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Association between Polymorphisms in DNA Base Excision Repair Genes *XRCC1, APE1*, and *ADPRT* and Differentiated Thyroid Carcinoma

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Abstract

Purpose: DNA BER pathway is related with carcinogenesis. We hypothesized that functional polymorphisms of three BER genes, *XRCC1, apurinic/apyrimidinic endonuclease* (*APE1*), and *ADPRT*, confer risks for DTC and its progression.

Experimental Design: Five common nonsynonymous single nucleotide polymorphisms (*Arg194Trp, Arg280His,* and *Arg399Gln* for *XRCC1*; *Asp148Glu* for *APE1*; and *Val762Ala* for *ADPRT*) were genotyped in Chinese DTC cases and controls.

Results: The *XRCC1-194Trp/Trp* genotype showed a significantly increased risk for DTC (odds ratio, 1.85; 95% confidence interval, 1.11-3.07; $P = 0.018$). Subset analysis based on regional LN metastasis showed that the genetic effect came primarily from the subjects with LN metastasis (odds ratio, 4.54; 95% confidence interval, 2.11-9.79; $P = 0.0001$), but no significant association for subjects without LN metastasis. The other four single nucleotide polymorphisms did not show significant results. Haplotype analysis of *XRCC1* polymorphisms yielded a significant result ($P = 0.004$), especially in the subjects with LN metastasis ($P = 0.0002$). Moreover, we found that *XRCC1-194Trp* and *ADPRT-762Ala* variants collectively contributed to an increased risk of the disease and LN metastasis, with the combined variant homozygotes exhibiting the highest 3.18-fold risk for DTC ($P = 0.046$) and 9.25-fold risk for DTC with LN metastasis ($P = 0.004$).

Conclusions: The *XRCC1* polymorphisms, especially the *194Trp* allele, may have an effect on DTC development and progression. This variant can interact with *ADPRT-762Ala* variant to further substantially increase susceptibility to the disease and regional LN metastasis. Identifying these risk genetic markers could provide more insight into the DTC pathogenesis and may also provide information to develop better prevention and therapeutic strategies.

Thyroid cancer is the most prevalent endocrine malignancy (1), and the incidence rate has increased over recent decades (2–4). Differentiated thyroid carcinoma (DTC), including papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), accounts for >90% of thyroid malignancies. Exposure to ionizing radiation is the only verified cause of thyroid carcinogenesis in human, especially radiation exposure at young ages (5). However, not everyone exposed to radiation develops thyroid cancer, and most patients with thyroid cancer have no histories of high-dose radiation exposure. This suggests that potential predisposing genetic factors may have an effect on an individual's susceptibility to thyroid cancer.

Ionizing radiation causes various DNA damages, including single- and double-strand breaks, and the generation of reactive oxygen species that cause base damage (6, 7). In the thyroid, reactive oxygen species and free radicals can be generated through physiologic or pathologic processes, and excessive oxidative stress can cause DNA damage and somatic mutation (8, 9). DNA damage consequently initiates DNA repair process in the cells. Genetic variations in the DNA repair genes can modulate the DNA repair capacity, leading to individual susceptibility to cancer risk (10, 11). Among the DNA repair pathways, the base excision repair (BER) specifically removes alterations of a single base when it is methylated, oxidized, or reduced, and it thus rectifies single-strand breaks in DNA (10, 11). Therefore, interindividual variation in the BER

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Translational Relevance

In thyroid cancer, DNA base excision repair (BER) pathway is related with carcinogenesis. In this study, we showed that genetic polymorphisms in the *X-ray repair cross-complementing 1* (*XRCC1*) and *ADP-ribosyltransferase* (*ADPRT*) genes leading to deficient BER may play a role in the development of differentiated thyroid carcinoma (DTC) and its regional lymph node (LN) metastasis.

