

## Relationship between XPD Lys 751 Gln polymorphism and colorectal cancer risk: a case-control study in a population-based study

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### ABSTRACT

**Aim:** In our study, we analyzed the allelic frequency of XPD Lys751Gln polymorphism of the XPD gene and the correlation between its variant alleles with colorectal cancer in patients and control groups.

**Background:** Human cells are routinely exposed to mutagenic and carcinogenic aromatic amines via smoking, pollution areas and other sources. These chemicals can form DNA adducts in vivo and thus lead to DNA damage. Amongst the known genetic polymorphisms of the DNA-repair genes the xeroderma pigmentosum group D (XPD, also known as ERCC2) has been the most extensively studied most commonly.

**Patients and methods:** This study has examined the relationship between the XPD Lys 751 Gln polymorphism and colorectal cancer in 88 patients and their 88 age and sex-matched controls. Genomic DNA from peripheral whole blood was extracted using Miller method to determine the genotype of subjects with RFLP-PCR analysis.

**Results:** This study shows cancer patients have more of the heterozygous genotype (XPD Lys 751 Gln) compared to control group. However the results are not statistically significant. Furthermore, colorectal cancer was less common in individuals with recessive homozygous genotype ( $P < 0.0001$ ).

**Conclusion:** This study suggests that individuals with heterozygous polymorphism (Lys/Gln) may have an increased susceptibility to colorectal cancer compared to other polymorphisms (Lys/Lys and Gln/Gln).

**Keywords:** Colorectal cancer, Nucleotide excision repair, XPD gene, polymorphism.

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### Introduction

Colorectal cancer is the third most common cancer in industrialized countries and is a major cause of cancer-related death in these countries (1). There are no accurate statistics on the colorectal cancer mortality in Iran, but after gastric cancer, colorectal cancer is the most common

gastrointestinal cancer (2). About 10 to 15 percent of colorectal cases are due to hereditary syndromes (3). Environmental factors could also have role in developing colorectal cancer, possibly through direct binding of metabolites (DNA adduct) or oxidative stress leads to damage to DNA. The primary mechanism for removal of this type of DNA damage is nucleotide excision repair (NER). If these DNA lesions are left without repair, they cells are at increased risk of mutagenesis and

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oncogenesis. Variation in repair capacity may be due to polymorphisms in genes responsible for DNA repair. Therefore, a polymorphism in a DNA repair gene may affect an individual's genetic predisposition for cancer development. This relationship has been shown in NER pathway, for polymorphisms including XPD Asp312Asn, XPA G23A, XPC Lys939Gln, ERCC1 Asn118Asn and particularly XPD Lys751Gln polymorphism. The excision repair cross-complementing rodent repair deficiency, group 2 (*ERCC2*) gene, also called the xeroderma pigmentosum group D (*XPD*) gene, is located at chromosome 19q13.3. It comprises 23 exons and spans about 54,000 base pairs (4) The *XPD* gene encodes *XPD* protein with helicase activity that participates in unwinding of helix around DNA damages site during NER and transcription (5). Also it has been reported that inactivation of the *XPD* gene is lethal for mice embryos (6).

*XPD* gene polymorphisms may act as genetic susceptibility factors because this gene is involved in several cellular activities and rare mutations in this gene causes genetic disease. Recently several studies have shown that variant alleles of polymorphism for XPD Lys751Gln has been associated with increased DNA adduct levels (7, 8, and 9) and with reduced capacity to DNA repair (10) and with cancer risk (11-13).

However, the association between variant alleles of this polymorphism and colorectal cancer is a matter of debate (14-17). We have selected this polymorphism to study the frequency of this polymorphism variants in Iranian population and examine whether the association between this polymorphism variants and risk of colorectal cancer.

In our study we analyzed the allelic frequency of XPD Lys751Gln polymorphism of the *XPD* gene and the correlation between its variant alleles with colorectal cancer in patients and control groups.

