EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 4
729-733
Year: 2018

ASSOCIATION OF XPD LYS751GLN POLYMORPHISM WITH GASTRIC CANCER RISK IN KASHMIRI POPULATION

Bushra Nissar[1], Showkat A. Kadla[2], Falaque UL Afshan[1], Misbah[1], Idrees A. Shah[1], and Prof. Bashir Ahmad Ganai[3]*

[1]Department of Biochemistry, University of Kashmir, Srinagar, India.
[2]Department of Gastroenterology, Government Medical College and Hospital, Srinagar, India.
[3]Centre of Research for Development, University of Kashmir, Srinagar, J&K, India.

***Corresponding Author: Prof. Bashir Ahmad Ganai**
Centre of Research for Development, University of Kashmir, Srinagar, J&K, India.

Article Received on 05/02/2017 **Article Revised on 26/02/2018** **Article Accepted on 19/03/2018**

ABSTRACT
Genetic polymorphisms in several DNA repair genes can affect the capacity of DNA repair and are therefore associated with susceptibility to many cancers, including gastric cancer. In the present study, polymorphic variants of the DNA repair gene, XPD Lys751Gln was studied in association with the gastric cancer risk in Kashmiri population. A total of 60 histologically confirmed GC patients and matched controls were recruited for the study who underwent surgical resection. The genotyping of XPD gene was carried out by polymerase chain reaction-restriction fragment length polymorphism. In conclusion, polymorphic variants of XPD Lys751Gln (O.R=1.041, 95% C.I=0.25 - 4.26) were not found to be associated with the risk of gastric cancer in Kashmiri population. However, further studies will be needed with a larger sample size to authenticate these findings.

KEYWORDS: DNA repair, Gastric cancer, Kashmir, Polymorphism, XPD.

INTRODUCTION
Gastric cancer (GC) is the fifth most common cancer as regards the prevalence and second leading cause of cancer related deaths among both the sexes, remaining a major clinical health challenge with a poor prognosis.[1,2,3] In India gastric cancer is the fifth most common cancer among males and ranks seventh in females.[4] In Kashmir valley GC is the most frequently encountered cancer among males and third most commonly reported cancer among females.[5] Kashmir, that possesses different geographic location follows a unique pattern of dietary practices and is exposed to specific set of environmental and dietary carcinogens that can lead to DNA damages and eventually to cancer.[6]

The existing DNA repair systems, however maintain the genomic integrity and provide protection against the DNA damaging exposures. At least four different DNA repair pathways exist that function on specific types of DNA damages. Evidences suggest that the NER is amongst the most versatile mechanisms of DNA repair. It is a multipurpose DNA damage removal pathway that removes a wide variety of DNA damages including bulky mono-adducts, UV-induced photoproducts, cross-links and oxidative damage.[7] One of the most important proteins of the NER pathway is Xeroderma Pigmentosum D (XPD), which is also known as Excision Repair Cross Complementation group 2 (ERCC2). XPD is an ATP dependant helicase that exists as a part of multi-subunit, TFIIH (transcription repair factor II helicase) complex, participating in unwinding of the DNA during NER and transcription.[8] Several SNPs have been reported in the XPD gene, of which Lys751Gln (rs13181) SNP results in an amino acid change in a domain that is important for the interaction with p44 helicase activator and result in a change in the XPD function.[9,10]

A number of existing studies have suggested that the polymorphisms in the DNA repair genes may contribute to the susceptibility to cancers by altering the repair capacity.[11] Evidences also suggest that XPD polymorphism is associated with an increased gastric cancer risk.[12-16] However, analysis of the same XPD variants showed lack of any association in other population based studies.[17-20] Therefore, the present case-control study was carried out to investigate whether the polymorphism in the DNA repair gene XPD/ERCC2 (Lys751Gln) is associated with the risk of developing GC in Kashmir.

