DOI: 10.7860/JCDR/2014/10061.5132

Original Article

Biochemistry Section

XRCC-1 Gene Polymorphism (Arg399Gln) and Susceptibility to Development of Lung Cancer in Cohort of North Indian Population: A Pilot Study

VIBHA UPPAL[1], MOHIT MEHNDIRATTA[2], DEBABRATTA MOHAPATRA[3], RAJESH K GROVER[4], DINESH PURI[5]

ABSTRACT

Background: Smoking has been considered to be the major cause of lung cancer. However, only a fraction of cigarette smokers develop this disease. This suggests the importance of genetic constitution in predicting the individual's susceptibility towards lung cancer. This genetic susceptibility may result from inherited polymorphisms in genes controlling carcinogen metabolism and repair of damaged deoxyribonucleic acid (DNA). These repair systems are fundamental to the maintenance of genomic integrity. X-ray repair cross complimenting group I (XRCC1), a major DNA repair gene in the base excision repair (BER) pathway. It is involved in repair by interacting with components of DNA at the site of damage. Inconsistent results have been reported regarding the associations between the Arg399Gln polymorphism of XRCC1.

This study demonstrates the importance of recognition of this relationship of lung carcinoma and genetic constitution of the person which will help guide clinicians on the optimal screening of this disease.

Aim: To assess the role of XRCC1 gene polymorphism (Arg399Gln) directly on the variation in susceptibility to development of lung cancer in North Indian subjects.

Materials and Methods: One hundred males with diagnosed cases of lung cancer were recruited from Delhi State Cancer Institute (DSCI). Hundred healthy volunteers were taken as controls. DNA isolation was done and Polymerase chain reaction–Restriction Fragment Length Polymorphism (PCR–RFLP) procedure undertaken to amplify the region containing Arg/Gln substitution at codon 399 (in exon 10).

Results: XRCC1 gene polymorphism is associated with increased risk of lung cancer when the Arg/Arg genotype was used as the reference group. The Arg/Gln and Gln/Gln was associated with statistically increased risk for cancer.

Conclusion: Arg399Gln polymorphism in XRCC1 gene polymorphism is associated with lung cancer in North Indian subjects and screening for this polymorphism will help in targeting predisposed individuals and its prevention.

Keywords: Base excision repair, Deoxyribonucleic acid repair, Polymorphism, Polymerase chain reaction–Restriction fragment length polymorphism

INTRODUCTION

Lung cancer has been considered as a disease caused solely by exposure to environmental carcinogens. Smoking, along with occupational exposure, is considered, to be the major cause of lung cancer [1]. All smokers do not develop lung cancer suggesting inter-individual differences in development of the disease. This is due to the differences in the DNA repair mechanism because of the genetic constitution of the person [2].

Tobacco smoke contains an array of potent chemical carcinogens and reactive oxygen species that produce DNA damage. In humans, >70 genes are involved in the five major DNA repair pathways: direct repair, base excision repair (BER), nucleotide excision repair (NER), mismatch repair and double-strand break (DSB) repair [3,4]. NER targets bulky, helix-distorting adducts, such as benzo(a) pyrene-guanine adduct, whereas BER removes small base adducts produced by oxidation, methylation, and radiation [4]. Among the several major DNA repair pathways that operate on specific types of damaged DNA by cigarette smoking, BER is involved in repair of DNA base damage and single-strand breaks (SSB) [5].

The NER pathway involves several genes termed Xeroderma Pigmentosum (XP) or Excision Repair Cross Complementing (ERCC) where XP, complementation group A (XPA), ERCC1, ERCC2/XPD, ERCC4/XPF and ERCC5/XPG are most important [6]. BER involves XRCC1, methylpurine glycosylase (MPG), polymerase B (POLB), ligase 3 (LIG3), exonuclease 1 (EXO1) and proliferating cell nuclear antigen (PCNA) genes [7].

