RESEARCH ARTICLE

Interaction of XRCC1 and XPD Gene Polymorphisms with Lifestyle and Environmental Factors Regarding Susceptibility to Lung Cancer in a High Incidence Population in North East India

Bhaskar Jyoti Saikia, Rup Kumar Phukan*, Santanu Kumar Sharma, Gaganpreet Singh Sekhon, Jagadish Mahanta

Abstract

Background: This study aimed to explore the role of XRCC1 (Arg399Gln) and XPD (Lys751Gln) gene polymorphisms, lifestyle and environmental factors as well as their possible interactions in propensity to develop lung cancer in a population with high incidence from North East India. Materials and Methods: A total of 272 lung cancer cases and 544 controls were collected and XRCC1 (Arg399Gln) and XPD (Lys751Gln) genotypes were analyzed using a polymerase chain reaction based restriction fragment length polymorphism assay. Conditional multiple logistic regression analysis was used to calculate adjusted odds ratios and 95% confidence intervals after adjusting for confounding factors. Results: The combined Gln/Gln genotype of XRCC1 and XPD genes (OR=2.78, CI=1.05-7.38; p=0.040) was significantly associated with increased risk for lung cancer. Interaction of XRCC1Gln/Gln genotype with exposure of wood combustion (OR=2.56, CI=1.16-5.66; p=0.020), exposure of cooking oil fumes (OR=3.45, CI=1.39-8.58; p=0.008) and tobacco smoking (OR=2.54, CI=1.21-5.32; p=0.014) and interaction of XPD with betel quid chewing (OR=2.31, CI=1.23-4.32; p=0.009) and tobacco smoking (OR=2.13, CI=1.12-4.05; p=0.022) were found to be significantly associated with increased risk for lung cancer. Conclusions: Gln/Gln alleles of both XRCC1 and XPD genes appear to amplify the effects of household exposure, smoking and betel quid chewing on lung cancer risk in the study population.

Keywords: XRCC1 - XPD - environmental factors - interaction - lung cancer - high risk population - North-East India

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Introduction

Exposure to tobacco smoke, fumes and airborne particulates in the indoor environment and ionizing radiations are regarded as triggering factors for DNA damages (Sterpone and Cozzi, 2010; Tang et al., 2010). Converging lines of evidence suggest that cancer can be initiated by DNA damage, which if not repaired, can cause errors during DNA synthesis (Maynard et al., 2009). Humans are routinely exposed to mutagenic and carcinogenic aromatic amines via smoking, cooking of food and other sources (Zheng and Lee, 2009). DNA so-damaged is typically repaired by certain DNA-repairing enzymes. These enzymes are fundamental for the maintenance of genomic integrity in case of replication errors. Therefore individuals with impairment in DNA repair capability are often at an elevated risk of cancer development (Berwick and Vineis, 2000). In humans more than 100 proteins are involved in DNA repair system (Lopez-Cima et al., 2007). These proteins are implicated in various DNA repair pathways, including base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR) (Li et al., 2011). The X-ray repair cross-complementing group 1 (XRCC1) gene plays an important role in the development of lung cancer (Wang et al., 2014). XRCC1 protein plays a central role in base excision repair (BER) pathway by interacting with other DNA repair proteins (Yin et al., 2009). XRCC1 interacts with polynucleotide kinase enzyme, DNA pol-β, PARP1 and DNA ligase IIIα (Pramanik et al., 2011; Mutairi et al., 2013). Three coding polymorphisms in the XRCC1 gene are at codons 399 (Arg to Gln), 280 (Arg to His) and 194 (Arg to Trp) (Shen et al., 1998). In particular, 399 Gln/Gln allele is found to be significantly associated with higher level of DNA adducts, somatic mutations, sister chromatid exchanges and chromosomal damages (Lunn et al., 1999). Xerodermapigmentosum group D (XPD) encodes an evolutionary conserved ATP dependent helicase, a subunit of transcription factor II H (TFIIH) which is essential for transcription and NER (Coin et al., 1999; Li et al., 2013).

*Regional Medical Research Centre, N.E. Region (ICMR), Dibrugarh, Assam, India. *For correspondence: phukanrk@gmail.com*

Mutation of XPD codons 312 and 751 increases the risk of lung cancer (Zhou et al., 2012). XPD 751Gln/Gln has been demonstrated to have suboptimal DNA-repair capacity to remove UV photoproducts when compared to the XPD 751 Lys/Lys and Lys/Gln genotypes (Qiao et al., 2002).

Lung cancer (LC) is leading cause of cancer death worldwide with an annual mortality of 18.2 % cancer death (Ferlay et al., 2010a). India contributes 6.2% cases of LC with approximately 58,000 incidence cases reported in 2008 (Ferlay et al., 2010b). North Eastern (NE) parts of India represent a unique, strategic geographic location with a demographic diverse population. Manipur and Mizoram are two states from NE parts of India. LC is mostly predominant in NE parts of India, with highest age-adjusted rate (AAR) in Mizoram (28.3 per 10^5 in male and 28.7 per 10^5 in female). Manipur also contributes a very high incidence of LC (with AAR of 14.1 per 10^5 in males and 11.9 per 10^5 in females) (NCRP, 2013). These areas are also reported for a unique consumption of tobacco, betel quids and cooking habits that are different from other places (Phukan et al., 2001, 2005, 2006).

High risk of LC in the study population may be an outcome of genetic and environmental risk factors or a complex interaction of both. Studies have also reported association of XRCC1 and XPD allelic polymorphisms for LC (De-Ruyck et al., 2007; Li et al., 2013; Natukula et al., 2013). Lack of data on XRCC1 and XPD polymorphisms and high incidence of LC in NE parts of India prompted us to explore and evaluate any relevance of these polymorphisms in the study population. We also wished to explore the interaction of XRCC1 and XPD gene with smoking, betel quid chewing, alcohol consumption, exposure of wood combustion during cooking and cooking oil fumes (COF).

