Chinese Journal of Physiology 58(1): 64-71, 2015
DOI: 10.4077/CJP.2015.BAD279

Contribution of Double Strand Break Repair Gene *XRCC3* Genotypes to Nasopharyngeal Carcinoma Risk in Taiwan

Juhn-Cherng Liu[1, 2, *], Chia-Wen Tsai[1, 2, *], Chin-Mu Hsu[2, *], Wen-Shin Chang[1, 2], Chi-Yuan Li[1], Shih-Ping Liu[2], Wu-Chung Shen[2], and Da-Tian Bau[1, 2, 3, *]

[1]*Graduate Institute of Clinical Medical Science, China Medical University, Taichung 40402*
[2]*Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung 40458*
and
[3]*Graduate Institute of Basic Medical Science, China Medical University, Taichung 40402*
Taiwan, Republic of China

Abstract

The DNA double strand break repair protein XRCC3 plays a central role in removing double strand breaks from the genome and defects in cellular repair capacity is closely related to human cancer initiation. Therefore, we aimed to investigate the contribution of *XRCC3* genotypes to individual nasopharyngeal carcinoma (NPC) susceptibility. In this hospital-based population research, the genotyping and analyzing of *XRCC3* rs1799794, rs45603942, rs861530, rs3212057, rs1799796, rs861539, rs28903081 in a large Taiwanese population was performed. Totally, 176 NPC patients and 880 age- and gender-matched healthy controls were genotyped and analyzed by PCR-RFLP method. The results showed that there was a differential distribution among NPC and control subjects in the genotypic ($P = 0.000488$) and allelic ($P = 0.0002$) frequencies of *XRCC3* rs861539. As for the gene-environment interaction, we have firstly provided evidence showing that there is an obvious joint effect of *XRCC3* rs861539 CT and TT genotypes with individual smoking habits on increased NPC risk. In conclusion, the T allele of *XRCC3* rs861539, interacts with smoking habit in increasing NPC risk, may be an early detection marker for NPC.

Key Words: genotype, nasopharyngeal carcinoma, polymorphism, Taiwan, XRCC3

Introduction

Nasopharyngeal carcinoma (NPC) is rare cancer which happened in most countries around the world with an incidence rate generally less than 1 per 100,000 person-years. However, in record the NPC incidence is extremely high in Southern China (25-30 per 100,000 person-years) (4, 6, 40). In Taiwan the annual incidence rates for males and females were 8.41 and 2.93 per 100,000 person-years, respectively[†]. Compared with Western countries, the incidence rate is significantly higher in Taiwan with a very high genetic conservation. Thus, the genetic studies for Taiwanese are very useful, especially for NPC susceptibility evaluation. In addition to Epstein-Barr virus (EBV) infection (27, 42), certain dietary factors (18) and genetic differences such as single nucleotide polymorphisms (SNPs) which may all contribute to NPC carcinogenesis (28, 31, 32), environmental factors such as smoking, may also play a role in the etiology of NPC (5, 19, 36, 37).

Environmental carcinogens such as tobacco smoke may induce double strand breaks (DSBs) in the cells.

Corresponding author: Dr. Da-Tian Bau, Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Rd., Taichung 40458, Taiwan, R.O.C. Tel. +886-4-22052121 ext. 7534, E-mail: artbau2@gmail.com; datian@mail.cmuh.org.tw
*These authors contributed equally to this study.
[†]Cancer Registration System Annul Report in Taiwan, ROC. Department of Health Executive Yuan, 2010.
Received: February 27, 2014; Revised: May 14, 2014; Accepted: June 5, 2014.
 ISSN : 0304-4920. http://www.cps.org.tw

Table 1. The demographic and clinical characteristics of nasopharyngeal carcinoma patients and controls

Characteristics	Controls (n = 880)			Patients (n = 176)			*P*-value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (year)			51.3 (8.8)			48.2 (11.1)	0.5104
Gender							0.5692
Male	660	75.0%		128	72.7%		
Female	220	25.0%		48	27.3%		
Indulgence							
Cigarette smoking	253	28.8%		77	43.8%		0.0001*
Betel quid chewing	238	27.0%		55	31.3%		0.2688
Alcohol drinking	331	37.6%		80	45.5%		0.0622

[a] Based on *Chi*-square test, and * Statistically identified as significant.

