NAT2, *XRCC1* and *hOGG1* Polymorphisms, Cigarette Smoking, Alcohol Consumption and Risk of Upper Aerodigestive Tract Cancer

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Abstract. *Aim: To evaluate associations between polymorphisms of the N-acetyltransferase 2 (NAT2), human 8-oxoguanine glycosylase 1 (hOGG1) and X-ray repair cross-complementing protein 1 (XRCC1) genes and risk of upper aerodigestive tract (UADT) cancer. Patients and Methods: A case–control study involving 117 cases and 224 controls was undertaken. The NAT2 gene polymorphisms were genotyped by automated sequencing and XRCC1 Arg399Gln and hOGG1 Ser326Cys polymorphisms were determined by Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism (PCR-RFLP) methods. Results: Slow metabolization phenotype was significantly associated as a risk factor for the development of UADT cancer (p=0.038). Furthermore, haplotype of slow metabolization was also associated with UADT cancer (p=0.014). The hOGG1 Ser326Cys polymorphism (CG or GG vs. CC genotypes) was shown as a protective factor against UADT cancer in moderate smokers (p=0.031). The XRCC1 Arg399Gln polymorphism (GA or AA vs. GG genotypes), in turn, was a protective factor against UADT cancer only among never-drinkers (p=0.048). Conclusion: Interactions involving NAT2, XRCC1 Arg399Gln and hOGG1 Ser326Cys polymorphisms may modulate the risk of UADT cancer in this population.*

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Upper aerodigestive tract (UADT) cancer corresponds to tumors of the oral cavity, larynx and pharynx. These tumors represent the eighth most common cause of cancer-related death worldwide, accounting for 5% of all new diagnosed cancers (1). The main risk factors associated with UADT are smoking and alcohol consumption (2, 3). Polymorphisms that affect the function of genes involved in activation and de-toxification of carcinogenic compounds, bioactivation of pro-carcinogens and which repair DNA damage can influence the risk of cancer (4). Genetic association studies have demonstrated a relationship between polymorphisms in *N*-acetyltransferase 2 (*NAT2*), X-ray repair cross-complementing protein 1 (*XRCC1*) and human 8-oxoguanine glycosylase 1 (*hOGG1*) genes and risk for various types of cancer such as bladder, colorectal, breast, prostate, pancreatic and lung (5-7). NAT2 is a key phase II enzyme that participates in the bioconversion of aromatic and heterocyclic amines present in, for example, cigarette smoke (8). According to the activity and stability determined by DNA sequence variations, the *NAT2* gene may be classified into slow, intermediate or rapid alleles (9). Previous studies demonstrated that different rates of acetylation may be associated with various tobacco-associated cancers (8, 10). The DNA repair enzyme XRCC1 plays a central role in the base excision repair pathway (11). The *XRCC1 Arg399Gln*

polymorphism (exon 10, base G > A, amino acid Arg > Gln, rs25487) results in a non-conservative amino acid change and may alter XRCC1 function (12). In turn, the human *hOGG1* gene, an important multifunctional gene involved in BER, plays a role in repairing damaged DNA (13). The most extensively studied *Ser326Cys* polymorphism (rs1052133) is located in exon 7 of *hOGG1*, and results in a substitution of serine (Ser) to cysteine (Cys) at codon 326, which has been reported to affect hOGG1 function (14). The characterization of mutations and genetic polymorphisms may be useful in determining individual and population susceptibility to cancer, leading to new opportunities for prevention, early diagnosis and genetic counseling. The aim of the present study was to evaluate the association of polymorphisms in *NAT2, XRCC1* and *hOGG1* genes with the risk of development of UADT cancer.

Patients and Methods

Participants. The cases (n=117) were recruited at the High Complexity Oncology Center at Itabuna and at the Oncology Clinic at Ilhéus, both cities located in the state of Bahia, Brazil, from February 2008 to August 2009. Healthy population-based controls (n=224) were recruited in the same region of origin of cases, matched for age, sex and self-reported skin color. Demographic information on lifestyle and dietary habits were obtained from a socioeconomic questionnaire answered by both groups. Those who reported consuming at least one cigarette per day for a minimum period of one year were reported as smokers. Pack-year calculations were performed by multiplying the number of packs of cigarettes smoked per day by the total exposure time in years. According to this criterion, cases and controls were categorized as never-smokers (pack-years=0), moderate-smokers (≤20 pack-years), and heavy-smokers (>20 pack-years). Regarding alcohol intake, individuals who reported drinking alcohol at a frequency of twice week or more, for a minimum of one year, were considered drinkers. From each participant enrolled in the study approximately 3 ml of peripheral blood was collected during therapy for DNA extraction. The study was approved by the Ethics Committees of participating institutions (protocol number 134/07).