Materials and Methods

Study design

Present study was an age (±5 years), sex and ethnicity matched population based case-control study. The study duration was from June 2010-May 2013. The work was carried out at Regional Medical Research Centre (RMRC) NE Region, Indian Council of Medical Research (ICMR); India in collaboration with Population Based Cancer Registry (PBCR), Imphal, Manipur and Aizawl, Mizoram, India. Incident cases and control subjects willing to participate in the study were indigenous people of Manipur and Mizoram. Histopathologically or cytologically confirmed LC cases with no evidence of pulmonary inflammation or benign lung tumors were included in the study. Cases too old to be interviewed elaborately and who refused to be interviewed were excluded from this study. Cancer free control subjects with age (±5 years), sex and ethnicity matched were selected from healthy population of the states. None of the controls subjects had consanguinity with the cases or had any non-communicable diseases. Information of smoking, betel quid chewing, consumption of alcohol, exposure

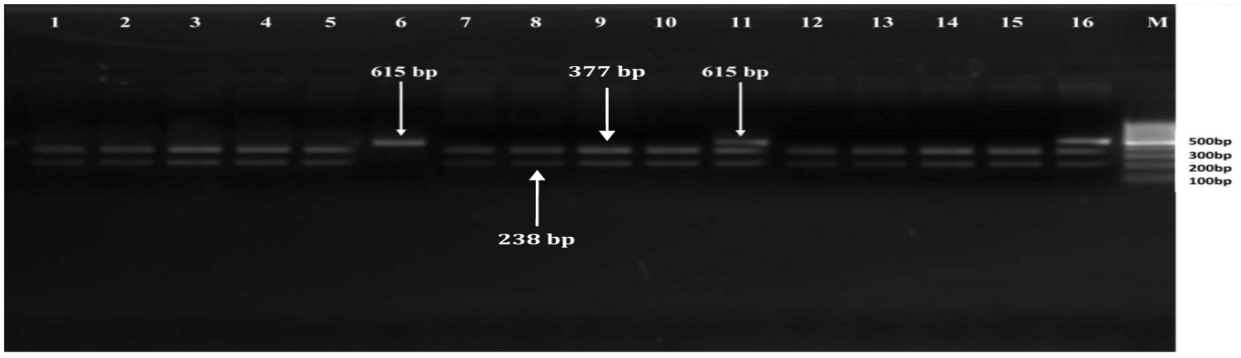

Figure 1. RFLP Photograph of 2% Agarose Gel Electrophoresis for XRCC1 Genotype. Lane M represents 100 bp DNA Ladder. Lane 6 is characterised by 615bp that represents XRCC1 Gln/Gln genotype. Lane 1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14 and 15 are characterised by 377bp and 238bp that represents XRCC1 Arg/Arg genotype. While, Lane 11 and 16 are characterised by 615bp, 377bp and 238bp that represents XRCC1 Arg/Gln genotype

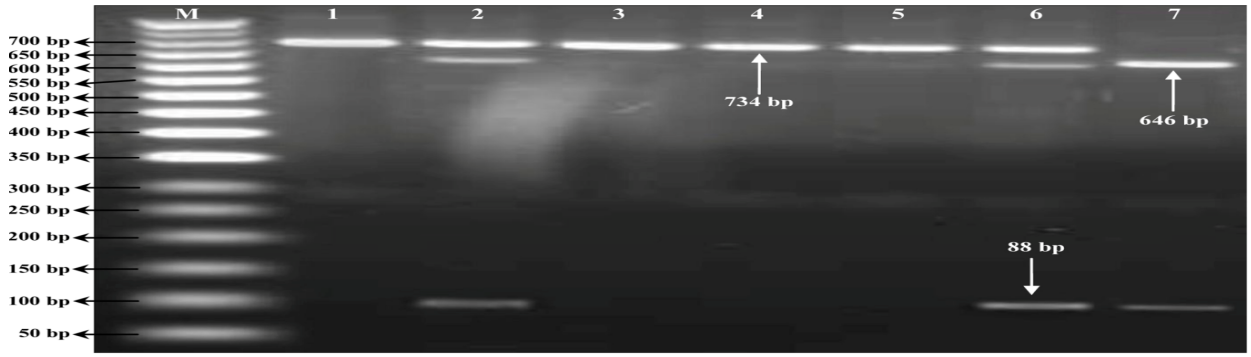

Figure 2. RFLP Photograph of 2% Agarose gel electrophoresis for XPD Genotype. Lane M represents 50 bp DNA Ladder. Lane 1, 3, 4 and 5 are characterised by 734bp that represents XPD Lys/Lys genotype. Lane 2 and 6 are characterised by 734bp, 646bp and 88bp that represents XPD Lys/Gln genotype. While, Lane 7 is characterised by 646bp and 88bp that represents XPD Gln/Gln genotype

to household combustion and COF were recorded in a structured pre-designed questionnaire. The time period set for exposure of wood combustion during cooking and COF was 25 years, the participants were asked whether they have been exposed to the aforesaid time period or not. Those who were found to be exposed were taken as 'yes' for exposure of wood combustion and COF where as those who were not exposed for the set time period of 25 years were taken as 'no'. Written informed consent was taken from all subjects for participation in a protocol approved by the Institutional ethical committee of RMRC, NE Region (Indian Council of Medical Research). Thus a total of confirmed 272 LC cases and 544 controls matched for age (±5 years), sex and ethnicity were enrolled in the study.

DNA extraction

Four ml. of blood was collected from all subjects in EDTA vials. DNA was extracted by standard phenol chloroform method (Landi et al., 2006) and stored at -80º C till further analysis.

