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ORIGINAL ARTICLE

Haplotype analysis of *XRCC1* gene polymorphisms and the risk of thyroid carcinoma

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Summary

Purpose: *Variants in DNA repair genes may alter the repair mechanisms that make the persons vulnerable to DNA damage. These polymorphic variants in the DNA repair pathway genes, such as XRCC1, have been associated with susceptibility of several types of cancer including thyroid cancer. This study was designed to explore the link between XRCC1 polymorphisms and modulation of thyroid cancer risk.*

Methods: *Our study consisted of 456 thyroid cancer patients and 400 controls. For XRCC1 polymorphisms analyses, three single nucleotide polymorphisms (SNPs) (rs25489, rs25487 and 1799782) were selected and genotyped by ARMS-PCR.*

Results: *The homozygous mutant (AA) of rs25489 SNP showed highly significant association with thyroid cancer risk (OR=0.17; 95%CI=0.10-0.31; p=0.0001). In the rs25487 polymorphism all genotypes showed no significantly increased risk of thyroid cancer in patients compared to controls (p>0.05). In the rs1799782 of XRCC1 gene, the homozygous mutant (TT) significantly decreased the risk of thyroid cancer (OR=0.71; 95%CI=0.50-1.01; p=0.05). Eight haplotypes were generated for three selected SNPs (rs25489, rs25487 and rs1799782) of XRCC1 gene among thyroid cases and controls. The haplotype GAT (OR=1.69; 95%CI=1.25-2.30; p=0.0005) and GGC (OR=2.75; 95%CI=2.11-3.58; p=1.29e-014) showed highly significant association with increased risk of thyroid cancer. The haplotypes AAC (OR=0.31; 95%CI=0.17-0.57; p=6.68e-005), AAT (OR= 0.51; 95%CI=0.34-0.78; p=0.001), AGT (OR=0.46; 95%CI=0.29-0.71; p=0.0003) and GGT (OR=0.80; 95%CI=0.64-0.98; p=0.03) had significant reducing effect in thyroid cancer patients.*

Conclusions: *XRCC1 Arg280His and Arg194Trp were associated with thyroid cancer in Pakistani population. These genetic markers may provide an insight into the disease pathogenesis and help open novel therapeutic strategies for thyroid cancer.*

Key words: *DNA repair, haplotypes, polymorphism, thyroid carcinoma, XRCC1*

Introduction

Thyroid cancer is the most common endocrine malignancy. It accounts for more than 90% of all endocrine malignancies and 50% mortality attributable to endocrine cancers [1,2]. The incidence of thyroid cancer is 1-2% worldwide and is alarming in Europe [3,4], Canada [5,6], United Kingdom [7], and United States of America [8]. Now it is considered as fastest growing and sixth most common cancer type [9]. In Pakistan, the incidence of thyroid cancer is 1.2% of all malignancies, among which 69-71% are papillary thyroid carcinoma, 11-13% follicular thyroid carcinoma, 3-5% medullary thyroid carcinoma and 1-2% anaplastic carcinoma [10].

The development of thyroid cancer is a multifactorial process associated with a variety of risk factors. Exposure to ionizing radiation in childhood, family history, different thyroid diseases, dietary iodine and environmental pollutants (endo-

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crine disruptors) are the main risk factors involved in thyroid carcinogenesis [11]. Additionally, it is also a well-established fact that many gene polymorphisms especially in DNA repair genes are involved in thyroid carcinogenesis [12]. In many of the earlier studies, variations in DNA repair pathways and their mechanisms have been related with increased risk of cancers [13,14]. Particularly the genetic polymorphisms in DNA repair genes cause the substitution of amino acids which may lead to differential capacity of repair DNA damage. These genetic polymorphisms in DNA repair genes may be associated with genetic instability and carcinogenesis [15]. In humans, four DNA repair mechanism have been found which include base excision repair (BER), nucleotide excision repair (NER), double strand break repair (DSBR) and mismatch repair (MMR) [16,17]. All these DNA repair mechanisms are involved in the maintenance of genome integrity and DNA damage repair. These DNA damaged bases are produced due to exogenous or endogenous mutagens [18,19]. It has been speculated that 10^4 lesions of modified bases are repaired by the BER pathway in each cell per day [20].

One of the major representative of BER pathway is X-ray Cross Complementing group 1 (*XRCC1*) which acts as scaffold to recruit BER for excision or strand break repair [21]. *XRCC1* is involved in the recruitment of other BER proteins in the first steps of BER pathway [22,23] and helps correcting errors during DNA replication and recombination [24]. The location of this gene is at 19q13.2. DNA ligase, DNA polymerase, endonuclease AP and PARP interact with *XRCC1*. The most important function of *XRCC1* protein is to coordinate the BER pathway among various components. More than 300 SNPs have been reported in *XRCC1* gene, of which most studied polymorphisms in the coding region are rs25489 (Arg280His), rs25487 (Arg399Gln) and rs1799782 (Arg194Trp) [25].

The selected SNPs of *XRCC1* have the ability to change the structure of protein which may impair the function of enzymes associated with the BER pathway [26]. The present study was designed to investigate possible associations of BER pathway gene *XRCC1* polymorphisms with thyroid cancer.