## Patients and Methods

### Study groups, extraction of genomic DNA and genotyping

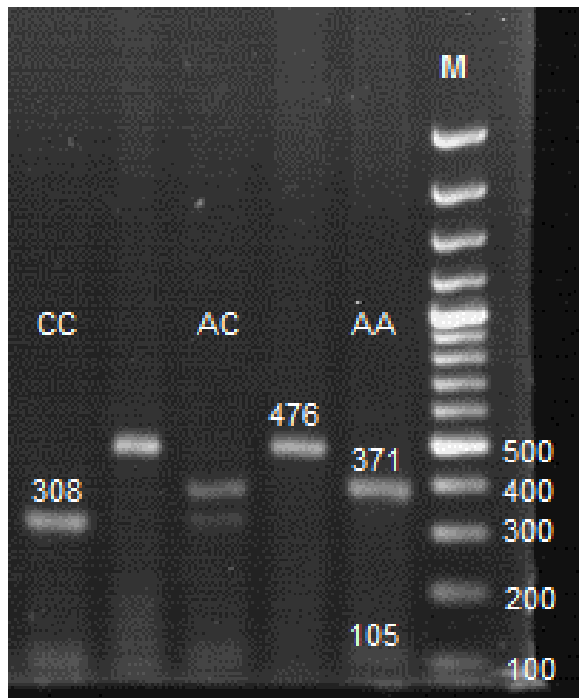
Eighty-eight colorectal cancer patients (age 28-74 years with mean  $\pm$  SE =  $54 \pm 1.4$ ) and 88 age and gender-matched controls (age 34-81 years with mean  $\pm$  SE =  $52.39 \pm 1.318$ ) were enrolled in this study. From each individual 5 ml blood was collected and stored in  $-20^{\circ}\text{C}$ . Genomic DNA was extracted using Miller procedure (18).

After designing the forward primer 5'ATCCTGTCCCTACTGGCCATTC and the reverse primer 5'CCACTAACGTCCAGTGAAGTGC using NCBI and SGD data base (19, 20) the polymorphic sites in exon 23 was amplified (476 bp fragments).

RFLP-PCR analysis was used to genotype all samples. Briefly, the selected fragments from 23 exons were amplified using PCR and then digested with *PstI* enzyme. Agarose gel electrophoresis was performed to determine the type of polymorphism. The PCR reaction mixture contained 0.75 mM MgCl<sub>2</sub>, mixture of 0.5 mM deoxynucleotide triphosphates, 0.5 nM of each primer, 0.5 units of Taq DNA polymerase and 100–200 ng of genomic DNA. PCR program consisted of a 4min denaturation step at  $94^{\circ}\text{C}$  followed by 40 cycles of 30s at  $94^{\circ}\text{C}$ , 35s at  $61.8^{\circ}\text{C}$ , 40s at  $72^{\circ}\text{C}$  and 10min at  $72^{\circ}\text{C}$ . The *PstI* used to digestion PCR amplicon for 16 h at  $37^{\circ}\text{C}$  and digested product was separated using agarose gels (*PstI* enzyme according to Spitz et al (10). Table 1 displays details of RFLP-PCR reactions.

To ensure the proper functioning of restriction enzyme, primers were designed to cut a natural site, and create bands of 105 bp and 371 bp (Fig 1, AA, when there aren't any polymorphisms). In the presence of polymorphism, restriction enzyme makes 63 bp, 105 bp, 308 bp and 371bp in the heterozygous state (Fig 1, AC) and 63bp, 105 bp, 308 bp in the homozygote state (Fig1, CC). Finally, to demonstrate reproducibility, 27

samples (case and control) (15.34%) were randomly re-genotype.



**Figure 1.** RFLP analysis of the XPD Lys751Gln polymorphism. Fragment sizes (bp) for each genotype with cut PCR product by *PstI* enzyme, along with the Marker (molecular size standard as M) are shown. Notably, the 63 bp on the gels used are not resolved.

