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Genetic Polymorphisms in DNA Base-excision Repair Genes *ADPRT*, *XRCC1*, and *APE1* and the Risk of Squamous Cell Carcinoma of the Head and Neck

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BACKGROUND. Tobacco smoke contains numerous carcinogens that cause DNA damage, including oxidative lesions that are removed effectively by the base-excision repair (BER) pathway, in which adenosine diphosphate ribosyl transferase (ADPRT), x-ray repair cross-complementing 1 (XRCC1), and apurinic/apyimidinic endonuclease (APE1) play key roles. Genetic variations in the genes encoding for these DNA repair enzymes may alter their functions. Although there have been several studies that generated mixed results on the association between *XRCC1* variants and the risk of squamous cell carcinoma of the head and neck (SCCHN), no reported studies have investigated the association between *ADPRT* and *APE1* variants and SCCHN risk.

METHODS. In a hospital-based, case-control study of 830 non-Hispanic white patients with SCCHN and 854 cancer-free, matched control participants, the authors genotyped the *ADPRT* alanine 762 valine (*Ala*762*Val*) single-nucleotide polymorphism (SNP), the *XRCC1* arginine 399 glutamine (*Arg*399*Gln*) SNP, and the *APE* aspartic acid 148 glutamic acid (*Asp*148*Glu*) SNP and assessed their associations with the risk of SCCHN in multivariate logistic regression models.

RESULTS. The findings indicated that a significantly decreased risk of SCCHN was associated with the *ADPRT* 762*Ala*/*Ala* genotype (adjusted odds ratio [OR], 0.51; 95% confidence interval [95% CI], 0.27–0.97) and the combined *ADPRT* 762*Ala*/*Val* and *Ala*/*Ala* genotypes (OR, 0.79; 95% CI; 0.63–1.00) compared with the *ADPRT* 762*Val*/*Val* genotype, but no altered risk was associated with the *XRCC1 Arg*399*Gln* or *APE Asp*148*Glu* polymorphisms, and no evidence of interactions was observed between the 3 selected SNPs and age, sex, smoking status, drinking status, or tumor site.

CONCLUSIONS. The *ADPRT Ala*762*Val* polymorphism may play a role in the etiology of SCCHN or in linkage disequilibrium with other untyped protective alleles. Larger studies with more SNPs in the BER genes will be needed to verify these findings. ***Cancer* 2007;110:867–75.** © *2007 American Cancer Society.*

KEYWORDS: DNA repair, genetic variant, head and neck cancer, genetic susceptibility, molecular epidemiology.

Genetic variations in DNA repair genes can modulate DNA repair capacity and, consequently, alter cancer risk.[1,2] To date, >150 human DNA repair genes in several distinct DNA repair pathways have been identified.[3] Among these pathways, the base-excision repair (BER) pathway, which possibly handles the largest number of cytotoxic and mutagenic base lesions, recently has been associated with the risk of human cancer.[4] The BER pathway specifically removes alterations of a single base that has been methylated, oxidized, or reduced and thus rectifies single-strand interruptions in

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DNA.[5] Tobacco smoke is associated with the increased formation of DNA lesions,[6] including those induced by oxidative damage, which can be repaired through the BER pathway. Therefore, an individual variation in BER is one of the host factors that may influence tobacco smoking-related cancer risk, such as squamous cell carcinoma of the head and neck (SCCHN) (including the oral cavity, pharynx, and larynx), which is one of the most common cancers in the developed world.[7] Recently, several studies have demonstrated that sequence variations in BER genes may contribute to SCCHN susceptibility.[8]

A number of DNA repair enzymes are involved in the BER process, of which apurinic/apyimidinic endonuclease (APE), adenosine diphosphate ribosyl transferase (ADPRT), and x-ray repair cross-complementing 1 (XRCC1) play key roles.[8] APE1 (also called APEX1) processes the abasic sites left from the incision 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.[9] ADPRT (also called poly [ADP-ribose] polymerase family, member 1 [PARP1]) specifically binds to DNA strand breaks and play roles in the long-patch repair process[10]; and XRCC1 interacts with a complex of DNA repair proteins, including poly (ADP-ribose) polymerase, DNA ligase 3, and DNA polymerase β, and coordinates the gap-sealing process in the short-batch BER.[11,12]

Genetic polymorphisms have been identified in the *ADPRT*, *XRCC1*, and *APE1* genes, and studies have suggested that some of these polymorphisms may be associated with cancer risk.[8,13] However, most of the published studies of the association between the *ADPRT*, *XRCC1*, and *APE1* polymorphisms and cancer risk were confined to cancers of the breast,[14] lung,[15] esophagus,[16] and skin.[17] Several studies have investigated their association with SCCHN in Caucasians, but the sample sizes in those studies were relatively small, and the studies included only *XRCC1* polymorphisms.[18–23] Therefore, we hypothesized that common, nonsynonymous, single-nucleotide polymorphisms (nsSNPs) of the BER genes may contribute to the risk of SCCHN, and we tested this hypothesis in a hospital-based study of 830 patients with incident SCCHN and 854 cancer-free control participants who were frequency-matched by age, sex, and ethnicity. We also explored possible gene-gene and gene-environment interactions in this study population.

