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Polymorphisms of *XPG/ERCC5* and risk of squamous cell carcinoma of the head and neck

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Abstract

Objectives—Xeroderma pigmentosum group G (XPG) protein is essential for the nucleotide excision repair (NER) system, and genetic variations in *XPG/ERCC5* that affect DNA repair capacity may contribute to the risk of tobacco-induced cancers, including squamous cell carcinoma of the head and neck (SCCHN). We investigated the association between *XPG/ERCC5* polymorphisms and risk of squamous cell carcinoma of the head and neck (SCCHN).

Methods—We genotyped 12 tagging and potentially functional single nucleotide polymorphisms (SNPs) of *XPG/ERCC5* in a case-control study of 1,059 non-Hispanic white patients with SCCHN and 1,066 cancer-free age-and sex matched controls and evaluated their associations with SCCHN risk.

Results—Multivariate logistic regression showed that only an intronic tagging SNP (rs4150351A/C) of *XPG/ERCC5* was associated with a decreased risk of SCCHN (adjusted OR=0.76, 95% CI=0.62–0.92 for AC *vs*. AA; adjusted OR=0.81, 95% CI=0.67–0.98 for AC/CC *vs*. AA), but this association was nonsignificnant after corrections by the permutation test (empirical *P*=0.105). In the genotype-phenotype correlation analysis using peripheral lymphocytes from 44 SCCHN patients, we found that rs4150351 AC/CC was associated with a statistically significant increase in *XPG/ERCC5* mRNA expression.

Conclusion—These findings suggest that genetic variation in *XPG/ERCC5* may not affect the SCCHN risk, although rs4150351 C variant genotypes were associated with the increased expression of *XPG/ERCC5* mRNA and nonsignificantly decreased risk of SCCHN. Larger population-based and additional functional studies are warranted to validate our findings.

Keywords

ERCC5; polymorphism; SCCHN; risk

Introduction

DNA repair plays a critical role in protecting the genome from insults of environmental carcinogens, such as tobacco smoke and ultraviolet (UV) radiation [1, 2]. To date, more than 150 genes are involved in at least five distinct DNA repair pathways in humans: nucleotide

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excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination (HR) and non-homologous end joining (NHEJ) [3, 4]. Of those pathways, NER is the most versatile repair mechanism responsible for many different forms of DNA damage, including bulky adducts cross-links, oxidative DNA damage, thymidine dimers, and alkylating damage [5]. Studies have shown that there are inter-individual variations of DNA repair capacity (DRC) in the general population and that a suboptimal DRC has an effect on risk of smoking-related cancers, such as lung cancer and squamous cell carcinoma of the head and neck (SCCHN) [6, 7]. Furthermore, accumulated evidence has also shown that genetic variants in one or more NER genes contribute to phenotypic variation of DRC, thereby modifying the susceptibility to cancer [8-12].

Xeroderma pigmentosum group G (*XPG*), also known as *ERCC5*, is one of eight NER core genes (i.e., *ERCC1, XPA, XPB/ERCC3, XPC, XPD/ERCC2, XPE/DDB1, XPF/ERCC4*, and *XPG/ERCC5*) in humans, which mainly functions as a structure-specific endonuclease that cleaves the damaged DNA strand on the 3’ endside [13, 14]. In addition, XPG stimulates BER of oxidative DNA damage to facilitate efficient transcription by RNA polymerase II [15, 16]. As one of the key factors of the NER pathway, *XPG/ERCC5* has been widely explored for its role in carcinogenesis with various tumor cell lines or tissues. For example, its expression levels were found to be correlated with risk and prognosis of several human cancers, including SCCHN [17-21]. Recent studies further suggested that *XPG/ERCC5* expression levels were related to cellular NER activity and response to cisplatin and irofulven of therapeutic agents, potentially making it an attractive therapeutic target for human cancers [17, 21, 22].

Located on chromosome 13q32.3-q33.1, *XPG/ERCC5* contains 15 exons, spanning 32 kb in length. At least 446 single nucleotide polymorphisms (SNPs) in the *XPG/ERCC5* gene have been identified (http://www.ncbi.nlm.nih.gov/projects/SNP/); however, only few are potentially functional, which may affect gene expression, protein functions, or cellular DRC [23, 24]. Of those SNPs, the His1104Asp polymorphism (rs17655) located in the exon 15 has been largely investigated in genetic and epidemiologic studies of susceptibility to cancers of the breast [25], lung [26], stomach [27], bladder [28], colorectum [29], and head and neck [30-33]. Additionally, it has been reported that rs17655 of *XPG/ERCC5* together with SNPs of several other NER genes jointly contributed to the variability of DRC [34]. These support the hypothesis that variants of *XPG/ERCC5* may be associated with development of human cancers. However, to our knowledge, few studies have comprehensively investigated associations between SNPs of *XPG/ERCC5* and SCCHN risk [30-33].

