucial in increased CYP1A2 activity of smokers, measured by the urinary caffeine metabolic ratio (CMR). The same polymorphisms were found to play a major role in rising urinary mutagenicity of smokers [8]. Moreover, CYP1A2 –2467T/delT polymorphism was found to be a significant risk modifier of smoking-induced lung cancer [9]. CYP1A2 activity may therefore be a modulating factor along the continuum from the exposure to relevant environmental and occupational exposures and certain cancers. However, to the best of our knowledge, no molecular epidemiology study has been published about the role of CYP1A2 polymorphisms on the interaction between occupational and environmental exposures and risk of BC.

BC is among the most frequent diagnosed cancer in the world [10]. In Italy, BC is the 4th incident cancer among males and the 11th among females, with an estimated number of about 16,000 new BC diagnosed among males during 1998–2002 [11, 12]. Tobacco smoking and occupations involving AAs and PAHs exposures are the main known causes of BC [12, 13].

Case-controls and cohort studies overall support a protective effect of vegetables or, more consistently, fruit consumption on BC risk, generally reporting a small decrease of risk significant only for fruit intake and a modification of the association by variants of metabolic genes [14–17]. On the other hand, two recent prospective cohort studies do not find any association between fruit and vegetable consumption, combined or separately, and BC risk [18, 19].

Coffee drinking has been classified as a possible human carcinogen (group 2B) by IARC [20] on the basis of overall limited evidence in human carcinogenicity data and considering bladder among the target organs; caffeine is oxidatively demethylated by CYP1A2. Recent epidemiological studies found negative, dubious or slightly positive association between coffee drinking and BC [21–26].

Results, of the relationships among some covariates and variation of other metabolic genes (i.e., *GST*, *NAT*, *SULT*, *CYP1B1*), and genes that modulate oxidative stress and DNA repair on BC risk, were reported in our previous papers [21, 27–29]. Aims of this study were to search for new genetic characteristics which might contribute to individual susceptibility to BC, and to further understand the role of CYP1A2 genetic polymorphisms –2467T/delT (rs35694136) and –163C/A (rs762551), in modulating the

relationship between occupational and environmental exposures and BC risk. We analyzed the association between CYP1A2 genetic polymorphisms –2467 T/delT and –163 C/A within the framework of a hospital-based case-control study conducted in Brescia, a highly industrialized area in Northern Italy, with a case-only study design.

Materials and methods

Study population

The design was a hospital-based case-control study. The inclusion criteria were being male, aged between 20 and 80, resident in the Brescia province. The cases were newly diagnosed, histologically confirmed BC patients, admitted in the Urology departments of the two main hospitals of Brescia. The controls were patients affected by various urological non-neoplastic diseases (i.e., hydronephrosis, urolithiasis, malformative urological diseases, prostatic adenoma and hypertrophy, urological traumas, orchiepididymitis, hydrocele), frequency matched to cases by age (± 5 years), period and hospital of admission. From July 1997 to December 2000, 216 incident BC cases were admitted in the two hospital and 220 hospital eligible controls were enrolled. The response rate was 93% in cases and 97% in controls, leading to 201 cases and 214 controls recruited in the study. DNA to evaluate CYP1A2 genetic polymorphism was available for 185 cases and 180 controls; the –2467T/delT variant was determined in 167 cases and 141 controls and the –163C/A variant in 155 cases and 148 controls.

Cases were affected mainly by transitional cell carcinoma (181, 98%); 3 were squamous cell carcinoma and 1 was a neuroendocrine tumor. Controls included patients with ICD-9 code 591 (4%), 592 (39%), 223 (1%), 252 (1%), 596 (1%), 598 (5%), 599 (2%), 600 (39%), 603 (3%), 788 (2%), 959 (3%).

