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Alteration of the risk of oral pre-cancer and cancer in North Indian population by XPC polymorphism genotypes and haplotypes

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ABSTRACT

Chewing and smoking of tobacco have been reported to cause DNA damage in oral mucosa which can be repaired by Xeroderma Pigmentosum Group C (XPC) protein. This study aimed to evaluate the association of XPC gene polymorphisms with the risk of oral diseases including oral pre cancer and cancer. In the present study we genotyped 302 patients with oral diseases and 300 healthy controls for XPC PAT (D > I), C > T and A > C polymorphisms with PCR-RFLP and PCR method. Haplotypes were constituted from different XPC genotypes with SNPstats programme. Our results show that individuals with D/I genotype for XPC D > I polymorphism were significantly protected from developing Oral submucous fibrosis (OSMF) and Leukoplakia (p = .029 and 0.031 and respectively). Risk of oral cancer was also significantly lower with the I/I genotype of the same XPC polymorphism (p = .048). However, once oral cancer is established, individual with I allele were at significantly increased risk for having high stage and metastatic tumor (p = .011 and 0.03 respectively). Carrier of T allele for XPC C > T polymorphism were significantly protected from development of OSMF (p = .004) but did not show any association with the development of other pre oral cancerous lesions or oral cancer. In contrast, CC genotype as well as C allele for XPC A > C polymorphism was significantly associated with increased risk of Lichenplanus (p = .006 and 0.004 respectively). XPC C > T and A > C polymorphisms did not show any association with clinical parameters of oral cancer. Haplotype D/T/A and I/T/A showed protective association with development of oral disease (P-value = .001 and 0.0001 respectively). In conclusion, results from the present study demonstrate association of XPC polymorphisms with oral pre cancer and cancer.

1. Introduction

Compared to the U.S. oral cancer development is high in India (Kekatpure and Kuriakose, 2010). In India, 20 per 100,000 people are affected by oral cancer which accounts for about 30% of all types of cancers. Invasive oral cancer is usually introduced by the presence of clinically known dysplasia of the oral mucosa or oral pre cancerous lesions. These oral pre cancers include, Lichen Planus, Leukoplakia, and Oral submucous fibrosis (OSMF). An oral pre cancerous lesion is defined as a benign, morphologically altered tissue that has a greater than normal risk of cancer transformation (Warnakulasuriya et al., 2007). Caffeine, tobacco, alcohol are known to be associated with a high number of cases of OSMF which are potentially malignant. Carcinogenic molecules from such substances are known to cause oxidative DNA damage to epithelial cells, and can be repaired by cell's own DNA repair mechanisms (Frosina, 2000; Wood et al., 2001). However, faulty DNA repair systems, primarily occurring due to genetic polymorphisms may play a role in the development of oral pre cancer as well as oral cancer (Scully and Bagan, 2009).

DNA repair genes are of four different categories depending on the repair pathways in which they are involved; Nucleotide Excision repair (NER), Base Excision repair (BER), Mismatch Repair (MMR) and double strand break repair. Xeroderma Pigmentosum group C (XPC) belongs to the NER pathway and polymorphisms in this gene are reported to be associated with oral pre cancerous lesion as well as oral cancer (Bootsma et al., 2002). The NER participates in the elimination of variety of stocky DNA-like ultra violet light-induced pyrimidine dimers, other photoproducts, larger chemical adducts and crosslinks. The Xeroderma Pigmentosum group C is involved in the recognition and initiation of nucleotide excision repair pathway and binds to HR23B to

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form a stable XPC-HR23B complex, which identifies and binds to damaged DNA (Sugasawa et al., 1998). XPC polymorphisms may change the NER ability and affect genetic predisposition to cancer. It has been reported that polymorphisms in XPC may play an important role in lung cancer (Hollander et al., 2005),lymphomagenesis (Soufir et al., 2002) and urinary bladder neoplasm (Sanyal et al., 2004).

The present study was conducted to investigate any association of XPC A > C (Lys939Gln, rs2228001), C > T (Ala499Val, rs2228000) and intron 9 PAT (D > I) polymorphisms with the risk of oral pre cancer and cancer.

