CHAPTER 4

Role of XRCC1 in BER pathway

And Genetic susceptibility to oral cancer

XRCC1 and Base Excision Repair

The base excision repair (BER) pathway detects and repairs damage from stimuli such as reactive oxygen species, alkylating agents, and ionizing radiation (74,75). 8-Hydroxyguanine DNA glycosylase (OGG1) initiates the process by cleaving the damaged base, leaving an unmatched base on the opposite strand. Apurinic/ apyrimidinic endonuclease (APE1) then cleaves the associated sugar-phosphate chain. At this point, two pathways are possible: a single-nucleotide repair pathway (major pathway) or a long-patch repair pathway of a few base pairs (minor pathway). In the major pathway, polymerase β interacts with X-ray repair cross-complementing group 1 (XRCC1) in heterodimers with DNA ligase III to complete the repair process. In the minor pathway, a flap of several bases is constructed by polymerase δ/ε; the extraneous flap is removed by flap endonuclease I, and DNA ligase I completes the repair by using proliferating cell nuclear antigen as a scaffold (75–77) (Fig 4.1).

XRCC1 (X-ray repair cross complementing group I), is a remarkable protein in the BER pathway which is a polypeptide that interacts with PARP-1 (78, 79), PNK (80), Pol β (78-80) and Lig 3α (82,83). The interaction of XRCC1 with PNK stimulates both the 5'-kinase and 3'-phospatase activities of this enzyme and the interaction with Lig3α increases the intracellular stability of the ligase. Studies have shown a role for XRCC1 both in vitro and in vivo during the repair of either direct SSBs or those arising indirectly during BER.

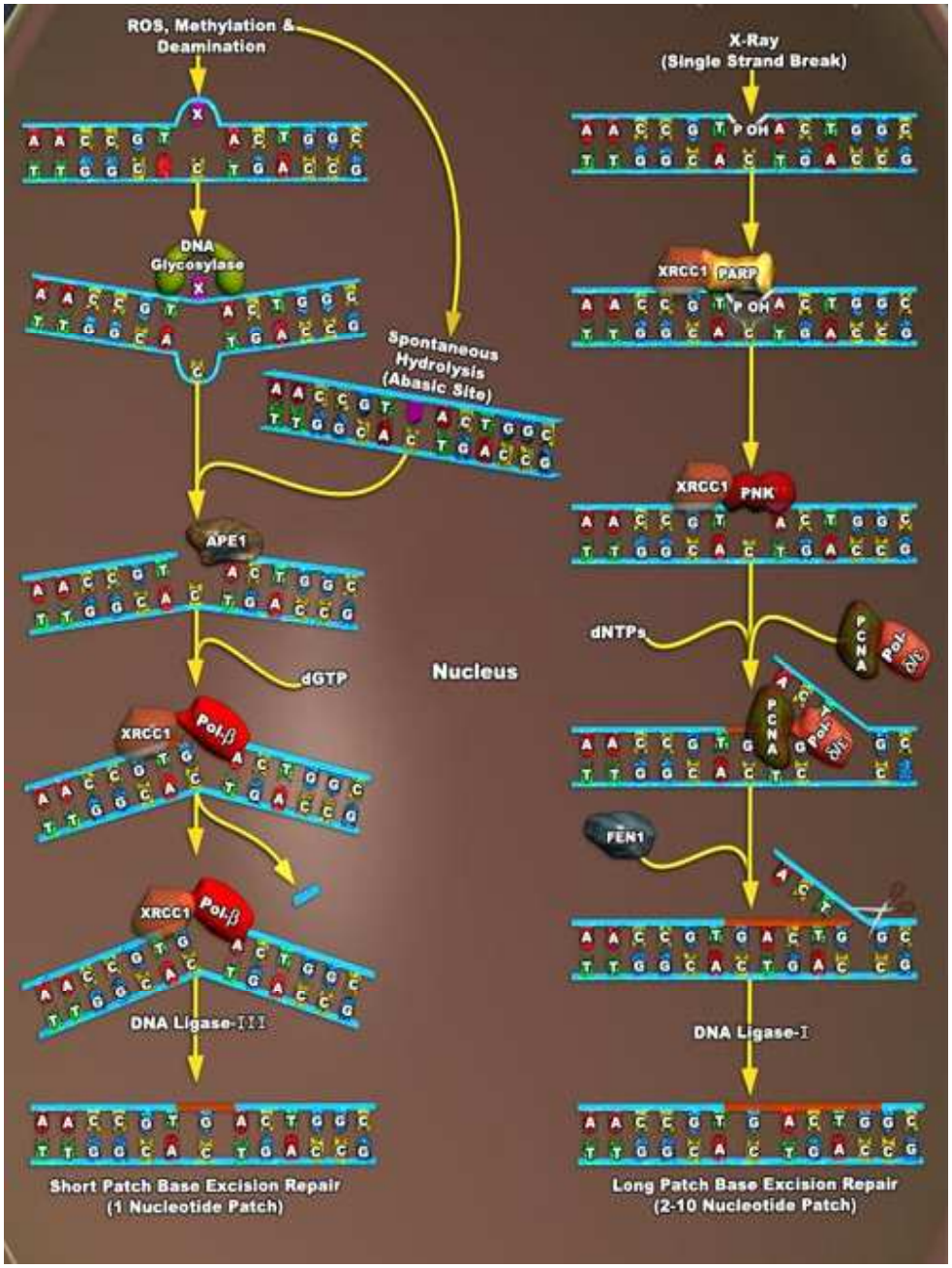


Fig 4.1 The Base Excision Repair Pathway

(Source : Applied Biosytems)

Loss of XRCC1 also results in decreased genetic stability, including increased frequencies of spontaneous and/or induced chromosome translocations and deletions. (84-87). Polymorphisms of the XRCC1 gene occur at residues that are identical in human, hamster and mouse suggesting that these amino acids are evolutionarily conserved (88,89).

The human XRCC1 gene was the first mammalian gene isolated that affects cellular sensitivity to ionizing radiation (90). Human XRCC1 maps to chromosome 19q13.2, spans a genomic distance of 32 kb, and is composed of 17 exons (91,92). DNA sequencing of the human XRCC1 gene has shown three non-conservative changes (PC thesis).Shen et al (93) found three polymorphisms of the *XRCC 1* gene, which resulted in amino acid changes at evolutionary conserved regions:

- C→T substitution at position 26304 in codon 194 of exon 6.
- G→A substitution at position 27466 in codon 280 of exon 9.
- G→A substitution at position 28152 in codon 399 of exon 10.

