

Gastric Cancer Risk and XPD/ERCC2 SNPS (LYS751GLN, ASP312ASN) Gene Polymorphism—An Experimental Study in Kashmir Valley of India

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Abstract:

ERCC2/XPD, is one of the core genes involved in transcription- coupled NER pathway (a fundamental defense mechanism against the carcinogenic effects of sunlight and certain genetic defects in the DNA repair pathways); and its functional polymorphisms may be associated with the risk of different types of cancers. The present investigation includes Lys751Gln & Asp312Asn polymorphisms in a case-control hospital based study in 100 Kashmiri GC patients & 100 age & sex matched controls. It was observed that most of the cases were males, numbering 72 with a maximum number falling in the age group of 56-65 years; whereas female GC cases were only 28 with a maximum number in the age group of 56-65 years as in the males. The male GC cases were all smokers especially hooka smokers. All female cases were non-smokers. Statistically significant results were observed in GC cases who were smokers with a p-value of <0.0001; $\chi^2 = 63.15$; O.R. = 7.427 and 3.919 to 14.081 of 95% C.I., suggesting a strong correlation between smoking and the risk of GC. Among the dietary factors the intake of salt tea that was consumed by both the GC cases as well controls was found to be statistically insignificant (Fishers exact test = 0.134; O.R. = 1.644 and 95% C.I. = 0.90 to 2.976). Statistically significant values were found in GC cases who included preserved dried foods in their diet. (Fishers exact test = <0.0001; O.R. = 11.643; and 95% C.I. = 5.542 to 24.46). The intake of too much of spices and chillies in the diet of GC cases was also found to be statistically significant. (Fishers exact test = 0.007; O.R. = 2.257 and 95% C.I. = 1.28 to 3.979) suggesting the role of these dietary foods in the GC risk. Family

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history of carcinogenesis although found in the small number of GC cases and none in the control group was moderately statistically significant. (Fishers exact test =0.029).

The genotypic frequencies of ERCC2codon 751 showed a significant difference between cases and controls ($\chi^2 =16.85$ $p \leq 0.0001$). Allelic frequencies revealed statistically significant excess of Gln allele in cases as compared to controls and a low percentage of Lys allele in cases as compared in controls (Fishers exact test = ≤ 0.0001 , OR =0.213, 95% CI 0.106 to 0.426). The codon 312 of ERCC2 studied also showed statistically significant genotypic frequencies between cases and controls (χ^2 8.75 p value of 0.013). Allelic frequencies revealed statistically significant excess of Asn allele in cases as compared to controls and a low percentage of Asp allele in cases as compared in controls (Fishers exact test = 0.001, O.R = 0.384, 95% C.I = 0.218 to 0.675). The magnitude and statistical significance of Asp312Asn polymorphism was smaller and weaker than the Lys751Gln polymorphism studied in the present study of GC cases vs controls.

Abbreviations: ERCC –excision repair cross-complementing rodent repair deficiency; XPD- xeroderma pigmentosum group D; GC- gastric cancer; SNP-single nucleotide polymorphism; GMC government medical college Srinagar Kashmir; NIMS- National institute of medical sciences Rajasthan.

Key words: Gastric cancer, Gene polymorphism, Hooka Smokers, ERCC, Jammu and Kashmir

Introduction

Gastric cancer is one of leading causes of cancer-related deaths in both sexes and thus remains a worldwide burden (1–3). In India, gastric cancer is the fifth most common cancer among males and seventh most common cancer among females (4). The incidence of gastric cancer in India is high in certain geographical areas (southern part and north-eastern states of the country) where the incidence is comparable to high-incidence areas of the world (5). There has been a decline in the incidence of gastric cancer but this declining trend has not been seen in certain parts of India (6). In the valley of Kashmir J&K, situated in the northern part of India, the incidence of Gastric Cancer has been reported to be a highly prevalent malignancy and together with esophageal carcinoma it accounts for about 60% of all cancers, which is higher than in other parts of the region. Kashmir is a high prevalence zone of gastric cancer. The incidence of gastric cancer in Kashmir has been reported to exceed 40% of all cancers, and the incidence is three to six times higher than that at various metropolis cancer registries in India (7). Cancer of the stomach is amongst the first five cancers in the Kashmir valley, with a M: F ratio of 3.17:1. (8).