2. Material and methods

2.1. Study subjects

This study evaluated a total of 302 patients (with previously treated and pathologically confirmed oral pre cancer and cancer) who were registered at the department of Oral Pathology & Microbiology, King George's Medical University and 300 healthy controls after obtaining ethical clearance from the Institutional Ethics Committee of the King George's Medical University, Lucknow. Informed written consents were obtained from all subjects. Venous blood samples were collected in EDTA tubes and stored at −80 °C till DNA extraction. Genomic DNA extraction from blood samples was carried out by salting out method.

2.2. Genotyping

Genotyping for XPC PAT (D > I) polymorphisms were done by PCR method, while, genotypes for XPC A > C and, C > T polymorphisms were studied by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) technique. PCR products (both undigested and digested) were resolved on a 2% agarose gel and stained with ethidium bromide for visualization under UV light. PCR products were generated in 10 µl reaction volumes containing 10 ng of genomic DNA, 2.0 mM MgCl2, 0.11 mM each dNTP, 0.3 mM each primer (Table 1) and 0.5 U Taq DNA polymerase (Sigma Aldrich, USA). PCR products were digested with restriction enzymes listed in Table 1 and gel image of representative genotypes for each polymorphisms are shown in Fig 1, 2 and 3.

2.3. Statistical analysis

To determine the difference between the cases and controls with respect to genotype distributions and allele frequencies Chi^2-test (Yates corrected) was used. It was also used to find out difference in distribution of genotypes between different disease categories. Odds ratios (OR), 95% confidence interval (CI) and P-values for the assessment of associated risk due to genotypes and variant allele of studied polymorphisms were calculated by the Epi-Info programme (http://wwwn.cdc.gov/epiinfo/). SNPstats was employed to construct haplotypes. The extent of linkage disequilibrium (LD) was expressed in the maximum likelihood estimate of disequilibrium, D'.

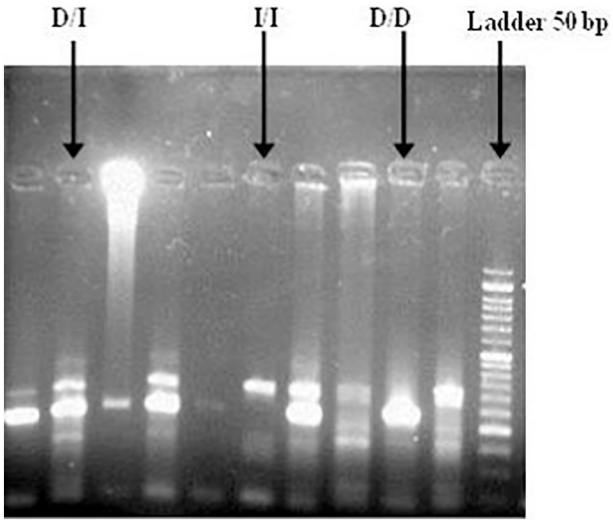

Fig. 1. Genotypes of XPC (polyAT) on 2.0% agarose gel. Lane 1,3,5,9 deletion, Lane 2,4,7 insertion/deletion, Lane 6,10 insertion, Lane 11 ladder 50 p.

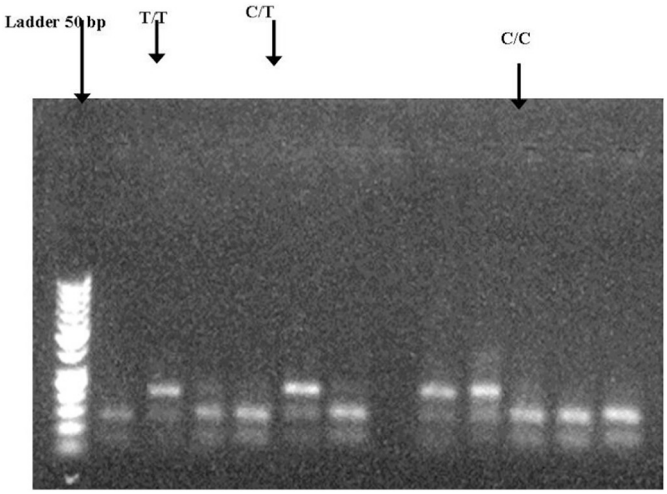

Fig. 2. Genotypes of XPC (exon 9) on 2.0% agarose gel. Lane 1 50 bp Ladder, Lane 2,4,5,7,11,12,13 CC genotype and this 107bp,64bp, Lane 3 TT genotype and this 171 bp, Lane 6,9,10 CT genotype and this 171bp,107bp,64bp.

