Contribution of DNA Double-strand Break Repair Gene *XRCC3* Genotypes to Oral Cancer Susceptibility in Taiwan

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Abstract. *The DNA repair gene X-ray repair cross complementing protein 3 (XRCC3) is thought to play a major role in double-strand break repair and in maintaining genomic stability. Very possibly, defective double-strand break repair of cells can lead to carcinogenesis. Therefore, a case–control study was performed to reveal the contribution of XRCC3 genotypes to individual oral cancer susceptibility. In this hospital-based research, the association of XRCC3 rs1799794, rs45603942, rs861530, rs3212057, rs1799796, rs861539, rs28903081 genotypes with oral cancer risk in a Taiwanese population was investigated. In total, 788 patients with oral cancer and 956 age- and gender-matched healthy controls were genotyped. The results showed that there was significant differential distribution among oral cancer and controls in the genotypic (p=0.001428) and allelic (p=0.0013) frequencies of XRCC3 rs861539. As for the other polymorphisms, there was no difference between case and control groups. In gene–lifestyle interaction analysis, we have provided the first evidence showing that there is an obvious joint effect of XRCC3 rs861539 genotype with individual areca chewing habits on oral cancer risk. In conclusion, the T allele of XRCC3 rs861539, which has an interaction with areca chewing habit in oral carcinogenesis, may be an early marker for oral cancer in Taiwanese.*

Oral cancer, which is the tenth most commonly diagnosed cancer worldwide (1), has the highest incidence of all head and neck cancers in Taiwan (2). Three major lifestyle factors, namely the use of tobacco, alcohol and betel nuts, are main causes of oral cancer in Taiwan, while the genomic etiology of oral cancer is of great interest but largely unknown. Human DNA repair mechanisms protect the genome from various insults caused by endogenous and exogenous DNA-damaging agents (3) and defects in the DNA repair system are thought to be essential in tumorigenesis (4, 5). Therefore, it is logical to suspect that some genetic variants of DNA repair genes might contribute to oral cancer pathogenesis.

Environmental carcinogens such as UV light, ionizing radiation or chemical agents, contained for instance in tobacco smoke, may induce double-strand breaks (DSBs) in DNA. DSBs are a severe type of DNA damage which should be repaired by the DNA DSB repair system (6, 7). If cells cannot remove DSBs immediately by means of homologous recombination and non-homologous end-joining, these DNA DSBs may induce pre-cancerous lesions and even cancer itself (8, 9). Genetic polymorphisms in DNA DSB repair genes influences the DNA repair capacity and confers predisposition to several types of cancer, including skin (10), breast (11, 12) liver (13), gastric (14), and oral (15, 16) cancer. The X-Ray repair cross-complementing group 3 (*XRCC3*; 14q32.3) is a member of the RAD51 recombinase (*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 (17).

The most common genetic polymorphism of the *XRCC3* gene is the rs861539 C/T polymorphism (also named Thr241Met, T241M, C18067T and C722T). Some studies were performed to investigate the association between *XRCC3* rs861539 and oral pre-malignant (9) and oral cancer risk (18-22), however no consistent finding was reported. The inconsistency may be caused by small sample sizes and different genetic backgrounds among different ethnicities. To identify the contribution of the XRCC3

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Table I. *Summary of the characteristics for all the patients with oral cancer and healthy controls.*

Characteristic	Controls (n=956)			Patients (n=788)			*p*-Value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			56.6 (8.7)			55.8 (9.9)	0.7951
Gender							1.0000
Male	727	76.0%		599	76.0%		
Female	229	24.0%		189	24.0%		
Indulgence							
Betel quid chewers	506	52.9%		661	83.9%		<0.0001*
Cigarette smokers	667	69.8%		595	75.5%		0.0084*
Alcohol drinkers	641	67.1%		560	71.1%		0.0773
Histology							
Tongue				325	41.2%		
Buccal mucosa				294	37.3%		
Mouth floor				30	3.8%		
Retromolar trigone				26	3.3%		
Alveolar ridge				18	2.3%		
Palate				18	2.3%		
Lip				39	4.9%		
Others				38	4.9%		

[a]Based on Chi-square test,*statistically significant at $p<0.05$.

genotype to oral cancer risk in Taiwan, we determined the genotypic frequencies of seven single nucleotide polymorphisms (SNPs) of the *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–lifestyle interaction.