DSBs are a very severe type of DNA damage which should be repaired by the DNA DSB repair system as soon as possible (35, 41). Tobacco smoking accounts for 5% of cancer cases overall in the world, and several lines of evidence have linked tobacco smoking to NPC risk including those from case-control studies conducted in China, the United States, Southeast Asia, Europe, Singapore, China (Guangdong), and Taiwan (31, 37). Mechanically, if cells cannot remove them immediately by means of homologous recombination (HR) and non-homologous end-joining (NHEJ), those DNA DSBs may induce precancerous lesions and cancer itself as well (21, 38). Genetic polymorphisms in DNA DSB repair genes influence DNA repair capacity and confer predisposition to several cancers including skin (15), breast (1, 2), liver (17), gastric (11), and oral cancer (3, 10). The X-ray repair cross-complementing group 3 (XRCC3; 14q32.3) is a member of the rad51 DNA repair family, which has been shown to interact directly with rad51 and is essential with respect to the proper accumulation of rad51 at sites of DNA DSBs in the nucleus (29).

The most commonly studied *XRCC3* genetic polymorphic site is the rs861539 C/T polymorphism (also named Thr241Met, T241M, C18067T and C722T). However up to now no finding of any *XRCC3* SNP about NPC was reported. To identify the contribution of *XRCC3* genotyping to NPC risk in Taiwan, we determined the genotypic frequencies of seven polymorphisms of *XRCC3* gene at promoter A-315G (rs1799794), promoter C-280T (rs45603942), intron5 (rs861530), exon6 (rs3212057), intron7 (rs1799796), exon8 (rs861539) and exon10 (rs28903081), and evaluate the gene-environment interaction.

Materials and Methods

Study Population and Sample Collection

One hundred and seventy-six NPC patients were diagnosed and recruited at the outpatient clinics of general surgery between 2003-2009 at the China Medical University Hospital, Taichung, Taiwan. The clinical characteristics of patients including histological details were all graded and defined by expert surgeons. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. At the same time 880 (five-fold of the cases) healthy volunteers as controls were selected by matching for age, gender and habits after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Both groups completed a short questionnaire which included personal habits. Smokers were defined as daily or almost daily smokers who had smoked at least five packs of cigarettes in their lifetime. Smokers were recorded for their age of smoking initiation, whether they were currently smoking or had already quit, and if so, when they had quit, and on average, how many cigarettes they smoked or had smoked daily. As for the 880 non-cancer healthy people, the ratio of male versus female was both 75% versus 25% in each group. The mean age of the NPC patients and the controls were 48.2 (SD = 11.1) and 51.3 (SD = 8.8) years, respectively (see Table 1 for more details). Our continuous study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consents were obtained from all participants.

Genotyping Conditions

Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and kept as previous literature (9, 30). A total of seven polymorphic sites were analyzed in all the subjects in control and case groups. Briefly, all of the seven polymorphic sites were geno-

Table 2. Summary of the rs numbers, primer sequences, restriction enzymes, amplicon lengths before and after enzyme digestion, for the *XRCC3* SNPs investigated in this study

XRCC3 SNP	Primer Sequence	Restriction Enzyme	Amplicon Length	Genotypes and Enzymatic Fragment Sizes
rs1799794	F: 5'-CACACTGCGGTCTTGCAGTG-3' R: 5'-CAGGCTGGGTCTGGATACAA-3'	*Bts*CI	505 bp	G: 505 bp A: 289 + 216 bp
rs45603942	F: 5'-GGGATGCAGGTTCAACTGAC-3' R: 5'-AACTTGGACTGTGTCAAGCA-3'	*Alu*I	352 bp	C: 352 bp T: 187 + 165 bp
rs861530	F: 5'-CCGAGGAACGTGCTGAACTT-3' R: 5'-CTCCCTAACAGCCTCCATGT-3'	*Fat*I	497 bp	G: 497 bp A: 293 + 204 bp
rs3212057	F: 5'-CCATGACCGCAGGCACTTGT-3' R: 5'-AGAACGCGACAAGGATGGTA-3'	*HpyCH4*III	455 bp	G: 455 bp A: 235 + 220 bp
rs1799796	F: 5'-GG AACCAGTTGT GTGAGCCT-3' R: 5'-CCTGGTTGATGCACAGCACA-3'	*Alu*I	430 bp	G: 430 bp A: 226 + 204 bp
rs861539	F: 5'-GACACCTTGT TGGAGTGTGT-3' R: 5'-GTCTTCTCGATGGTTAGGCA-3'	*Fat*I	358 bp	C: 358 bp T: 200 + 158 bp
rs28903081	F: 5'-CTGCTTCCTGTTTCTCAGGT-3' R: 5'-GCACTGATCGTGTAGGAACA-3'	*BstU*I	198 bp	A: 198 bp G: 102 + 96 bp

typed by means of a PCR-restriction fragment length polymorphism (PCR-RFLP), and further processed as previous genotyping publications (7, 8, 23). PCR was performed on BioRad's Mycycler (BioRad, Hercules, CA, USA) following the normal manufacturer's instructions. Each PCR reaction consisted of 5 min initial cycle at 94°C for 5 min; 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 10 min. Then the SNP-containing DNA amplicons were subjected to individual overnight digestion by restriction endonucleases following the manufacturer's instructions (see Table 2 for more details). Following digestion, each sample was immediately analyzed by 2% agarose gel electrophoresis. Details such as the primer sequences, and enzymatic digestion conditions for each SNP analyzed in this study were summarized in Table 2.