**Table 1.** Details of RFLP-PCR analysis of the XPD Lys751Gln polymorphism.

PCR primers	
Forward	5'ATCCTGTCCCTACTGGCCATTC
Reverse	5'CCACTAACGTCCAGTGAAGTGC
<b>PCR fragment</b>	476
Restriction enzyme	<i>PstI</i>
Fragment size	
Wild type	105,371
Heterozygote (AC)	63,105, 308, 371
Varian homozygote	63, 105, 308
Agarose gel	1.5%

### Statistical analysis

To calculate the difference in the distribution of the genotype frequency between patients with colorectal cancer and control group Pearson's  $\chi^2$  test was used. To compare observed and expected genotype frequencies between patients and control

subjects Hardy-Weinberg equilibrium was tested by the goodness-of-fit  $\chi^2$  test. In all cases, a colorectal cancer patient vs. controls odd ratios (ORs) with 95% confidence intervals (CIs) was estimated. In all cases  $P < 0.05$  was considered statistically significance.

### Results

In this study, male to female ratios were similar in both groups, 0.568 and 0.59 for patients and controls, respectively. Demographic characteristics of two groups have been shown in Tables 2. Forty-five percent of patients had a positive family history for colorectal cancer in their first-degree relatives (55% with AA genotype and 45% with AC genotype). The frequency of genotypes of the XPD Lys751Gln polymorphism has been shown in Table 3 and Fig. 2 depicts the frequencies of analyzed genotypes. The frequency of alleles A and C in the patients was 0.68 and 0.32 and in controls was 0.48 and 0.52, respectively.

**Table 2.** Demographic characteristics of study groups

	Patients	Control	P-Value
<b>Total</b>	88(100%)	88(100%)	
<b>Gender</b>			0.760
Men	52(59%)	50(56.8%)	
Female	36(40.9%)	38(43.1%)	
<b>Mean age (SD), years</b>	54.9± 1.4	52.39±1.318	0.140

In this case-control study we observed a difference in genotypic frequency between patients and controls (Table 3). Heterozygous genotype XPD Lys751Gln was more common in cancer patients compare to cases in controls (OR: 1.33, 95% CI: 0.679-2.620) but this difference was not statistically significant ( $P = 0.406$ ) and it could be due natural variation or a significant association concealed by a small sample size. Notably, patients with colorectal cancer are less frequently carriers of homozygous recessive genotype XPD Gln751Gln compared to controls and we observed a negative correlation, but

statistically significant between XPD Gln751Gln genotype and colorectal cancer risk (OR: 0.097, 95%CI: 0.032-0.297,  $P < 0.0001$ ). Moreno has found higher risk of colorectal cancer among variant allele carriers versus for those with homozygous wild genotype (OR: 1.40, 95%CI: 1.08-1.81) (16). Also, Stern and colleagues have demonstrated lower risk for developing colorectal cancer in homozygous genotype carriers (OR=0.7, 95%CI: 0.4-1.0) (21).

In subgroup analysis we analyzed genotypes according to gender in both groups. In this study, although women with the heterozygous genotype were (OR=2.18) more common in cancer patient group compared to women in control group, this difference was not statistically supported accordingly by the 95%CI for OR and interestingly no women in patient group showed a homozygous genotype (Table 3). A similar analysis carried out among men, but it did not show statistically significant results.