In the future, our results may be applied to develop better prevention programs and therapeutic regimens. For example, a regular thyroid ultrasound evaluation should be arranged for risky individuals with disadvantageous genotypes. Patients with goiter and the risk genotypes may need a more comprehensive survey and more aggressive surgical treatment because of the increased risk for thyroid malignancy and its LN metastasis. In addition, the *XRCC1* and *ADPRT* genes can become targets for drug development.

pathway is one of the host factors that may influence thyroid cancer risk.

The BER pathway is a multistep process that requires the activation of several proteins, among which X-ray repair cross-complementing 1 (XRCC1), apurinic/apyrimidinic endonuclease (APE1, also known as APEX1), and ADP-ribosyltransferase (ADPRT, also known as PARP1) play important roles (12). *XRCC1* is located at chromosome 19q13.2-13.3 and is thought to act as a scaffold protein for both BER and single-strand break repair (13). XRCC1 also interacts with several important repair proteins through its different domains (14, 15). *APE1* is located at chromosome 14q11.2-q12, which bridges the abasic sites of the damaged base by cleaving the DNA backbone at the 5′ side to the abasic site, leaving a 3′-hydroxyl group and a 5′-deoxyribose phosphate group flanking the nucleotide gap, and initiates the BER process (16). *ADPRT* is located at chromosome 1q41-q42, specifically binds to DNA strand breaks, and plays a role in the long-patch BER (17).

Several functional genetic polymorphisms have been identified in the *XRCC1*, *APE1*, and *ADPRT* genes, and studies have suggested that some of these polymorphisms may be associated with cancer risk (12, 18). There are reports of association between the *XRCC1*, *APE1*, and *ADPRT* polymorphisms and various cancers, including lung (19), esophagus (20), breast (21), and skin (22) cancers. From the literature review, the association with thyroid cancer risk has been reported in one small study (105 cases and 105 controls) for *XRCC1* polymorphisms in Chinese (23), but no report for *APE1* and *ADPRT*. Therefore, we hypothesized that common, nonsynonymous single nucleotide polymorphisms (nsSNP) of the *XRCC1*, *APE1*, and *ADPRT* genes may affect the capacity of thyroid cells to repair reactive oxygen species–induced DNA damage and thus may interact to contribute collectively to the development and regional lymph node (LN) metastasis of DTC. We tested this hypothesis using a case-control study among a Chinese population living in Taiwan.

Materials and Methods

Subjects. We recruited 283 patients with DTC and 469 controls at the Kaohsiung Medical University Hospital in Taiwan from November 2005 to February 2007. Among the DTC subjects, 217 were retrospectively recruited (initial diagnosis was made between 1980 and October 2005) and 66 were incident cases between 2005 and 2007. All subjects were unrelated ethnic Chinese and residents in Taiwan. The diagnosis with DTC and the presence of neck regional LN metastasis were both confirmed by pathologic examination. The control subjects were recruited from the Department of Otolaryngology (n = 198) or the healthy subjects (n = 271) who participated in regular health examination at the same hospital. All of the control subjects received thyroid physical examination by a single endocrinologist or otolarygologist. None of the controls had been diagnosed with cancer or thyroid disease on enrollment. The self-administrated questionnaire included the demographic characteristics, family history of cancer, and history of ionizing radiation exposure. After we obtained informed consent, each participant provided 5 mL of blood. The study was approved by Institutional Review Board of Kaohsiung Medical University Hospital.

SNP selections. We searched the databases[9] and the related literatures to identify all reported nsSNPs at the *XRCC1*, *APE1*, and *ADPRT* genes with a minor allele frequency >0.05 in the Han Chinese population. We identified three common nsSNPs for the *XRCC1* gene and only one common nsSNP for the *APE1* and *ADPRT* genes, respectively. The five SNPs selected for genotyping were *Arg194Trp* [*C21935T*; reference SNP no. (rs) 1799782], *Arg280His* (*G23098A*; rs25489), and *Arg399Gln* (*G23885A*; rs25487) for *XRCC1*; *Asp148Glu* (*T1349G*; rs3136820) for *APE1*; and *Val762Ala* (*T40336C*; rs1136410) for *ADPRT*.

