Association of *hOGG1* and *XPD* Polymorphisms with Urothelial Carcinoma in Taiwan

YUAN-HUNG WANG[1,2], SHAUH-DER YEH[3,4], KUN-HUNG SHEN[5], CHENG-HUNG SHEN[6], MIN-CHE TUNG[7], CHI-TUNG LIU[1] and HUNG-YI CHIOU[1,2]

[1]School of Public Health and [2]Center of Excellence for Cancer Research, Taipei Medical University, Taipei, Taiwan, R.O.C.;
[3]Department of Urology, Taipei Medical University Hospital, Taipei; Taiwan, R.O.C.;
[4]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, R.O.C.;
[5]Department of Urology, Chi-Mei Medical Center, Tainan, Taiwan, R.O.C.;
[6]Department of Urology, Chia-Yi Christian Hospital, Chiayi, Taiwan, R.O.C.;
[7]Department of Urology, Tung's Taichung MetroHarbor Hospital, Taichung County, Taiwan, R.O.C.

Abstract. *The aim of this study was to investigate the association of human oxoguanine glycosylase (hOGG1) and xeroderma pigmentosum group D (XPD) polymorphisms with urothelial carcinoma (UC) in Taiwan. Patients and Methods: This hospital-based case-control study included 460 UC cases and 540 cancer-free controls, who had been frequency matched by age and gender, between August 2006 and October 2009. The joint effects of cigarette smoking, alcohol consumption and risk genotypes of the hOGG1 and XPD genes on UC risk was estimated using an unconditional logistic regression. Results: Individuals carrying both the hOGG1 (C/G or G/G) and XPD (A/C or C/C) risk genotypes had a significantly higher UC risk (OR=1.8, 95% CI=1.01-3.0) than the hOGG1 (C/C) and XPD (A/A) reference group. Those who had a history of cigarette smoking and alcohol consumption carrying both the hOGG1 and XPD risk genotypes had the highest UC risk (OR=9.9, 95% CI= 4.5-21.8). The UC cases carrying both the hOGG1 and XPD risk genotypes had a significantly increased risk (OR=5.2, 95% CI=1.2-22.3) of high grade tumor. Conclusion: A significant joint effect of cigarette smoking, alcohol consumption and both hOGG1 and XPD risk genotypes increases UC risk and UC cases carrying both hOGG1 and XPD risk genotypes have a significantly greater risk of high grade tumor.*

Urothelial carcinoma (UC) is derived from the urothelium of the urinary tract including the renal pelvis, ureter and bladder. One of the most prevalent UC is bladder cancer, which is also the eighth most common malignant cancer among men in Taiwan (1). Cigarette smoking is a major risk factor for UC and can result in a 2- to 4-fold increased risk among those who have ever smoked (2, 3). Polycyclic aromatic hydrocarbons, heterocyclic aromatic amines, N-nitroso compounds and reactive oxygen species (ROS) contained in cigarette smoke are thought to be carcinogenic constitutes which can lead to direct or indirect DNA damage (4). Acetaldehyde, an alcohol metabolite, is a carcinogen that interferes with DNA repair enzymes (5). A meta–analysis of 16 epidemiological studies suggested a higher UC risk of 1.3-fold for male alcohol drinkers (6). However, the association between alcohol consumption and UC was still controversial in various studies (7, 8).

Chemical carcinogens can lead to an increased risk of UC and induce DNA damage through the formation of small DNA lesions, such as oxidized bases or bulky DNA adducts which can result in genomic instability, accumulation of mutation and the development of cancer (9-11). Base excision repair (BER) and nucleotide excision repair (NER) are two major DNA damage repair pathways, which are responsible for repairing base damage and bulky DNA adducts, respectively (10). Human oxoguanine glycosylase (hOGG1) is a key enzyme in the BER pathway and can remove 8-hydroxydeoxyguanosine (8-OHdG) from damaged DNA induced by free oxygen radicals (12). The xeroderma pigmentosum group D (XPD), also known as excision repair cross-complementing group 2 (ERCC2), codes a component of the transcription factor IIH (TFIIH) essential for the NER pathway and controls cell cycle progression (13, 14).

