INTERNATIONAL JOURNAL OF MEDICAL AND APPLIED SCIENCES
Earthjournals Publisher
E-ISSN:2320-3137
www.earthjournals.org

RESEARCH ARTICLE

THE ASSOCIATIONS OF DNA REPAIR GENE POLYMORPHISMS WITH CARCINOMA PROSTATE

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Abstract:

Objective: The aim of the study is to determine the risk of carcinoma (ca) prostate due to polymorphisms in genes regulating the DNA Repair pathway , the X ray repair Cross complementary 1 gene (XRCC1) and Xeroderma pigmentosum group D gene (XPD). Methods: Fifty patients (25 ca prostate patients and 25controls) were included in the study, conducted between November 2008 and March 2011. XRCC1 and XPD gene were isolated from blood, processed, studied under UV transillumination and genotypes were identified. The association with risk of carcinoma prostate was determined by calculating the Odds Ratio. Any possible effect modification by age, gleason score and serum PSA was also evaluated. Results: For XRCC1 gene, the Arg/Gln genotype was seen in 60% of carcinoma prostate patients compared to Arg/Arg (16%) and Gln/Gln (24%). The Arg/Gln genotype was found to have statistically significant association with carcinoma prostate ($p<0.05$) when comparing with the genotype of controls. For XPD gene, the Lys/Gln genotype was seen in 52% of ca prostate patients compared to Lys/Lys (36%) and Gln/Gln (12%) . The Lys / Gln genotype was found to have statistically significant association with Prostate cancer ($p<0.05$). No significant association was observed between the genotypes when stratified by age, gleason score and serum PSA. Conclusions: The XRCC 1 Arg/Gln genotype and XPD Lys/Gln genotype were significantly associated with an increased risk of developing Prostate cancer. The present study would enable identification of genetically predisposed individuals.
Key-words: DNA repair, Carcinoma prostate, Gene polymorphisms

INTRODUCTION

Prostate cancer is the most common noncutaneous malignancy in men in the United States. In India, the incidence is relatively less. Risk of disease varies most prominently with age, ethnicity, family history, and diet. A familial pattern account for only 5–10% of prostate cancers, whereas a larger percentage of prostate cancers may be due to common polymorphisms in genes giving rise to a low penetrance risk of disease [1-3.] Malignant transformation of prostate cells is accompanied by somatic genomic changes[4, 5.]

In vitro studies of human prostate tissue have demonstrated that DNA adducts form in prostate tissue, after exposure to environmental toxins [6, 7.] Moreover, intake of antioxidants via the diet or

as supplements may decrease prostate cancer risk through the inactivation of reactive oxygen species, thereby protecting the DNA from oxidative damage [8].

This evidence suggests that DNA repair capacity may play an important role in prostate carcinogenesis, but little is known about what direct effect DNA repair capacity has on prostate cancer risk.

XRCC1gene is involved in DNA repair in the base excision repair pathway and appears to play a scaffolding role in bringing together a complex of DNA repair proteins, including poly (ADP-ribose) polymerase (PARP), DNA ligase 3 (LIG3), and DNA polymerase [9 –11]. Codons 194 and 399 contain polymorphisms that result in amino acid substitutions within evolutionarily conserved regions [12, 13]. Several studies have linked XRCC1 polymorphisms with biomarkers of DNA damage, including aflatoxin B1-DNA adducts and glycophorin A variants in erythrocytes [14], polyphenol-DNA adducts[15], and DNA repair capacity in lymphocyte[16].

The XPD gene codes for a DNA helicase involved in transcription and nucleotide excision repair. Mutations in the XPD gene can completely prevent DNA opening and dual incision, steps that lead to the repair of DNA adducts[17]. The DNA repair function of XPD is critical to reparation of genetic damage from tobacco and other carcinogens [18]. Several common single bp substitution polymorphisms in the XPD gene have been identified.