Using a comprehensive approach of selecting 12 SNPs of *XPG/ERCC5* that tag all common (minor allele frequency [MAF] ≥0.05) of the gene from the NIEHS database (http://egp.gs.washington.edu/) and the HapMap database (http://www.hapmap.org/), we conducted a large case-control study of 1,059 non-Hispanic white patients with SCCHN and 1,066 cancer-free age-and-sex matched controls to investigate associations between these SNPs and SCCHN risk and to evaluate modification effects of both the tagging and potentially functional SNPs in *XPG/ERCC5* on SCCHN risk.

Materials and Methods

Study population

Participant recruitment was described previously [33, 35, 36]. Briefly, all patients with histopathologically confirmed SCCHN were consecutively recruited from The University of Texas M.D. Anderson Cancer Center between October 1999 and October 2007. Among all patients initially contacted for participation, approximately 90% of eligible cases agreed to

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participate in the study. There was no age, sex, histology or stage restrictions, but patients with second SCCHN primary tumors, primary tumors of the nasopharynx or sinonasal tract, metastasized cancer from other organs, or any histopathologic diagnosis other than SCCHN, were excluded. Cancer-free controls, frequency-matched to cases on age (±5 years) and sex, were recruited from those visitors to outpatients at M.D. Anderson Cancer Center. These individuals were not related genetically to the enrolled cases or to each other. The overall response rate of controls was approximately 85%. The designed questionnaires were used to acquire subjects' information on demographic data and environmental exposure history, such as age, sex, smoking and alcohol consumption. After the interview, approximately 30-ml venous blood sample was collected from each study participant. Among all cases, only 44 subjects had some left-over frozen PBMCs (blood mononuclear cells) available for culture, which had different genotypes for SNPs, and were used for evaluating mRNA expression levels. All subjects were non-Hispanic whites and a total of 1,059 cases and 1,066 controls that completed the interview and donated a one-time blood sample were included in the analysis. This study was approved by the institutional review board of M. D. Anderson. Informed consent was obtained from all study subjects.

SNPs selection and genotyping

Polymorphisms of the *XPG/ERCC5* gene were selected by a comprehensive approach combining potentially functional or tagging SNPs. The NIEHS database (http://egp.gs.washington.edu/) and the HapMap database (http://www.hapmap.org/) were used to search for all common SNPs (MAF ≥ 0.05 in European populations) located in or within a 3-kb region centered around the *XPG/ERCC5* gene, potentially functional significance of which was then predicted by the online software, Pupasuite 2 (http://pupasview.bioinfo.ochoa.fib.es/) and FuncPred (http://manticore.niehs.nih.gov/snpfunc.htm). In this study, potentially functional SNPs included all those related to amino acid changing, transcription factor binding sites (TFBS), exonic splicing enhancers (ESE), exonic splicing silencers (ESS) and miRNA binding sites. Furthermore, common tagging SNPs (Hardy-Weinberg equilibrium $P \geq 0.05$ and call rate ≥85%) were identified by the Haploview software on the basis of pairwise linkage disequilibrium (LD) (r^2 threshold: 0.8) and with a priority of forcing the potentially functional SNPs in the selection. As a result, 12 SNPs (Table 1 and Supplementary Fig. 1) were selected for genotyping by using the SNPlex assay in the DNA Core Facility at MD Anderson Cancer Center, according to the protocol of the manufacturer (Applied Biosystems, Foster City, CA). The output data from the SNPlex assays were analyzed using the GeneMapper software (Applied Biosystems) to determine the genotypes. In addition, the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method was also used to revaluate the genotypes of samples that failed in the SNPlex assays. Approximately 10% of the samples were randomly selected to perform the repeated genotyping assays, and the results were 100% concordant.