Epidemiological studies have indicated that single nucleotide polymorphisms (SNPs) in DNA repair genes can modulate repair capability and may be associated with the

Correspondence to: Hung-Yi Chiou, School of Public Health, Taipei Medical University, 250 Wu-Hsing St., Taipei 110, Taiwan. Tel: +886 223779189, Fax: +886 223779188, e-mail: hychiou@tmu.edu.tw

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individual susceptibility to malignancy (15). A functional polymorphism, C to G transversion, located in exon 7 of the *hOGG1* gene resulting in an amino acid change from serine (Ser) to cysteine (Cys) at codon 326 (Ser326Cys, rs1052133) has indicated a lower activity *in vitro* with the G allele and has been shown to be associated with a higher cancer risk of lung, prostate and bladder (16-18). However, the correlation between the *hOGG1* Ser326Cys polymorphism and its enzyme activity is still unresolved. Polymorphisms in the *XPD* gene can not only lower the helicase activity but also cause a deficiency in NER capacity although the biological function of XPD polymorphisms has not been elucidated completely (12, 19). The A to C transversion in exon 23 of the *XPD* gene causing an amino acid change from lysine (Lys) to glutamine (Gln) at codon 751 (Lsy751Gln, rs28365048) has been examined in relation to lung, breast and bladder malignancies (20-22). This *XPD* Lsy751Gln polymorphism is thought to be related to decreased DNA repair capability and increased DNA adducts.

UC is a complex and multi-step malignancy and may be associated with the joint effects of exposure to environmental risk factors and individual genetic susceptibility (23). Therefore, the effects of polymorphisms in DNA repair genes and environmental exposures on the risk of UC should be evaluated simultaneously. It was hypothesized that *hOGG1* Ser326Cys and *XPD* Lsy751Gln might modify individual susceptibility to UC. To test this hypothesis, a hospital-based case-control study was conducted to examine the joint effects of cigarette smoking, alcohol consumption, *hOGG1* Ser326Cys and *XPD* Lys751Gln polymorphisms on UC risk in Taiwan.

Patients and Methods

Subjects and data collection. This was a hospital-based case-control study including 1,000 subjects. A total of 460 histologically confirmed UC cases were recruited from the Department of Urology of Taipei Medical University Hospital, Chi-Mei Medical Center, Chiayi Christian Hospital and Tung's Taichung MetroHarbor Hospital between August 2006 and October 2009. Pathological confirmation of UC was performed by regular urological practice including endoscopic biopsy and surgical resection of urinary tract tumors. Staging and grading of the tumors were determined by the 1997 TNM classification system and the 1973 World Health Organization classification. Tumor stage was classified into two groups, non-muscle-invasive (≤T1) and muscle-invasive (T2-T4). Tumor grade was recorded as two groups, low (G1) and high (G2 and G3) grade. A total of 540 cancer-free controls, frequency-matched on age and gender, were collected from individuals who were admitted to the same hospitals as the UC cases for a health examination and had no history of urological diseases. The response rate was 90% for the UC cases and 85% for the controls. Written informed consent was obtained from all the participants. This protocol was approved by the institutional review boards of the collaborating hospitals. All the participants were interviewed by a well-trained interviewer using a structured questionnaire to collect information including demographic characteristics, history of cigarette smoking and alcohol consumption. According to the definition of U.S. Centers for Disease Control, the study subjects who consumed more than 100 cigarettes during their lifetime were defined as ever smokers, while those who consumed less than 100 cigarettes in their lifetime were defined as never smokers (24). For alcohol consumption, ever drinkers were recognized as individuals who had consumed alcohol three days or more per week for at least six months, while the others were regarded as never drinkers (25, 26).