3. Results

3.1. Demographics of the study population

The demographic profile including age, gender, habitual risk factors and tumor clinical characteristics which may contribute to the progression of oral lesions and OSCC, are shown in Table 2. In brief, the study recruited 302 oral disease cases, including 203 (67%) males and 99 (33%) females. Calculated mean age of cases was 46.67. The mean age of 300 healthy controls [73% males and 27% females] was 38.02.

Table 1
Primers and restriction enzymes used for genotyping of various polymorphisms in oral pre cancer & cancer patients and controls.

Gene	Primer sequence	Annealing temp. (°C)	Restriction enzyme
XPC (PolyAT) D/I	F-"GTAGTCGGGAGAAAGCCTGTT" R-"TGGTTTGGGAAGAGGAAAAGA"	58	NA
XPC (Exon9) C/T	F-"GACAAAGGCTGGGTCCAAGA R-"CCACTTTTCCTCCTGCTCAC"	63.1	*AciI*
XPC (Exon15) A/C	F-"GATGCAGGAGGTGGACTCTCT" R-"GTAGTGGGGCAGCAGCAACT"	67	PuvII

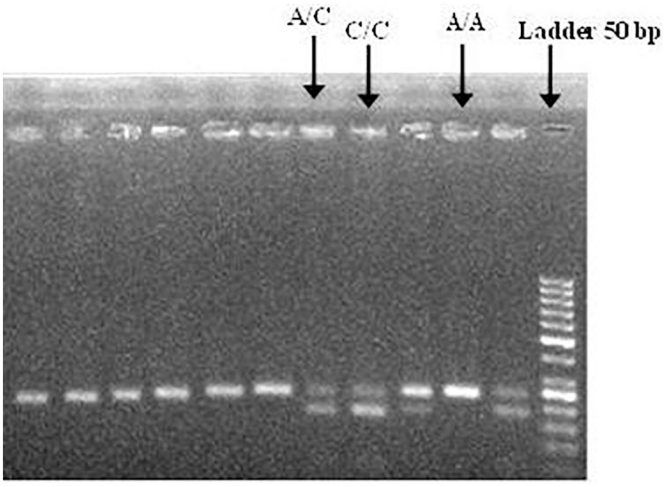

Fig. 3. Genotypes of XPC (exon 15) on 2.0% agarose gel. Lane 2,3,4,5,6,7,11 AA genotype and this 244 bp, Lane 8,9,10 AC genotype and this 244bp,189bp,55bp, Lane 12 CC genotype and this 189bp, 55 bp, Lane 13 ladder 50bp.

Table 2
Demographic in patient and controls.

Demographic character	Cases (n = 302) (%)	Control (n = 300) (%)	P- value
Male	203 (67%)	219 (73%)	Ref
Female	99 (33%)	81 (27%)	0.144
Age distribution			
21–40	89 (29%)	160 (53%)	Ref
41–90	213 (71%)	140 (47%)	< 0.0001*
Mean age	46.67	38.02	
Median age	47	37.5	
Habitual risk			
Alcohol consumption			
Yes	41 (14%)	11 (04%)	Ref
No	261 (86%)	289 (96%)	< 0.0001*
Smoking			
Yes	141 (46%)	78 (26%)	Ref
No	161 (54%)	222 (74%)	< 0.0001*
Tobacco chewing			
Yes	134 (44%)	73 (25%)	Ref
No	168 (56%)	227 (75%)	< 0.0001*
Type of oral diseases			
Leukoplakia	70 (23.33%)	–	–
O.S.M.F	90 (30.00%)	–	–
Lichen planus	70 (23.33%)	–	–
Malignancy	72 (23.33%)	–	–
Clinical features of oral cancer subjects			–
Tumor Stage		–	–
I	8 (11%)		
II	11 (15%)		
III	18 (25%)		
IV	35 (49%)		
Tumor T Status		–	–
T1 + T2	10(13%)		
T3 + T4	62 (87%)		
Lymph Node		–	–
N0	25 (34%)		
N1 + N2	47 (66%)		
Metastasis		–	–
M0	54 (75%)		
M1	18 (25%)		
Cell differentiated grade		–	–
Grade 1	31 (45%)		
> Grade 1	39 (55%)		

* Significant value.