Genotyping. Genomic DNA was extracted from the peripheral blood by the standard methods. Genotyping was done using the Taqman fluorogenic 5′ nuclease assay (Applied Biosystems). Briefly, PCR primers and Taqman minor groove binder probes were designed and reactions were done in 96-well microplates with ABI 9700 thermal cyclers (Applied Biosystems). Fluorescence was measured with an ABI 7500 Real-Time PCR System (Applied Biosystems) and analyzed with its System SDS software version 1.2.3.

Statistical analysis. Continuous variables were analyzed by independent t test and were presented as mean ± SD. Allele frequencies were estimated by direct gene counting. Observed numbers of each genotype were compared with those expected for Hardy-Weinberg equilibrium using the χ^2 test. Hardy-Weinberg equilibrium test was done for cases and controls separately. Genetic effects were compared by χ^2 test or Fisher's exact test. A trend test (P_{trend}) assuming a dose response with increasing number of the risk allele was also done. Multivariate logistic regression analysis was done to obtain age- and sex-adjusted odds ratios (OR) and 95% confidence intervals (95% CI) while assessing the genetic effects. For the purpose of these calculations, age for controls was the age at enrollment, whereas age for cases was the age at cancer diagnosis. All analyses were done using Statistical Package for the Social Sciences for Windows 13.0 version (SPSS, Inc.). All statistical tests were two sided and significant level was set at $P < 0.05$. Haploview was applied to calculate linkage disequilibrium and define haplotype blocks. Haplotype analysis was done using the Hap-Clustering program (24). The PHASE program was used to infer haplotypes and test for the association between phenotypes and haplotypes/diplotypes (25). We tested for multiplicative gene-gene interactions by evaluating departures from multiplicative joint effect models (26). A more than multiplicative

[9] http://www.hapmap.org and http://www.ncbi.nlm.nih.gov/SNP/

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Table 1. Baseline demographics of study characteristics

Variables	Cases (*n* = 283)	Controls (*n* = 469)	*P**
Age (y), mean (SD)	45.3 (13.2) †	43.9 (13.5) ‡	0.50
Sex (male:female)	1:4.34	1:3.69	0.39
Male, *n* (%)	53 (18.7%)	100 (21.3%)	
Female, *n* (%)	230 (81.3%)	369 (78.7%)	
Histologic type			
Papillary carcinoma, *n* (%)	259 (91.5%)	—	
Follicular carcinoma, *n* (%)	24 (8.5%)	—	
LN metastasis			
Positive, *n* (%)	65 (23.0%)	—	
Negative, *n* (%)	218 (77.0%)	—	

*Two-sided χ^2 test.
† Age at diagnosis.
‡ Age at enrollment.

joint effect was suggested when $OR_{11} > OR_{10} \times OR_{01}$. Departures from these multiplicative models were assessed by including main effect variables and their product terms in the logistic model.

Results

Subject characteristics. Table 1 shows the baseline characteristics of the subjects. No significant difference in age or sex was found between the case and control groups. Females were predominant among our subjects, which was in agreement with the gender distribution in patients with thyroid cancer. Histologic classification of cases included 259 with PTC (91.5%) and 24 with FTC (8.5%). Regional neck LN metastasis was detected in 64 PTC patients and 1 FTC patient. Except for the diagnostic medical X-rays, all the subjects denied previous exposure to other ionizing radiation sources (either accidental or therapeutic).

Association with individual SNP. The allele and genotype frequencies of the five polymorphisms and their associations with DTC risks are shown in Table 2. The distribution of genotypes was in agreement with Hardy-Weinberg equilibrium in both cases and controls for each SNP.

