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The role of Ile3434Thr *XRCC7* gene polymorphism in Differentiated Thyroid Cancer risk in an Iranian population

Maryam Rahimi[1], Shima Fayaz[2], Armaghan Fard-Esfahani[3], Mohammad Hossein Modarressi[4], Seyed Mohammad Akrami[4] and Pezhman Fard-Esfahani[*2]

[1]Science and Research Branch of Islamic Azad University, Tehran; [2]Dept. of Biochemistry, Pasteur Institute of Iran, Tehran; [3]Research Institute for Nuclear Medicine, Tehran University of Medical Sciences, Tehran; [4]Dept. of Medical Genetics, Tehran University of Medical Science, Tehran, Iran

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ABSTRACT

Background: The aim of this study was to understand any association between differentiated thyroid carcinoma (DTC) and *Ile3434Thr XRCC7* gene polymorphism (GenBank accession number: rs7830743). DTC is the most prevalent thyroid neoplasm, which includes papillary and follicular cell carcinoma. *XRCC7* gene encodes a protein that functions in non-homologous end joining DNA repair pathway. Non-synonymous polymorphisms in this gene may alter DNA repair capacity of the cell and change the risk of developing cancers. **Methods:** DTC patients (n = 173) and cancer free individuals (n = 204) were enrolled in a case-control study. The Ile3434Thr polymorphic alleles were discriminated by using amplification refractory mutation system-PCR method. The frequencies of this single nucleotide polymorphism in case and control groups were compared. Also, risk ratio for developing DTC in dichotomized genotypes was estimated by multivariate logistic regression analysis. **Results:** Dichotomized genotypes into those with and without the 3434Thr allele showed that this allele was associated with DTC (OR [odd ratio]: 1.89, 95% confidence interval (CI) = 1.29-2.79, $P<0.001$). Also, TC genotype was significantly associated with increased risk of DTC (OR: 2.42, 95% CI = 1.55-3.81, $P = 0.0001$) in individuals carrying this genotype. **Conclusion:** Allele 3434Thr in *XRCC7* gene might be associated with differentiated thyroid cancer risk. Further studies with larger samples are needed to verify these initial findings. *Iran. Biomed. J. 16 (4): 218-222, 2012*

Keywords: DNA repair enzymes, Thyroid neoplasms, Genetic polymorphism

INTRODUCTION

Thyroid cancer is the most prevalent endocrine cancer [1] and it is more prevalent in females. The worldwide estimated new case of thyroid cancer is 163,000 in females [2]. Differentiated thyroid carcinoma (DTC) mostly includes papillary thyroid carcinoma and follicular thyroid carcinoma. Exposure to radiation especially at young ages is a risk factor in thyroid cancer [3], though in most cases, specific risk factors for DTC cannot be identified [4]. As double-strand breaks (DSB) are produced by replication errors and exogenous agents such as ionizing radiation, it has been suggested that genetic variants in DNA repair pathways may be involved in DTC cancer risk [5]. There are two major DSB repair pathways in mammalian cells: homologous recombination and non-homologous end joining (NHEJ) [6]. NHEJ joins the ends of a broken DNA without need for homology sequence and it is an important repair mechanism for DSB in mammalian cells. This pathway requires DNA-dependent protein kinase (DNA-PK), so-called XRCC7 [7, 8]. This is a holoenzyme consisting of a Ku DNA-Binding domain and a catalytic subunit, which starts the NHEJ process. Ku binds a DSB and uses the catalytic subunit, DNA-PKc, to bind DNA termini. This process activates serine/threonine protein kinase enzymatic activity [9].

The association of DNA-PK variants with different kinds of cancer including thyroid [10] has been reported [11-13]. Up to now, more than 300 genetic variants have been reported in *XRCC7* gene [14]. Among them, I3434T poly-morphism (GenBank accession number: rs7830743) is the most prevalent variant with mean allele frequency of 0.1439. The relationship between non-synonymous polymorphism of *XRCC7* gene and occurrence of DTC was the subject of our case-control study, as the relevant amount of mean allele frequency provides smaller sample size for case-control study [15].