Table 3
Distribution of different genotypes and alleles of XPC PAT D > I, C > T and A > C polymorphisms among subjects of oral diseases (pre cancer and cancer) and healthy controls.

XPC PAT D/I genotypes	Cases (Oral diseases)	Controls	p- value	Odds ratio	95% CI
D/D	163 (36%)	138 (46%)	Ref	1	1
D/I	90 (31%)	129 (43%)	0.004*	0.590	0.415-0.839
I/I	37 (13%)	33 (11%)	0.949	0.949	0.563–1.598
D	416 (72%)	405 (68%)	Ref	1	1
I	164 (28%)	195 (32%)	0.130	0.818	0.638–1.050
XPC C > TGenotypes					
CC	102 (34%)	69 (22%)	Ref	1	1
CT	126 (43%)	145 (49%)	0.009*	0.587	0.398-0.866
TT	69 (23%)	83 (28%)	0.014*	0.562	0.036-0.874
C	330 (56%)	283 (48%)	Ref	1	1
T	264 (44%)	311 (52%)	0.007*	0.728	0.579-0.914
XPC A > C Genotypes					
AA	133 (45%)	141 (47%)	Ref	1	1
AC	109 (37%)	123 (41%)	0.794	0.939	0.661–1.333
CC	55 (18%)	36 (12%)	0.064*	1.619	0.999-2.623
A	375 (63%)	405 (67%)	Ref	1	1
C	219 (37%)	195 (33%)	0.127	1.212	0.955–1.539

* Significant association.

Smoking and tobacco chewing have been observed as two significant risk enhancers for the development of oral diseases (p value < .001). Types of oral diseases included in this study are also documented in Table 2, which includes 23.33% leukoplakia, 30% OSMF, 22.33% Lichen planus and 23.33% Oral Squamous Cell Carcinoma cases. Clinical parameters of oral cancer patients at the time of diagnosis including, tumor stage, size (Tumor T status), grade, nodal involvement, metastasis are also documented in Table 2.

3.2. Genotypes of XPC polymorphisms and risk of oral diseases

Distribution of different genotypes and alleles for XPC A > C (Lys939Gln, rs2228001), C > T (Ala499Val, rs2228000) and intron 9 PAT (D > I) polymorphisms among the cases of oral diseases (pre oral and oral cancer) and control are shown in Table 3. In control population all the genotypes were at Hardy-Weinberg equilibrium. With reference to D/D genotype, frequency of D/I genotypes for intron 9 PAT (D > I) polymorphism was significantly lesser in cases than in control population indicating a protective association of D/I genotype with the development of oral diseases (OR = 0.590, p value = .004). A similar protective association was also documented with CT and TT genotypes of XPC C > T polymorphism. As compared to CC genotype, the frequency of CT and TT genotype was significantly lower in cases than in control (OR = 0.587 and 0.582 respectively). In contrast, with reference to AA genotype odds for developing oral diseases was marginally higher with the CC genotype of XPC A > C polymorphism (OR = 1.619).

3.3. Genotypes of XPC polymorphisms and risk of pre malignant lesions and oral cancer

As the study showed association of studied XPC polymorphism with the oral disease, we assessed whether they were associated with oral premalignant lesions or oral cancer and thus further stratified our cases in two groups: (i) patients with pre oral cancer lesion and (ii) patients with oral cancer. Distribution of different genotypes and alleles for XPC polymorphisms among the patients of pre oral cancer lesions and histologically confirmed oral cancer patients are documented in Table 4. The protective association of variant allele genotype (D/I and I/I) for XPC intron 9 polymorphism was documented both with pre malignant

Table 4
Frequency distribution of different genotypes and alleles of XPC PAT D > I, XPC C > T, XPC A > C polymorphisms among oral pre cancer (Oral submucous fibrosis + Lichenplanus, + Leukoplakia), cancer and controls.