**Table 3.** Distribution of XPD genotypes by case and control status

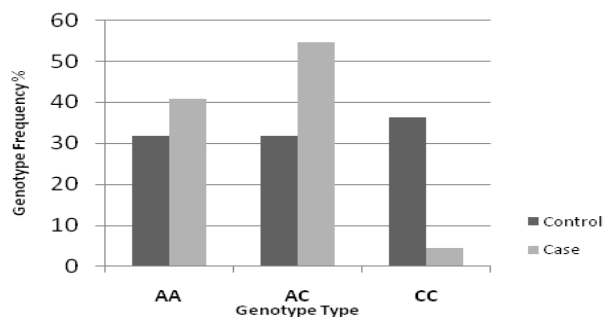
Genotype	case/control	P-value	OR	95%CI
<b>Total subjects</b>	88/88			
AA	36/28	1.0	1.0*	
AC	48/28	0.406	1.333	0.679-2.620
CC	4/32	0.000	0.097	0.032-0.297
<b>Male</b>	52/50			
AA	20/14	1.0	1.0	
AC	28/20	0.965	0.980	0.405-2.372
CC	4/16	0.006	0.175	0.051-0.616
<b>Female</b>	36/38			
AA	16/14	1.0	1.0	
AC	20/8	0.156	2.18	0.747-6.384
CC	0/16	- <sup>†</sup>	- <sup>†</sup>	

\* Reference value

<sup>†</sup>No Case were observed in this group; therefore, it is not possible to calculate an P-value and OR

The age difference was another issue that was investigated in this study. In order to better evaluate the impact of age on the risk of colorectal cancer, patients based on the average age were divided in two groups of  $\leq 54$  years old (group I)

and  $>54$  years (group II) (Table 2). In this study, statistical analysis showed three times (OR=3, 95%CI=1.411-7.870,  $P=0.025$ ) increased risk of colon cancer with increasing age in women compared to men, but for rectal cancer, increasing age is associated with increased cancer risk more than doubles in females compared to male, but not statistical significance (OR=2.4, 95%CI=0.673-8.411,  $P=0.183$ ). For colon cancer there is approximately 6-fold increased risk of cancer with advancing age in women compared to men (OR=6, 95%CI= 1.206-29.887,  $P=0.027$ ) (data not shown). Patients in this study have had significant positive history of colorectal cancer in their first degree relatives (OR=4.5; 95%CI= 2.010-10.044,  $P < 0.0001$ ).



**Figure 2.** Frequencies of analyzed genotypes in control and colorectal cancer groups.

## Discussion

XPD protein is an ATP-dependent helicase, absolutely essential nucleotide excision repair. Studies have shown that the activity of this protein is essential for life, complete elimination of XPD gene leads to embryonic lethality (22). Point mutations in this gene play a decisive role in diseases associated with the repair-defect such as xeroderma pigmentosum, trichothiodystrophy, and Cockayne syndrome. (23). Most of these mutations are located in the interaction domain (C terminal) with the p44 protein, in the transcription

factor IIIH complex, that is essential for activating the helicase activity (24).

The aim of this study was to investigate if there was any relationship between XPD polymorphisms and the risk of colorectal cancer. Many epidemiological studies with the aim of identifying the role of XPD polymorphisms on the risk of various cancers have been done and different association between two polymorphisms (Asp312Asn and Lys751Gln) and the risk of various cancers such as melanoma, lung, breast, basal cell carcinoma, bladder, prostate, head and neck and lung has been reported.

In this study, the heterozygous variant was more common in patients with colorectal cancer than in the control group, these results are consistent with the studies from Skjelbred (17) and Moreno (16). Some studies show reduced capacity to repair the heterozygous genotype (Lys751Gln) (10, 25 and 26).

There are some reports that denote higher levels of mRNA of the XPD gene (27) and higher repair capacity in women (28) in sub-cohort studies. In this study, gender differences in cancer incidence have also been observed, but it was not statistically significant and this result is contrary to other previously mentioned studies. These results show that a gender difference might have a role in predisposition to colorectal cancer in view of genotype polymorphism.