MATERIALS AND METHODS

Study Participants

Recruitment for our ongoing SCCHN study has been described previously.[24] Briefly, all patients had newly diagnosed, untreated SCCHN that was confirmed histologically at The University of Texas M. D. Anderson Cancer Center between May 1995 and October 2003. Patients with second primary SCCHN tumors, primary tumors of the nasopharynx or sinonasal tract, primary tumors outside the upper aerodigestive tract, cervical metastases of unknown origin, or histopathologic diagnoses other than squamous cell carcinoma were excluded. Because genotype frequencies vary between ethnic groups, the few minority patients who were enrolled were excluded from this analysis. Consequently, this study included 830 non-Hispanic white patients with primary tumors of the oral cavity (253 patients; 30.5%), the pharynx (425 patients; 51.2%; 387 oropharyngeal tumors and 38 hypopharyngeal tumors), and the larynx (152 patients; 18.3%). The response rate among patients who were approached for participation was approximately 95%. Self-reported cancer-free control participants were recruited from unrelated hospital visitors who accompanied the patients to the clinics but did not seek medical care. First, we surveyed potential control participants at the clinics using a short questionnaire to determine their willingness to participate in research studies and to obtain demographic and risk factor information. Then, we matched the control participants by frequency to the patients by age (±5 years), sex, and ethnicity. The response rate among those we approached for recruitment was approximately 90%. We interviewed each eligible participant to obtain data on tobacco smoking and alcohol use. Those participants who had smoked <100 cigarettes in their lifetime were defined as never smokers; otherwise, they were considered ever smokers. Those smokers who had quit for >1 year were considered former smokers, and the remaining smokers were defined as current smokers. Similarly, participants who had consumed alcoholic beverages at least once a week for >1 year previously were defined as ever drinkers. Ever drinkers who had quit drinking for >1 year previously were defined as former drinkers, and the others were defined as current drinkers. After we obtained informed consent, each participant provided 30 mL of blood. The research protocol was approved by The University of Texas M. D. Anderson Cancer Center Institutional Review Board.

SNP Selections

We searched the National Institute of Environmental Health Science database (available at URL: http://egp.gs.washington.edu) and related literature to identify all reported nsSNPs in the *ADPRT*, *XRCC1*, and *APE1* genes with a minor allele frequency >0.05 in

European descendents. We identified only 1 common nsSNP for each of the *ADPRT* and *APE1* genes and identified 3 common nsSNPs for the *XRCC1* gene. In a recent article, it was reported that the *XRCC1* 399 glutamine (399*Gln*) allele was in complete linkage disequilibrium (LD) with the 280 histidine (280*His*) allele ($D' = 1.0$) and that the 280*His* allele was in complete LD with the 194 arginine (194*Arg*) allele ($D' = 1.0$) in both whites and African Americans.[14] In addition, *XRCC1 Arg*399*Gln* has been well studied and appears more likely to be associated with a risk of various types of cancer. Consequently, we selected to genotype *ADPRT* alanine 762 valine (*Val*762*Ala*) (reference SNP no. [rs] 1136410), *XRCC1 Arg*399*Gln* (rs25487), and *APE1* aspartic acid 148 glutamic acid (*Asp*148*Glu*) (rs3136820) polymorphisms in this study.

Genotyping

A leukocyte cell pellet was obtained from the buffy coat by centrifugation of 1 mL of whole blood. The cell pellet was used for genomic DNA extraction by using the Qiagen DNA Blood Mini Kit (Valencia, Calif). The DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 nanometers (nm) and 280 nm. Polymerase chain reaction (PCR) was used to amplify the fragments of *ADPRT, XRCC1*, and *APE1* that contained the sites of *Val*762*Ala*, *Arg*399*Gln*, and *Asp*148*Glu*, respectively. The primers, PCR annealing times, and restriction enzyme (New England Biolabs, Beverly, Mass) conditions for *ADPRT Val*762*Ala*, *XRCC1 Arg*399*Gln*, and *APE1 Asp*148*Glu* have been described previously.[17] The genotyping assays for approximately 10% of the samples were repeated, and the results were 100% concordant.

