Association Studies Between XRCC1, XRCC2, XRCC3 Polymorphisms and Differentiated Thyroid Carcinoma

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Key Words
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Abstract
Background/Aims: DNA HRR pathway and BER pathway play vital roles in differentiated thyroid cancer (DTC) development, thus we supposed that polymorphisms of XRCC1, XRCC2, XRCC3 DNA repair genes are associated with thyroid cancer risk and progression. Methods: We searched the NCBI database for relevant literatures to determine eight SNPs to be included in our study (XRCC1: rs25487, rs25489, rs1799782; XRCC2: rs3218536; XRCC3: rs1799794, rs56377012, rs1799796, rs861539). Results: SNP of rs25487 was linked with a 53% decrease in DTC risk (OR: 0.47; 95%CI: 0.268-0.82; P = 0.01). For SNP of rs1799782, the homozygous TT genotype indicated a statistically significant 2-fold increased risk of DTC (OR: 2.09; 95%CI: 1.27-3.43; P < 0.001) after multivariate adjustment. For SNP of rs861539, the homozygous TT genotype suggested statistically significant 3-fold increased risk of DTC (OR: 3.02; 95%CI: 1.68-5.42; P < 0.001). No significant association between the other five SNPs and DTC risk. Besides that, female was linked with 47% increase in DTC risk (OR: 1.47; 95%CI: 1.062-2.04; P = 0.02) after multivariate adjustment. Similar results for most of the SNPs were obtained from subgroup analysis by different histological types of DTC. Haplotype analysis revealed that AGC and GGT haplotypes of XRCC1 polymorphisms were associated with DTC. Moreover, results from gene-gene interaction showed that XRCC1-rs25487, XRCC1- rs1799782 and XRCC3- rs861539 variants jointly contributed to a significantly increased risk of DTC, with the combination variant of rs1799782-CT heterozygote and rs861539-TT homozygote exhibiting a higher 3.66-fold risk of DTC (OR: 3.66; 95% CI: 1.476-9.091, P = 0.005). Conclusion: Polymorphisms of XRCC1 (rs25487, rs1799782) and XRCC3 (rs861539), may play a critical role in DTC development and progression. Furthermore, XRCC1 variant can interact with XRCC3 variant to significantly increase DTC susceptibility. Identifying these genetic risk markers could provide evidence for exploring the insight pathogenesis and develop novel therapeutic strategies for DTC.
Introduction

Thyroid cancer is a common cancer in the world and there has been a steady increase in its incidence since the past decades [1-3]. Differentiated thyroid cancer (DTC) is a popular subtype of thyroid cancer and it accounts for over 90% of all thyroid carcinomas, which includes several other subtypes such as follicular thyroid cancer (FTC) and papillary thyroid carcinoma (PTC) [4-7]. The incidences of FTC and PTC are two to four times higher in females than in males, as a consequence, thyroid cancer becomes the eighth most common cancer occurred in females [6, 8]. So far, ionizing radiation (IR) is the only established risk factor for thyroid cancer [6, 8]. However, ionizing radiation does not always trigger thyroid cancer because both environmental agents and genetic factors could exert important roles in thyroid carcinogenesis [3, 9]. Moreover, the identification of genetic factors enables us to clarify the mechanisms of thyroid carcinogenesis.

Since radiation is able to induce severe DNA lesions and is associated with thyroid carcinogenesis, people who are genetically sensitive to radiation may experience an increased risk of thyroid cancer [10]. Furthermore, DNA damage may contribute to both genetic instability and carcinogenesis, which could occur through different pathways (e.g., exogenous carcinogens, endogenously-produced reactive oxygen metabolites). DNA damage repair systems play vital roles in maintaining normal physiological functions, particularly for sustaining genome integrity in humans [11]. Since genetic variations within DNA repair genes may increase cancer risk [12, 13], DNA repair genes have been surmised as a candidate gene that is related to cancer susceptibility. Among various DNA repair pathways, the base excision repair (BER) could particularly eliminate methylation, oxidation or alteration of a single base, which further restore DNA single-strand breaks [14], while the homologous recombination repair (HRR) could restore DNA double-strand breaks [15]. Therefore, variations in the BER pathway or the HRR pathway may trigger thyroid cancer.