Genotypes	Pre-oral cancer	P-value	Odd ratio (95% CI)	Oral cancer	P- value	Odd ratio (95% CI)	Controls
XPC PAT D/I Genotypes							
D/D	123 (55%)	Ref	1	40 (57%)	Ref	1	138 (46%)
D/I	62 (29%)	0.0024*	0.5392 (0.3657–0.79520	28 (40%)	0.3592	0.7488 (0.4366–1.284)	129 (43%)
I/I	35 (16%)	0.6154	1.190 (0.6975–2.030)	2 (3%)	0.0408*	0.2091 (0.048–0.909)	33 (11%)
D	306 (69%)	Ref	1	108 (77%)	Ref	1	405 (68%)
I	134 (31%)	0.5265	0.9095 (0.6975–1.186)	32 (23%)	0.0335*	0.6154 (0.4003–0.946)	195 (32%)
XPC C > T Genotypes							
CC	80 (35%)	Ref	1	22 (31%)	Ref	1	69(22%)
CT	104 (45%)	0.0274*	0.6186 (0.4110–0.9311)	22 (31%)	0.038*	0.475 (0.246–0.917)	145(49%)
TT	43 (18%)	0.0018*	0.4468 (0.2739–0.7289)	26 (38%)	0.957	0.9825 (0.512–1.885)	83(28%)
C	264 (58%)	Ref	1	66 (47%)	Ref	1	283 (48%)
T	190 (41%)	0.0009*	0.6549 (0.5119–0.83790	74 (53%)	0.990	1.020 (0.7055–1.475)	311(52%)
XPC A > C Genotypes							
AA	110 (48%)	Ref	1	23 (34%)	Ref	1	141 (47%)
AC	73 (32%)	0.1913	0.7608 (0.5191–1.115)	36 (54%)	0.0629	1.794 (1.008–3.193)	123 (41%)
CC	47 (20%)	0.0576	1.673 (1.014–2.761)	8 (12%)	0.6533	1.362 (0.5628–3.298)	36 (12%)
A	293 (63%)	Ref	1	82 (61%)	Ref	1	405 (67%)
C	167 (37%)	0.2190	1.184 (0.9168–1.529)	52 (39%)	0.195	1.317 (0.8942–1.940	195 (33%)

* Significant association.

lesions and oral cancer (Table 4). Compared to the D/D genotype, the risk of both pre malignant lesions and oral cancer was lower (OR = 0.539, 95% CI 0.365–0.795 and OR = 0.209, 95% CI 0.048–0.909 respectively) with variant allele genotypes of intron 9 polymorphism. However, the protective association of TT genotypes of C > T polymorphism was only observed with pre malignant lesions (OR = 0.446, 95% CI 0.273–0.728) and not with oral cancer (Table 4). With reference to AA genotype, odds for developing both pre malignant and malignant lesions were marginally higher with the CC genotype of XPC A > C polymorphism (OR = 1.673, 95% CI 1.014–2.761 and OR = 1.794, 95% CI 1.008–3.193 respectively).

As association of several genotypes with the development of pre malignant lesion were observed, it appeared important to assess if it is associated with the decreased or increased risk of any of the pre malignant lesions in particular. A distribution of different genotypes and alleles for XPC polymorphisms among the patients of pre oral cancer lesions are shown in Table 5. In particular, the D/I of PAT polymorphism was found to decrease the risk of OSMF (OR = 0.523, 95% CI 0.300–0.910) and Leukoplakia (OR = 0.509, 95% CI 0.284–0.913). Similarly, compared to the common allele genotype CC, both the variant allele genotypes (CT and TT) of C > T polymorphism were observed to significantly decrease the risk for OSMF (for CC vs CT OR = 0.294, 95% CI 0.167–0.519 and for CC vs TT OR = 0.376, 95% CI 0.200–0.705). Compared to allele C the T allele of C > T polymorphism was also found to decrease the risk of OSMF (OR = 0.529, 95% CI 0.373–0.749). In contrast, a threefold higher risk for Lichenplanus was observed with the CC genotype of A > C polymorphism compared to AA genotype (OR = 2.895, 95% CI 1.401–1.598). The C allele alone was found to be responsible for 2 fold risk enhancement of Lichenplanus compared to A allele (OR = 1.749, 95% CI 1.203–2.542).

3.4. Haplotypes of XPC polymorphisms and risk of oral diseases

Haplotyping of XPC A > C (rs2228001) C > T (rs2228000) and intron 9 PAT (D > I) (rs77907221) polymorphisms generated 8 different haplotypes as detailed in Table 6. Compared to the A/C/D haplotype the frequency of A/T/D and A/T/I haplotype were significantly higher among the control population than in patients with oral diseases (Table 6) indicating a protective association of these haplotypes with the susceptibility to develop any kind of oral disease including pre malignant lesions and oral cancer. The frequencies of other haplotypes were almost similar between cases and controls. Further, the above polymorphisms of XPC were not in significant LD (D = 0.0089 for D/I and C > T; 0.0477, for D/I and A > C and 0.0411 for C > T and A > C polymorphism).