Statistical Analysis

Differences in selected demographic variables, smoking status, and drinking status between the cases and controls were evaluated by using the chi-square test. The associations between genotypes of the selected polymorphisms and SCCHN risk were estimated by computing the odds ratios (ORs) and 95% confidence intervals (95% CIs) from both univariate and multivariate logistic regression analyses with adjustment for age, sex, smoking status, and drinking status. Homogeneity tests were performed to evaluate the differences in stratum variable-related ORs.[25] The potential locus-locus and gene-environment interactions were evaluated by logistic regression analysis and were tested by comparing the changes in deviance (-2 log likelihood) between the models of main effects with or without the interaction term. All statistical analyses were performed with Statistical Analysis System software (version 9.1.3; SAS Institute Inc, Cary, NC) and Review Manage (version 4.2; Oxford, U.K.).

To summarize published case-control association studies of *XRCC1* polymorphisms and SCCHN risk, we performed a meta-analysis[13] on those studies that had an appropriate case-control design with detailed genotyping information on the *XRCC1 Arg*399*Gln* polymorphism and clearly identified ethnicity (ie, Caucasians and Asians). We attempted to include all case-control association studies of SCCHN that had genotyping data for the *XRCC1 Arg*399*Gln* polymorphism. Eligible studies were identified by searching the electronic literature Pub-Medline for relevant reports (last search update, February 20, 2007). Consequently, data for the current analysis were available from 7 published case-control studies,[18–22,26–28] including 1135 SCCHN cases and 1795 controls, for the *XRCC1 Arg*399*Gln* polymorphism. Two investigators extracted data independently and reached a consensus on all of the items. We sought the following information from each article: author name, journal and year of publication, country of origin, selection and characteristics of SCCHN cases and controls, demographics, ethnicity, and genotyping information for the *XRCC1 Arg*399*Gln* polymorphism. The risks (ORs) of SCCHN associated with the *XRCC1 Arg*399*Gln* polymorphism were estimated for each study. We estimated the risk of the variant genotype *Gln*/*Gln*+*Arg*/*Gln* compared with the wild-type *Arg*/*Arg* homozygote as well the *Gln*/*Gln* genotype compared with the *Arg*/*Arg* genotype. In addition to comparisons for total participants, studies were also categorized into different subgroup analyses according to ethnicity. For each subgroup, we estimated the between-study heterogeneity across eligible comparisons using the chi-square-based Q test.[13] All analyses were performed with Review Manage (version 4.2). All P values were 2-sided.

RESULTS

Because we used frequency matching, there was no difference in the frequency distribution for age ($P = .956$) or sex ($P = .339$) between patients (cases) and control participants (controls) (Table 1). Compared with the controls, the cases were more likely to be smokers (current smokers: 34.7% vs 15.8%; former smokers: 39.6% vs 37.1%) and drinkers (current drinkers: 51.0% vs 40.4%; former drinkers: 25.8% vs 19.3%; $P < .001$ for both smoking and drinking status). Therefore, these risk factors appeared to be involved in the etiology of SCCHN in this study

TABLE 1
Frequency Distributions of Selected Variables in Non-Hispanic White Patients With Squamous Cell Carcinoma of the Head and Neck (Cases) and Control Participants

	Cases (n = 830)		Controls (n = 854)		
Variable	No.	%	No.	%	P*
Age, y					.956
≤55	410	49.4	423	49.5	
>55	420	50.6	431	50.5	
Sex					.339
Men	206	24.8	195	22.8	
Women	624	75.2	659	77.2	
Smoking status					<.001
Current	288	34.7	135	15.8	
Former	329	39.6	317	37.1	
Never	213	25.7	402	47.1	
Drinking status					<.001
Current	423	51.0	345	40.4	
Former	214	25.8	165	19.3	
Never	193	23.2	344	40.3	

* Chi-square test.

population, but they were adjusted for in the multivariate regression analyses and were used in the later stratification and gene-environment interaction analysis.

The genotype and allele frequencies of *ADPRT Val*762*Ala*, *XRCC1 Arg*399*Gln*, and *APE1 Asp*148*Glu* polymorphisms and their associations with risk of SCCHN are shown in Table 2. The genotype distribution in the control group was in agreement with the Hardy-Weinberg equilibrium (*ADPRT*: chi-square statistic = 3.183; $P = .074$; *XRCC1*: chi-square statistic = 0.147; $P = .701$; *APE1*: chi-square statistic = 0.626; $P = .429$).