For the *XRCC1-Arg194Trp* polymorphism, we found that the allelic and genotypic frequencies were significantly different between cases and controls ($P = 0.03$ and 0.04, respectively; Table 2). Moreover, when we further divided the cases by the presence of LN metastasis, the genotype distribution became more significantly different [$P = 0.004$; degrees of freedom (*df*), 4] among the three affected status groups (Table 3). The frequency of the risk *Trp/Trp* genotype was highest in patients

Table 2. Allele and genotype frequencies of selected BER SNPs and their associations with DTC

Polymorphisms	MAF	*P**	Major homozygote, *n* (%)	Heterozygote, *n* (%)	Minor homozygote, *n* (%)	*P* †
XRCC1-Arg194Trp	Trp	0.03				0.04
Control	0.29		234 (49.9)	199 (42.4)	36 (7.7)	
Case	0.34		127 (44.9)	119 (42.1)	37 (13.1)	
OR (95% CI) ‡			1.0 (Reference)	1.08 (0.79-1.48)	1.85 (1.11-3.07)	
XRCC1-Arg280His	His	0.21				0.27
Control	0.14		349 (74.4)	113 (24.1)	7 (1.5)	
Case	0.11		224 (79.2)	54 (19.1)	5 (1.8)	
OR (95% CI) ‡			1.0 (Reference)	0.74 (0.51-1.07)	1.07 (0.33-3.41)	
XRCC1-Arg399Gln	Gln	0.07				0.19
Control	0.23		277 (59.1)	165 (35.2)	27 (5.8)	
Case	0.28		150 (53.0)	110 (38.9)	23 (8.3)	
OR (95% CI) ‡			1.0 (Reference)	1.25 (0.91-1.71)	1.58 (0.87-2.86)	
APEX1-Asp148Glu	Glu	0.45				0.76
Control	0.39		179 (38.2)	214 (45.6)	76 (16.2)	
Case	0.41		102 (36.0)	130 (45.9)	51 (18.0)	
OR (95% CI) ‡			1.0 (Reference)	1.08 (0.78-1.49)	1.17 (0.76-1.80)	
ADPRT-Val762Ala	Ala	0.09				0.24
Control	0.41		168 (35.8)	221 (47.1)	80 (17.1)	
Case	0.45		86 (30.4)	139 (49.1)	58 (20.5)	
OR (95% CI) ‡			1.0 (Reference)	1.21 (0.87-1.70)	1.39 (0.91-2.14)	

Abbreviation: MAF, minor allele frequency.
*Two-sided χ^2 test for distribution of allelic frequencies (*df*, 1).
† Two-sided χ^2 test for distribution of genotypic frequencies (*df*, 2).
‡ Comparing by multivariate logistic regression model with adjustment for sex and age for cases and controls.

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Table 3. Age- and gender-adjusted association between XRCC1-Arg194Trp and thyroid cancer

Genotype	Controls (n = 469)	Overall cases (n = 283)			Cases with LN metastasis (n = 65)			Cases without LN metastasis (n = 218)		
	n (%)	n (%)	OR (95% CI)*	P*	n (%)	OR (95% CI)*	P*	n (%)	OR (95% CI)*	P*
Arg/Arg	234 (49.9)	127 (44.9)	1.0 (Reference)		21 (32.3)	1.0 (Reference)		106 (48.6)	1.0 (Reference)	
Arg/Trp	199 (42.4)	119 (42.0)	1.08 (0.79-1.48)	0.614	30 (46.2)	1.74 (0.96-3.15)	0.066	89 (40.8)	0.95 (0.68-1.34)	0.788
Trp/Trp	36 (7.7)	37 (13.1)	1.85 (1.11-3.07)	0.018	14 (21.5)	4.54 (2.11-9.79)	0.0001	23 (10.6)	1.34 (0.75-2.38)	0.318
		P = 0.044 †			P = 0.0004 †			P = 0.456 †		

*OR and P value were from multivariate logistic regression model with adjustment for sex and age.
† Two-sided χ^2 test for distribution of genotype frequencies between control and case groups (df, 2).

with positive LN metastasis followed by patients without LN metastasis and was lowest in controls with a trend test P value of 0.002 (data not shown). More analyses showed that the comparison between the cases with the presence of LN metastasis and controls yielded a χ^2 P value of 0.0004 for the genotypic effect (Table 3). Age- and sex-adjusted logistic regression analysis showed that when compared with the wild *Arg/Arg* genotype, the *Trp/Trp* genotype had a significantly increased risk of DTC (OR, 1.85; 95% CI, 1.11-3.07; P = 0.018), especially in cases with LN metastasis (OR, 4.54; 95% CI, 2.11-9.79; P = 0.0001). For the cases without LN metastasis, the *Arg194Trp* polymorphism did not seem to have a significant effect (Table 3). Because females were predominant among our subjects, we also divided the study subjects by gender and examined the sex-specific genetic effect, but we did not find any significant sex-specific effect in the *XRCC1-Arg194Trp* polymorphism.