Previous researches have reported that X-ray repair cross-complementing group 1 (XRCC1) is involved in the BER pathway through repairing DNA single-strand breaks, while both X-ray repair cross-complementing group 2 (XRCC2) and X-ray repair cross-complementing group 3 (XRCC3) participate in the HRR pathway by repairing DNA double-strand breaks [16-19]. As suggested by earlier studies, polymorphisms of these genes are related to thyroid cancer risk [6, 20-24]. However, no conclusive result has been reported due to the conflicting findings among different studies. The inconsistency may be caused by factors related to heterogeneity including limited samples and different ethnic groups.

In this study, we carried out a hospital-based case-control study in a Chinese population to assess the possible impacts of genetic polymorphisms in XRCC1 (rs25487, rs25489, rs1799782), XRCC2 (rs3218536), and XRCC3 (rs1799794, rs56377012, rs1799796, rs861539) on the susceptibility to DTC.

Materials and Methods

Study subjects

We conducted a case-control study of 679 participants, including 276 patients with thyroid carcinoma and 403 healthy subjects as the control group. Patients were admitted in the Department of Thyroid and Breast Surgery, the Second Hospital of Hebei Medical University between May 2010 and October 2014. All patients were diagnosed and confirmed by histopathology examination of tissue section. The control group included subjects who were conducted by normal health examination in the same hospital during the same period. Thyroid physical examination was performed for all subjects in the control group by two endocrinologists or pathologist and none of them was confirmed with thyroid disease or other cancer. Baseline information (demographic characteristics, smoking status, alcohol consumptions, exposure to radiation and so on) was obtained from all subjects using a self-administered questionnaire. Subjects who smoke over 150 cigarettes in their life were considered as smokers and those who had quitted smoking for more than 1 year were non-smokers. Subjects with alcohol consumption of at least once a week for more
than 1 year were classified as drinkers, and those who no longer consume alcohol for more than 1 year were considered as non-drinkers. DNA was extracted from 5mL peripheral blood provided by every subject in the study. All subjects have signed the informed consent and the study was approved by the ethical committee of the Second Hospital of Hebei Medical University.

**SNPs selection**

We searched the NCBI database for relevant literatures and initially selected SNPs on XRCC1, XRCC2, and XRCC3 genes based on the selection criteria that the minor allele frequency (MAF) is greater than 0.05 in the Chinese population. The selected eight SNPs for genotyping were listed as: rs25487, rs25489, rs1799782 for XRCC1; rs3218536 for XRCC2; rs1799794, rs56377012, rs1799796, rs861539 for XRCC3.

**DNA extraction and SNP genotyping**

For each blood sample, whole blood of 1mL was used for genomic DNA extraction using the QIAamp DNA Blood Kit (QIAGEN Inc., Valencia, CA) according to the approved guidelines. SNP genotyping was operated with the Sequenom MassARRAY iPLEX platform (Sequenom Inc., San Diego, CA, USA). Briefly, PCR primers were designed using MassARRAY Assay Design Version 3.1 (Sequenom Inc., San Diego, CA, USA), and PCR reactions were performed in 96 well plates with iPLEX thermal cyclers. The iPLEX reaction products were desalted using water and resin treatment, and then were handled by a SpectroCHIP (Sequenom, Inc., San Diego, CA). Data was analyzed using SpectroTYPER 4.0 software (Sequenom, Inc., San Diego, CA).

**Statistical analysis**

Continuous variables were expressed in the form of mean ± standard deviation and analyzed by the Student’s t-test. The chi-squared test was performed to assess significant difference of counted data in sex, smoking status, alcohol consumption, family history of thyroid cancer and radiation exposure between the control and case group. Hardy-Weinberg equilibrium test was used to compare the actual genotypes with the expected number and it is based on the Hardy-Weinberg equilibrium theory (\( p^2 + q^2 = 1 \)). The difference in allele frequencies and genotypes between the control and case group were analyzed by the Chi-squared tests. Logistic regression with or without the adjustment of sex and alcohol consumptions was applied to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). Apart from that, haploview software was used for investigating the linkage disequilibrium and the haplotype blocks [25]. SHESIS program was implemented to construct haplotypes and evaluate the relationship between constructed haplotypes and DTC risk [26]. We also assessed multiplicative gene-gene interactions using joint effect models and all significance levels were set at \( P < 0.05 \).