Quantitative measurement of ERCC5 mRNA expression—Quantitative real-time reverse transcription-PCR (qRT-PCR) was used to determine the expression level of *XPG/ERCC5* in peripheral blood mononuclear cells (PBMCs). Total RNA was isolated from phytohemagglutinin-stimulated peripheral blood lymphocytes from 44 SCCHN patients by using the TRIzol reagent (Invitrogen™, USA). Expression levels of the target and reference genes were analyzed using an ABI7900 sequence detection system (Applied Biosystems, Foster City, CA) with a final volume of 5 µl containing 5 ng of the cDNA, 0.25 µl primers, and 2.5 µl Master mix. The thermal cycling conditions were: 95°C for 5 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. 18S RNA was measured as an endogenous control to normalize for differences in the amount of cDNA used in each reaction. *XPG/ERCC5* and 18S mRNA levels were quantified in separate tubes in duplicates, and

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expression level of *XPG/ERCC5* relative to that of 18S was calculated using the equation ratio = $Ct_{ERCC5}/Ct_{18S}*100\%$.

Statistical analysis—Deviation of genotype frequencies for each SNP from the Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ^2 test. Distributions of demographic characteristics, selected variables, and frequencies of genotypes of *XPG/ERCC5* between cases and controls were evaluated by using the χ^2 test. The associations between *XPG/ERCC5* genotypes and SCCHN risk were estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) in different genetic models from both univariate and multivariate Logistic regression with adjustment for age, sex, smoking status and alcohol use. We also corrected multiple testing on associations of all SNPs by using Stata 10.0 through 1000 permutations that randomly permutated the case/control status independent of genotypes. The SAS TTEST procedure was used to compare the expression levels of *XPG/ERCC5* between cases with different genotypes. All of the statistical analyses were two-sided with a significance level of 0.05 and performed with Statistical Analysis System software (v.9.2 SAS Institute, Cary, NC).

Results

Characteristics of study subjects

The details of 1,059 cases and 1,066 controls enrolled in this study are shown in Table 2. The mean age was 57.0 years (±11.1) for cases and 56.6 years (±11.0) for controls, and the frequency-matching on age and sex between cases and controls was adequate (P=0.522 and 0.660, respectively). However, cases were more likely to be smokers (71.9% *vs*. 51.1%, P< 0.001) and drinkers (72.6% *vs*. 56.7%, P< 0.001) than controls. Among all SCCHN cases, 317 (29.9%) had primary tumors of the oral cavity, 538 (50.8%) of the oropharynx and 204 (19.3%) of the hypopharynx/larynx. In addition, 259 cases (24.5) were of I-II stages, and 800 cases (75.5%) were of III-IV stages.

Associations between XPG/ERCC5 variants and SCCHN risk

The position, MAF and genotyping rate of the 12 selected SNPs are presented in Table 1. The observed genotype frequencies for these 12 SNPs were all in Hardy-Weinberg equilibrium in the controls (P>0.004, 0.05/12), and the SNP calling rates were all >98.0%. The genotype frequencies of *XPG/ERCC5* SNPs in the cases and the controls are summarized in Table 3. In the single locus analyses, only the genotype frequencies of rs4150351 were significantly different between the cases and the controls (P=0.005). After adjustment for age, sex, smoking and alcohol status, multivariate Logistic regression analysis further revealed that variant genotypes of rs4150351A/C were significantly associated with a decreased risk of SCCHN (adjusted OR=0.76, 95% CI=0.62−0.92 for AC *vs*. AA and adjusted OR=0.81, 95% CI=0.67–0.98 for AC/CC *vs*. AA). To reduce the false discovery rate, we further used permutation to asses statistical significance of SNPs (1000 permutations) and found that the P value for rs4150351 was the smallest among of all 12 SNPs, but the p value changed to non-significant (Empirical P=0.105). No significant associations with SCCHN risk were identified for other 11 SNPs examined in this study (Table 3).

We further evaluated the effect of rs4150351AC/CC genotypes on SCCHN risk stratified by selected variables including age, sex, smoking status, alcohol status, tumor site and stage (Table 4). Although the protective effect of rs4150351AC/CC genotypes were statistically significant in some groups, heterogeneity test showed that there was no significant heterogeneity (P>0.05) between every two strata, suggesting no risk effect modification by the variables under investigation.

Because 10 of 12 SNPs (rs2094258, rs2296147, rs4771436, rs1047768, rs2227869, rs4150351, rs4150355, rs4150383, rs4150386 and rs17655) are in the same block and in incomplete LD ($0<r^2<0.8$) (Supplementary Fig.1), we conducted the haplotype analysis for these 10 SNPs. A total of 53 haplotypes were derived from the observed genotypes, of which CCTCGATGAG was the most common haplotype in cases and controls with the frequencies of 26.3 and 24.3%, respectively. However, no significant associations were found between all other haplotypes and risk of SCCHN, compared with the common CCTCGATGAG haplotype (data not shown).