MATERIALS AND METHODS
Study subjects and data collection
A total of 60 histologically confirmed GC patients and matched Controls were recruited for the study who

underwent surgical resection, from SKIMS, Srinagar. All the patients duly signed the informed consent and the recruitment was initiated following the approval from the Institutional Ethics committee. The tissue samples were collected during surgical resection, properly labelled and stored in – 80° C prior to use. Genomic DNA was isolated from the tissue samples of all the subjects manually using the standard phenol/ chloroform method.[21]

Polymerase Chain Reaction

The XPD Lys751Gln genotypes were determined by the PCR amplification and restriction digestion of the products with restriction enzyme *Pst*I (Thermo Fischer Scientific Inc. (EU), Lithuania. The primers used for the amplification of a region of 436 bp carrying the restriction site for *Pst*I were as follows: Forward primer (5'- GCC CGC TCT GGA TTA TAC G-3') and Reverse primer (5'- CTA TCA TCT CCT GGC CCC C-3') (Integrated DNA Technologies, Coralville, Lova). All the reactions were carried out in a total reaction volume of 25µl containing 50-60 ng of genomic DNA template, and 1U of Taq DNA polymerase. The PCR conditions were as follows: Initial denaturation for 10 minutes at 94℃, followed by 35 cycles, each of 1 minute denaturation at 94℃, 30 seconds annealing at 65℃ and 45 seconds extension at 72℃, followed by a final extension at 72℃ for 7 minutes. The amplified PCR product was resolved on 2% agarose gel containing 0.5 µg/ml ethidium bromide, and visualized under Gel documentation system (Figure 1).

Restriction Fragment Length Polymorphism

The PCR amplicon of 436bp was digested with *Pst*I overnight at 37℃. The digestion products were resolved on 3% agarose gel containing 0.5 µg/ml ethidium bromide, using a gel electrophoresis system at 100 V for 30-40 min and visualized under UV light. The *Pst*I digestion resulted in two fragments of 290 bp and 146 bp for homozygous wild genotype (Lys/Lys), three fragments of 227 bp, 146 bp, and 63 bp for homozygous variant genotype (Gln/Gln) and four fragments of 290 bp, 227 bp, 146 bp and 63 bp for heterozygous genotype (Lys/Gln) (Figure 2).

Statistical Analysis

Numbers and percentages were calculated and presented for categorical variables, as well as means and standard deviations (SD). Genotype frequencies of the XPD gene polymorphism in Kashmiri population were determined according to Hardy–Weinberg equilibrium and data were analyzed using the computer software SPSS (version 20).

RESULTS

The mean age of cases was 61.24±10.22 years of which 44 were males and 16 were females. In both the cases and controls predominant age group was 60-70 years followed by 50-60 years. Homozygous wild genotype AA was present 25% of cases and 20% of controls. The control population exhibited a similar frequency of wild AA genotype as compared to the cases. 71.7% of the controls and 65% cases carried heterozygous AC genotype respectively. The CC homozygous variant gene was present in 10% cases and 8.3% controls respectively. The frequency of wild (A) allele was observed in 57.5% and 55.83% in cases and controls respectively. The distribution of minor allele frequency (O.R=1.07, 95% CI=0.64-1.78) and genotypic frequency (O.R=1.041, 95% C.I=0.25 - 4.26) was not significantly different between the cases and controls (Table 1). The correlation of XPD Lys751Gln polymorphism of XPD gene with various studied parameters of study population is presented in table 2.