**Results**

**Subject characteristics**

The baseline characteristics of 276 case subjects and 403 control subjects are showed in Table 1. There are no significant differences in age, alcohol consumptions, family history of thyroid cancer, and radiation exposure between the case and control group. Females were more frequent in the whole subjects than males (\( P < 0.05 \)). Smoking status also showed statistically differences between the case and control group (\( P < 0.05 \)). Histological classification of cases included 235 PTC cases (91.5%) and 41 FTC cases (8.5%).

**Association between SNPs and risk of DTC**

The genotype and allele frequencies of the eight studied SNPs and their associations with DTC risk are listed in Table 2. For the eight SNPs, the genotype frequencies in the control group were complied with the Hardy-Weinberg equilibrium. For SNP of rs25487, difference in genotype frequencies was statistically significant between the control and case group (\( P = 0.02 \)). The A allele of rs25487 was more frequently observed in the case group compared to the control group (27.5% vs. 27.1%, \( P = 0.004 \)). For the SNP of rs1799782, the genotype frequencies and allelic frequencies were also significantly different between the case and control group (\( P = 0.005 \) and 0.012, respectively; Table 2). We also discovered that the ge-
notype frequency distribution of rs861539 between the case and control group showed significantly difference ($P = 0.0004$). The T allele frequency of rs861539 was more frequently observed in the case group compared with the control group (21.1% vs. 30.6%, $P < 0.0001$). For SNPs of XRCC1-rs25489, XRCC2-rs3218536, XRCC3-rs1799794, XRCC3-rs56377012 and XRCC3-rs1799796, there is no significant difference in allele frequencies and genotype distributions between the control and case group (Table 2).

The association between eight SNPs of XRCC1, XRCC2, XRCC3 and thyroid cancer was presented in Table 2. For the SNP of rs25487, the homozygous AA genotype was linked with a statistically significant 53% decrease in DTC risk ($P = 0.009$) and this trend remained as statistically significant with multivariate adjustment (OR: 0.47; 95%CI: 0.268-0.82; $P = 0.01$, Table 3). For SNP of rs1799782, the homozygous TT genotype had a statistically significant 2-fold increase in DTC risk (OR: 2.09; 95%CI: 1.27-3.43; $P < 0.001$; Table 3) after multivariate adjustment. For SNP of rs861539, the homozygous TT genotype was associated with a statistically significant 3-fold increased DTC risk (OR: 3.02; 95%CI: 1.68-5.42; $P < 0.001$; Table 3) after multivariate adjustment. When the genotype was heterozygous TC, the variant T allele was associated with a 42% increase in DTC risk (OR: 1.42; 95%CI: 1.006-1.99; $P = 0.045$; Table 3) after multivariate adjustment. For the XRCC1-rs25489, XRCC2-rs3218536, XRCC3-rs1799794, XRCC3-rs56377012 and XRCC3-rs1799796 polymorphisms, no significant association was presented. Moreover, females were associated with 47% increase in DTC risk compared to males (OR: 1.47; 95%CI: 1.062-2.04; $P = 0.02$; Table 3) after multivariate adjustment.

Subgroup analysis by different histological types of DTC was further performed on the association between SNPs and DTC risk (PTC: 91.5%; FTC: 8.5%). Similar results were obtained for most of the SNPs genotypes in papillary carcinoma cases, while no significant association existed between SNP of rs25487, rs861539 and follicular carcinoma risk (Table 4).