For the *XRCC1-Arg280His*, *XRCC1-Arg399Gln*, *APE1-Asp148Glu*, and *ADPRT-Val762Ala* polymorphisms, we did not find any significantly different distribution in either allele or genotype frequencies between cases and controls (Table 2). Stratifying the data by the status of LN metastasis or sex group did not yield significant results for any of these four SNPs.

Haplotype analysis of XRCC1 polymorphisms with DTC risk. The frequencies of the four common haplotypes were significantly different between the DTC and control groups (overall P = 0.004; Table 4). In the case group, the frequencies of haplotype *194Trp-280Arg-399Arg* and *194Arg-280Arg-399Gln* were more predominant (P = 0.046 and 0.021, respectively), whereas the frequency of wild *194Arg-280Arg-399Arg* haplotype was significantly lower (29.5% versus 37.3%; P = 0.003). The other four possible haplotypes, including *194Trp-280His-399Arg*, *194Trp-280Arg-399Gln*, *194Arg-280His-399Gln*, and *194Trp-280His-399Gln*, were predicted to be too rare for meaningful statistical analysis. The distributions of the four common haplotypes between cases with LN metastasis and controls were significantly different (overall P = 0.0002, and the haplotype-specific P values are shown in Table 4). We also found that among the 576 subjects genotyped in our study, all of the 59 subjects carrying the *194Trp/Trp* genotype had the *399 Arg/Arg* genotype exclusively and all of the 43 subjects with the *399 Gln/Gln* genotype had the *194 Arg/Arg* genotype exclusively.

Gene-gene interaction. We further evaluated the joint effect between any combination of the *XRCC1*, *ADPRT*, and *APE1* genotypes in the risks of DTC. An interaction between *XRCC1-Arg194Trp* and *ADPRT Val762Ala* polymorphisms was observed. When the combined wild-type homozygote *XRCC1* (*Arg/Arg*)/*ADPRT* (*Val/Val*) was used as the reference, the *XRCC1-194Trp* and *ADPRT-762Ala* variants collectively contributed to an increased risk for all cases (P_{trend} = 0.011) and for cases with LN metastasis (P_{trend} < 0.001). The combined variant homozygotes exhibited the highest 3.18-fold risk for DTC and 9.25-fold risk for DTC with LN metastasis (Table 5). No significant result was found for the cases without LN metastasis. Evidence for a more than multiplicative joint effect came from the fact that the OR of combined (*XRCC1-Trp/Trp*)/(*ADPRT-Ala/Ala*) genotype (Table 5) was larger than the product of the OR of (*XRCC1-Trp/Trp*) and the OR of (*ADPRT-Ala/Ala*) [i.e., for all cases, $OR^{(Trp/Trp)}$ = 1.85, $OR^{(Ala/Ala)}$ = 1.39 (Table 2), 1.85 × 1.39 = 2.57 < 3.18]. Similarly, departures from the multiplicative joint effect for cases with LN metastasis could be shown as follows: $OR^{(Trp/Trp)}$ = 4.54 (Table 3),

Table 4. Haplotype frequencies of XRCC1 polymorphisms with DTC risk

Haplotypes	Controls (n = 469)	Overall cases (n = 283)		Cases with LN metastasis (n = 65)	
XRCC1-194-280-399	(%)	(%)	P	(%)	P
Arg-Arg-Arg (CGG)	37.3	29.5	0.003	21.6	<0.001
Trp-Arg-Arg (TGG)	28.0	32.9	0.046	42.8	0.001
Arg-Arg-Gln (CGA)	21.2	26.4	0.021	24.0	0.366
Arg-His-Arg (CAG)	10.4	8.4	0.177	6.3	0.188
		overall P = 0.002		overall P < 0.001	

NOTE: Haplotype-specific P value was from Hap-Clustering program.