Protein structure and function

Although no enzyme activity has been attributed to XRCC1, discrete domains of interaction with three other enzymes involved in BER are documented. An N-terminal end interacts with DNA polymerase β (94). Pol β knockout cells show high sensitivity to methylating agents, indicating its role in protecting cells against killing due to apoptosis or chromosomal aberrations (95). XRCC 1 also associates with PARP [poly (ADP-ribose) polymerase] (96), a nuclear zinc-finger DNA-binding

protein that detects DNA strand breaks (97). Both XRCC1 and PARP have BRCT domains, which are weakly conserved motifs, which mediate protein interactions (98). LIG3 is a third protein that interacts with XRCC 1 (99) and has two forms LIG α and LIG β (100). The complex formation of LIG α with the BRCT2 domain of XRCC1 is mediated through its BRCT domain (101). Thus the XRCC 1 protein involved in the base excision repair pathway, acts apparently as a scaffolding protein, facilitating the repair reaction by binding DNA ligase III at its carboxy and DNA polymerase β to its amino terminus (102).

Significance of genetic polymorphisms in XRCC1 gene and cancer

Shen *et al.* (93) reported five polymorphisms in the *XRCC1* gene, three of which occur at conserved sequences and resulted in amino acid substitutions. These three coding polymorphisms were detected at codons 194 (*Arg-Trp*), 280 (*Arg-His*), and 399 (*Arg-Gln*). XRCC1 is involved in the repair of single stranded breaks following base excision repair resulting from exposure to endogenously reactive oxygen species, ionizing radiation or alkylating agents (103). Because amino acid residues at the protein-protein interfaces of multi protein complexes and those involved in the active sites play a role in the enzyme function, it is possible that the *XRCC1* polymorphisms may result in altered efficiency of the protein. Codon 399 in *XRCC1 is* located with in the BRCT domain that interacts with PARP (104). A recent report by Lunn et al (105) suggested that the *XRCC1* codon 399 polymorphism may result in deficient DNA repair. *XRCC1* mutants cells have increased sensitivity to ionizing radiation, UV, hydrogen peroxide and mitomycin C (90). Individuals with the *XRCC1*

codon 399 *Gln* variant were more likely to have detectable poly phenol DNA adducts and higher levels of adducts; in addition smokers with these variants were found to have a greater mean sister chromatid frequency than the wild type allele carriers (103). Potential sites of phosphorylation represent a means by which the function of XRCC1 could be modulated in response to DNA damage. In addition, XRCC1 might also have a regulatory function in transcription since BRCT domains are known to play a role in transcriptional activation in response to DNA damage (106).

Aim of the study

The current study investigated the hypothesis that the genetic polymorphisms of DNA repair gene *XRCC1* resulting in three non conservative amino acid substitutions at codons 194 (Arg→Trp), 280 (Arg→His) and 399 (Arg→Gln) may increase the susceptibility to oral cancer by modifying individual DNA repair capability.

Specific objectives

The present study was conducted with the following specific aims:

- To evaluate the frequency of *XRCC1* codon 194 (Arg↔Trp), codon 399 (Arg↔Gln) and codon 280 (Arg↔His) polymorphic variants among the study subjects.
- To assess the individual and combined effect of these gene polymorphisms on oral cancer risk

Materials and methods

Study population

The present study included 110 oral cancer patients, 44 subjects with hyperplastic leukoplakia and 40 with dysplastic leukoplakia, being seen at the Head and Neck Clinic of the Regional Cancer Centre, Thiruvananthapuram. A total of 110 normal controls were also included. Controls were mostly from the same geographical area and socioeconomic background as the cases and included visitors and relatives of the patients. Control subjects were apparently normal and gave no history of any malignancy or any systemic disease. All controls were age and sex matched and all as far as possible also matched for habits. The study was approved by the Institutional Review Board and Human Ethics Committee of the Regional Cancer Centre. Informed consent was obtained from all subjects.

DNA Extraction

DNA was extracted from the whole blood using the Genomic Prep Blood DNA Isolation kit (Amersham Pharmacia Biotech Inc, USA). The detailed procedure for genomic DNA extraction is given in Appendix 1. Briefly, to isolate DNA from whole blood, red blood cells, which lack genomic DNA, must first be lysed to facilitate their separation from the white blood cells. Remaining white blood cells are then lysed in the presence of a DNA preservative using an anionic detergent that solubilizes the cellular components. The DNA preservative (EDTA) limits the activity of DNases that are present in the cells and elsewhere in the environment. The contaminating RNA is then removed by treatment with RNase. The cytoplasmic and nuclear proteins are removed by salt precipitation. Genomic DNA is finally isolated by precipitation with

alcohol and dissolved in Tris-EDTA buffer solution. The extracted DNA was then quantitated by measuring the optical density at 260nm using a spectrophotometer (Shimadzu, Japan) and stored at 4ºC until genotype analysis was done. (Appendix1).

PCR analysis of XRCC1 gene polymorphisms

The genomes of codon 194,280 and 399 were amplified in a PCR using the following primers by the method of Lee et al (2001).

XRCC1 194	:	5'- GTT CCG TGT GAA GGA GGA GGA -3' 5'- CGA GTC TAG GTC TCA ACC CTA CTC ACT –3'
XRCC1 280 &	:	5'- TTG ACC CCC AGT GGT GCT AA –3'
XRCC1 399		5'- AGT CTG CTG GCT CTG GGC TGG –3'

The PCR reactions were started with a reaction volume of 50 µl containing 100 ng of genomic DNA, 0.4 mM dNTPs, 5 pmol of each primer, 1.25 unit of Taq DNA polymerase (Bangalore Genei, Bangalore, India) and 1 X PCR buffer, [50 mM KCl, 10mM Tris-HCl (pH 9.0), 1.5 mM MgCl2 and 0.1% Triton X-100]. The reactions were carried out in the following thermocycler conditions: denaturation at 94 °C for 5 minutes, 35 cycles of 40 seconds at 94°C, 30 seconds at 55°C and 40 seconds at 72°C, subsequently followed by a 10 minute extension period at 72°C. There was slight modification in PCR conditions for *XRCC1* codon 280 and 399. The annealing temperature was increased from 55 °C to 65°C to get the desired band with out non-specific binding. Negative controls in all PCR assays consisted of a similar reaction mixture with the template replaced with sterile water. The PCR products were

visualized using a UV transilluminator after ethidium bromide staining. Detailed protocol is provided in Appendix 1.