Materials and Methods

Study population and sample collection. Seven hundred and eighty-eight patients diagnosed with oral cancer were recruited at the China Medical University Hospital in central Taiwan during 1998 to 2010. All patients voluntarily participated, completed a self-administered questionnaire and provided a 5 ml peripheral blood sample. The questionnaire administered to the participants included questions on history and frequency of alcohol consumption, areca chewing and smoking habits. Self-reported alcohol consumption, areca chewing and smoking habits were evaluated and classified as categorical variables, with use more than twice a week for years as 'ever'. A total of 956 non-cancer healthy people as controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of the hospital. The ratio of males *versus* females was 76% *versus* 24% in each group. The mean age of the patients with oral cancer and the controls was 55.8 (SD=9.9) and 56.6 (SD=8.7) years, respectively (see Table I for more details). Our continuous study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

Genotyping conditions. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC) and further processed as per our previous articles (23, 24). A total of seven polymorphic sites were analyzed in all participants of the control and case groups. Briefly, all of the seven polymorphic sites were genotyped by means of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was performed on a BioRad Mycycler (BioRad, Hercules, CA, USA) following the 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 II 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 are summarized in Table II.

Statistical analyses. Matched SNP data and clinical characteristics (case/control=788/956) were analyzed. 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 controls from those expected under the Hardy–Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's chi-square test 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 were recognized as significant when the statistical *p*-value was less than 0.05.

Table II. *Summary of the rs numbers, primers, amplicon length before and after enzyme digestion, and restriction enzymes for all the X-ray repair cross complementing protein 3 (XRCC3) single nucleotide polymorphisms investigated in this study.*

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

Table III. *Distribution of X-ray repair cross complementing protein 3 (XRCC3) genotypes among patients with oral cancer and controls.*

Genotype	Controls		Patients		*p*-Value[a]	Odds ratio (95% CI)[b]
	(n)	%	(n)	%		
rs1799794					0.8932	
GG	229	24.0%	195	24.7%		1.00 (Reference)
AG	532	55.6%	438	55.6%		0.97 (0.77-1.22)
AA	195	20.4%	155	19.7%		0.93 (0.70-1.24)
rs45603942					0.9967	
CC	886	92.7%	731	92.8%		1.00 (Reference)
CT	64	6.7%	52	6.6%		0.98 (0.67-1.44)
TT	6	0.6%	5	0.6%		1.01 (0.31-3.32)
rs861530					0.3101	
AA	284	29.7%	247	31.3%		1.00 (Reference)
AG	519	54.3%	435	55.2%		0.96 (0.78-1.19)
GG	153	16.0%	106	13.5%		0.80 (0.59-1.08)
rs3212057					1.0000	
GG	956	100.0%	788	100.0%		1.00 (Reference)
AG	0	0.0%	0	0.0%		1.00
AA	0	0.0%	0	0.0%		1.00
rs1799796					0.8184	
AA	438	45.8%	370	47.0%		1.00 (Reference)
AG	468	49.0%	381	48.3%		0.96 (0.79-1.17)
GG	50	5.2%	37	4.7%		0.88 (0.56-1.37)
rs861539					0.001428*	
CC	878	91.9%	684	86.8%		1.00 (Reference)
CT	73	7.6%	92	11.7%		1.62 (1.17-2.23)*
TT	5	0.5%	12	1.5%		3.08 (1.08-8.79)*
rs28903081					1.0000	
GG	956	100.0%	788	100.0%		1.00 (Reference)
AG	0	0.0%	0	0.0%		1.00
AA	0	0.0%	0	0.0%		1.00

[a]Based on chi-square test. [b]CI: Confidence interval; *statistically identified as significant.