SNP selection and genotyping. A 6-8 ml sample of peripheral blood was collected from each participant into an ethylene-diaminetetraacetic acid (EDTA)-coated tube. Genomic DNA was extracted from the peripheral blood lymphocytes and was stored at –80˚C. Genotyping was determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Briefly, the following primers were designed for the *hOGG1* Ser326Cys polymorphism: 5'-AGACCCAGTGGACTCTTCCACCACCG-3' (sense) and 5'-CAACATGAGACTGGGTGGGGATGGGGAGAG-3' (antisense) and for the *XPD* Lys751Gln polymorphism: 5'-CCCCTCTCCCTTTCC TCTGTT-3' (sense) and 5'-GCTGCC TTCTCCTGCGATTA-3' (antisense). The PCR conditions used for the *hOGG1* Ser326Cys polymorphism were: one cycle at 94˚C for 5 min; 30 cycles of 94˚C for 40 sec, 60˚C for 40 sec and 72˚C for 40 sec and a final extension at 72˚C for 10 min and for the *XPD* Lys751Gln polymorphism were: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 sec, 61˚C for 30 sec and 72˚C for 30 sec and a final extension at 72˚C for 10 min. The genotypes were determined after digestion with Fnu4HI and PstI restriction enzymes for the *hOGG1* Ser326Cys polymorphism (C/C: 376bp; C/G: 376, 227 and 149bp and G/G: 227 and 149bp) and *XPD* Lys751Gln polymorphism (A/A: 273bp; A/C: 273, 207 and 66bp and C/C: 207 and 66bp), respectively. To ensure quality control, a random 10% of the samples were genotyped repeatedly.

Statistical analysis. Selected characteristics among the UC cases and controls were compared using the Chi-square test for categorical variables and the Student's *t*-test for continuous variables. Hardy-Weinberg Equilibrium (HWE) was examined using a goodness-of-fit Chi-square test to compare the observed and expected frequencies among the control subjects. The effects of the *hOGG1* Ser326Cys and *XPD* Lys751Gln polymorphisms on UC risk were estimated by odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional multivariate-adjusted logistic regression adjusted for age, gender, cigarette smoking and alcohol consumption. In addition, the joint effects of environmental exposure including cigarette smoking and alcohol consumption and *hOGG1* and *XPD* risk genotypes on UC risk was further estimated using an unconditional logistic regression analysis adjusted for age and gender. *P*-values of <0.05 were considered statistically significant. All the statistical analyses were performed using Statistical Analysis Software for Windows, version 9.1 (SAS Institute, Cary, NC, USA).

Results

Characteristics of study population. The distribution of selected characteristics among the UC cases and controls are shown in Table I. No significant differences were observed in age between the UC cases (62.7±10.9 years) and controls (61.9±11.0 years) ($p=0.247$) or in gender and education level

Table I. *The distribution of selected characteristics among urothelial carcinoma cases and controls.*

Variable		UC cases (n=460)	Controls (n=540)	OR[a] (95%CI)
Age (years)				
≤50		69 (15.0)	97 (17.9)	1.0 (Referent)
51-69		256 (55.6)	294 (54.5)	1.2 (0.9-1.7)
≥70		135 (29.4)	149 (27.6)	1.3 (0.9-1.9)
Mean (SD) age, years		62.7 (10.9)	61.9 (11.0)	0.247[b]
Gender				
Female		130 (28.3)	172 (31.8)	1.0 (Referent)
Male		330 (71.7)	368 (68.2)	1.1 (0.9-1.6)
Education level				
Illiterate		87 (18.9)	99 (18.3)	1.0 (Referent)
Elementary		218 (47.4)	226 (41.9)	1.1 (0.8-1.5)
Junior/Senior high school		110 (23.9)	150 (27.8)	0.8 (0.6-1.2)
College		45 (9.8)	65 (12.0)	0.8 (0.5-1.3)
Cigarette smoking/ Alcohol consumption				
Smoke	Alcohol			
Never	Never	207 (45.0)	313 (58.0)	1.0 (Referent)
Never	Ever	9 (2.0)	16 (3.0)	1.1 (0.9-1.6)
Ever	Never	141 (30.7)	159 (29.4)	1.6 (1.1-2.2)*
Ever	Ever	103 (22.3)	52 (9.6)	3.5 (2.3-5.4)***
				p for trend <0.05
Tumor stage				
Non-muscle-invasive (≤T1)		281 (61.1)		
Muscle-invasive (T2-T4)		179 (38.9)		
Tumor grade				
Low grade (G1)		73 (15.9)		
High grade (G2-G3)		387 (84.1)		

Data is shown as numbers with percentages in parentheses. [a]Crude OR (odds ratio) and 95% CI (confidence interval) estimated by univariate logistic regression. [b]Student *t*-test. *p<0.05; ***p<0.001.