DNA repair systems play crucial role in maintaining the normal functioning of cells and in turn maintain the genomic integrity of an organism. This genomic stability can be altered by exposure to endogenous &/ exogenous agents damaging the cellular DNA which may lead to the accumulation of the probable mutations and may result in carcinogenesis. At

least four main partly overlapping DNA repair pathways exist in humans for repairing the DNA damage(9). Several polymorphisms in genes that encode for DNA repair proteins have been described and most of these are participants in the four major DNA repair pathways: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and double-strand break/recombination repair. SNPs of common DNA repair genes have been identified and demonstrated to be linked to sporadic carcinogenesis(10).

In mammals, nucleotide excision repair (NER) is the most versatile DNA repair mechanism responsible for removing a wide variety of helix-distorting lesions that intervene in base pairing and generally destruct transcription and normal replication(9). NER is also associated with the transcription coupled repair pathway(11). NER involves 25 proteins that locate the damaged strand, introduce incisions on each side of lesions, excise an oligonucleotide of 24 to 32 residues and fill in the gap by repair synthesis and ligation. The DNA helicase ERCC2 (XPD), subunit of the basal transcription factor TFIIH that is required for transcription initiation by RNA polymerase II, is also one of the core genes involved in transcription-coupled NER(9,12,13). The importance of ERCC2 is highlighted by the existence of three different disorders that are caused by hereditary defects in this protein, including cancer-prone syndrome Xeroderma Pigmentosum, Cockayne syndrome and Trichothiodystrophy (13).

Various studies have reported the associations of XPD/ERCC2 Lys751Gln; Asp312Asn; and /or Arg156Arg with the susceptibility to gastric cancer among different ethnicities, but with mixed or conflicting results(2,3,14-25). The role of the ERCC2 Asp312Asn and Lys751Gln was evaluated in several cancer case-control studies. Presence of variant alleles was reported to be associated with the risk of several types of cancers such as melanoma, prostate, bladder, esophagus among others (26-29). Genetic polymorphisms in the ERCC2 gene in thyroid cancer was reported and the results showed that the ERCC2 polymorphism Asp312Asn and Lys751Gln are not separately associated with a significant increase for thyroid cancer. A statistically significant proportion carrying the variant allele Asp312Asn also had the Lys751Gln allele. The subjects homozygous for both variant alleles were overrepresented in thyroid cancer group and the combination of the two variant forms of both polymorphisms [Asn312Asn and Gln751Gln] are associated with a significant increase risk to thyroid cancer suggesting an interaction between these two polymorphisms (30).

In one of the study of systematic review and meta-analysis, statistically significant findings were apparently noted in Asians but not in Caucasians for both XPD/ERCC2 Lys751Gln and XPD/ERCC2 Asp312Asn polymorphisms. The study suggested that Gln751Gln (CC) and Asn312Asn (AA) genotypes may be important biomarkers of GC susceptibility for Asians, the assumption that needs to be further confirmed in future well designed studies in Asian populations (31).