When the *ADPRT Val*/*Val* genotype was used as the reference group, a significantly decreased risk was associated with the *Ala*/*Ala* genotype (adjusted OR, 0.51; 95% CI, 0.27–0.97), and a borderline significantly decreased risk was associated with the combined *Val*/*Ala*+*Ala*/*Ala* genotypes (adjusted OR, 0.79; 95% CI, 0.63–1.00). When the *XRCC1 Arg*/*Arg* genotype was used as the reference group, however, no significantly altered risk was associated with the *Arg*/*Gln*, *Gln*/*Gln* or combined *Arg*/*Gln*+*Gln*/*Gln* genotypes. Similarly, when the *APE1 Asp*/*Asp* genotype was used as the reference group, no significantly altered risk for SCCHN was associated with the *Asp*/*Glu*, *Glu*/*Glu*, or combined *Asp*/*Glu*+*Glu*/*Glu* genotypes (Table 2).

Although it is known that ADPRT, XRCC1 and APE1 play roles in the BER pathway, only the *ADPRT* polymorphism exhibited the main effect on SCCHN risk in this study. Thus, it is possible that there may be some locus-locus interactions that may have masked the main effects of certain genotypes of these genes. Therefore, we examined possible effect modification in data stratified by the covariates and further analyzed the interactions between the dichotomized *ADPRT* genotypes and the dichotomized *XRCC1* or *APE1* genotypes. We observed that, although a borderline significantly decreased risk associated with the combined *ADPRT Val*/*Ala*+*Ala*/*Ala* genotypes was evident only among those who carried the combined *XRCC1 Arg*/*Gln*+*Gln*/*Gln* genotypes (adjusted OR, 0.74; 95% CI, 0.55–1.00), there was no evidence of a locus-locus interaction between *ADPRT* and *XRCC1* genotypes (P for interaction = .516) or between *ADPRT* and *APE1* genotypes (P for interaction = .496).

Next, we performed stratification analysis for the association between the *ADPRT* variant genotypes by age, sex, smoking and drinking status, and tumor site. Table 3 shows that the protective effect associated with the combined *ADPRT Val*/*Ala*+*Ala*/*Ala* genotypes was more pronounced among older individuals, women, and patients with oral cancer. Further homogeneity tests suggested that differences in strata-related ORs were statistically significant for the strata of age ($P = .030$) and sex ($P = .021$) as well as among tumor sites ($P = .001$). These results suggest possible gene-environment interactions between these risk factors and genotypes of the selected polymorphisms in the etiology of SCCHN. However, further analyses revealed no statistical evidence of such interactions on a multiplicative scale, as assessed in the multivariate logistic regression models (Table 3).

Finally, we performed a meta-analysis of available published studies[18–22,26–28] on the association between the *XRCC1 Arg*399*Gln* polymorphism and the risk of SCCHN, because we found no published studies that had investigated the association between 2 other SNPs and the risk of SCCHN. Because each of these studies was small, we combined all participants by ethnicity. In the combined analysis of 448 cases and 735 controls among Caucasians, the OR for SCCHN associated with the *XRCC1 Gln*/*Gln* genotype was 0.97 (95% CI, 0.65–1.44) compared with the *Arg*/*Arg* genotype, whereas the risk was 1.44-fold (95% CI, 0.26–7.83) for Asians (201 cases and 260 controls); overall, individuals in both groups (649 cases and 995 controls) who carried the *Gln*/*Gln* genotype did not have a significantly altered risk, compared with individuals who carried the *Arg*/*Arg* genotype (OR, 1.11; 95% CI, 0.65–1.91) (Fig 1A). Similar results were observed for the combined *Gln*/*Gln*+*Gln*/*Arg* genotypes (Caucasians: OR, 0.89; 95% CI, 0.74–1.06; 790 cases and 1364

TABLE 2
Genotype and Allele Frequencies of the *ADPRT, XRCC1,* and *APE1* Polymorphisms Among Non-Hispanic White Patients With Squamous Cell Carcinoma of the Head and Neck (Cases) and Controls and Their Associations With the Risk of Squamous Cell Carcinoma of the Head and Neck*