SUBJECTS AND METHODS:

Study population : 50 patients (seen in Outpatient Department or those admitted in Urology ward)

Nature of Study : Retrospective study

No. of Ca.Prostate cases: 25

No. of Controls : 25, (BPH: 19, Age matched controls: 6]

Study Period : November 2008 to March 2011

The age of the subjects included in the study ranged from 55 to 87 years. The cases and controls were similar in ethnicity and nutritional status. Clinical characteristics, including Gleason score, PSA, and tumor stage were obtained. The controls were unrelated individuals with normal serum PSA and normal digital rectal examination .

Outline of methodology:

DNA was isolated from the blood sample obtained. Then exon 10 of XRCC1 gene and exon 23 of XPD gene are amplified using polymerase chain reaction (PCR) and then purified. Then they were made to react with enzymes Msp1 for XRCC1 and MboII for XPD gene and agarose gel electrophoresis was done. Then they were visualized under ultraviolet (uv) illumination. The genotypes were studied based on RFLP polymorphisms.

METHODOLOGY:

Blood sample collection:

5 ml of peripheral blood was obtained by direct venipuncture and transferred into EDTA containing vacutainer tubes, which were kept in thermos ice box and transferred immediately to the Department of Endocrinology, IBMS, Taramani. The buffy coat was separated and stored at –20°C until isolation of dna was done.

DNA isolation and purification:

The phenol chloroform method of DNA isolation was used in this study. This frequently used method for DNA isolation removes proteins and other cellular components from nucleic acids, resulting in relatively pure DNA preparations. The concept of isolation of DNA is that all the other components of the cell and chromatin are removed using suitable methods to leave behind the DNA. In general the isolation of DNA from mammalian tissues follow four different steps they were 1.lysis of cells with a detergent like sodium dodecyl sulphate (SDS), 2. Digestion of proteins with enzymes (Proteinase – K), 3. Extraction of DNA by phenol chloroform method, 4. Precipitation of DNA with isopropyl alcohol or 100% ethanol.

Quality check & quantification of DNA:

The integrity of the DNA was assessed by running it in 0.7% Agarose gel. Further the quantification and quality check of DNA was performed by subjecting the DNA to spectrophotometry. The concept of quality check of DNA is to find out the purity of the extracted DNA. The extracted DNA may contain impurities like phenol, proteins and others. The integrity of the DNA is checked by agarose gel electrophoresis. The DNA is mixed with loading dye and run electrophoretically on 0.7% agarose gel in TAE buffer, the high molecular weight DNA appeared as sharp band without smearing.

PCR amplification:

The primers were commercially procured. The primers from XPD exon 23 are forward (5’-CAGGTGAGGGGGACATCTGG-3’) and reverse (5’-CTCTCCCTTTCCTCTGTTCTCTGC-3’).The primers from XRCC1 exon 10 are forward (5'- GCTTTCTCTGTGTCCACTATGCTGC-3') and reverse (5'-TCTGATAAGCAGGCTTCACAGAGCC-3'). The primers were mixed with the isolated DNA and other reaction elements and subjected to a thermal cycle. After the completion of the thermal cycles, the PCR product was resolved on 2% Agarose gel in Mupid-ex electrophoresis tank (TAKARA, Japan), and the amplification was visualized and documented using UVP- UV Trans-illuminator. Then this mix was subjected to restriction fragment length polymorphism.

RFLP **(**restriction fragment length polymorphism):

The PCR Fragment was digested by the enzyme MboII , which digests the 733 bp fragment of XPD Exon 10 and enzyme Msp I which digests the PCR fragment of XRCC 1.

RFLP reaction was done under ideal conditions of 37°C- 16 hours/Overnight, 4°C- ∞. After the completion of the restriction digestion, 20 µl of the digest product and 2 µl of 6X DNA gel loading dye was mixed well and resolved by electrophoresis in Mupid-ex electrophoresis tank (TAKARA, Japan) in 2.0% agarose gel. The resolved gel was visualized using UVP- UV Trans-illuminator and studied for the genotype.