DNA Extraction and polymorphisms identification. Peripheral blood (3 ml) was collected from each participant and subjected to DNA extraction. Polymorphisms *XRCC1* Arg399Gln and *hOGG1* Ser326Cys were analyzed using Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism (PCR-RFLP) as described previously (15). Fourteen *NAT2* gene single nucleotide polymorphisms (SNPs) were analyzed by sequencing: C190T, G191A, C282T, T341C, G363A, A411T, A434C, C481T, G499A, G590A, C759T, A803G, G857A and A845C. The *NAT2* PCR reaction was carried out with the primers NAT2_1F (5'AAAAGGGATTCA TGCAGTAGA3') and NAT2_1R (5'AAA TAACGTGAGGGTA GAGAGG3'). The PCR product (1141 bp) was sequenced in ABI3500 equipment (Applied Biosystems, Foster City, CA, USA) with the same PCR primers and additionally with two internal primers, NAT2_1seq (5'GTTAACA AATACAGCACTGGCA3') and NAT2_2seq (5'TGCC AGTGC TGTATTTGTTAAC3'), for full coverage of the fragment. Eight ancestry informative markers (AIMs) were genotyped to correct possible effect of population stratification between cases and controls (SB, APO, AT3, PV92, LPL, MID93, MID52, MID575 (16).

Statistical analysis. To assess differences in genotypic and allelic frequencies between cases and controls, homogeneity tests using statistic χ^2 were performed. Fisher's exact test was applied for the same purpose when the expected value for a cell of the contingency table was less than 5. Odds ratios (ORs) with confidence intervals of 95% (95% CI) were calculated to estimate the risk of UADT cancer associated with different genetic and lifestyle factors evaluated. A conditional forward logistic regression, with a 0.05 probability of inclusion and a 0.10 probability of exclusion in the model, was applied to assess the risk of UADT cancer attributed to polymorphisms of *NAT, XRCC1* and *hOGG1* after adjusting for sex, age, smoking, drinking and the first two principal components (PC1 and PC2) of ancestry informative markers (AIMs). Principal components analysis (PCA) was performed using SNPRelate package of the free software R (17). The gametics phases binding SNPs and NAT2 haplotype frequencies were estimated using a pseudo-Bayesian approach (Excoffier–Laval–Balding - ELB algorithm) and expectation maximization (EM) algorithm, respectively, through the software Arlequin version 3.11 (18). The genotypic frequencies recorded for *NAT2, XRCC1* and *hOGG1* were tested for Hardy–Weinberg equilibrium among cases and controls. Analysis of gene–environment interaction were conducted from stratified variables (genotype × smoking) that were introduced in logistic regression models with other selected variables. To test the significance of observed interactions, we applied a likelihood ratio test using the χ^2 distribution. All statistical analyzes were performed with statistical package SPSS version 10.0 (SPSS, Chicago, IL, USA) adopting a significance level of 5% (α=0.05).

Results

The general characteristics of cases and controls are described in Table I. There were no significant differences between the groups with regard to sex, age and self-reported skin color. Individuals classified as ever drinkers had a 4.14-fold (95% CI 2.47-6.92, p<0.001) higher risk of developing UADT cancer compared to never drinkers. Smoking was also significantly associated with this type of cancer, with a 17.67-fold (95% CI 7.45-41.90, p<0.001) increase in the risk of disease observed among ever smokers. The number of cigarettes consumed was also significantly associated with UADT cancer, with a risk of 10.26 (95% CI 4.06-25.96, p<0.001) for individuals who reported consuming up to 20 pack-years and a risk of 21.24 (95% CI 8.47-53.23, p<0.001) for individuals who reported consuming more than 20 pack-years.

With respect to the anatomical site of the primary tumor, there was a predominance of tumors of the oropharynx (32.8%), followed by the larynx (31.9%) and oral cavity (25.0%); hypopharyngeal tumors corresponded to 8.6%, while nasopharyngeal tumor was observed for a single case (1%). For one case (1%), the primary anatomical tumor site could not be determined. None of the patients had distant metastases.

