No association between XRCC3 *Thr241Met and* XPD *Lys751Gln polymorphisms and the risk of colorectal cancer in West Algerian population: a case–control study*

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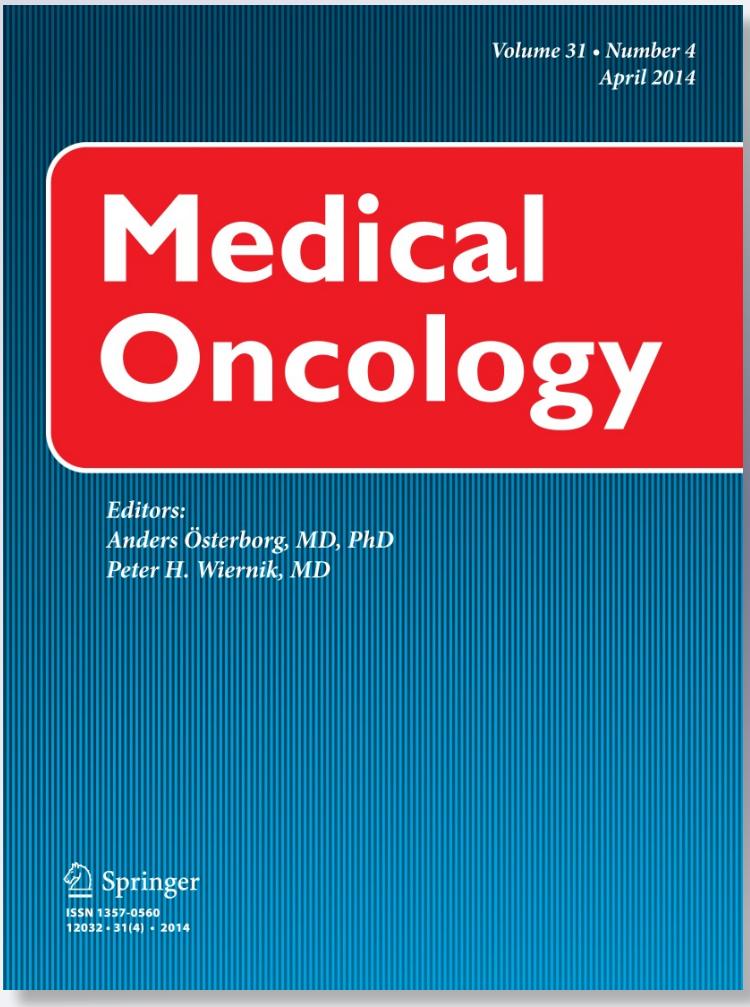
Medical Oncology

ISSN 1357-0560
Volume 31
Number 5

Med Oncol (2014) 31:1-7
DOI 10.1007/s12032-014-0942-3



Springer

Med Oncol (2014) 31:942
DOI 10.1007/s12032-014-0942-3

ORIGINAL PAPER

No association between *XRCC3* Thr241Met and *XPD* Lys751Gln polymorphisms and the risk of colorectal cancer in West Algerian population: a case–control study

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Received: 20 February 2014 / Accepted: 25 March 2014

Abstract Colorectal cancer (CRC) is a complex and multifactorial disease, in which genetic and environmental factors both seem to play a part. Many epidemiological studies have explored the association between genetic polymorphisms of X-ray repair cross-complementing group 3 (*XRCC3*) (Thr241Met) and Xeroderma pigmentosum group D (*XPD*) lysine to glutamine at codon 751 (Lys751Gln) and risk of CRC in various populations; however, the results are controversial. We conducted this case–control study in a West Algerian population to assess the potential role of this genetic polymorphism on the risk of CRC in this population. Genomic DNA was extracted from blood samples collected from 129 sporadic CRC patients and 148 normal controls. The polymorphisms were determined by pyrosequencing technique. The distribution of *XRCC3* Thr241Met and *XPD* Lys751Gln genotypes among controls did not differ significantly from those predicted by the Hardy–Weinberg distribution ($p > 0.05$). There were no significant differences in the genotypes distribution and allele frequencies between CRC patients and controls. A significant association was found between the combined heterozygous of *XRCC3* and homozygous variant of *XPD* gene and CRC. This is the first study on DNA repair genetic polymorphisms in West Algerian population, and it suggests that the XRCC3 Thr241Met and XPD Lys751Gln polymorphisms may not be associated with the CRC risk in this population.

