Original Paper

Haplotype Based Analysis of XRCC3 Gene Polymorphisms in Thyroid Cancer

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Key Words

Thyroid cancer • Polymorphisms • DNA repair • Haplotypes

Abstract

Background/Aims: In mammalian cells, XRCC3 plays an important role in the DNA double-strand breaks (DSBs) repair by homologous recombination. Genetic polymorphisms in XRCC3 gene may potentially affect the repair of DSBs and thus confer susceptibility to thyroid cancer. In this study, we used a haplotype-based approach to investigate whether 5 selected SNPs i.e. rs1799796, rs1799794, rs861539, rs709399 and rs861530 of XRCC3 gene are associated with thyroid cancer risk in 456 cancer patients and 400 cancer-free controls. **Methods:** Genotyping was performed using Allele-specific PCR followed by sequencing. Statistical analysis was performed to analyse gene and haplotype association. **Results:** After analysis, frequency of mutant genotype/alleles of SNPs (rs1799796, $p < 0.0001$; rs1799794, $p < 0.0001$; rs861539, $p < 0.001$; rs709399, $p < 0.0001$; rs861530, $p < 0.002$) was found significantly higher in thyroid cancer patients compared to controls. Significant associations were found for most of the variant genotypes in SNPs of rs1799794, rs1799796, rs861539, rs861530 and rs709399 in papillary thyroid and follicular cancer patients compared to other histologic subtypes of thyroid carcinoma. Additionally, haplotype analysis revealed that haplotypes, AACGA ($p = 0.0005$), AGTAA ($p = 0.008$), GATAA ($p = 0.001$), GGCAA ($p = 0.001$) were linked with significant increase in thyroid cancer risk. However, haplotype AGCGG ($p = 0.0009$) was associated with a significant reduced thyroid cancer risk. **Conclusion:** Our results suggest that common genetic variants in the XRCC3 gene of DSB repair pathway may modulate thyroid cancer risk.

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Introduction

Thyroid cancer is the most frequent malignancy of thyroid gland and the most increasing endocrine cancer in Pakistan and globally [1]. Its increasing incidence is the main reason for considerable attention of recent researches [2]. In Pakistan, thyroid cancer is responsible for 1.2% cases of all malignancies, comprising 69-71% papillary carcinoma, 11-13% follicular carcinoma, 3-5% medullary carcinoma and 1-2% anaplastic carcinoma [3]. Its increasing incidence despite of good survival rate is still alarming in Europe [4, 5], Canada [6, 7], United Kingdom, UK [8] and United States of America, USA [9]. Now it is considered as fastest growing and sixth most common cancer type [10].

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The DNA double strand breaks (DSBs) are the most lethal type of DNA lesions involved in many tumorigenesis initiation and progression and cause cellular genomic instability [11, 12]. These double strand breaks arise due to exposure to exogenous agents such as ionizing radiations (IRs) and alkylating agents [13]. Homologous recombination repair (HRR) is essentially an error-proof mechanism that occurs during the S or G₂ phases of the cell cycle, when the sister chromatid can provide a template for accurate repair [14]. Accumulating evidence indicates that a reduced DNA damage repair capacity can lead to a predisposition to DNA damage, mutations and subsequently results in cancer [15]. The precise mechanisms of regulation in the DNA DSBs repair is not well understood, but a number of genes are involved in regulation of DNA repair pathways.

X-ray repair cross-complementing group 3 (*XRCC3*) is one of the central protein, playing an important role in HRR pathway. *XRCC3* interacts and stabilizes RAD51 and involves in HRR for DNA DSBs and cross-link repair in mammalian cells [16]. *XRCC3* is a paralog of RAD51, and similar to RAD51, it is essential for genetic stability [17].

Genetic polymorphisms have now been identified in a number of genes involved in repairing DSBs and including genes belonging to the HRR repair pathway. The *XRCC3* SNP rs861539 leads to amino acid substitution that may affect the function and/or its interaction with other proteins involved in DNA damage and repair [18]. The SNP rs1799794 (4541 A > G) is located in 5'UTR and the SNP rs1799796 (17893 A > G) is located in exon 5 splice site [19]. Previous studies have examined the relationship between different *XRCC3* polymorphisms with the risk of different cancers such as lung cancer, breast cancer, ovarian cancer and head and neck cancer [20-25]. The association between *XRCC3* polymorphisms and thyroid cancer risk has not been studied in Pakistani population however, published studies in other populations remain confusing and focussed on single variant rather than haplotype-based gene-gene interaction analysis. Thus we conducted this case-control study to investigate the association of the *XRCC3* polymorphism on the risk for thyroid cancer.

Material and Methods

Study subjects and ethical approval

The subjects included in this study consisted of 456 diagnosed thyroid cancer patients and 400 age and sex-matched controls. The diagnosis of the thyroid cancer patients were all histologically confirmed at Nuclear medicine department of NORI (Nuclear oncology radiation institute) and PIMS (Pakistan institute of Medical Sciences), Islamabad. Controls were selected from individuals receiving routine medical examinations in these hospitals, and individuals with a previous diagnosis of any type of cancer were excluded. Blood samples from study participants were collected in EDTA vacutainer tubes and a structured questionnaire was filled by interviewing each participant at the time of sample collection about demographic factors, smoking, treatment and dietary habits. This study was approved by the institutional ethical review boards of COMSATS institute of information and technology, Islamabad and both collaborating hospitals, from which patients and controls were recruited. Additionally, all experiments were performed in accordance with relevant guidelines and regulations.