Restriction Fragment Length Polymorphism

The PCR products were digested with specific restriction enzymes for detecting the codon 194,280 and 399 polymorphisms of the XRCC1 gene. 10 µl of the PCR products were digested separately with 10 units of *Pvu*II (for codon 194), *Rsa I* (for codon 280), and *Msp*1 (for codon 399) [NEB, Beverly, MA, USA] at 37°C for 1 hour. The products were then resolved on 2% agarose gels. 100 bp DNA molecular weight marker was used to assess the size of the PCR – RFLP products.

Evaluation of RFLP

The PCR amplification produced a *Pvu*II restriction site for the *Trp* allele of codon 194. After digestion, the *Arg* allele gave a segment of 138 bp , while the *Trp* allele gave the digested products of 75 and 63 base pair fragments (Fig 4.2). For the PCR product (Fig 4.3) of codon 280 a restriction site of *Rsa I* was created in the *Arg* allele and yielded products of 63,201 and 576 bps, while the *His* allele gave products of 201 and 660 bps (Fig 4.4). For the PCR product of codon 399, a restriction site of Msp1 was created in the *Arg* allele and gave the products of 115, 285 and 461bp, while the *Gln* allele gave the products of 285 and 576 bp (Fig 4.4).

DNA Sequencing

XRCC1 194 codon, 280 codon and 399 codon PCR products were eluted out from the gel using GFX Gel Band Purification kit, by following the manufacturer's instructions. Sequencing of all the samples was carried out in an ABI 3730 capillary sequencer (Fig 4.5).

XRCC1 gene polymorphism mapping

We wanted to know whether any of the polymorphic positions analyzed in our study could be mapped onto XRCC1 domains, BRCT I and BRCT II. The BRCT-1 domain is a region with extensive homology to BRCA1 and includes a binding site for PARP. Amino acid differences in these repair enzymes could therefore result in changes in repair proficiency (107). Two of the variant positions namely 194 and 280 codon do not map to BRCT domains within XRCC1. However, A399G falls within XRCC1 BRCT1 domain. To investigate whether this polymorphism would radically change the protein structure, we attempted to build a model of BRCT1 domain (which has not been solved experimentally) using comparative modeling techniques. Although the target-template sequence identity is low (~21%), the model is of sufficient quality to predict whether the polymorphic position maps to the inside or onto the domain surface.

Comparative modeling and polymorphism mapping

The human XRCC1 BRCT1 domain sequence was downloaded from SWISS-PROT (primary accession number: P18887). Appropriate template for modeling was found by using 3D-PSSM server (http://www.sbg.bio.ic.ac.uk/~3dpssm/). The template chosen for modeling was the crystal structure of NAD-dependent DNA ligase from *T. filiformis* (PDB id: 1DGS, chain A), which shared 25% identity and 48% similarity with the target sequence.

The target sequence was manually threaded through the crystal structure using Deep View (spdbv 3.7) and the resulting project file was sent to SWISS-MODEL for model building and energy minimization (108). The polymorphic position was mapped onto the surface of the resulting model. PyMOL was used for structural representations.

Data analysis

Data analysis was performed using SAS software version 8.2. The 2x2 contingency cross-tabulation tables provide distribution of cases and controls by genotype status for each set of genes. Normal genotype was considered as the referent group. Odds ratio was calculated to quantify the measure of association with corresponding 95% confidence interval. PROC FREQ was used to obtain the results to meet the objectives. The CMH option provides adjusted odds ratio for 2x2 tables. PROC FREQ further computes the odds ratio estimate using the Mantel Haenszel and logit methods. The proc freq procedure presents both the Mantel Haenszel and logit

estimate for the odds ratio. When any one of the cells in the contingency table was found to have zero counts, then value 0.5 needed to be added to each cell of the contingency tables and thus logit estimate of the common odds ratio was reported. In cases where cell counts were non-zeros the Mantel Haenszel estimate was presented. The chi-square or Fisher's exact test was used to measure the extent to which the observed data differ from those expected if the two odds of exposure are equal. Fisher's exact test was performed when the expected frequencies was less than 5 in any cell. For the confounding factor gender (strata level at female and male), stratum specific estimates and their confidence interval were estimated. The summarization of overall results of the study in a way that removes the confounding effect of exposure was also calculated. The CMH option produces the Cochran-Mantel-Haenszel statistics (general association statistic). For this stratified 2×2 table after adjusting for gender, estimates of the common odds ratio and the Breslow-Day test for homogeneity of the odds ratios were also computed.

Results

Genotype frequencies in cases and controls

This study analyzed the distribution of XRCC1 genotypes in 304 subjects, which included 110 cases of oral carcinoma, 84 cases of leukoplakia (44 hyperplasia and 40 dysplasia) and 110 normal controls. Table I shows the distribution of XRCC1 codons 194, 280 and 399 polymorphisms in the four groups of subjects. The frequency of both 194Trp and 399Gln variant alleles were more pronounced among cases compared to controls.

Table I

GENOTYPE FREQUENCIES OF XRCC1 AND XPD IN SUBJECTS WITH ORAL LESIONS AND CONTROLS

GENOTYPE	ORAL CANCER	DYSPLASTIC LEUKOPLAKIA	HYPERPLASTIC LEUKOPLAKIA	CONTROLS
	N=110 (%)	N=40 (%)	N=44 (%)	N=110 (%)
XRCC1				
Exon 6, codon194				
Arg/Arg	66(60.0%)	21(52.5%)	28(63.6%)	90(81.8%)
Arg/Trp	37(33.6%)	16(40.0%)	13(29.5%)	19(17.3%)
Trp/Trp	7(6.4%)	3(7.5%)	3(6.8%)	1(0.9%)
Exon 9,codon 280				
Arg/Arg	77(70.0%)	22(55.0%)	32(72.7%)	83(75.5%)
Arg/His	31(28.2%)	15(37.5%)	11(25.0%)	26(23.6%)
His/His	2(1.8%)	3(7.5%)	1(2.3%)	1(0.9%)
Exon10, codon 399				
Arg/Arg	46(41.8%)	15(37.5%)	27(61.4%)	73(66.4%)
Arg/Gln	48(43.6%)	20(50.5%)	10(2.7%)	33(30.0%)
Gln/Gln	16(14.5%)	5(12.5%)	7(15.9%)	4(3.6%)