Repair of DNA DSB involves homologous and non-homologous recombination repair pathways. These pathways include several proteins such as RAD51 (a eukaryote gene), ataxia telangiectasia mutated (ATM), ATM- and Rad3-related (ATR) and XRCC, which are important for maintenance of genomic stability [8]. Lung cancer patients have been found to have lower DNA repair capacity compared with healthy individuals. Molecular epidemiology studies have demonstrated that the variant DNA repair genotypes may alter susceptibility to lung cancer [9]. XRCC1, a major DNA repair gene in the BER pathway, acts as a scaffold of different activities involved in repair by interacting with components at the site of damage. Human XRCC1 is located on chromosome 19q13.2-13.3 [10] and two known polymorphisms at codon 280 (exon 9, base 27466 G to A, Arg to His) and codon 399 (exon 10, base 28152 G to A, Arg to Gln) leads to amino acid substitution [11].

XRCC1 has multiple roles in repairing DNA base damage and single-strand DNA breaks. XRCC1 acts as a facilitator or coordinator in BER, through its interaction with poly (ADP-ribose) polymerase, DNA polymerase β, and DNA ligase III [12-14]. Shen et al., identified three coding polymorphisms in the XRCC1 gene at the codon 194 (Arg to Trp), 280 (Arg to His), and 399 (Arg to Gln) [11].

Inconsistent results have been reported regarding the associations between the Arg399Gln polymorphism of XRCC1 and either functional significance or the risk of tobacco associated cancers. The Gln allele of this polymorphism was associated with higher levels of DNA adducts however, studies found no association between this polymorphism and elevated DNA adduct levels. Association

	Cases	Controls	p-value
Age (years) mean±SD	63.70 ± 3.91	62.08 ± 2.88	0.001*
Smokers : non smokers	76:24	56:44	0.003*
Male : female	64:36	76:24	0.064

[Table/Fig-1]: Demographic characteristics of cancer patients (cases) and controls **Significant*

Subjects	Genotype			p-value
	Arg/Arg	Arg/Gln	Gln/Gln	
Cases (n=100)	18	32	50	<0.001*
Controls (n=100)	12	65	23	

[Table/Fig-2]: XRCC genotypes distribution amongst cases and controls **Significant*

Subjects		Genotype			p-value
		Arg/Arg	Arg/Gln	Gln/Gln	
Cases (n=100)	Smokers (76)	9 (11.8%)	45 (59.2%)	22 (28.9%)	0.002*
	Non-Smokers (n=24)	9 (37.5%)	5 (20.8%)	10 (41.7%)	
Controls (n=100)	Smokers (56)	6 (10.7%)	14 (25%)	36 (64.3%)	0.686
	Non-Smokers (n=44)	6 (13.6%)	8 (18.2%)	30 (68.2%)	

[Table/Fig-3]: Association of polymorphism with smoking, **Significant*

between XRCC1 variants in DNA repair gene and cancer risk was reported along with the fact that amino acid substitution (Arg399Gln) may alter the phenotype of the XRCC1 protein resulting in deficient DNA repair capacity leading to risk in Head and Neck cancer, but this relationship is complex [11,15]. A study by Vogel et al., reported no association of XRCC1 gene Arg399Gln polymorphism in lung cancer risk [16].

This study demonstrates the importance of recognition of this relationship of lung carcinoma and genetic constitution of the person which will help guide clinicians on the optimal screening of this disease. Three coding polymorphisms at conserved sites have been reported in the XRCC1 gene. In this study, we focused on the codon 399 polymorphism.

MATERIALS AND METHODS

A bi-institutional pilot study was conducted at University College of Medical Sciences (UCMS), Guru Teg Bahadur (GTB) Hospital, and Delhi State Cancer Institute (DSCI), Delhi, India. A total of 100 primary lung cancer patients were recruited in the study. These patients were pathologically confirmed and were under treatment by the oncologists at DSCI. Patients with primary cancer of other sites apart from lung were not included. Equal number of control subjects (n=100) were enrolled. The smoking status of all the subjects were noted. The study was approved by the Institutional Ethics Committee, UCMS. Informed written consent was taken from all subjects.