Sample size estimation

For the case-control study, the required sample size was estimated assuming: p_0 (prevalence of controls exposed to AAs) = 0.05 (this percentage was chosen because the carcinogenic potential of AA exposure was the main hypothesis of the case-control study); OR (relative risk of bladder cancer associated with AA exposure) = 4.50; α (type I error rate) = 0.05; β (type II error) = 0.80. Using the method suggested by Schlesselman [30] each sample (cases or controls) had to include 83 individuals. The actual sample was enlarged (actually including 185 cases and 185 controls) to take into the alpha level, the desired statistical

power level, the anticipated effect size, and the number of predictors in the multiple logistic regression analysis [31].

The required sample size for the case-only study was estimated according to Yang [32]. Since nonsmokers and exsmokers were excluded from this analysis, the actual number of bladder cancer cases was 67. The corresponding minimum sample size had to be 14 or 118, respectively, if packyears were dichotomized as: 25–99 and ≥ 100 ; or 25–49 and ≥ 50 (see below Table 4). These estimates were obtained using the formula 5 of Yang [32], where the variance of the logarithm of OR for interaction under the alternative hypothesis was based on the frequencies of our Table 4, and the null variance was calculated from the marginal totals of the same Table 4.

Data collection

A written informed consent was obtained from each recruited subject and the study was approved by the local Ethical Committee. All subjects were administered a face-to-face questionnaire by trained interviewers. Data were collected on demographic variables, active and passive lifetime smoking history, alcohol and coffee consumption (all types), fluid intake, diet via a food-frequency questionnaire (fruits, vegetables, PAHs containing food, such as toasted, fried, smoked and grilled food and pizza), lifetime occupational history, including each job lasting for at least 1 year, and leisure activities. Job titles and industrial activities were coded according to the International Standard Classification of Occupation (ISCO, International Labour Office, 1968) and International Standard Classification of all Industrial Activities (ISIC, United Nations, 1968) by two occupational physicians, blind to case/control status; major disagreement (i.e., 1st or 2nd digit), was discussed and resolved. For each specific job title, the subject's exposure to PAHs or AAs was assessed based on the information collected in the questionnaire (including plant activity, type of production, exposures to chemicals, detailed description of workplaces, job tasks, use of personal protective devices and hygienic behaviours in workplaces) or provided by records on risk assessments and personal knowledge of local industries and literature data. According to the methodology described in previous studies, the occupational exposure to AAs and PAHs in each job, was classified as possible, probable or definite (reliability) and scored for mode of contact (respiratory only, dermal only, or both respiratory and dermal), intensity (low, medium or high), and frequency (low as 1–5% of the working time, medium as 5.1–30%, high as more than 30%). More detailed information regarding study population and data collection are described in previous publications [21, 27–29, 33] and is available upon request.

Genotyping

Genotype assays on DNA were carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for two CYP1A2 polymorphic sites ($-2467T/delT$ and $-163C/A$) as previously described [8]. Briefly all PCR reactions (25 μ l) were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Italy), with each mastermix (Applied Biosystems, Italy) comprising 0.2 mM dNTPs, 1 U of Taq polymerase, the appropriate concentration of MgCl₂ (1.75 and 1.25 mM) and 0.4 μ M of each primer (Invitrogen Life Technologies, Italy). Variants $-2467T/delT$ and $-163C/A$ were identified by NdeI and ApaI restriction enzymes purchased from New England Biolabs (Italy) [9]. Quality-control measures were adopted in genotyping, such as validation of results by using the RT-PCR method and blind repeat of 10% of samples.