Statistical Analyses

The subjects matched with all SNPs data and clinical (case/control = 176/880) were taken into final analyzing. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *XRCC3* SNPs in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's *Chi*-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the *XRCC3* genotypes between cases and controls. Cancer risk associated with the genotypes was estimated as odds ratio (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. Data was recognized as significant when the statistical *P*-value was less than 0.05. All statistical tests were performed using SAS, Version 9.1.3 (SAS Institute Inc., Cary, NC, USA) on two-sided probabilities.

Results

The basic characteristics of recruited 176 NPC patients and 880 non-cancer controls are shown in Table 1. Since the controls are age-, gender-matched with the cases, there was no significant difference between the two groups as in their age and gender (Table 1). As for the individual habit status, there was a significant difference that the case group seemed to have more cigarette smokers ($P = 0.0001$), but not alcohol drinkers or betel quid chewers (Table 1). The frequency distributions of the genotypes for the seven *XRCC3* polymorphic sites between controls and NPC patients are shown in Table 3. Among them, the genotypic distribution pattern of *XRCC3* rs861539 was significantly different between NPC and control groups ($P = 0.000488$), while those for rs1799794, rs45603942, rs861530, rs3212057, rs1799796 and rs28903081 were not significant ($P > 0.05$) (Table 3). In detail, distributions of *XRCC3* rs861539 CC homozygote/heterozygote/TT homozygote in controls and oral cancer patients were 91.9/7.6/0.5% and 86.8/11.7/1.5%, respectively (Table 3). The ORs for CT and TT genotypes versus CC wild-type were 1.85 (95%CI = 1.10-3.09) and 5.47 (95%CI = 1.74-17.18), respectively. There was no heterozygote or homozy-

Table 3. Distribution of *XRCC3* genotypes among nasopharyngeal carcinoma patients and controls

Genotype	Controls		Patients		*P*-value[a]	Odds ratio (95% CI)[b]
	(n)	%	(n)	%		
rs1799794					0.8838	
GG	212	24.1%	44	25.0%		1.00 (Reference)
AG	489	55.6%	99	56.3%		0.98 (0.66-1.44)
AA	179	20.3%	33	18.7%		0.89 (0.54-1.45)
rs45603942					0.7883	
CC	818	93.0%	161	91.5%		1.00 (Reference)
CT	54	6.1%	13	7.4%		1.22 (0.65-2.29)
TT	8	0.9%	2	1.1%		1.27 (0.27-6.04)
rs861530					0.7630	
AA	264	30.0%	55	31.3%		1.00 (Reference)
AG	477	54.2%	97	55.1%		0.98 (0.68-1.40)
GG	139	15.8%	24	13.6%		0.83 (0.49-1.40)
rs3212057					1.0000	
GG	880	100.0%	176	100.0%		1.00 (Reference)
AG	0	0.0%	0	0.0%		
AA	0	0.0%	0	0.0%		
rs1799796					0.8702	
AA	399	45.3%	83	47.2%		1.00 (Reference)
AG	435	49.5%	85	48.3%		0.94 (0.67-1.31)
GG	46	5.2%	8	4.5%		0.84 (0.38-1.84)
rs861539					**0.000488***	
CC	809	91.9%	148	84.1%		1.00 (Reference)
CT	65	7.4%	22	12.5%		**1.85 (1.10-3.09)***
TT	6	0.7%	6	3.4%		**5.47 (1.74-17.18)***
rs28903081					1.0000	
GG	880	100.0%	176	100.0%		1.00 (Reference)
AG	0	0.0%	0	0.0%		
AA	0	0.0%	0	0.0%		

[a]*P*-value based on *Chi*-square test (Fisher's Exact test when n < 5).
[b]CI: confidence interval.
*Statistically identified as significant.

gote variant for *XRCC3* rs3212057 and rs28903081 among Taiwanese subjects (Table 3). To sum up, the genotype of *XRCC3* rs861539, not rs1799794, rs45603942, rs861530, rs3212057, rs1799796 or rs28903081, is associated with NPC risk and may be a biomarker for the early detection and prediction of NPC.