RESULTS:

Statistical analysis:

Statistical analysis was done using the SPSS Software. The association between XRCC1 and XPD Polymorphisms and risk of ca prostate was determined by calculating the Odds Ratio (OR) at 95% Confidence interval(CI). Any possible effect modification by age was also evaluated by stratifying the age at diagnosis as (<65 *versus* >65). In addition, to investigate the potential effect of genotype on disease aggressiveness, stratified the analyses of the cases' clinical characteristics at diagnosis was done. The cancer risk was also analysed by stratifying the patients based on Gleason score and Sr.PSA levels.

Table-1: Association of XRCC 1 genotype and prostate cancer

Genotype	Cases (%) N- 25	Controls(%) N – 25	OR (95% CI)	P
Arg/Arg	4 (16%)	10 (40%)	0.29 (0.06 – 1.27)	0.115
Arg/Gln	15(60%)	7 (28%)	3.86 (1.02 – 15.17)	0.046
Gln/Gln	6 (24%)	8 (32%)	0.67 (0.16 - 2.74)	0.753

Arg- arginine, Gln- glycine, OR- odds ratio, CI- confidence interval.

Table- 1: The Arg/Gln genotype was the predominant genotype and was found to have statistically significant association with ca prostate ($p<0.05$) when comparing with the genotype of controls

The Association of XRCC 1 genotype and Prostate cancer was assessed in 25 Prostate cancer patients. 4 (16%) were Arg/Arg, 15 (60%) were Arg/Gln and 6 (24%) were Gln/Gln. Among the 25 controls, 10 (40%) were Arg/Arg, 7(28%) were Arg/Gln, 8 (32%) were Gln/Gln.

With Arg/Arg as the reference genotype , the Odds Ratio (95% CI) of the Homozygosity and heterozygosity of the Gln allele was assessed. It was found that the Arg/ Gln genotype was found to have statistically significant association with Prostate cancer ($p<0.05$). The Arg/Arg genotype and Gln/Gln genotype did not have a significant association with Prostate cancer.

Figure-1: XRCC 1 genotype among prostate cancer patients and controls stratified by age

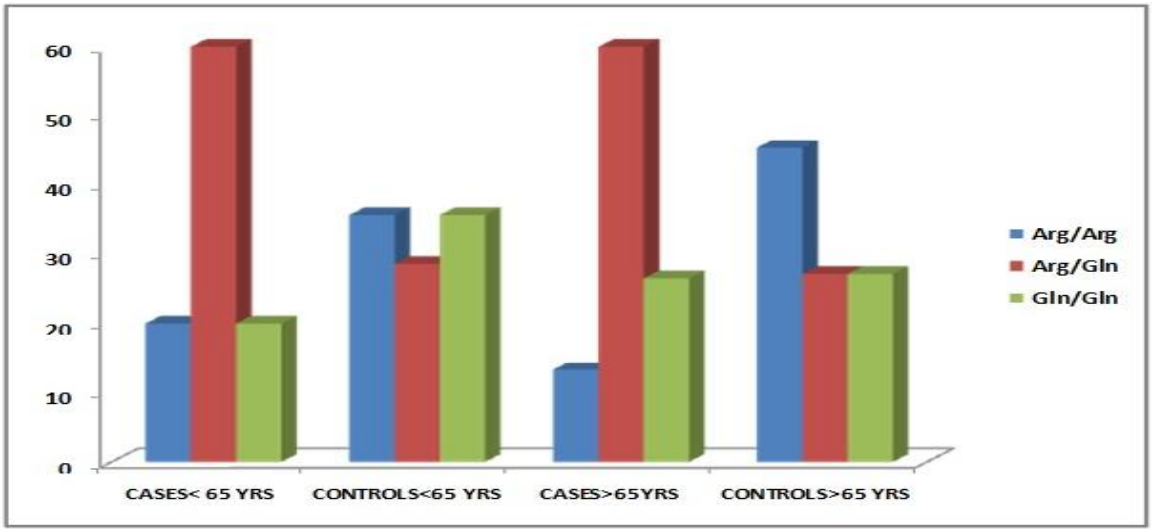

Figure-1: No significant association was observed between the XRCC 1 genotype stratified by age and risk of Prostate cancer