Materials & Methods

Study Population

The research work was the hospital based case control study, undertaken in order to understand the etiology of gastric cancer (GC) in the North-Indian state of Kashmir (J&K) India. The study subjects in this hospital based case-control study included 100 histopathologically confirmed GC cases and equally matched for age & sex, apparently healthy, normal controls who were not having any previous history of the disease. The study was carried over a period of two and a half years from Aug 2012, in the Research Laboratories of Department of Biochemistry, Government Medical College /Shri Maharaja Hari Singh ji Hospital, Karan Nagar, Srinagar in collaboration with the Division of Gastroenterology & Hepatology GMC/S.M.H.S. Hospital Srinagar Kashmir after approval of the study by the Ethical Clearance Committee of the Department of Biochemistry GMC Sgr. The study was designed in order to evaluate the role of XPD/ERCC2 (Lys751Gln and (Asp312Asn) gene polymorphism in GC. Association of various risk factors in GC, like smoking, dietary food habits and family history of the disease was also taken into consideration. Information on demographic characteristics, family history of cancer, lifestyle habits (smoking) and dietary factors was collected and properly maintained using a questionnaire. Blood samples of all the patients and controls were collected in 5ml properly labelled EDTA tubes, transported immediately and stored at -80 °C (until DNA extraction)

DNA Extraction

Genomic DNA was extracted by kit method. The kit used was GeNei™ supplied by MERCK SPECIALITIES PRIVATE LIMITED. Protocol followed was as per the kit instructions for the DNA Extraction from frozen blood. DNA extracted was qualitatively & quantitatively assessed by gel electrophoresis & and by measuring optical density at 260nm and 280 nm by double beam spectrophotometer. The ratio of 260/280nm was calculated and only those DNA samples for which the ratio was 1.7-1.9 were considered for the experimental use. DNA was aliquoted into three to four tubes so as to protect damage from freeze thawing and stored in -80°C Deep freezer until use.

Genotyping:

For determining ERCC2 Lys751Gln and Asp312Asn polymorphisms, PCR was performed followed by RFLP analysis. DNA samples for A35931C polymorphism (Lys751Gln) in exon 23 were amplified with the follow primers, 5'-CCCTCTCCCTTCTCTGTCTCTGC-3' (upstream) and 5'-GGAACAGTGCAGGAGGGATGGG-3' (downstream). The PCR was done in a 50-µL reaction containing 1× PCR buffer, 2.5 mmol/L MgCl₂, 0.8 mmol/L deoxynucleotide triphosphate, 1.0 µmol/L of each primer, and 1.25 units of

AmpliTaq Gold (Applied Biosystems, Foster City, CA). PCR was performed with initial denaturation at 94°C for 7 minutes followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 62°C, 30 seconds at 72°C and final extension step of 10 minutes at 72°C. Ten microliters of amplified fragment was digested with 5 units of PstI (Fermentas, Vilnius, Lithuania) for 90 minutes at 37°C. The fragments obtained were analyzed in a 2% agarose gel with ethidium bromide (0.5 mg/mL). The homozygous wild-type (Lys751) produced one restriction site (28 and 331 bp); whereas the homozygous variant allele (Gln751) produced three bands (28, 63 and 268 bp) and heterozygous (Lys/Gln) displayed all four bands (28, 63, 268 and 331 bp). The amplification fragment for G23591A polymorphism (Asp312Asn) in exon 10 was obtained with the primers, 5'-CTGTTGGTGGGTGCCCGTATCTGTTGGTCT-3' (upstream) and 5'-TAATATCGGGGCTCACCCCTGCAGCACTTCCT-3' (downstream)(32). PCR was performed in 25 µl reaction mixtures containing 1.5 mM MgCl₂, 0.2 mM deoxynucleotide triphosphates, 3% DMSO, 0.2 µM primers, 1 µg of template DNA, and 1.5 units of Taq polymerase in PCR buffer [10 mM Tris-HCl (pH 9.0 at 25°C), 50 mM KCl, and 0.1% Triton X-100 (Promega). PCR was performed with initial denaturation at 94°C for 4 min followed by 30 cycles of 30 s at 94°C, 30 s at 60°C, 60 s at 72°C and then by a final extension step of 5 min at 72°C. The restriction analysis was done in which 10 µl of PCR product was digested with 5 units of Eco130I (Fermentas) for 120 minutes at 37°C. The fragments obtained were analyzed on a 2% agarose gel with ethidium bromide (0.5 mg/mL). The homozygous wild-type allele (Asp312) produced one restriction site (245 and 506 b.p.); whereas the homozygous variant allele (Asn312) produced three bands (32, 245, and 474 b.p.) and heterozygous (Asp/Asn) displayed all four bands (32, 245, 474 and 506 b.p.). The Genotypic determinations were carried out in independent experiments strictly following the PCR conditions. Samples that failed to amplify were repeated and reanalyzed.