**Results from Haplotype analysis**

The frequencies of the six frequent haplotypes in case group were specifically different from control group ($P = 0.003$; Table 5). Six common haplotypes (frequency > 0.03 in either the control or case group has been selected) accounted for most of the haplotypes in the case and control group. For those commonly observed haplotypes, the AGC haplotype was associated with a 35% reduction in DTC risk (OR: 0.65; 95%CI: 0.505-0.86, $P = 0.002$), while the GGT haplotypes were linked with significantly increase in DTC risk (OR: 1.8; 95%CI: 1.316-2.543; $P = 0.0002$). The other four common haplotypes, including GGC, GAC, AGT and AAC, were not significantly associated with DTC risk.

### Table 1. Baseline demographics for controls (N= 403) and thyroid cancer (N= 276) groups. *Two-sided χ test*

| Variables                      | Case (n=276) | Control (n=403) | $P^*$ |
|--------------------------------|-------------|-----------------|------|
| Age(year)                      | 46.18±14.345| 44.95±15.38     | 0.2352|
| Sex                            |             |                 | 0.0303|
| Male, n (%)                    | 93(33.8)    | 170(42.2)       |      |
| Female, n (%)                  | 183(66.2)   | 233(57.8)       |      |
| Smoking status                 |             |                 | 0.008 |
| Non-smoker, n (%)              | 184(66.6)   | 276(68.6)       |      |
| Smoker, n (%)                  | 92(33.4)    | 127(31.4)       |      |
| Alcohol consumption            |             |                 | 0.1786|
| No, n (%)                      | 187(67.7)   | 232(57.5)       |      |
| Yes, n (%)                     | 89(32.3)    | 171(42.5)       |      |
| Family history of thyroid cancer|            |                 | 0.0899|
| No, n (%)                      | 199(72.2)   | 309(76.7)       |      |
| Yes, n (%)                     | 77(27.8)    | 94(23.3)        |      |
| Radiation exposure             |             |                 | 0.4841|
| No, n (%)                      | 270(98)     | 390(96.8)       |      |
| Yes, n (%)                     | 6(2)        | 13(3.2)         |      |
| Histological type              |             |                 |      |
| Papillary carcinoma, n (%)     | 235         |                 |      |
| Follicular carcinoma, n (%)    | 41          |                 |      |
Gene-gene interaction

We further assessed how the interaction in any joint of the XRCC1 (rs25487, rs1799782) and XRCC3 (rs861539) genotypes affects DTC risk. As suggested by the combination effect of XRCC1 (rs25487) and XRCC3 (rs861539) which combined wild-type homozygote XRCC1 (GG)/XRCC3 (CC) as the reference, we found that the combined rs25487-GG and rs861539-CC contributed to a 3.08-fold increased DTC risk (OR: 3.08; 95% CI: 1.32-7.180, \( P = 0.008 \); Table 6). When we combined the XRCC1 (rs1799782) and XRCC3 (rs861539), with the wide type homozygote as the reference, the combination of rs1799782-CT variant heterozygote and rs861539-CC variant homozygote showed a significantly 3.66-fold DTC risk (OR: 3.66; 95% CI: 1.476-9.091, \( P = 0.005 \); Table 6). The combination of rs1799782-CC and rs861539-TT was also associated with an increased DTC risk (OR: 3.13; 95% CI: 1.343-7.277, \( P = 0.008 \); Table 6). The mutated homozygote of rs1799782-TT along with the heterozygote of rs861539-CT exhibited a 2.5-fold increased DTC risk (OR: 2.54; 95% CI: 1.057-6.106, \( P = 0.039 \); Table 6).

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**Table 2.** Allele and genotype frequencies of selected SNPs in case and control and their associations with DTC risk. *Two-sided \( \chi^2 \) test for distribution of allelic frequencies (df, 1). *Two-sided \( \chi^2 \) test for distribution of genotypic frequencies (df, 2). Comparing by multivariate logistic regression model.