Genotyping of XRCC1 and XPD gene

Genotyping of XRCC1 and XPD were done by polymerase chain reaction based restriction fragment length polymorphism. All of the PCR reactions were carried out by a Master cycler gradient thermo cycler (Bio-Rad, United States) in a final volume of 25 ul containing 200 ng of each primer (Sigma, United States), 50 ng genomic DNA, 1.0mM $MgCl_2$ (Roche, Germany), 200 ul dNTPs (Roche, Germany) and 2.0 unit of Taq DNA Polymerase (Roche). The PCR product was visualized in 2% agarose gel electrophoresis by gel documentation system (Cell Biosciences). XRCC1 PCR products were amplified with primers 5'-GCCCCGTCCCAGGTAAG-3' (sense) and 5'-AGCCCCAAGACCCTTTCACT-3'(antisense) (Park et al., 2002) followed by MspI (Promega) restriction digestion. The homozygous Gln allele was determined by presence of an uncut 615-bp band (indicative of absence of MspI cutting site), homozygous Arg allele was determined by presence of two bands at 377 and 238 bp while the heterozygous Arg/Gln allele was characterized by presence of three bands at 615, 377 and 238 bp (Figure 1). XPD PCR products were amplified with the primers 5'-CCTCTCCCTTTCCTCTGTTC-3' (sense) and 5'-CAGGTGAGGGGGACATCT-3' (antisense) (Vettriselvi et al., 2007) and digested with PstI (New England BioLabs, Inc.). The homozygous Lys/Lys allele was characterized by an undigested band of 734bp, homozygous Gln/Gln allele determined by 646bp and 88bp, while heterozygous Lys/Gln genotype had three bands of 734bp, 646bp and 88bp (Figure 2). 10% of

Table 1. Distribution of Demographic Characteristic and Risk of Lung Cancer

Category	Case n (%)	Control n (%)	Crude OR (95% CI)	p-value	Multivariate OR (95% CI)	p-value
Sample size	272 (100)	544 (100)	---	---	---	---
Sex			0.529†			
Male	130 (47.8)	260 (47.8)	---	---	---	---
Female	142 (52.2)	284 (52.2)	---	---	---	---
Age (years)						
Range	21-88	21-89	---	---	---	---
Means±SD	61.96±11.91	61.79±12.21	---	0.851‡	---	---
Histological type						
Non-small squamous	160 (58.8)	---	---	---	---	---
Non-small adenocarcinoma	65 (23.9)	---	---	---	---	---
Small cell carcinoma	26 (9.6)	---	---	---	---	---
Other §	21 (7.7)	---	---	---	---	---
Exposure of wood combustion						
No	147 (54.0)	346 (63.6)	1.0 (Reference)	---	1.0 (Reference)	---
Yes (Overall)	125 (46.0)	198 (36.4)	1.49 (1.11-1.20)	0.008	1.32 (0.96-1.81)	0.088
Yes (Males)	44 (16.2)	85 (15.6)	1.05 (0.67-1.65)	0.819	1.03 (0.64-1.63)	0.917
Yes (Females)	81 (29.8)	113 (20.8)	2.01 (1.34-3.02)	0.001	1.58 (1.01-2.48)	0.046*
Exposure of cooking oil fumes						
No	136 (50.0)	321 (59.0)	1.0 (Reference)	---	1.0 (Reference)	---
Yes (Overall)	136 (50.0)	223 (41.0)	1.44 (1.07-1.93)	0.015	1.27 (0.93-1.75)	0.133
Yes (Males)	49 (18.0)	99 (18.2)	0.98 (0.64-1.52)	0.941	0.94 (0.60-1.48)	0.786
Yes (Females)	87 (32.0)	124 (22.8)	2.04 (1.35-3.08)	0.001	1.61 (1.02-2.53)	0.039*
Betel-quid chewing						
Nonchewer	85 (31.3)	208 (38.2)	1.0 (Reference)	---	1.0 (Reference)	---
Chewer (Overall)	187 (68.7)	336 (61.8)	1.36 (1.00-1.86)	0.05	1.36 (0.99-1.87)	0.056
Chewer (Males)	83 (30.5)	158 (29.1)	1.14 (0.74-1.76)	0.556	1.09 (0.69-1.70)	0.718
Chewer (Females)	104 (38.2)	178 (32.7)	1.63 (1.05-2.54)	0.03	1.62 (1.02-2.56)	0.041*
Tobacco smoking						
Nonsmoker	75 (27.6)	203 (37.3)	1.0 (Reference)	---	1.0 (Reference)	---
Smoker (Overall)	197 (72.4)	341 (62.7)	1.56 (1.14-2.15)	0.006	1.62 (1.17-2.24)	0.004*
Smoker (Males)	108 (39.7)	191 (35.1)	1.77 (1.04-3.03)	0.034	1.86 (1.07-3.23)	0.027*
Smoker (Females)	89 (32.7)	150 (27.6)	1.50 (0.99-2.27)	0.053	1.56 (1.02-2.39)	0.042*

*Significant; †Based on Chi-square test; ‡For independent samples T-test; § Other includes large cell carcinoma, bronchioalveolar carcinoma

randomly selected samples were randomly sequenced to verify genotyping results and 100% concordance was found.

Statistical analysis

Difference in demographic characteristics, tobacco smoking, betel quid chewing and genotype frequencies between cases and controls were evaluated using Chi Square (χ^2) test. Estimates of LC risk, imparted by genotypes were determined by deriving odds ratio (OR) and its corresponding 95% confidence interval (95% CI) using multivariable conditional logistic regression after adjusting for potential confounders. For all tests, a two sided p≤0.05 was considered statistically significant. All statistical analysis were done using SPSS version 17.0.Tests for Hardy-Weinberg equilibrium amongst cases and control were conducted using observed genotype frequencies and a chi-square test featuring one degree of freedom.