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Table 5. Adjusted ORs and 95% CIs for the joint effects of XRCC1-Arg194Trp and ADPRT-Val762Ala

Genotypes		Controls (n = 469)	Overall cases (n = 283)			Cases with LN metastasis (n = 65)		
XRCC1	ADPRT	n (%)	n (%)	OR (95% CI)	P	n (%)	OR (95% CI)	P
Arg/Arg	Val/Val	83 (17.7)	34 (12.0)	1.0 (Reference)		7 (10.8)	1.0 (Reference)	
Arg/Arg	Val/Ala	112 (23.9)	66 (23.3)	1.43 (0.86-2.36)	0.165	10 (15.4)	1.10 (0.40-3.01)	0.858
Arg/Arg	Ala/Ala	39 (8.3)	27 (9.5)	1.67 (0.88-3.14)	0.114	4 (6.2)	1.27 (0.35-4.61)	0.716
Arg/Trp	Val/Val	76 (16.2)	45 (15.9)	1.43 (0.83-2.46)	0.202	13 (20.)	2.13 (0.80-5.64)	0.128
Arg/Trp	Val/Ala	88 (18.8)	51 (18.0)	1.38 (0.81-2.34)	0.236	12 (18.5)	1.75 (0.65-4.69)	0.266
Arg/Trp	Ala/Ala	35 (7.5)	23 (8.1)	1.57 (0.81-3.05)	0.181	5 (7.7)	1.79 (0.53-6.06)	0.348
Trp/Trp	Val/Val	9 (1.9)	7 (2.5)	1.92 (0.66-5.58)	0.231	3 (4.6)	3.88 (0.84-17.8)	0.081
Trp/Trp	Val/Ala	21 (4.5)	22 (7.8)	2.47 (1.20-5.08)	0.014	7 (10.8)	4.31 (1.35-13.8)	0.014
Trp/Trp	Ala/Ala	6 (1.3)	8 (2.8)	3.18 (1.02-9.87)	0.046	4 (6.2)	9.25 (2.07-41.4)	0.004
				P_{trend} = 0.011			P_{trend} < 0.001	

NOTE: OR, 95% CI, and P values were obtained from a multivariate logistic regression model with adjustment for sex and age.

$OR^{(Ala/Ala)}$ = 1.22 (data not shown), and 4.54 × 1.22 = 5.54 < 9.25. These results clearly indicated that a more than multiplicative joint effect between the *XRCC1-Trp/Trp* and *ADPRT-Ala/Ala* genotypes existed in the risk of developing thyroid cancer and its LN metastasis.

Discussion

In the present study, we examined the relationship between the five nsSNPs at the three BER genes and the risk of DTC and regional LN involvement. Our results suggested a potential role of the *XRCC1* nsSNP *Arg194Trp* in the development of thyroid cancer and LN metastasis. The *Trp* allele increased the risk of DTC development and LN metastasis. In addition, a gene-gene interaction between the *XRCC1-194Trp* and *ADPRT-762Ala* variants was observed. This interaction makes mechanistic sense because these two genes interact in the long-patch BER pathway. In response to DNA damage, ADPRT specifically binds to DNA strand breaks where it is autoactivated and recruits XRCC1-ligase complex (19).