Correlation analysis for XPG/ERCC5 expression and XPG/ERCC5 genotypes

To further explore the functional relevance of *XPG/ERCC5* polymorphisms, we conducted genotype-phenotype correlation analysis between variant genotypes of rs4150351 and levels of *XPG/ERCC5* mRNA expression in lymphocytes from 44 SCCHN cases. The means±SD of relative levels were 241.6±19.2, 231.3±16.2 and 213.0±24.1 for subjects with genotypes AA (n=28), AC (n=14) and CC (n=2), respectively. The results showed that cases with rs4150351 AC/CC genotypes (n = 16) had significantly lower normalized Ct values (i.e., high initial concentration), indicating higher levels of *ERCC5* expression, compared to cases with the AA genotypes (n = 28) ($P = 0.037$; Fig. 1).

Discussion

In the present study, we assessed the effect of genetic variation in *XPG/ERCC5* on susceptibility to SCCHN by genotyping 12 tagging or potentially functional SNPs of the gene. After rigorous corrections for multiple tests, we found that none of SNPs in *ERCC5/XPG* was significantly associated with SCCHN risk, though variant genotypes of *XPG/ERCC5* rs4150351 were associated with an increased expression of *ERCC5/XPG* mRNA and nondignificantly reduced SCCHN risk; the latter is likely due to limited statistical power of the present study.

XPG/ERCC5 acts as a structure-specific endonuclease that is critical to both NER subpathways of the transcription-coupled repair (TCR), which specifically targets and repairs DNA damage on the transcribed strand of actively expressed genes, and the global genomic repair (GGR), which removes DNA damage from the remaining genome [37]. In addition, patients with large truncations in the XPG protein frequently present features of combined XP and Cockayne syndrome (XP-CS). In animal experiments, complete inactivation of the *XPG/ERCC5* gene leads to severe developmental defects in mice; these suggest that the XPG protein is involved in additional housekeeping functions besides the role in the NER pathway [38, 39]. It has been reported that some genetic mutations in *XPG/ERCC5* affect the NER endonuclease activity [18, 41] and that decreased expression of *XPG/ERCC5* in lymphocytes has been associated with increased risk of some cancers, including SCCHN [18, 40]. Our previous study also found that the combined effects of select functional SNPs in the core NER genes including *ERCC5* were significantly associated with the DRC phenotype in nonmelanoma skin cancer cases and healthy controls [41].

In the present study, the results showed that *XPG/ERCC5* rs4150351 was associated with *ERCC5/XPG* expression levels but with non-significantly reduced risk of SCCHN. Rs4150351 is an intronic SNP of *XPG/ERCC5*, and its functional significance has not been elucidated. Some studies have reported that an intronic SNP may alter mRNA levels of genes by affecting transcription, RNA elongation, splicing, or maturation [42-44]. Interestingly, we found that variant (AC/CC) genotypes of *XPG/ERCC5* rs4150351, compared with the AA genotype, were indeed associated with increased mRNA levels of *XPG/ERCC5* in PBMCs from SCCHN patients. These data suggest a potentially functional

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impact of this intronic SNP on the mRNA levels, but the underlying molecular mechanism needs further investigation.

Several studies have investigated the associations of SNPs in *XPG/ERCC5* with risk of various cancers [11, 25-33]. Of these SNPs, the most frequently studied one was His1104Asp (rs17655, G/C) located in the XPG C-terminus, which is required for its interactions with other components of the NER pathway, such as XPB, XPD and TFIIH subunits [45]. The His1104Asp amino acid change may influence these protein-protein interactions; however, such a potentially functional relevance has not been tested in published reports, and studies on the association between His1104Asp and risk of human cancers have generated inconsistent results [25-33], possibly because of differences in study design, sample size, tumor sites or ethnicity. For example, Abbasi *et al*. found an increased risk of laryngeal cancer only for 1104Asp/His heterozygous carriers [31], whereas another two studies reported no associations between His1104Asp genotypes and risk of SCCHN [30, 32]. Our previous analysis with 829 SCCHN patients and 854 cancer-free controls [33] and the current analysis with a larger sample size did not find an association between His1104Asp and risk of SCCHN in non-Hispanic whites. The possible discrepancy of results between different studies may be caused by different genetic background by ethnicity. For example, the frequency of rs17655 C allele was 0.47 in the study by Wen *et al* (in Chinese populations) [32], but 0.22 in our study (non-Hispanic white populations). Furthermore, some studies also investigated the effect of other two *XPG/ERCC5* SNPs (rs1047768 and rs4771436) on SCCHN risk [11, 31] but found no significant associations, consistent with the findings in the present study.