Statistical analysis

The statistical analysis was performed including several categorical and interval variables. Tobacco smoking was categorized as never smokers, former smokers, and current smokers and packyears (life-long consumption of cigarettes) were calculated. As regards occupational exposure to AAs and/or PAHs, an index of cumulative exposure (CE) was calculated as the product of duration of each job entailing the exposure (l) and the reliability (r), intensity (i) and frequency (f) scores ($r \times i \times f \times l$), summing up as many products as were necessary to take into account all the different jobs done. The time (years) since first exposure (TSFE) and time (years) since last exposure (TSLE) to AAs and PAHs, separately, were also determined. The life-long time-weighted average of cups/day of coffee was recoded as 0 (never drinkers), ≤ 3 , 4, ≥ 5 cups/day. PAHs containing food, fruits, large leaf vegetable and other vegetables consumption was divided into four categories (less than once/month; less than once/week; 1–3 times/week; more than 3 times/week).

Using STATA 10, univariable logistic regression models were fitted to obtain the Odds Ratio (OR) and the 95% confidence interval (CI) for BC, in the different classes or categories of explanatory variables. Explanatory variables (those proved to be associated with the outcome) were forced in the model and then selected by backwards stepwise selection (with $P < 0.05$ as criteria) using the multivariable unconditional logistic regression analysis. Nevertheless, some of the variables whose likelihood ratio (LR) test was not statistically significant ($P > 0.05$) were still retained in the model if resulting in being a substantial confounder (i.e., a variable whose omission from the model resulted in at least one stratum specific OR of one variable changing by at least 10% of its original value).

To find out whether the genotype was in Hardy–Weinberg equilibrium, distribution of the observed and expected genotype frequencies were compared using a chi-square test. Bladder cancer cases only were classified in a table according to environmental factors (rows) and genotypes (columns), and an unconditional logistic regression model was used, to assess the interaction. The OR (with CI, and *P*-value) expresses the linear trend of bladder cancer risk with increasing environmental exposures in subjects with high activity compared to those with low activity of CYP1A2. As suggested by our previous studies on the relationship between CYP1A2 activity and genotype [8], we grouped subjects in two categories those with and without increasing CYP1A2 activity alleles (*CYP1A2* –2467*delT* and –163A).

Results

The mean age was 63 years among cases (N. 185) and 62 among controls (N. 180); age of cases and controls did not differ significantly (*P* = 0.218). With regard to BC risk factors, on average cases smoked 23.1 (±24.3) packyears, drunk 1.1 (±0.9) cups of coffee/day, had 18.2 (±40.4) years of cumulative occupational exposure to PAHs and 5.6 (±26.9) years to AAs. Among controls, mean tobacco smoking was 14.7 (±22.4) packyears, coffee drinking 0.8 (±0.7) cups/day, cumulative occupational exposure to PAHs was 18.2 (±40.4) years and 1.0 (±6.7) years to AAs. Univariable logistic regression models show significant association between BC and smoking, packyears, coffee, large leaf vegetables, occupational CE and TSLE to AAs (Table 1). No association was detected for age, and exposure to PAHs (CE, TSFE, TSLE; data not shown).

Smoking categories, packyears, coffee intake (cups/day), vegetable intake, CE_AAs, TSFE_AAs, and TSLE_AAs, proved to be associated with the outcome (Table 1), were forced in a model of multivariable unconditional logistic regression and showed a significantly increased risk of BC with packyears (especially for ≥50 packyears) and heavy coffee drinkers (especially for ≥5 cups/day). A decreased BC risk was noted for large leaf vegetable consumption (especially, for consumption > once/week). Even though the confidence intervals of OR cross 1, here also appears a non-significant association between occupational AAs cumulative exposure and BC risk, after taking into account the effect of all the other variables. Neither interaction nor effect modification were found (data not shown).

The multivariable logistic regression analysis showed that the only potential confounder of the association between BC risk and cumulative exposure to AAs was heavy tobacco smoking (packyears; *P* < 0.000), while the *P*-values of coffee (*P* = 0.05), and vegetables (*P* = 0.05)

were all borderline, suggesting weak evidence about their confounding role.