The frequencies of the alleles for the *XRCC3* rs1799794, rs45603942, rs861530, rs3212057, rs1799796, rs861539 and rs28903081 of all the recruited subjects are shown in Table 4. Among them, the carriers of *XRCC3* rs861539 allele T were of higher risk for NPC (P = 0.0002), while genotypes of *XRCC3* rs1799794, rs45603942, rs861530, rs3212057, rs1799796 and rs28903081 were not associated with individual NPC susceptibility (Table 4).

In Taiwan, the habit of cigarette smoking is a risky factor for increased NPC risk. Therefore, the risk of NPC related to *XRCC3* genotypes was further examined with stratification by personal smoking status. Table 5 showed the interaction of *XRCC3* genotype and smoking status on personal NPC susceptibility (Table 5). The results showed that compared with C/C genotype, the C/T plus T/T significantly enhanced the risk only in the smoker group (P = 0.0015, OR = 3.30, 95% CI = 1.63-6.69), not in the non-smoker group (P > 0.05, OR = 1.41, 95% CI = 0.71-2.81) (Table 5).

Discussion

In recent years, there were a few papers investigated the contribution of genetic variations on DSB

Table 4. Distribution of *XRCC3* alleles among nasopharyngeal carcinoma patients and controls

Allele	Controls	%	Patients	%	*P*-value[a]
rs1799794					0.6828
Allele G	913	51.9%	187	53.1%	
Allele A	847	48.1%	165	46.9%	
rs45603942					0.4627
Allele C	1,690	96.0%	335	95.2%	
Allele T	70	4.0%	17	4.8%	
rs861530					0.5952
Allele A	1,005	57.1%	207	58.8%	
Allele G	755	42.9%	145	41.2%	
rs1799796					0.6554
Allele A	1,233	70.1%	251	71.3%	
Allele G	527	29.9%	101	28.7%	
rs861539					**0.0002***
Allele C	1,683	95.6%	318	90.3%	
Allele T	77	4.4%	34	9.7%	

[a]*P* based on *Chi*-square test.
*Statistically identified as significant.

Table 5. Odds ratios for *XRCC3* rs861539 genotype and nasopharyngeal carcinoma stratified by personal smoking status

Genotypes	Non-smokers		*P*-value	OR (95% CI)[a]	Smokers		*P*-value	OR (95% CI)[a]
	Controls	Patients			Controls	Patients		
CC	576	88	0.3330	1.00 (Reference)	233	60	**0.0015***	1.00 (Reference)
CT+TT	51	11		1.41 (0.71-2.81)	20	17		**3.30 (1.63-6.69)***
Total	627	99			253	77		

[a]OR: Odds ratio, CI: confidence interval; ORs were estimated with multivariate logistic regression analysis.
*Statistically identified as significant.

repair genes to head and neck risk (3, 10, 12, 13, 16, 22, 24-26, 33, 34, 38, 39). However, those investigated the contribution of genetic variations on DSB repair genes to NPC risk were very few. The present study is to investigate the role of *XRCC3* gene polymorphisms in NPC risk in Taiwan, where the NPC prevalence density in high due to an over-exposure to environmental factors such as betel quid, smoke and alcohol. Among these polymorphisms of *XRCC3* we investigated, the rs861539 located in the exon region and the T allele on it was associated with increased NPC risk in Taiwan (Table 3 and 4), while the other polymorphisms were not associated. The rs861539 genetic variation direct results in an amino acid coding alteration from Thr to Met which may indicate *XRCC3* rs861539 genetic polymorphism also result in functional alteration and predisposing to NPC cancer progression. Physiologically speaking, the cells with risky TT or CT genotypes may of less DNA repair capacity than those with CC wild-type genotype. Exposed to the same dose of exogenous or endogenous DNA damage, these cells with risky genotypes would leave more DNA adducts induced by the DNA damaging agents in the genome of these people. Years by years, these people with TT or CT genotypes at *XRCC3* rs861539 would accumulated more genomic instability and be attacked by all types of cancer. Thus, the effects of variant genotypes would be stronger in the smoker group than in the non-smoker group since the genome of smokers are exposed to higher exogenous DNA damaging agents, especially those carcinogens in cigarette components (Table 5).

In 2012, *XRCC3* rs3212057 was reported to be associated with head and neck cancer in Poland (14). There were some groups reporting negative association between *XRCC3* genotype and oral cancer in Brazil (12), Belgium (33), and India (24). Consistent with ours, *XRCC3* rs861539 was reported to be associated with oral cancer risk in Thailand. However the sample size of the report was rather small with

only 112 oral cancer cases and 119 controls (22).