Results

The details of demographic characteristics among cases and controls enrolled in this study are shown in Table 1. There was no statistically significant difference in term of mean age of cases (61.96±11.91 years, range 21-88) and controls (61.79±12.21 years, range 21-89) (p=0.711) of study subjects. 58.8% of cases were of non small squamous cell carcinoma, 23.9% were of non small adenocarcinoma and 9.6% were of small cell carcinoma and 7.7% others. Significant risk was observed for smoking (OR=1.62, CI=1.17-2.24; p=0.004). Risk was observed for betel-quid chewing (OR=1.36, CI=0.99-1.87; p=0.056), exposure of wood combustion (OR=1.32, CI=0.96-1.81; p=0.088) and COF (OR=1.27, CI=0.93-1.75; p=0.133) but results are not statistically significant. Tests for Hardy-Weinberg equilibrium amongst cases and controls were conducted using observed genotype frequencies and a chi-square test featuring one degree of freedom. The distribution of genotypes for both XRCC1 and XPD genes among cases and controls were in Hardy-Weinberg equilibrium (Table 1).

Gln/Gln genotype was higher among cases than control groups in both XRCC1 (8.5% vs 6.3%) and XPD genes (12.9% vs 10.5%). Risk for LC was higher for individuals carrying Gln/Gln genotype in both XRCC1 (OR=1.37, CI=0.77-2.43; p=0.289) and XPD (OR=1.26, CI=0.78-2.04; p=0.352) genes, but results are not statistically significant. However a significant risk association was observed for combined effect Gln/Gln genotype of XRCC1 and XPD gene (OR=2.78, CI=1.05-7.38; p=0.040) after adjusting for potential confounders (Table 2).

Table 3. XRCC1 Arg399Gln and XPD Lys751Gln Allele Frequencies and Risk of Lung Cancer

Allele	Case n=544(%)	Control n=1088 (%)	Crude OR (95% CI)	p-value†
XRCC1 Arg399Gln				
Allele Arg	395 (72.6)	832 (76.5)	0.82 (0.65-1.03)	0.089
Allele Gln	149 (27.4)	256 (23.5)	1.23 (0.97-1.55)	0.089
XPD Lys751Gln				
Allele Lys	358 (65.8)	749 (68.8)	0.87 (0.70-1.08)	0.216
Allele Gln	186 (34.2)	339 (31.2)	1.15 (0.92-1.43)	0.216

†Based on Chi-square test

Table 2. Genetic Interaction and Distributions of XRCC1 Arg399Gln and XPD Lys751Gln Genotypes and Risk of Lung Cancer

Genotypes		Case n(%)	Control n (%)	Crude OR (95% CI)	p-value	Adjusted OR (95% CI) †	p-value
XRCC1 Arg399Gln							
Arg/Arg		146 (53.7)	322 (59.1)	1.0 (Reference)	---	1.0 (Reference)	---
Arg/Gln		103 (37.8)	188 (34.6)	1.21 (0.89-1.65)	0.231	1.08 (0.78-1.48)	0.654
Gln/Gln		23 (8.5)	34 (6.3)	1.49 (0.85-2.62)	0.165	1.37 (0.77-2.43)	0.289
Hardy-Weinberg equilibrium test‡							
$\chi2$		0.63	0.86	---	---	---	---
p-value		0.43	0.35	---	---	---	---
XPD Lys751Gln							
Lys/Lys		121 (44.5)	262 (48.2)	1.0 (Reference)	---	1.0 (Reference)	---
Lys/Gln		116 (42.6)	225 (41.3)	1.12 (0.82-1.52)	0.488	1.04 (0.76-1.43)	0.795
Gln/Gln		35 (12.9)	57 (10.5)	1.33 (0.83-2.13)	0.238	1.26 (0.78-2.04)	0.352
Hardy-Weinberg equilibrium test‡							
$\chi2$		0.75	0.7	---	---	---	---
p-value		0.39	0.4	---	---	---	---
XRCC1	XPD						
Arg/Arg	Lys/Lys	68 (25.0)	163 (30.0)	1.0 (Reference)	---	1.0 (Reference)	---
Arg/Arg	Lys/Gln	67 (24.6)	129 (23.7)	1.25 (0.83-1.87)	0.294	1.19 (0.78-1.80)	0.416
Arg/Arg	Gln/Gln	11 (4.0)	30 (5.5)	0.88 (0.42-1.85)	0.735	0.86 (0.40-1.83)	0.691
Arg/Gln	Lys/Lys	52 (19.1)	84 (15.4)	1.48 (0.95-2.32)	0.083	1.37 (0.87-2.16)	0.179
Arg/Gln	Lys/Gln	38 (14.1)	85 (15.6)	1.07 (0.67-1.72)	0.776	0.87 (0.53-1.43)	0.583
Arg/Gln	Gln/Gln	13 (4.8)	19 (3.5)	1.64 (0.77-3.51)	0.202	1.47 (0.68-3.19)	0.334
Gln/Gln	Lys/Lys	1 (0.4)	15 (2.8)	0.16 (0.02-1.23)	0.079	0.14 (0.02-1.07)	0.058
Gln/Gln	Lys/Gln	11 (4.0)	11 (2.0)	2.40 (0.99-5.79)	0.052	2.29 (0.93-5.62)	0.07
Gln/Gln	Gln/Gln	11 (4.0)	8 (1.5)	3.30 (1.27-8.55)	0.014	2.78 (1.05-7.38)	0.040*

