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# Association of Promoter Polymorphisms in *Xrcc2* Gene Involved in DNA Double Strand Break Repair and Increased Susceptibility to Thyroid Cancer Risk in Pakistani Population

**Sarwar R, Bashir K, Saeed S, Mahjabeen I and Kayani MA***

*Cancer Genetics and Epigenetics Lab, Department of Biosciences COMSATS Institute of Information Technology, Islamabad, Pakistan*

***Corresponding author:** Mahmood Akhtar Kayani, Cancer Genetics & Epigenetics Research Group, Department of Biosciences, COMSATS Institute of Information Technology, Park Road Chak shahzad Islamabad, Pakistan, Tel: +92-321-5357981; E-mail: mkayani@comsats.edu.pk

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## Abstract

**Introduction:** The incidence of thyroid cancer (TC) has rapidly increased globally in recent decades. It is the most frequent endocrine malignancy which is fifth most common cancer in females. Double strand break repair (*DSBR*) pathway gene, X-Ray Repair Complementing Defective Repair in Chinese Hamster Cells 2 (*XRCC2*) has high rate of polymorphisms and may cause individual's susceptibility towards carcinogenesis including thyroid cancer.

**Objective:** Main objective of present study is to find the association of hotspot promotor polymorphisms in *XRCC2* gene with thyroid cancer risk.

**Methods:** In this study, we performed genetic association studies in 856 individuals (456 cases and 400 controls) for three promoter region SNPs of *XRCC2* gene i.e., G4234C (rs3218384), G4088T (rs3218373) and G3063A (rs2040639). Genotyping was performed by amplification refractory mutation system (ARMS-PCR) followed by direct sequencing.

**Results:** We found association of G4234C with thyroid cancer risk in stage I and II ($p>0.0004$) cancer patients while no association was observed with other parameters. Significant increased risk of developing thyroid cancer risk was observed in patients for G4088T with variant heterozygote T/G (OR=1.65, 95% CI=1.20-2.24; $p<0.001$) and polymorphic homozygote G/G (OR=1.66, 95% CI=1.16-2.36; $p=0.005$) compared with healthy controls. For G3063A polymorphism, a significant difference in genotypes distributions was observed for heterozygous variant G/A (OR=2.11; 95% CI=1.52-2.94; $p<0.0001$) and A/A variant genotype (OR=2.02; 95% CI=1.37-2.97; $p<0.0003$). When stratified for different parameters, significant increased risk was observed in female patients, patients with age ≥ 42 years, smoking and stage I and II patients for G4088T and G3063A in comparison to controls.

**Conclusion:** Present study concluded that G4234C, G4088T and G3063A SNPs in *XRCC2* gene may modify the risk of thyroid cancer development.

**Keywords:** Promotor polymorphism; *XRCC2*; DNA repair; Thyroid cancer; Smoking; ARMS-PCR; Carcinogenesis

## Introduction

Thyroid cancer is the most prevalent endocrine malignancy with increasing incidence rate in recent years [1]. Females are more likely to have thyroid cancer at a ratio of 3:1 [2]. The main risk factors of thyroid cancer are genetic factor, environmental factors and exposure to ionization radiations at childhood [3]. Exposure to ionization radiations cause single strand and double strand breaks and can produce chromosomal damage and release of reactive oxygen species that causes genomic instability [4,5]. In human there are many pathways to repair this DNA damage, out of which double strand break repair (DSBR) pathway is an important and preferred pathway to repair such lesion [6]. This pathway has two types, non-homologous end joining (NHEJ) and homologous recombinant repair (HRR) pathway. HRR is an error prone pathway which is template specific and considered to play a significant role in the repair of DNA double strand damage produced by ionization radiations [7]. HR encompasses many genes, but major role is performed by RAD51 and RAD51-like genes such as *XRCC2* and *XRCC3* [8].

X-ray repair complementing defective repair in Chinese hamster cells 2 (*XRCC2*) is very important gene which plays a basic role in DNA repair in conjunction with Rad51 paralogs (Rad51B, Rad51C, Rad51D and *XRCC3*) [9]. *XRCC2* protein is a RAD51-related protein, essential for efficient homologous recombinant repair of DNA double strand breaks [10-12]. It is thus essential for maintenance of chromosome stability, forming part of a nucleoprotein filament acting as a cofactor for the RAD51 strand invasion and exchange activities [13-15].

So far, only limited studies have examined the association between DNA- repair gene *XRCC2* polymorphisms and thyroid cancer [16-20]. However the selected hot spot promoter polymorphisms in this study have not been investigated in thyroid cancer. Therefore in this study, we have performed a case-control study to investigate three important promoter gene polymorphisms (SNPs) as G4234C (rs3218384),

G4088T (rs3218373) and G3063A (rs2040639) in the *XRCC2* gene in thyroid cancer patients and age and sex matched healthy controls in Pakistani population. Additionally we also determined the association of these selected polymorphisms with different risk factors such as age, gender and smoking in order to elucidate the gene-environment interaction in carcinogenesis of thyroid gland.

