

XRCC1 Polymorphisms and Risk of Papillary Thyroid Carcinoma in a Korean Sample

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Received: 2 December 2010
Accepted: 9 May 2011

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Polymorphisms of DNA repair genes, *X-ray repair cross-complementing group 1 (XRCC1)* might contribute to individual susceptibility to different types of cancers. We analyzed the relationship between *XRCC1* polymorphisms and the risk of papillary thyroid carcinoma in a Korean sample. A hospital-based case-control study was performed in 111 papillary thyroid carcinoma patients and 100 normal control subjects. *XRCC1* Arg194Trp and Arg399Gln single nucleotide polymorphisms (SNPs) were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The *XRCC1* Arg194Trp Arg/Trp genotype was significantly associated with a decreased risk of papillary thyroid carcinoma compared to that of Arg/Arg genotype (odds ratio [95% confidence intervals]; 0.550 [0.308-0.983]). There was no significant association between *XRCC1* Arg399Gln genotypes and risk of papillary thyroid carcinoma. Based on these results, the *XRCC1* Arg194Trp Arg/Trp genotype could be used as a useful molecular biomarker to predict genetic susceptibility for papillary thyroid carcinoma in Koreans.

Key Words: Polymorphisms; *XRCC1*; SNP; Papillary Thyroid Carcinoma; Susceptibility

INTRODUCTION

Papillary thyroid carcinoma is rising worldwide and accounted for 13.1% of cancers registered in Korea in 2007 (1, 2). Etiologic risk factors for papillary thyroid cancer remain largely unknown, although childhood exposure to ionizing radiation and a history of benign thyroid nodules are known to be related to thyroid cancer (1, 3). Generally, human cancer is believed to be induced by both environmental and host factors, including individual genetic susceptibility. Genetic susceptibility is related to chromosomal aberrations or genetic polymorphisms of various genes, including those involved in DNA repair (4-7). Accumulating evidence suggests that genetic polymorphisms influence the risk of environmental carcinogenesis, and that genetic susceptibility plays an important role in the development of human cancer (8-10).

DNA repair is critical for the maintenance of genome integrity and corrects DNA damage caused by either exogenous carcinogens or endogenously-produced reactive oxygen metabolites. *X-ray repair cross-complementing group 1 (XRCC1)* encodes a protein that plays an important role in the base excision repair (BER) pathway for repairing single-strand DNA breaks. *XRCC1* acts by interactive conjugation with poly (ADP-ribose), DNA polymerase beta, and DNA ligase III. Many studies have suggested that *XRCC1*-lacking cells have increased sensitivity to ionizing radiation, alkylating agents, ultra-violet light and hydrogen

peroxide (11-14). Numerous single-nucleotide polymorphisms (SNPs) of *XRCC1* have been identified, and *XRCC1* polymorphisms have been found to be associated with increased risk for various cancers including head and neck, lung, gastric, breast, esophageal, and thyroid cancers (8, 15-20).

To our knowledge, there have been only four previous reports examining *XRCC1* SNPs and the risk of thyroid carcinoma (19-22), and these studies have reported conflicting data. Furthermore, up to date, a genetic study of *XRCC1* SNPs in papillary thyroid cancer has not been performed in a Korean sample. In an effort to evaluate the genetic influence on risk of papillary thyroid carcinoma, we performed a genetic analysis of two known polymorphisms of the *XRCC1* gene (*XRCC1* Arg194Trp and *XRCC1* Arg399Gln) in a Korean sample.

MATERIALS AND METHODS

Study subjects

The hospital-based case-control study was performed from January 2004 to August 2005. The patient group consisted of 111 patients who underwent thyroidectomy and who were confirmed as having papillary thyroid carcinoma by pathologic examination. The control group consisted of 100 volunteers who visited our health promotion center for medical examinations and who were free of thyroid nodules. The control group had normal thyroid ultrasonographies and function. Subjects who had been ex-

posed to radiation or had a prior history of cancer were excluded from the control group. The ethnicity of all subjects was Korean. The patient group consisted of 28 males and 83 females with a mean age of 46.6 yr, and the control group consisted of 57 males and 43 females with mean age of 46.7 yr (Table 1). Female was significantly higher in the case group than in the control group ($P < 0.001$). Peripheral blood specimens were taken from all participants and stored at -80°C for DNA isolation.