Statistical Analysis

Corresponding data were tabulated and results were statistically analyzed. For gender data, subjects with gastric cancer and healthy control groups were compared by the chi-square (χ^2) tests in contingency tables. Genotypic and allelic frequencies were compared between groups using the χ^2 test/Fishers exact test. Odds ratios (OR) and the 95% confidence interval (CI) were calculated using SPSS software version 20. A p-value of <0.05 was used as a criterion for statistical significance.

Results

Out of the 100 confirmed cases of GC, 72 were males and 28 were of female gender. Keeping in view the prevalence of GC in different age groups, the patients were categorized in 5 age groups of; 26-35 years/36-45 years/46-55years/56-65years/66-75 years and 76-85 years. From the age group of 26-35 years & 36-45 years there were no male patients, whereas there were 2 females from 26-35 years & 2 from 36-45 age group. From the age group of 46-55 years there were 16 males and 10 females. From 56-65 years the number of GC cases was largest, at 32 for males and 14 females. It was second largest for males in the age group of 66-75 years the number of cases were 20 in this age group for males and 4 in the 76-85 years whereas no female GC cases were in these last two age groups 66-75 years & 76-85 years. Equal number of controls from each age group and gender were taken .The controls from each age group & gender taken, were healthy with no known history of any malignancy or any other disease. Consent was taken from both patients and control subjects during the recruitment of patients and subjects in the study. Complete information of the patients as per the questionnaire was obtained and recorded.

Majority of the GC patients in this study were in the age group of 56-65 years with the largest number of males falling in this age group. Females under study were highest in the age group of 46-55 years. There were no males in the age groups of 26-35 years & 36-45 years; and no females in the age groups of 66-75 years & 76-85 years.

Table 1: Number of Males & Females in GC Patients and Respective Controls.

AGE (YEARS)	GC PATIENTS (MALE)	CONTROLS (MALES)	GC PATIENTS (FEMALES)	CONTROLS (FEMALES)
26-35	0	0	2	2
36-45	0	0	2	2
46-55	16	16	10	10
56-65	32	32	14	14
66-75	20	20	0	0
76-85	4	4	0	0
TOTAL	72	72	28	28

Risk Factors (Smoking.)

As observed in the study, out of 72 male GC cases 65 were smokers and among them 51 were hooka smokers and least number were snuff smokers. Among the male controls, maximum number of subjects were non- smokers with 16 persons consuming smoke in the form of cigarettes and only 4 were hooka smokers. The females GC cases were 28, all of them being non-smokers. The female controls in the study were also non-smokers.

Table 2:- Risk Factor (Smoking)

RISK FACTOR	CASES	CONTROLS	χ^2	p-value
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(SMOKING)	(n=100)	(n=100)		
HOOKA	51	4	63.15	≤0.0001
CIGARETTE	10	16		
SNUFF	4	0		
NON-SMOKERS	35	80		

RISK FACTOR (SMOKING)	CASES (n=100)	CONTROLS (n=100)	p-value	O.R (ODDS RATIO)	95% C.I. (CONFIDENCE INTERVAL)
SMOKERS	65	20	≤0.0001	7.429	3.919 to 14.081
NON-SMOKERS	35	80			

Statistically significant findings were noted in the GC patients who were smokers with a p-value of <0.0001; $\chi^2=63.15$; O.R. =7.427 and 3.919 to 14.081 of 95% C.I., suggesting a strong correlation between smoking and the risk of GC.