Keywords *XRCC3* · *XPD* · Polymorphism · Susceptibility · Colorectal cancer · West Algerian population

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Introduction

Colorectal cancer (CRC) is one of the most frequent common types of malignancy as well as leading cause of death around the world, accounting for nearly one million new cases diagnosed and half a million deaths annually [1]. Incidence rates vary widely according to region: it is high in developed countries and seems to be low in developing countries [2]. This type of cancer is obviously a significant public health problem in Algeria. In Western Algeria, the annual incidence of CRC approaches 10 cases per 100,000 inhabitants (8.4/100,000 males and 9.5/100,000 females) and it ranks third for all cases of cancer in both men and women [3].

Published online: 01 April 2014

Most cases of CRCs arise sporadically, indicating that cancer occurs in individuals without a family history of the disease. The sporadic CRC has a complex etiology, believed to be caused by a combination of multiple genetic and environmental factors that expose certain individuals at a higher risk [4]. Numerous association studies of CRC susceptibility genes have revealed many critical genes, including those involved in DNA damage repair [5, 6].

DNA repair plays an important role in the maintenance of genomic stability by correcting DNA alterations caused by endogenous and exogenous genotoxic agents. Depending on the type of the damage, five major DNA repairing mechanisms have been described in mammalian cells: base excision repair (BER), nucleotide excision repair (NER), double-strand break repair (DSBR), mismatch repair (MMR) and inter-strand crosslink repair (ICLR) [7, 8]. Single nucleotide polymorphisms (SNPs) in DNA repair genes may be associated with reduced DNA repair capacity and may therefore be related to the development of cancer. Among known genetic polymorphisms in DNA repair genes, X-ray repair cross-complementing group 3 (*XRCC3*) and the Xeroderma pigmentosum group D (*XPD, ERCC2*) have been the most frequently studied.

The *XRCC3* gene (MIM:600675), which is located at chromosome 14q32.3, encodes a member of an emerging family of Rad-51 related proteins that participates in the repair of DNA double-strand breaks via homologous recombination DSBR to maintain chromosome stability [9, 10]. *XRCC3* is a polymorphic gene where many SNPs have been already described. The most common polymorphism consists in a C18607T transition (rs861539) at exon 7 resulting in an amino acid change at codon 241 (Thr241Met) [11]. This polymorphism has been reported to be associated with the development of some cancers such as bladder [12], skin [13], breast [14], lung and colorectal [15] cancers.

The *XPD* gene (MIM: 126340), also known as excision repair cross-complementation group 2 (*ERCC2*), is mapped at chromosome 19q13.3 and is involved in transcription initiation and in the NER pathway which repairs bulky adducts and UV-induced DNA damage. The *XPD* gene product has ATP-DNA dependant helicase activity and also acts as a subunit of the basal transcription factor TF2/TFIIH complex [16]. Mutations in *XPD* can alter its helicase activity, indicating relatively low DNA repair capacity and thus contribute to the carcinogenesis [17]. A SNP at the exon 23 (A35931C, rs13181), which causes a non-synonymous substitution of Lysine to Glutamine at codon 751 (Lys751Gln) [11], has been studied extensively for its association with various types of cancer [18–21].

A large number of epidemiological studies in different populations have investigated the role of these polymorphisms on CRC risk, and the results are conflicting. Since no reported studies are available from Algerian population, we conducted this case–control study to determine the possible effect of *XRCC3* Thr241Met and *XPD* Lys751Gln polymorphisms on the CRC risk in a West Algerian population.