Table I. *General characteristics of cases and controls.*

Variable	Cases (n=117)	Controls (n=224)	OR (95% CI)	*p*-Value
Gender n (%)				0.373
Male	101 (86.3)	185 (82.6)	–	
Female	16 (13.7)	39 (17.4)		
Age mean (SD)	60.9 (12.44)	69.6 (11.30)	–	0.341
Skin color n (%)				
White	17 (14.5)	36 (16.1)	–	0.709
No White	100 (85.5)	188 (83.9)		
Drinkers n (%)				
Never	27 (23.1)	108 (55.4)	Ref.	
Ever	90 (76.9)	87 (44.6)	4.14 (2.47-6.92)	<0.001
Smokers n (%)				
Never	6 (5.1)	106 (51.2)	Ref.	
Ever	111 (94.9)	111 (57.8)	17.67 (7.45-41.90)	<0.001
Pack-years				
0	6 (6.2)	95 (50.0)	Ref.	
<20	35 (36.5)	54 (28.4)	10.26 (4.06-25.96)	<0.001
>20	55 (57.3)	41 (21.6)	21.24 (8.47-53.23)	<0.001
Primary sites n (%)				
Oral cavity	29 (25.0)			
Oropharynx	38 (32.8)			
Hipofaringe	10 (8.6)			
Nasopharynx	1 (0.9)			
Larynx	37 (31.9)			
Ocult primary	1 (0.9)			
Stage (UICC) n (%)				
I + II	11 (10.9)			
III + IV	80 (89.1)			

OR : Odds ratio

Regarding stage, most patients (89.1%) were diagnosed with non-metastatic locally-advanced disease (stages II and IV), while only 10.9% had a diagnosis of early-stage disease (stage I and II).

Table II presents the results of bivariate and multivariate logistic regression involving different risk variables for UADT cancer. As described in the table, associations between *XRCC1* Arg399Gln and *hOGG1* Ser326Cys polymorphisms with UADT cancer were statistically non-significant in both crude and adjusted analysis. For the *NAT2* gene, in turn, although the slow acetylator phenotype was not associated as a risk factor for UADT cancer in unadjusted analysis (crude OR=1.60, 95% CI 0.95-2.69; p=0.076), in multivariate analysis, this phenotype was positively associated with UADT cancer (adjusted OR=1.92, 95% CI 1.03-3.57, p=0.038). Furthermore, by analyzing the distribution of different NAT2 haplotypes between cases and controls we observed significant differences for NAT2*5D allele (8.3% and 3.1% in cases and controls, respectively, p=0.014), which confers a slow acetylation profile (data not shown).

In Table III interaction analysis between *XRCC1* Arg399Gln, *hOGG1* Ser326Cys, *NAT2* acetylation phenotypes and smoking for UADT cancer risk is described. In multivariate analysis, *NAT2* slow acetylation phenotype was a risk factor for UADT cancer only among moderate smokers (adjusted OR=3.70, 95% CI 1.12-12.19, p=0.032). Assuming a dominant genetic model, carrying at least one C allele for *hOGG1* Ser326Cys was protective against developing UADT among moderate smokers (OR=0.35, 95% CI 0.12-0.99, p=0.044), but not heavy smokers and never smokers. This result was confirmed after adjustment for other variables in the multivariate logistic regression model (OR=0.31, 95% CI 0.11-0.89, p=0.031).

Interaction analysis of *XRCC1* Arg399Gln, *hOGG1* Ser326Cys and *NAT2* acetylation phenotypes with alcohol drinking in UADT cancer risk are described in Table IV. The Arg399Gln polymorphism of *XRCC1* was significantly associated as a protective factor for UADT cancer only among never drinkers with at least one A allele, adopting the dominant genetic model (OR=0.37, 95% CI=0.14 to 0.96, p=0.037). This result remained significant after adjustment by multivariate logistic regression model (OR=0.34, 95% CI 0.11-0.99; p=0.048).