Age-related increase in cancer of the cooperation between the accumulation of mutations and age-related changes is driven (29). The NER system has the ability to repair some oxidative damages and bulky photo-products induced by UV (30) and DNA oxidation rate and with increasing age can dramatically increase in activity due to the decreased ability of cells to repair DNA (31). Studies have shown that older tissues are more sensitive to oxidative damage and some studies have shown that younger cells DNA are more damaged by UV radiation compare to older cells (32). In summary, some studies

indicate that the aging process increases the susceptibility to developing cancer and this study also shows that aging (in group II than group I) increases the risk of colorectal cancer to three times.

The goal of this study was to analyze the relationship between the XPD Lys751Gln polymorphism and colorectal cancer. This study demonstrates that heterozygous genotype is more common in patients with colorectal cancer and homozygous genotype is not associated with this cancer. This correlation may translate that carriers of heterozygous genotypes are more prone to develop colorectal cancer compared to the people with wild or homozygous genotype because individual with heterozygote genotype have reduced capacity repair and it can be diagnostic biomarker in colorectal patients. Although this study has not demonstrated statistical significance, this hypothesis warrants further cohort and prospective studies with more cases in the future.

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## References

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1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics. *CA Cancer J Clin* 2006;56:106-30.
2. Pourhoseingholi MA, Faghihzadeh S, Hajizadeh E, Gatta G, Zali MR, Abadi AR. Trend analysis of gastric cancer and colorectal cancer mortality in Iran. *Iranian Journal of Cancer Prevention* 2011;1:38-43.
3. Schottenfeld D, Winawer S, Fraumeni JF. Cancers of the large intestine. In: Schottenfeld D, Fraumeni JF Jr, Editors. *Cancer epidemiology and prevention*. New York: Oxford University Press; 1996. P.813-40.