Materials and methods

Study population and sample collection

In this case–control genetic association study, two comparable groups were selected. The case group included a total of 129 unrelated patients diagnosed with sporadic CRC (84 male and 45 female subjects with age range of 27–72 years) treated at the Oran University Hospital Center (CHU) and the Anti-Cancer Center of Oran (CAC), recruited from 2007 to 2012. A questionnaire was administered to each patient in order to gather basic demographic and clinical data smoking habits, alcohol use and personal/family medical history. The control group, composed of 148 normal healthy individuals (94 male and 54 female subjects aged between 25 and 64 years), came from the general population and had no history of any type of cancer. All participants included in the present study were from the Western Algeria and gave their written informed consent before investigation.

Approximately 10 ml of peripheral blood samples were collected in EDTA tubes and stored at −20 °C until analysis.

DNA Extraction

Genomic DNA was isolated from peripheral blood leukocytes using standard salting out procedure, involving sodium dodecylsulfate (SDS)/proteinase K digestion followed by ethanol precipitation [22].

SNP genotyping by pyrosequencing

We genotyped all patients and control samples for the *XRCC3* and *XPD* polymorphisms using pyrosequencing technology. First, fragments were directly amplified from genomic DNA with one of the primers biotinylated. Primers for amplification and sequencing were designed using PSQ Assay Design software version 1 (Biotage AB); primer sequences are listed in Table 1.

The polymerase chain reaction (PCR) was carried out in a 96-well plates using a final volume of 50 µl containing 1X PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer and 1U Taq DNA polymerase (Invitrogen). Thermal cycling conditions for the *XRCC3* were as follows: 95 °C for 5 min, 45 cycles with

Table 1 Primer sequences

SNP	Forward primer (5′–3′)	Reverse primer (5′–3′)	Sequencing primer (5′–3′)
XRCC3 Thr241Met	[a]CGCCTGGTGGTCATCATCGACT	ATACGAGCTCAGGGGTGCAAC	TGCTCAGCTCACGCA
XPD Lys751Gln	[a]TGGAGCAGCTAGAATCAGA	CCTGCGATTAAAGGCTGTGGA	GCAATCTGCTCTATCCTC

[a] Biotinylated (Biotin group added to the 5′ primer)

denaturation at 95 °C for 40 s, annealing at 64 °C for 40 s, extension at 72 °C for 20 s and a final extension at 72 °C for 10 min. For *XPD,* the initial denaturation step was performed at 95 °C for 5 min followed by 45 cycles at 95 °C for 20 s, 58 °C for 20 s, 72 °C for 20 s and finally 5 min at 72 °C.

The biotinylated PCR products were then captured on streptavidin–sepharose-coated beads denatured and washed. The pyrosequencing primer was added and annealed to the captured strand. The pyrosequencing reaction was performed as recommended by the manufacturer's instruction, using PyroMark MD system (Biotage AB) in combination with a SNP Reagent Kit which contained the enzyme, substrate and nucleotides. The sequencing data were analyzed with PSQ 96MA SNP software (Biotage AB).

Statistical analysis

Polymorphic genotypes were categorized into homozygous wild, heterozygous and homozygous variant. A chi-square ($\chi 2$) test was performed for each polymorphism to determine whether the control sample demonstrated Hardy–Weinberg equilibrium and to compare the distribution of genotypes frequencies between CRC cases and controls. The odds ratios (OR) and 95 % confidence interval (CI) were determined with logistic regression analysis in order to evaluate the association between *XRCC3* (Thr241Met) and *XPD* (Lys751Gln) polymorphisms and CRC susceptibility. A p value < 0.05 was considered as statistically significant. All statistical analyses were performed with Epi-Info™ version 7 software.