Methods

Study subjects and ethical approval

The study consisted of 456 diagnosed thyroid cancer patients and 400 healthy controls (Table 1). Diagnosed thyroid cancer patients were histologically confirmed at the Nuclear Medicine Department of Nuclear Oncology Radiation Institute (NORI) and Pakistan Institute of Medicine Sciences (PIMS), Islamabad. Controls were selected from individuals receiving routine medical examinations in these hospitals, while individuals with a previous diagnosis of any type of cancer were excluded. Peripheral blood samples were collected from all subjects. This study was approved by the institutional ethical review boards of COMSATS Institute of Information Technology, Islamabad and both collaborating hospitals. Additionally, all experiments were performed in accordance with relevant guidelines and regulations.

DNA extraction

Approximately 3-4ml blood samples were collected in vacutainer tubes from all recruited patients and controls. DNA was extracted from whole blood by the phenol-chloroform method with minor modifications [27]. The extracted DNA was visualized by 1% agarose gel stained with ethidium bromide. The DNA quality was measured spectrophotometrically using Nano Drop (Thermoscientific, USA) and stored at -20°C until used.

Table 1. Demographic characteristics for cases and controls

Variables	*Cases (n=456) n (%)*	*Controls (n=400) n (%)*	*OR(95%CI)*	*p value (x^2)*
Age, years			1.03(0.79-1.35)	0.79
<42	208 (45.6)	179 (44.75)		
>42	248 (54.38)	221 (55.25)		
Sex			1.44(1.03-2.02)	0.03
Male	107 (23)	70 (18)		
Female	349 (77)	330 (82)		
Smoking status			0.55(0.41-0.72)	0.0001
Smokers	109 (24)	119 (30)		
Non-smokers	347 (76)	281 (70)		
Family history			3.5(1.65-7.39)	0.001
Yes	34 (7)	9 (2.25)		
No	422 (93)	391 (97.75)		

SNPs selection

Three polymorphisms in DNA repair gene *XRCC1* were selected from dbSNP database. The SNP selection criteria were based on minor allele frequency (MAF) greater than 0.05. The selected SNPs included Arg-399Gln (rs25487), Arg280His (rs25489) and Arg194Trp (rs1799782).

Genotyping

Genotyping was performed by allele-specific polymerase chain reaction (ARMS-PCR). Primers for PCR amplification were designed using WASP (web based allele specific primer designing tool). Primers specific for each polymorphism are given in Supplementary Table 1. PCR reaction was carried out in a reaction volume of 10µl 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 5mins and 35 cycles at 94°C for 30sec, optimized annealing temperature for 45sec, 72°C for 1min and final extension for 10min. The PCR products were visualized on a 2% agarose gel electrophoresis (100V, 300A for 45mins). Presence or absence of expected product size bands, specific for wild or mutant primers, were evaluated using UV trans illuminator (Gel Doc BioRad, USA). β-Actin was used as internal control in each reaction. PCR products with homozygous wild, homozygous mutant and heterozygous mutant genotypes were further confirmed with DNA sequence analysis.

Statistics

For each polymorphism, demographical characteristics were compared between cancer cases and healthy controls using x^2 test. Odds ratio (ORs) and 95% confidence intervals (CI) were calculated after adjusting for gender, age, smoking status and family history of cancer. P value of <0.05 was statistically significant. Haplotypes were generated from the genotype data. The linkage disequilibrium (LD) and haplotype analysis were performed using Haploview 4.2.

Results

Demographic data obtained from thyroid cancer patients (n=456) and healthy controls (n=400) is shown in Table 1. Based on 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 controls.

The genotypic distribution for three *XRCC1* SNPs (rs25489, rs25487 and 1799782) and thyroid cancer risk are given in Table 2. The results showed that heterozygous (GA) genotype of rs25489 did not show significant association with thyroid cancer (OR=1.05; 95%CI=0.74-1.47; p=0.77), while homozygous mutant (AA) of the same SNP significantly decreased the risk of thyroid cancer in patients compared to controls (OR=0.17; 95%CI=0.10-0.31; p=0.0001). In the rs25487 polymorphism, the heterozygous (GA) genotype significantly decreased the risk of thyroid cancer (OR=0.24; 95%CI=0.17-0.34; p=0.0001). However, the homozygous mutant of this SNP significantly increased the risk (OR=4.80; 95%CI=2.54-9.07; p=0.0001). In the rs1799782 SNP *XRCC1* heterozygous (CT) genotype did not show significant association (OR=0.88; 95%CI=0.66-1.16; p=0.38), while the homozygous mutant (TT) significantly decreased the risk (OR=0.71; 95%CI=0.50-1.01; p= 0.05), in patients compared to controls.

Table 2. Genotype frequencies of selected SNPs of XRCC1 gene in cases and controls

Polymorphisms of XRCC1	*Cases n (%)*	*Controls n (%)*	*OR (95% CI)*	*p value*
rs25489				
GG	351(76.9)	257(64.25)	1	
GA	89(19.5)	75(18.7)	1.05(0.74 to 1.47)	0.77
AA	16(3.5)	68(17.0)	0.17(0.10 to 0.31)	0.0001
GA+AA	105 (23.0)	143 (35.7)	0.53 (0.39 to 0.72)	0.0001
rs25487				
GG	150(32.89)	140(35.0)	1	
GA	166(36.4)	138(34.5)	1.08(0.82 to 1.43)	0.56
AA	140(30.7)	122(30.5)	1.09(0.75 to 1.35)	0.94
GA+AA	306 (67.1)	260 (65.0)	1.09(0.82 to 1.45)	0.51
rs1799782				
CC	93(20.3)	50(12.5)	1	
CT	288(63.1)	264(66.0)	0.88(0.66 to 1.16)	0.38
TT	75(16.44)	86(21.5)	0.71(0.50 to 1.01)	0.05
CT +TT	363 (79.6)	350 (87.5)	0.55 (0.38 to 0.81)	0.002