4. Weber CA, Salazar EP, Stewart SA, Thompson LH. ERCC2: cDNA cloning and molecular characterization of a human nucleotide excision repair gene with high homology to yeast RAD3. *EMBO J* 1990;9:1437-47.
5. Reardon JT, Sancar A. Molecular anatomy of the human excision nuclease assembled at sites of DNA damage. *Mol Cell Biol* 2002;22:5938-45.
6. de Boer J, Donker I, de Wit J, Hoeijmakers JH, Weeda G. Disruption of the mouse xeroderma pigmentosum group D DNA repair/basal transcription gene results in preimplantation lethality. *Cancer Res* 1998;58: 89-94.
7. Hou SM, Falt S, Angelini S, Yang K, Nyberg F, Lambert B, Hemminki K. The XPD variant alleles are associated with increased aromatic DNA adduct level and lung cancer risk. *Carcinogenesis* 2002;23:599-603.
8. Matullo G, Palli D, Peluso M, Guarrera S, Carturan S, Celentano E, et al. XRCC1, XRCC3 XPD gene polymorphisms, smoking and (32) P-DNA adducts in a sample of healthy subjects. *Carcinogenesis* 2001;22:1437-45.
9. Palli D, Russo A, Masala G, Saieva C, Guarrera S, Carturan S, et al. DNA adduct levels and DNA repair polymorphisms in *UFA*-exposed workers and a general population sample. *Int J Cancer* 2001;94:121-27.
10. Spitz MR, Wu X, Wang Y, Wang LE, Shete S, Amos CI, et al. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. *Cancer Res* 2001; 61: 1354-57.
11. Wiencke JK. DNA adduct burden and tobacco carcinogenesis. *Oncogene* 2002; 21: 7376-91.
12. Tang D, Cho S, Rundle A, Chen S, Phillips D, Zhou J, et al. Polymorphisms in the DNA repair enzyme XPD are associated with increased levels of PAH-DNA adducts in a case-control study of breast cancer. *Breast Cancer Res Treat* 2002; 75: 59-166.
13. Pastorelli R, Cerri A, Mezzetti, M, Consonni, E, Airoldi L. Effect of DNA repair gene polymorphisms on BPDE-DNA adducts in human lymphocytes. *Int J Cancer* 2002; 100: 9-13.
14. Yeh CC, Sung FC, Tang R, Chang-Chieh CR, Hsieh LL. Polymorphisms of the XRCC1, XRCC3, & XPD genes and colorectal cancer risk: a case-control study in Taiwan. *BMC Cancer* 2005; 5: 12.
15. Mort R, Mo L, McEwan C, Melton DW. Lack of involvement of nucleotide excision repair gene polymorphisms in colorectal cancer. *Br J Cancer* 2003; 89: 333-37.
16. Moreno V, Gemignani F, Landi S, Gioia-Patricola L, Chabrier A, Blanco I, et al. Polymorphisms in genes of nucleotide and base excision repair: risk and prognosis of colorectal cancer. *Clin Cancer Res* 2006;12:2101-108
17. Skjelbred CF, Saebo M, Wallin H, Nexo BA, Hagen PC, Lothe IM et al. Polymorphisms of the XRCC1, XRCC3 and XPD genes and risk of colorectal adenoma and carcinoma, in a Norwegian cohort: a case control study. *BMC Cancer* 2006; 6: 67.
18. Millerr SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Res* 1988;16:1215
19. <http://www.ncbi.nlm.nih.gov/SNP>
20. <http://www.yeastgenome.org/cgi-bin/web-primer>
21. Stern MC, Siegmund KD, Conti DV, Corral R, Haile RW. XRCC1, XRCC3, and XPD polymorphisms as modifiers of the effect of smoking and alcohol on colorectal adenoma risk. *Cancer* 2006;15:2384-90.
22. Friedberg EC. DNA damage and repair. *Nature* 2003;4:421-36.
23. Stary A, Sarasin A. The genetics of the hereditary xeroderma pigmentosum syndrome. *Biochimie* 2002;84:4960.
24. Tirode F, Busso D, Coin F. Reconstitution of the transcription factor TFIIH: assignment of functions for the three enzymatic subunits, XPB, XPD, and cdk7. *Mol Cell* 1999;3:8795
25. Qiao Y, Spitz MR, Shen H, Guo Z, Shete S, Hedayati M, et al. Modulation of repair of ultraviolet damage in the host-cell reactivation assay by polymorphic XPC and XPD/ERCC2 genotypes. *Carcinogenesis* 2002;23:295-299.
26. Wu X, Zhao H, Wei Q, Amos CI, Zhang K, Guo Z, et al. XPA polymorphism associated with reduced lung cancer risk and a modulating effect on nucleotide excision repair capacity. *Carcinogenesis* 2003;24:505-509.
27. Vogel U, Nexo BA, Tjonneland A, Wallin H, Hertel O, Raaschou-Nielsen O. ERCC1, XPD and RAI mRNA levels in lymphocytes are not associated with lung cancer risk in a prospective study of Danes. *Mutation Research* 2006;593:88-96.
28. Errico D, Calcagnile A, Iavarone I, Sera F, Baliva G, Chinni LM, et al. Factors that influence the DNA repair capacity of normal and skin cancer-affected individuals. *Cancer Epidemiol Biomarkers Prev* 1999;8:553-59.

## ***24 Relationship between XPD Lys 751 Gln polymorphism and colorectal cancer risk***

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29. Krtolica A, Campisi J. Cancer and aging: a model for the cancer promoting effects of the aging stroma. *Int J Biochem Cell Biol* 2002;34:1401-14.

30. Lombard DB, Chua KF, Mostoslavsky R, Gostissa M. DNA Repair, Genome Stability, and Aging. *Cell* 2005;120:497–512.

31. Hamilton ML, Remmen HV, Drake JA, Yang H, Guo ZM, Kewitt K, et al. Does oxidative damage to DNA increase with age? *PNAS* 2001;98:10469-74.

32. Guo ZM, Heydari A, Richardson A. Nucleotide excision repair of actively transcribed versus nontranscribed DNA in rat hepatocytes: effect of age and dietary restriction. *Exp Cell Res* 1998;245:228–38.