Table II. *Associations between X-ray repair cross-complementing protein 1 (XRCC1) Arg399Gln, human 8-oxoguanine glycosylase 1 (hOGG1) Ser326Cys and the N-acetylation phenotypes and the risk of upper aerodigestive tract (UADT) cancer.*

Variable	Cases n (%)	Controls n (%)	OR[a] (95% CI)	*p*-Value	OR[b] (95% CI)	*p*-Value
N-Acetylation phenotype						
Rapid or intermediate	53 (52.5)	90 (63.8)	Ref.	–		
Slow	48 (47.5)	51 (36.2)	1.60 (0.95-2.69)	0.076	1.92 (1.03-3.57)	0.038
XRCC1 Arg399Gln						
GG	69 (59.0)	112 (53.3)	Ref.	–		
GA	39 (33.3)	86 (41.0)	0.73 (0.45-1.19)	0.213	NS	>0.05
AA	9 (7.7)	12 (5.7)	1.21 (0.48-3.04)	0.673	NS	>0.05
GA ou AA	48 (41.0)	98 (46.7)	0.79 (0.50-1.25)	0.325	NS	>0.05
G	177 (75.6)	310 (73.8)	Ref.	–		
A	57 (24.4)	110 (26.2)	0.90 (0.63-1.31)	0.607		
hOGG1 Ser326Cys						
CC	87 (74.4)	137 (65.6)	Ref.	–		
CG	25 (21.4)	62 (29.7)	0.63 (0.37-1.06)	0.096	NS	>0.05
GG	5 (4.2)	10 (4.7)	0.78 (0.26-2.38)	0.671	NS	>0.05
CG ou GG	30 (25.6)	72 (34.4)	0.65 (0.39-1.06)	0.1	NS	>0.05
C	199 (85.0)	336 (80.4)	Ref.	–		
G	35 (15.0)	82 (19.6)	0.72 (0.46-1.11)	0.137		

[a]OR not adjusted; [b]OR adjusted for age, gender, smoking, drinking and genetic ancestry (PC1 and PC2) by multiple logistic regression; NS: Not significant to enter the logistic model by forward variable selection method.

Discussion

We evaluated 14 *NAT2* SNPs and seven of these (C190T, G363A, A411T, A434C, G499A, C759T and A845C) were monomorphic for the wild-type allele and thus were not included in the analysis of association with UADT. The other *NAT2* SNPs (G191A, C282T, T341C, C481T, G590A, A803G and G857A) were tested for association with UADT. *NAT2* SNP A803G was not found to be in Hardy–Weinberg equilibrium, and so was not used for the determination of haplotypes.

In the present study, the most frequent haplotypes in both cases and controls were *NAT2**4, *NAT2**6A and *NAT2**5A, which accounted for 75.0% of haplotypes in controls and 81.0% in cases. These data corroborate the study by Talbot *et al.* (19) in the same population, in which the most frequent haplotype was *NAT2**4. These data are also consistent with another study conducted in Latin America (20) that found greater frequency of haplotypes as the *NAT2**4 and *NAT2**5B. All haplotypes were tested for association with UADT cancer, however, only *NAT2**5D was associated with cancer risk. This haplotype is responsible for encoding a slow metabolizing enzyme (21). Several studies in different countries reported association between haplotypes that confer a slow metabolism profile and UADT cancer risk (22-25), suggesting that inefficiency in the elimination of carcinogenic compounds by *NAT2* in individuals carrying these haplotypes could increase the risk for these malignancies. However, some studies have not confirmed these associations, and even opposite results, with genotypes for fast or intermediate acetylation profiles associated as risk factors for UADT cancer, have been described by others (26, 27). These conflicting results suggest that ethnic and geographic factors, in addition to differences in lifestyle between these populations may modify the impact of this polymorphism on the risk of disease. Another factor that can influence these results is the number of SNPs analyzed in the different studies. The selection of SNPs to determine the *NAT2* haplotypes is not standardized by researchers, which makes comparisons between different studies difficult. The number of SNPs used to determine the *NAT2* haplotypes differs greatly, ranging from one (26) to 13 (27). These data suggest that the use of a greater number of polymorphic SNPs for inference of *NAT2* acetylation profiles may be required, particularly in populations where these polymorphisms did not show a pattern of strong linkage disequilibrium.

In association analysis between *NAT2* acetylation profile and UADT cancer, the fast or intermediate acetylator phenotypes were considered as the reference group. There were significant differences for the distribution of these phenotypes between cases and controls. Individuals classified as slow acetylators had a significantly higher risk of developing UADT cancer compared to fast or intermediate acetylators. These data agree with those reported by others, suggesting that the phenotype of *NAT2* based on the metabolic profile is associated with the risk of UADT cancer (22, 23).