To more fully explore the SNPs of *ERCC5*, we also imputed genotypes by using these 12 SNPs within a ±3-kb region around the *XPG/ERCC5* on chromosome 13q22. The imputation used IMPUTE 2 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html) and the CEU data from 1000 genomes (June 2010 release, pilot1) as a reference panel (http://www.1000genomes.org/). Finally, only 36 imputed SNPs (MAF>0.05 and an estimate of r^2 between imputed and true genotypes above 0.3 as thresholds of quality) were evaluated for the association with SCCHN risk, but we did not any significant associations. These findings further suggested that it is likely that SNPs of *ERCC5* may play a very limited role in the etiology of SCCHN, if any.

Our study has a number of strengths. This large SCCHN case-control study provided us sufficient statistical power to detect a moderate effect of *ERCC5* SNPs on SCCHN risk. Furthermore, we comprehensively selected 12 tagging and potentially functional SNPs of *XPG/ERCC5* that covered all common SNPs of the gene by using database searching and functional prediction *in silico*. However, the present study also has several potential limitations. Firstly, it was a hospital-based, and thus the genotype frequency in the control group may not represent the true frequency in the general population because of potential selection bias. However, the agreement with Hardy-Weinberg equilibrium for all 12 SNPs and similar allele frequencies of our controls to those reported in CEU populations from the HapMap database suggested the minimal selection bias, if any, in terms of genotype frequencies. Secondly, although our sample size was relatively large, the small sample size in subgroup analysis may have limited statistical power. Therefore, additional larger studies in different populations are needed to validate our findings. Finally, we may have missed some potentially functional SNPs that are not available in the existing databases we used for SNPs selection. For example, recent 1000 genome database has identified that another SNP (rs76871136) of *ERCC5/XPG* has a MAF of 14% for Europeans and may change a Gly to a stop codon; however, frequency information of this SNP is currently unavailable in the Hapmap database. Thus, additional studies need to focus on such SNPs and provide more comprehensive information for the association between SNPs of *ERCC5* and SCCHN risk.

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In conclusion, we comprehensively evaluated the effect of all available common genetic variants in *XPG/ERCC5* on SCCHN risk but found a weak association, which disappeared after corrections for multiple testing, between *XPG/ERCC5* rs4150351 variant genotypes (AC/CC) and SCCHN risk in this non-Hispanic white study population. Although this *XPG/ERCC5* polymorphism may be functional, its role in susceptibility to SCCHN remains to be confirmed in larger epidemiological studies and in-depth mechanistic studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

OR	odds ratios
CI	confidence interval

ERCC5	excision repair cross complement group 5
XPG	xeroderma pigmentosum group G
SNP	single nucleotide polymorphism
DRC	DNA repair capacity
LD	linkage disequilibrium
MAF	minor allele frequency
SCCHN	squamous cell carcinoma of the head and neck

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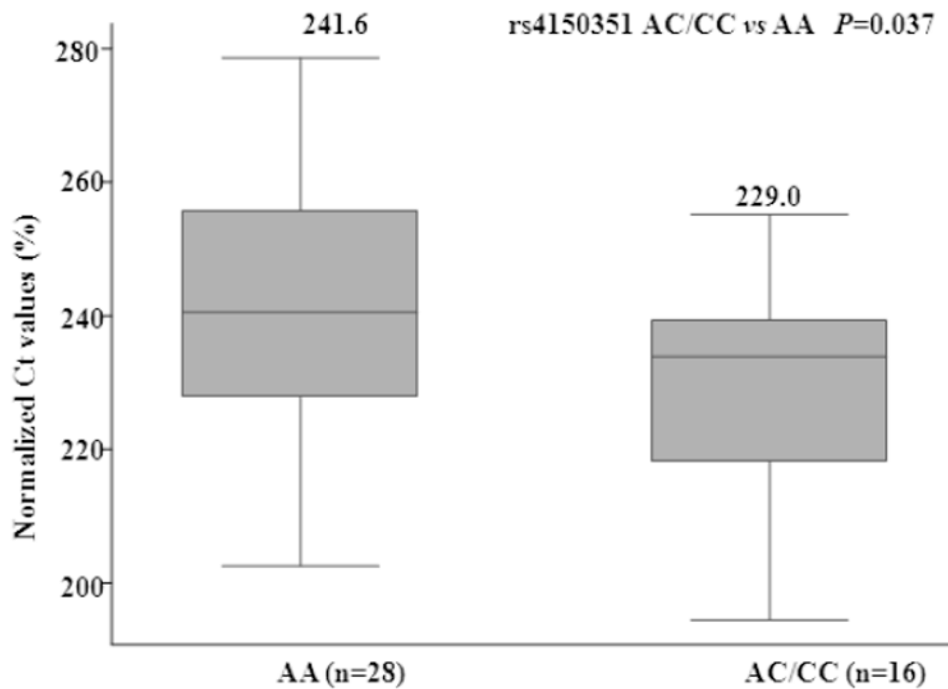