The present study has several advantages. First is its large enough sample size. Although the case is limited to 176, we have recruited 5-fold (n = 880) age- and gender-matched controls and the overall analyzing power is satisfying. Second, all the analysis without adjustment strengthen the accuracy and reliability of our findings. Third, the frequencies of *XRCC3* polymorphisms variant alleles were similar to those reported in the NCBI website in the Asian population studies, for example the T minor allele frequencies of *XRCC3* rs861539 is 4.4% (Table 4) in our 880 controls, a little smaller than 4.7 to 11.0% for Asian population recorded on NCBI website. In 2005, Jin and his colleaguesstudy reported that the T minor allele frequencies of *XRCC3* rs861539 was 0.36% in 280 controls and 0.71% in 140 colorectal cancer patients in Taiwan (20). All of the above data suggested that no selection bias for the subject enrolments in our work, and the verifying of our findings in further larger studies is not so urgently necessary in the same issue. The paper has several disadvantages such as the limited sample size. Since NPC only was responsible for the prevalence of less than 1/100,000 per year all over the world and in Taiwan the annual incidence rates for males and females were 8.41 and 2.93 per 100,000 person-years, it is not easily to collect a large sample size within a single hospital. To fulfill this, the authors boosted the analyzing power by increasing the number of control to 880, and the total sample size is up to more than 1,000 and very representative.

The Table 1 suggested that the cigarette smoking is a risky factor for NPC in Taiwan (Table 1). Also, the results in this study have shown that there was positive interaction of variant DNA double strand break gene *XRCC3* rs861539 genotypes with individual smoking habits in NPC risk (Table 5). People with smoking habit and carrying the T allele of *XRCC3* rs861539 have an increased risk of NPC among our stratified subgroups (Table 5). These findings strengthened the theory of NPC carcinogenesis that genetic variants in DNA double strand break repairing system may enhance the genomic vulnerability to smoking-related DNA attacks, leading to NPC cancer progression.

To sum up, we found the genotype of *XRCC3* rs861539, but not those of rs1799794, rs45603942, rs861530, rs3212057, rs1799796 or rs28903081, was associated with increased NPC risk. In addition, the elevated NPC risk by variant genotypes of *XRCC3* rs861539 was more obviously enhanced among smokers, but not among none-smokers. Individual betel quid chewing and alcohol drinking habits could not enhance the risky genotype in increasing NPC susceptibility (data not shown). The Taiwanese are of conserved genetic background, combined Eastern-Western lifestyle and diet, and specific environmental risk factors, such as betel quid chewing for (oral) cancer progression. To realize the personalized medicine and therapy, the biomarkers for Taiwanese are in urgent need since NPC is much prevalent in Taiwan and south Asia. Up to now, there is early screening methodology for oral cancer, colorectal cancer, breast cancer, hepatoma, but not NPC. The *XRCC3* rs861539 CT or TT risk genotype found in this paper might serve as a useful biomarker for early detection and prediction of NPC in Taiwan.

Acknowledgments

We thank Tsai-Ping Ho, Chieh-Lun Hsiao, Tzu-Chia Wang, Yun-Ru Syu, Lin-Lin Hou, Chia-En Miao and Tissue-bank of China Medical University Hospital for technical assistance. This study was supported by research grants from Terry Fox Cancer Research Foundation of China Medical University and the National Science Council (NSC101-2320-B-039-045 and NSC102-2320-B-039-045).