3.5. XPC gene polymorphisms in relation to different clinical parameters of patients with oral cancer

Distribution of genotypes for different XPC polymorphisms among different disease categories are listed in Table 7. Frequency of patients with variant allele genotypes (D/I + I/I) for XPC intron 9 PAT polymorphism were higher (63%) among high stage (stage III + IV) diseases compared to low stage disease (sate I + II; 16%). Similarly, risk of being diagnosed with larger size tumor were significantly higher (OR = 3.5; Table 7) with D/I + I/I genotypes than D/D genotypes. Compared to D/D genotypes, patients with D/I + I/I genotypes were also found to harbour metastatic disease at the time of diagnosis. Rest of the

Table 5
Frequency distribution of different genotypes and alleles of XPC PAT D > I, XPC C > T, XPC A > C polymorphisms among cases of Oral submucous fibrosis, Lichenplanus, Leukoplakia and healthy controls.

Genotypes	Oral submucous fibrosis	P- value	Lichenplanus	P- value	Leucoplakia	P- value	Controls
XPC PAT D/IGenotypes							
D/D	47 (53%)	Ref	34 (56.67%)	Ref	42 (60%)	Ref	138 (46%)
D/I	23 (25%)	0.0294*	19 (31.66%)	0.1306	20 (29%)	0.0318*	129 (43%)
I/I	20 (22%)	0.1126	07 (12%)	0.9165	8 (11%)	0.7482	33 (11%)
D	117 (65%)	Ref	87 (72%)	Ref	102 (73%)	Ref	405 (68%)
I	63 (35%)	0.5927	33 (28%)	0.3334	38 (27%)	0.2594	195 (32%)
XPC C > T Genotypes							
CC	42 (48%)	Ref	19 (27%)	Ref	19 (27%)	Ref	69(22%)
CT	26 (30%)	0.001*	40 (57%)	0.995	38 (54%)	0.875	145(49%)
TT	19 (22%)	0.0032*	11 (16%)	0.1103	13 (19%)	0.2134	83(28%)
C	110 (64%)	Ref	78 (56%)	Ref	76 (54%)	Ref	283 (48%)
T	64 (36%)	0.004*	62 (44%)	0.104	64 (46%)	0.186	311(52%)
XPC A > C Genotypes							
AA	50 (56%)	Ref	23 (33%)	Ref	37 (53%)	Ref	141 (47%)
AC	25 (27%)	0.056*	30 (43%)	0.237	18 (26%)	0.0829	123 (41%)
CC	15 (17%)	0.775	17 (24%)	0.006*	15 (21%)	0.2684	36 (12%)
A	125 (70%)	Ref	76 (54%)	Ref	92 (66%)	Ref	405 (67%)
C	55 (30%)	0.689	64 (46%)	0.004*	48 (44%)	0.760	195 (33%)

* Significant association.

Table 6
Distribution of different haplotypes of XPC A > C, C > T & PAT D > I polymorphism in Oral diseases and controls.

SNP1	SNP2	SNP3	Cases	Controls	p- value	Odds ratio	95% CI
D	C	A	89 (30.58%)	61 (20.38%)	Ref	1	1
D	T	A	48 (16.43%)	76 (25.51%)	0.001*	0.432	0.26-0.70
D	C	C	40 (13.97%)	38 (12.99%)	0.306	0.721	0.41–1.25
I	C	A	33 (11.20%)	26 (08.61%)	0.769	0.869	0.47–1.59
D	T	C	31 (10.64%)	26 (08.62%)	0.626	0.817	0.44–1.51
I	T	A	15 (04.96%)	39 (13.00%)	0.0001*	0.263	0.13-0.51
I	C	C	23 (07.90%)	17 (05.64%)	0.977	0.927	0.45–1.87
I	T	C	13 (04.31%)	15 (05.24%)	0.289	0.594	0.26–1.33

* Significant association.

genotypes for XPC A > C and C > T polymorphisms did not show any association with any clinical parameters of oral cancer (Table 7).