Genotyping of *XRCC1* SNPs

DNA was extracted from peripheral blood using the Wizard™ Genomic DNA purification kit (Promega, Madison, WI, USA). Two SNPs of *XRCC1*, Arg194Trp and Arg399Gln, were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The dimorphic sites were *XRCC1* codon 194 (C→T, Arg→Trp, exon 6), and *XRCC1* codon 399 (G→A, Arg→Gln, exon 10). The primers were as follows: 5'-GCC CCG TCC CAG GTA-3' and 5'-AGC CCC AAG ACC CTT TCA CT-3' to amplify a 491 bp fragment of *XRCC1* covering the Arg194Trp polymorphism, and 5'-TTG TGC TTT CTC TGT GTC CA-3' and 5'-TCC TCC AGC CTT TTC TGA TA-3' to amplify a 615 bp fragment of *XRCC1* covering the Arg399Gln polymorphism. Polymerase chain reaction (PCR) was performed in a mixture containing 1 × PCR buffer, 50 ng genomic DNA, 3 mM MgCl₂, 200 μM dNTP mixture, 0.5 units *Taq* DNA polymerase, and 0.6 μM primer for codon 194 or 0.8 μM primer for codon 399. A thermal cycler (GeneAmp PCR system 2400, Perkin Elmer, MA, USA) was used with the following specifications: a denaturing step of 94°C for 4 min, followed by 30 cycles of 94°C for 30 sec and 68°C for 90 sec. The PCR products were verified on 3% Metaphor agarose gels (FMC Bioproducts, Rockland, ME, USA). The restriction enzyme *MspI* was used to identify *XRCC1* Arg194Trp and Arg399Gln SNPs. PCR products were digested with *MspI* restriction enzyme at 37°C overnight, and the digested products were then separated on a 3% Metaphor agarose gels (FMC Bioproducts) containing ethidium bromide. The 491 bp fragment of codon 194 always yielded a 174 bp band, acting as an indicator of complete digestion. *XRCC1* Arg194Trp genotypes Arg/Arg, Arg/Trp, and Trp/Trp generated 292 bp, 292 and 313 bp, or 313 bp DNA bands, respectively (Fig. 1). *XRCC1* 399Arg allele generated 221 bp and 374 bp bands upon *MspI* restriction, whereas *XRCC1* 399Gln allele generated only a 615 bp uncut band (Fig. 2).

Table 1. Demographic characteristics in the papillary thyroid carcinoma patients and controls

Characteristics	Case (%)	Control (%)	P value
Age (yr)	46.6 ± 15.9 (range, 17-83)	46.7 ± 10.4 (range, 30-73)	0.897
Gender			< 0.001
Male	28 (25.2)	57 (57.0)	
Female	83 (74.8)	43 (43.0)	

Statistical analysis

The chi square test was used to compare *XRCC1* polymorphism frequencies of the papillary thyroid carcinoma and normal control groups. A multivariate logistic regression model was used to obtain odds ratios (OR) adjusted for age and sex, with a 95% confidence interval (95% CI) for a genetic polymorphism and its association. All statistical data were obtained using SPSS (SPSS version 12.9, Chicago, IL, USA).

Ethics statement

The study protocol was approved by the institutional review board of College of Medicine, Hanyang University (August 26, 2009). All participants provided informed consent.

RESULTS

The genotype frequencies of the two *XRCC1* SNPs are shown in Table 2. Genotype distributions of all loci were in Hardy-Weinberg equilibrium (data not shown). The frequencies of the *XRCC1* Arg194Trp genotypes Arg/Arg, Arg/Trp, and Trp/Trp were 53.2%, 38.7%, and 8.1% in the cancer group and 37.0%, 49.0%, and 14.0% in the control group, respectively. The ORs (95% CI) for the *XRCC1* Arg194Trp genotypes Arg/Trp and Trp/Trp relative to Arg/Arg were 0.550 (0.308-0.983) and 0.403 (0.159-1.025), respectively (Table 1). The *XRCC1* Arg194Trp Arg/Trp genotype was significantly associated with a decreased risk for papillary thyroid carcinoma compared with that of the Arg/Arg genotype.