| Polymorphisms | Control N (%) | Case N (%) | \( \chi^2 \) df, 1 | OR (95% CI) | \( P \) |
|---------------|---------------|------------|----------------|--------------|--------|
| XRCC1 rs25487 |               |            | 0.02           |              |        |
| GG           | 174 (43.7)    | 146 (53)   | 1.0 (Reference) |              |        |
| AG           | 173 (43)      | 108 (39)   | 0.75 (0.54-1.04) | 0.0983       |        |
| AA           | 54 (13.3)     | 22 (8)     | 0.49 (0.28-0.84) | 0.0098       |        |
| AG+AA        | 227           | 130        | 0.69 (0.50-0.93) | 0.0191       |        |
| A allele frequency | 0.271        | 0.275      | 0.0041         | 0.27         |        |
| rs25489      |               |            |                |              |        |
| GG           | 298 (74)      | 218 (79)   | 1.0 (Reference) |              |        |
| GA           | 97 (24)       | 52 (19)    | 0.73 (0.50-1.07) | 0.1296       |        |
| AA           | 8 (2)         | 6 (2)      | 1.02 (0.35-2.99) | 1            |        |
| AA+GA        | 105           | 58         | 0.75 (0.52-1.08) | 0.1436       |        |
| A allele frequency | 0.140        | 0.116      | 0.192           | 0.005        |        |
| rs1799782    |               |            |                |              |        |
| CC           | 202 (50)      | 124 (45)   | 1.0 (Reference) |              |        |
| CT           | 173 (43)      | 112 (41)   | 1.118 (0.80-1.54) | 0.5077       |        |
| TT           | 28 (7)        | 40 (14)    | 2.327 (1.36-3.96) | 0.0018       |        |
| T allele frequency | 0.284        | 0.348      | 0.012           | 0.005        |        |
| XRCC2 rs3218536 |             |            |                |              |        |
| GG           | 324 (80)      | 218 (79)   | 1.0 (Reference) |              |        |
| GA           | 76 (19)       | 55 (20)    | 1.076 (0.73-1.58) | 0.7663       |        |
| AA           | 3 (1)         | 3 (1)      | 1.480 (0.29-7.43) | 0.6893       |        |
| AA+GA        | 79            | 58         | 1.091 (0.74-1.59) | 0.6972       |        |
| A allele frequency | 0.102        | 0.111      | 0.605           | 0.29         |        |
| XRCC3 rs1799794 |             |            |                |              |        |
| AA           | 202 (50)      | 116 (42)   | 1.0 (Reference) |              |        |
| AG           | 161 (40)      | 127 (46)   | 1.374 (0.99-1.90) | 0.057        |        |
| GG           | 40 (10)       | 33 (12)    | 1.437 (0.85-2.40) | 0.1824       |        |
| AG+GG        | 201           | 160        | 1.386 (1.01-1.88) | 0.0418       |        |
| G allele frequency | 0.299        | 0.350      | 0.0493          | 0.51         |        |
| rs6377012    |               |            |                |              |        |
| AA           | 351 (87)      | 238 (86)   | 1.0 (Reference) |              |        |
| AG           | 47 (12)       | 30 (11)    | 0.94 (0.57-1.53) | 0.9018       |        |
| GG           | 5 (1)         | 8 (3)      | 2.360 (0.76-7.30) | 0.1562       |        |
| AG+GG        | 52            | 38         | 1.078 (0.68-1.69) | 0.8179       |        |
| G allele frequency | 0.071        | 0.093      | 0.388           | 0.51         |        |
| rs1799796    |               |            |                |              |        |
| AA           | 213 (53)      | 136 (50)   | 1.0 (Reference) |              |        |
| AG           | 159 (40)      | 113 (40)   | 1.113 (0.80-1.53) | 0.5636       |        |
| GG           | 3 (1)         | 27 (10)    | 1.364 (0.77-2.38) | 0.3117       |        |
| AG+GG        | 190           | 140        | 1.154 (0.84-1.56) | 0.39         |        |
| G allele frequency | 0.274        | 0.303      | 0.2561          | 0.0004       |        |
| rs861539     |               |            |                |              |        |
| CC           | 255 (63)      | 143 (52)   | 1.0 (Reference) |              |        |
| TC           | 126 (32)      | 97 (35)    | 1.373 (0.98-1.91) | 0.0713       |        |
| TT           | 22 (5)        | 36 (13)    | 2.918 (1.65-5.15) | 0.0003       |        |
| CT+TT        | 148           | 133        | 1.602 (1.17-2.10) | 0.0033       |        |
| T allele frequency | 0.211        | 0.306      | <0.01           | <0.01        |        |
Radiation is a significant factor for thyroid cancer due to its impacts on DNA lesions and increasing evidence has unveiled that radiation triggers different DNA repair capacities in humans. Thus, polymorphisms in DNA repair genes are likely to influence thyroid cancer risk [27-31]. As a result, a hospital-based case-control study was carried out to assess the