Fig. 1. Boxplot for levels of *XPG/ERCC5* mRNA expression in peripheral blood lymphocytes from 44 SCCHN cases with known *XPG/ERCC5* variant genotypes (rs4150351)

Data represented median and quartiles. The relative levels of *XPG/ERCC5* were normalized to that of *18S* using the equation ratio = Ct_{ERCC5} / Ct_{18S} * 100%. A lower normalized Ct value represents higher expression. The means±SD of relative levels were 241.6±19.2 and 229.0±17.5 for subjects with AA and AC/CC genotypes, respectively.

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Table 1

Primary information and genotyping results of selected SNPs in *XPG/ERCC5*

Gene and locus	NCBI rs #	Location	Type	Base change	MAF in Hapmap database (European)	MAF in our cases	MAF in our controls	HWE	Genotyping rate (%)
XPG/ERCC5	rs2094258	5’ near gene	TFBS	C>T	0.22	0.18	0.18	0.09	98.4
13q22	rs2296147	5’UTR	TFBS	T>C	0.38	0.49	0.47	0.44	99.8
	rs4771436	intron_1	tagging	T>G	0.23	0.23	0.23	0.94	99.8
	rs1047768	exon_2 (H46H)	ESE or ESS	C>T	0.45	0.41	0.41	0.67	99.9
	rs2227869	exon_8 (C529S)	non-synonymous	G>C	0.05	0.04	0.04	0.95	100
	rs4150351	intron_12	tagging	A>C	0.18	0.17	0.19	0.11	99.9
	rs4150355	intron_12	tagging	C>T	0.32	0.38	0.36	0.91	99.5
	rs4150383	intron_14	tagging	G>A	0.17	0.18	0.17	0.97	99.9
	rs4150386	intron_14	tagging	A>C	0.12	0.12	0.12	0.72	100
	rs17655	exon_15 (D1104H)	non-synonymous	G>C	0.27	0.22	0.22	0.10	100
	rs873601	3’UTR	miRNA binding	A>G	0.38	0.26	0.27	0.45	99.9
	rs4150393	3’ near gene	tagging	A>G	0.10	0.12	0.13	0.10	100

NCBI = National Center for Biotechnology Information; MAF= minor allele frequency; HWE= Hardy-Weinberg equilibrium; TFBS=transcription factor binding sites; ESE=exonic splicing enhancers; ESS=exonic splicing silencers

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Table 2

Distributions of selected variables in SCCHN cases and cancer-free controls.

Variables	Cases N (%)	Controls N (%)	p^a
All subjects	1,059 (100)	1,066 (100)	
Age, yr			0.522
≤57(median)	570 (53.8)	559 (52.4)	
>57(median)	489 (46.2)	507 (47.6)	
Sex			0.660
Females	262 (24.7)	255 (23.9)	
Males	797 (75.3)	811 (76.1)	
Smoking status			<0.001
No	298 (28.1)	521 (48.9)	
Yes	761 (71.9)	545 (51.1)	
Alcohol status			<0.001
No	290 (27.4)	462 (43.3)	
Yes	769 (72.6)	604 (56.7)	
Tumor site			
Oral cavity	317 (29.9)		
Oropharynx	538 (50.8)		
Hypopharynx/ Larynx	204 (19.3)		
Stage			
I-II	259 (24.5)		
III-IV	800 (75.5)		

[a]Two-sided χ^2 test

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Table 3

Logistic regression analysis for associations between *XPG/ERCC5* polymorphisms and SCCHN risk.