DNA Extraction

Approximately 3-4ml blood sample was collected in vacutainer tubes from all enrolled subjects in this study. DNA was extracted by Phenol-Chloroform method with some modifications [26]. The extracted DNA was quantified by 2% ethidium bromide gels and spectrophotometrically using Nano Drop (Thermoscientific, USA) and stored at 4°C until used.

Selection of polymorphisms

The five polymorphisms studied were selected from X-ray repair complementing defective repair in Chinese hamster cells 3 (*XRCC3*) gene. Selected SNPs included one intronic polymorphism 17893A/G (rs1799796), one 5'UTR splice site polymorphism 4541A/G (rs1799794), one missense mutation in exon 7, 18067C/T or Thr241Met, (rs861539) and two polymorphisms from 3'UTR region 19279C/T (rs709399)

and 12701G/A (rs861530). The selection of these polymorphisms were based on their wide evaluation in association with different forms of cancer. Primers for PCR amplification were designed by WASP (web based allele specific primer designing tool).

Genotyping

Selected *XRCC3* polymorphisms were genotyped in 456 thyroid cancer patients and 400 controls. ARMS-Polymerase chain reaction was employed to determine the genotypes of all selected hot spot polymorphisms in this study. PCR reaction was carried out in a reaction volume of 10µl containing 50-100ng genomic DNA, 100µM of each primers and Solis BioDyne master mix. The thermal cycling conditions used was: 94°C for 1min, 35 cycles of 94°C for 30 sec, optimized annealing temperature for 45 sec, 72°C for 1 min and final extension for 7 minutes. The amplified product was visualized on 2% agarose gel electrophoresis (100V for 45min). ARMS-PCR method was tested with internal control, beta actin as a positive control. PCR products with homozygous wild, homozygous mutant and heterozygous genotype were further confirmed by DNA sequencing analysis and duplicate PCR with random samples.

Statistical analysis

Categorical data were summarized using frequencies and percentiles. Comparison of two groups of categorical variables was performed using Chi- square (χ^2) test or Fisher's exact test if the number was <5. All reported p values were two-sided and $p \leq 0.05$ was considered to be statistically significant. Logistic regression analyses were used to calculate the adjusted odds ratios (ORs) and 95% confidence intervals (CIs) to determine the risk inflation of different allele/genotype in cancer. Analysis was performed using GraphPad prism software version 6.0.

Haplotype combinations were estimated from gene data. The haplotype analysis and linkage disequilibrium (LD) were performed using Haploview 4.2, in which expectation maximization (EM) algorithm was used to infer haplotypes from the observed genotypes with an unknown linkage phase. The LD coefficient (D') <0.2 was considered to have no LD, $D' > 0.5$ had a LD, $D' > 0.8$ had a strong LD and $D' = 1$ is in complete LD. r^2 was the relevant factor. The difference in the distribution of the haplotype frequencies between cases and control groups were assessed with χ^2 test. Allele combination analysis was adopted to examine joint effects of five *XRCC3* polymorphisms on thyroid cancer risk after adjusting the covariates (age, gender and smoking). Additionally, to address the multiple comparison analysis, a Bonferroni correction was applied to the comparisons. The Bonferroni correction is a conservative method that is free of independency and distribution assumptions. The Bonferroni correction set the significance level at α (alpha)/n (number of comparisons). The specific multiple comparisons that were used for each Bonferroni-corrected P value are stated below each table.

Results

Characteristics of Patients and Controls

In this study, 456 thyroid cancer patients and 400 control subjects were analyzed for five selected SNPs of *XRCC3* gene i.e. rs1799796, rs1799794, rs861539 (Thr241Met), rs709399 and rs861530 and demographic data of these thyroid cancer patients and controls is given in Table 1.

Associations between *XRCC3* SNPs and thyroid cancer risk

The genotypes and allele frequencies distribution for five *XRCC3* SNPs and thyroid cancer risk are given in Table 2. The homozygous wild-type genotype was used as the reference group to calculate the odd ratios (ORs) and 95% confidence interval (CI). We found a significant association between *XRCC3* SNPs and thyroid cancer risk. For first selected SNP (rs1799794) of *XRCC3* gene, frequency of variant G allele (OR: 1.70, 95% CI: 1.35-2.15, $P < 0.0001$) was significantly higher in patients compared to controls. For the second selected SNP (rs1799796) of *XRCC3* gene, the frequency of variant G allele (OR: 1.56, 95% CI: 1.27-1.91, $P < 0.0001$) was statistically higher in patients compared to controls as shown in Table 2.

Genotyping of third selected SNP (rs861539) of *XRCC3* showed that frequency of homozygous mutant TT (OR: 2.08, 95% CI: 1.34-3.24, $p = 0.001$) along with variant T allele frequen-

cy (OR: 1.40, 95% CI: 1.12-1.75, $p = 0.003$) were found significantly higher in thyroid cancer patients group compared to controls. For fourth selected SNP (rs861530) of *XRCC3* gene, frequencies of the heterozygous mutant (AG) genotype (OR: 2.45, 95% CI: 1.33-3.61, $P = 0.0008$), homozygous mutant (GG) genotype (OR: 2.39, 95% CI: 1.52-3.75, $P = 0.002$) and variant G allele frequency (OR: 1.40, 95% CI: 1.12-1.75, $P < 0.0001$) were observed statistically significantly higher in patients compared to control group. Genotyping of fifth SNP (rs709399) of *XRCC3* gene showed that frequencies of homozygous mutant (AA) genotype (OR: 1.94, 95% CI: 1.44-2.62, $P < 0.0001$) and variant A allele (OR: 2.01, 95% CI: 1.65-2.44, $P < 0.0001$) were significantly associated with increased risk of thyroid cancer compared to controls as shown in Table 2.