Results

The distribution of gender and age group among study subjects are given in Table 2. The mean age of CRC cases was 53.89 ± 12.75 years while the mean age of controls was 43.33 ± 11.49 years. CRC incidence was higher among individuals more than 50 years compared to those less than 50 years. Comparing the incidence of CRC between males and females, males were more commonly affected than females (65 vs. 34 %). There was no statically significant difference in mean age and gender distributions between cases and controls, indicating a well matched study population ($p > 0.05$).

We have determined the frequencies of two non-synonymous polymorphisms: *XRCC3* Thr241Met, *XPD* Lys751Gln in CRC patients and matched controls in order to evaluate their possible association with the risk of CRC. Tables 3 and 4 summarize the distribution of the allele and genotype frequencies between cases and control subjects;

Table 2 Distribution of sex and age among cases and controls

Variable	Controls ($n = 148$) n (%)	CRC Patients ($n = 129$) n (%)	p value
Gender			
Male	94 (63.51)	84 (65.12)	0.78
Female	54 (36.49)	45 (34.88)	
Age (in years)			
<50	72 (48.65)	49 (37.98)	0.07
≥50	76 (51.35)	80 (62.02)	
Mean ± SD	43.3 ± 11.5	53.9 ± 12.7	

n number, % frequency

Table 3 Distribution of genotypes and frequency of alleles of the *XRCC3* Thr241Met polymorphism and its association with risk of colorectal cancer

	Controls ($n = 148$) n (%)	CRC Patients ($n = 129$) n (%)	OR (95 % CI)	p value
Genotype				
Thr/Thr	55 (37.2)	45 (34.9)	1[a]	
Thr/Met	72 (48.6)	68 (52.7)	1.15 (0.69–1.93)	0.58
Met/Met	21 (14.2)	16 (12.4)	0.93 (0.43–1.99)	0.85
Allele				
Thr	182 (61)	158 (61)	1[a]	
Met	114 (38)	100 (38)	1.01 (0.72–1.42)	0.95

n number, % frequency, *OR* odds ratio, *CI* confidence interval, p significance

[a] Genotype saved as reference category

Table 4 Distribution of genotypes and frequency of alleles of the XPD Lys751Gln polymorphism and its association with risk of colorectal cancer

	Controls ($n = 148$) n (%)	CRC Patients ($n = 129$) n (%)	OR (95 % CI)	p value
Genotype				
Lys/Lys	82 (55.4)	64 (49.6)	1[a]	
Lys/Gln	54 (36.5)	56 (43.4)	1.33 (0.81–2.18)	0.26
Gln/Gln	12 (8.1)	9 (7.0)	0.96 (0.38–2.42)	0.93
Allele				
Lys	218 (74)	184 (71)	1[a]	
Gln	78 (26)	74 (29)	1.124 (0.77–1.63)	0.54

n number, % frequency, *OR* odds ratio, *CI* confidence interval, *p* significance

[a] Genotype saved as reference category

Table 5 Combined effect of *XRCC3* and XPD genotypes on risk colorectal cancer

Genotype *XRCC3–XPD*	Controls ($n = 148$) *n*	CRC patients ($n = 129$) *n*	OR (95 % CI)	p value
Thr/Thr–Lys/Lys	11	6	1[a]	
Thr/Thr–Lys/Gln	9	9	1.83 (0.47–7.12)	0.38
Thr/Thr–Gln/Gln	1	1	1.83 (0.096–34.8)	0.68
Thr/Met–Lys/Lys	40	37	1.67 (0.57–5.04)	0.34
Thr/Met–Lys/Gln	30	23	1.40 (0.45–4.36)	0.55
Thr/Met–Gln/Gln	2	8	7.33 (1.16–46.23)	0.02*
Met/Met–Lys/Lys	31	21	1.24 (0.40–3.88)	0.71
Met/Met–Lys/Gln	15	23	2.81 (0.86–9.22)	0.08
Met/Met–Gln/Gln	9	1	0.20 (0.02–2.01)	0.14

n number, % frequency, *OR* odds ratio, *CI* confidence interval, *p* significance

* $p < 0.05$ considered as statistically significant

[a] Genotype saved as reference category

all distributions were in Hardy–Weinberg equilibrium (Thr241Met: $\chi 2 = 0.109$, $p = 0.741$; Lys751Gln: $\chi 2 = 0.533$, $p = 0.465$). There were no significant differences in the genotypes distribution and allele frequencies between cases and controls ($p > 0.05$).