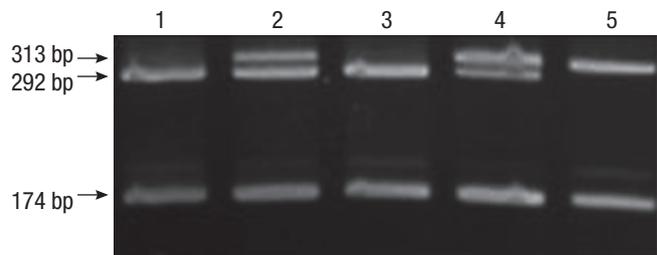


Fig. 1. *XRCC1* Arg194Trp single nucleotide polymorphism (SNP) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Arg/Arg genotype, lane 1 and 3; Arg/Trp genotype, lane 2 and 4; Trp/Trp genotype, lane 5.

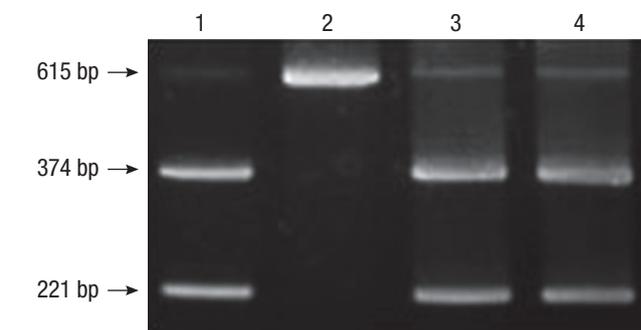


Fig. 2. *XRCC1* Arg399Gln single nucleotide polymorphism (SNP) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Arg/Arg genotype, lane 1; Arg/Gln genotype, lane 3 and 4; Gln/Gln genotype, lane 2.

Table 2. Genotype frequencies of *XRCC1* SNPs and risk estimates of papillary thyroid carcinoma patients and control subjects

Genotypes	Cancer patients (%) (n = 111)	Controls (%) (n = 100)	Total	P value	Adjusted OR (95% CI)	P value
<i>XRCC1</i> Arg194Trp				0.077		
Arg/Arg	59 (53.2)	37 (37.0)	96		1.00	
Arg/Trp	43 (38.7)	49 (49.0)	92		0.550 (0.308-0.983)	0.044
Trp/Trp	9 (8.1)	14 (14.0)	23		0.403 (0.159-1.025)	0.056
<i>XRCC1</i> Arg399Gln				0.118		
Arg/Arg	87 (78.4)	72 (72.0)	159		1.00	
Arg/Gln	17 (15.3)	19 (19.0)	36		0.740 (0.359-1.529)	0.510
Gln/Gln	7 (6.3)	9 (9.0)	16		0.644 (0.228-1.814)	0.405

*Adjusted for the other covariates in a logistic regression model for each stratum. SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

The frequencies of the *XRCC1* Arg399Gln genotypes Arg/Arg, Arg/Gln and Gln/Gln were 78.4%, 15.3%, and 6.3% in the cancer group and 72.0%, 19.0%, and 9.0% in the control group, respectively. The ORs (95% CI) for *XRCC1* Arg399Gln genotype Arg/Gln and Gln/Gln relative to Arg/Arg were 0.740 (0.359-1.529) and 0.644 (0.228-1.817), respectively (Table 1). The *XRCC1* Arg-399Gln genotypes showed a trend of decreased risk of papillary thyroid carcinoma; however, it was not significantly different from those of the other genotypes.

There were no gender- or age-associated statistically significant differences with regard to the risk of papillary thyroid carcinoma for the two *XRCC1* polymorphisms (data not shown).

