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SHORT REPORT

Haplotype Analysis of the *XRCC1* Gene and Laryngeal Cancer

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Objective: Several polymorphisms in DNA repair genes have been extensively studied in association with various human cancers, including laryngeal cancer. The present study aimed to investigate the association between polymorphisms of the *XRCC1* gene and laryngeal cancer in a Chinese population. *Methods:* Five polymorphisms of the *XRCC1* gene (rs3213403, rs1799778, rs1001581, rs3213282, and rs3810378) were genotyped by TaqMan in 234 patients with larynx cancer and 230 age- and sex-matched controls without cancer. *Results:* The rs3213403, rs1799778, and rs3213282 polymorphisms of *XRCC1* were associated with larynx cancer. Haplotype analysis indicated that CCA (odds ratio [OR], 5.707; 95% confidence interval [CI], 3.277–9.938; $p < 0.001$), TGG (OR, 4.344; 95% CI, 2.804–6.732; $p < 0.001$), ACA (OR, 1.615; 95% CI, 1.159–2.250; $p = 0.004$), and GCG (OR, 1.702; 95% CI, 1.164–2.489; $p = 0.005$) were associated with an increased risk for larynx cancer, respectively. However, TGA (OR, 0.518; 95% CI, 0.398–0.673; $p < 0.001$) and ACC (OR, 0.314; 95% CI, 0.215–0.457; $p < 0.001$) were associated with a decreased risk for larynx cancer. *Conclusions:* The results indicated that *XRCC1* genetic polymorphisms were associated with larynx cancer in a Chinese population.

Introduction

Larynx cancer is a complex disease resulting form interaction among several environmental factors, such as smoking, high alcohol consumption, and occupational and environmental exposure to carcinogens (Parkin *et al.*, 2002; Pawlowska *et al.*, 2009; Kupisz *et al.*, 2010) and genetic polymorphisms (Wang *et al.*, 2013; Ziao *et al.*, 2013). Accumulated evidence suggested that DNA damage, if not repaired or misrepaired, may result in genomic instability, cancer transformation, and/or cell death (Futreal *et al.*, 1994; Khanna and Jackson, 2001; O'Driscoll and Jeggo, 2006).

The x-ray repair cross-complementing group 1 gene (*XRCC1*) encodes a protein that interacts with nicked DNA and participates with poly-adenosine diphosphate-ribose polymerase, DNA ligase III, and DNA polymerase B to repair single-strand DNA breaks (Thompson and West, 2000). The *XRCC1* gene is polymorphic, and dozens of variants have been identified, including several nonsynonymous single-nucleotide polymorphisms (SNPs) in the coding region. In the past decade, a huge amount of studies have investigated the association between *XRCC1* polymorphism and cancer, but much of the research has focused on SNPs rather than haplotypes, which better characterize the common patterns of variation in a population (Crawford and Nickerson, 2005).

In the present study, we established haplotypes of the *XRCC1* gene, consisting of 5 SNPs (rs3213403, rs1799778, rs1001581, rs3213282, and rs3810378), and assessed the association between these haplotypes and larynx cancer.

Material and Methods

Patients

Blood samples were obtained from 234 patients with laryngeal cancer from the Department of Otolaryngology-Head and Neck Surgery, Jinling Hospital, Nanjing Clinical Medical College, the Second Military Medical University in 2005–2013 and 230 cancer-free age- and sex-matched controls. The patients ranged in age from 40 to 80 years (mean age ± standard deviation, 62.2 ± 8.0 years). There were 112 cases of grade 1, 105 cases of grade 2, and 17 cases of grade 3 in total. According to TNM staging, there were 55 cases of stage I, 38 cases of stage II, 88 cases of stage III, 43 cases of stage IVA, and 11 cases of stage IVB. The study was