OR: odds ratio, CI: confidence interval. OR, CI and p value calculated by regression analysis

Subgroup analysis of different histological types of thyroid cancer was further performed to evaluate the association between SNPs and thyroid cancer risk. Among all the four subtypes, homozygous mutant (AA) of rs25489 significantly decreased the risk of thyroid cancer in papillary thyroid carcinoma (OR=0.23; 95%CI=0.13-0.41; p=0.00001). In rs25487, heterozygous (GA) significantly decreased the risk of thyroid carcinoma in all four subtypes (OR=0.25; 95%CI=0.18-0.35; p=0.0001, OR=0.29; 95%CI=0.17-0.49; p=0.0001, OR=0.19; 95%CI=0.06-0.52; p=0.001, and OR=0.14; 95%CI=0.03-0.65; p=0.01, respectively). However, mutant (AA) rs25487 significantly increased the risk of thyroid cancer in three subtypes i.e. papillary (OR=5.49; 95%CI=2.87-10.49; p=0.0001), follicular (OR=6.09; 95%CI=2.66-13.90; p=0.0001) and medullary thyroid carcinoma (OR=7.46; 95%CI=1.87-29.67; p=0.004). In case of rs1799782, mutant (TT) allele significantly increased the risk in follicular (OR=1.69; 95%CI=1.00-2.85; p=0.04) and anaplastic thyroid carcinoma (OR=4.86; 95%CI=1.06-22.16; p=0.04) but in papillary thyroid carcinoma it significantly decreased the risk of thyroid cancer (OR=0.62; 95%CI=0.42-0.90; p=0.01). Relevant data is given in Table 3.

Association of thyroid cancer with treatment types was evaluated in patients compared to controls. Heterozygous (GA) rs25489 significantly increased the risk of thyroid cancer among all patients treated with chemotherapy and radiotherapy (OR=2.38; 95%CI=1.09-5.18; p=0.02, and OR=8.66; 95%CI=3.74-20.04; p=0.0001, respectively). However, in the case of patients treated with iodine therapy, it significantly decreased the risk of thyroid cancer (OR=0.21; 95%CI=0.12-0.37; p=0.0001). Heterozygous (GA) rs25487 significantly decreased the risk of thyroid cancer in all patients treated with iodine therapy, chemotherapy and radiotherapy (OR=0.26; 95%CI=0.18-0.36; p=0.0001, OR=0.23; 95%CI=0.10-0.49; p=0.0001, and OR=0.06; 95%CI=0.02-0.16; p=0.0001, respectively), while homozygous (AA) of the same SNP significantly increased the risk of thyroid cancer in all patients treated with iodine therapy, chemotherapy and radiotherapy (OR=4.43; 95%CI=2.31-8.48; p=0.0001, OR=6.21; 95%CI=2.03-18.98; p=0.001 and OR=13.6; 95%CI=4.97-37.32; p=0.0001). Heterozygous (CA) mutant of rs1799782 polymorphism showed significantly decreased risk of thyroid cancer in patients treated with radiotherapy (OR=0.25; 95%CI=0.11-0.58; p=0.001). The results are shown in Table 4.

Haplotype analysis results of XRCC1 SNPs

Eight haplotypes were generated for three selected SNPs (rs25489, rs25487 and rs1799782) of

Table 3. Distribution of genotypes and odds ratios (OR) for different histological subtypes of thyroid carcinoma and controls in XRCC1

Genotypes XRCC1	*Controls (n=400)*	*Papillary carcinoma (n=351)*			*Follicular carcinoma (n=82)*			*Medullary carcinoma (n=16)*			*Anaplastic carcinoma (n=7)*		
		n	*OR (95% CI)*	*p value*	*n*	*OR (95% CI)*	*p value*	*n*	*OR (95% CI)*	*p value*	*n*	*OR (95% CI)*	*p value*
rs25489													
GG	257	264	1.00 (REF)		56	1.00 (REF)		8	1.00 (REF)		3	1.00 (REF)	
GA	75	71	1.09 (0.76-1.57)	0.60	19	1.30 (0.73-2.71)	0.35	6	2.60 (0.91-7.37)	0.07	1	0.72 (0.08-6.08)	0.76
AA	68	16	0.2 (0.13-0.41)	0.00001	07	0.45 (0.20-1.03)	0.45	2	0.69 (0.15-3.13)	0.63	3	3.66 (0.80-16.73)	0.09
rs25487													
GG	52	99	1.00 (REF)		19	1.00 (REF)		5	1.00 (REF)		3	1.00 (REF)	
GA	336	201	0.25 (0.18-0.35)	0.0001	50	0.29 (0.17-0.49)	0.0001	8	0.19 (0.06-0.52)	0.001	3	0.14 (0.03-0.65)	0.01
AA	12	51	5.49 (2.87-10.49)	0.0001	13	6.09 (2.66-13.90)	0.0001	3	7.46 (1.87-29.67)	0.004	1	5.03 (0.60-48.32)	0.13
rs1799782													
CC	50	87	1.00 (REF)		19	1.00 (REF)		4	1.00 (REF)		1	1.00 (REF)	
CT	264	213	0.79 (0.59-1.07)	0.13	47	0.69 (0.42-1.12)	0.13	9	0.66 (0.24-1.81)	0.42	2	0.20 (0.03-1.07)	0.06
TT	86	51	0.62 (0.42-0.90)	0.01	26	1.69 (1.00-2.85)	0.04	3	0.84 (0.23-3.02)	0.79	4	4.86 (1.06-22.16)	0.04

XRCC1 among cases and controls. Haplotype GAT (OR=1.69; 95%CI=1.25-2.30; p=0.0005) and GGC (OR=2.75; 95%CI=2.11-3.58; p=1.29e-014) showed highly significant association with increased risk of thyroid cancer. Decreased risk was observed in haplotypes AAC (OR=0.31; 95%CI=0.17-0.57; p=6.68e-005), AAT (OR=0.51; 95%CI=0.34-0.78; p=0.001), AGT (OR=0.46; 95%CI=0.29-0.71; p=0.0003) and GGT (OR=0.80; 95%CI=0.64-0.98; p=0.03), as shown in Table 5.