Subgroup analysis by different histological types of thyroid cancer was further performed on the association between SNPs and thyroid cancer risk. Significant associations was found for most of the variant genotypes in SNPs of rs1799794, rs1799796, rs861539, rs861530 and rs709399 in papillary thyroid cancer patients. Also significant association existed with follicular thyroid carcinoma in SNPs of rs1799796, rs861539 and rs709399. While only significant association with heterozygous genotype, GA; $p = 0.02$ of rs709399 with medullary thyroid cancer and for heterozygous genotype AG; $p = 0.04$ of rs1799796 with anaplastic thyroid cancer was observed as shown in Table 3.

Table 1. Frequency distribution analysis of selected demographic and risk factors in thyroid cancer cases and controls. ^aFor Chi square test; ^bFischer exact test. OR=odds ratio and CI= confidence interval. * $P \leq 0.05$ =significant value

Variables	Cancer cases (N=456)	Control (N=400)	OR (95%CI)	*P-value
1. Gender				
Males	107 (23.4%)	70 (17.5%)		
Females	349 (76.5%)	330 (82.5%)	1.44 (1.03-2.02)	0.03 ^a
2. Age (years)				
Median (Range)	42.5±13 (15-75)	42±13.2(20-65)		
≤42	208 (45%)	179 (44.75%)		
>42	248 (55%)	221 (55.25%)	1.03 (0.79-1.35)	0.79 ^a
3. Family History of Cancer				
Yes	34 (8%)	9 (2.25%)		
No	422 (92%)	391 (97.75%)	3.5 (1.65-7.39)	0.001 ^b
4. Smoking History (cigarette, paan, betel quid, moist sniff)				
Smokers	109 (24%)	119 (30%)		
Non-Smokers	347 (76%)	281 (70%)	0.55 (0.41-0.72)	< 0.0001 ^a
5. Type of thyroid cancer				
Papillary	351 (77%)	-		
Follicular	82 (17%)	-		
Medullary	16 (3.5%)	-		
Anaplastic	7 (1.5%)	-		

Table 2. Genotypic and allelic frequency distribution of *XRCC3* SNPs between thyroid cancer cases and controls. Abbreviation: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; *P-value calculated by χ^2 -test

Genotype / Allele	Cases (456)	Controls (400)	OR (95% CI)	*P- value
4541A/G(rs1799794)				
AA	289 (63%)	297 (74.3%)	1.00 (Ref)	
AG	90 (20%)	65 (16%)	1.54 (1.06-2.22)	0.02
GG	77 (17%)	38 (9.7%)	1.27 (0.82-1.98)	0.27
A allele frequency	668 (73%)	659 (82%)	1.00 (Ref)	1
G allele frequency	244 (27%)	141 (18%)	1.70 (1.35-2.15)	<0.0001
17893A/G(rs1799796)				
AA	284 (55.7)	212 (53%)	1.00 (Ref)	
AG	104 (22.8)	128 (32%)	0.78 (1.02-1.84)	0.03
GG	68 (21.5)	60 (15%)	0.55 (1.08-2.20)	0.01
A allele frequency	672 (73)	552 (69)	1.00 (Ref)	
G allele frequency	240 (27)	248(37)	0.56(1.27-1.91)	<0.0001
18067C/T or T241M, (rs861539)				
CC	277 (60.8)	273 (68.2%)	1.00 (Ref)	
CT	109 (23.9)	85 (21.2%)	1.16(0.84-1.60)	0.35
TT	70 (15.3)	42 (10.5%)	2.08(1.34-3.24)	0.001
C allele frequency	663 (73)	631(79)	1.00 (Ref)	
G allele frequency	249 (27)	169(21)	1.40(1.12-1.75)	0.003
12701G/A (rs861530)				
AA	199 (44%)	222 (55.5%)	1.00 (Ref)	
AG	150 (33%)	98 (24.5%)	2.45 (1.33-3.61)	0.0008
GG	107(23%)	80 (20%)	2.39 (1.52-3.75)	0.002
A allele frequency	548 (60%)	572 (71.5%)	1	
G allele frequency	364 (40%)	228 (28.5)	1.66(1.36-2.04)	<0.0001
19279G/A (rs709399)				
GG	132(28.9)	198 (49.5%)	1	
GA	155(34)	109 (27.2%)	1.37(1.02-1.84)	0.03
AA	169(37.1)	93 (23.2%)	1.94(1.44-2.62)	<0.0001
G allele frequency	419(46)	505(63)	1	
A allele frequency	493(54)	295(37)	2.01 (1.65-2.44)	<0.0001

Haplotype analysis of the *XRCC3* SNPs

Haplotype analysis was performed to investigate the effects of all possible haplotypes associated with thyroid cancer disease and to see whether the five SNPs were in linkage disequilibrium. The rare haplotypes (with frequency <5%) were excluded from the associa-