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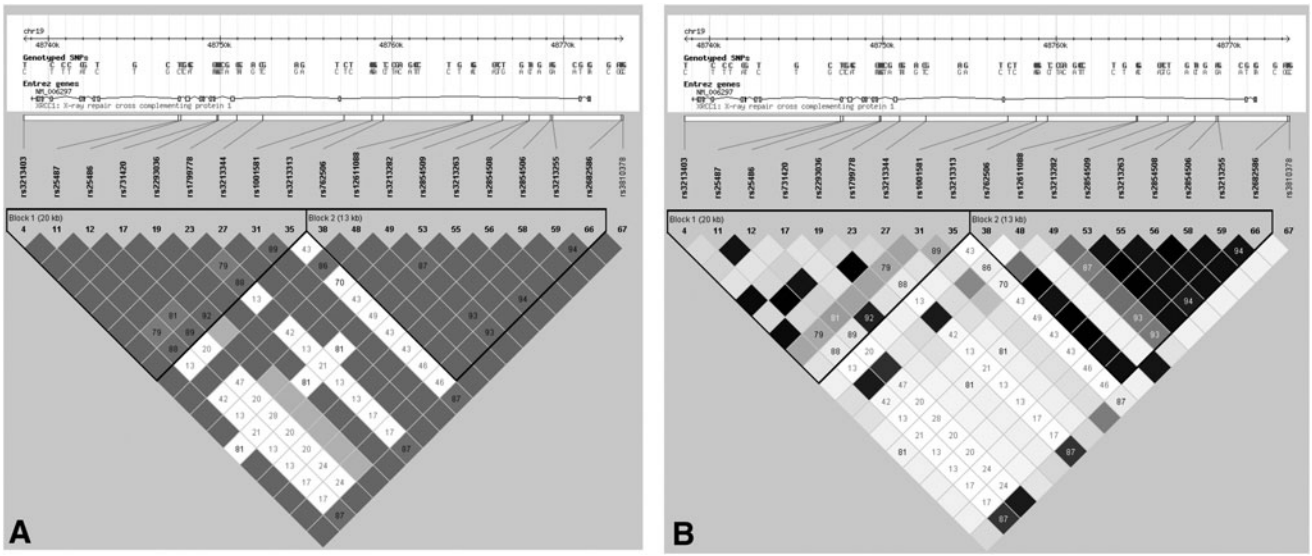

FIG. 1. Genetic variation at human *XRCC1* gene. By using Haploview 4.2 software and the HapMap phrase II database, 19 genotyped single-nucleotide polymorphisms (SNPs) from Chinese Han were scanned. Linkage disequilibrium (LD) blocks across the locus in Chinese Han were derived by solid spline method in Haploview 4.2. **(A)** LD value shown: $|D'| \times 100$; $|D'|$ color scheme: $|D'| = 0$: white; $0 < r^2 < 1$: shades of gray, $|D'| = 1$: black; **(B)** LD value shown: $r^2 \times 100$; r^2 color scheme: $r^2 = 0$: white; $0 < r^2 < 1$: shades of gray; $r^2 = 1$: black.

TABLE 1. GENOTYPE DISTRIBUTION OF *XRCC1* TAG SINGLE-NUCLEOTIDE POLYMORPHISMS BETWEEN PATIENTS WITH LARYNGEAL CANCER AND CONTROLS

SNPs	*Genotype and allele*	*Patients with laryngeal cancer* (n = *234*)	*Controls* (n = *230*)	p *value*
rs3213403	AA	139 (0.594)	160 (0.696)	0.073
	AG	86 (0.368)	63 (0.274)	
	GG	9 (0.038)	7 (0.030)	
	A	364 (0.778)	383 (0.833)	0.035
	G	104 (0.222)	77 (0.167)	
rs1799778	AA	21 (0.090)	15 (0.065)	0.071
	AC	89 (0.380)	69 (0.300)	
	CC	124 (0.530)	146 (0.635)	
	A	131 (0.280)	99 (0.215)	0.022
	C	337 (0.720)	361 (0.785)	
rs1001581	CC	16 (0.068)	11 (0.048)	0.580
	CT	81 (0.346)	82 (0.357)	
	TT	137 (0.585)	137 (0.596)	
	C	113 (0.241)	104 (0.226)	0.638
	T	355 (0.759)	356 (0.774)	
rs3213282	CC	24 (0.103)	31 (0.135)	0.018
	CG	105 (0.449)	125 (0.543)	
	GG	105 (0.449)	74 (0.322)	
	C	153 (0.327)	187 (0.407)	0.012
	G	315 (0.673)	273 (0.593)	
rs3810378	CC	13 (0.056)	15 (0.065)	0.506
	CG	85 (0.363)	72 (0.313)	
	GG	136 (0.581)	143 (0.662)	
	C	111 (0.237)	102 (0.222)	0.576
	G	357 (0.763)	358 (0.778)	

SNP, single-nucleotide polymorphism.