Specimen collection: Two ml of venous blood was drawn from each subject. It was dispensed into EDTA vial and was used for genetic analysis.

DNA isolation: DNA isolation was done on the same day of collection of blood sample. It was done using a DNA extraction kit (Zymo research) as per the manufacturer's protocol. Briefly, genomic lysis buffer was added to the samples, vortexed and mixture transferred to the Zymo spin column. Using the fast spin technology purified DNA was eluted.

PCR procedure: The region containing Arg→Gln substitution at codon 399 (in exon 10) was amplified by PCR to obtain an undigested fragment of 242 bp using the forward primer 5'-CCCCAAGTACAGCCAGGTC-3' and the reverse primer 5'-TGTCCCGCTCCTCTCAGTAG-3' [17].

RFLP procedure: The PCR product was digested by MspI (….C↓CGG….) at 37°C for 1 hour according to the protocol provided by the manufacturer (Bangalore Genei). It was resolved on 2% agarose using horizontal gel electrophoresis and staining with ethidium bromide. Arg/Arg genotype was digested to form 94 and 148 bp fragments while the Gln/Gln missed the MspI restriction site producing only one band. Heterozygous genotypes gave three bands.

Genotype	Crude OR (95% CI)	Adjusted OR
Arg/Arg	1.610 (0.731– 3.547), p=0.238	2.001 (0.858-4.670), p=0.109
Arg/Gln	0.242 (0.134-0.437), p<0.001*	0.276(0.150-0.507), p<0.001*
Gln/Gln	3.545 (1.918–6.554), p<0.001*	2.862 (1.504-5.447), p=0.001*

[Table/Fig-4]: Logistic regression analysis of XRCC1 codon 399 polymorphism in lung cancer., *OR: adjusted for Age and Smoking; *Significant*

STATISTICAL ANALYSIS

Statistical testing was conducted with the statistical package for the social science system version SPSS 17.0. Results are expressed as mean ± SD, or numbers and percentages. The comparison of normally distributed continuous variables between the groups was performed using Student's t- test. Nominal categorical data between the groups were compared using Chi-square test or Fisher's exact test as appropriate. For all statistical tests, a p-value less than 0.05 was taken to indicate a significant difference.

RESULTS

The details of cases and controls enrolled in this study are shown in [Table/Fig-1]. The mean age was significantly more in cases. Age factor was significant (p=0.001) with respect to the presence of disease, cases were more in older age group. There were more males in case population as compared to females. Cases showed a higher prevalence of smoking as compared to controls. The distribution of XRCC1 genotype among control and cases are shown in [Table/Fig-2]. The Gln allele is significantly present in cancer cases. There was a positive association of smoking with the polymorphism as shown in [Table/Fig-3]. [Table/Fig-4] shows the crude and adjusted OR for lung cancer by XRCC1 genotype. When the Arg/Arg genotype was used as the reference groups the Arg / Gln and Gln/ Gln was associated with statistically increased risk for cancer. The adjusted odds ratio is used to remove the effect of age and smoking.

DISCUSSION

There is a complex gene environment interaction leading to lung cancer. In the present study among the 100 lung cancer subjects investigated, Arg399Gln polymorphism in XRCC1 gene is associated with lung cancer.

All our cases were pathologically and clinically confirmed. The main limitations of our study were hospital-based subjects, recall bias due to the fact that information on smoking exposure was obtained retrospectively, and especially possible false positive associations, due to multiple comparisons made. To limit selection bias, we carefully selected controls from patients admitted for various diagnoses that were thought to be unrelated to exposures of interest.