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

Genotypes XRCC3	Controls (n=400)	n	Papillary carcinoma (n=351) OR (95% CI)	*P-value	n	Follicular carcinoma (n=82) OR (95% CI)	*P-value	n	Medullary carcinoma (n=16) OR (95% CI)	*P-value	n	Anaplastic (n=7) OR (95% CI)	*P-value
rs1799794													
AA	297	229	1.00(Ref)		60	1.00(Ref)		9	1.00(Ref)		3	1.00(Ref)	
AG	65	79	1.49 (1.03-2.15)	0.03	12	0.88 (0.45-1.72)	0.71	5	2.34 (0.78-6.96)	0.12	2	2.06 (0.39-10.8)	0.39
GG	38	47	1.47 (0.93-2.31)	0.09	10	1.32(0.63-2.77)	0.45	2	1.36 (0.29-6.21)	0.69	2	3.81 (0.71-20.31)	0.11
rs1799796													
AA	212	133	1.00(Ref)		62	1.00(Ref)		8	1.00(Ref)		1	1.00(Ref)	
AG	128	131	1.26 (0.93-1.71)	0.12	14	0.43 (0.23-0.80)	0.008	4	0.70 (0.22-2.23)	0.55	5	5.31 (1.01-27.7)	0.04
GG	60	87	1.86 (1.29-2.69)	0.0008	6	0.44 (0.18-1.07)	0.07	4	1.88 (0.58-6.05)	0.28	1	0.94 (0.11-7.98)	0.95
rs861539													
CC	273	191	1.00(Ref)		78	1.00(Ref)		6	1.00(Ref)		2	1.00(Ref)	
CT	85	96	1.39 (0.99-1.95)	0.05	3	0.14 (0.04-0.45)	0.001	6	2.22 (0.78-6.29)	0.13	4	4.94 (1.08-22.50)	0.03
TT	42	64	1.90 (1.25-2.89)	0.002	1	0.10 (0.01-0.77)	0.02	4	2.84 (0.87-9.20)	0.08	1	1.42 (0.16-12.08)	0.74
rs861530													
AA	222	147	1.00(Ref)		61	1.00(Ref)		5	1.00(Ref)		1	1.00(Ref)	
AG	98	138	1.99 (1.46-2.72)	<0.0001	9	0.37 (0.18-0.78)	0.08	7	2.39 (0.86-6.60)	0.09	3	2.31 (0.50-10.50)	0.27
GG	80	66	0.92 (0.64-1.33)	0.67	12	0.68 (0.35-1.32)	0.26	4	1.33 (0.41-4.24)	0.62	3	3.01 (0.65-13.6)	0.15
rs709399													
GG	198	51	1.00(Ref)		67	1.00(Ref)		9	1.00(Ref)		5	1.00(Ref)	
GA	109	143	1.83 (1.35-2.49)	0.0001	7	0.24 (0.11-0.55)	0.0007	3	0.23 (0.06-0.83)	0.02	2	1.06 (0.20-5.58)	0.93
AA	93	157	0.93 (0.68-1.28)	< 0.0001	8	0.35 (0.16-0.76)	0.008	4	1.10 (0.34-3.49)	0.87	0	0.21 (0.01-3.87)	0.01

tion analyses. The most common haplotypes of the five polymorphisms, calculated by Haploview 4.2, are summarized in Table 4.

The haplotypes were generated using the five XRCC3 intragenic SNPs, i.e. rs1799796, rs1799794, rs861539 (Thr241Met), rs709399 and rs861530 in the thyroid cancer patients and controls, and 32 different haplotypes were generated which accounted for most of the haplotypes in the case and control group. To decrease the type I error for relatively small sample size, we applied Bonferroni correction and after this correction, only five haplotypes maintained their significance. Among these five haplotypes, AACGA haplotype (P=0.0005), AGTAA haplotype (P=0.008), GATAA haplotype (P=0.001), GGCAA haplotype (P=0.001) were linked with significant increased risk of thyroid cancer in patient com-

Table 4. Frequency distribution of XRCC3 haplotypes in thyroid cancer cases and controls. Abbreviation: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; *P-value calculated by Chi square, χ^2 -test

XRCC3 haplotypes (SNPs)					Frequency		χ^2	*P-value
rs1799794	rs1799796	rs861539	rs709399	rs861530	Cases	Controls		
A	A	C	A	G	0.08	0.201	41.7	Ref
A	A	C	A	A	0.11	0.122	0.31	0.57
A	A	C	G	A	0.07	0.033	12.0	0.0005
A	A	C	G	G	0.06	0.095	4.80	0.02
A	A	T	A	A	0.039	0.041	0.02	0.87
A	A	T	A	G	0.037	0.060	4.48	0.03
A	A	T	G	A	0.025	0.013	3.27	0.07
A	A	T	G	G	0.025	0.021	0.34	0.56
A	G	C	A	A	0.067	0.043	5.15	0.02
A	G	C	A	G	0.048	0.057	0.56	0.45
A	G	C	G	A	0.055	0.032	5.68	0.01
A	G	C	G	G	0.021	0.052	10.9	0.0009
A	G	T	A	A	0.026	0.009	6.94	0.008
A	G	T	A	G	0.014	0.027	-	0.98
A	G	T	G	A	0.016	0.006	3.18	0.07
A	G	T	G	G	0.038	0.010	1.12	0.28
G	A	C	A	A	0.022	0.037	0.03	0.86
G	A	C	A	G	0.024	0.038	3.29	0.06
G	A	C	G	A	0.020	0.010	5.29	0.02
G	A	C	G	G	0.015	0.024	0.16	0.68
G	A	T	A	A	0.021	0.001	10.2	0.001
G	A	T	A	G	0.009	0.008	2.33	0.12
G	A	T	G	A	0.014	0.001	-	-
G	A	T	G	G	0.028	0.010	0.59	0.44
G	G	C	A	A	0.025	0.007	0.98	0.001
G	G	C	A	G	0.009	0.022	0.24	0.62
G	G	C	G	A	0.018	0.009	-	-
G	G	C	G	G	0.009	0.006	5.44	0.01
G	G	T	A	A	0.006	0.004	-	-
G	G	T	A	G	0.004	0.000	-	-
G	G	T	G	A	0.012	0.000	-	-
G	G	T	G	G	0.022	0.000	9.50	0.002