Table II (a) gives the Odds ratio (OR) estimates for the association between the genetic variants of XRCC1 genes and risk of oral cancer. A very small proportion of the subjects were homozygous for XRCC1 codon 194 and 280 and 399 polymorphism. However there was a significant variation between distribution of the polymorphic variants between cases and controls. There was wide variation between the risk estimates of the three codons. No significant differences were observed between the crude ORs for oral cancer and the ORs obtained when adjusted for age and gender. Subjects with an XRCC1 194 *Trp/Trp* and *Arg/Trp* variant had an increased risk of being a case (OR =9.5, 95%CI= 1.14-79.46 p value=0.02). The wide range of confidence interval may be due to the relatively less number of homozygous polymorphic subjects. A similar positive association was seen in subjects who carried the *Arg*399*Gln* polymorphic variant (OR=6.34, 95% CI=1.9-20.17 p value=0.001). The codon 280 polymorphic variant exhibited no statistical significance (p value=0.61) and a borderline risk (OR=2.15, 95%CI=0.92-24.2). The homozygous variants in all cases except *Arg*399*Gln* and exhibited a higher risk than heterozygous variants when compared with the wild type genotype, which was taken as the referent category.

Table II (a)

DISTRIBUTION OF XRCC1 POLYMORPHISM IN ORAL CANCER CASES AND CONTROLS

GENOTYPE/ POLYMORPHISM	CASE* N=110	CONTROL* N=110	OR[A] (95% CI)	P VALUE
XRCC1 Codon194				
Trp/Trp	7	1	9.5 (1.14-79.46)	0.02
Arg/Trp	37	19	2.65 (1.40-5.02)	
Arg/Arg	66	90	1(referent)	0.003
Codon280				
His/His	2	1	2.15 (0.92-24.2)	0.61
Arg/His	31	26	1.28 (0.70-2.35)	
Arg/Arg	77	83	1(referent)	0.44
Codon399				
Gln/Gln	16	4	6.34 (1.99-20.17)	0.001
Arg/Gln	48	33	2.30 (1.29-4.10)	
Arg/Arg	46	73	1(referent)	0.006

*Cases include oral cancer

*Controls include normal population

OR^{A} =age, gender and habits adjusted Odds

When individual risk of dysplastic leukoplakia subjects was studied as against normal population (Table II b) it was seen that only XRCC1 codon 194 and 399 allele variants gave significant results (OR=12.8, and 6.0 respectively)

Table II (b)

DISTRIBUTION OF XRCC1 POLYMORPHISM IN DYSPLASTIC LEUKOPLAKIA CASES AND CONTROLS

GENOTYPE/ POLYMORPHISM	CASE* N=40	CONTROL* N=110	OR^A (95% CI)	P VALUE	OR^B (95%CI)
XRCC1 Codon194					
Trp/Trp	3	1	12.8 (1.27-129.8)	0.02	12.8 (1.27-129.9)
Arg/Trp	16	19	3.60 (1.59-8.17)	0.003	3.60 (1.59-8.8
Arg/Arg	211	90	1(referent)		1(referent)
Codon280					
His/His	3	1	11.3 (1.12-114.1)	0.03	11.4 (1.12-114.3)
Arg/His	15	26	2.17 (0.98-4.79)	0.05	2.18 (0.98-4.80)
Arg/Arg	22	83	1(referent)		1(referent)
Codon399					
Gln/Gln	5	4	6.08 (1.46-25.35)	0.01	6.08 (1.46-25.35)
Arg/Gln	20	33	2.94 (1.34-6.47)		2.94 (1.34-6.47)
Arg/Arg	15	73	1(referent)	0.09	1(referent)

Cases include dysplastic leukoplakia

Controls include normal population

OR^A =Crude Odds; OR^B =age, gender and habits adjusted Odds

In the case of leukoplakia, which showed hyperplasia, the risk of cancer as against normal controls was statistically significant for 399Gln allele only (OR= 4.73, 95% CI= 1.28-17.4, p value= 0.03). The wild type genotype was taken as the referent category.

All groups with lesions (hyperplasia, dysplasia and squamous cell carcinoma) were then categorized into cases and the normal population was ranked as controls to assess the cumulative risk of all these conditions together. It was observed that the XRCC1 194 codon, and 399 codon gave significant results. Table III shows that the variant homozygous genotypes of these two codons were more frequent in cases than in controls. The homozygous *Trp/Trp* variant of XRCC1 codon 194 had a ten fold increased risk (OR=10.7, 95% CI=1.3-79.2) of oral cancer as compared to the homozygous *Arg/Arg* wild genotype. Similarly the homozygous *Gln/Gln* genotype had a 5.8 fold increased risk (95%CI=1.94-17.3) of oral cancer compared to its wild genotype.

On combining the homozygous and heterozygous variants of each codon, as the individual genotypes were few in number, codon 194 and 399 exhibited nearly three-fold increased risk compared to the wild genotype. The risk estimates for combined analysis is given in Table IV.

Table III

RISK ESTIMATES OF CASES AND CONTROLS

GENOTYPE/ POLYMORPHISM	CASE* N=194	CONTROL* N=110	OR^A (95% CI)	P VALUE	OR^B (95%CI)
XRCC1 Codon194					
Trp/Trp	13	1	10.7 (1.30-79.2)	0.009	10.7 (1.30-79.4)
Arg/Trp	66	19	2.71 (1.52-4.85)	0.001	2.71 (1.52-4.87)
Arg/Arg	115	90	1(referent)		1(referent)
Codon280					
His/His	6	1	3.80 (0.45-32.1)	0.25	3.81 (0.45-32.3)
Arg/His	57	26	1.38 (0.81-2.38)	0.28	1.39 (0.81-2.39)
Arg/Arg	131	83	1(referent)		1(referent)
Codon399					
Gln/Gln	28	4	5.80 (1.94-17.3)	0.001	5.80 (1.94-17.4)
Arg/Gln	78	33	1.96 (1.17-3.27)	0.01	1.96 (1.17-3.28)
Arg/Arg	88	73	1(referent)		1(referent)