Table 2 shows the observed and expected genotypes for *CYP1A2* –2467*delT* (determined in 167 cases and 141 controls) and –163C/A (determined in 155 cases and 148 controls). The expected genotype frequencies were not significantly different from the observed frequencies in BC cases and controls separately considered, indicating that they were in Hardy–Weinberg equilibrium. The frequencies are in line with those found in our previous study on healthy Caucasian volunteers in which *CYP1A2* –2467*delT* and –163A frequency were 24 and 67% respectively [9]. The distributions of the *CYP1A2* –2467*delT* or –2467*delT/delT* and –163A/C or A/A genotypes among BC cases and controls was similar, indicating no overall effect of these polymorphisms in BC risk.

No interaction/modification of BC risk was detected between polymorphisms (*CYP1A2* –2467*delT* and –163A/C genotypes) and smoking status, coffee consumption, large leaf vegetable consumption, cumulative exposure to AAs and PAHs (Table 3).

Table 4 shows a significant interaction (case-only study) between the increasing *CYP1A2* activity genotype (*CYP1A2* –2467*delT* or –*delT/delT*) and tobacco consumption (>25 packyears) in current-smoker (*P* = 0.04). No significant interaction/modification of effect was shown analyzing the –163C/A genotype (Table 4).

Discussion

This work, besides sustaining the role of known personal habits such as cigarette smoking (especially for ≥50 packyears) in increasing BC risk, suggest a probable role of occupational exposure to AAs and heavy coffee drinking (especially for >5 cups/day) and the significant protective effect for large leaf vegetables consumption on BC risk, confirming previous observations in the literature [14–17]. The associations of AAs cumulative exposure and heavy coffee drinking with BC risk seem, however, to be weakened the first by smoking habit, that appears to be an important confounder, and the latter might be attributed to residual confounding by inadequate adjustment for cigarette smoking (which is over-represented among those who drink the most coffee/caffeine) [23]. Moreover our results suggest that the effect of heavy cigarette smoking on the risk of BC is modulated by *CYP1A2* –2467*delT* polymorphism.

To the best of our knowledge, this is the first study showing that functional *CYP1A2* –2467*delT* polymorphism modifies the effect of cigarette smoking on the risk of BC, indicating that high CYP activity might be a susceptibility factor for BC in smokers. The present work is in

Table 1 Distribution of bladder cancer cases and controls by risk indicators

	Cases	Controls	Univariate analysis OR (95% CI)	P-trend	Multivariate analysis OR ^o (95% CI)
<i>Smoking classes</i>					
No smokers	13	36	Reference	<0.001	
Ex smokers	81	93	2.41 (1.20–4.86)		
Smokers	91	51	4.94 (2.40–10.2)		
<i>Packyears</i>					
0	13	37	Reference	<0.001	Reference
1–24.9	32	60	1.52 (0.71–3.26)		1.36 (0.63–2.98)
25–49.9	51	43	3.38 (1.59–7.15)		2.95 (1.37–6.35)
≥50	89	40	6.33 (3.04–13.2)		5.63 (2.54–12.5)
<i>Coffee drinking (cups/day)</i>					
0	43	55	Reference	0.002	Reference
≤3	103	109	1.21 (0.75–1.96)		1.20 (0.71–2.03)
4	16	8	2.56 (1.00–6.53)		2.52 (0.93–6.83)
≥5	23	8	3.68 (1.50–9.03)		3.05 (1.17–7.94)
<i>Large leaf vegetables intake (No. servings)</i>					
<1/month	15	3	Reference	0.008	Reference
<1/week	13	7	0.37 (0.08–1.74)		0.33 (0.06–1.69)
1–3/week	66	70	0.19 (0.05–0.68)		0.19 (0.05–0.74)
>3/week	91	100	0.18 (0.05–0.65)		0.20 (0.05–0.76)
<i>AAs: cumulative exposure^a</i>					
Not exposed	169	173	Reference	0.042	Reference
1–24	7	5	1.43 (0.45–4.60)		1.04 (0.29–3.78)
≥25	9	2	4.61 (0.98–21.6)		4.29 (0.86–21.5)
<i>AAs: TSFE^b (years)</i>					
Not exposed	169	173	Reference	0.086	
1–36	7	3	2.39 (0.61–9.39)		
≥37	9	4	2.30 (0.70–7.62)		
<i>AAs: TSLE^c (years)</i>					
Not exposed	171	175	Reference	0.052	
1–16	7	3	2.39 (0.61–9.39)		
≥17	7	2	3.58 (0.73–17.5)		
<i>PAHs: cumulative exposure^a</i>					
Not exposed	119	117	Reference	0.966	
1–24	35	31	1.11 (0.64–1.92)		
≥25	31	32	0.95 (0.55–1.66)		
<i>PAHs: TSFE^b (years)</i>					
Not exposed	119	117	Reference	0.727	
1–36	32	33	0.95 (0.55–1.56)		
≥37	34	30	1.11 (0.64–1.94)		
<i>PAHs: TSLE^c (years)</i>					
Not exposed	123	119	Reference	0.987	
1–16	31	31	0.97 (0.55–1.69)		
≥17	31	30	1.00 (0.57–1.75)		