It is of particular interest that the genetic risk in our findings was more predominant in DTC cases that showed neck LN metastasis. In this study, we showed that the genetic effect of the *XRCC1-194Trp/Trp* genotype came primarily from the subjects with LN metastasis, although cases without LN metastasis also had a higher prevalence of the *194Trp/Trp* genotype than controls (Table 3). This pattern was even more prominent by the combined effect of *ADPRT-762Ala/Ala* genotype (Table 5). A high incidence of neck regional LN metastasis is one of the characteristics of PTC, which has been identified as an independent risk factor of recurrence (27–29). In some studies, a deleterious effect of LN metastasis on survival has been reported (28–30). Evidence showed that attenuated DNA repair capacity is involved not only in carcinogenesis but also in tumor growth and survival rate. The genetic polymorphisms in the DNA BER pathway are related to tumor progression, therapy resistance, or survival in various cancers (31–33). One study showed that the *194Trp/Trp* genotype of the *XRCC1* gene was associated with significantly decreased overall survival (multivariate hazard ratio, 4.64; P = 0.011) in pancreatic cancer (32). Cancer progression is associated with multiple gene defects or mutations. It is possible that the *194Trp/Trp* genotype itself, or along with the *ADPRT* gene, leads to attenuated DNA repair capacity and increased tumor progression, resulting in acceleration of thyroid cancer development and metastasis.

XRCC1 protein is exclusively required for DNA BER and single-strand break repair (15). Although lines of evidence have shown the potential biological significance of the three common nsSNPs at the *XRCC1* gene (the *Arg194Trp*, *Arg280His*, and *Arg399Gln*), the genetic epidemiologic studies of cancer risk have not reached a conclusive result (12, 18). Consistent with our result, the genotype *194Trp/Trp* of *XRCC1* was found to be associated with increased risk of lung (34), esophageal (35), and cervical cancers (36) in the Chinese population, although it was reported as a protective factor against gastric carcinoma in a small study (37). Similarly, contradictory data also existed with regard to *Arg280His* and *Arg399Gln* polymorphisms (12, 18), and the observation of these two SNPs in the current study did not show significant results. The association between *XRCC1* polymorphisms and thyroid cancer has also been reported in a noninternational journal (23). Different from our results, they showed that *399Gln/Gln* but not *194Trp/Trp* increased risk of developing PTC. However, the sample size was small (105 cases and 105 controls), and the distribution of genotypes in *Arg194Trp* polymorphism was not in agreement with Hardy-Weinberg equilibrium for both cases and overall subjects in their study. Therefore, their result should be taken with caution.

It is conceivable that genes involved in the same pathway may have a collective effect on DNA repair outcomes. Previous studies have suggested that *XRCC1* may interact with *ADPRT* to increase the risk of cancers of lung (19), esophagus (20), and stomach (38) in the Chinese population. Indeed, we found that *XRCC1-194Trp* and *ADPRT-762Ala* variants collectively had an OR of 9.25 for DTC with LN metastasis. Although the interaction results were intriguing, these gene-gene joint effect findings should be interpreted with caution, given the modest sample size of the present study to evaluate the joint effects.

There are limitations in the present study. First, this was a hospital-based case-control study; the control group in this study might not provide a good representation of the general population. However, the allele and genotype frequencies in our controls were consistent with the previous studies in Chinese population in Taiwan (39–41) or other places

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(19, 20, 34–37, 42), which suggested a clue to the nonbiased sampling of our control subjects. Second, retrospectively recruiting patients might lead to survival bias. To test for this potential bias and its effect on our conclusion, we divided the subjects into two groups by the follow-up period of more than 5 years and less than 5 years. We found that the frequency of the risk *194Trp* allele of *XRCC1* was 34.3% in the long follow-up group and 33.8% in the short follow-up group. Therefore, our ascertainment was not confounded by survival rate. We used common and functional rather than tagging SNPs of the selected BER genes, which may not provide a systematic evaluation of these candidate genes. The significant result was mainly driven by the 65 patients with LN metastasis. Therefore, our result needed further validation in independent samples.

In conclusion, the current results indicate that the *XRCC1* polymorphisms, especially the *194Trp* variant, may have an effect on DTC with LN metastasis. *XRCC1* may interact with *ADPRT 762Ala* variant to further increase susceptibility to the disease and LN metastasis. These findings suggest that deficient BER may play an important role in the development and progression of DTC. Identifying these risk genetic markers could provide more insight into the disease pathogenesis and may also provide information to develop better prevention programs and therapeutic regimens.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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