XPG/ERCC5 Locus	Genotype	Cases [a] N (%)	Controls [a] N (%)	P^b	Crude OR (95% CI)	Adjusted OR (95% CI) [c]	P^c
		N=1,038	N=1,053				
rs2094258	CC	706 (68.0)	721 (68.5)	0.808 [d]	1.00	1.00	
	CT	295 (28.4)	291 (27.6)		1.04 (0.85-1.26)	1.00 (0.82-1.22)	0.991
	TT	37 (3.6)	41 (3.9)		0.92 (0.58-1.46)	0.93 (0.58-1.48)	0.749
	CT/TT	332 (32.0)	332 (31.5)	0.851 [e]	1.02 (0.85-1.23)	0.99 (0.82-1.20)	0.934
		N=1,056	N=1,065				
rs2296147	TT	280 (26.5)	294 (27.6)	0.619 [d]	1.00	1.00	
	CT	532 (50.4)	543 (51.0)		1.03 (0.84-1.26)	1.02 (0.83-1.25)	0.876
	CC	244 (23.1)	228 (21.4)		1.12 (0.88-1.43)	1.13 (0.88-1.45)	0.346
	CT/CC	776 (73.5)	771 (72.4)	0.591 [e]	1.06 (0.87-1.28)	1.06 (0.87-1.28)	0.572
		N=1,057	N=1,064				
rs4771436	TT	630 (59.6)	637 (59.9)	0.971 [d]	1.00	1.00	
	GT	374 (35.4)	372 (34.9)		1.02 (0.85-1.22)	1.01 (0.84-1.22)	0.920
	GG	53 (5.0)	55 (5.2)		0.97 (0.66-1.44)	0.98 (0.65-1.46)	0.908
	GT/TT	427 (40.4)	427 (40.1)	0.929 [e]	1.01 (0.85-1.20)	1.01 (0.84-1.20)	0.954
		N=1,059	N=1,065				
rs1047768	CC	369 (34.8)	379 (35.6)	0.911 [d]	1.00	1.00	
	CT	506 (47.8)	507 (47.6)		1.03 (0.85-1.24)	0.98 (0.81-1.19)	0.855
	TT	184 (17.4)	179 (16.8)		1.06 (0.82-1.36)	1.07 (0.82-1.38)	0.630
	CT/TT	690 (65.2)	686 (64.4)	0.720 [e]	1.03 (0.87-1.23)	1.00 (0.84-1.21)	0.971
		N = 1,059	N = 1,066				
rs2227869	GG	987 (93.2)	974 (91.4)	0.278 [d]	1.00	1.00	
	CG	70 (6.6)	90 (8.4)		0.77 (0.56-1.06)	0.73 (0.52-1.01)	0.060
	CC	2 (0.2)	2 (0.2)		0.99 (0.14-7.02)	0.84 (0.12-6.16)	0.864
	CG/CC	72 (6.8)	92 (8.6)	0.123 [e]	0.77 (0.56-1.06)	0.73 (0.52-1.01)	0.060

XPG/ERCC5 Locus	Genotype	Cases [a] N (%)	Controls [a] N (%)	P^b	Crude OR (95% CI)	Adjusted OR (95% CI) [c]	P^c
		N = 1,057	N = 1,065				
rs4150351	AA	740 (70.0)	693 (65.1)	0.005 [d]	1.00	1.00	
	AC	275 (26.0)	342 (32.1)		0.75 (0.62-0.91)	0.76 (0.62-0.92)	0.006
	CC	42 (4.0)	30 (2.8)		1.31 (0.81-2.12)	1.46 (0.89-2.39)	0.138
	AC/CC	317 (30.0)	372 (34.9)	0.016 [e]	0.80 (0.67-0.96)	0.81 (0.67-0.98)	0.030
		N = 1,054	N = 1,061				
rs4150355	CC	408 (38.7)	436 (41.1)	0.323 [d]	1.00	1.00	
	CT	488 (46.3)	487 (45.9)		1.07 (0.89-1.29)	1.06 (0.88-1.28)	0.550
	TT	158 (15.0)	138 (13.0)		1.22 (0.94-1.60)	1.22 (0.93-1.61)	0.155
	CT/TT	646 (61.3)	625 (58.9)	0.267 [e]	1.11 (0.93-1.32)	1.10 (0.92-1.31)	0.323
		N = 1,059	N = 1,063				
rs4150383	GG	720 (68.0)	732 (68.9)	0.906 [d]	1.00	1.00	
	AG	308 (29.1)	300 (28.2)		1.04 (0.86-1.26)	1.02 (0.84-1.24)	0.822
	AA	31 (2.9)	31 (2.9)		1.02 (0.61-1.69)	1.04 (0.61-1.76)	0.886
	AG/AA	339 (32.0)	331 (31.1)	0.674 [e]	1.04 (0.87-1.25)	1.02 (0.85-1.24)	0.804
		N = 1,059	N = 1,066				
rs4150386	AA	823 (77.7)	825 (77.4)	0.974 [d]	100	1.00	
	AC	223 (21.1)	227 (21.3)		0.99 (0.80-1.21)	0.98 (0.79-1.22)	0.862
	CC	13 (1.2)	14 (1.3)		0.93 (0.44-1.99)	0.87 (0.40-1.89)	0.716
	AC/CC	236 (22.3)	241 (22.6)	0.876 [e]	0.98 (0.80-1.20)	0.97 (.79-1.20)	0.806
		N = 1,059	N = 1,066				
rs17655	GG	648 (61.2)	654 (61.4)	0.608 [d]	1.00	1.00	
	CG	359 (33.9)	350 (32.8)		1.04 (0.86-1.24)	1.02 (0.84-1.23)	0.860
	CC	52 (4.9)	62 (5.8)		0.85 (0.58-1.24)	0.85 (0.57-1.27)	0.434
	CG/CC	411 (38.8)	412 (38.6)	0.965 [e]	1.01 (0.85-1.20)	0.99 (0.83-1.19)	0.937
		N = 1,058	N = 1,066				