Dietary/Risk Factors:

Various dietary factors were considered in the study. Both male and female GC patients consumed about 400-600ml of the salt tea daily. Besides this the intake of preserved dried foods as well as the consumption of too much of spices/ chillies in the diet of cases as well as controls were included in the study. The sun-dried vegetables were also consumed by cases as well as took high amount of spices/ chillies in their diet. Family history of the cases as well as controls was taken into consideration. Majority of the patients from both males and females in cases as well as controls did not had any significant family history of the disease.

Table 3:-Dietary/Risk Factors

Dietary Factors	Cases (n=100)	Controls (n=100)	Fishers Exact Test	O.R. (Odds Ratio)	95% C.I. (Confidence Intervals)
1.SALT TEA (1 cup= 100 ml)					
a) 5-10cups/day	39	28	0.134	1.644	0.90 to 2.976
b) 2-4cups/day	61	72			
2.PRESERVED DRIED FOODS					
a)Yes	89	41	≤0.0001	11.643	5.542 to 24.46
b)No	11	59			
3.SPICES/CHILLIES					
a) High	57	37	0.007	2.257	1.28 to 3.979
b) Moderate	43	63			
4.FAMILY HISTORY			0.029	-	-
a)Yes	6	0			
b)No	94	100			

Statistically significant values were obtained for the GC patients who took preserved dried foods in their diet with Fishers exact test of ≤0.0001 which is

statistically highly significant. O.R. calculated was 11.643 with 5.542 to 24.46 of 95% C.I. Inclusion of high amount of spices/ chillies among the patients was also statistically significant, with Fishers exact test of 0.007. O.R. calculated was 2.257 and 95% C.I. calculated was 1.28 to 3.979. Family history of the patients as well as the controls was not there, only 6 patients had family history of any type of cancer which too is statistically significant with Fishers exact test of 0.029. The consumption of salt tea by the GC patients was found to be statistically insignificant with Fishers exact test of 0.134; O.R. of 1.644 & 95% C.I. of 0.90 to 2.976 respectively.

Table 4. Genotypic & Allelic Frequencies of ERCC2 Gene Codon 751 among Cases & Controls and Their Association with Risk of Gastric Cancer.

ERCC2 GENE (CODON751)	Variants	Cases (n=100)	Control (n=100)	Fishers Exact test	O.R. (Odds Ratio)	95% C.I. (Confidence Interval)	χ^2 (p-value)
Genotypic Frequency	Lys/Lys	69	92	0.001	0.197	0.070to0.555	16.85(\leq 0.0001)
	Lys/Gln	19	5	1.00	0.950	0.191to4.722	
	Gln/Gln	12	3	0.007	0.188	0.051to0.690	
Allelic Frequency	Lys	157(78.5)	189(94.5)	\leq 0.0001	0.213	0.106to0.426	
	Gln	43(21.5)	11(5.5)				

The genotypic frequencies of ERCC2 gene of codon 751 showed a significant difference between cases and the controls ($\chi^2 = 16.85$ $p \leq 0.0001$) which is statistically very significant. The Lys/Lys homozygous wild type was found in both the cases and controls with a frequency of 69% cases Vs 92% in controls. Heterozygous Lys/Gln variant was found in most of cases as compared to controls with 19% in cases vs. 5% in controls. The homozygous Gln/Gln variant was similarly found in most cases with 12% vs 3% in controls. Allelic frequencies revealed statistically significant excess of Gln allele in cases as compared to controls and a low percentage of Lys allele in cases as compared in controls (Fishers exact test = ≤ 0.0001 , OR = 0.213, 95% CI 0.106to0.426)

Table 5. Genotypic & Allelic Frequencies of ERCC2 Gene Codon 312 among Cases & Controls and Their Association with Risk of Gastric Cancer.