Table 5. The distribution of rs1799794 and rs1799796 combined genotypes in thyroid cancer cases and controls. Abbreviations: OR, odds ratios; CI, confidence intervals; n, number of individuals. *P-value calculated by Chi square, χ^2 -test. P value after Bonferroni correction i.e. P = 0.006 (calculated as 0.05/8). P values in bold have still maintained their significance after Bonferroni correction

rs1799794-rs1799796	Cases, n	Controls, n	OR (95% CI)	*P-value
AA/AA	130	169	1.00	
AA/AG	102	86	1.05(0.76-1.45)	0.75
AA/GG	57	42	1.21(0.79-1.85)	0.36
AG/AA	44	38	1.01(0.64-1.60)	0.94
AG/AG	24	15	1.42(0.73-2.75)	0.29
AG/GG	22	12	1.64(0.80-3.37)	0.12
GG/AA	30	25	1.05(0.61-1.82)	0.84
GG/AG	28	7	3.70(1.58-8.50)	0.002
GG/GG	19	6	2.85(1.12-7.22)	0.02

pared to controls when AACAG haplotype was taken as reference. However, fifth haplotype AGCGG ($P=0.0009$) was associated with a significant reduced risk of thyroid cancer. Following Bonferroni correction, the haplotype analysis did not show any significance for the other haplotypes of XRCC3 gene polymorphisms. Furthermore, no linkage disequilibrium was observed for selected SNPs of XRCC3 gene.

Combined genotype analysis of XRCC3 SNPs

Tables 5-14 summarize the association studies among the combined genotypes of the five XRCC3 SNPs and overall risk for thyroid cancer. Statistical significant association is considered for each combined genotype after Bonferroni correction. The combined GG/AG genotype frequency (OR: 3.70, 95% CI: 1.58-8.50, $p=0.002$) of rs1799794 and rs1799796 maintained its statistical significance to ~4 fold increase in thyroid cancer risk in patients compared to controls as shown in Table 5. Four fold significant increase in thyroid cancer risk was also observed for rs1799794 and rs861539 in combined AG/TT genotype (OR: 4.19, 95% CI: 1.58-11.14, $p=0.004$) in thyroid cancer patients compared to controls (Table 6). For rs1799794-rs861530 genotype to genotype interaction, ~5 fold increased risk was observed in combined GG/AG genotype (OR: 4.77, 95% CI: 1.81-12.56, $p=0.001$) in thyroid cancer patients compared to controls when combined AA/AA wild genotype was taken as reference (Table 7).

The combined AA/AA genotype (OR: 1.64, 95% CI: 1.17-2.30, $p=0.003$) and GG/GA genotype frequencies (OR: 4.29, 95% CI: 1.76-10.4, $p=0.001$) for rs1799794 and rs709399 polymorphisms were significantly higher in thyroid cancer patients compared to control group when compared with combined AA/GG genotype as reference. However combined AG/GA ($p=0.03$) and GG/AA genotype frequencies lost their significant values after Bonferroni correction (Table 8). For rs1799796 and rs861539, combined AG/TT genotype frequency (OR: 2.94, 95% CI: 1.47-5.86, $p=0.002$) was ~3 fold higher in thyroid cancer patients versus control group when compared with combined AA/CC wild genotype as reference (Table 9).

Table 6. The distribution of rs1799794 and rs861539 combined genotypes in thyroid cancer cases and controls. Abbreviations: OR, odds ratios; CI, confidence intervals; n, number of individuals. *P-value calculated by Chi square, χ^2 -test. P value after Bonferroni correction i.e. $P=0.006$ (calculated as 0.05/8). P values in bold have still maintained their significance after Bonferroni correction

rs1799794-rs861539	Cases	Controls	OR (95% CI)	*P-value
AA/CC	178	192	1.00	
AA/CT	11	71	0.72(0.50-1.05)	0.09
AA/TT	49	34	1.29(0.81-2.05)	0.26
AG/CC	53	50	0.92(0.60-1.39)	0.69
AG/CT	14	10	1.23(0.54-2.81)	0.61
AG/TT	23	5	4.19(1.58-11.14)	0.004
GG/CC	46	31	1.33(0.82-2.15)	0.23
GG/CT	16	3	4.00(1.19-10.8)	0.02
GG/TT	15	3	4.50(1.29-15.6)	0.01