Odds ratio (OR) and 95% confidence interval (95% CI) estimated at univariate (with *P* value for trend) and multivariate logistic regression analysis after backward selection of the independent variables

^oOdds ratio adjusted each for the effect of other factors present in the final model of logistic regression

^a Sum of products ($r \times i \times f \times l$); where *r* reliability, *i* intensity, *f* frequency; and *l* length of exposure

^b TSFE: time since first exposure

^c TSLE: time since last exposure

Table 2 *CYP1A2* $-2467T/delT$ and $-163C/A$ distribution and OR in bladder cancer cases and controls

	Cases		Controls		OR	95% CI
	Observed number	Expected number ^a	Observed number	Expected number ^a		
$-2467T/T$	95	94	69	70	Ref. 0.73	0.46–1.14
$-2467T/delT$ or $delT/delT$	72	74	72	72		
$-163C/C$	10	15	16	15	Ref. 1.75	0.77–4.15
$-163A/C$ or A/A	145	140	132	133		

^a According to Hardy–Weinberg

Table 3 Distribution of bladder cancer cases according to environmental variables (rows) and genotypes (columns), and results (OR, 95% CI, and *P*-value) of unconditional logistic regression to assess interaction between the two

Environmental variables	Levels	Genotypes			
		$-2467T/delT$ or $delT/delT$	T/T	$-163A/C$ or A/A	C/C
Smoking	Never smokers	0	5	11	2
	Former smokers	36	42	65	2
	Current smokers	36	48	69	6
		OR ^a = 1.16; 95% CI 0.67–2.02; <i>P</i> = 0.58		OR ^a = 1.0; 95% CI 0.36–2.72; <i>P</i> = 1.0	
Coffee	Never drinkers	21	14	28	4
	<4 cups/day	37	56	81	6
	4 cups/day	10	16	23	0
	≥5 cups/day	4	9	13	0
		OR ^a = 0.79; 95% CI 0.60–1.04; <i>P</i> = 0.09		OR ^a = 2.74; 95% CI 0.94–8.01; <i>P</i> = 0.06	
Vegetables	Never	9	5	12	0
	<1 times/week	5	6	11	1
	1–3 times/week	25	36	47	4
	>3 times/week	33	48	75	5
		OR ^a = 0.78; 95% CI 0.56–1.10; <i>P</i> = 0.16		OR ^a = 0.84; 95% CI 0.39–1.83; <i>P</i> = 0.67	
CE_AAs ^b	0	66	86	132	9
	1	3	3	7	0
	2	3	6	6	1
		OR ^a = 0.86; 95% CI 0.45–1.66; <i>P</i> = 0.66		OR ^a = 0.75; 95% CI 0.23–2.47; <i>P</i> = 0.64	
CE_PAHs ^b	0	51	56	88	7
	1	11	21	30	1
	2	10	18	27	2
		OR ^a = 0.74; 95% CI 0.49–1.12; <i>P</i> = 0.15		OR ^a = 1.14; 95% CI 0.48–2.68; <i>P</i> = 0.75	