Genotype-genotype interaction

After haplotype analysis, interaction between the selected SNPs of *XRCC1* was calculated by the joint effect model. The combined effects of rs25489 *vs* rs25487 are shown in Table 6, and increase in thyroid cancer risk was observed in the combined effect of GG/AA genotype (OR=11.37; 95% CI=4.06-31.87; p=0.0001), GA/GG genotype (OR=2.85; 95%CI=1.20-6.76; p=0.01) and GA/AA genotype (OR=2.44; 95%CI=0.77-7.74; p=0.12), whereas decreased thyroid cancer risk was observed in the combined effect of GG/GA genotype (OR=0.43; 95%CI=0.32-0.57; p=0.0001) and AA/GA genotype (OR=0.16; 95%CI=0.08-0.31; p=0.0001) and AA/AA genotype (OR=0.21; 95%CI= 0.02-1.95; p=0.17).

The combined effect of rs25489 *vs* rs1799782 is shown in Table 6. A significant increase in risk

Table 4. Distribution of genotypes and odds ratios (OR) of XRCC1 polymorphisms for different treatments of thyroid carcinoma and controls

Genotypes XRCC1	*Controls (n=400)*	*Radioactive I_{131}-therapy (n=398)*			*Chemotherapy (n=31)*			*Radiotherapy (n=27)*		
		n	*OR (95% CI)*	*p value*	*n*	*OR (95% CI)*	*p value*	*n*	*OR (95% CI)*	*p value*
rs25489										
GG	257	297	1.00 (REF)		18	1.00 (REF)		7	1.00 (REF)	
GA	75	84	1.15 (0.81-1.64)	0.40	11	2.38 (1.09-5.18)	0.02	18	8.66 (3.74-20.04)	0.0001
AA	68	17	0.21 (0.12-0.37)	0.00001	02	0.33 (0.07-1.44)	0.14	2	0.39 (0.09-1.68)	0.20
rs25487										
GG	52	119	1.00 (REF)		09	1.00 (REF)		12	1.00 (REF)	
GA	336	231	0.25 (0.18-0.35)	0.0001	17	0.29 (0.17-0.49)	0.0001	07	0.19 (0.06-0.52)	0.001
AA	12	48	5.49 (2.87-10.49)	0.0001	05	6.09 (2.66-13.90)	0.0001	08	7.46 (1.87-29.67)	0.004
rs1799782										
CC	50	87	1.00 (REF)		08	1.00 (REF)		12	1.00 (REF)	
CT	264	240	0.79 (0.59-1.07)	0.13	16	0.69 (0.42-1.12)	0.13	09	0.66 (0.24-1.81)	0.42
TT	86	71	0.62 (0.42-0.90)	0.01	07	1.69 (1.00-2.85)	0.04	06	0.84 (0.23-3.02)	0.79

Table 5. Distribution of haplotype analysis in the study cohort

Haplotype	*Cases (freq)*	*Controls (freq)*	*x^2*	*Fisher's p value*	*Pearson's p value*	*OR (95% CI)*
AAC	15.25 (0.017)	40.93 (0.051)	15.919	6.71e-005	6.68e-005	0.31 (0.17–0.57)
AAT	39.56 (0.43)	64.31 (0.080)	10.24	0.001380	0.001378	0.51 (0.34–0.78)
AGC	33.22 (0.036)	46.64 (0.058)	4.58	0.032271	0.32244	0.61 (0.38–0.96)
AGT	32.97 (0.036)	60.12 (0.075)	12.60	0.000387	0.000387	0.46 (0.29–0.71)
GAC	189.07 (0.207)	184.78 (0.231)	01.39	0.237105	0.237028	0.87 (0.69–1.09)
GAT	131.12 (0.144)	71.98 (0.090)	11.79	0.000599	0.000598	1.69 (1.25–2.30)
GGC	237.46 (0.260)	90.65 (0.113)	58.488	1.47e-014	1.29e-014	2.75 (2.11–3.58)
GGT	233.35 (0.256)	240.58 (0.301)	4.284	0.38525	0.038493	0.80 (0.64–0.98)

OR: odds ratio, CI: confidence interval. OR, CI and p value calculated by regression analysis. Haplotypes follow the SNPs as: rs25489 (Arg280His), rs25487 (Arg399Gln) and rs1799782 (Arg194Trp)

was observed in the combined effect of GG/CT genotype (OR=1.33; 95%CI=1.02-1.75; p=0.03) and GA/CC genotype (OR=2.84; 95%CI=1.20-6.73; p=0.01), while significantly decreased thyroid cancer risk was observed in the combined effect of AA/CT genotype (OR=0.18; 95%CI=0.09-0.36; p=0.0001) and AA/TT genotype (OR=0.32; 95%CI=0.12-0.82; p=0.018).