## Materials and Methods

### Patient recruitment and ethical issues

This case-control study included total 856 individuals (456 thyroid cancer patients and 400 cancer free subjects as a control group), all of Pakistani ethnicity. Blood from cancer patients was collected from Nuclear medicine department of NORI (Nuclear oncology radiation institute) Islamabad and Jinnah Hospital, Lahore from 2012 to 2014. Control subjects were collected from NORI and PIMS (Pakistan institute of medicine Sciences), Islamabad from the healthy attendants of patients. These control subjects were recruited after diagnostic exclusion of any cancer or cancer-related diseases and were frequency matched to the thyroid cancer patients with respect to age and gender. Patients suffering from goiter and other benign thyroid diseases were excluded. Demographic data, age at diagnosis, tumor type, grade, type of treatment were recorded with signed written consent of patients. Sample size was estimated by WHO sample size calculator (**http://www.who.int/chp/steps/resources/sampling/en/**). Our study was approved by ethical review boards of COMSATS institution of information and technology Islamabad and both hospitals, from which patients and controls were recruited.

### DNA extraction

Approximately 3-4ml blood sample was collected in vacutainer tubes from all enrolled subjects in this study. DNA was extracted from whole blood by Phenol chloroform method with some modifications [21]. The extracted DNA was quantified by 2% ethidium bromide gels and spectrophotometrically using Nano Drop (Thermoscientifiv, USA) and stored at -20°C until used.

### SNPs selection

Three polymorphisms in DNA repair gene *XRCC2* were selected from dbSNP database. These reported polymorphisms were present in the promoter region i.e., *XRCC2* G4234C (rs3218384), G4088T (rs3218373) and G3063A (rs2040639). The global minor allele frequency for all of these are greater than 0.1.

### Genotyping and DNA sequencing

Genotyping was performed by Allele-specific polymerase chain reaction (ARMS-PCR). Primers for PCR amplification were made by WASP (web based allele specific primer designing tool) [22]. Primers specific for each polymorphism are given (Table 1).

| Gene/Allele | Sense primer | Antisense primer | Amplicon size (bp) | Annealing temp (°C) |
|---|---|---|---|---|
| *XRCC2* | | | | |
| *G4234C* **(rs3218384)** | ACTCTACGGC CAGTCAAATG | GCCTGCTTGTG CAATACAATA | 234 | 58 |
| | ACTCTACGGC CAGTCAAATC | | 234 | |
| *G4088T* **(rs3218373)** | TAAAGCCCAT TTGTTTCAAG | AAACGCTAGGA AAGAGCATA | 137 | 54 |
| | TAAAGCCCAT TTGTTTCAAT | | 137 | |
| *G3063A* **(rs2040639)** | GTTGTAAACC AGCCTAGGAA C | GCACACCTGTT CGTGTGACT | 252 | 60 |
| | | GCACACCTGTT CGTGTGACC | 252 | |
| **Beta-Actin Internal control)** | CGAGAAGATG ACCCAGGTGA | TACATGGCTGG GGTGTTGAA | 496 | 55 |

**Table 1:** Oligos for selected *XRCC2* gene polymorphisms with optimized annealing temperature and expected product size.

Last base pair in oligo specific to change in respective polymorphism is given in bold letter whereas underlined base is deliberate mismatch inserted in primer sequence to increase specificity.

In order to optimize PCR conditions, we varied annealing temperature, concentration of primers and $MgCl_2$ concentration. PCR reaction was carried out in a reaction volume of 10 µl containing 50-100 ng genomic DNA, 100 µM of each primers and Solis BioDyne master mix. The thermal cycling protocol used was: 94°C for 30 sec, optimized annealing temperature for 45 sec, 72°C for 1 min and final extension for 7 minutes. The PCR products were visualized on a 2% agarose gel electrophoresis (100 V, 300 A for 45 min). To identify products by presence or absence of bands specific for wild or mutant primers in each well was thus evaluated using UV trans illuminator (Gel Doc BioRad, USA). Internal control β-Actin was used in each reaction as a positive control for PCR. Genotyping results were further confirmed by sequencing of samples with homozygous wild, polymorphic homozygous and heterozygous pattern for respective polymorphism.

### Statistical analyses

For each polymorphism, demographical characteristics were compared between cases and controls using $\chi^2$ test. Odd ratios (ORs) and 95% confidence intervals (CIs) after adjusting for gender, age, ionization radiation and family history of cancer were calculated. P value <0.05 was considered to be statistical significant. Statistical analysis was performed using GraphPad prism software v 6.0.