* Significant; † Adjusted OR were estimated by adjusting exposure of wood combustion, cooking oil fumes, betel-quid chewing and tobacco smokingin conditional multiple logistic regression model; ‡Hardy–Weinberg equilibrium test is calculated for 1 (one) degree of freedom and values rounded to two decimals

Table 4. Interaction of XRCC1 Arg399Gln and XPD Lys751Gln Genotypes with Exposure of Wood Combustion, Cooking Oil Fumes, Betel-Quid Chewing and Tobacco Smoking

Model	Interaction Parameters		Case n (%)	Control n(%)	Crude OR (95% CI)	p-value	Adjusted OR (95% CI) #	p-value
1†	XRCC1	Exposure of wood combustion						
	Arg/Arg	No	81 (29.8)	211 (38.7)	1.0 (Reference)	---	1.0 (Reference)	---
	Arg/Arg	Yes	65 (23.9)	111 (20.4)	1.53 (1.02-2.27)	0.038	1.38 (0.91-2.10)	1.129
	Arg/Gln	No	59 (21.7)	114 (21.0)	1.35 (0.90-2.02)	0.149	1.24 (0.82-1.87)	0.313
	Arg/Gln	Yes	44 (16.2)	74 (13.6)	1.55 (0.99-2.44)	0.058	1.28 (0.80-2.05)	0.31
	Gln/Gln	No	7 (2.6)	21 (3.9)	0.87 (0.36-2.12)	0.757	0.93 (0.38-2.30)	0.881
	Gln/Gln	Yes	16 (5.9)	13 (2.4)	3.21 (1.48-6.96)	0.003	2.56 (1.16-5.66)	0.020*
2‡	XRCC1	Exposure of cooking oil emission						
	Arg/Arg	No	71 (26.1)	195 (35.8)	1.0 (Reference)	---	1.0 (Reference)	---
	Arg/Arg	Yes	75 (27.6)	127 (23.3)	1.62 (1.09-2.41)	0.016	1.44 (0.95-2.18)	0.082
	Arg/Gln	No	56 (20.6)	101 (18.5)	1.52 (0.99-2.33)	0.052	1.38 (0.89-2.12)	0.148
	Arg/Gln	Yes	47 (17.3)	87 (16.0)	1.48 (0.95-2.32)	0.084	1.22 (0.76-1.94)	0.413
	Gln/Gln	No	9 (3.3)	25 (4.6)	0.99 (0.44-2.22)	0.978	0.91 (0.40-2.05)	0.811
	Gln/Gln	Yes	14 (5.1)	9 (1.7)	4.27 (1.77-10.30)	0.001	3.45 (1.39-8.58)	0.008*
3§	XRCC1	Betel-quid chewing						
	Arg/Arg	Nonchewer	56 (20.6)	124 (22.8)	1.0 (Reference)	---	1.0 (Reference)	---
	Arg/Arg	Chewer	90 (33.1)	198 (36.4)	1.01 (0.67-1.51)	0.975	1.01 (0.67-1.51)	0.98
	Arg/Gln	Nonchewer	26 (9.6)	72 (13.2)	0.80 (0.46-1.38)	0.424	0.72 (0.41-1.25)	0.241
	Arg/Gln	Chewer	77 (28.3)	116 (21.3)	1.47 (0.96-2.25)	0.077	1.36 (0.88-2.10)	0.166
	Gln/Gln	Nonchewer	3 (1.1)	12 (2.2)	0.55 (0.15-2.04)	0.374	0.56 (0.15-2.09)	0.388
	Gln/Gln	Chewer	20 (7.4)	22 (4.1)	2.01 (1.02-3.99)	0.045	1.89 (0.94-3.79)	0.074
4¶	XRCC1	Tobacco smoking						
	Arg/Arg	Nonsmoker	51 (18.8)	137 (25.2)	1.0 (Reference)	---	1.0 (Reference)	---
	Arg/Arg	Smoker	95 (34.9)	185 (34.0)	1.38 (0.92-2.07)	0.12	1.46 (0.96-2.21)	0.075
	Arg/Gln	Nonsmoker	19 (7.0)	50 (9.2)	1.02 (0.55-1.89)	0.948	1.01 (0.54-1.88)	0.983
	Arg/Gln	Smoker	84 (30.9)	138 (25.4)	1.64 (1.07-2.49)	0.022	1.66 (1.08-2.54)	0.021*
	Gln/Gln	Nonsmoker	5 (1.8)	16 (2.9)	0.84 (0.29-2.41)	0.745	0.86 (0.30-2.47)	0.773
	Gln/Gln	Smoker	18 (6.6)	18 (3.3)	2.69 (1.30-5.56)	0.008	2.54 (1.21-5.32)	0.014*
5†	XPD	Exposure of wood combustion						
	Lys/Lys	No	77 (28.3)	176 (32.4)	1.0 (Reference)	---	1.0 (Reference)	---
	Lys/Lys	Yes	44 (16.2)	86 (15.8)	1.17 (0.75-1.84)	0.497	1.03 (0.64-1.66)	0.904
	Lys/Gln	No	55 (20.2)	138 (25.3)	0.91 (0.60-1.38)	0.657	0.85 (0.56-1.30)	0.459
	Lys/Gln	Yes	61 (22.4)	87 (16.0)	1.60 (1.05-2.45)	0.029	1.36 (0.87-2.12)	0.174
	Gln/Gln	No	15 (5.5)	32 (5.9)	1.07 (0.55-2.09)	0.84	1.09 (0.55-2.16)	0.797
	Gln/Gln	Yes	20 (7.3)	25 (4.6)	1.83 (0.96-3.49)	0.067	1.55 (0.80-3.00)	0.193
6‡	XPD	Exposure of cooking oil emission						
	Lys/Lys	No	71 (26.1)	152 (27.9)	1.0 (Reference)	---	1.0 (Reference)	---
	Lys/Lys	Yes	50 (18.4)	110 (20.2)	0.97 (0.63-1.51)	0.903	0.87 (0.55-1.38)	0.547
	Lys/Gln	No	45 (16.5)	137 (25.2)	0.70 (0.45-1.09)	0.116	0.66 (0.42-1.02)	0.064
	Lys/Gln	Yes	71 (26.1)	88 (16.2)	1.73 (1.13-2.63)	0.011	1.43 (0.91-2.23)	0.118
	Gln/Gln	No	20 (7.4)	32 (5.9)	1.34 (0.72-2.50)	0.362	1.28 (0.68-2.43)	0.442
	Gln/Gln	Yes	15 (5.5)	25 (4.6)	1.29 (0.64-2.59)	0.483	1.04 (0.50-2.15)	0.915
7§	XPD	Betel-quid chewing						
	Lys/Lys	Nonchewer	46 (16.9)	107 (19.7)	1.0 (Reference)	---	1.0 (Reference)	---
	Lys/Lys	Chewer	75 (27.6)	155 (28.6)	1.13 (0.72-1.75)	0.6	1.17 (0.75-1.83)	0.495
	Lys/Gln	Nonchewer	34 (12.5)	73 (13.4)	1.08 (0.64-1.85)	0.769	1.06 (0.62-1.82)	0.836
	Lys/Gln	Chewer	82 (30.1)	152 (27.9)	1.26 (0.81-1.94)	0.309	1.20 (0.77-1.87)	0.424
	Gln/Gln	Nonchewer	5 (1.8)	28 (5.1)	0.42 (0.15-1.14)	0.089	0.41 (0.15-1.14)	0.087
	Gln/Gln	Chewer	30 (11.0)	29 (5.3)	2.41 (1.30-4.46)	0.005	2.31 (1.23-4.32)	0.009*
8¶	XPD	Tobacco smoking						
	Lys/Lys	Nonsmoker	35 (12.9)	105 (19.3)	1.0 (Reference)	---	1.0 (Reference)	---
	Lys/Lys	Smoker	86 (31.6)	157 (28.9)	1.64 (1.03-2.61)	0.036	1.76 (1.09-2.82)	0.020*
	Lys/Gln	Nonsmoker	31 (11.4)	77 (14.2)	1.21 (0.69-2.13)	0.513	1.16 (0.65-2.05)	0.614
	Lys/Gln	Smoker	85 (31.3)	148 (27.1)	1.72 (1.08-2.75)	0.022	1.70 (1.06-2.73)	0.028*
	Gln/Gln	Nonsmoker	9 (3.3)	21 (3.9)	1.29 (0.54-3.07)	0.571	1.32 (0.55-3.18)	0.537
	Gln/Gln	Smoker	26 (9.6)	36 (6.6)	2.17 (1.15-4.08)	0.017	2.13 (1.12-4.05)	0.022*