Table 7. The distribution of rs1799794 and rs861530 combined genotypes in thyroid cancer cases and controls. Abbreviations: OR, odds ratios; CI, confidence intervals; n, number of individuals. *P-value calculated by Chi square, χ^2 -test. P value after Bonferroni correction i.e. $P=0.006$ (calculated as 0.05/8). P values in bold have still maintained their significance after Bonferroni correction

rs1799794-rs861530	Cases	Controls	OR (95% CI)	*P-value
AA/AA	128	164	1.00	
AA/AG	90	80	0.98(0.70-1.37)	0.92
AA/GG	71	53	1.20(0.82-1.77)	0.33
AG/AA	39	34	1.00(0.62-1.62)	0.97
AG/AG	34	13	2.39(1.24-4.61)	0.008
AG/GG	17	18	0.82(0.41-1.61)	0.56
GG/AA	32	24	1.18(0.68-2.04)	0.54
GG/AG	26	5	4.77(1.81-12.56)	0.001
GG/GG	19	9	1.88(0.84-4.22)	0.12

Table 8. The distribution of rs1799794 and rs709399 combined genotypes in thyroid cancer cases and controls. Abbreviations: OR, odds ratios; CI, confidence intervals; n, number of individuals. *P-value calculated by Chi square, χ^2 -test. P value after Bonferroni correction i.e. $P=0.006$ (calculated as 0.05/8). P values in bold have still maintained their significance after Bonferroni correction

rs1799794-rs709399	Cases	Controls	OR (95% CI)	*P-value
AA/GG	80	148	1.00	
AA/GA	94	81	1.02(0.73-1.42)	0.89
AA/AA	115	68	1.64(1.17-2.30)	0.003
AG/GG	29	31	0.80(0.47-1.36)	0.42
AG/GA	33	22	1.34(0.76-2.33)	0.30
AG/AA	28	12	2.11(1.06-4.21)	0.03
GG/GG	23	19	1.06(0.57-1.98)	0.84
GG/GA	28	6	4.29(1.76-10.4)	0.001
GG/AA	26	13	1.80(0.91-3.55)	0.01

No statistical significance was maintained for all combined genotype combinations for rs1799796 and rs861530 compared to combined AA/AA genotype after Bonferroni correction (Table 10). For the rs1799796 and rs709399 combined genotypes distribution, the combined AG/GG genotype (OR: 2.05, 95% CI: 1.26-3.33, $p = 0.003$) and combined GG/AA genotype frequencies (OR: 3.02, 95% CI: 1.59-5.71, $p = 0.0007$) were significantly higher in patients compared to controls. The combined AA/GG wild genotypes were used as reference (Table 11). For rs861539 and rs861530 combined genotype distribution, only combined TT/GG genotype frequency (OR: 3.16, 95% CI: 1.42-7.03, $p = 0.004$) increased ~4 fold risk in thyroid cancer patients versus controls when compared with combined CC/GG genotype (Table 12). For rs861539 and rs709399 combined genotype analysis when compared with wild combined CC/GG genotype as reference, combined CC/AA genotype (OR: 1.61, 95% CI: 1.15-2.27, $p = 0.005$) and combined TT/AA genotype frequency (OR: 3.39, 95% CI: 1.45-7.90, $p = 0.004$) were significantly higher in thyroid cancer patients compared to controls as shown in Table 13. After Bonferroni correction, for combined genotype analysis of rs861530 and rs709399, only combined AG/AA genotype frequency maintained its significance and ~3 fold increased risk of thyroid cancer was observed in patients compared to controls when combined AA/GG genotype was taken as reference for comparison (Table 14).

Discussion

Targeting DNA damage becomes an effective approach to increase the efficiency of radiation therapy [27]. X-ray repair complementing defective repair in Chinese hamster cells 3 (*XRCC3*) belongs to a family of genes responsible for repairing DNA double strand breaks caused by normal metabolic processes or exposure to ionizing radiation [20]. *XRCC3* proteins are recruited to a DSB during the S and G2/M phase of the cell cycle. Considering the importance of *XRCC3* to maintain chromosomal stability by repairing most lethal DSBs and inhibiting tumorigenesis, we hypothesized that change in *XRCC3* expression affected by

Table 9. The distribution of rs1799796 and rs861539 combined genotypes in thyroid cancer cases and controls. Abbreviations: OR, odds ratios; CI, confidence intervals; n, number of individuals. *P-value calculated by Chi square, χ^2 -test. P value after Bonferroni correction i.e. $P = 0.006$ (calculated as 0.05/8). P values in bold have still maintained their significance after Bonferroni correction

rs1799796-rs861539	Cases	Controls	OR (95% CI)	*P-value
AA/CC	122	153	1.00	
AA/CT	48	53	0.77(0.50-1.16)	0.21
AA/TT	34	26	1.15(0.68-1.96)	0.58
AG/CC	94	79	1.05(0.74-1.47)	0.75
AG/CT	25	18	1.23(0.66-2.29)	0.51
AG/TT	35	11	2.94(1.47-5.86)	0.002
GG/CC	61	41	1.35(0.88-2.05)	0.15
GG/CT	19	14	1.19(0.59-2.42)	0.61
GG/TT	18	5	3.24(1.19-8.82)	0.02

Table 10. The distribution of rs1799796vs rs861530 combined genotypes in thyroid cancer cases and controls. Abbreviations: OR, odds ratios; CI, confidence intervals; n, number of individuals. *P-value calculated by Chi square, χ^2 -test. P value after Bonferroni correction i.e. $P = 0.006$ (calculated as 0.05/8). P values in bold have still maintained their significance after Bonferroni correction

rs1799796-rs861530	Cases	Controls	OR (95% CI)	*P-value
AA/AA	90	141	1.00	
AA/AG	65	46	1.27(0.85-1.91)	0.23
AA/GG	49	45	0.94(0.61-1.45)	0.81
AG/AA	63	59	0.92(0.63-1.35)	0.69
AG/AG	53	29	1.68(1.04-2.70)	0.03
AG/GG	38	20	1.72(0.98-3.02)	0.05
GG/AA	46	22	1.92(1.13-3.26)	0.01
GG/AG	32	23	1.23(0.71-2.15)	0.45
GG/GG	20	15	1.17(0.59-2.33)	0.63