Table III. *Stratified associations by categories of smokers between polymorphisms in XRCC1 Arg399Gln, hOGG1 Ser326Cys and N-acetylation phenotypes and UADT cancer risk.*

Variable	Cases n (%)	Controls n (%)	OR[a] (95% CI)	*p*-Value	OR[b] (95% CI)	*p*-Value
Never smokers						
N-Acetylation phenotype						
Rapid or intermediate	2 (40.0)	33 (62.3)	Ref.	–		
Slow	3 (60.0)	20 (37.7)	2.47 (0.38-16.11)	0.376	NS	>0.05
XRCC1 Arg399Gln						
GG	5 (83.3)	51 (53.7)	Ref.	–		
GA ou AA	1 (16.7)	44 (46.3)	0.23 (0.02-2.06)	0.222	NS	>0.05
G	11 (91.7)	143 (75.3)	Ref.	–		
A	1 (8.3)	47 (24.7)	0.27 (0.03-2.20)	0.301		
hOGG1 Ser326Cys						
CC	5 (83.3)	61 (66.6)	Ref.	–		
CG ou GG	1 (16.7)	32 (34.4)	0.38 (0.04-3.40)	0.66	NS	>0.05
C	11 (91.7)	149 (80.1)	Ref.	–		
G	1 (8.3)	37 (19.9)	0.36 (0.04-2.92)	0.468		
≤20 Pack-years						
N-Acetylation phenotype						
Rapid or intermediate	17 (53.1)	23 (74.2)	Ref.	–		
Slow	15 (46.9)	8 (25.8)	2.53 (0.87-7.34)	0.082	3.70 (1.12-12.19)	0.032
XRCC1 Arg399Gln						
GG	20 (57.1)	30 (56.6)	Ref.	–		
GA or AA	15 (42.9)	23 (43.4)	0.98 (0.41-2.32)	0.96	NS	>0.05
G	53 (75.7)	78 (73.6)	Ref.	–		
A	17 (24.3)	28 (26.4)	0.89 (0.44 -1.79)	0.751		
hOGG1 Ser326Cys						
CC	29 (82.9)	34 (63.0)	Ref.	–		
CG or GG	6 (17.1)	20 (37.0)	0.35 (0.12-0.99)	0.044	0.31 (0.11-0.89)	0.031
C	63 (90.0)	86 (79.6)	Ref.	–		
G	7 (10.0)	22 (20.4)	0.43 (0.17-1.06)	0.067		
>20 Pack-years						
N-Acetylation phenotype						
Rapid or intermediate	25 (56.8)	17 (56.7)	Ref.	–		
Slow	19 (43.2)	13 (43.3)	0.99 (0.39-2.53)	0.990	NS	> 0.05
XRCC1 Arg399Gln						
GG	34 (61.8)	19 (47.5)	Ref.	–		
GA or AA	21 (38.2)	21 (52.5)	0.56 (0.24-1.27)	0.165	NS	> 0.05
G	84 (76.4)	55 (68.8)	Ref.	–		
A	26 (23.6)	25 (31.3)	0.68 (0.35-1.30)	0.242		
hOGG1 Ser326Cys						
CC	38 (69.1)	25 (62.5)	Ref.	–		
CG or GG	17 (30.9)	15 (37.5)	0.74 (0.31-1.76)	0.502	NS	> 0.05
C	89 (80.9)	63 (78.8)	Ref.	–		
G	21 (19.1)	17 (21.3)	0.87 (0.43-1.79)	0.713		

[a]OR not adjusted; [b]OR adjusted for age, gender, smoking, drinking and genetic ancestry (PC1 and PC2) by multiple logistic regression; NS: Not significant to enter the logistic model by forward variable selection method

In the present study neither *XRCC1* Arg399Gln nor *hOGG1* Ser326Cys polymorphisms were significantly associated with UADT cancer risk. These results agree with those reported in a meta-analysis published recently that investigated the association between the *XRCC1* Arg399Gln polymorphism and UADT cancer (12). However, two different meta-analyses have demonstrated the existence of a significant association between the *hOGG1* Ser326Cys polymorphism and UADT cancer (28, 29), in disagreement with that found in the present study.

Analysis of gene–gene interaction were conducted, however no significant results were obtained (data not

Table IV. *Stratified associations by categories of alcohol drinkers between polymorphisms in XRCC1 Arg399Gln, hOGG1 Ser326Cys and N-acetylation phenotypes and UADT cancer risk.*