*Corresponding Author; Tel./Fax: (+98-21) 6640 2770; E-mail: fard-esfahani@pasteur.ac.ir

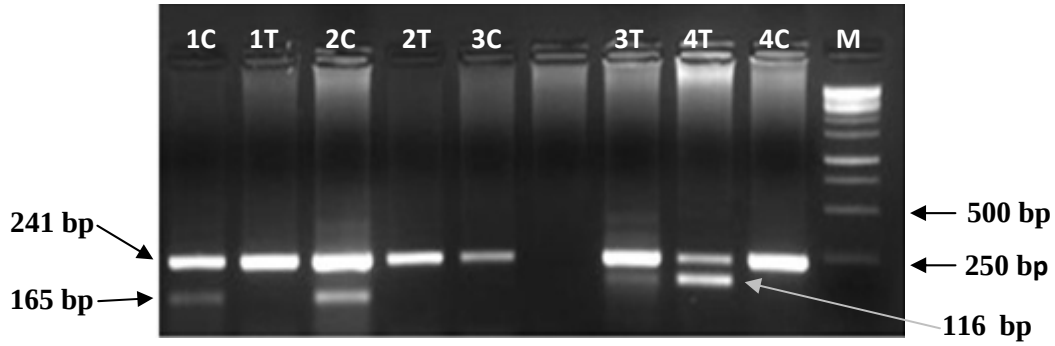


Fig. 1. Amplification refractory mutation system PCR for allele discrimination of Ile3434Thr polymorphism in *XRCC7* gene on 2.5% gel electrophoresis. Two PCR reactions were performed for each sample: C and T; C allele and T allele reactions, respectively. Samples numbers 1 and 2, CC genotype; samples 3 and 4, TT genotype; M, 1 kb DNA markers. The 241, 165 and 116 bp bands correspond to common, C allele and T allele PCR products, respectively.

MATERIALS AND METHODS

Patients and controls. We recruited 173 patients with DTC (134 females and 39 males) and 204 controls (153 females and 51 males) from Research Institute for Nuclear Medicine of Shariati Hospital (Tehran, Iran) for GenBank (accession number: rs7830743) single nucleotide polymorphism (SNP) analysis during September 2008-2009. The individuals signed a written consent form for genetic tests. None of the following history was included in the selection criteria: cervical lymph node involvement, other cancer(s), being heavy smoker or alcoholism and a radiation exposure.

XRCC7 genotyping. Amplification refractory mutation system-PCR technique was used for discriminating the C and T alleles. A pair of common primers (CF: 5'-CAA GCC AAA AAG GGA AAG TG-3' and CR: 5'-GGC TCA AAG TCT CCT CTG GA-3') was used based on previous work in the literature [16] to produce non-allele-specific amplicon and designated as common. Two allele-specific primers (SC: 5'-TGC AGT TCT GCA GAA TCA G-3' and ST: 5'-CTT TGG TGT CCT TGA TAG TTA T-3') were designed for production of allele-specific amplicons. A 241-bp DNA segment was amplified using CF and CR primers, while 116 bp and 165 bp allele-specific amplicons were amplified using CF-SC and CR-ST primer pairs, respectively. For each sample, two PCR reactions with three primers were performed: CF and CR were common in both reactions, while, SC and ST were specific primers for each one (designated as "C" and "T" reactions, respectively). The PCR mixture (25 µl) contained 2.5 µl PCR 10X buffer, 0.5 µl dNTP, 0.75 µl $MgCl_2$, 10 pmol common primers, 24 pmol C or T primers, 0.1 unit Taq DNA polymerase, 19.25 µl double-distilled water, and 10-15 ng genomic DNA. The cycling conditions were at 95°C for 10 min, followed by 35 cycles (94°C, 30 s; 56°C, 30 s and 72°C, 60 s) and a final cycle at 72°C for 7 min. PCR products were separated by standard electrophoresis on 2.5% agarose gel containing ethidium bromide. For confidence, a few of samples were subjected to DNA sequencing.