Table IV. *Distribution of X-ray repair cross complementing protein 3 (XRCC3) alleles among patients with oral cancer and controls.*

Allele	Controls	%	Patients	%	*p*-Value[a]
rs1799794					0.6548
Allele G	990	51.8%	828	52.5%	
Allele A	922	48.2%	748	47.5%	
rs45603942					1.0000
Allele C	1836	96.0%	1514	96.1%	
Allele T	76	4.0%	62	3.9%	
rs861530					0.2124
Allele A	1087	56.9%	929	58.9%	
Allele G	825	43.1%	647	41.1%	
rs1799796					0.5891
Allele A	1344	70.3%	1121	71.1%	
Allele G	568	29.7%	455	28.9%	
rs861539					0.0013*
Allele C	1829	95.7%	1460	92.6%	
Allele T	83	4.3%	116	7.4%	

[a]Based on chi-square test. *Statistically identified as significant.

Results

The clinical characteristics of 788 patients recruited with oral cancer and 956 age- and gender-matched controls are shown in Table I. Since the controls are age-, gender-matched with the cases, there was no significant difference between the two groups as to their age and gender (Table I). However, for the personal habits, there was a significant difference in that the case group seemed to have more smokers and betel quid chewers (Table I). The frequency distributions of the genotypes for the *XRCC3* rs1799794, rs45603942, rs861530,

Table V. *Odds ratios for X-ray repair cross complementing protein 3 (XRCC3) rs861539 genotype and oral cancer risk after stratification by areca chewing habit.*

Genotypes	Non-betel quid chewers		*p*-Value	OR (95% CI)[a]	Betel quid chewers		*p*-Value	OR (95% CI)
	Controls	Patients			Controls	Patients		
CC	411	115	0.8594	1.000 (Reference)	467	569	0.0010*	1.000 (Reference)
CT+TT	39	12		1.10 (0.56-2.17)	39	92		1.94 (1.31-2.87)*
Total	450	127			506	661		

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

rs3212057, rs1799796, rs861539 and rs28903081 polymorphic sites between controls and patients with oral cancer are shown in Table III. Genotypic distribution pattern of *XRCC3* rs861539 was significantly different between oral cancer and control groups ($p<0.05$), while those for rs1799794, rs45603942, rs861530, rs3212057, rs1799796 and rs28903081 were not significant ($p>0.05$) (Table III). In detail, distributions of *XRCC3* rs861539 CC homozygotes/heterozygotes/TT homozygotes in controls and patients were 91.9/7.6/0.5% and 86.8/11.7/1.5%, respectively (Table III). There was no heterozygote or homozygote variant for *XRCC3* rs3212057 and rs28903081 among Taiwanese subjects (Table III). To sum up, the genotype of *XRCC3* rs861539, not rs1799794, rs45603942, rs861530, rs3212057, rs1799796 or rs28903081, appears to be associated with oral cancer risk and may be a biomarker for oral cancer.

The frequencies of the alleles for the *XRCC3* rs1799794, rs45603942, rs861530, rs3212057, rs1799796, rs861539 and rs28903081 of all the recruited participants are shown in Table IV. Among them, the carriers of *XRCC3* rs861539 T allele were at higher risk for oral cancer ($p=0.0013$), while genotypes of *XRCC3* rs1799794, rs45603942, rs861530, rs3212057, rs1799796 and rs28903081 were not associated with oral cancer susceptibility (Table IV).

We were interested to investigate the potential gene–lifestyle interactions between the *XRCC3* gene and oral cancer-related habits. In Taiwan, the habits of smoking, alcoholism and betel quid chewing are believed to significantly increase oral cancer risk. Therefore, the risk of oral cancer related to *XRCC3* genotypes was further examined with stratification by areca chewing, smoking and alcohol drinking status. Table V shows the interaction of XRCC3 genotype and betel quid chewing status on personal oral cancer susceptibility (Table V). Compared with the CC genotype, having the CT or TT genotype significantly increased oral cancer risk only in the areca chewers ($p=0.0010$, OR=1.94, 95% CI=1.31-2.87), not in the non-areca chewers ($p>0.05$, OR=1.10, 95%CI=0.56-2.17) (Table V). With the same strategy, the interactions among *XRCC3* genotype and smoking or alcohol drinking statuses were also analyzed, but no significant interaction was found (data not shown).