Cases include hyperplastic leukoplakia, dysplastic leukoplakia and SCC

Controls include normal population

OR^A =Crude Odds; OR^B =age, gender and habits adjusted Odds

Table IV

COMBINED HOMOZYGOUS AND HETEROZYGOUS XRCC1 AND XPD POLYMORPHISM AND RISK OF ORAL CANCER

GENOTYPE/ POLYMORPHISM	CASE* N=194	CONTROL* N=110	OR[A] (95% CI)	P VALUE
XRCC1 Codon194				
Trp/Trp +Arg/Trp	79	20	3.09 (1.76-5.42)	<0.0001
Arg/Arg	115	90	1(referent)	
Codon280				
His/His+ Arg/His	63	27	1.47 (0.87-2.50)	0.18
Arg/Arg	131	83	1(referent)	
Codon399				
Gln/Gln+ Arg/Gln	106	37	2.37 (1.46-3.85)	0.0007
Arg/Arg	88	73	1(referent)	

Cases include hyperplastic leukoplakia, dysplastic leukoplakia and SCC

Controls include normal population

OR[A] =age, gender and habits adjusted Odds

Effects of genotype and habits on oral cancer risk

The combined effects of genotypes and covariates like smoking, betel quid chewing and alcoholism on estimates of risk are shown in Table V. Smokers, alcoholics and betel quid users were classified into two groups: ever (users) and never (non-users). The XRCC1 *Arg*399*Gln* genotype was shown to modify the effects of smoking and betel quid chewing but not the use of alcohol. XRCC1 *Arg*194*Trp* codon, *Arg/Gln+Gln/Gln* ever group gave an increased risk of 4.2 (95% CI=1.63-11.2, p

value=0.012) for smoking when compared to the *Arg/Gln+Gln/Gln* of the never group, whereas alcoholism and betel quid gave no significant results. Similarly, the XRCC1 *Arg*399*Gln* exhibited a risk of 3.9 (95% CI= 1.76-9.05) for smoking and 4.62 (95% CI= 1.24-17.2) for betel quid chewing. The polymorphic genotype conferred an increased risk compared to the wild type genotype (Table V).

Table V

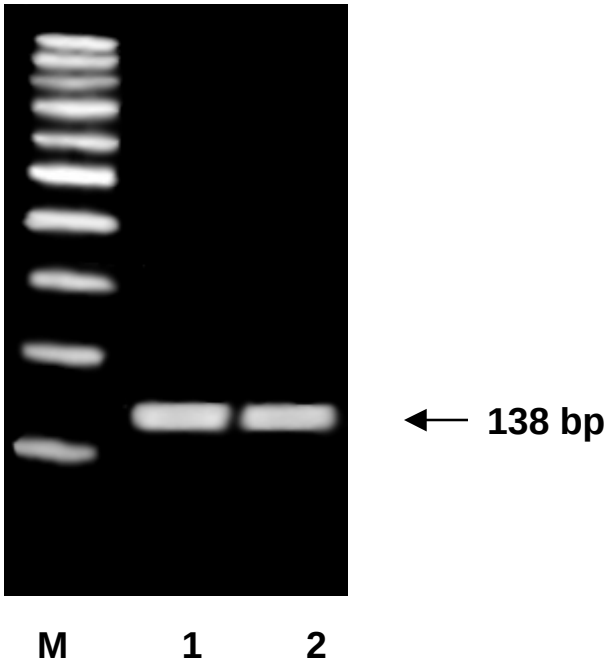
EFFECT OF XRCC1 *Arg*399*Gln* GENOTYPE AND HABITS ON ORAL CANCER RISK

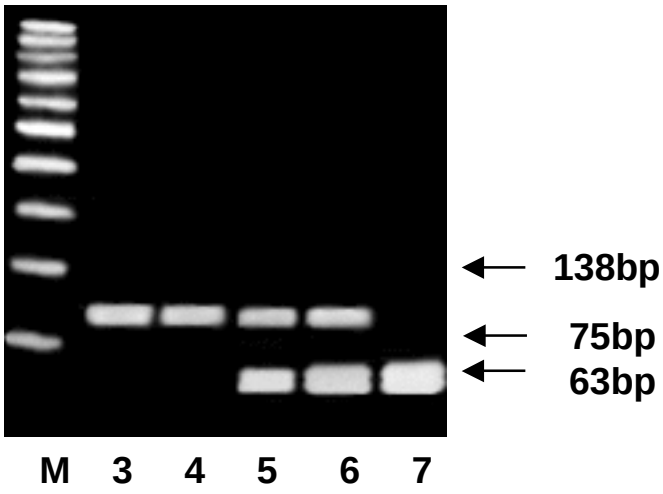
HABITS	GENOTYPE	CASES (n)	CONTROL (n)	OR^A (95%CI)	P Value
Smoking					
Never	*Arg/Gln+Gln/Gln*	49	26	1.63 (0.88-3.03)	0.12
	Arg/Arg	53	46	1(referent)	
Ever	*Arg/Gln+Gln/Gln*	57	11	3.99 (1.76-9.05)	
	Arg/Arg	35	27	1(referent)	0.001
Alcohol					
Never	*Arg/Gln+Gln/Gln*	70	23	2.42 (1.32-4.45)	0.005
	Arg/Arg	59	47	1(referent)	
Ever	*Arg/Gln+Gln/Gln*	35	14	2.24 (0.99-5.06)	0.006
	Arg/Arg	29	26	1(referent)	
Betel quid chewing					
Never	*Arg/Gln+Gln/Gln*	90	32	2.13 (1.26-3.61)	0.004
	Arg/Arg	75	60	1(referent)	
Ever	*Arg/Gln+Gln/Gln*	16	5	4.62 (1.24-17.2)	0.03
	Arg/Arg	9	13	1(referent)	