Table 11. The distribution of rs1799796 and rs709399 combined genotypes in thyroid cancer cases and controls. Abbreviations: OR, odds ratios; CI, confidence intervals; n, number of individuals. *P-value calculated by Chi square, χ^2 -test. P value after Bonferroni correction i.e. $P = 0.006$ (calculated as 0.05/8). P values in bold have still maintained their significance after Bonferroni correction

rs1799796-rs709399	Cases	Controls	ORs (95% CI)	*P-value
AA/GG	54	124	1.00	
AA/GA	80	54	1.36(0.93-1.98)	0.10
AA/AA	70	54	1.16(0.79-1.70)	0.44
AG/GG	49	43	0.99(0.64-1.54)	0.99
AG/GA	48	39	1.08(0.69-1.70)	0.70
AG/AA	57	26	2.05(1.26-3.33)	0.003
GG/GG	29	31	0.80(0.47-1.36)	0.42
GG/GA	27	16	1.51(0.80-2.84)	0.20
GG/AA	42	13	3.02(1.59-5.71)	0.0007

polymorphisms in promoter, exonic and intronic region might modulate the pathogenesis of thyroid cancer. Present study was designed to assess the association of five selected SNPs with thyroid carcinogenesis. We also investigated whether or not the five SNPs were in linkage disequilibrium or association of common haplotypes of these SNPs with thyroid carcinogenesis. Additionally, we also estimated the association among the combined genotypes of these SNPs and overall risk of thyroid cancer.

In this study, the variant allele frequencies for selected *XRCC3* polymorphisms (rs1799794, rs861539, rs861530 and rs709399) were found to increase the risk of thyroid cancer, however GG genotype frequency of rs1799796 was found to decrease the thyroid cancer risk. First two SNP rs1799794, rs861530 included in this study are located in 3' UTR region of *XRCC3* and their functional significance has not yet been elucidated. However, their 3' UTR location can affect polyadenylation and alterations in regulatory protein-mRNA and miRNA-mRNA interactions. It can cause disease directly, modifying the severity of the disease phenotype, or be linked with disease susceptibility [28-30]. Third SNP with increased risk of thyroid cancer, rs861539 (Thr241Met in exon 5 coding region) is the most extensively studied genetic variant of *XRCC3* [31-33] and evidence indicates that the Thr/Met variant resides in the adenosine triphosphate-binding domain of *XRCC3*, the only domain with known functional activity [34]. This fact suggests that the change caused by the *XRCC3* Thr241Met polymorphism is associated with other proteins, especially *RAD51* [35]. The product of *XRCC3* is a *RAD51* paralog necessary for the binding and stabilization of the latter during DNA repair. In our study, the increased risk of rs861539 polymorphism in thyroid cancer is comparable to previous results reporting significant associations with thyroid cancer risk [36-40]. Nevertheless, many studies have found no evident correlation between *XRCC3* polymorphisms and thyroid cancer [41, 42]. The rs861539 polymorphism has also been reported associated with other cancers such as colorectal cancer [43, 44], ovarian cancer [19], glioma [45], bladder cancer [32, 46] and acute myeloid leukemia [47, 48].

Table 12. The distribution of rs861539 and rs861530 combined genotypes in thyroid cancer cases and controls. Abbreviations: OR, odds ratios; CI, confidence intervals; n, number of individuals. *P-value calculated by Chi square, χ^2 -test. P value after Bonferroni correction i.e. P = 0.006 (calculated as 0.05/8). P values in bold have still maintained their significance after Bonferroni correction

rs861539-rs861530	Cases	Controls	OR (95% CI)	*P-value
CC/AA	121	147	1.00	
CC/AG	93	68	1.25(0.88-1.76)	0.20
CC/GG	63	58	0.94(0.64-1.38)	0.77
CT/AA	38	54	0.58(0.37-0.90)	0.01
CT/AG	33	17	1.75(0.96-3.20)	0.06
CT/GG	21	14	1.33(0.66-2.65)	0.41
TT/AA	40	21	1.78(1.00-2.99)	0.04
TT/AG	24	13	1.65(0.83-3.29)	0.15
TT/GG	23	8	3.16(1.42-7.03)	0.004

Table 13. The distribution of rs861539vs rs709399 combined genotypes in thyroid cancer cases and controls. Abbreviations: OR, odds ratios; CI, confidence intervals; n, number of individuals. *P-value calculated by Chi square, χ^2 -test. P value after Bonferroni correction i.e. P = 0.006 (calculated as 0.05/8). P values in bold have still maintained their significance after Bonferroni correction

rs861539-rs709399	Cases	Controls	OR (95% CI)	*P-value
CC/GG	74	134	1.00	
CC/GA	94	74	1.14(0.81-1.60)	0.43
CC/AA	109	65	1.61(1.15-2.27)	0.005
CT/GG	25	45	0.45(0.27-0.76)	0.002
CT/GA	33	19	1.56(0.87-2.79)	0.13
CT/AA	34	21	1.45(0.82-2.54)	0.19
TT/GG	33	19	1.56(0.87-2.79)	0.13
TT/GA	28	16	2.94(1.57(0.83-)	0.16
TT/AA	26	7	3.39(1.45-7.90)	0.004