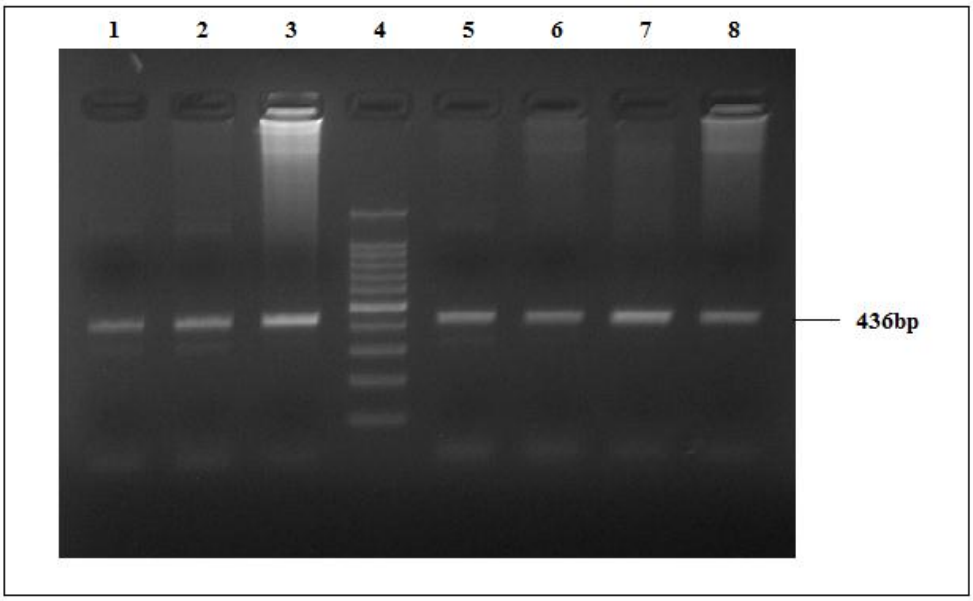

Figure 1: Representative gel picture showing PCR product of ERCC2/XPD codon 751 run on 2% agarose gel.
Lane 4: 100bp ladder
Lane 1-3, 5-8: 436bp PCR product.

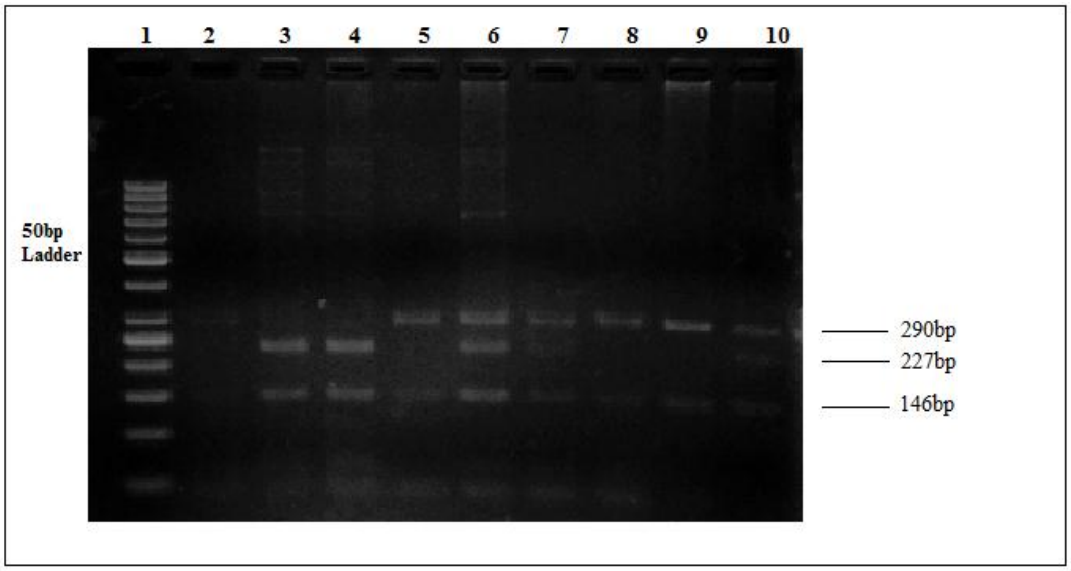

Figure 2: Representative gel of showing Pst I digested amplicons of XPD codon 751 run on 3% agarose gel.
Lane 1: 50-bp DNA ladder.
Lane 2, 5, 8, 9: 146bp and 290bp Lys/Lys genotype.
Lanes 6, 7, 10:146 bp, 227bp, 290 bp and 63 bp Lys/Gln genotype.
Lanes 3 and 4: 146 bp, 227bp and 63 bp Gln/Gln genotype.

Table 1: Genotype analysis and Allele frequency of Lys751Gln (AC) polymorphism in XPD gene.