The association of XRCC 1 genotype with Prostate cancer patients based on the age was calculated. Among the subjects less than 65 yrs, Arg/Arg was observed in 20% and 35.7%, Arg/Gln observed in 60% and 28.6%, Gln/Gln observed in 20 % and 35.7% of cases and controls respectively. Among the subjects more than 65 yrs, Arg/Arg was observed in 13.3% and 45.4%, Arg/Gln in 60% and 27.2% , Gln/Gln in 26.6% and 27.2% of cases and controls respectively. No significant association was observed between the genotypes stratified by age and risk of Prostate cancer.

Table - 2: Association of XRCC 1 gene with gleason score in patients

Genotype	Patients(%)		OR	95% CI	P
	Gleason Score≥7 N- 12	Gleason Score<7 N-13			
Arg/Arg	2(16.6)	2(15.3)	1.10	0.09 – 14.18	1.000
Arg/Gln	8(66.6)	7(53.8)	1.71	0.26 – 11.92	0.688
Gln/Gln	2(16.6)	4(30.7)	0.45	0.04 – 4.10	0.645

Arg- arginine, Gln- glycine, OR- odds ratio, CI- confidence interval.

Table-2: No significant association was observed between the genotypes when stratified by gleason score

The XRCC 1 genotype distribution based on the Gleason score was assessed. Arg/Arg genotype was seen in 16.6% and 15.3% , Arg/Gln genotype in 66.6% and 53.8%, Gln/Gln in 16.6% and 30.7% of patients with Gleason score less than 7 and more than 7 respectively. No significant association between the genotypes and Histologic grade of Prostate cancer. Statistical assessment is limited by the small number of patients in each group.

Distribution of XRCC 1 genotype in patients based on PSA levels:

The XRCC 1 Genotype association with Prostate cancer based on the Sr.PSA levels were assessed. Arg/ Arg genotype was observed in 20% and 13.3 % , Arg/Gln in 60% and 60% , Gln/Gln in 20% and 26.6% of patients with Sr. PSA levels of more than 50 ng/ml and less than 50 ng/ml respectively. No significant association was observed between the genotypes stratified by Serum PSA levels and Carcinoma Prostate.

Table- 3: Association of XPD genotype and prostate cancer

Genotype	Cases (%) N- 25	Controls(%) N - 25	OR (95% CI)	P
Lys/Lys	9 (36%)	12 (48%)	0.61(0.17 – 2.19)	0.567
Lys/Gln	13(52%)	5 (20%)	4.33 (1.06 – 18.63)	0.039
Gln/Gln	3(12%)	8(32%)	0.29 (0.05 – 1.49)	0.172

Lys- lysine, Gln- glycine, OR- odds ratio, CI- confidence interval

Table-3: The Lys / Gln genotype was the predominant genotype and was found to have statistically significant association with Prostate cancer ($p<0.05$).

The Association of XPD genotype and Prostate cancer was assessed in 25 Prostate cancer patients . 9 (36%) were Lys/Lys , 13(52%) were Lys/Gln and 3 (12%) were Gln/Gln. Among the 25 controls, 12 (48%) were Lys/Lys , 5(20%) were Lys/Gln , 8 (32%) were Gln/Gln.With Lys/Lys as the reference genotype , the Odds Ratio (95% CI) of the Homozygosity and heterozygosity of the Gln allele was assessed .It was found that the Lys / Gln genotype was found to have statistically significant association with Prostate cancer ($p<0.05$). The Lys/Lys genotype and Gln/Gln genotype did not have a significant association with Prostate cancer.