Statistical analysis: Hardy-Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$, p = frequency of the variant allele, q = 1-p) was tested by χ^2 test to compare the observed genotype frequencies with the estimated ones within the control group. Risk ratio for developing DTC in dichotomized genotypes was estimated by multivariate logistic regression analysis, and A P value of less than 0.05 indicated statistical significance. In this analysis, data for genotype frequencies, without sex or age adjustment, were used for calculation of the of odd ratio (OR). The statistical analyses were performed by SPSS version 13.

RESULTS AND DISCUSSION

Allele discrimination was performed by amplification refractory mutation system PCR method (Fig. 1). Presence of bands with expected size in relevant PCR product reactions showed presence of one or two allele(s). Common amplicon was used as internal PCR control. The results of DNA sequencing of a wild type and a variant carrier have been shown in Figure 2.

The demographic characteristics of the study subjects have been summarized in Table 1. There was no significant difference in gender and age between DTC cancer patients and controls.

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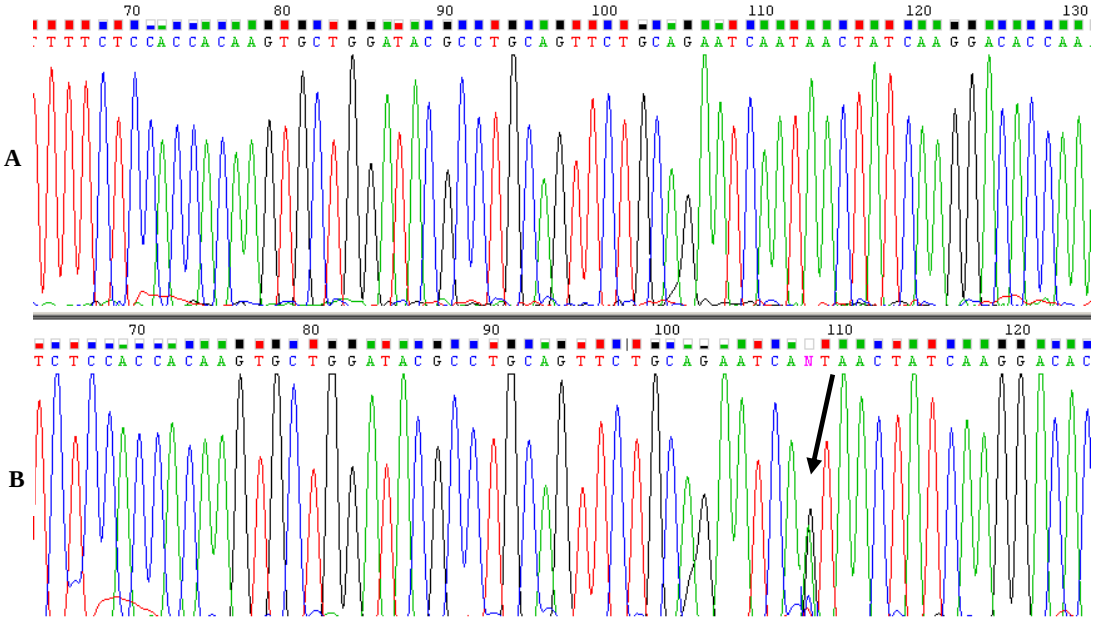


Fig. 2. Electropherograms of *XRCC7* gene PCR amplicons using CF and CR primers (see text). (A) Homozygous wild allele and **(B)** heterozygous variant allele. Solid arrow shows nucleotide position change.

The observed genotype distributions of *XRCC7* gene among cases and controls have been shown in Table 2. Based on Hardy-Weinberg equation, the observed genotypes showed no deviation from that expected. The frequency of C allele observed in DTC patients was about 75%, which was significantly higher than that in controls (52%, $P = 0.001$). Also, the calculated OR (1.89, 95% CI = 1.29-2.79) demonstrated a higher risk in DTC patients for having T allele. On the other hand, a significant difference in genotype distribution was found among DTC cancer and control groups. Furthermore, different OR were calculated to evaluate the risk of DTC in different genotypes (Table 3).