Table II. *hOGG1 Ser326Cys and XPD Lys751Gln polymorphisms and risk of urothelial carcinoma.*

Genotype	UC cases (n=460)	Controls (n=540)	OR[a] (95% CI)
hOGG1 Ser326Cys			
C/C	55 (11.9)	82 (15.1)	1.0 (Referent)
C/G	227 (49.4)	246 (45.6)	1.4 (0.94-2.1)[§]
G/G	178 (38.7)	212 (39.3)	1.3 (0.8-1.9)
C/C	55 (11.9)	82 (15.1)	1.0 (Referent)
C/G, G/G	405 (88.1)	458 (84.9)	1.3 (0.91-1.9)
XPD Lys751Gln			
A/A	390 (84.8)	472 (87.4)	1.0 (Referent)
A/C	70 (15.2)	67 (12.4)	1.3 (0.9-1.9)
C/C	0 (0.0)	1 (0.2)	-
A/A	390 (84.8)	472 (87.4)	1.0 (Referent)
A/C, C/C	70 (15.2)	68 (12.6)	1.3 (0.9-1.8)
Number of combined risk genotypes[b]			
0	40 (8.7)	71 (13.1)	1.0 (Referent)
1	365 (79.4)	412 (76.3)	1.6 (1.02-2.4)*
2	55 (11.9)	57 (10.6)	1.8 (1.01-3.0)*
			p for trend =0.0404

Data is shown as numbers with percentages in parentheses. [a]Adjusted for age, gender, cigarette smoking, alcohol consumption and cigarette smoking × alcohol consumption. [b]Risk genotypes: *hOGG1* Ser326Cys-C/G+G/G and *XPD* Lys751Gln-A/C+C/C. *p<0.05.

between these two groups. In combination analysis, subjects with a habit of both cigarette smoking and alcohol consumption had the significantly highest UC risk (OR=3.5, 95% CI=2.3-5.4). Ever smokers without a habit of alcohol consumption had a significantly increased UC risk of 1.6-fold compared to the reference group. However, ever drinkers without a habit of cigarette smoking had no increased risk of UC. Among the UC cases, 38.9% were muscle- invasive and 84.1% were high grade tumors.

hOGG1 Ser326Cys and XPD Lys751Gln polymorphisms and UC risk. The genotype distributions of the *hOGG1* Ser326Cys polymorphism (Chi-square=1.13, p=0.57) and *XPD* Lys751Gln polymorphism (Chi-square=1.02, p=0.60) in the controls were in Hardy-Weinberg equilibrium. The distribution of the *hOGG1* Ser326Cys and *XPD* Lys751Gln polymorphisms between the UC cases and controls are shown in Table II. Compared with those who carried the C/C genotype of *hOGG1* Ser326Cys as reference, the study subjects with the C/G or G/G genotype had an increased UC risk (OR=1.3). The study subjects with the A/C or C/C genotype of *XPD* Lys 751Gln had a 1.3-fold increased UC risk as compared to those with the A/A genotype. The combined effect of the *hOGG1* (C/G or G/G) and *XPD* (A/C or C/C) risk genotypes on UC risk was also determined in the subsequent analysis. Compared with the study subjects without any of the risk genotypes, significantly increased UC risks of 1.6 and 1.8 were found for those with one or two risk genotypes, respectively, showing a significant dose-response relationship.