Discussion

In recent years, some researchers investigated the contribution of genetic variations in genes of DSB repair to oral cancer risk (9, 15, 16, 18-22, 25-29). Among them, several studies had analyzed the interaction of genetic variations and behavioral factors on oral cancer (19, 20, 26, 28). The present study investigated the role of *XRCC3* gene polymorphisms in oral cancer risk in Taiwan, where oral cancer prevalence is the highest in the world due to use of betel quid, tobacco and alcohol. Among the seven polymorphisms of *XRCC3*, rs861539 located in the exon 8 region and the T allele for it was associated with oral cancer in Taiwan (Tables III and IV), but the other six polymorphisms were not. The direct result of rs861539 genetic variation is an amino acid coding alteration from Thr to Met. Possibly, this *XRCC3* rs861539 genetic polymorphism may also result in functional polymorphism and predisposing to oral carcinogenesis. Up to now, the studies investigating the association of *XRCC3* polymorphism with oral cancer risk did not reach a consistent conclusion. This may be due to different recruited populations with different genetic background, various behavioral and environmental factors were taken into consideration, and inconsistent polymorphic sites were chosen. In 2012, *XRCC3* rs3212057 was found to be associated with head and neck cancer in Poland (30). There were also some negative findings reporting no association between *XRCC3* genotype and oral cancer in Brazil (18), Belgium (20), India (19). As for *XRCC3* rs861539, a positive finding was reported in Thailand, but the sample size of the study was rather small, with only 112 oral cancer cases and 119 controls (21).

Thus, a relatively large sample size (controls:cases=956:788) and concise data analysis without adjustment strengthen the accuracy and reliability of our finding. Moreover, the frequencies of *XRCC3* polymorphisms variant alleles were similar to those reported in the National Center for Biotechnology Information (NCBI) website for the Asian population studies, for example the minor T allelic frequency

of *XRCC3* rs861539 is 4.3% (Table IV) in our control group and 4.7 to 11.0% for the Asian population according to the NCBI. In 2005, in Jin and colleagues' work, the minor T allelic frequency of *XRCC3* rs861539 was reported as 0.36% in 280 controls and 0.71% in 140 patients with colorectal cancer in Taiwan (31). The data suggested that there was no selection bias for enrolments in terms of genotype. Therefore, verifying of our findings in further larger studies is not so urgently needed.

There were three behavioral factors reported to be closely-related to oral carcinogenesis in Taiwan, cigarette smoking, alcohol consumption and betel quid chewing. Previously, our group has provided evidence for interaction between DNA DSB genes and betel quid chewing habit for *XRCC4* and *XRCC5* (15, 29). Again, the results in this study have shown that there was positive interaction of variant DNA DSB gene *XRCC3* rs861539 genotypes with betel quid chewing habits in oral cancer risk (Table V). People with betel quid chewing habit and carrying the T allele of *XRCC3* rs861539 have a higher risk of oral cancer among our stratified sub-groups. These findings strengthen the theory for oral carcinogenesis that genetic variants in DNA double strand break system may enhance genomic vulnerability to DNA caused by areca chewing, leading to oral carcinogenesis.

In conclusion, we found that the genotype of *XRCC3* rs861539, but not those of rs1799794, rs45603942, rs861530, rs3212057, rs1799796 or rs28903081, was associated with higher oral cancer risk through the T allele. In addition, the increased oral cancer risk due to variant genotypes of *XRCC3* rs861539 was more obviously enhanced among betel quid chewers, but not among non-chewers. Individual smoking and alcohol drinking habits did not appear to enhance oral cancer susceptibility. The *XRCC3* rs861539 polymorphism might become a potential biomarker for the early detection and prediction of oral oncology and further investigation of the phenotypic effects determined by this genotypic variation on oral carcinogenesis are needed.

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