The combined effects of rs25487 *vs* 1799782 are shown in Table 6. A significant increase in thyroid cancer risk was observed in the combined effect of GG/CT genotype (OR=4.77; 95%CI=1.81-12.56; p=0.0015), AA/CC genotype (OR=8.94; 95%CI= 1.14-70.19; p=0.03) and AA/CT genotype (OR=4.06; 95%CI=2.01-8.19; p=0.0001), whereas significantly decreased risk was observed in the combined effect of GA/CT genotype (OR=0.42; 95%CI=0.31-0.55; p=0.0001) and GA/TT genotype (OR=0.47; 95%CI=0.31-0.70; p=0.0002).

Discussion

In this study, the possible association of *XRCC1* gene polymorphisms with thyroid cancer risk was evaluated in thyroid cancer patients and healthy controls. *XRCC1* is an important BER pathway repair protein which repairs single strand breaks in combination with three other DNA repair genes i.e DNA ligase III, PARP and DNA polymerase [28]. *XRCC1* is located on chromosome 19q13.2-13 [29] and has 22 exons and produces about 2.2kb transcript. Evidence has indicated that DNA damage and impaired DNA repair system may cause various diseases and alterations in DNA structure [30]. DNA repair efficiency in cells is a determining factor for preventing the development of thyroid cancer [31]. Polymorphisms in *XRCC1* gene disturb the interaction of proteins with other enzymes of the BER pathway which ultimately decrease the

Table 6. Risk of thyroid cancer in relation to combined genotype

Genotypes	*Cases*	*Controls*	*OR (95% CI)*	*p value*
rs25489 *vs* rs25487				
GG/GG	115	35	1	
GG/GA	189	217	0.43(0.32 to 0.57)	**0.0001**
GG/AA	47	4	11.37(4.06 to 31.87)	**0.0001**
GA/GG	22	7	2.85(1.20 to 6.76)	**0.016**
GA/GA	56	65	0.72(0.49 to 1.06)	0.097
GA/AA	11	4	2.44(0.77 to 7.74)	0.1280
AA/GG	3	8	0.32(0.08 to 1.23)	0.0982
AA/GA	12	56	0.16(0.08 to 0.31)	**0.0001**
AA/AA	1	4	0.21(0.02 to 1.95)	0.1733
rs25489 *vs* rs1799782				
GG/CC	72	35	1	
GG/CT	229	172	1.33(1.02 to 1.75)	**0.0349**
GG/TT	50	49	0.88(0.58 to 1.34)	0.5577
GA/CC	22	07	2.84(1.20 to 6.73)	**0.0173**
GA/CT	48	47	0.88(0.57 to 1.35)	0.5697
GA/TT	19	22	0.74(0.39 to 1.40)	0.3635
AA/CC	00	08	15.18(0.87 to 263.86)	0.0619
AA/CT	10	44	0.18(0.09 to 0.36)	**0.0001**
AA/TT	06	16	0.32 (0.12 to 0.82)	**0.0185**
rs25487 *vs* rs1799782				
GG/CC	26	05	1	
GG/CT	90	34	4.77(1.81 to 12.56)	**0.0015**
GG/TT	24	11	1.96(0.94 to 4.06)	0.0685
GA/CC	58	44	1.17(0.77 to 1.78)	0.4389
GA/CT	154	219	0.42(0.31 to 0.55)	**0.0001**
GA/TT	45	75	0.47(0.31 to 0.70)	**0.0002**
AA/CC	10	01	8.94(1.14 to 70.19)	**0.0371**
AA/CT	43	10	4.06(2.01 to 8.19)	**0.0001**
AA/TT	06	01	5.32(0.63 to 44.38)	0.1225

OR: odds ratio, CI: confidence interval. OR, CI and p value calculated by regression analysis and then confirmed by Bonferroni correction. P values in bold have still maintained their significance after Bonferroni correction

Supplementary Table 1. Primers for XRCC1 gene SNPs with product size and annealing temperature

Name of SNPs	*Primer sequence*	*Product size (bp)*	*Annealing temp. (°C)*
rs25489		227	56
WR*	CCAGTGCCAGCTCCAACTAA		
MR**	CCAGTGCCAGCTCCAACTAG		
CR***	TACTTGGCCCCAAGCTCTAG		
rs25487		171	58
WR	GCGTGTGAGGCCTTACCTAT		
MR	GCGTGTGAGGCCTTACCTAC		
CF	CACACCTAACTGGCATCTTC		
rs1799782		253	57
WF	GGGGGCTCTCTTCTTCAGAC		
MF	GGGGGCTCTCTTCTTCAGAT		
CR	AGTGATCCAGGAGTCCCAGC		

SNPs: single nucleotide polymorphisms, *Wild forward, **Mutant forward, ***Common reverse

DNA repair capacity and start the carcinogenic process [32].

In *XRCC1* gene, several SNPs are present. Three common SNPs in the coding region of *XRCC1* gene have been frequently studied which include Arg399Gln (rs25487), Arg280His (rs25489) and Arg194Trp (rs1799782) [33-36]. *XRCC1* SNPs have previously been studied in many cancers including prostate cancer [34], glioma [37] and endometrial cancer [38]. These polymorphisms have also been reported in thyroid cancer in different populations and in different ethnic groups with inconsistent results [35,39-43] In this study, association of three SNPs i.e Arg399Gln (rs25487), Arg280His (rs25489) and Arg194Trp (rs1799782) in *XRCC1* have been evaluated with modulated thyroid cancer risk.