References

1. Bau, D.T., Fu, Y.P., Chen, S.T., Cheng, T.C., Yu, J.C., Wu, P.E. and Shen, C.Y. Breast cancer risk and the DNA double-strand break end-joining capacity of nonhomologous end-joining genes are affected by BRCA1. *Cancer Res*. 64: 5013-5019, 2004.
2. Bau, D.T., Mau, Y.C., Ding, S.L., Wu, P.E. and Shen, C.Y. DNA double-strand break repair capacity and risk of breast cancer. *Carcinogenesis* 28: 1726-1730, 2007.
3. Bau, D.T., Tseng, H.C., Wang, C.H., Chiu, C.F., Hua, C.H., Wu, C.N., Liang, S.Y., Wang, C.L., Tsai, C.W. and Tsai, M.H. Oral cancer and genetic polymorphism of DNA double strand break gene Ku70 in Taiwan. *Oral Oncol*. 44: 1047-1051, 2008.
4. Cao, S.M., Simons, M.J. and Qian, C.N. The prevalence and prevention of nasopharyngeal carcinoma in China. *Chinese J. Cancer* 30: 114-119, 2011.
5. Cao, Y., Miao, X.P., Huang, M.Y., Deng, L., Liang, X.M., Lin, D.X., Zeng, Y.X. and Shao, J.Y. Polymorphisms of methylenetetrahydrofolate reductase are associated with a high risk of nasopharyngeal carcinoma in a smoking population from Southern China. *Mol. Carcinog*. 49: 928-934, 2010.
6. Chan, A.T., Teo, P.M. and Johnson, P.J. Nasopharyngeal carcinoma. *Ann. Oncol*. 13: 1007-1015, 2002.
7. Chang, C.H., Wu, H.C., Ke, H.L., Lin, H.H., Shen, W.C., Sun, S.S., Tsai, C.W., Tsai, R.Y. and Bau, D.T. The genetic role of cyclooxygenase 2 in ureter cancer in Taiwan. *Adapt. Med.* 3: 99-105, 2011.
8. Chang, W.S., Tsai, C.W., Cheng, K.H., Lien, C.S., Huang, C.P., Liao, W.L., Lee, M.H., Wu, H.C., Chang, C.H., Lin, C.C. and Bau, D.T. The Role of APEX1/Ref-1 (apurinic/apyrimidic endonuclease DNA-repair gene) in Renal Cell Carcinoma. *Adapt. Med.* 4: 216-223, 2012.
9. Chang, W.S., Yang, M.D., Tsai, C.W., Cheng, L.H., Jeng, L.B., Lo, W.C., Lin, C.H., Huang, C.Y. and Bau, D.T. Association of cyclooxygenase 2 single-nucleotide polymorphisms and hepatocellular carcinoma in Taiwan. *Chinese J. Physiol*. 55: 1-7, 2012.
10. Chiu, C.F., Tsai, M.H., Tseng, H.C., Wang, C.L., Wang, C.H., Wu, C.N., Lin, C.C. and Bau, D.T. A novel single nucleotide polymorphism in XRCC4 gene is associated with oral cancer suscep-