Joint effect of environmental exposures and hOGG1 and XPD risk genotypes on UC risk. The joint effects of exposure to environmental risk factors including cigarette smoking and alcohol consumption and individual genetic susceptibility of the *hOGG1* Ser326Cys and *XPD* Lys751Gln polymorphisms on UC risk are shown in Table III. Subjects without any of the studied risk factors were identified as the reference group. Those with one risk factor of environmental exposure or genotypic susceptibility were defined as group I. Individuals

Table III. *Joint effects of cigarette smoking, alcohol consumption and hOGG1 Ser326Cys and XPD Lys751Gln risk genotypes on urothelial carcinoma risk.*

Group of risk factors	Cigarette smoking		Alcohol consumption		No. of risk genotypes[b]		No. of cases/ controls	OR[a] (95% CI)
	Never (–)	Ever(+)	Never (–)	Ever(+)	0 (–)	≥1(+)		
Reference	–		–		–		10/46	1.0 (Referent)
I	+		–		–		217/286	3.4 (1.7-6.9)***
I	–		+		–		217/286	
I	–		–		+		217/286	
II	+		+		–		140/162	4.2 (1.9-8.8)***
II	+		–		+		140/162	
II	–		+		+		140/162	
III	+		+		+		93/46	9.9 (4.5-21.8)*** p for trend <0.0001

[a]Adjusted by age and gender. [b]Risk genotypes: *hOGG1* Ser326Cys-C/G+G/G and *XPD* Lys751Gln-A/C+C/C. ***$p<0.001$.

with two risk factors were recognized as group II. While, subjects having three risk factors formed group III. The significantly highest UC risk of 9.9-fold was observed in group III. Group II and group I individuals exhibited significantly higher UC risks, 4.2 and 3.4, respectively, when compared to those in the reference group. A significant joint effect from the exposure to environmental risk factors and genetic susceptibility on UC risk was observed in this study ($p<0.0001$).

Association of risk genotypes with tumor stage, grade and recurrence. The associations between the *hOGG1* Ser326Cys and *XPD* Lys751Gln polymorphisms and tumor stage, grade and recurrence are shown in Table IV. No significant differences in the frequency of the risk genotypes of the *hOGG1* Ser326Cys polymorphism were found in tumor stage, grade and recurrence. The frequency of the risk genotypes of the *XPD* Lys751Gln polymorphism was significantly greater in the high grade (17.1%) than in the low grade (5.5%) tumors ($p=0.012$), showing a significantly increased risk of high grade tumor (OR=3.2, 95% CI=1.2-9.4). Non-significant higher risks of 1.3 and 1.3-fold were also observed for tumor stage and recurrence. Furthermore, the UC cases carrying both the *hOGG1* Ser326Cys and *XPD* Lys751Gln risk genotypes had a significantly increased risk (OR=5.2, 95% CI=1.2-22.3) of high grade tumor.

Discussion

Consistent with previous studies (3, 4, 25), cigarette smoking and alcohol consumption were significantly associated with UC risk, implying a significant joint effect of cigarette smoking and alcohol consumption on UC risk. Some epidemiological studies have shown that cigarette smoking and alcohol consumption interact in a multiplicative way on cancer risk (27).

In a Korean study, subjects carrying the C/C or C/G genotype of *hOGG1* Ser326Cys had an increased risk of recurrence in bladder cancer as compared to those with the G/G genotype (28), while a Japanese study found that subjects carrying the G/G genotype had a significantly higher bladder cancer risk (OR=1.85) (18). Consistent with the Japanese study, the present findings showed that subjects carrying the G allele of the *hOGG1* gene had a higher UC risk (OR=1.3) with borderline significance. In addition, the genotype distribution of *hOGG1* Ser326Cys polymorphism has shown ethnic variation with frequency of the G variant allele of 52.3% in Korean, 42% in Japanese, 25% in Turkish populations and 21.7% in Caucasians (16, 18). The frequency for the G variant allele found among the cancer-free controls in the present study was 62%, which was similar to the reported Korean population.