^a ORs and 95% CI were calculated by unconditional logistic regression

^b Sum of products ($r \times i \times f \times l$); where *r* reliability, *i* intensity, *f* frequency; and *l* length of exposure

line with our previous results demonstrating that *CYP1A2* $-2467T/delT$, but not the other analyzed intronic variants ($-3860G/A$, $-734C/A$, $-163C/A$), was a significant risk modifier of tobacco smoking-induced lung cancer [9]; moreover, recent works, which, however, did not analyse $-2467T/delT$, reported no influence of variants $-163C/A$ and $-734C/A$ on risk of BC [34, 35]. A possible explanation of such results may be related to the specific site of our examined deletion variant that is located in a key position -2467 intron 1– of *CYP1A2* induction mechanism, i.e., within the binding region (positions -2495 and

-2000 [36]) of the xenobiotic responsive element (XRE). Pathway of *CYP1A2* induction is complex and still not fully known. It involves polyaromatic chemicals, such as those present in cigarette smoke, that act as ligands for the Ah receptor (AhR) [36, 37]. In the nucleus, the stimulated ligand-AhR, forming a heterodimer with the Ah receptor nuclear translocator (Arnt), activates the transcription of *CYP1A2*, in its control region [37, 38], by binding the XRE actually in positions -2495 and -2000 [36]. An increased transcription rate and, partly, the increased stability of mRNA, are the result of this *CYP1A2* inductive binding

Table 4 Distribution of bladder cancer cases according to packyears (rows) and genotypes (columns), and results (OR, 95% CI, and *P*-value) of unconditional logistic regression to assess interaction between the two

Packyears	−2467T/delT or delT/delT	T/T	Totals	−163A/C or A/A	C/C	Totals
25–49	0	2	2	2	0	2
50–99	17	28	45	36	4	40
≥100	12	8	20	15	2	17
Total	29	38	67	53	6	59
	OR ^a = 2.83			OR ^a = 0.71		
	95% CI = 1.02–7.80			95% CI = 0.14–3.68		
	P = 0.045			P = 0.688		

^a ORs and 95% CI were calculated by unconditional logistic regression

effect [37, 38]. Our results would suggest that *CYP1A2* −2467T/delT polymorphism located inside the binding region of AhR/Arnt heterodimer may modify the binding sites (chromatin structure) [36], thereby increasing the *CYP1A2* induction mechanism by tobacco smoke components. A rising in mRNA expression level has already been reported for similar polymorphisms in the 5′-promoter region of the *CYP2E1* gene [39].

Our data are in line with the assumption that high *CYP1A2* activity genetically determined could be a susceptible factor for BC in smokers [1]. Tobacco smoke is a very complex mixture containing hundreds of human carcinogens [40]. The excess of BC in smokers seems to be mainly attributed to arylamines, in particular to the well-established human carcinogen the arylamine 4-aminobiphenyl (4-ABP) [41–43]. 4-ABP requires metabolic activation to form reactive electrophiles, whose binding to human bladder epithelium DNA [44, 45], inducing p53 mutations [46] and genetic instability [47], was detected and thought to be essential for its carcinogenic effect. The N-oxidation of ABP to N-hydroxy-ABP, catalyzed primarily by hepatic cytochrome P4501A2 [48], is believed to be the first critical step in this bioactivation [49]. Although *CYP1A2* is primarily expressed in human liver, the activated N-hydroxyl metabolites generated in the liver was found to be transported to the bladder where, under acidic conditions [50, 51] or, enzymatically, by O-acetylation of N-hydroxy arylamine (predominantly by the N-acetyltransferase 1 (NAT1) isozyme), the compound is further activated to the ultimate carcinogenic DNA-reactive metabolite [52]. Extensive *CYP1A2* activity was significantly related to the increase in body content of mutagens [1, 53–55], and in some other indicators of genotoxic risk, such as DNA and protein adducts [41, 56] when exposure to AAs occurs. Moreover, experimental studies also showed that in dogs treated with ABP, N-hydroxy-ABP and the subsequent ABP-DNA adduct formation in bladder epithelium are directly dependent [57]. Our data would suggest that higher *CYP1A2*