### Table 3. Multivariate logistic regression model

| Variables                               | OR    | 95% CI       | P   |
|-----------------------------------------|-------|--------------|-----|
| Sex(female vs. male)                    | 1.47  | 1.06-2.04    | 0.02|
| Smoking state(smoker vs. non-smoker)    | 1.13  | 0.80-1.58    | 0.49|
| rs25487(AG vs.GG)                       | 0.75  | 0.53-1.04    | 0.09|
| rs25487(AA vs.GG)                       | 0.47  | 0.268-0.82   | 0.01|
| rs1799782(CT vs.CC)                     | 1.21  | 0.79-1.83    | 0.37|
| rs1799782(TT vs.CC)                     | 2.09  | 1.27-3.43    | 0.00|
| rs861539(TC vs.CC)                      | 1.42  | 1.006-1.99   | 0.05|
| rs861539(TT vs.CC)                      | 3.02  | 1.68-5.42    | <0.001|

### Table 4. Distribution of genotypes and odds ratios (OR) for papillary carcinoma or follicular carcinoma and controls. OR and P value were from multivariate logistic regression model with adjustment for sex and alcohol consumption

| XRCC1    | Control (n=403) | Papillary carcinoma (n=235) | Follicular carcinoma (n=41) |
|----------|-----------------|----------------------------|----------------------------|
|          | n  | n   | OR(95%CI)  | P    | n  | n   | OR(95%CI)  | P    |
| rs25487  |     |     |            |      |     |     |            |      |
| GG       | 176 | 125 | 1.0(Reference) | 21   |     |     |            |      |
| AG       | 173 | 91  | 0.74(0.52-1.04) | 0.0992 | 17 | 0.8236(0.42-1.61) | 0.611 |
| AA       | 54  | 19  | 0.57(0.33-0.99) | 0.0488 | 3  | 0.4656(0.13-1.62) | 0.305 |
| rs1799782|     |     |            |      |     |     |            |      |
| CC       | 202 | 154 | 1.0(Reference) | 27   |     |     |            |      |
| CT       | 169 | 46  | 0.357(0.24-0.52) | <0.001 | 6  | 0.265(0.10-0.65) | 0.002 |
| TT       | 32  | 35  | 1.435(0.85-2.42) | 0.182 | 8  | 3.434(1.39-8.45) | 0.010 |
| XRCC3    |     |     |            |      |     |     |            |      |
| rs861539 |     |     |            |      |     |     |            |      |
| CC       | 255 | 123 | 1.0(Reference) | 21   |     |     |            |      |
| TC       | 126 | 80  | 1.04(0.72-1.49) | 0.854 | 17 | 1.63(0.8348-3.215) | 0.154 |
| TT       | 22  | 32  | 3.016(1.682-5.40) | 0.000 | 3  | 1.65(0.4576-5.992) | 0.434 |

### Table 5. Haplotype frequencies of XRCC1 polymorphisms with DTC risk. Overall P for distribution of haplotype frequencies between control and case groups was calculated by Two-sided χ² test. Haplotype-specific P value and OR were from SHESIS program

| Haplotypes                          | control(freq) | case(freq) | P     | OR(95%CI)     |
|-------------------------------------|---------------|------------|-------|---------------|
| XRCC1-rs25487-rs25489-rs1799782     |               |            |       |               |
| GGC                                 | 370(0.459)    | 267(0.483) | 0.325958 | 1.117(0.896-1.393) |
| AGC                                 | 211(0.262)    | 105(0.189) | 0.00207 | 0.659(0.505-0.860) |
| GGT                                 | 76(0.094)     | 88(0.159)  | 0.000282 | 1.830(1.316-2.542) |
| GAC                                 | 62(0.077)     | 33(0.060)  | 0.243554 | 0.771(0.498-1.195) |
| AGT                                 | 35(0.044)     | 29(0.053)  | 0.410797 | 1.235(0.747-2.041) |
| AAC                                 | 26(0.032)     | 10(0.018)  | 0.123825 | 0.564(0.269-1.181) |