There are many factors in human carcinogenesis; one of which is genetic susceptibility that is involved at different stages of the cancer process [17]. In the present study, we examined the relationship between *XRCC7* gene polymorphism and DTC patients in a subpopulation in Iran. The product of this gene participates in NHEJ pathway, which is one of the DSB repair pathways [18]. It has been shown that cells lacking *XRCC7* are sensitive to ionizing radiation and other DNA damaging agents and display impaired DNA repair [19]. It can be assumed that variants of *XRCC7* gene may alter the function of the so-called product [20], which *per se* may contribute to change the risk of cancer development.

There are only limited studies evaluating the association of different SNPs in NHEJ genes with the risk of different types of cancer. One report from China revealed a negative association of *XRCC7* 6721G allele (GenBank accession number: rs7003908, OR = 0.70, 95% CI = 0.47-1.03) with risk of bladder cancer (OR = 1.53, 95% CI = 1.04-2.25) [13]. However, the association of the same SNP with urothelial bladder cancer (OR = 4.45, $P = 0.001$) and prostate cancer (OR = 1.529, $P = 0.002$) showed an opposite manner in northern population of India [12, 21]. Also, a significant relationship between *XRCC7* 6721GG genotype with hepatocellular cell carcinoma has been shown [11]. However, no significant difference was noted in genotypes of several XRCC7 SNPs between glioma patients and controls [22].

Table 1. Demographic information of case and control subjects.

Variable	DTC (n = 173)		*P* value	Controls (n = 204)	
	n	%		n	%
Sex			0.577		
Male	39	22.54		51	25.00
Female	134	77.45		153	75.00
Age			0.301		
<=50	142	82.08		159	77.94
>50	31	17.91		45	22.05

$P<0.05$ is significant.

Table 2. Genotype frequencies of case and control subjects.

Variable	DTC (n = 173)		*P* value	Controls (n = 204)	
	n	%		n	%
Genotypes			0.0005		
TT	101	58.38		156	76.47
TC	69	39.88		44	21.56
CC	3	1.73		4	1.96
Alleles			0.001		
C	75			52	

$P<0.05$ is significant.

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Table 3. Odd ratio (OR) and confidence interval (CI) of case and control subjects.

Variable	Crude OR	95% CI	*P* value
CC vs TT	1.16	0.25- 5.28	0.8490
TC vs TT	2.42	1.55- 3.81	0.0001
(CC or TC) vs TT**	2.32	1.49-3.61	0.0002
CC vs (TC or TT)***	0.88	0.19-4.00	0.8710

$P<0.05$ is significant; **dominant C allele; ***recessive C allele

Here, we performed a case-control study to analyze the association of Ile3434Thr change (rs: 7830743, T>C) in *XRCC7* gene with DTC in an Iranian subpopulation. Our study shows a significant association of Ile3434Thr polymorphism in *XRCC7* gene with DTC in Iranian population. The risk of DTC was increased in a dose-response manner as the number of C alleles (Thr) was increased. Also, when we used TT as the reference, the dominant model (CC or TC) was associated with 2.32 increased risk of DTC ($P<0.0002$). However, the relationship between this SNP with papillary thyroid carcinoma has not been shown in a similar study in Saudi Arabia with 223 cases and 229 controls [16]. This discrepancy between the results may probably be due to different populations and different factors, which influence developing thyroid carcinomas, small sample size or other unknown phenomena.

This non-synonymous polymorphism results in substitution of isoleucine with threonine in position 3434 in the FRAP-ATM-TRRAP domain of DNA-PK protein. This domain interacts with kinase domain to stabilize the C-terminus region of enzyme. It can be assumed that substitution of a non-polar isoleucine with a polar threonine may alter the function of this domain and eventually the DSB repair capacity of the genome.

Although the potential involvement of defects in the *XRCC7* gene in inducing chromosomal instability is conceivable, to date, no direct clinical evidence has been obtained in patients with thyroid cancer. Therefore, to our knowledge, this is the first report that shows the relationship between *XRCC7* gene and thyroid cancer.

In summary, this study provides the evidences that the polymorphism of *XRCC7* rs: 7830743 may be a risk factor for development of DTC in an Iranian population. Because this is the first study which reports the significant association between *XRCC7* polymorphism and DTC cancer susceptibility, to confirm our findings, additional studies with larger sample size are recommended in different ethnic populations.

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