	Cases (n = 830)		Controls (n = 854)		OR (95% CI)	
Genotype	**No.**	**%**	**No.**	**%**	**Crude**	**Adjusted†**
ADPRT Val762Ala						
Val/Val	632	76.2	609	71.3	1.00	1.00
Val/Ala	182	21.9	216	25.3	0.81 (0.65–1.02)	0.83 (0.66–1.05)
Ala/Ala	16	1.9	29	3.4	0.53 (0.29–0.99)	0.51 (0.27–0.97)
Val/Ala+Ala/Ala	198	23.9	245	28.7	0.78 (0.63–0.97)	0.79 (0.63–1.00)
XRCC1 Arg399Gln						
Arg/Arg	335	40.3	360	42.1	1.00	1.00
Arg/Gln	374	45.1	385	45.1	1.04 (0.85–1.28)	1.08 (0.87–1.34)
Gln/Gln	121	14.6	109	12.8	1.19 (0.89–1.61)	1.16 (0.85–1.58)
Arg/Gln+Gln/Gln	495	59.6	494	57.9	1.08 (0.89–1.31)	1.10 (0.90–1.35)
APE1 Asp148Glu						
Asp/Asp	217	26.1	234	27.4	1.00	1.00
Asp/Glu	428	51.6	437	51.2	1.06 (0.84–1.33)	1.02 (0.80–1.29)
Glu/Glu	185	22.3	183	21.4	1.09 (0.83–1.44)	1.05 (0.78–1.39)
Asp/Glu+Glu/Glu	613	73.9	620	72.6	1.07 (0.86–1.32)	1.03 (0.82–1.29)

OR indicates odds ratio; 95% CI, 95% confidence interval; *ADPRT,* adenosine diphosphate ribosyl transferase gene; Val, valine; Ala, alanine; *XRCC1*, x-ray repair cross-complementing 1 gene; Arg, arginine; Gln, glutamine; *APE1*, apurinic/apyrimidinic endonuclease gene; Asp, aspartic acid; Glu, glutamic acid.

* The observed genotype frequency among the control participants was in agreement with Hardy-Weinberg equilibrium (*ADPRT*: chi-square = 3.183, P = .074; *XRCC1*: chi-square = 0.147, P = .701; *APE1*: chi-square = 0.626, P = .429). In a 2-sided chi-square test for either genotype distribution, P = .222 for *ADPRT Val762Ala,* P = .447 for *XRCC1 Arg399Gln,* and P = .913 for *APE1 Asp148Glu.*

† Adjusted by age, sex, smoking status, and drinking status.

TABLE 3
Stratification Analysis of the Effects of Combined *ADPRT* Genotypes on the Risk of Squamous Cell Carcinoma of the Head and Neck

	ADPRT genotypes (cases/controls)							
	Val/Val		*Val/Ala+Ala/Ala*		OR (95% CI)			
Variable	**No.**	**%**	**No.**	**%**	**Crude**	**Adjusted***	***P*†**	***P*‡**
Age, y								
≤55	304/303	48.1/49.8	106/120	53.5/49.0	0.89 (0.65–1.21)	0.92 (0.67–1.27)	.030	.067
>55	328/306	51.9/50.2	92/125	46.5/51.0	0.70 (0.52–0.96)	0.67 (0.48–0.94)		
Sex								
Men	157/142	24.8/23.3	49/53	24.8/21.6	0.84 (0.53–1.31)	0.95 (0.59–1.54)	.021	.727
Women	475/467	75.2/76.7	149/192	75.2/78.4	0.76 (0.60–0.98)	0.77 (0.59–0.99)		
Smoking status								
Current	230/99	36.4/16.3	58/36	29.3/14.7	0.69 (0.43–1.12)	0.67 (0.40–1.10)	.958	.951
Former	236/222	37.3/36.4	93/95	47.0/38.8	0.92 (0.66–1.29)	0.91 (0.65–1.28)		
Never	166/288	26.3/47.3	47/114	23.7/46.5	0.72 (0.48–1.06)	0.71 (0.48–1.05)		
Drinking status								
Current	154/247	24.4/40.5	39/97	19.7/39.6	0.65 (0.42–0.98)	0.67 (0.44–1.03)	.083	.949
Former	151/118	23.9/19.4	63/47	31.8/19.2	1.05 (0.67–1.64)	1.05 (0.66–1.66)		
Never	327/244	51.7/40.1	96/101	48.5/41.2	0.71 (0.51–0.98)	0.73 (0.52–1.03)		
Tumor site								
Oral cavity	202/609	32.0/100	51/245	25.8/100	0.63 (0.45–0.88)	0.67 (0.47–0.96)	.001	NC
Pharynx	323/609	51.1/100	102/245	51.5/100	0.79 (0.60–1.03)	0.78 (0.60–1.03)		
Larynx	107/609	16.9/100	45/245	22.7/100	1.05 (0.72–1.53)	1.12 (0.75–1.69)		

ADPRT indicates adenosine diphosphate ribosyl transferase gene; Val, valine; Ala, alanine; OR, odds ratio; 95% CI, 95% confidence interval; NC: not calculated.

* Adjusted by age, sex, and smoking and drinking status.