4. Discussion

Premalignant oral lesions often lead to the development of oral cancer and prevention of oral lesion could therefore reduce the incidence of oral cancer. This indicates that identification of susceptibility markers such as SNP for pre malignant as well as malignant oral lesion will be helpful in proper management of oral diseases. SNPs of DNA repair genes, particularly of XPC have been previously reported to alter the risk of many different cancers. However, the association of XPC polymorphisms with oral cancer and especially with pre malignant oral lesions are not well explored. In the present study we analysed the association of XPC A > C (Lys939Gln, rs2228001), C > T (Ala499Val, rs2228000) and intron 9 PAT (D > I) polymorphisms with the risk for development of pre malignant oral lesions as well as oral cancer.

Compared to the D/D genotype, the PAT I/I homozygous subjects of intron 9 PAT (D > I) polymorphisms showed lower DNA Repair Capacity (DRC) and hence this polymorphism may modulate DRC and could be a useful biomarker for identifying individuals at risk of developing cancer (Qiao et al., 2002a; Qiao et al., 2002b). This XPC-PAT polymorphism has also been reported to be in linkage disequilibrium with another single nucleotide polymorphism C > A located at the −5 position of the XPC intron 11 splice acceptor site (intron 11C/A polymorphism) which has been reported to be associated with increased skipping of exon 12. The abnormally spliced XPC mRNA isoform has diminished DNA repair activity and may contribute to cancer susceptibility (Khan et al., 2002). The present study reports an association of XPC intron 9 PAT (D > I) polymorphism with the susceptibility of OSMF, Leukoplakia as well as oral cancer. In particular the heterozygote, i.e. the D/I genotype was found to lower the risk of above mentioned oral diseases. We have already reported similar association of D/I genotype with the risk of oral pre malignant lesions in a study

Table 7
Distribution of different genotypes of XPC A > C, C > T & PAT D > I polymorphism among different disease categories.*

XPC A>C, C>T & D/IGenotypes/Alleles					
Tumor T Status	**T3+T4**	**T1+T2**	**P-value**	**Odds Ratio**	**95% CI**
XPC PAT D/I					
DD	16 (43%)	24 (73%)	Ref	1	1
DI+II	21 (57)	09 (27)	0.024*	3.5	1.28-9.56
XPC C>T					
CC	13 (36%)	09 (27%)	Ref	1	1
CT+TT	23 (64%)	24 (73%)	0.59	0.66	0.23-1.84
XPC A>C					
AA	15 (40%)	08 (26%)	Ref	1	1
AC+CC	22 (60%)	22 (74%)	0.35	0.53	0.18-1.51
Lymph Node	**N1+N2+N3**	**N0**			
XPC PAT D/Ins					
DD	22 (50%)	17 (68%)	Ref	1	1
DI+II	22 (50%)	08 (32%)	0.231	2.12	0.76-5.93
XPC C>T					
CC	12 (27%)	10 (40%)	Ref	1	1
CT+TT	33 (73%)	15 (60%)	0.37	1.83	0.64-5.17
XPC A>C					
AA	16 (37%)	07 (29%)	Ref	1	1
AC+CC	27 (63%)	17 (71%)	0.69	0.69	0.23-2.03
Metastasis	**M1**	**M0**			
XPC PAT D/Ins					
DD	5 (31%)	35(65%)	Ref	1	1
DI+II	11 (69%)	19 (35%)	0.03*	4.05	1.12-13.39
XPC C>T					
CC	4 (25%)	18 (33%)	Ref	1	1
CT+TT	12 (75%)	36 (67%)	0.74	1.5	0.43-5.31
XPC A>C					
AA	06 (35%)	17 (34%)	Ref	1	1
AC+CC	11 (65%)	33 (66%)	0.92	0.94	0.29-2.99
Tumor stage	**III+1V**	**I+II**			
XPC PAT D/I					
DD	24 (47%)	16 (84%)	Ref	1	1
DI+II	27 (53%)	03 (16%)	0.011*	6.0	1.55-23.14

(*continued on next page*)

Table 7 (*continued*)