Overall P = 0.003

**Discussion**

Radiation is a significant factor for thyroid cancer due to its impacts on DNA lesions and increasing evidence has unveiled that radiation triggers different DNA repair capacities in humans. Thus, polymorphisms in DNA repair genes are likely to influence thyroid cancer risk [27-31]. As a result, a hospital-based case-control study was carried out to assess the
relationship between eight SNPs of three DNA repair genes and DTC susceptibility in a Chinese population. Our results showed that allele distributions and genotype of XRCC1-rs25489, XRCC2-rs3218536, XRCC3-rs1799794, XRCC3-rs56377012 and XRCC3-rs1799796 were not associated with DTC risk. However, we discovered that other polymorphisms XRCC1-rs1799782 and XRCC3-rs861539 were associated with an increased risk of DTC, while XRCC1-rs25487 was related to a decreased risk of DTC. Subgroup analysis by different histological types of DTC also showed similar results for most of the SNPs in papillary carcinoma cases. Besides that, haplotype analysis revealed that the AGC and GGT haplotypes of XRCC1 polymorphisms (rs25487, rs25489, rs1799782) were associated with risk of DTC. Our results of gene-gene interaction showed that the four combinations (rs25487-GG with rs861539-TT, rs1799782-CT with rs861539-TT, rs1799782-TT with rs861539-TC, rs1799782-CC with rs861539-TT) were associated with an increase in DTC risk. It has been suggested that mutations in XRCC1, XRCC2 and XRCC3 genes may contribute to decreased or lost DNA repair capacity. Furthermore, SNP of XRCC1, XRCC2 and XRCC3 may affect the risk of several types of cancer, including thyroid carcinoma, glioma and breast cancer [21, 28, 32-34].

**Table 6.** The joint effects of XRCC1-rs25487, XRCC1-rs1799782 and XRCC3-rs861539. OR, 95% CI, and P values were calculated by a multivariate logistic regression model

| XRCC1    | XRCC3               | Control | Case          |          |          | P        |
|----------|---------------------|---------|---------------|----------|----------|----------|
| rs25487  | rs861539            | n       | n             | OR(95%CI)|          |          |
| GG       | CC                  | 111     | 76            | 1.0 (Reference) |          |          |
| GG       | TC                  | 56      | 51            | 1.33(0.82-2.147) | 0.2714  |          |
| GG       | TT                  | 9       | 19            | 3.083(1.32-7.180) | 0.0081  |          |
| AG       | CC                  | 109     | 56            | 0.7504(0.4858-1.159) | 0.2251  |          |
| AG       | TC                  | 55      | 38            | 1.009(0.6083-1.674) | 1        |          |
| AG       | TT                  | 9       | 14            | 2.272(0.9358-5.516) | 0.0759  |          |
| AA       | CC                  | 34      | 11            | 0.4725(0.2254-0.9904) | 0.0585  |          |
| AA       | TC                  | 17      | 8             | 0.6873(0.2823-1.673) | 0.5153  |          |
| AA       | TT                  | 3       | 3             | 1.461(0.2870-7.433) | 0.6902  |          |
| rs1799782| rs861539            |         |               |          |          |          |
| CC       | CC                  | 127     | 65            | 1.0 (Reference) |          |          |
| CC       | TC                  | 64      | 43            | 1.313(0.8053-2.140) | 0.3153  |          |
| CT       | CC                  | 10      | 16            | 3.126(1.343-7.277) | 0.0088  |          |
| CT       | TT                  | 107     | 60            | 1.096(0.7090-1.693) | 0.7392  |          |
| CT       | TC                  | 54      | 41            | 1.483(0.8959-2.456) | 0.1527  |          |
| CT       | TT                  | 8       | 15            | 3.663(1.476-9.091) | 0.0053  |          |
| TT       | CC                  | 20      | 19            | 1.856(0.9258-3.721) | 0.0999  |          |
| TT       | TT                  | 10      | 13            | 2.540(1.057-6.106) | 0.0398  |          |
| TT       | TT                  | 2       | 5             | 4.885(0.9220-25.88) | 0.0536  |          |