Variables	Cases n (%)	Controls n (%)	OR[a] (95% CI)	*p*-Value	OR[b] (95% CI)	*p*-Value
Never drinkers						
N-acetylation Phenotype						
Rapid or Intermediate	13 (52.0)	48 (72.7)	Ref.	–		
Slow	12 (48.0)	18 (27.3)	2.46 (0.95-6.38)	0.06	NS	>0.05
XRCC1 Arg399Gln						
GG	20 (74.1)	49 (51.6)	Ref.	–		
GA ou AA	7 (25.9)	46 (48.4)	0.37 (0.14 > 0.05 0.96)	0.037	0.34 (0.11-0.99)	0.048
G	46 (85.2)	139 (73.2)	Ref.	–		
A	8 (14.8)	51 (26.8)	0.47 (0.21 > 0.05 1.07)	0.069		
hOGG1 Ser326Cys						
CC	20 (74.1)	58 (61.1)	Ref.	–		
CG ou GG	7 (25.9)	37 (38.9)	0.55 (0.21 > 0.05 1.42)	0.214	NS	>0.05
C	46 (85.2)	150 (78.9)	Ref.	–		
G	8 (14.8)	40 (21.1)	0.65 (0.28 > 0.05 1.49)	0.309		
Ever drinkers						
N-acetylation Phenotype						
Rapid or Intermediate	40 (52.6)	33 (57.9)	Ref.	–		
Slow	36 (47.4)	24 (42.1)	1.24 (0.62 > 0.05 2.47)	0.546	NS	>0.05
XRCC1 Arg399Gln						
GG	49 (54.4)	46 (53.5)	Ref.	–		
GA ou AA	41 (45.6)	40 (46.5)	0.96 (0.53 > 0.05 1.74)	0.899	NS	>0.05
G	131 (72.8)	128 (74.4)	Ref.	–		
A	49 (27.2)	44 (25.6)	1.07 (0.67 > 0.05 1.75)	0.727		
hOGG1 Ser326Cys						
CC	67 (74.4)	60 (69.8)	Ref.	–		
CG ou GG	23 (25.6)	26 (30.2)	0.79 (0.41 > 0.05 1.53)	0.489	NS	>0.05
C	153 (85)	141 (82.0)	Ref.	–		
G	27 (15.0)	31 (18.0)	0.80 (0.45 > 0.05 1.41)	0.445		

[a]OR not adjusted; [b]OR adjusted for age, sex, smoking, drinking and genetic ancestry (PC1 and PC2) by multiple logistic regression; NS: Not significant to enter the logistic model by forward variable selection method.

shown). In the analysis of interaction with smoking, carrying a C allele for *hOGG1* Ser326Cys was a protective factor against developing UADT among moderate smokers, while the slow acetylation phenotype for *NAT2* was significantly associated as a risk factor in this smoking group. Interestingly, carrying an A allele for the *XRCC1* Arg399Gln polymorphism was also associated as a protective factor against UADT cancer among never-drinkers. In combination, this result suggests that, similarly to the polymorphisms in bio-transformation genes such as *NAT2*, variants of the mismatch repair genes could influence susceptibility to cancer development under lower levels of exposure to carcinogens (30). One possible explanation for the protective effect conferred by mutant alleles of these DNA repair genes is that increased levels of DNA damage might increase the level of apoptosis during cell division (5). Thus, the apoptosis pathway would favor a protective effect for the development of cancer among carriers of mutant alleles, since mutations that could give rise to carcinoma have a lower probability of being accumulated in the cell. Furthermore, the A allele of *XRCC1* Arg399Gln was associated as a protective factor for non-melanoma skin cancer (31) and a protective effect of the G allele of *hOGG1*Ser326Cys was shown for UADT (32) and breast (33) cancer. Unlike the significant interaction between *NAT2* and smoking observed in this study, others have reported that this association did not vary across strata of pack-year cigarette smoking (34).

An important limitation of the present study is its small sample size, which leads to a considerable lack of statistical power. Thus, it is possible that our results may be due to chance, as several gene–environment interactions were examined. Therefore, these findings should be considered with caution and need to be confirmed in larger, well-designed studies.

In conclusion, the haplotype *NAT2**5D, which confers a slow acetylation profile, and slow metabolization phenotype of NAT2 were significantly associated as risk factors for the development of UADT cancer, especially among moderate

smokers. Carrying a G allele for *hOGG1* Ser326Cys was associated with protection against UADT cancer among moderate-smokers. Carrying an A allele for *XRCC1* Arg399Gln polymorphism, in turn, was a protective factor for these types of cancer among never-drinkers. These gene–enviroment interactions suggest that these polymorphisms have a greater effect on the risk of UADT cancer under lower levels of exposure to environmental carcinogens.

Conflicts of Interest

The Authors state no conflict of interest and have received no payment for preparation of this article.

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