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XPG/ERCC5 Locus	Genotype	Cases [a] N (%)	Controls [a] N (%)	P^b	Crude OR (95% CI)	Adjusted OR (95% CI) [c]	P^c
rs873601	AA	565 (53.4)	572 (53.7)	0.323 [d]	1.00	1.00	
	AG	427 (40.4)	411 (38.5)		1.05 (0.88-1.26)	1.08 (0.90-1.30)	0.397
	GG	66 (6.2)	83 (7.8)		0.81 (0.57-1.14)	0.82 (0.57-1.17)	0.265
	AG/GG	493 (46.6)	494 (46.3)	0.931 [e]	1.01 (0.85-1.20)	1.04 (0.87-1.24)	0.678
		N = 1,059	N = 1,066				
rs4150393	AA	821 (77.5)	814 (76.4)	0.787 [d]	1.00	1.00	
	AG	217 (20.5)	228 (21.4)		0.94 (0.77-1.16)	0.94 (0.76-1.17)	0.574
	GG	21 (2.0)	24 (2.2)		0.87 (0.48-1.57)	0.87 (0.47-1.60)	0.649
	AG/GG	238 (22.5)	252 (23.6)	0.537 [e]	0.94 (0.77-1.15)	0.93 (0.76-1.15)	0.514

[a] The numbers were not the same for each SNP because of their different calling rates due to few uncalled samples.

[b] Chi square test for genotype distributions between cases and controls.

[c] Adjusted by age, gender, smoking and alcohol status in Logistic regress models

[d] for genotyping distribution

[e] for dominant genetic models

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Table 4

Stratified analysis for *XPG/ERCC5* rs4150351 genotypes and SCCHN risk

Variables	rs4150351 (Cases/ Controls)		Crude OR (95% CI)	Adjusted OR (95% CI)[a]	*P* for Heterogeneity
	AA	AC/CC			
XPG/ERCC5	740/693	317/372	0.80 (0.67-0.96)	0.81 (0.67-0.98)	
Age					
≤57(median)	401/365	167/194	0.78 (0.61-1.01)	0.79 (0.61-1.02)	0.653
>57(median)	339/328	150/178	0.82 (0.63-1.06)	0.85 (0.65-1.12)	
Sex					
Females	177/164	85/91	0.87 (0.61-1.25)	0.92 (0.63-1.34)	0.424
Males	563/529	232/281	0.78 (0.63-0.96)	0.77 (0.62-0.96)	
Smoking status					
Never	202/332	96/189	0.84 (0.62-1.13)	0.84 (0.62-1.14)	0.756
Ever	538/361	221/183	0.81 (0.64-1.03)	0.79 (0.62-1.00)	
Alcohol status					
Never	199/306	91/155	0.90 (0.66-1.24)	0.90 (0.66-1.24)	0.399
Ever	541/387	226/217	0.75 (0.59-0.94)	0.76 (0.60-0.96)	
Tumor site					
Oral cavity	220/693	97/372	0.82 (0.63-1.08)	0.83 (0.63-1.10)	0.953
Oropharynx	378/693	160/372	0.79 (0.63-0.99)	0.78 (0.62-0.99)	
Hypopharynx/Larynx	142/693	60/372	0.79 (0.57-1.09)	0.80 (0.57-1.14)	
Stage					
I-II	175/693	83/372	0.88 (0.66-1.18)	0.89 (0.66-1.21)	0.522
III-IV	565/693	234/372	0.77 (0.63-0.94)	0.79 (0.64-0.96)	

[a]Adjusted for age, sex, smoking status and alcohol status in Logistic regression models