OR^A= age and gender adjusted Odds

FIG. 4.2

PCR-RFLP analysis of codon 194 genotype





Lane M:DNA marker

Lane 1&2: 138 bp PCR product

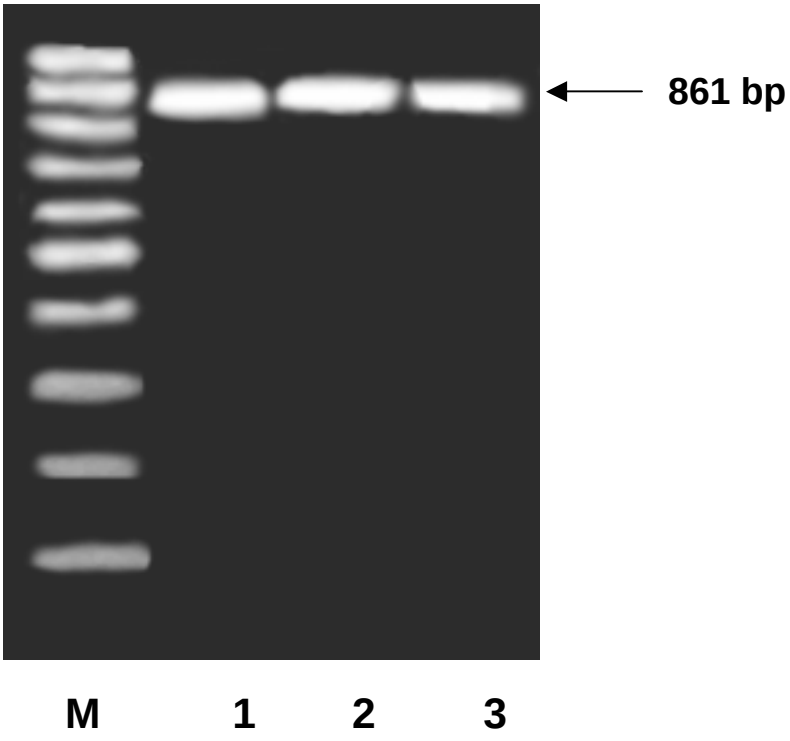
Lane 3&4: Arg/Arg wild genotype

Lane 5&6: Arg/Try heterozygous polymorphic genotype

Lane 7: Arg/Gln homozygous polymorphic genotype

FIG 4.3

PCR product of XRCC1 codon 280/399 genotype analysis

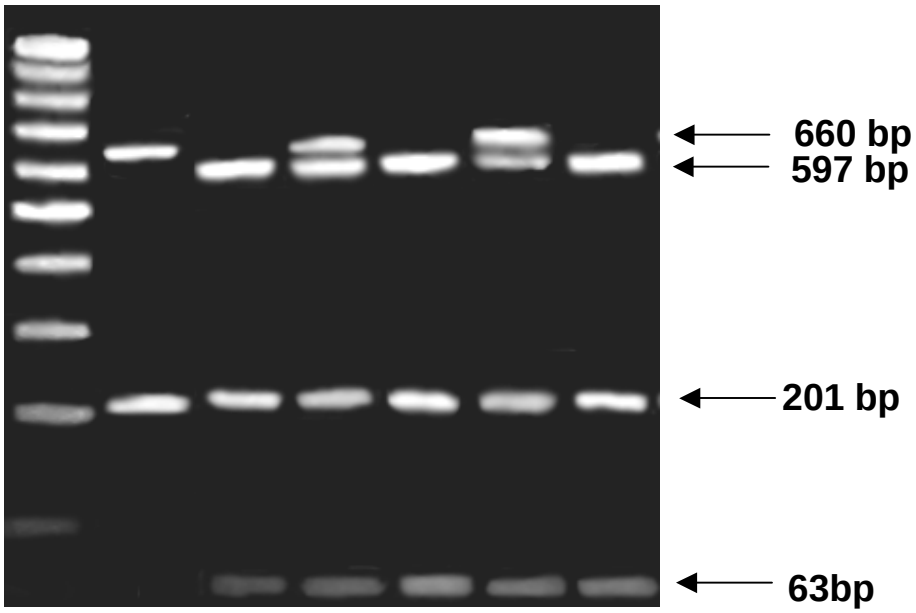


Lane M:DNA marker

Lane ,2&3: 861 bp PCR product

FIG. 4.4

Rsa I RFLP of XRCC1 codon 280 genotype analysis



Msp I RFLP of XRCC1 codon 399 genotype analysis

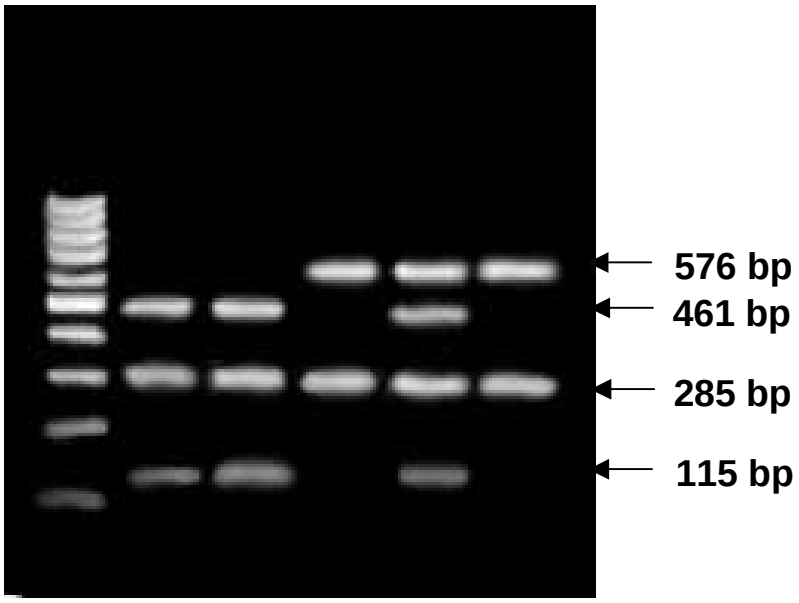
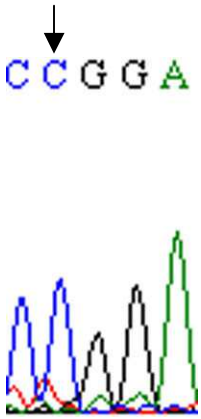