## Results

All demographic data collected for both thyroid cancer patients (456) and healthy controls (400) are presented (Table 2).

| Variables | Patients (N=456) | Controls (N=400) | OR(95%CI) | p-value |
|---|---|---|---|---|
| **1. Age (years)** | | | | |
| Median (Range) | 42.5(15-75) | 42(20-65) | | |

| | | | | |
|---|---|---|---|---|
| **Gender** | | | | |
| Males | 107(23.4%) | 70(17.5%) | 1.44(1.03-2.02) | 0.03* |
| Females | 349(76.5%) | 330(82.5%) | | |
| Age | | | | |
| ≤ 42 | 208(45%) | 179(44.75%) | 1.03(0.79-1.35) | 0.79 |
| >42 | 248(55%) | 221(55.25%) | | |
| **2. Family History of Cancer** | | | | |
| Yes | 34(8%) | 9(2.25%) | 3.5(1.65-7.39) | 0.001*** |
| No | 422(92%) | 391(97.75%) | | |
| **Smoking History (cigarette, paan, bidi, betel quid, moist sniff )** | | | | |
| Smokers | 109(24%) | 119(30%) | 0.55(0.41-0.72) | <0.0001*** |
| Non-Smokers | 347(76%) | 281(70%) | | |
| **Type of thyroid cancer** | | | | |
| Papillary | 355(78%) | - | | 0.25 |
| Follicular | 82(18%) | - | | |
| Medullary | 16(3%) | - | | |
| Anaplastic | 5(1%) | - | | |
| **Grade of cancer** | | | | |
| Grade I | 211(46%) | - | | 0.08 |
| Grade II | 162(36%) | - | | |
| Grade III | 72(15%) | - | | |
| Grade IV | 11(3%) | - | | |
| **Type of treatment** | | | | |
| Radioactive iodine | 398(87%) | - | | 0.34 |
| **Chemotherapy** | 31(7%) | - | | |
| External beam radiation | 27(6%) | - | | |

**Table 2:** Frequency distribution analysis of selected demographic and risk factors in thyroid cancer cases and controls.

OR - Odds ratio; CI - Confidence interval;* p-value ≤ 0.05, **p-value ≤ 0.01 and ***p-value ≤ 0.001 by $\chi^2$ -test.

According to demographic data, frequencies of gender (OR=1.44; 95% CI=1.03-2.02; p=0.03), family history (OR=3.5; 95% CI=1.69-7.39; p=0.001) and smoking status (OR=1.55; 95% CI=1.41-1.72; p<0.0001) were found significantly higher in patients compared to healthy controls. There was no statistical difference between histopathological type of cancer, cancer staging and treatment type (p=0.25; 0.08 and 0.34 respectively). The genetic distributions of three SNPs (G4234C (rs3218384), G4088T (rs3218373) and G3063A (rs2040639) in promoter region (5'UTR) of *XRCC2* gene was calculated in total thyroid cancer patients and healthy controls. Genotyping was performed using Allele refractory mutation system ARMS-PCR and sequencing analysis as shown in (Figures 1,2 and 3).

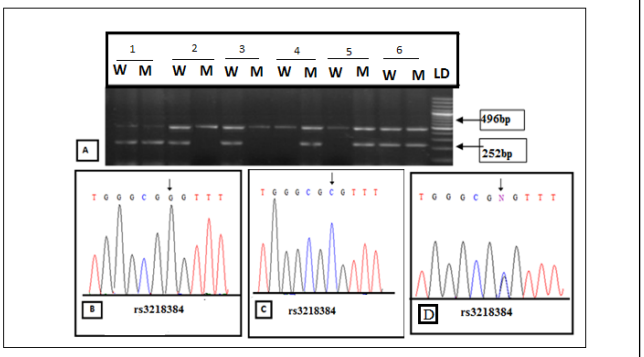


**Figure 1:** (A) ARMS-PCR genotyping of G4234C (rs3218384) in thyroid cancer patients in 2% agarose gel electrophoresis. Sample 1 and 6 showing heterozygous genotype (G/C), sample 2 and 3 showing homozygous wild genotype (G/G), samples 4 and 5 representing homozygous mutant genotype (C/C) (product size 252bp). Amplification of internal control (β-Actin) in all samples was observed (product size 496bp). Sequencing electropherogram of respective SNP (B) showing homozygous wild type (G/G), (C) showing homozygous mutant genotype (C/C), (D) showing heterozygous genotype (G/C) in thyroid cancer patients. W stands for wild type, M stands for mutant and LD stands for ladder.

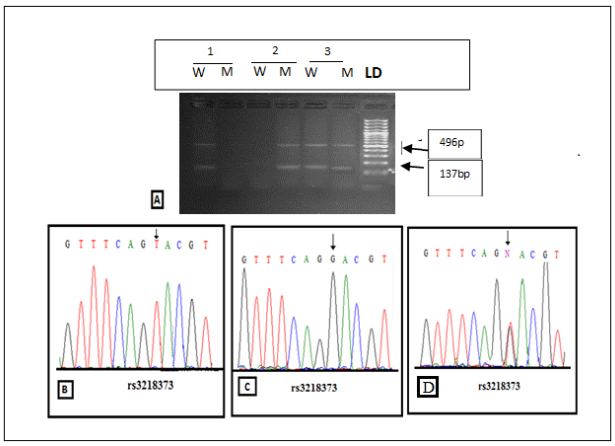


**Figure 2:** (A) ARMS-PCR genotyping of G4088T (rs3218373) in thyroid cancer patients in 2% agarose gel electrophoresis. Sample 1 showing homozygous wild genotype (T/T), sample 2 showing homozygous mutant genotype (G/G) and sample 3 showing heterozygous genotype (T/G) (product size 137bp). Amplification of internal control (β-Actin) in all samples was observed (product size 496bp). Sequencing electropherogram of respective SNP (B) showing homozygous wild type (T/T), (C) showing homozygous mutant genotype (G/G), (D) showing heterozygous genotype (T/G) in thyroid cancer patients. W stands for wild type, M stands for mutant and LD stands for 100bp ladder (Fermentas).