*Significant; †Exposure of cooking oil fumes, betel-quid chewing and tobacco smokingwere adjusted to estimate adjusted OR in each model; ‡Exposure of wood combustion, betel-quid chewingand tobacco smokingwere adjusted to estimate adjusted OR in each model; §Exposure of wood combustion, exposure of cooking oil fumesand tobacco smokingwere adjusted to estimate adjusted OR in each model; ¶Exposure of wood combustion, exposure of cooking oil fumes and betel-quid chewing were adjusted to estimate adjusted OR in each model; #Adjusted OR were estimated through conditional multiple logistic regression model

Allele frequency Gln was also higher in cases than control groups in both XRCC1 (27.4% vs 23.5%) and XPD (34.2 vs 31.2%) genes. Risk was observed for Gln Allele in both XRCC1 (OR=1.23, CI=0.97-1.55; p=0.089) and XPD (OR=1.15, CI=0.92-1.43; p=0.216) genotype; however result are not statistically significant (Table 3).

Interaction of XRCC1Gln/Gln genotype with exposure of wood combustion (OR=2.56, CI=1.16-5.66; p=0.020), exposure of COF (OR=3.45, CI=1.39-8.58; p=0.008) and tobacco smoking (OR=2.54, CI=1.21-5.32; p=0.014) were also significantly associated with increased risk of LC. Similarly interaction of XPD with betel quid chewing (OR=2.31, CI=1.23-4.32; p=0.009) and tobacco smoking (OR=2.13, CI=1.12-4.05; p=0.022) were also significantly associated with increased risk for LC after adjusting for potential confounders (Table 4).

Discussion

In this study, we examined whether association of XRCC1 and XPD genes polymorphisms and their interaction with indoor household exposure during cooking, tobacco smoking and betel quid chewing are implicated in development of LC in population with high incidence of lung cancer from North East India. Observation on association of XRCC1 and XPD on LC was inconsistent in different ethnic and geographical region with varying allele frequency (Lopez-Cima et al., 2007; Improta et al., 2008; Karkucak et al., 2012). In present study, no significant independent association of XRCC1 and XPD polymorphisms for LC was observed. These findings are concordant with some of the previous reports over different ethnic population (David-Beabes et al., 2001; Huang et al., 2008; Sun et al., 2013). However present study reveals significant association when XRCC1 Gln/Gln genotype interact with exposure of wood combustion, exposure of COF and tobacco smoking, while XPD Gln/Gln genotype with betel quid chewing and tobacco smoking. Combined effect of XRCC1 (Arg399Gln) and XPD (Lys751Gln) were also analysed. Result suggested that individuals with both XRCC1 Gln/Gln and XPD Gln/Gln genotype seemed to have synergistically increased risk for LC compared with those of either of them. Environmental exposure primarily tobacco smoke and other household exposure contain complex mixture of certain substances, it is plausible that repair of DNA damage intrigued by these mixed substances either by BER pathway or NER pathway. The failure or diminished on either side may cause LC risk. As expected, our study also confirmed the well established association between tobacco smoking and LC. Because of the traditional culture of study population, responsibility of cooking lies mostly with women; they are more exposed to COF and other household exposure. Interestingly in the present study significantly higher risk was observed in women than in males for LC in terms of exposure of wood combustion (OR=1.58, CI=1.01-2.48; p=0.046) and COF (OR=1.61, CI=1.02-2.53; p=0.039). Study conducted in Shenyang also observed a positive association between COF and LC risk among women (Li et al., 2008). Another study conducted by Hung et al. (2007) reported that COF is capable of causing cellular destruction of genetic material.