Polymorphism rs25487 (Arg399Gln) is in non-conserved region having higher sister chromatid exchange frequency and lengthy cell cycle delay in response to ionizing radiations [44]. Our results revealed that rs25487polymorphism of *XRCC1* gene do not show any association with thyroid cancer in Pakistani population. Similar to our findings, no association between thyroid cancer and rs25487 polymorphism have earlier been observed in different populations [38,43]. Similar results were obtained with breast cancer in Saudi population [44,45], gastric cancer in Korean population [46,47] and pancreatic cancer in Chinese population [48]. Nevertheless, increased association of Arg399Gln (rs25487) has been observed with primary open-angle glaucoma (POAG) in Polish population [49].

In the present study, our findings with rs25489 (Arg280His) polymorphism of *XRCC1* showed significantly decreased risk with variant genotypes in thyroid cancer patients compared to controls. In consistence with our observations, protective association with thyroid cancer has also been found in Chinese population [31]. It has been suggested that Arg280His might contribute towards differentiated thyroid carcinoma (DTC) in Caucasians and provide protective effect in Asian population [50]. Protective effects against the risk of thyroid cancer among Asians have been observed with heterozygous genotype of Arg280His, but this did not reach statistical significance [39,51]. In contrast to our findings, variant genotypes of Arg280His have significantly been associated with DTC among Caucasians by different researchers [28,38,52]. A similar trend was also observed in two other studies with DTC but with no statistical significance [53,54]. In a meta-analysis, no positive association has been reported with rs25489 polymorphism in thyroid cancer [43].

In the rs1799782 (Arg194Trp) polymorphism, variant 194Trp allele has been reported with increased protein function which led to enhanced DNA repair capacity and decreased sensitivity to genotoxins. Therefore, decreased risk of certain cancers has been observed in different populations [55]. Our results with rs1799782 (Arg194Trp) have also indicated decreased risk of thyroid cancer in patients compared to controls. Like our results, decreased thyroid cancer risk with papillary thyroid cancer has been reported with mutant Trp allele of Arg194Trp in Kazakhstan with OR of 0.55 [41] and in Korean population [56]. Similarly, a protective effect against thyroid cancer with OR of 0.76 has been observed for heterozygous genotype Arg/Trp in Caucasian population which is in agreement with our observed risk of heterozygous genotype (OR=0.71). Protective effect of rs1799782 (Arg194Trp) has been observed with other cancers

such as breast cancer [57] and lung cancer [38]. Nevertheless, increased risk of thyroid cancer with Arg194Trp polymorphism has also been reported in Taiwanese population [39] and in United States [40].

Subtype analysis in the present study showed 2-fold significantly increased risk of follicular thyroid carcinoma in homozygous mutant (TT) and 31% decreased risk in heterozygous genotypes (CT) of rs1799782 polymorphism. However, decrease in papillary thyroid carcinoma was found in both the homozygous minor (AA) and heterozygous genotypes (GA) of rs25487. These results are consistent with earlier findings of one study, but the values in that study did not reach statistical significance [31].

Haplotype analysis of rs25489 (Arg280His), rs25487 (Arg399Gln) and rs1799782 (Arg194Trp) in our study has shown significant association with thyroid cancer risk. Similarly, Yan et al. [31] revealed that haplotypes of *XRCC1*, rs25489 (Arg280His), rs25487 (Arg399Gln) and rs1799782 (Arg194Trp) polymorphisms are associated with DTC risk in Chinese population. In contrast to this result, Fard-Esfahani et al. [54] reported no significant risk association between *XRCC1* haplotypes and DTC patients in Iranian population. Previous findings among Asian countries concluded that Arg194-Gln399+Trp194 and Arg399 haplotypes have been associated with increased risk of breast cancer [58]. Multiple haplotypes of *XRCC1* polymorphisms thus have been associated with significantly increased risk of DTC [39]. Therefore, it is of interest to elucidate the utility of *XRCC1* haplotypes in the prediction of thyroid cancer risk.

Based on our results, it is concluded that the *XRCC1* Arg280His and Arg194Trp polymorphisms might contribute to individual susceptibility to thyroid carcinoma in Pakistani population. Therefore, change in protein biochemistry of *XRCC1* gene leads to the hypothesis that such variant alleles could potentially be used as a molecular biomarker for thyroid cancer susceptibility. However, studies using large case-control datasets in different ethnic populations are required. Further investigation of these polymorphisms in association with exposure to different environmental factors is needed to explore the role of these SNPs in thyroid cancer.

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Conflict of interests

The authors declare no conflict of interests.

References

1. Gilfillan CP. Review of the genetics of thyroid tumours: diagnostic and prognostic implications. ANZ J Surg 2010; 80: 33-40.
2. Aschebrook-Kilfoy B, Ward MH, Sabra MM, Devesa SS. Thyroid cancer incidence patterns in the United States by histologic type, 1992–2006. Thyroid 2011;21:125-34.
3. Smailyte G, Miseikyte-Kaubriene E, Kurtinaitis J, Sakoda L, Horn-Ross P, Feldt-Rasmussen U. Increasing thyroid cancer incidence in Lithuania in 1978–2003. BMC Cancer 2006;6:284.
4. Kilfoy BA, Zheng T, Holford TR et al. International patterns and trends in thyroid cancer incidence, 1973–2002. Cancer Causes Control 2009;20:525-31.
5. Shaw A, Semenciw R, Mery L. Cancer in Canada Fact Sheet Series# 1: Thyroid cancer in Canada. Chronic Dis Inj Can 2014;34:64-8.
6. Brown P, Jiang H, Ezzat S, Sawka AM, Guay B, Johnson-Obaseki S. A detailed spatial analysis on contrasting cancer incidence patterns in thyroid and lung cancer in Toronto women. BMC Public Health 2016;16:950.
7. McNally RJQ, Blakey K, James PW, Gomez Pozo B, Basta NO, Hale J. Increasing incidence of thyroid cancer in Great Britain, 1976–2005: age-period-cohort analysis. Eur J Epidemiol 2012;27:615-22.
8. Giri S, Pathak R, Aryal MR, Karmacharya P, Bhatt VR, Martin MG. Seasonal variation in the presentation of thyroid cancer in the USA: an analysis of the Surveillance, Epidemiology and End Results Registry. Cancer Causes Control 2014;25:1583.
9. Nikiforov YE. Is ionizing radiation responsible for the increasing incidence of thyroid cancer? Cancer 2010;116:1626-28.
10. Bukhari MH, Niazi S, Hanif G et al. An updated audit of fine needle aspiration cytology procedure of solitary thyroid nodule. Diagn Cytopathol 2008;36:104-12.
11. Carpi A, Mechanick JI, Saussez S, Nicolini A. Thyroid