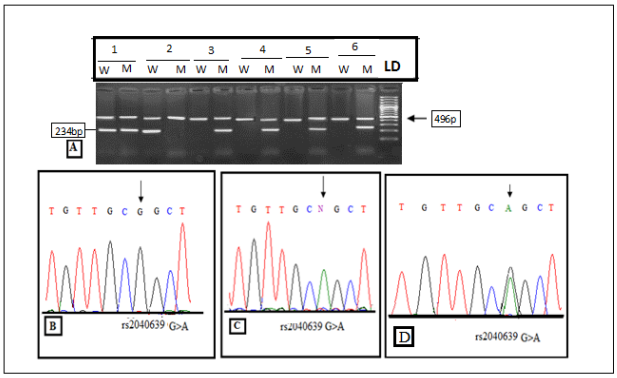


**Figure 3:** (A) ARMS-PCR genotyping of G3063A (rs2040639) in thyroid cancer patients in 2% agarose gel electrophoresis. Sample 1 showing heterozygous genotype (G/A), sample 2 showing homozygous wild genotype (G/G) and samples 3, 4, 5 and 6 repesnting homozygous mutant genotype (A/A) (product size 137bp). Amplification of internal control (β-Actin) in all samples was observed (product size 496bp). Sequencing electropherogram of respective SNP (B) showing homozygous wild type (G/G), (C) homozygous showing mutant genotype (A/A), (D) showing heterozygous genotype (G/A) in thyroid cancer patients. W stands for wild type, M stands for mutant and LD stands for ladder.

For the first selected polymorphism in *XRCC2* gene, G4234C (rs3218384), no significant difference in genotype frequency was observed in thyroid cancer patients compared to healthy controls (p>0.05) (Table 3).

| Gene/ polymorphism | Model | Genotype | Cases N (%) | Control N (%) | OR (95% CI) | p-value |
|---|---|---|---|---|---|---|
| *XRCC2* **G4234C (rs3218384)** | Codominant | G/G | 178(39) | 165(42) | Ref(1) | |
| | | G/C | 158(34.6) | 122(29.8) | 1.20(0.90-1.61) | 0.19 |
| | | C/C | 120(26.3) | 113(28.2) | 0.96(0.67-1.37) | 0.52 |
| | Dominant | G/G | 178(39) | 215(53.8) | Ref(1) | |
| | | G/C +C/C | 278(61) | 235(46) | 1.09(0.83-1.44) | 0.50 |
| **G4088T (rs3218373)** | Codominant | T/T | 216(48) | 257(64) | Ref(1) | |
| | | T/G | 140(31) | 85(21) | 1.65(1.20-2.24) | 0.001*** |
| | | G/G | 100(22) | 58(15) | 1.66(1.16-2.36) | 0.005*** |
| | Dominant | T/T | 217(47) | 257(65) | Ref (1) | |
| | | T/G-G/G | 240(53) | 143(35) | 2.01(1.51-2.62) | <0.0001*** |
| **G3063A (rs2040639).** | Codominant | G/G | 227(49.8) | 288(72) | Ref(1) | |
| | | G/A | 136(30) | 67(17) | 2.11(1.52-2.94) | <0.0001*** |
| | | A/A | 93(20) | 45(11) | 2.02(1.37-2.97) | 0.0003*** |
| | Dominant | G/G | 227(49.8) | 288(72) | Ref(1) | |
| | | G/A +A/A | 229(50.2) | 112(28) | 2.60(1.95-3.45) | <0.0001*** |

**Table 3:** Genotype frequencies of three SNPs of *XRCC2* gene, G4234C (rs3218384), G4088T (rs3218373) and G3063A (rs2040639) in study cohort.