Table 14. The distribution of rs861530 and rs709399 combined genotypes in thyroid cancer cases and controls. Abbreviations: OR, odds ratios; CI, confidence intervals; n, number of individuals. *P-value calculated by Chi square, χ^2 -test. P value after Bonferroni correction i.e. P = 0.006 (calculated as 0.05/8). P values in bold have still maintained their significance after Bonferroni correction

rs861530- rs709399	Cases	Controls	OR (95% CI)	*P-value
AA/GG	49	100	1.00	
AA/GA	75	64	1.03(0.71-1.48)	0.85
AA/AA	75	58	1.16(0.79-1.68)	0.43
AG/GG	54	56	1.03(0.71-1.48)	0.85
AG/GA	41	25	1.48(0.88-2.48)	0.13
AG/AA	55	17	3.09(1.76-5.41)	0.0001
GG/GG	29	42	0.57(0.35-0.94)	0.03
GG/GA	39	20	1.77(1.01-3.10)	0.04
GG/AA	39	18	2.00(1.11-3.52)	0.01

Fourth studied SNP of *XRCC3*, rs1799794, is located in the 5' UTR region and significant association of rs1799794 was observed with increased risk of thyroid cancer in this study. The underlying mechanism of its function may be related to its alteration in local DNA secondary structure or functional motif, thereby affecting the binding affinities of the relevant transcription factors [49]. In earlier studies, rs1799794 has been observed associated with increased risk of overall cancer [50], breast cancer [23], bladder cancer [51, 52]. Nevertheless, decreased risk of ovarian cancer has also been observed in some of the studies [20].

The results of haplotype analysis were consistent with the results of genotypic analysis, carriers of *XRCC3* haplotypes containing one of high risk alleles AACGA, AGTAA, GATAA and GGCAA had a significantly increased risk of thyroid cancer compared with those of the common haplotype AACAG. However haplotype AGCGG was associated with decreased risk of thyroid cancer. The possible reason for this protective role may be due to presence of G allele of rs1799796. It has earlier been reported that rs1799794/rs1799796/rs861539 haplotypes may decrease the risk of thyroid cancer and behave as a protective factor [53]. In one of the earlier haplotype based case-control study in hepatocellular carcinoma (HCC) risk in Chinese population, concluded that carriers with T allele of rs861539, G allele of rs861530 and rs3212092 T allele (TTG haplotype) have increased risk of HCC [54] which is comparable to our results. Two previous studies of haplotypes inferred from three *XRCC3* SNPs (rs1799794/rs1799796/rs861539) concluded that breast cancer risk is increased among individuals with "GAT" haplotype [55, 56]. It has been observed that *XRCC3* 241Met allele and its associated haplotype (4552A/18067T) were significantly inversely associated risk of squamous and basal cell carcinoma [57]. Smedby et al. [58] showed that the *XRCC3* haplotype-TCGTAAC (rs32120 24/rs3212038/rs3212057/ rs3212068/rs3212090) was more common for follicular lymphoma cases than controls. Finally, Zhou et al. [45] reported that rs861530 G and 3212092 T high-risk alleles contributed independently to risk of gliomas.

Our results of SNP-SNP interaction of *XRCC3* showed that the twelve combinations (rs1799794-GG with rs1799796-AG, rs1799794-AG with rs861539-TT, rs1799794-GG with rs861530-AG, rs1799794-AA with rs709399-AA, rs1799794-GG with rs709399-AG, rs1799796-AG with rs861539-TT, rs1799796-AG with rs709399-AA, rs1799796-GG with rs709399-AA, rs861539-TT with rs861530-GG, rs861539-CC with rs709399-AA, rs861539-CT with rs709399-GG, rs861539-TT with rs709399-AA and rs861530- AG with rs709399-AA) are associated with an increased thyroid cancer risk. The present combined genotype frequency and their significance in thyroid carcinogenesis may show the synergistic effect of the selected polymorphisms on the said disease. So far only a few studies have been conducted regarding the combined genotypes of *XRCC3* SNPs and their involvement in cancer, [59, 20] but the results are not comparable because they genotyped different SNPs.

Before making any conclusion, several potential limitations of this study should be addressed. First of all future studies, should incorporate a large sample size with various ethnics to confirm the association between *XRCC3* SNPs and thyroid carcinogenesis. Secondly, few well known risk factors for thyroid cancer have been discovered, therefore other genetic and environmental factors should be assessed. Thirdly, subjects in this case control study came from two hospitals and this may cause selection bias that could have substantial impact on overall conclusions. As a result, large scale studies that adjusting for a wide range of factors should be recommended to validate our findings.

The current available data suggest that the identification of patterns of genetic variations, in the form of haplotypes rather than single or point variations, may present a more promising approach to predict a person's response to drug therapy, and allow for highly individualized treatment of patients with complex diseases, such as cancer and their associated risk factors. In conclusion, our findings suggest a potential association between *XRCC3* (rs1799794, rs1799794, rs861539, rs861530 and rs709399) genetic variants and thyroid cancer disease susceptibility. Nevertheless, these conclusions should be re-confirmed with large samples and various ethnicities.

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Disclosure Statement

The authors declare that they have no competing interests.

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