† P value from a test for homogeneity between strata of *ADPRT* (*Val/Ala+Ala/Ala* genotypes vs *Val/Val* genotypes).

‡ P value from a test for interaction between strata of *ADPRT* (*Val/Ala+Ala/Ala* genotypes vs *Val/Val* genotypes).

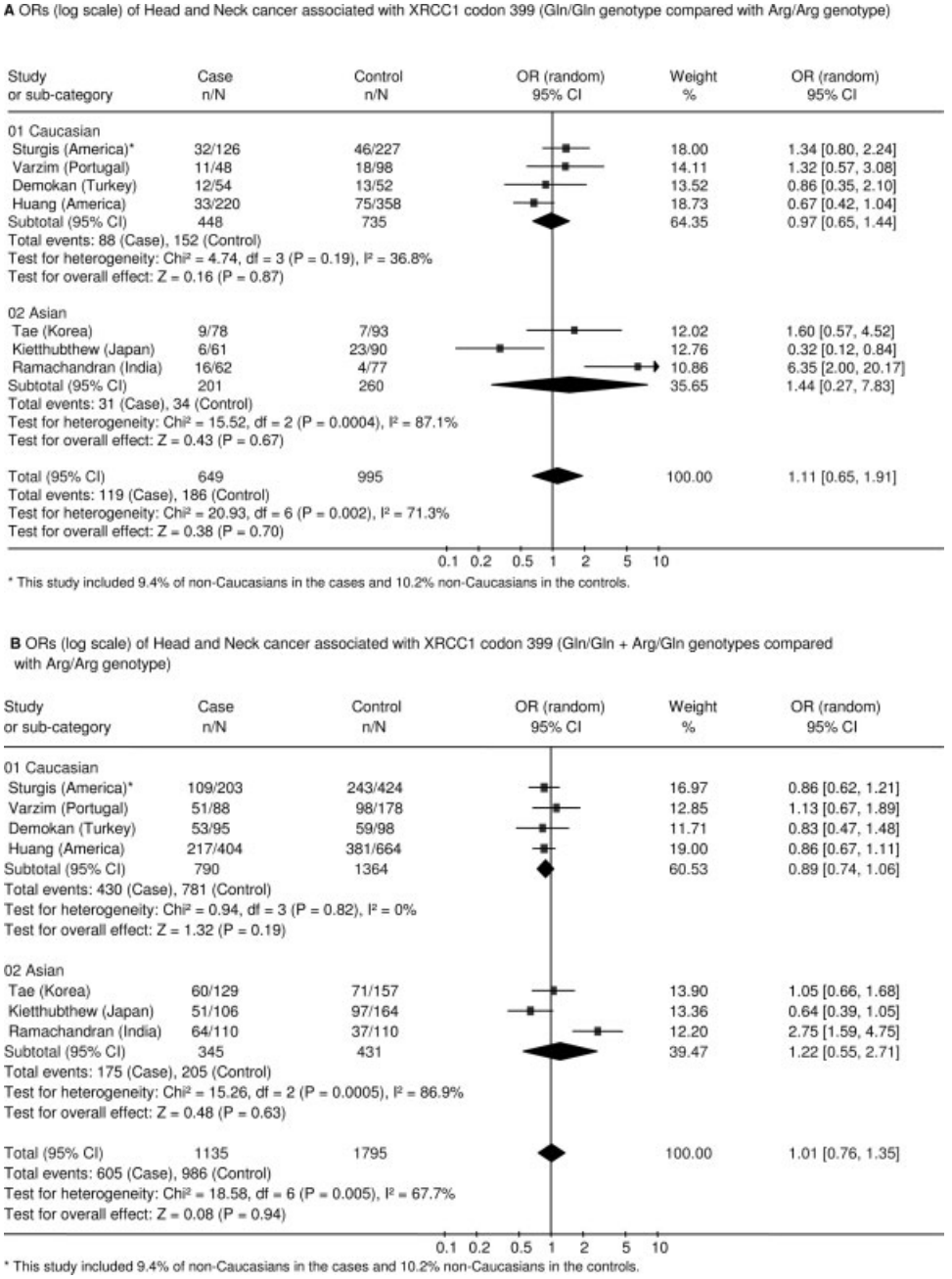

FIGURE 1. Meta-analysis of 7 published studies and odds ratios (ORs) (log scale) associated with the X-ray cross-complementing 1 gene (*XRCC1*) codon arginine 399 glutamine (*Arg*399*Gln*) genotypes in different ethnic groups. (A) ORs associated with 399*Gln*//*Gln* compared with *Arg*/*Arg*. (B) ORs associated with 399*Gln*/*Gln*+*Gln*/*Arg* compared with *Arg*/*Arg*. For each study, the estimated OR and its 95% confidence interval (95% CI) is plotted with a box and a horizontal line. Quadrangles indicate pooled ORs and 95% CIs; Chi^2, chi-square; df, degrees of freedom; I^2, index of heterogeneity.