ORs=odd ratios and 95% CI=95% confidence interval; ***p-value ≤ 0.001 by $\chi^2$ -test

Stratification analysis according to age, gender and smoking also showed no association with any genotype frequency (p>0.05) (Table 4).

| *XRCC2* **G4234C (rs3218384)** | | Genotype | Cases N (%) | Control N (%) | OR (95% CI) | p-value |
|---|---|---|---|---|---|---|
| **Gender** | | | | | | |
| **Male** | General | G/G | 42(39) | 29(41) | Ref (1) | |
| | | G/C | 38(36) | 19(27) | 1.47(0.77-2.85) | 0.24 |
| | | C/C | 28(26) | 22(31) | 0.77(0.39-1.50) | 0.44 |
| | Dominant | G/C +C/C | 56(52) | 32(46) | 1.30(0.71-2.38) | 0.38 |
| **Female** | General | G/G | 136(39) | 146(44) | Ref (1) | |
| | | G/C | 120(34) | 93(28) | 0.70(0.48-1.02) | 0.08 |
| | | C/C | 92(26) | 91(28) | 0.92(0.62-1.35) | 0.72 |
| | Dominant | G/C +C/C | 212(61) | 184(56) | 1.22(0.90-1.66) | 0.18 |
| **Age of diagnosis** | | | | | | |
| **≤ 42** | General | G/G | 81(39) | 78(44) | Ref (1) | |
| | | G/C | 72(35) | 50(28) | 1.36(0.88-2.10) | 0.15 |
| | | C/C | 55(26) | 51(28) | 0.90(0.57-1.91) | 0.65 |
| | Dominant | G/C +C/C | 127(61) | 101(56) | 1.21(0.80-1.81) | 0.35 |
| **≥ 42** | General | G/G | 97(39) | 97(44) | Ref (1) | |
| | | G/C | 86(35) | 62(28) | 1.36(0.91-2.01) | 0.12 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | C/C | 65(26) | 62(28) | 0.91(0.60-1.36) | 0.65 |
| | Dominant | G/C +C/C | 114(46) | 102(46) | 0.99 (0.68-1.42) | 0.96 |
| **Smoking status** | | | | | | |
| **Smokers** | General | G/G | 38(35) | 54(45) | Ref (1) | |
| | | G/C | 37(34) | 34(29) | 1.39(0.98-1.96) | 0.05 |
| | | C/C | 34(31) | 31(26) | 0.79(0.56-1.13) | 0.21 |
| | Dominant | G/C +C/C | 58(53) | 53(45) | 1.41(0.84-2.38) | 0.19 |
| **Non Smokers** | General | G/G | 140(40) | 121(43) | Ref(1) | |
| | | G/C | 121(35) | 78(28) | 0.71(0.48-1.06) | 0.38 |
| | | C/C | 86(25) | 82(29) | 1.10(0.73-1.65) | 0.39 |
| | Dominant | G/C +C/C | 152(44) | 132(47) | 0.87(0.64-1.20) | 0.42 |

**Table 4:** Determination of *XRCC2* G4234C (rs3218384) association with mean age of diagnosis, gender and smoking status in thyroid cancer patients vs. respective controls using general and dominant models.

Frequencies are represented as number and percentages N(%); p-values were calculated using Chi- square test and bold values were statistical significant as *, **, *** representing $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$; OR, Odd ratio; CI, Confidence interval.

However significant increase in stage I and II thyroid cancer patients was observed for heterozygote genotype G/C (OR=2.57, 95% CI=1.43-4.61; p=0.001) and combined genotype G/C+C/C (OR=2.41, 95% CI=1.48-3.91; p=0.0004) in thyroid cancer patients compared to controls (Table 5).

| **Gene/ Polymorphism** | **Genotyping** | **Stagel +II N=373** | **Stage III +IV N=83** | **OR (95% CI)** | **p-value** |
|---|---|---|---|---|---|
| *XRCC2* | | | | | |
| G4234C (rs3218384) | G/G | 131 | 47 | 1.00(Ref) | |
| | G/C | 142 | 16 | 2.57(1.43-4.61) | 0.001*** |
| | C/C | 100 | 18 | 1.32(0.74-2.33) | 0.33 |
| | G/C+C/C | 242 | 36 | 2.41(1.48-3.91) | 0.0004*** |
| G4088T (rs3218373) | T/T | 160 | 56 | 1.00(Ref) | |
| | T/G | 127 | 13 | 2.78(1.48-5.21) | 0.001*** |
| | G/G | 86 | 14 | 1.48(0.79-2.75) | 0.21 |
| | T/G+G/G | 213 | 27 | 2.76(1.66-4.56) | 0.0001*** |
| G3063A (rs2040639) | G/G | 171 | 56 | 1.00(Ref) | |
| | G/A | 122 | 14 | 2.39(1.26-4.42) | 0.005** |
| | A/A | 79 | 14 | 1.32(0.70-2.47) | 0.37 |
| | G/A+A/A | 171 | 28 | 1.66(1.01-2.73) | 0.04* |

**Table 5:** Genotyping distribution of G4234C (rs3218384), G4088T (rs3218373) and G3063A (rs2040639) with respect to clinical staging of thyroid cancer.

All bold values are statistically significant, * $p \leq 0.05$, **p-value $\leq$ 0.01 and ***p-value $\leq 0.001$ by $\chi^2$ -test.