activity, smoking-induced and −2467T/delT genetically related/modified, may increase the formation of the key N-hydroxylamine [43].

Among the strengths of the research, the good characterization of the population with thorough and reliable collection of several personal, occupational, environmental variables and genetic endpoints. The case-only study design is also an issue; it is a powerful method for studying gene-environment interactions because it enables to achieve greater statistical power than a case-control study of the same sample size [32]. Moreover, it should be underlined that not only the *CYP1A2* polymorphisms we studied are common in the general population and BC is a frequent cancer especially in males, but also exposure to AAs and PAHs, known bladder carcinogens, might be still significantly prevalent in male occupations, and *CYP1A2* is involved in their metabolism. Therefore, such polymorphisms may have an impact in public health and it should be characterized in high risk population. Finally the result of this study appear to be biologically plausible and the direction of the effects is consistent with the a priori expectations also based on available literature data on both polymorphisms. In this regard, we should mention that the results of this research are in the same direction of our previous studies [9] on *CYP1A2* −2467T/delT polymorphism as significant risk modifier of smoking-induced lung cancer [8, 9].

Among the limitations of the present study is the small sample size, in particular of the case-only study. Here, the actual number of 67 cases (showed in Table 4) was higher than 14 but lower than 118, the minimum required sample sizes estimated according to different assumptions. Therefore it is uncertain whether the case-only study had a sufficient statistical power. However, it should be noticed, that, opposing heavy smokers (≥100 packyears) to light/medium smokers, the required sample size was 14 and therefore study power could be sufficient.

The case-control study, including 365 observations, was presumed to possess sufficient statistical power to detect a

significant effect [30]. However, the influence of AAs in bladder carcinogenesis is still uncertain from the final model of multivariable logistic regression. Heavy tobacco smoking was the only potential confounder in the association between BC risk and cumulative exposure to AAs. This confounding effect could also have a biological significance since AAs are present in the inhaled smoke of cigarettes [40]. The biological significance of our findings might also be uncertain, due to the complex mechanism of CYP1A2 induction, still not fully known. Moreover, since few observations would suggest the existence of gene–gene interactions in bladder carcinogenesis [58–60], the hypothesis of an interaction of this polymorphism with other susceptible genetic variants relevant for carcinogen metabolism or DNA repair will be tested in our study population.

Finally, in this case-control study, the hospital controls were selected among patients affected by non-neoplastic urological diseases. This selection might introduce a bias if the occurrence of the diseases eligible for the control status is associated with the genotype of interest. However, the distribution of the genetic polymorphisms among our control group were under HWE, arguing against selection bias. Moreover, the variability of diseases among controls could minimize the occurrence of such a bias. To the best of our knowledge, there are no evidence of association of the diseases of the controls with CYP1A2 variability nor with the variables analyzed.

In conclusion, this work confirms the role of cigarette smoking (especially for ≥ 50 packyears), in increasing BC risk and propose a significant protective effect for large leaf vegetables consumption.

The new finding stemming from this study, that sustains *CYP1A2 -2467T/delT* as modifier of the effect of heavy cigarette smoking also on BC risk, generates the hypothesis that this new genetic functional characteristic contributes to individual susceptibility to BC. This hypothesis should be considered in a larger study together with gene–gene interaction analysis of this polymorphism with the other susceptible genetic variants for BC.

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