A slight decrease in bladder cancer risk for the C/C genotype as compared to individuals with the A/A or A/C genotype of *XPD* Lys751Gln has been reported (29). Additionally, ever smokers carrying the A/A or A/C genotype were twice as likely to have bladder cancer as those with the C/C genotype. Furthermore, some previous studies indicated no significant relationship between *XPD* Lys751Gln polymorphism and bladder cancer risk (19, 20). In the present study, the subjects carrying the C allele (A/C or C/C genotype) of the XPD gene had a 1.3-fold higher though not statistically significant UC risk. The frequency of the C/C genotype of *XPD* gene has been reported to be 10.0% in Caucasians, 7.6% in Indian and 5.6% in Turkish populations (12, 13, 20), however, among the controls in the present study the frequency was only 0.2%. Thus the Lys751Gln polymorphism of the *XPD* gene may have various ethnic-related effects on UC risk. Recently, an G to A transition in the *XPD* gene causing an amino acid change from aspartate

Table IV. *Association between tumor stage, grade, recurrence and genotypes of hOGG1 Ser326Cys and XPD Lys751Gln polymorphisms.*

Genotypes	Tumor stage[a]		OR (95%CI)[b]	Tumor grade		OR (95%CI)[b]	Recurrence		OR(95%CI)[b]
	NMI	MI		Low	High		No	Yes	
hOGG1 Ser326Cys									
C/C	34	21	1.0 (Referent)	8	47	1.0 (Referent)	35	20	1.0 (Referent)
C/G, G/G	247	158	1.0 (0.5-1.8)	65	340	0.9 (0.4-2.1)	245	160	1.0 (0.5-1.4)
XPD Lys751Gln									
A/A	243	147	1.0 (Referent)	69	321	1.0 (Referent)	162	228	1.0 (Referent)
A/C, C/C	38	32	1.3 (0.8-2.2)	4	66	3.2 (1.2-9.4)*	24	46	1.3 (0.7-2.4)
Number of combined risk genotypes[c]									
≤1	249	156	1.0 (Referent)	71	334	1.0 (Referent)	246	159	1.0 (Referent)
2	32	23	1.1 (0.6-1.9)	2	53	5.2 (1.2-22.3)*	34	21	1.1 (0.6-1.9)

[a]NMI=Non-muscle-invasive; MI= Muscle-invasive. [b]Adjusted for age, gender, cigarette smoking, alcohol consumption and cigarette smoking × alcohol consumption. [c]Risk genotypes: *hOGG1* Ser326Cys-C/G+G/G and *XPD* Lys751Gln-A/C+C/C. *$p<0.05$.

(Asp) to asparagine (Asn) at codon 312 (Asp312Asn, rs1799793) has been tested (30, 31). A meta-analysis suggested that both Lys751Gln and Asp312Asn polymorphisms affect smoking-related cancer risk by modulating DNA repair capability (20). Further examination of the association between these two polymorphisms of the *XPD* gene and UC risk should be undertaken. In the present study, the subjects who carried both the *hOGG1* Ser326Cys and XPD Lys751Gln risk genotypes had a significantly higher UC risk indicating a synergistic gene-gene interaction.

In the combination analysis, the study subjects who had a history of both cigarette smoking and alcohol consumption and also carried one or more of the *hOGG1* and XPD risk genotypes had the highest UC risk of 9.9-fold. Thus the epidemiological evidence suggested a significant synergistic effect on UC risk of the *hOGG1* Ser326Cys and *XPD* Lys751Gln risk genotypes and exposure to environmental risk factors including cigarette smoking and alcohol consumption.

In addition, the UC cases carrying the *XPD* Lys751Gln risk genotypes had a significantly increased risk of high grade tumors, implying that the deficient DNA repair capacity still had an independent effect on UC risk after the adjustment for environmental risk factors. The *XPD* Lys751Gln polymorphism has also been shown to be significantly associated with histological grade of breast cancer (22) and ovarian cancer (32).

Limitations of this study included the relatively high incidence of muscle-invasive and high grade tumors in the study population and the fact that frequencies of the selected polymorphisms show ethnic variation. Thus conservative interpretation of the findings is necessary. More detailed information and a larger sample size might be included in further study to validate the significant findings.

In conclusion, the risk genotypes of the *hOGG1* Ser326Cys and *XPD* Lys751Gln polymorphisms result in the lack of DNA repair capability, which can modify individual susceptibility to UC. A significant finding is the joint effect of cigarette smoking, alcohol consumption and both risk genotypes on UC risk. Moreover, the UC cases with both the *hOGG1* Ser326Cys and *XPD* Lys751Gln risk genotypes have a significantly greater risk of high grade tumor.

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