For the second selected polymorphism of *XRCC2* gene, G4088T (rs3218373) a significant increase in thyroid cancer risk was observed in patients for variant heterozygote T/G (OR=1.65, 95% CI=1.20-2.24; p=0.001) and polymorphic homozygote G/G (OR=1.66, 95% CI=1.16-2.36; p=0.005) as shown (Table 3). When stratified for gender, ~2 fold increase in female thyroid cancer risk was observed for the heterozygote genotype T/G (OR=1.54, 95% CI=1.09-2.16; p=0.01), polymorphic homozygote G/G (OR=1.59, 95% CI=1.05-2.39; p=0.02) and combined genotype T/G+G/G in thyroid cancer patients compared to controls. For age of diagnosis ($\leq 42$ and $\geq 42$), marginal increase in thyroid cancer risk was observed with polymorphic genotype G/G (OR=1.11, 95% CI=0.71-1.75; p=0.05) in the groups of age $\geq 42$ in thyroid cancer patients as compared to controls. In case of smoking, significant increase in thyroid cancer risk was observed in the heterozygote genotype T/G (OR=1.23, 95% CI=0.67-2.25; p=0.04) and combined genotype (OR=2.00, 95% CI=1.17-3.37; p=0.01) of respective polymorphism in thyroid cancer patients compared to controls as shown (Table 6).

| ***XRCC2* G4088T (rs3218373)** | | **Genotype** | **Cases N (%)** | **Control N (%)** | **OR (95% CI)** | **p-value** |
|---|---|---|---|---|---|---|
| Gender | | | | | | |
| Male | General | T/T | 52(49) | 38(54) | Ref(1) | |
| | | T/G | 26(24) | 16(23) | 1.08(0.53-2.20) | 0.82 |
| | | G/G | 30(28) | 16(23) | 1.31(0.65-2.64) | 0.44 |
| | Dominant | T/G +G/G | 56(52) | 32(46) | 1.30(0.71-2.38) | 0.38 |
| Female | General | T/T | 165(47) | 219(66) | Ref(1) | |
| | | T/G | 114(33) | 69(21) | 1.54(91.09-2.16) | 0.01** |
| | | G/G | 70(20) | 45(14) | 1.59(1.05-2.39) | 0.02* |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Dominant | T/G +G/G | 184(53) | 114(34) | 1.70(1.27-2.26) | 0.0003*** |
| Age of diagnosis | | | | | | |
| ≤ 42 | General | T/T | 112(54) | 96(54) | Ref(1) | |
| | | T/G | 50(24) | 47(26) | 0.88(0.56-1.40) | 0.61 |
| | | G/G | 46(22) | 36(20) | 1.09(0.67-1.78) | 0.7 |
| | Dominant | T/G +G/G | 96(46) | 83(46) | 1.01(0.73-1.41) | 0.91 |
| ≥ 42 | General | T/T | 134(54) | 119(54) | Ref(1) | |
| | | T/G | 60(24) | 58(26) | 0.89(0.59-1.36) | 0.6 |
| | | G/G | 54(22) | 44(20) | 1.11(0.71-1.75) | 0.05* |
| | Dominant | T/G +G/G | 114(46) | 102(46) | 1.01(0.68-1.42) | 0.96 |
| Smoking status | | | | | | |
| Smokers | General | T/T | 51(47) | 66(55) | Ref (1) | |
| | | T/G | 29(27) | 27(23) | 1.23(0.67-2.25) | 0.04* |
| | | G/G | 38(35) | 26(22) | 1.66(0.93-2.95) | 0.08 |
| | Dominant | T/G +G/G | 58(53) | 53(45) | 2.00(1.17-3.37) | 0.01** |
| Non Smokers | General | T/T | 195(56) | 149(53) | Ref(1) | |
| | | T/G | 81(23) | 78(28) | 1.28(0.86-1.91) | 0.2 |
| | | G/G | 71(20) | 54(19) | 1.02(0.66-1.58) | 0.16 |
| | Dominant | T/G +G/G | 152(44) | 132(47) | 0.82(0.64-1.20) | 0.42 |

**Table 6:** Determination of *XRCC2* G4088T (rs3218373) association with mean age of diagnosis, gender and smoking status in thyroid cancer patients vs. respective controls using general and dominant models.

Frequencies are represented as number and percentages N(%); p-values were calculated using Chi- square test and bold values were statistical significant as *, **, *** representing $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$; OR, Odd ratio; CI, Confidence interval.

With respect to staging, ~3 fold increase in stage I and II patients was observed for heterozygous T/G (OR=2.78, 95% CI=1.48-5.21; p=0.001) and combined genotype, T/G+G/G (OR=2.76, 95% CI=1.66-4.56; p=0.0001) compared to stage III and IV patients.