XPC C>T					
CC	14 (27%)	08 (42%)	Ref	1	1
CT+TT	37 (73%)	11 (58%)	0.37	1.92	0.64-5.76
XPC A>C					
AA	37 (53%)	06 (33%)	Ref	1	1
AC+CC	32 (47%)	12 (66%)	0.20	0.43	0.14-1.28
Cell differentiated grade	**>Grade 1**	**Grade 1**			
XPC PAT D/I					
DD	21 (52%)	19 (63%)	Ref	1	1
DI+II	19 (48%)	11 (37%)	0.50	1.56	0.59-4.11
XPC C>T					
CC	12 (31%)	10 (32%)	Ref	1	1
CT+TT	27 (69%)	21 (68%)	0.89	1.07	0.38-2.95
XPC A>C					
AA	15 (38%)	8 (28%)	Ref	1	1
AC+CC	24 (62%)	20 (72%)	0.56	0.64	0.22-1.81

* Significant association.

with smaller sample size (Tripathi et al., 2017) as well as with squamous cell carcinoma of head and neck (Yadav et al., 2018).The I allele of this XPC polymorphism is associated with reduced DNA repair capacity and hence accumulation of DNA damage (Qiao et al., 2002c). It is possible that too much DNA damage induces the otherwise healthy cells to undergo apoptosis and hence prevents the development or oral diseases. However, once disease has been established, the same variant allele genotypes i.e. D/I + I/I of PAT (D > I) polymorphism are found to be associated with higher stage and metastatic oral cancer. Which probably might occur due to contribution of those damaged cells which have evaded apoptosis to the development of aggressive disease in a person where cancer has already initiated or established.

Subjects with the variant alleles (CT and TT) of XPC C > T (Ala499Val, rs2228000) polymorphisms reported to exhibit a 8.6% and 13.1% decrease in DNA damages induced by BPDE and gamma-radiation respectively (Zhu et al., 2008). In the present report the variant allele T for this XPC polymorphism were observed to give protection against the development of pre malignant lesions like OSMF but not oral cancer. Wang et al. also reported similar protective association of T allele with the risk of oral pre malignant lesions (Wang et al., 2007). Huang et al. reported that individuals with XPC T allele exhibited a significantly reduced risk for advanced colorectal adenoma in their case-control study and this finding was supported by a haplotype analysis (Huang et al., 2006). Similar results of the variant T alleles for XPC (Ala499Val) were also reported by Zhu et al. and Zhou et al. (Zhu et at., 2007; Zhou et al., 2006). A plasmid based NERC assay reported a probable effect of the XPC C > T polymorphism on the function of XPC protein (Lockett et al., 2005). Moreover, this polymorphism is in LD with another polymorphism located in the putative transcription factor binding site at the 5' UTR of the XPC gene. This UTR polymorphism has been predicted to alter the function of XPC gene by affecting its transcription and is reported to enhance the risk of SCC of lung (Lee et al., 2005).

Zhu et al. showed that BPDE-induced DNA damage was significantly higher in carrier of C allele for XPC A > C (Lys939Gln, rs2228001) polymorphism than the carrier of A allele. Similarly, subjects carrying at least one variant C allele exhibited significantly higher γ-radiation-induced DNA damage than the wild-type genotype. (Zhu et al., 2007). However, in vitro studies measuring DNA repair capacity of C allele for XCP A > C polymorphism has shown inconclusive and contradictory results (Qiao et al., 2002d). In the present study the common allele genotype AA for XPC A > C polymorphism was found to be protective for the development of oral diseases especially of oral cancer and Lichenplanus, while it increased the risk of OSMF. Such contrasting association is possibly due to the fact that Lichenplanus and OSMF are two different diseases. We have previously reported association of this polymorphism with risk of PMOL. Beside oral and pre oral cancer the C allele of XPC A > C polymorphism is also documented to enhance the risk of colorectal cancer, especially in Asian population (Peng et al., 2014).

Haplotypes constituted from the three XPC polymorphisms studied here showed that the haplotypes with T and A allele respectively from of XPC C > T and A > C polymorphisms were protective against the development of oral diseases which further strengthen our observation that the T allele and A allele for these two XPC polymorphisms give protection against the development of oral diseases.

In conclusion, the present study demonstrates association of XPC A > C (Lys939Gln, rs2228001), C > T (Ala499Val, rs2228000) and intron 9 PAT (D > I) polymorphisms with the risk for development of oral precancerous lesion as well as oral cancer. The C > T polymorphism not only modifies the risk for development of oral cancer but also influences the clinical characteristics of the same.

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