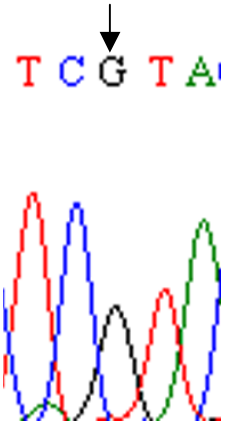
Fig 4.5

SEQUENCING OF XRCC1 GENE POLYMORPHISMS

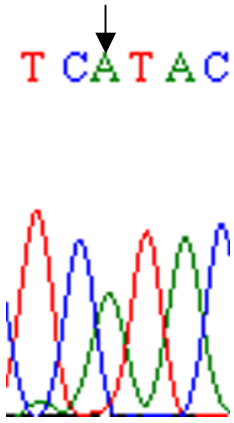


Codon 194 Arg

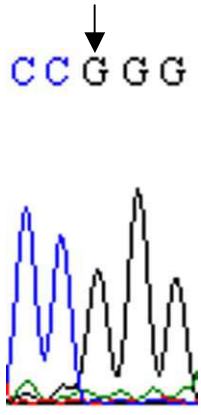
Codon 194 Trp



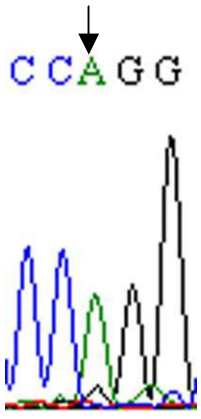
Codon 280 Arg



Codon 280 His



Codon 399 Arg

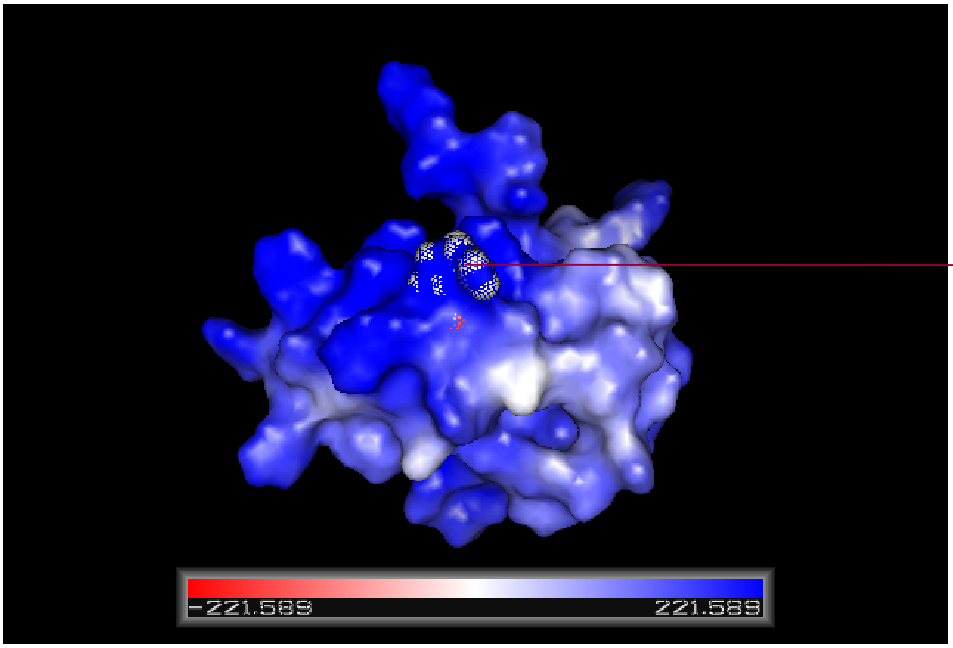


Codon 399 Gln

FIG 4.6

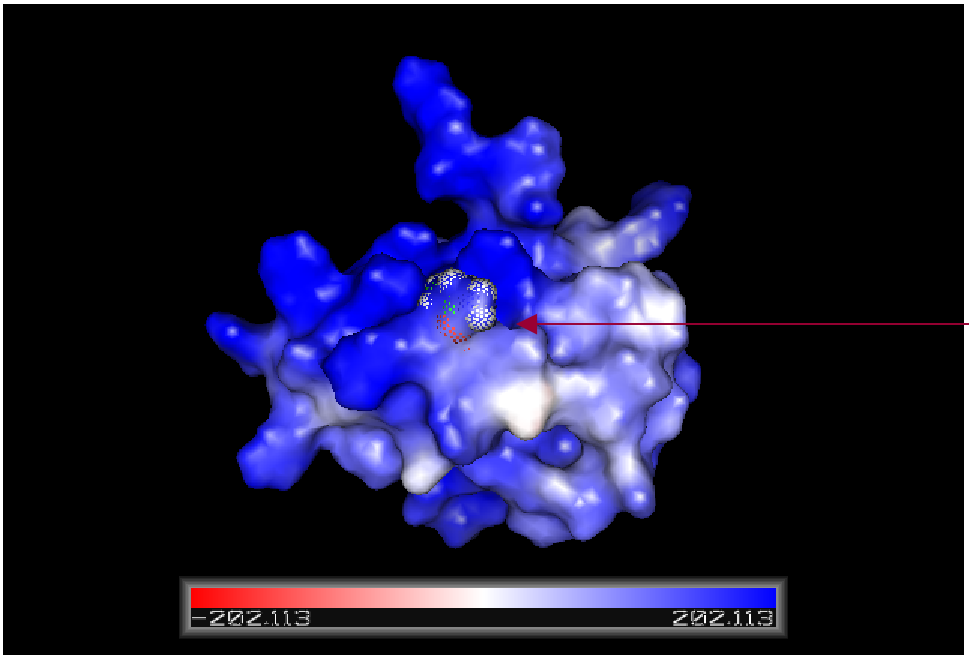
POLYMORPHISM MAPPING OF XRCC1 GENE

BRCT 1 domain of XRCC1



Arg399

Mutated BRCT 1 domain of XRCC1



Gln399

Polymorphism mapping

Figure 4.6 shows the molecular model of human XRCC1 BRCT1 domain. As depicted in the figure, position 399 in the BRCT1 domain maps to the protein surface. The accuracy of the model (based on percentage target-template sequence similarity) is good enough to predict whether a particular residue is located on the surface or inside the 'core'.

Discussion

DNA repair is a system of defenses designed to protect the integrity of the genome. Deficiencies in this system are likely to lead to development of cancer. A large number of low risk genes modulate the carcinogenic process in humans. The epidemiology of DNA repair capacity and of its effects on cancer susceptibility in humans is therefore an important area of investigation.

Squamous cell carcinoma of the oral cavity is a serious health problem both in developing and underdeveloped nations as a consequence of long-term tobacco and alcohol use. However, since not all exposed individuals actually develop cancer, variations in genetic susceptibility may be equally important in the disease etiology. Each year after diagnosis between 3-5% of head and neck cancer patients develop a second malignancy and likewise such patients may be more genetically susceptible to cancer (109). The major reason for high mortality rates is late diagnosis with lesions that are large deeply invasive and often metastatic to regional lymph nodes. However, oral cancer also satisfies the criteria to be a suitable disease

for screening as well as prognostication since it has clinically recognizable precancerous lesions (leukoplakia) and asymptomatic early invasive lesions.