For the third selected polymorphism, G3063A (rs2040639), a significant difference in genotypes distributions was observed for heterozygous variant G/A (OR=2.11; 95% CI=1.52-2.94; p<0.0001) and A/A variant genotype (OR=2.02; 95% CI=1.37-2.97; p=0.0003) in thyroid cancer patients as compared to controls. Combined genotype (GA+AA) of respective polymorphism also showed ~3 fold increase cancer risk in patients compared to controls as shown (Table 3). When data was stratified for clinicopathological parameters, for gender, in females patients, significant difference was observed in heterozygote G/A (OR=2.01, 95% CI=1.42-2.96; p=0.0001), polymorphic homozygote A/A (OR=2.12, 95% CI=1.37-3.27, $p \leq 0.0001$) and combined genotype G/A+A/A (OR=2.60, 95% CI=1.89-3.58, $p \leq 0.0001$) in cancer patients as compared to controls. For age of diagnosis (≤ 42 and ≥ 42), ~2 fold increase in thyroid cancer risk was observed for heterozygous variant G/A (OR=1.96, 95% CI=1.24-3.06; p=0.003), polymorphic homozygous A/A (OR=1.93, 95% CI=1.14-3.24; p=0.01) and combined genotype G/A+A/A (OR=2.56, 95% CI=1.74-3.76; p<0.001) in patients of age ≥ 42 years when compared with controls. For smoking status, ~3 fold increase in thyroid cancer risk was observed for heterozygote variant G/A (OR=3.01, 95% CI=1.52-5.93; p=0.001), polymorphic homozygous A/A (OR=2.06, 95% CI= 0.98-4.33; p=0.05) and combined genotype (G/A+A/A) (OR=3.31, 95% CI=1.52-5.93; p=0.001) of respective polymorphism in patients with smoking status when compared with healthy controls as shown (Table 7).

| ***XRCC2* G3063A (rs2040639)** | | **Genotype** | **Cases N (%)** | **Control N (%)** | **OR (95% CI)** | **p-value** |
|---|---|---|---|---|---|---|
| **Gender** | | | | | | |
| **Male** | General | GG | 53(50) | 30(43) | 1.00(Ref) | |
| | | GA | 33(31) | 21(30) | 1.04(0.54-2.00) | 0.90 |
| | | AA | 21(20) | 19(27) | 0.65(0.32-1.33) | 0.24 |
| | Dominant | GA+AA | 54(50) | 40(57) | 0.76(0.41-1.40) | 0.38 |
| **Female** | General | GG | 174(50) | 238(72) | 1.00(Ref) | 1.0 |
| | | GA | 103(30) | 56(17) | 2.01(1.42-2.96) | 0.0001*** |
| | | AA | 72(21) | 36(11) | 2.12(1.37-3.27) | <0.0001*** |
| | Dominant | GA+AA | 175(50) | 92(28) | 2.60(1.89-3.58) | <0.0001*** |
| **Age of diagnosis** | | | | | | |
| **≤ 42** | General | GG | 104(50) | 109(61) | 1.00 (Ref) | 1.0 |
| | | GA | 63(30) | 40(22) | 1.50(0.95-2.39) | 0.07 |
| | | AA | 41(20) | 30(17) | 1.21(0.72-2.05) | 0.45 |
| | Dominant | GA+AA | 104(50) | 50(28) | 1.58(1.68-3.94) | 0.52 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **≥ 42** | General | GG | 129(52) | 159(72) | 1.00 (Ref) | 1.0 |
| | | GA | 70(28) | 37(17) | 1.96(1.24-3.06) | 0.003*** |
| | | AA | 49(20) | 25(11) | 1.93(1.14-3.24) | 0.01** |
| | Dominant | GA+AA | 124(50) | 62(28) | 2.56(1.74-3.76) | <0.0001*** |
| **Smoking status** | | | | | | |
| **Smokers** | General | GG | 55(50) | 72(61) | 1.00(Ref) | 1.0 |
| | | GA | 33(30) | 15(13) | 3.01(1.52-5.93) | 0.001*** |
| | | AA | 22(20) | 13(11) | 2.06(0.98-4.33) | 0.05* |
| | Dominant | GA+AA | 55(50) | 28(24) | 3.31(1.87-5.83) | <0.0001*** |
| **Non Smokers** | General | | | | | |
| | | GG | 172(50) | 168(60) | 1.00(ref) | |
| | | GA | 103(30) | 91(32) | 0.88(0.62-1.23) | 0.46 |
| | Dominant | AA | 71(20) | 78(28) | 1.21(0.75-1.96) | 0.41 |
| | | GA+AA | 124(35) | 109(39) | 1.19(0.85-1.67) | 0.44 |

**Table 7:** Determination of *XRCC2* 3063 G/A (rs2040639) association with mean age of diagnosis, gender and smoking status in thyroid cancer patients vs. respective controls using general and dominant models.

Frequencies are represented as number and percentages N(%); p-values were calculated using Chi-square test and bold values were statistical significant as *, **, *** representing p<0.05, P<0.01 and P<0.001; OR, Odd ratio; CI, Confidence interval.

For staging of thyroid cancer ~3fold increase in stage I and II patients was observed for heterozygous G/A (OR=2.39, 95% CI=1.26-4.42; p=0.005) and combined genotype, G/A+A/A (OR=1.66, 95% CI=1.01-2.73; p=0.04) compared to stage III and IV patients.