Wood combustion, cooking oil emission, tobacco, betel quids primarily constitute large number of polycyclic aromatic hydrocarbon (PAH), alkaloids and other phenolic compounds which are considered as a prime risk factors of LC (Seow et al., 2000; Pfeifer et al., 2002; Li et al., 2008; Hosgood et al., 2010; Mandal et al., 2013). Individuals differ widely in their capacity to repair DNA damage from both exogenous agents, such as wood and tobacco smoke, exposure to COF as well as endogenous reactions. Present study reports for XRCC1 and XPD gene polymorphisms, their interaction with exposure of COF, wood combustion, betel quid chewing, tobacco smoking and alcohol consumption and its association with LC in a high risk area from NE parts of India. Though no significant association for XRCC1 and XPD genotype on LC was observed, the Gln/Gln allele of XRCC1 seems to contribute significant risk modifiers for exposure of wood combustion, exposure of COF and tobacco smoking while the Gln/Gln allele of XPD with betel quid chewing and tobacco smoking. Studies conducted by Lunn et al. (1999) reveals that Gln allele is associated with higher DNA adduct level or lower DNA repair efficiency. PAH-induced bulky DNA adducts, such as benzo[a]pyrenediol epoxide-DNA adducts, which are the most potent premutagenic adducts are mainly repaired by NER. A variety of reactive oxygen species, such as hydroxyl radical and hydrogen peroxide are generated during enzymatic oxidation of PAH (Park et al., 2002). These reactive oxygen species can lead to DNA damages which may be quantitatively a predominant PAH-induced DNA damage. Oxidative DNA damages are primarily removed via BER, including XRCC1.

Our study has several strengths and findings. It was a population based case-control study with a high participation rates. Our cases were incident; therefore possibility of observer or recall bias can be nullified. Also case–control matching was done in reference to age (±5 years), sex and ethnicity, thereby controlling for any confounding effect on account of these variables.

Present study indicates that there is no significant relationship between XRCC1, XPD polymorphisms in study population. Significant risk was observed for interaction of these genes with some environmental factors. However a validation of these results will require its replication in a larger sample size. Taking into account other factors such as susceptibility differences of familial aggregation studies, epigenetic mechanism and infection (Human papilloma virus) will gives us more probable factors for increase risk of LC in NE parts of India.

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References

Berwick M, Vineis P (2000). Markers of DNA Repair and Susceptibility to Cancer in Humans: an Epidemiologic Review. *J Natl Cancer Inst*, **92**, 874-97.

Coin F, Bergmann E, Tremeau-Bravard A, et al (1999). Mutations in XPB and XPD helicases found in xerodermapigmentosum patients impair the transcription function of TFIIH. *The EMBO Journal*, **18**, 1357-66.

David-Beabes GL, Lunn RM, London SJ (2001). No association between the xpd (lys751g1n). polymorphism or the xrcc3 (thr241met). polymorphism and lung cancer risk. *Cancer Epidemiol Biomarkers Prev*, **10**, 911-2.

De Ruyck K, Szaumkessel M, De Rudder I,et al (2007). Polymorphisms in base-excision repair andnucleotide-excision repair genes in relation to lung cancer risk. *Mutat Res*, **631**, 101-10.

Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, **127**, 2893-917.

Ferlay J, Shin HR, Bray F, et al (2010). Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer. GLOBOCAN, 2008 [October, 2012; 2011 20 June] Available from: http://globocan.iarc.fr.

Hosgood HD, Boffetta P, Greenland S, et al (2010). In-home coal and wood use and lung cancer risk: a pooled analysisof the international lung cancer consortium. *Environ Health Perspect*, **118**, 1743-7.

Huang G, Cai S, Wang W, et al (2008). Association between xrcc1 and xrcc3 polymorphisms with lung cancer risk: a meta-analysis from case-control studies. *Plos One*, **8**, 68457.

Hung HS, Wu WJ, Cheng YW, et al (2007). Association of cooking oil fumes exposure with lung cancer: involvement of inhibitor of apoptosis proteins in cell survival and proliferation *in vitro*. *Mutat Res*, **628**, 107-16.

Improta G, Sgambato A, Bianchino G, et al (2008). Polymorphisms of the DNA Repair Genes XRCC1 and XRCC3 and Risk of Lung and Colorectal Cancer: A Case-Control Study in a Southern Italian Population. *Anticancer Research*, **28**, 2941-6.

Karkucak M, Yakut T, Evrensel T, et al (2012). XRCC1 gene polymorphisms and risk of lung cancer in Turkish patients. *Int J Hum Genet*, **12**, 113-7.

Landi S, Gemignani F, Canzian F, et al (2006). DNA Repair and Cell Cycle Control Genes and the Risk of Young-Onset Lung Cancer. *Cancer Res*, **66**, 11062-9.

Li M, Yin Z, Guan P, et al (2008). XRCC1 polymorphisms, cooking oil fume andlung cancer in Chinese women nonsmokers. *Lung Cancer*, **62**, 145-51.