DNA repair pathways play a vital role in maintaining genetic integrity and it is becoming clear that defects in repair pathways are connected to many different types of diseases including cancers. DNA repair systems maintain genomic integrity, in the face of environmental insults [18].

The XRCC1 protein is considered to play an important role in DNA damage repair. It encodes a multi-domain protein that interacts with nicked DNA. This is involved in SSB and BER pathway. Different XRCC1 mutations have been shown to disrupt the function of the protein by affecting its binding to the substrate or by introducing changes in the catalytic domain [19].

XRCC1 Arg194Trp and Arg399Gln polymorphisms were the commonest one among more than 60 validated SNPs in XRCC1 gene and showed no major variations by ethnicity [20]. This nucleotide polymorphism (SNP; c.1316G>A; p.Arg399Gln; rs25487), is located near the breast cancer C-terminal domain (BRCT) which is involved in cell cycle checkpoint functions. This BRCT domain interacts with the poly-ADP-ribose polymerase binding domain of Poly(ADP-ribose) polymerase-1, which is involved in the BER pathway [21] BRCT has the potential of detecting the DNA damage and hence activating the BER pathway [22].

Three coding polymorphisms at conserved sites have been reported in the XRCC1 gene [14]. In this study, we focused on the codon 399 polymorphism because two other polymorphisms (codons 194 and 280) reside in functionally insignificant regions [14,16-18]. These results are consistent with previous studies that the Gln/Gln genotype is the risk genotype for various smoking related cancers [23-25]. Divine et al., reported that the Gln/Gln genotype was associated with an increased risk of adenocarcinoma [23]. Butkiewicz et al., found no association of lung cancer with this polymorphism in the Polish population [26]. The different results in different populations may be because of genetic and environmental differences [15].

Genetic susceptibility to lung cancer may depend on the level of exposure to tobacco smoke [27]. In our study we found a positive association with Gln/Gln and Arg/Gln genotype with lung cancer where $p<0.05$. Previous studies conducted in south Indian population by Kiran et al., also supports our data where genotyping of Gln/Gln and Arg/Gln have shown a high risk in cancer cases [28].

Cigarette smoke contains large quantities of carcinogens, including polycyclic aromatic hydrocarbons, such as benzo(a) -pyrene, which damage DNA by covalent binding or oxidation, following activation in vivo into benzo(a)pyrene-diol epoxide [29]. Although extensive prospective epidemiologic data have clearly established cigarette smoking as the major cause of lung cancer, only a fraction of cigarette smokers develop smoking-related lung cancer [30]. So in the present study the susceptibility to lung carcinoma could be attributed to genetic causes which synergistically act along with smoking leading to variation in carcinogen metabolism and/or in the capacity of DNA repair, which is essential in protecting the genome of cells.

The 399Gln polymorphism resulting from a guanine to adenine nucleotide occurs in the poly (ADP-ribose) polymerase binding domain and affects complex assembly or repair efficiency [11]. Lunn reported that the 399Gln allele was significantly associated with higher levels of aflatoxin B1-DNA adducts and glycophorin A somatic mutations [31]. Duell reported that sister chromatid exchange frequencies were higher in carriers of the 399Gln allele than in homozygous carriers of the 399Arg allele [17]. These studies suggest that individuals with the 399Gln allele are less able to repair DNA damage. In our study also the Gln/Gln polymorphism was predominant followed by Gln/Arg in the lung cancer patients.

It is for the first time that we have shown that Arg399Gln polymorphism in XRCC1 gene is associated with lung cancer in North Indian subjects, but due to small sample size more studies need to be conducted. It is possible that polymorphisms of other genes not evaluated in this study could play a role in lung cancer risk, but evaluation of more polymorphisms would require larger sample sizes. Although the exact biological mechanisms for the gene-environment (smoking) interaction related to the XRCC1 phenotypes as consequences of these polymorphisms could not be clarified, this study did provide important additional evidence of gene-environment interactions between XRCC1 polymorphisms and smoking.