ERCC2 GENE (CODON 312)	Variants	Cases (n=100)	Controls (n=100)	Fishers Exact test	O.R.	95% C.I.	χ^2 (p-value)
Genotypic Frequency	Asp/Asp	73	89	0.039	0.389	0.166 to 0.910	8.75 (0.013)
	Asp/Asn	19	9	0.690	0.528	0.093 to 3.00	
	Asn/Asn	8	2	0.048	0.205	0.042 to 0.996	
Allelic Frequency	Asp	165	187	0.001	0.384	0.218 to 0.675	
	Asn	46	20				

The genotypic frequencies of ERCC2 gene of codon 312 showed a difference between cases and the controls ($\chi^2 8.75$ p value of 0.013) which being less than

0.05 is statistically significant. The Asp/Asp homozygous wild type was found in both the cases and controls with a frequency of 73% cases Vs 89% in controls. Heterozygous Asp/Asn variant was found in most of cases as compared to controls with 19% in cases vs. 9% in controls which was statistically insignificant. The homozygous Asn/Asn variant was similarly found in most cases with 8% vs 2% in controls. Allelic frequencies revealed statistically significant excess of Asn allele in cases as compared to controls and a low percentage of Asp allele in cases as compared in controls (Fishers exact test = 0.001, O.R = 0.384, 95% C.I = 0.218 to 0.675).

Discussion

In our case-control study, SNPs in the ERCC2 gene Codon Lys751Gln and codon Asp312Asn and their association with the risk of GC in Kashmiri population of India was investigated in 100 GC cases and 100 normal controls. Polymorphism assessed was present in both the codons. Each polymorphism in the 751 codon of the human ERCC2 gene was composed of the following genotypes – the wild type or polymorphic variant type with different RFLP size distributions. Lys/Lys was the homozygous wild type allele; Lys/Gln the heterozygous variant whereas Gln/Gln represents the homozygous mutant. Out of these the homozygous wild type Lys/Lys was found in 69 of cases and higher in the control group with 92, but was statistically significant. Heterozygous variant Lys/Gln though was found to be higher in cases than in the controls with 19 GC cases and 5 controls but was found to be statistically insignificant ,whereas the homozygous mutant Gln/Gln was significantly higher in cases with 12 GC cases as compared to 3 controls which is again statistically significant. The genotypic frequencies of ERCC2 gene of codon 751 showed a significant difference between cases and the controls ($\chi^2 = 16.85$ $p \leq 0.0001$) which is statistically very significant suggesting that SNP in the codon 751 that results in Lys to Gln substitution may be associated with the risk of GC in the Kashmiri population of J&K India. Allelic frequencies revealed statistically significant excess of Gln allele in cases as compared to controls and a low percentage of Lys allele in cases as compared in controls. In all the three variant forms the Lys/Lys homozygous wild type was taken as a referent category.

Polymorphism in codon 312 of the ERCC2 gene was composed of the genotypes; Asp/Asp the homozygous wild type found in both cases as well as controls with 73% in cases vs 89% in controls which again being higher in controls than in the cases but was statistically significant. Heterozygous variant Asp/Asn was though higher in cases than in the controls with 19% of GC cases vs 9% controls but was statistically insignificant, whereas the homozygous mutant type Asn/Asn was found higher in cases than in the controls with 8% of GC cases vs 2% controls and is statistically significant. For this codon also the genotypic frequencies showed statistical

difference between cases and controls ($\chi^2 = 8.75$; p-Value = 0.013) which being statistically significant, thereby, suggesting that the polymorphism in this codon may also be associated with the risk of GC in the Kashmiri population of J&K India. Allelic frequencies revealed excess of Asn in cases as compared to controls, whereas Asp allele was predominant in controls as compared in cases. For this codon also the homozygous wild type Asp/Asp was taken as a referent category.