## Discussion

Double strand breaks in DNA is the most lethal form of DNA damage causing chromosomal translocations, deletions and amplification resulting in instability of genome leading to cancer formation [23,24]. This lethal type of DNA damage is repaired by Homologous recombination repair (HRR) pathway. Among many HRR pathway genes, *XRCC2* is essential for the efficient repair of DSB by homologous recombination between sister chromatids induced by ionization radiations, reactive oxygen species and alkylating agents. Studies in human and mice with *XRCC2* disruption confirm that this gene, if defective in gene function results in 100 fold decrease in HR repair activity and can be involved in cancer induction and transformation [8,25].

In Pakistan, like several other countries of the world, thyroid cancer has highest increasing incidence rate amongst all endocrine cancer [26,27]. Despite this increasing incidence of thyroid cancer, the exact cause of its pathogenesis is not well understood. Interest in the molecular genetics of cancer regarding association of different polymorphisms and different DNA repair genes are now the main focus of research studies. Promoter regions are important to study as they act as regulatory elements which control translation and mRNA decay and are also sites for RNA interference [28-30]. Here we identified three SNP's in the 5′UTR of the *XRCC2* gene such as G4234C (rs3218384), G4088T (rs3218373) and G3063A (rs2040639) and genotyped by ARMS-PCR. Although the functional consequences of these polymorphisms are unknown, however their location in important domain of *XRCC2* is believed to regulate the expression of *XRCC2* gene and its mRNA levels. Thus these polymorphisms may have an important consequence on disease state.

Direct evidence to support or contradict our findings is lacking, since there are no previous published studies of selected promoter polymorphisms in thyroid cancer. However eleven studies have been identified with *XRCC2* G4234C (rs3218384) polymorphism, five studies in lung carcinoma [31-35] three in breast cancer [9,36,37], one in epithelial ovarian cancer [38], one in esophageal squamous cell carcinoma and gastric cardiaadenocarcinoma [36]. For G4088T (rs3218373) polymorphism, one study is found in Spanish population for bladder Cancer [39]. Finally, for G3063A (rs2040639) polymorphism, two studies are found for oral cancer [40,41] and one for colorectal cancer [42]. Contradictory results with respect of different selected parameters have been observed which may be population specific and additionally specific for cancer type.

In this study on thyroid cancer patients and healthy controls, we observed no significant difference in overall genotypic frequency between thyroid cancer patients and controls for *XRCC2* G4234C (rs3218384) even when stratified the data for age, gender and smoking status (p>0.05). However there is significant higher risk of developing thyroid cancer in stage I and II patients for heterozygous G/A and combined genotype G/A+A/A. For other two studied SNP's i.e., G4088T (rs3218373) and G3063A (rs2040639) polymorphisms, we observed significantly higher risk of developing thyroid cancer in patients as compared to healthy controls and this risk may be enhanced in female patients, particularly in those with age of diagnosis ≥ 42 years, with smoking and staging of thyroid cancer for both respective polymorphisms. It is obvious that women are two to three times more likely to develop thyroid cancer due to involvement of female hormonal factors and these selected polymorphisms may enhance this risk factor in female thyroid cancer patients. Smoking contains many chemical substances which can release free radicals and reactive oxygen and cause damage to cells in smokers [43]. The DNA damage caused by these pre-carcinogenic compounds may require homologous recombination particularly, the role of *XRCC2* [44]. Smoking is considered as an important environmental risk factor for thyroid cancer progression [45]. Carcinogenesis of the thyroid is a multifactorial process, usually involving an interaction between multiple genetic and environmental events. Interestingly we observed that heterozygous variant and combined genotype, in all three selected polymorphisms, play a significant role in increasing thyroid cancer risk in stage I and II patients (well differentiated) compared to stage III and IV (undifferentiated) thyroid cancer patients. Thus genotype in

homozygous mutant alone does not manifest any role in increasing thyroid cancer risk; nevertheless combined effect with heterozygous genotype may play a significant role towards thyroid carcinogenesis.

In summary, our findings indicate association of G4234C (rs3218384) with thyroid cancer staging only while we observed significant association of two promoter polymorphisms, G4088T (rs3218373) and G3063A (rs2040639) with elevated risk of developing thyroid cancer and this risk may be enhanced with smoking in female thyroid cancer patients, with age ≥ 42 years and staging of thyroid cancer with both polymorphisms. These two promoter polymorphisms, G4088T (rs3218373) and G3063A (rs2040639) in *XRCC2* gene may act as independent biomarker risk of thyroid cancer in Pakistani population. Additionally, data from present study suggests that combination of polymorphisms in promoter regions or other variations linked to it in the same gene or gene in vicinity could conceivably play a role in the process of developing thyroid cancer in Pakistani population. Furthermore, therapeutic measures may be directed towards promoter SNP's that influence gene expression. A larger case-control study with larger sample size with more clinical outcomes may be helpful to get a final conclusion about the genetic impact of *XRCC2* polymorphisms. Results obtained from our findings so far may be helpful for future investigation in pathogenesis of thyroid cancer. Nevertheless, these findings set a foundation for the subsequent studies on thyroid carcinogenesis, particularly in Pakistani population.

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