Li XD, Han JC, Zhang YJ, Li HB, Wu XY (2013). Common variations of DNA repair genes are associated with response to platinum-based chemotherapy in NSCLCs. *Asian Pac J Cancer Prev*, **14**, 145-8.

Li Z, Guan W, Li MX, et al (2011). Genetic polymorphism of DNA base-excision repair genes (APE1, OGG1 and XRCC1). and their correlation with risk of lung cancer in a Chinese population. *Arch Med Res*, **42**, 226-34.

Lopez-Cima MF, Gonzalez-Arriaga P, Garcia-Castro L, et al (2007). Polymorphisms in XPC, XPD, XRCC1 and XRCC3 DNA repair genes and lung cancer risk in a population of Northern Spain. *BMC Cancer*, **7**, 162.

Lunn RM, Langlois RG, Hsieh LL, et al (1999). XRCC1 Polymorphisms: Effects on Aflatoxin B1-DNA Adductsand Glycophorin A Variant Frequency. *Cancer Res*, **59**, 2557-61.

Mandal SK, Singh TT, Sharma TD, Amrithalingam V (2013). Clinico-pathology of Lung Cancer in a Regional Cancer Center in Northeastern India. *Asian Pac J Cancer Prev*, **14**, 7277-81.

Maynard S, Schurman SH, Harboe C, et al (2009). Base excision repair of oxidative DNA damage and association with cancer and aging. *Carcinogenesis*, **30**, 2-10.

Mutairi FMA, Alanazi M, Shalaby M, et al (2013). Association of XRCC1 gene polymorphisms with breast cancer susceptibility in Saudi Patients. *Asian Pac J Cancer Prev*, **14**, 3809-13.

Natukula K, Jamil K, Pingali UR, Attili VSS, Madireddy URN (2013). The Codon 399 Arg/Gln XRCC1 Polymorphism is Associated with Lung Cancer in Indians. *Asian Pac J Cancer Pre*v, **14**, 5275-9.

NCRP (2013). National Cancer Registry Programme, Three-year report of the population based cancer registries 2009-2011, (Incidence and Distribution of Cancer: Report of 25 PBCRs in India). Indian Council of Medical Research, Bangalore, India, pp 154-79.

Park JY, Lee SY, Jeon H, et al (2002). Polymorphism of the DNA repair gene XRCC1 and risk of primary lung cancer. *Cancer Epidemiol Biomarkers Prev*, **11**, 23-7.

Pfeifer GP, Denissenko MF, Olivier M, et al (2002). Tobacco smoke carcinogens, DNA damage and p53 mutations insmoking-associated cancers. *Oncogene*, **21**, 7435-51.

Phukan RK, Ali MS, Chetia CK, Mahanta J (2001). Betel nut and tobacco chewing; potential risk factors of cancer of oesophagus in Assam, India. *Brit J Cancer*, **85**, 661-7.

Phukan RK, Narain K, Zomawia E, Hazarika NC, Mahanta J (2006). Dietary habits and stomach cancer in Mizoram, India. *J Gastroenterol*, **41**, 418-24.

Phukan RK, Zomawia E, Narain K, Hazarika NC, Mahanta J (2005). Tobacco use and stomach cancer in Mizoram, India. *Cancer Epidemiol Biomarkers Prev*, **14**, 1892-6.

Pramanik S, Devi S, Chowdhary S, et al (2011). DNA repair gene polymorphisms at XRCC1, XRCC3, XPD, and OGG1 loci in Maharashtrian population of central India. *Chemosphere*, **82**, 941-6.

Qiao Y, Spitz MR, Shen H, et al (2002). Modulation of repair of ultraviolet damage in the host-cell reactivation assay by polymorphic XPC and XPD/ERCC2 genotypes. *Carcinogenesis*, **23**, 295-9.

Seow A, Poh W, The M, et al (2000). Fumes from meat cooking and lung cancer risk in Chinese women. *Cancer Epidemiol Biomarkers Prev*, **9**, 1215-21.

Shen MR, Jones IM, Mohrenweiser H (1998). Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer Res*, **58**, 604-8.

Sterpone S, Cozzi R (2010). Influence of XRCC1 genetic polymorphisms on ionizing radiation-induced DNA damage and repair. *J Nucleic Acids*, **10**, 1-6.

Sun Y, Zhang YJ, Kong XM (2013). No association of XRCC1 and CLPTM1L polymorphisms with non-small cell lung cancer in a non-smoking Han Chinese population. *Asian Pac J Cancer Prev*, **14**, 5171-4.

Tang L, Lim W, Eng P, et al (2010). Lung Cancer in Chinese Women: Evidence for an Interaction between Tobacco Smoking and Exposure to Inhalants in the Indoor Environment. *Environ Health Perspect*, **118**, 1257-60.

Vettriselvi V, Vijayalakshmi K, Paul SFD, et al (2007). XRCC1 and XPD Gene Polymorphisms in a South Indian Population. *Asian Pac J Cancer Prev*, **8**, 283-6.

Wang L, Chen Z, Wang Y, et al (2014). The association of c.1471G>A genetic polymorphism in XRCC1 gene with lung cancer susceptibility in Chinese Han population. *Tumour Biol*. [Epub ahead of print]

Yin Z, Zhou B, He Q, et al (2009). Association between polymorphisms in DNA repair genes and survival of non-smoking female patients with lung adenocarcinoma. *BMC Cancer*, **9**, 439.

Zheng W, Lee S (2009). Well-done Meat Intake, Heterocyclic Amine Exposure, and Cancer Risk. *Nutr Cancer*, **61**, 437-46.

Zhou M, Wan HY, Gao BL, Ding YJ, Jun RX (2012). Genetic polymorphisms of XPD and CDA and lung cancer risk. *Oncol Lett*, **4**, 247-51.