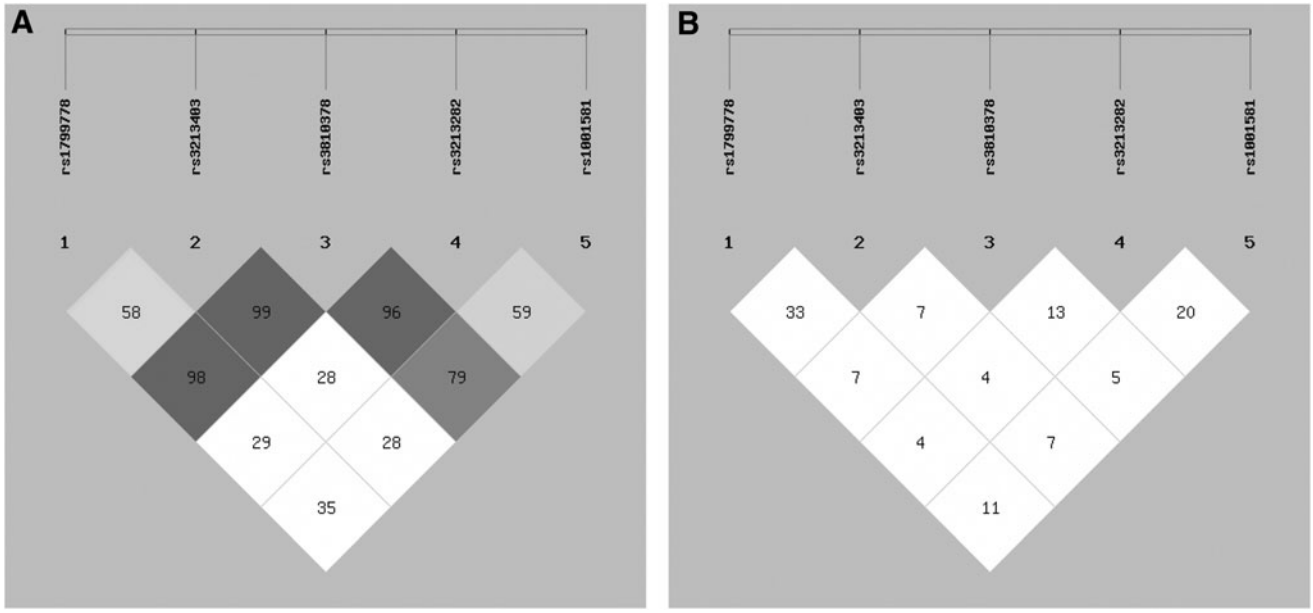

FIG. 2. The patterns of linkage disequilibrium in the *XRCC1* gene, with their | D'| **(A)** and r^2 values **(B)**.

approved by the Bioethics Committee of the Second Military Medical University, and each patient gave written consent.

Genotyping

There are 953 SNPs for the human *XRCC1* gene listed in the National Center for Biotechnology Information SNP database (http://www.ncbi.nlm.nih.gov/SNP). We also screened the data for the Tag SNPs on the International HapMap Project website (http://www.hapmap.org/). Using the Haploview 4.2 software and the HapMap phrase II database, we obtained five tagging SNPs (rs3213403, rs1799778, rs3810378, rs1001581, and rs3213282) for Han Chinese using minor allele frequency ≥ 0.10 and linkage disequilibrium patterns with $r^2 \geq 0.8$ as a cutoff. As shown in Figure 1, these five SNPs were located in two haplotype blocks.

Genomic DNA was extracted from the peripheral blood leukocytes using a DNA extraction kit (Beijing Bioteke Co. Ltd, Beijing, China). Genotyping was confirmed by TaqMan method, as described previously (Xiang *et al.*, 2009).

Statistical analysis

For each polymorphism, departure of the genotype distribution from that expected from Hardy-Weinberg equilibrium was assessed using the standard χ^2 test or Fisher exact test. Genotype frequencies in cases and controls were compared by χ^2 tests. The genotype-specific risks were estimated as odds ratios (ORs). In all cases, wild-type genotype served as a reference group. On the basis of the genotype data of the genetic variations, we performed linkage disequilibrium (LD) analysis and haplotype-based case–control analysis, using SHEsis software (http://analysis2.bio-x.cn/myAnalysis.php) (Shi and He, 2005; Li *et al.*, 2009). In the haplotype-based case–control analysis, haplotypes with a frequency <0.03 were excluded. Statistical significance was established at $p<0.05$.

Table 2. Distribution of Haplotype

Haplotype	*Patients with laryngeal cancer*	*Controls*	*OR (95% CI)*	p *value*
Block 1				
C C A*	79.63 (0.170)	15.95 (0.035)	5.707 (3.277–9.938)	<0.001
C G A*	33.34 (0.071)	39.65 (0.086)	0.813 (0.503–1.313)	0.396
T C A*	31.37 (0.067)	37.42 (0.081)	0.811 (0.495–1.328)	0.404
T G A*	219.66 (0.469)	289.99 (0.630)	0.518 (0.398–0.673)	<0.001
T G G*	103.97 (0.222)	28.37 (0.062)	4.344 (2.804–6.732)	<0.001
Block 2				
A C A*	106.97 (0.229)	71.99 (0.157)	1.615 (1.159–2.250)	0.004
A C C*	43.92 (0.094)	115.01 (0.250)	0.314 (0.215–0.457)	<0.001
A G C*	211.19 (0.451)	195.98 (0.426)	1.126 (0.868–1.460)	0.372
G G A*	22.11 (0.047)	26.99 (0.059)	0.803 (0.451–1.430)	0.455
G G C*	79.77 (0.170)	50.01 (0.109)	1.702 (1.164–2.489)	0.005

OR, odds ratio; CI, confidence interval.