The present study also included the role of various factors such as smoking and dietary factors that may be associated with the risk of GC in Kashmir. Smoking increases the risk of developing gastric cancer significantly, from 40% increased risk for current smokers to 82% increase for heavy smokers. Based on solid evidence, smoking is associated with an increased risk of stomach cancer (33-35). In the present study there was a large number of GC cases, consuming smoke in the form of hooka 51% cases vs 4% controls ; cigarette smokers were higher in controls than in the cases 10% cases vs 16% controls; whereas there were only a small percentage of snuff smokers 4% case vs 0% controls. Overall the number of smokers in cases was higher than in the controls with 65% cases vs 20% controls. The number of non-smokers in the control group was higher as compared in the cases with 35% in cases vs 80% controls. This was statistically very significant, suggesting a strong correlation between the GC and smoking.

Dietary factors are thought to contribute to the large international variation in GC rates (36). The excess intake of hot salted tea , consumption of dried leafy vegetables, pickled vegetables, dried smoked fish, dried raw food, spice cakes (wur), use of red chillies are some of the distinctive dietary habits for the increased risk of GC in Kashmir (37,38). Our study included GC cases and controls both relishing the intake of the traditional salt tea which was statistically insignificant, suggesting that the intake of salt tea does not contribute to the incidence of GC in Kashmir. Dried foods were consumed by both the cases as well as controls with 89% cases vs 41% controls consuming dried foods in their diet. Among the non-consumers of the dried foods the number was higher in controls than in the cases with 11% cases vs 59% controls. This was found to be statistically very significant, suggesting that the intake of various dried foods can be an important risk factor in GC among the Kashmiri population. The intake of high amount of spices/chillies in the diet was found to be high in the cases than in the controls with 57% cases vs 37% controls, whereas very less or moderate amount of spices/chillies were consumed by a large proportion of controls than the cases with 43% cases vs 63% controls; which again was found to be statistically significant. Family history of cases as well as controls was also taken into consideration. Among the cases there were only 6 cases who had the family history of the GC or any other malignancy whereas maximum number of cases had no family history of the disease or any other malignancy. All the controls did not had any family

history of any malignancy and were apparently healthy. Statistically this was also found to be significant.

Conclusion

ERCC2 also known as the Xeroderma pigmentosum complementation group D, XPD, is one of the core genes involved in transcription- coupled NER pathway and its functional polymorphisms may be associated with the risk of different types of cancers. Several SNPs have been identified in the ERCC2 gene. Among them, polymorphism in the codon 751 results in a Lys to Gln substitution whereas polymorphism in the codon 312 results in Asp to Asn substitution. As these polymorphisms have been found to be associated with an increased risk of several types of cancers, the present study was also undertaken in order to see the possible role of ERCC2 gene polymorphism in the GC cases of Kashmiri population of J&K India. Two polymorphisms were investigated Lys751Lys and Asp312Asn. Besides the role of various risk factors like the smoking, family history and dietary factors was also considered in order to evaluate the role of these dietary/risk factors in the association of GC risk.

This is the first study reporting the association of SNPs in ERCC2 gene in the risk of GC in the Kashmiri population of J&K. Our data suggest an elevated risk of GC in individuals with the ERCC2 Lys751Gln and Asp312Asn polymorphisms, particularly the Lys751Gln polymorphism in the Kashmiri population of J&K India. The magnitude and statistical significance of Asp312Asn polymorphism was smaller and weaker than the Lys751Gln polymorphism. The study also revealed the role of various risk factors in GC. Among the associated risk factors, as has been documented in various studies and as per the results obtained from this study, Smoking was found to be associated with the risk of GC. Another significant finding in the current study was the role of various dietary habits that are peculiar to the Kashmiri population like preserved dried foods, too much of spices/chillies in the diet, statistically significant results were obtained suggesting these dietary factors to be associated with the risk of GC. The study being the first to report the association of ERCC2 gene polymorphisms in the GC risk in Kashmir, the results need to be further assessed and confirmed on a larger case-control study.

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