XPD Lys751Gln (AC) polymorphism		Cases n=60 (%)	Controls n=60 (%)	OR (95% CI)	P-value
Genotypes	**AA**	15 (25)	12 (20)	1 (Reference)	
	AC	39 (65)	43 (71.7)	1.378 (0.57-3.30)	0.470
	CC	6 (10)	5 (8.3)	1.042 (0.25-4.26)	1
AA vs. AC+CC	**Present**	15 (25)	12 (20)	1.33 (0.56-3.15)	0.512
	Absent	45 (75)	48 (80)		
Allele Frequency	**A**	69 (57.5)	67 (55.83)	1.070 (0.64-1.78)	0.791
	C	51 (42.5)	53 (44.16)		

Chi-Square analysis and odds ratio analysis was used for genotyping analysis where *P* value of <0.05 at 95% confidence interval was considered to be significant. AA (Wild homozygous): Reference allele

Table 2: Demographic characteristics of gastric cancer cases and controls.

Characteristics		Cases n (%)	Controls n (%)	P-value
Total		60	60	-
Gender	**Males**	44	48	-
	Females	16	12	
Age	**>50**	46	53	-
	≤50	14	7	
Residence	**Urban**	16	19	0.155
	Rural	44	41	

n number of individuals.
1 Chi-square test (χ^2) was used to calculate P values for categorical variables.

DISCUSSION

Gastric carcinogenesis is a multifactorial disease that results from an interaction between the environmental factors and the individual genetic susceptibility. The carcinogenic compounds from various environmental and/or dietary sources can induce DNA alterations, which can lead to the genomic instability.[22] This genomic instability and the resulting genetic alterations are the key molecular and genetic events that occur early in the multistep process of gastric carcinogenesis.[23] However, the DNA repair pathways maintain the genomic integrity[24,25,26] and provide protection against the carcinogenesis,[27] through the DNA damages reversal.[28] A large number of evidences suggest that the Single Nucleotide Polymorphisms (SNPs) in the DNA repair genes are associated with several sporadic cancers,[29,30] including GC.[31,26] In the current study, we studied the polymorphism of DNA repair gene XPD in association with gastric cancer development in Kashmiri population.

Xeroderma Pigmentosum D or ERCC2 (Excision Repair Cross Complementation group 2) is one of the genetic complementation groups that encodes for proteins involved in the NER pathway.[32] Located on chromosome no. 19q13.3, XPD is composed of 23 exons. It is a DNA helicase that is essential for both basal transcription and NER.[33] In the NER pathway, as a member of TFIIH complex XPD is responsible for the lesion demarcation and unwinding.[26] It participates in the DNA helix unwinding to allow the excision of DNA fragments with the damaged base.[10,34,35] About 17 SNPs have been detected in the XPD gene of which 6 are in exons and 11 are in introns.[9] Of these 6 coding gene polymorphisms two common SNPs occur in codon 312 and 751 of XPD have been thoroughly studied. The XPD 751 polymorphism modifies the amino acid in an important interaction domain of XPD that affects the interaction with helicase activator p44 and may produce a relevant change in the XPD function.[33]

In the present, study we detected the polymorphism of XPD genes in 60 gastric cancer tissue samples and normal adjacent samples from the same patients. The results showed no statistically significant differences in the allelic or in the genotypic frequencies of XPD Lys751Gln polymorphisms between the controls and cases with GC. These results are in agreement with the results from previous studies in Polish,[36] Turkish,[37,38] Italian,[19] and Chinese populations.[39] However, unlike our results some of the earlier studies reported XPD Lys751Gln polymorphism to be significantly associated with the risk of GC.[12-16] These differences in the findings may be due to difference in ethnicities, study design and smaller sample size. The study needs to be repeated in a larger cohort and with a more valid source of DNA from blood for polymorphic the study.

CONCLUSION

In conclusion, our study reports that the polymorphism of XPD Lys751Gln is not associated with the risk of gastric cancer in Kashmiri population. However, further replicative studies with a lager sample size are warranted to validate these findings.

ACKNOWLEDGEMENT

The authors acknowledge the financial support provided by the Council of Scientific and Industrial Research (CSIR), Pusa, New Delhi under the reference number: **09/251(0049)/2012-EMR-I**. The authors are extremely thankful to all the subjects who took part in this study, for their valuable collaboration and understanding. We are also grateful to all the consultants and all the members of paramedical staff at SMHS hospital, Kashmir, for their valued help during subject recruitment and sample collection.

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