DISCUSSION

DNA damage is widely induced by both normal, endogenous metabolic processes and environmental carcinogens. If such damage is not repaired, it could result in mutations and genomic instability, which could in turn cause cellular malignant transformation. DNA repair mechanisms include four pathways involving more than 80 different genes with direct roles in repairing damaged DNA (10). The mismatch repair (MMR) pathway corrects primarily mispaired nucleotides arising during DNA replication. The nucleotide excision repair (NER) pathway mainly removes damage induced by ultra-violet (UV) and chemical exposure. Double-strand breaks caused by exposure to ionizing radiation or incomplete repair of other damage are repaired via double-strand-break repair (DSBR) pathways. The BER pathway is involved in the repair of damaged bases and abasic sites induced by free radicals. Many enzymes play a role in each pathway, including MLH1, MSH2, PMS2, and MSH6 in the MMR pathway; XPC, XPD, and ERCC1 in the NER pathway; BRCA1, BRCA2, and XRCC3 in the DSBR pathway; and XRCC1 in the BER pathway (10).

The XRCC1 protein functions exclusively in DNA BER, strand-break repair, and maintenance of genetic stability. Although the functional consequences of the two non-synonymous polymorphisms in *XRCC1* that were analyzed in this study are not fully understood, the nature of the amino acid substitutions (Arg-194Trp and Arg399Gln) may be expected to cause functional

changes in the XRCC1 protein. Such functional differences could potentially affect the risk of developing cancer. However, previous studies have yielded conflicting data. For instance, the *XRCC1* 194Trp allele was found to be associated with an increased risk of certain cancers, but was also found to exert a protective effect against cancer (15, 16).

To our knowledge, *XRCC1* SNPs and the associated risks of thyroid carcinoma were examined in four previous reports that yielded conflicting data (19-22). Chiang et al. (19) reported that the *XRCC1* Arg194Trp genotype Trp/Trp has a significantly increased risk for differentiated thyroid carcinoma in Taiwanese samples (OR 1.85; 95% confidence interval, 1.11-3.07). In another study performed in the United States, Ho et al. (20) reported that the *XRCC1* Arg194Trp heterozygous Arg/Trp genotype is associated with increased risk of differentiated thyroid carcinoma, while the *XRCC1* Arg399Gln homozygous Gln/Gln genotype and variant Gln allele are associated with decreased risk. The risks of papillary thyroid carcinoma associated with *XRCC1* Arg194Trp and Arg399Gln were also examined in a Chinese sample (21). According to this study, the *XRCC1* 399Gln variant genotype was associated with increased risk (OR: 2.71, 95% CI: 1.22-6.05), but the *XRCC1* 194Trp variant genotype was not significantly correlated. In another study performed in Kazakhstan (22), thyroid cancer risk was decreased for the minor Trp allele of *XRCC1* Arg194Trp. In this study, individuals with *XRCC1* Arg194Trp Arg/Trp genotype had a 0.55-fold decreased risk of papillary thyroid carcinoma compared to that of the *XRCC1* Arg-194Trp Arg/Arg genotype.

Although it would be hard to decipher the reasons for these contradictory results, they could be ascribed to several factors that may impact the polymorphisms in different ways, including variation in carcinogen exposures in different populations and different types of DNA damage in the initiation of different cancers. Moreover, inadequate study design such as nonrandom sampling, limited sample size, and pitfalls of unknown confounding influences should also be considered.

There are some limitations in this study. First, there was a significant difference of the distribution of gender between the cancer and control groups, although we analyzed the ORs with adjustment for gender and age. The more accurate risk estimation

may be possible in gender-matched case-control samples in the future. Second, the selection bias of a hospital-based case-control study may be an issue. Specifically, the control group in our study may not have accurately represented the general population, although the genotype distribution was in Hardy-Weinberg equilibrium. Third, our study included a relatively small number of cases (n = 111) and controls (n = 100). A further study with using large, matched case-control samples is necessary to validate the genetic effects of XRCC1 polymorphisms in the Korean population.

In conclusion, this study is the first to analyze XRCC1 SNPs and their associated risk of papillary thyroid carcinoma in a Korean sample. In this study, the Arg/Trp genotype of the XRCC1 Arg194Trp polymorphism is significantly associated with a reduced risk for papillary thyroid carcinoma. The XRCC1 Arg194Trp Arg/Trp genotype could potentially be used as a molecular biomarker to predict lower risk for papillary thyroid carcinoma in Koreans.

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AUTHOR SUMMARY***XRCC1* Polymorphisms and Risk of Papillary Thyroid Carcinoma in a Korean Sample**

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