CONCLUSION

XRCC1 Arg399Gln polymorphisms appear to play an important role in modifying the direction and magnitude of the association between cigarette smoking exposure and lung cancer risk. XRCC1 polymorphism might prove to be promising predictive or prognostic marker for lung cancer patients.

ACKNOWLEDGEMENTS

We are grateful to the Oncologists for their cooperation and help in selecting our study group and for permitting us to collect their medical histories. We are thankful to all the patients for their cooperation and participation in this study. We thank Dr. Puja Post-graduate student, Department of Biochemistry, UCMS, Delhi for sample collection.

REFERENCES

[1] Khuder SA. Effect of cigarette smoking on major histological types of lung cancer: A meta-analysis. *Lung Cancer.* 2001;31(2-3):139-48.
[2] Spitz MR, Wei Q, Dong Q, Amos CI, Wu X. Genetic susceptibility to lung cancer: The role of DNA damage and repair. *Cancer Epidemiol Biomarkers Prev.* 2003;12:689-98.
[3] Sancar A. DNA excision repair. *Annu Rev Biochem.* 1996;65:43-81.
[4] Yu Z, Chen J, Ford BN, Brackley ME, Glickman BW. Human DNA repair systems: An overview. *Environ Mol Mutagen.* 1999;33(1):3-20.
[5] Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: An epidemiologic review. *J Natl Cancer Inst*. 2000;92(11):874-97.
[6] Hanawalt PC, Ford JM, Lloyd DR. Functional characterization of global genomic DNA repair and its implications for cancer. *Mutat Res.* 2003;544(2-3):107-14.
[7] Barnes DE, Lindahl T. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annu Rev Genet.* 2004; 38:445-76.
[8] Thacker J, Zdzienicka MZ. The XRCC genes: expanding roles in DNA double-strand break repair. *DNA Repair (Amst.).* 2004;3(8-9):1081-90.
[9] Hung RJ, Brennan P, Canzian F, Szeszenia-Dabrowska N, Zaridze D, Lissowska J, et al. Large-scale investigation of base excision repair genetic polymorphisms and lung cancer risk in a multicenter study. *J Natl Cancer Inst.* 2005;97:567-76.
[10] Thompson LH, Bachinski LL, Stallings RL, Dolf G, Weber CA, Westerveld, A et al. Complementation of repair gene mutations on the hemizygous chromosome 9 in CHO: A third repair gene on human chromosome 19. *Genomics.* 1989;5(4):670-79.
[11] Shen MR, Jones IM, Mohrenweiser H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer Res.* 1998;58: 604-08.
[12] Kubota Y, Nash RA, Klungland A, Schar P, Barnes DE, Lindahl T. Reconstitution of DNA base excision-repair with purified human proteins: Interaction between DNA polymerase beta and XRCC1 protein. *EMBO J.* 1996;15:6662-70.
[13] Caldecott KW, McKeown CK, Tucker JD, Ljungquist S, Thompson LH. An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III. *Mol Cell Biol.* 1994;14:68-76.
[14] Masson M, Niedergang C, Schreiber V, Muller S, Menissier-de Murcia J, de Murcia, G. XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. *Mol Cell Biol.* 1998;18(6):3563-71.
[15] Hu Z, Ma H, Chen F,Wei Q, Shen H. XRCC1 polymorphism and cancer risk: A meta analysis of 38 case-control studies. *Cancer Epidemiol Biomarkers Prev.* 2005;14(7):1810-18.
[16] Vogel U, Nexo BA, Wallin H, Overvad K, Tjonneland A, Raaschou-Nielsen O. No association between base excision repair gene polymorphisms and risk of lung cancer. *Biochem Genet.* 2004;42(11-12):453-60.
[17] Duell EJ, Wiencke, JK, Cheng TJ, Varkonyi A, Zuo ZF, Ashok TDS, et al . Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. *Carcinogenesis.* 2000;21(5):965-71.
[18] Ratnasinghe D, Yao SX, Tangrea JA, Qiao YL, Andersen MR, Barrett MJ, et al. Polymorphisms of the DNA repair gene XRCC1 and lung cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2001;10(2):119-23.
[19] Caldecott KW. XRCC1 and DNA strand break repair. *DNA Repair (Amst.)*. 2003; 2:955-69.
[20] Hung RJ, Hall J, Brennan P, Boffetta P: Genetic polymorphisms in the base excision repair pathway and cancer risk: A HuGE review. *Am J Epidemiol.* 2005;162:925-42.
[21] Ladiges W, Wiley J, MacAuley A. Polymorphisms in the DNA repair gene XRCC1 and age-related disease. *Mech Ageing Dev*. 2003;124:27-32.
[22] Matullo G, Peluso M, Polidoro S, Guarrera S, Munnia A, Krogh V, et al. Combination of DNA repair gene single nucleotide polymorphisms and increased levels of DNA adducts in a population-based study. *Cancer Epidemiol Biomarkers Prev.* 2003;12:674-77.
[23] Divine KK, Gilliland ED, Crowell RE, Stidley CA, Bocklage TJ, Cook DL, Belinsky SA. The XRCC1 399 glutamine allele is a risk factor for adenocarcinoma of the lung. *Mutat Res.* 2001; 461(4): 273-78.