tibility in Taiwanese patients. *Oral Oncol*. 44: 898-902, 2008.
11. Chiu, C.F., Wang, C.H., Wang, C.L., Lin, C.C., Hsu, N.Y., Weng, J.R. and Bau, D.T. A novel single nucleotide polymorphism in XRCC4 gene is associated with gastric cancer susceptibility in Taiwan. *Ann. Surg. Oncol*. 15: 514-518, 2008.
12. Dos Reis, M.B., Losi-Guembarovski, R., de Souza Fonseca Ribeiro, E.M., Cavalli, I.J., Morita, M.C., Ramos, G.H., de Oliveira, B.V., Mizuno, L.T., Rogatto, S.R. and de Syllos Colus, I.M. Allelic variants of XRCC1 and XRCC3 repair genes and susceptibility of oral cancer in Brazilian patients. *J. Oral Pathol. Med*. 42: 180-185, 2013.
13. Gal, T.J., Huang, W.Y., Chen, C., Hayes, R.B. and Schwartz, S.M. DNA repair gene polymorphisms and risk of second primary neoplasms and mortality in oral cancer patients. *Laryngoscope* 115: 2221-2231, 2005.
14. Gresner, P., Gromadzinska, J., Polanska, K., Twardowska, E., Jurewicz, J. and Wasowicz, W. Genetic variability of Xrcc3 and Rad51 modulates the risk of head and neck cancer. *Gene* 504: 166-174, 2012.
15. Han, J., Colditz, G.A., Samson, L.D. and Hunter, D.J. Polymorphisms in DNA double-strand break repair genes and skin cancer risk. *Cancer Res*. 64: 3009-3013, 2004.
16. Hsu, C.F., Tseng, H.C., Chiu, C.F., Liang, S.Y., Tsai, C.W., Tsai, M.H. and Bau, D.T. Association between DNA double strand break gene Ku80 polymorphisms and oral cancer susceptibility. *Oral Oncol*. 45: 789-793, 2009.
17. Hsu, C.M., Yang, M.D., Chang, W.S., Jeng, L.B., Lee, M.H., Lu, M.C., Chang, S.C., Tsai, C.W., Tsai, Y., Tsai, F.J. and Bau, D.T. The contribution of XRCC6/Ku70 to hepatocellular carcinoma in Taiwan. *Anticancer Res*. 33: 529-535, 2013.
18. Hsu, W.L., Pan, W.H., Chien, Y.C., Yu, K.J., Cheng, Y.J., Chen, J.Y., Liu, M.Y., Hsu, M.M., Lou, P.J., Chen, I.H., Yang, C.S., Hildesheim, A. and Chen, C.J. Lowered risk of nasopharyngeal carcinoma and intake of plant vitamin, fresh fish, green tea and coffee: a case-control study in Taiwan. *PLoS One* 7: e41779, 2012.
19. Ji, X., Zhang, W., Xie, C., Wang, B., Zhang, G. and Zhou, F. Nasopharyngeal carcinoma risk by histologic type in central China: impact of smoking, alcohol and family history. *Int. J. Cancer* 129: 724-732, 2011.
20. Jin, M.J., Chen, K., Song, L., Fan, C.H., Chen, Q., Zhu, Y.M., Ma, X.Y. and Yao, K.Y. The association of the DNA repair gene XRCC3 Thr241Met polymorphism with susceptibility to colorectal cancer in a Chinese population. *Cancer Genet. Cytogenet*. 163: 38-43, 2005.
21. Khanna, K.K. and Jackson, S.P. DNA double-strand breaks: signaling, repair and the cancer connection. *Nat. Genet*. 27: 247-254, 2001.
22. Kietthubthew, S., Sriplung, H., Au, W.W. and Ishida, T. Polymorphism in DNA repair genes and oral squamous cell carcinoma in Thailand. *Int. J. Hyg. Environ. Health* 209: 21-29, 2006.
23. Lin, C.H., Ho, C.Y., Liu, C.S., Lin, W.Y., Li, C.I., Yang, C.W., Bau, D.T., Li, T.C. and Lin, C.C. Influence of adiponectin gene polymorphisms on adiponectin serum level and insulin resistance index in taiwanese metabolic syndrome patients. *Chinese J. Physiol*. 55: 405-411, 2012.
24. Majumder, M., Sikdar, N., Paul, R.R. and Roy, B. Increased risk of oral leukoplakia and cancer among mixed tobacco users carrying XRCC1 variant haplotypes and cancer among smokers carrying two risk genotypes: one on each of two loci, GSTM3 and XRCC1 (Codon 280). *Cancer Epidemiol. Biomarkers Prev*. 14: 2106-2112, 2005.
25. Matullo, G., Dunning, A.M., Guarrera, S., Baynes, C., Polidoro, S., Garte, S., Autrup, H., Malaveille, C., Peluso, M., Airoldi, L., Veglia, F., Gormally, E., Hoek, G., Krzyzanowski, M., Overvad, K., Raaschou-Nielsen, O., Clavel-Chapelon, F., Linseisen, J., Boeing, H., Trichopoulou, A., Palli, D., Krogh, V., Tumino, R., Panico, S., Bueno-De-Mesquita, H.B., Peeters, P.H., Lund, E., Pera, G., Martinez, C., Dorronsoro, M., Barricarte, A., Tormo, M.J., Quiros, J.R., Day, N.E., Key, T.J., Saracci, R., Kaaks, R., Riboli, E. and Vineis, P. DNA repair polymorphisms and cancer risk in non-smokers in a cohort study. *Carcinogenesis* 27: 997-1007, 2006.
26. Mondal, P., Datta, S., Maiti, G.P., Baral, A., Jha, G.N., Panda, C.K., Chowdhury, S., Ghosh, S., Roy, B. and Roychoudhury, S. Comprehensive SNP scan of DNA repair and DNA damage response genes reveal multiple susceptibility loci conferring risk to tobacco associated leukoplakia and oral cancer. *PLoS One* 8: e56952, 2013.
27. Niedobitek, G., Hansmann, M.L., Herbst, H., Young, L.S., Dienemann, D., Hartmann, C.A., Finn, T., Pitteroff, S., Welt, A., Anagnostopoulos, I., Friedrich, R., Friedrich, H., Sam, C.K., Araujo, I., Rickinson, A.B. and Stein, H. Epstein-Barr virus and carcinomas: undifferentiated carcinomas but not squamous cell carcinomas of the nasopharynx are regularly associated with the virus. *J. Pathol*. 165: 17-24, 1991.
28. Shih, L.C., Tsai, C.W., Tsai, M.H., Tsou, Y.A., Chang, W.S., Li, F.J., Lee, M.H. and Bau, D.T. Association of cyclin D1 genotypes with nasopharyngeal carcinoma risk. *Anticancer Res*. 32: 1093-1098, 2012.
29. Thacker, J. The RAD51 gene family, genetic instability and cancer. *Cancer Lett*. 219: 125-135, 2005.
30. Tsai, C.W., Ho, C.Y., Shih, L.C., Ying, T.H., Hsieh, Y.H., Chen, Y.C., Chang, W.S., Huang, C.Y., Pan, S.B., Shui, H.A., Chen, C.P., Wang, P.S. and Bau, D.T. The Joint Effect of hOGG1 Genotype and Smoking Habit on Endometriosis in Taiwan. *Chinese J. Physiol*. 56: 263-268, 2013.
31. Tsai, C.W., Tsai, M.H., Shih, L.C., Chang, W.S., Lin, C.C. and Bau, D.T. Association of interleukin-10 (IL10) promoter genotypes with nasopharyngeal carcinoma risk in Taiwan. *Anticancer Res*. 33: 3391-3396, 2013.
32. Tsou, Y.A., Tsai, C.W., Tsai, M.H., Chang, W.S., Li, F.J., Liu, Y.F., Chiu, C.F., Lin, C.C. and Bau, D.T. Association of caveolin-1 genotypes with nasopharyngeal carcinoma susceptibility in Taiwan. *Anticancer Res*. 31: 3629-3632, 2011.
33. Werbrouck, J., De Ruyck, K., Duprez, F., Van Eijkeren, M., Rietzschel, E., Bekaert, S., Vral, A., De Neve, W. and Thierens, H. Single-nucleotide polymorphisms in DNA double-strand break repair genes: association with head and neck cancer and interaction with tobacco use and alcohol consumption. *Mutat. Res*. 656: 74-81, 2008.
34. Werbrouck, J., De Ruyck, K., Duprez, F., Veldeman, L., Claes, K., Van Eijkeren, M., Boterberg, T., Willems, P., Vral, A., De Neve, W. and Thierens, H. Acute normal tissue reactions in head-and-neck cancer patients treated with IMRT: influence of dose and association with genetic polymorphisms in DNA DSB repair genes. *Int. J. Radiat. Oncol. Biol. Phys*. 73: 1187-1195, 2009.
35. Wood, R.D., Mitchell, M., Sgouros, J. and Lindahl, T. Human DNA repair genes. *Science* 291: 1284-1289, 2001.
36. Xu, F.H., Xiong, D., Xu, Y.F., Cao, S.M., Xue, W.Q., Qin, H.D., Liu, W.S., Cao, J.Y., Zhang, Y., Feng, Q.S., Chen, L.Z., Li, M.Z., Liu, Z.W., Liu, Q., Hong, M.H., Shugart, Y.Y., Zeng, Y.X., Zeng, M.S. and Jia, W.H. An epidemiological and molecular study of the relationship between smoking, risk of nasopharyngeal carcinoma, and Epstein-Barr virus activation. *J. Natl. Cancer Inst*. 104: 1396-1410, 2012.
37. Xue, W.Q., Qin, H.D., Ruan, H.L., Shugart, Y.Y. and Jia, W.H. Quantitative association of tobacco smoking with the risk of nasopharyngeal carcinoma: a comprehensive meta-analysis of studies conducted between 1979 and 2011. *Am. J. Epidemiol*. 178: 325-338, 2013.
38. Yang, H., Lippman, S.M., Huang, M., Jack Lee, J., Wang, W., Spitz, M.R. and Wu, X. Genetic polymorphisms in double-strand break DNA repair genes associated with risk of oral premalignant lesions. *Eur. J. Cancer* 44: 1603-1611, 2008.