Although, a large number of studies have been done on various preventive strategies of oral cancer, little work is known on the clinical significance of SNPs in DNA repair genes and its possible role as a tool for identifying high risk (susceptible) subgroups that might benefit from intensive screening interventions.

In the present study we examined the DNA repair gene XRCC1 as candidate susceptibility genes for oral cancer in a population based case control study of the South Indian, Travancore population. This study was concentrated on three non-conservative amino acid substitutions of the XRCC1 gene. Such substitutions are expected to be more significant for protein function than conservative ones. The polymorphisms studied were *Arg*194*Trp* (a C→T substitution), *Arg*280*His* (G→A substitution) and *Arg*399*Gln* (G→A substitution)

Our findings suggest a positive association between polymorphism in the *Arg*194*Trp* and the *Arg*399*Gln* genotype and risk for oral cancer. The *Arg*194*Trp* and *Arg*280*His* amino acid substitutions reside in the linker region separating the DNA polymerase B domain from the poly (ADP-ribose polymerase interacting domain. The *Arg*399*Gln* change occurs in the COOH-terminal side of the poly (ADP-ribose) polymerase interacting domain and within a BRCT domain (93). Amino acid substitutions in the

BRCT domain and in the DNA polymerase B interacting domain in the Chinese hamster have been reported to disrupt the functionality of XRCC1 (110).

Our study has shown a 2-3 fold increased risk for subjects carrying the polymorphic variant as against the normal population in the case of XRCC1 194 codon and 399 codon. The codon 280 polymorphism was not found to be significantly higher in frequency in oral cancer cases compared to controls. Varying results have been previously reported in different forms of cancer. In a study on squamous cell carcinoma of the Head and Neck, elevated frequencies of polymorphic codon 194 and 399 have been observed (111). Abdel Rahman et al showed that the inheritance of codon 194 Trp variant and codon 399 Gln variants are associated with increased risk of early onset of colorectal carcinoma (112). Most of the published studies on codon 194 polymorphism have reported a reduced risk of cancer associated with the Trp allele (113-117). However our present results show a significant risk of 2.3 ($p=0.001$).

Functional studies of XRCC1 suggest that the codon 399 Gln allele may be associated with multiple DNA damage phenotypes in human cells and tissues. Lunn et al reported that the 399 Gln allele was associated with an increased aflatoxin DNA adduct burden in placental tissue and an elevated glycophorin A mutant frequency in erythrocytes(103). Duell at al reported a positive association between this allele and detection of polyphenol DNA adducts from blood mononuclear cells as well as a positive association between the variant 399 allele and baseline sister chromatid

exchange frequencies in lymphocytes from smokers (103). These studies therefore suggest a role for XRCC1 in the repair of multiple DNA damage end points in human cells and tissues and imply that the 399Gln allele of XRCC1 has an important potentially harmful phenotype. Two other studies have supported this hypothesis. Sturgis et al have reported an OR of 1.6(95% CI, 1.0-2.6) for the variant genotype in a case control study of cancer of the head and neck (116) and Divine et al observed an odds ratio of 2.8(95% CI 1.2-7.9) in a study of lung cancer (113). In a study by Ratnasinghe et al only the XRCC1 *Arg*280*His* polymorphism was seen to be associated with the risk of lung cancer. In a case control study involving 108 miners with lung cancer and 216 normal controls, individuals with the variant allele were at an 80% greater risk compared with those with the homozygous wild type genotype (93).

This study also investigated potential gene – environment interactions. Cigarette smoking, alcohol consumption and use of “pan” (betel quid) are associated with the production of free radical intermediates that induce base damage and single stranded breaks (118). Tobacco smoke contains an array of potent carcinogens including polycyclic aromatic hydrocarbons, aromatic amines and tobacco specific nitrosamines and BPDE, which form DNA adducts. Our study was able to show evidence for these gene-environment interactions. There was a modest positive association with smoking and betel quid users for subjects with XRCC1 399 codon and XPD 751 codon variant genotypes. Two-fold increase in risk was associated

with almost all genotypes studied, except the XRCC1 *Arg*280*His* genotype (OR=1.10).

A striking feature of XRCC1 is the presence of two BRCA1 carboxy –terminal (BRCT) domains, denoted BRCT1 and BRCTII that are located centrally and at the C terminus of this polypeptide respectively (119-120). The C terminal domain is responsible for binding and stabilizing Lig3α (121-123) and is required for SSBR specifically during the Go/G1 phase of the cell cycle (124,125) The BRCT I domain has become a site of considerable interest since it was identified as the site of a common human genetic polymorphism (*Arg*399*Gln*) that appears from a large number of epidemiological studies to impact significantly on cancer risk (126-128). Chinese hamster ovary cell lines with nonconservative amino acid substitutions in the BRCT –1 domain of XRCC1 show a reduced ability to repair single strand breaks and a hypersensitivity to ionizing radiation (114).

In an attempt to understand the functional significance of the polymorphisms, we mapped the XRCC1 variants to their respective domains. Two of the variant positions viz. *Arg*194*Trp* and *Arg*280*His* did not map to any important domains. However, the *Arg*399*Gln* variant falls within the BRCTI domain and our modeling studies reveal that it maps to the protein surface. It is interesting to note that this particular polymorphic position does not fall within the conserved hydrophobic ‘core’ of the protein and is therefore unlikely to have drastic effects on the protein structure. The polymorphism changes the large and basic side chain of Arg to a

medium sized and polar one of Gln. It is a well-known fact that the residues located on the protein solvent interface contribute to stabilizing the protein energetically. Also surface residues are involved in many protein-protein, protein-DNA and protein-ligand interactions. Hence the changes from Arg to Gln can potentially destabilize the protein and this variation might also affect any possible protein interaction involved. This provides a possible molecular mechanism as to how the polymorphism might affect protein function without affecting the protein structure drastically.

The results of molecular mapping together with the epidemiological data confirms our hypothesis that polymorphisms in functionally important repair genes like XRCC1 may alter the protein structure thus interfering in its function. Our data thus supports a role for DNA repair gene polymorphisms in increased oral cancer risk.