[24] Abdel-Rahman SZ, El-Zein RA. The 399Gln polymorphism in the DNA repair gene XRCC1 modulates the genotoxic response induced in human lymphocytes by the tobacco-specific nitrosamine NNK. *Cancer Lett.* 2000;159(1): 63-71.
[25] Sturgis EM, Castillo EJ, Lie L, Zheng R, Eicher SA, Clayman GL, et al. Polymorphisms of DNA repair gene XRCC1 in squamous cell carcinoma of the head and neck. *Carcinogenesis*. 1999;20(11): 2125-29.
[26] Butkiewicz D, Rusin M, Enewold L, Shields PG, Chora M, and Harris CC. Genetic polymorphisms in DNA repair genes and risk of lung cancer. *Carcinogenesis.* 2001;22: 593-97.
[27] Shen H, Xu Y, Qian Y, Yu R, Qin Y, Zhou L, et al. Polymorphisms of the DNA repair gene XRCC1 and risk of gastric cancer in a Chinese population. I*nt J Cancer.* 2000;88(4):601-06.
[28] Kiran M, Saxena R, Kaur J. Distribution of XRCC1 genotypes in north Indian population. *Indian J Med Res.* 2010;131:71-75.
[29] Hecht SS. Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst.* 1999;91:1194-1210.
[30] Mattson ME, Pollack ES, Cullen JW. What are the odds that smoking will kill you? *Am J Public Health.* 1987;77:425-31.
[31] Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA. XRCC1 polymorphisms: Effects on aflatoxin B-DNA adducts and glycophorin A variant frequency. *Cancer Res*. 1999;59: 2557-61.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, LHMC & Smt Sucheta Kriplani Hospital, New Delhi, India.
2. Assistant Professor, Department of Biochemistry, University College of Medical Sciences, New Delhi, India.
3. Intern, University College of Medical Sciences, New Delhi, India.
4. Director & CEO, Delhi State Cancer Institute, New Delhi, India.
5. Professor and Head, Department of Biochemistry, University College of Medical Sciences, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Vibha Uppal,
House No.25, Road No.7, East Punjabi Bagh, New Delhi-110026, India.
Phone : 09818773606, E-mail : vuppal_girotra@yahoo.co.in

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **May 05, 2014**
Date of Peer Review: **Jul 07, 2014**
Date of Acceptance: **Sep 09, 2014**
Date of Publishing: **Nov 20, 2014**