The results of the gene–gene interaction of *XRCC3* Thr241Met and *XPD* Lys751Gln genotypes of study subjects are shown in Table 5. There was a statistically significant increase in risk of CRC in cases carrying combined *XRCC3* Thr/Met and *XPD* Gln/Gln genotypes (OR = 7.33, 95 % CI 1.16–46.23, $p = 0.02$).

Discussion

In the present work, we conducted a case–control study consisting of 129 confirmed CRC cases and 148 controls to investigate the relationship between the functional polymorphisms *XRCC3* Thr241Met and *XPD* Lys751Gln and the risk of CRC in a Western Algerian population. In addition, we compared the allelic frequencies of the *XRCC3* and *XPD* genes variants in our population to those observed in different countries. To our knowledge, this is the first time that the association of these two polymorphisms with CRC is analyzed in an Algerian population sample.

XRCC3 protein facilitates the formation of a nucleoprotein filament complex with *RAD51-C* that assists *RAD51*-mediated strand invasion [9, 10, 23]. *XRCC3* mutant cells show a high frequency of multiple centrosomes and abnormal spindle formation, and present increased sensitivity to DNA damaging agents [24]. Previous studies have shown that a common polymorphism at codon 241 of *XRCC3* gene (Thr to Met) modifies the characteristics of the protein. The *XRCC3* Met^{241} variant allele has been reported to present relatively high DNA adduct levels in lymphocytes, which may result in reduced DNA repair capacity [25]. The *XRCC3* Met^{241} allele has been extensively studied and its frequency changes with ethnicity. In our population, the frequency of Met^{241} allele among healthy controls (0.38) was similar to those reported in Saudi Arabia [26], Spain [27] and American populations [28], whereas it was slightly higher than the frequency in Polish [29], Danish [30] Indian [31] and Italian populations [15]. This frequency was much higher than those reported in Asian populations: Taiwan [32], Thailand [33] and China [34]. On the other hand, it was slightly lower than the frequency in Tunisia [35], UK [36], Norway [21], Turkey [37] and France [38] populations.

On examining the risk, we found no significant association between the *XRCC3* Thr241Met polymorphism and CRC occurrence. Lack of association between this polymorphism and CRC is in accordance with data on Norwegian [21] and Polish [29] populations. In contrast, a case–control study conducted in a Southern Italian population reported that the Met allele was statistically associated with CRC risk [15]. Also, a Chinese study showed that the Met allele was moderately associated with CRC [34]. However, Mort et al. [36] observed that individuals with the Thr/Thr genotype had an increased risk of CRC in the British population. The results of Yeh et al. [32] have also shown that the *XRCC3* Thr^{241} allele was associated with a significantly increased risk of CRC in Taiwan. Likewise, the *XRCC3* Met^{241} allele showed a protective tendency against rectal cancer in Indian population (OR = 0.68, 95 % CI 0.46–1.02) [31]. Recently, Wang and Zhang performed a meta-analysis involving 15 case–control studies with a total of 4,475 cases and 6,373 controls [39]. The results from this pooled analysis concluded that the *XRCC3* Thr241Met polymorphism could represent a susceptibility gene for CRC with a low penetrance.