tumor marker genomics and proteomics: diagnostic and clinical implications. J Cell Physiol 2010;224:612-9.

12. Gudmundsson J, Sulem P, Gudbjartsson DF et al. Discovery of common variants associated with low TSH levels and thyroid cancer risk. Nat Gen 2012;44:319-22.
13. Johnson-Thompson MC, Guthrie J. Ongoing research to identify environmental risk factors in breast carcinoma. Cancer 2000;88:1224-9.
14. Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. Statist Med 2004;23:1111-30.
15. De Boer, J, Andressoo JO, de Wit J, Huijmans J, Beems RB, van Steeg H, Meradji M. Premature aging in mice deficient in DNA repair and transcription. Science 2007;296:1276-9.
16. Yu Z, Chen J, Ford BN, Brackley ME, Glickman BW. Human DNA repair systems: an overview. Environ Molec Mutagenesis 1999;33:3-20.
17. Christmann M, Tomicic MT, Roos WP, Kaina B. Mechanisms of human DNA repair: an update. Toxicology 2003;193: 3-34.
18. David SS, O'Shea VL, Kundu S. Base excision repair of oxidative DNA damage. Nature 2007;447:941-50.
19. Zharkov DO. Base excision DNA repair. Cell Mol Life Sci 2008;65:1544-65.
20. Lindahl T. Instability and decay of the primary structure of DNA. Nature 1993;362:709-15.
21. Maso, M, Niedergang C, Schreiber V, Muller S, de-Murcia G. XRCC1 is specifically associated with poly (ADP-ribose) polymerase and negatively regulates its activity following DNA damage. Mol Cell Biol 1998;18:3563.
22. Fan J, Wilson DM. Protein–protein interactions and posttranslational modifications in mammalian base excision repair. Free Radical Biol Med 2005;38:1121-38.
23. Al-Zoubi MS, Mazzanti CM, Zavaglia K et al. Homozygous T172T and Heterozygous G135C Variants of Homologous Recombination Repairing Protein RAD51 are related to Sporadic Breast Cancer Susceptibility. Biochem Gen 2016;54:83-94.
24. Rasouly A, Pani B, Nudler E. A magic spot in genome maintenance. Trends Genet 2017;33:58-67.
25. Duell EJ, Millikan RC, Pittman GS et al. Polymorphisms in the DNA repair gene XRCC1 and breast cancer. Cancer Epidemiol Prev Biomarkers 2001;10:217-22.
26. Hu Z, Ma H, Lu D et al. A promoter polymorphism (– 77T> C) of DNA repair gene XRCC1 is associated with risk of lung cancer in relation to tobacco smoking. Pharmacogenet Genom 2005;15:457-63.
27. Sarwar R., Bashir K, Saeed S, Mahjabeen I, Kayani MA. Association of Promoter Polymorphisms in Xrcc2 Gene Involved in DNA Double Strand Break Repair and Increased Susceptibility to Thyroid Cancer Risk in Pakistani Population. J Carcinog Mutagen 2016;7:3.
28. Hu Z, Hu X, Long J, Su L, Wei, B. XRCC1 polymorphisms and differentiated thyroid carcinoma risk: a meta-analysis. Gene 2013;528:67-73.
29. Kubota Y, Shimizu S, Yasuhira S, Horiuchi S. SNF2H interacts with XRCC1 and is involved in repair of H 2 O 2-induced DNA damage. DNA Repair 2016;43:69-77.
30. Lin YJ, Wan L, Huang CM et al. Polymorphisms in the DNA repair gene XRCC1 and associations with systemic lupus erythematosus risk in the Taiwanese Han Chinese population. Lupus 2009;18:1246-51.
31. Yan L, Li Q, Li X, Ji H, Zhang L. Association Studies between XRCC1, XRCC2, XRCC3 Polymorphisms and Differentiated Thyroid Carcinoma. Cell Physiol Biochem 2016;38:1075-84.
32. Forat-Yazdi M, Gholi-Nataj M, Neamatzadeh H, Nourbakhsh P, Shaker-Ardakani H. Association of XRCC1 Arg399Gln polymorphism with colorectal cancer risk: a HuGE meta analysis of 35 studies. Asian Pac J Cancer Prev 2015;16:3285-91.
33. Wei X, Chen D, Lv T. A functional polymorphism in XRCC1 is associated with glioma risk: evidence from a meta-analysis. Mol Biol Reports 2013;40:567-72.
34. Feng X, Miao G, Han Y, Xu Y, Wu H. Glioma risks associate with genetic polymorphisms of XRCC1 gene in Chinese population. J Cell Biochem 2014;115:1122-7.
35. Zhu W, Yao J, Li Y, Xu B. Assessment of the association between XRCC1 Arg399Gln polymorphism and glioma susceptibility. Tumor Biol 2014;35:3061-6.
36. Zhang XL, Lu Y, Yang S et al. An updated metaanalysis between the association of XRCC1 Arg399Gln polymorphism and hepatocellular carcinoma risk. Asian Pac J Cancer Prev 2014;15:3273-8.
37. He LW, Shi R, Jiang L, Zeng Y, Ma WL, Zhou JY. XRCC1 gene polymorphisms and glioma risk in Chinese population: a meta-analysis. PLoS One 2014;9:e111981.
38. Wang R, Hu X, Zhou Y et al. XRCC1 Arg399Gln and Arg194Trp polymorphisms in childhood acute lymphoblastic leukemia risk: a meta-analysis. Leukemia Lymphoma 2013;54:153-9.
39. Chiang FY, Wu CW, Hsiao PJ et al. Association between poly¬morphisms in DNA base excision repair genes XRCC1, APE1, and ADPRT and differentiated thyroid carcinoma. Clin Cancer Res 2008;14:5919-24.
40. Ho T, Li G, Lu J, Zhao C, Wei Q, Sturgis EM. Association of XRCC1 polymorphisms and risk of differentiated thyroid carcinoma: a case-control analysis. Thyroid 2009;19:129-35.
41. Sigurdson AJ, Land CE, Bhatti P et al. Thyroid nodules, polymorphic variants in DNA repair and RET-related genes, and interaction with ionizing radiation exposure from nuclear tests in Kazakhstan. Radiation Res 2009;171:77-88.
42. Gong W, Yang S, Yang X, Guo F. Blood preoperative neutrophil-to-lymphocyte ratio is correlated with TNM stage in patients with papillary thyroid cancer. Clinics 2016;71:311-4.
43. Jafari-Nedooshan J, Forat-Yazdi M, Neamatzadeh H, Zare Shehneh M, Kargar S, Seddighi N. Genetic Association of XRCC1 Gene rs1799782, rs25487 and rs25489 Polymorphisms with Risk of Thyroid Cancer: a Systematic Review and Meta-Analysis. Asian Pac J Cancer Prev 2017;18:263-70.
44. Matullo G, Dunning AM, Guarrera S et al. DNA repair polymorphisms and cancer risk in non-smokers in a cohort study. Carcinogenesis 2005;27:997-1007.
45. Al Mutairi FM, Alanazi M, Shalaby M, Alabdulkarim HA, Pathan AA, Parine NR. Association of XRCC1 gene