39. Yen, C.Y., Liu, S.Y., Chen, C.H., Tseng, H.F., Chuang, L.Y., Yang, C.H., Lin, Y.C., Wen, C.H., Chiang, W.F., Ho, C.H., Chen, H.C., Wang, S.T., Lin, C.W. and Chang, H.W. Combinational polymorphisms of four DNA repair genes XRCC1, XRCC2, XRCC3, and XRCC4 and their association with oral cancer in Taiwan. *J. Oral Pathol. Med*. 37: 271-277, 2008.
40. Yu, M.C. and Yuan, J.M. Epidemiology of nasopharyngeal carcinoma. *Semin. Cancer Biol*. 12: 421-429, 2002.
41. Yu, Z., Chen, J., Ford, B.N., Brackley, M.E. and Glickman, B.W. Human DNA repair systems: an overview. *Environ. Mol. Mutagen*. 33: 3-20, 1999.
42. Zong, Y.S., Sham, J.S., Ng, M.H., Ou, X.T., Guo, Y.Q., Zheng, S.A., Liang, J.S. and Qiu, H. Immunoglobulin A against viral capsid antigen of Epstein-Barr virus and indirect mirror examination of the nasopharynx in the detection of asymptomatic nasopharyngeal carcinoma. *Cancer* 69: 3-7, 1992.