controls; Asians: OR, 1.22; 95% CI, 0.55–2.71; 345 cases and 431 controls; all participants: OR, 1.01; 95% CI. 0.71–1.35; 1135 cases and 1795 controls) compared with the *Arg*/*Arg* genotype (Fig. 1B). In general, the grouped data for Caucasian studies were quite homogenous compared with the grouped data for Asian studies. The results from grouped Caucasian studies were consistent with our findings that both the *XRCC1 Gln*/*Gln* genotype and the combined *Gln*/*Gln*+*Gln*/*Arg* genotypes were not associated with a significantly altered risk of SCCHN compared with the *Arg*/*Arg* genotype (Table 2).

DISCUSSION

In this hospital-based, case-control study of 830 patients with SCCHN and 854 cancer-free controls, we demonstrated that a reduced risk of SCCHN was associated with the *ADPRT* variant genotypes (ie, *Ala*/*Ala* and *Ala*/*Ala*+*Val*/*Ala*); however, we did not observe any evidence of an interaction between the *ADPRT* 762*Ala* variant and the *XRCC1* 399*Gln* or *APE1* 148*Glu* variants in the etiology of SCCHN. Although exactly how the *ADPRT* 762*Ala* variant works to influence the risk of SCCHN is not clear, this variant either may be functional itself, or it may be in LD with other functional variants that are involved in the etiology of SCCHN. To the best of our knowledge, this is the largest case-control study on the association between these 3 BER nsSNPs and the risk of SCCHN.

Although it has been suggested that the *ADPRT Val*762*Ala* polymorphism contributes to carcinogenesis,[29] to our knowledge, no previously reported studies have investigated the association between *ADPRT* polymorphisms and the risk of SCCHN. Only 2 studies have investigated the association between the *ADPRT Val*762*Ala* polymorphism and the risk of cancer in Caucasians; in 1 study (488 cases and 524 controls), the *Ala*/*Ala* genotype was associated significantly with an increased risk of prostate cancer,[10] and in the other study, the combined *Ala*/*Ala*+*Ala*/*Val* genotypes were associated with a nonsignificantly reduced risk of melanoma.[17] In the current study, we observed that the *ADPRT* 762*Ala* variant genotypes (ie, *Val*/*Ala*+*Ala*/*Ala*) were associated with a significantly reduced risk of SCCHN. The discrepancies in findings between other studies and ours may reflect the differences in genetic background among different ethnic groups, carcinogen exposure in different populations, and study sample sizes. Moreover, it is possible that effect of the *ADPRT* variant genotypes may be tissue-specific. Indeed, studies have indicated that ADPRT expression levels appear to be low in breast cancer[30] but high in endometrial cancer,[31] indicating ADPRT may play different roles in different types of tumors.

It is likely that the *ADPRT* variant genotypes may cause altered BER functions,[29] leading to cumulative oxidative damage to DNA and, thereby, an increased frequency of mutations in the target tissues. According to this hypothesis, the *ADPRT* variant genotypes would be associated with an increased risk of SCCHN. However, our data do not appear to support this hypothesis. Alternatively, ADPRT dysfunction may modulate immune responses through affecting transcriptional regulation and also may influence normal mechanisms of cell death.[32] In fact, cells without efficient BER may be more prone to apoptosis in response to excessive damage to DNA, a mechanism that possibly may reduce the risk of carcinogenesis.[33] Our data appear to support this alternate hypothesis.

Although the results of the *XRCC1 Arg*399*Gln* polymorphism were conflicting, this polymorphism has been associated with measurably reduced DNA repair capacity, as assessed by the persistence of DNA adducts and elevated levels of sister chromatid exchanges.[34–36] To our knowledge to date, only 5 studies have investigated the association between the *XRCC1* 399 polymorphism and SCCHN risk in Caucasians, and the findings were not consistent.[18–22] In a pooled study of 555 patients with SCCHN (430 whites) and a group of 792 controls, the *XRCC1* 399*Gln*/*Gln* genotype was associated with decreased risk among whites.[20] Nevertheless, when all types of cancer were considered together, the results from 11,957 cancer cases and 14,174 control participants from 38 published case-control studies indicated that there was no main effect in either recessive or dominant modeling for the *Arg*399*Gln* polymorphism, and the variant *Gln*/*Gln* homozygote was not associated with overall cancer risk (OR, 1.01; 95% CI, 0.90–1.14).[13] In another meta-analysis, the 399*Gln*/*Gln* genotype was associated with an increased risk of lung cancer among Asians (OR, 1.34; 95% CI, 1.16–1.54) but not among Caucasians (OR, 0.99; 95% CI, 0.90–1.08), and that study included 11 Caucasian (or mostly Caucasian) studies with 5529 cases and 7127 controls.[37] Our current single, large study of 830 SCCHN cases and 854 controls indicated that the variant genotypes (ie, 399*Arg*/*Gln*+399*Gln*/*Gln*) were not associated with a significantly altered risk of SCCHN, a finding that is consistent with the results from our meta-analysis incorporating the 7 published studies on the association between the *XRCC1 Arg*399*Gln* polymorphism and the risk of SCCHN.