The XPD protein participates in the unwinding of the DNA double-stranded helix around the damaged site to allow the removal of DNA lesions [17]. In addition, it is also required for p53-dependent apoptosis and cell cycle regulation [40]. Mutations in the *XPD* gene may alter the protein function and abolish its enzymatic activity. Several polymorphic variants in this gene have been identified, and *XPD* Lys751Gln is the most common polymorphism in the gene; it is located in the important interaction domain (C-terminal) with the p44 protein, which stimulates *XPD* by activating its helicase activity [41]. It has been shown recently that the Lys751Gln reduces the ERCC2 protein expression by decreasing the ERCC2 mRNA stability [42]. Thus, it is the most widely studied polymorphism with regard to cancer association. In our study on Western Algerian CRC patients, we observed no association of the *XPD* Lys751Gln with CRC risk. These observations are similar to findings from several previous studies conducted in various ethnic groups [21, 31, 32, 36, 43–45]. Moreover, a recent meta-analysis of 15 case–control studies (including a total of 3,042 cases and 4,627 controls) also suggested that *XPD* Lys751Gln is not associated with CRC development [46]. However, an increased risk of low-risk adenomas was detected among individuals carrying the variant *XPD* Gln^{751} allele as compared to carriers of the Lys^{751} wild-type allele [21, 47]. On the other hand, pharmacogenetic analysis suggested that the *XPD* Lys751Gln variation may influence the susceptibility to 5-fluorouracil/oxalipatin chemotherapy among CRC patients [48]. In this last study, the Gln^{751} allele was significantly associated with a favorable survival of CRC patients. This is in contrast with another study in Taiwan which reported a lower response to the treatment among carriers of this allele [49]. Therefore, the *XPD* Lys751Gln polymorphism may be a useful pharmacogenetic determinant to define CRC patients who are more likely to benefit from platinum-based chemotherapy [50].

In comparing the allele frequency of *XPD* Lys751Gln to previous published data of different ethnic groups, our study indicates that the frequency of the Gln allele was 26 %; this frequency was lower than the values found for Indian [31], Tunisian [35], Iranian [51] and European populations [15, 21, 42, 52–54]. It appeared to be much higher than those reported in Taiwanese [32], Chinese [55] and South Korean populations [56].

We analyzed the combined effect of the *XRCC3* and *XPD* polymorphisms with regard to CRC risk. We found that the combination of variant homozygous *XPD* 751Gln/Gln and heterozygous variant *XRCC3* 241 Thr/Met genotypes was significantly associated with a higher risk of CRC (OR = 7.33, 95 % CI 1.16–46.23, $p = 0.02$). No reported studies have reached the same conclusion. Our results led us to hypothesis that the NER mechanism does not influence alone the occurrence of CRC in our population but might play a role in the development of this cancer when combined with certain DSBR genes genotypes. However, the possible explanation for this genotype combination on risk of CRC remains to be known. Indeed, the use of combined analysis of polymorphisms may represent an alternative way of analyzing the overall effect of the different genetic variants.

The difference in results between the present study and the previous studies describing associations between these polymorphisms studied and their allelic frequencies and the occurrence of CCR could be explained by several factors such as ethnicity, selection criteria of patients and controls, and genetic heterogeneity in the pathogenesis of CRC. It is also possible that these polymorphisms could be in linkage disequilibrium with other putative etiological variants, such as tobacco smoking and certain dietary components, which would likely differ across different ethnic populations. Unfortunately, information on these factors in our case–control study was not available for all patients. In addition, one of the limitations of our study is the relatively small sample size that influences the statistical power. In fact, the sample size is closely tied to statistical power: A larger sample size yields higher power.

In conclusion, our findings suggest that these polymorphisms are not involved in genetic susceptibility to CRC in our population. It is also likely that many variants or common susceptibility polymorphisms of DNA repair genes may jointly contribute to the susceptibility of CRC. Therefore, it is important to include more variants in the genes that participate in the same DNA repair pathways in

order to identify genetic markers as well as gene–gene and gene–environment interactions that may predict individual susceptibility to CRC. However, such studies should be performed on much larger populations in order to deal with the statistical limitations inherent to multiple testing.

Acknowledgments The authors of this article are grateful to all subjects for donating their blood for this study. In addition, they thank all the collaborators for collection of blood samples and information.

Conflict of interests The authors have declared that no conflict of interests exists.

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