**XRCC1** protein is involved in the BER pathway to repair DNA single-strand breaks combined with three DNA repair enzymes (DNA ligase III, DNA polymerase and PARP) [22, 24, 35, 36]. Recently, evidence has attempted to reveal the underlying biological meaning of common XRCC1 polymorphisms (rs25487, rs25489, rs1799782), but the results of genetic studies are still inconclusive [22, 24]. As suggested by earlier studies, XRCC1-rs1799782 was discovered to be linked with DTC risk in Chinese population [37, 38]. However, other studies suggested that XRCC1-rs1799782 polymorphism might have no effect on DTC susceptibility for different ethnicities [39]. Our data showed the homozygous TT genotype of rs1799782 had a significantly 2-fold increased risk of DTC after multivariate adjustment. A meta-analysis performed by Hu et al. showed that XRCC1-rs25487 polymorphism was not associated with DTC risk, while a reduced risk was found in Caucasian [22]. For XRCC1-rs25489 polymorphism, our data indicated that no significant association with DTC was observed, which was consistent with those reported by Chiang et al. in a Taiwan population [40]. However, a positive association has been found between SNP of rs25489 and DTC risk among Caucasian population [22]. Further studies with large sample size were recommended to verify our results.

The **XRCC2** gene is involved in the HR repair pathway. In line with our results, several studies provided evidence that there was no significant association between the XRCC2-rs3218536 polymorphism and thyroid cancer [20, 21]. Similarly, meta-analyses showed that
there was no significant association between SNP of rs3218536 and risk of breast cancer under any genetic models [6, 41]. However, the rs3218536 polymorphism could be a genetic adjuster for ovarian and colorectal cancer patients [42, 43].

XRCC3, a member of Rad51 family, is also involved in the HR pathway by repairing double-strand breaks, which further enhances the stability of chromosome. Previous studies discovered that the XRCC3-rs861539 polymorphism could affect DNA repair capacity and may be connected with cancer risk [6, 20, 23]. Several studies have investigated the association between XRCC3-rs861539 polymorphism and thyroid cancer susceptibility. Some studies with positive results are in line with our conclusions [6, 20, 23, 44], while others obtained negative results [45, 46]. This case-control study showed that XRCC3-rs1799794, XRCC3-rs56377012 and XRCC3-rs1799796 were not associated with DTC risk. Similarly, a previous study performed by Yuan et al. found that SNP of rs1799794, rs56377012 and rs1799796 polymorphisms were not related to PTC risk [6]. However, the association between 4 SNPs of XRCC3 (rs861539, rs1799794, rs56377012 and rs1799796) and DTC risk remains to be clarified by future studies.

Several potential limitations need to be considered in the current study. First of all, studies should incorporate a larger sample size with various ethnics to further confirm the association between SNPs of XRCC1, XRCC2, XRCC3 and DTC susceptibility. Secondly, few well-known risk factors for DTC has been discovered, therefore other genetic and environmental factors should be assessed. Thirdly, subjects in this case-control study came from one hospital and this may cause selection bias that could have substantial impact on the overall conclusions. As a result, large-scale studies that adjusting for a wide range of factors should be recommended to validate our findings.

In conclusion, our results indicate that eight polymorphisms of XRCC1, XRCC1, and XRCC3 gene might be related to individual susceptibility to DTC in Chinese population. Our study also suggests that XRCC1 (rs25487, rs25489, rs1799782), XRCC2 (rs3218536), and XRCC3 (rs1799794, rs56377012, rs1799796, rs861539) are novel genetic risk markers for DTC. Nevertheless, these conclusions should be confirmed by future genetic researches with large samples and various ethnics.

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Disclosure Statement

The authors declare no commercial or financial conflict of interest.

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