polymorphisms with breast cancer susceptibility in Saudi patients. Asian Pac J Cancer Prev 2013;14:3809-13.

46. Mao Y, Keller ET, Garfield DH, Shen K, Wang J. Stromal cells in tumor microenvironment and breast cancer. Cancer Metast Rev 2013;32:303-15.
47. Jin JM (Ed): The finite element method in electromagnetics. John Wiley & Sons, 2015 Feb 18.
48. Jiang T, Cui L, Chen L, Liu Z, Ren H. Genetic polymorphisms of XRCC1 gene and susceptibility to hepatocellular carcinoma in Chinese population. Med Oncol 2014;31:1-7.
49. Szaflik JP, Cuchra M, Przybylowska-Sygut K et al. Association of the 399Arg/Gln XRCC1, the 194 Arg/Trp XRCC1, the 326Ser/Cys OGG1, and the 324Gln/His MUTYH gene polymorphisms with clinical parameters and the risk for development of primary open-angle glaucoma. Mutat Res/Gen Toxicol Environm Mutagenesis 2013;753:12-22.
50. Bastos HN, Antao MR, Silva SN et al. Association of polymorphisms in genes of the homologous recombination DNA repair pathway and thyroid cancer risk. Thyroid 2009;19:1067-75.
51. Siraj-Blatchford I, Manni L. Would you like to tidy up now? An analysis of adult questioning in the English Foundation Stage. Early Years 2008;28:5-22.
52. García-Quispes WA, Pérez-Machado G, Akdi A et al. Association studies of OGG1, XRCC1, XRCC2 and XRCC3 polymorphisms with differentiated thyroid cancer. Mutat Res 2011;709-10: 67-72.
53. Akulevich NM, Saenko VA, Rogounovitch TI. Polymorphisms of DNA damage response genes in radiation-related and sporadic papillary thyroid carcinoma. Endocr Relat Cancer 2009;16:491-503.
54. Fard-Esfahani P, Fard-Esfahani A, Fayaz S et al. Association of Arg194Trp, Arg280His and Arg399Gln polymorphisms in X-ray repair cross-complementing group 1 gene and risk of differentiated thyroid carcinoma in Iran. Iranian Biomed J 2011;15:73.
55. Santos LS, Branko SC, Silva SN et al. Polymorphisms in base excision repair genes and thyroid cancer risk. Oncol Rep 2012;5:1859-68.
56. Patel AV, Calle EE, Pavluck AL, Feigelson HS, Thun MJ, Rodriguez C. A prospective study of XRCC1 (X-ray cross-complementing group 1) polymorphisms and breast cancer risk. Breast Cancer Res 2005;7:R1168.
57. Saadat M. Haplotype analysis of XRCC1 (at codons 194 and 399) and susceptibility to breast cancer, a meta-analysis of the literature. Breast Cancer Res Treat 2010;124:785-91.