Although it has been suggested that the *APE1 Asp*148*Glu* polymorphism may be associated with hypersensitivity to ionizing radiation,[38] to our knowledge, no previously reported study has investigated the association between *APE1* polymorphisms and the risk of SCCHN. Several studies have investigated the association between *APE1 Asp*148*Glu* and cancer risks; however, the findings were mixed among different ethnic populations and different cancer types. For example, in 1 study, the *APE1* 148*Glu*/*Glu* genotype was associated with the risk of lung cancer in a Japanese population[39]; whereas, in our previous study, we observed a significantly reduced risk of melanoma among Caucasians.[17] Conversely, none of the studies of other cancers reported any evidence of an association between the *APE1 Asp*148*Glu*

polymorphism and risk for cancers of the lung,[40] bladder,[41] and prostate.[42] Our current SCCHN data did not support an association between the *APE1* 148*Glu* variant genotypes (ie, 148*Glu*/*Asp*, 148*Glu*/*Glu*, and 148*Glu*/*Asp*+*Glu*/*Glu*) and an altered risk of SCCHN.

Because the *XRCC1*, *ADPRT*, and *APE1* genes are not located on the same chromosome, we cannot perform a further haplotype analysis. Nevertheless, genes that are involved in the same pathway may interact to influence DNA repair outcomes; indeed, some previous studies have suggested that *XRCC1* may interact with *ADPRT* to increase the risk of cancers of the lung,[15] stomach,[43] and esophagus[16] in Chinese populations. However, in the current study, we failed to identify any locus-locus interactions between *ADPRT* and *XRCC1* or *APE1* SNPs on the risk of SCCHN. Moreover, genetic polymorphisms and environmental risk factors sometimes interact to have an effect on cancer development. Tobacco smoking, which is a well-documented source of reactive oxygen species, is a major risk factor for cancers, including SCCHN. However, in the current study, we did not produce any evidence of gene-environment interactions between *ADPRT* polymorphisms and smoking or other risk factors. Therefore, further studies are necessary to validate our findings.

Similar to other case-control studies, inherent biases in the current study may have led to spurious findings. First, of the selected BER genes, we used common, functional, rather than tagging, SNPs. Therefore, we did not have the opportunity to evaluate genetic variations comprehensively either in the selected genes or in other BER genes that we did not study. Second, because this was a hospital-based, case-control study, the control group in this study may not have provided a good representation of the general population. However, we recruited a relatively large study population, applied a rigorous epidemiologic design in selecting study participants, and used further statistical adjustment to minimize potential biases. In addition, although the allele frequencies were not consistent with those from the SNP500Cancer database, which were derived from only 62 Caucasians (0.840 for the *Val* allele in *ADPRT*, 0.647 for the *Arg* allele in *XRCC1*, and 0.530 for the *Asp* allele in *APE1* in the current study compared with 0.887, 0.532, 0.565, respectively, according to the SNP500Cancer website [http://snp500cancer.nci.nih.gov]), the allele frequencies in our control participants approximated those from other studies that had large samples of cancer-free individuals.[8,10,13,20]

In summary, the current results indicated that individuals who had the *ADPRT* 762*Ala*/*Ala* and *Ala*/*Ala*+*Val*/*Ala* genotypes were at lower risk of developing SCCHN compared with individuals who had the *Val*/*Val* genotypes. Our data also indicated that the reduced SCCHN risk associated with *ADPRT* variant genotypes was more pronounced among older individuals, women, and patients with oral cancer. These findings suggest that *ADPRT* polymorphisms may have an effect on the risk of SCCHN. Because of uncontrolled biases in the selection of participants and the low penetrance role of the common nsSNPs in SCCHN susceptibility, it is likely that all of these findings were by chance. Therefore, our results should be validated further by other larger, population-based, preferably prospective studies.

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