Results

Table 1 shows the distribution of the genotypes and alleles of these five SNPs. The genotype distribution of each SNP did not show significant difference from the Hardy-Weinberg equilibrium values (data not shown). For total participants, the genotype and the allele distribution of rs3213282 differed significantly between the patients with laryngeal cancer and the control participants ($p = 0.018$ and 0.012, respectively). The GG genotype and G allele were more common in the patients with laryngeal cancer than in the control participants. For rs3213403 and rs1799778, although distributions of genotypes did not significantly differ between the two groups in distributions of genotypes, the allele frequencies were significantly different between the groups. However, the genotype and the allele distributions of rs1001581 and rs3810378 did not differ between the patients with laryngeal cancer and the control participants.

Figure 2 shows patterns of LD in the *XRCC1* gene, with their $|D'|$ and r^2 values. All 5 SNPs are located in 2 haplotype blocks. In the haplotype-based case–control analysis, for block one, haplotypes were established through the use of rs3213403, rs1799778, and rs3810378; for block 2, haplotypes were established through the use of rs3810378, rs1001581, and rs3213282. As shown in Table 2, CCA (OR, 5.707; 95% CI, 3.277–9.938; $p < 0.001$), TGG (OR, 4.344; 95% CI, 2.804–6.732; $p < 0.001$), ACA (OR, 1.615; 95% CI, 1.159–2.250; $p = 0.004$), and GCG (OR, 1.702; 95% CI, 1.164–2.489; $p = 0.005$) were associated with increased risk for laryngeal cancer. However, TGA (OR, 0.518; 95% CI, 0.398–0.673; $p < 0.001$) and ACC (OR, 0.314; 95% CI, 0.215–0.457; $p < 0.001$) were associated with decreased risk for laryngeal cancer.

Discussion

In the present study, we found *XRCC1* gene haplotypes were significantly associated with laryngeal cancer risk in a Chinese population.

Several studies have reported that the genes involved in DNA repair and in the maintenance of genome integrity play a crucial role in protecting against mutations that lead to cancer. SNPs have been identified in several DNA repair genes, such as *XRCC1*, *XRCC2*, and *RAD51*, and several previous studies indicated that these genes were associated with cancer risk, including laryngeal cancer. Cancer of the larynx is a head and neck cancer. It includes tumors of the nasal cavities, paranasal sinuses, oral cavity, nasopharynx, oropharynx, hypopharynx, and larynx. The contribution of polymorphisms of various DNA repair genes in development of head and neck carcinoma is controversial. Although Krupa *et al*. did not find an association of *XRCC1* polymorphism with laryngeal cancer in a Polish population (Krupa *et al.*, 2011), Mahjabeen and colleagues' finding suggests that *XRCC1* is associated with increased risk for head and neck cancer in a Pakistani population (Mahjabeen *et al.*, 2013). In a Chinese population, Yang *et al*. also found that *XRCC1* genetic polymorphism was associated with the increased risk for laryngeal cancer (Yang *et al.*, 2008).

In our study, we genotyped five SNPs in *XRCC1* in Chinese participants and assessed the association between *XRCC1* and laryngeal cancer using a haplotype-based case–control analysis. The rs3213282 significantly differed between patients with larynx cancer and control participants, indicating that the risk for laryngeal cancer is increased in participants with the G allele of rs3213282. Morris and Kaplan found that for genes with multiple susceptibilities, analysis based on haplotypes has advantages over analysis based on individual SNPs, particularly when LD between SNPs is weak (Morris and Kaplan, 2002). Consequently, in the present study, we successfully established haplotypes for the *XRCC1* gene from the different combination of the five SNPs. The frequency of CCA, TGG, ACA, and GCG was associated with increased risk for laryngeal cancer. However, both TGA and ACC were associated with decreased risk for laryngeal cancer.

The present study was limited by the relatively small sample size. This may have led to weak statistical significance and wide CIs when ORs were estimated.

In conclusion, the present results indicate that laryngeal cancer is associated with *XRCC1* gene polymorphisms. The CCA, TGG, ACA, and GCG haplotype appears to be a useful genetic marker, and the TGA and ACC haplotypes might be protective factors against laryngeal cancer in Chinese people.

Author Disclosure Statement

No competing financial interests exist.

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