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E-ISSN:2320-3137
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Figure-2: XPD genotype among prostate cancer patients and controls stratified by age

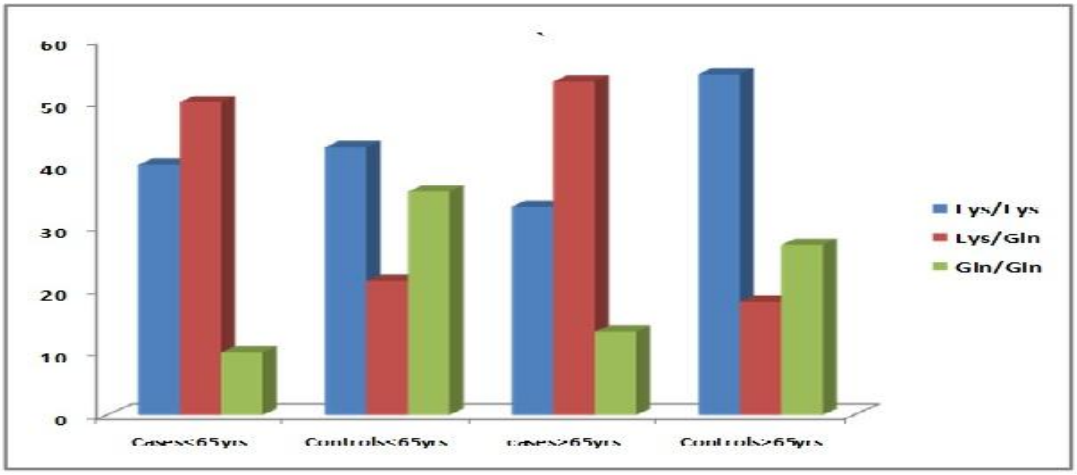

Figure-2: No significant association was observed between the XPD genotype stratified by age and risk of Prostate cancer

The association of XPD genotype with Prostate cancer patients based on the age was calculated. Among the subjects less than 65 yrs, Lys/Lys was observed in 40% and 42.8 % , Lys/Gln observed in 50% and 21.4% , Gln/Gln observed in 10 % and 35.7% of cases and controls respectively. Among the subjects more than 65 yrs , Lys/Lys was observed in 33.3% and 54.5% , Lys/Gln in 53.3% and 18.1% , Gln/Gln in 13.3% and 27.2% of cases and controls respectively. No significant association was observed between the genotypes stratified by age and risk of Prostate cancer.

Table – 4: Association of XPD gene and gleason score

Genotype	Patients(%)		OR	95% CI	P
	Gleason Score≥7 N- 12	Gleason Score<7 N-13			
Lys/Lys	5(41.6)	4(30.7)	1.61	0.23 – 11.45	0.688
Lys/Gln	6(50)	7(53.8)	0.86	0.13 – 5.47	0.835
Gln/Gln	1(8.3)	2(15.3)	0.50	0.02 – 8.95	1.000

Lys- lysine, Gln- glycine, OR- odds ratio, CI- confidence interval

Table-4: No significant association was observed between the genotypes when stratified by gleason score

The XPD genotype distribution based on the Gleason score was assessed. Lys/Lys genotype was seen in 41.6% and 30.7% , Ly/Gln genotype in 50% and 53.8% , Gln/Gln in 8.3% and 15.3% of patients with Gleason score less than 7 and more than 7 respectively. There was no significant association between the genotypes and Histologic grade of Prostate cancer. Statistical assessment is limited by the small number of patients in each group.

Distribution of XPD genotype in patients based on PSA levels:

The XPD Genotype association with Prostate cancer based on theSr.PSA levels were assessed. Lys/Lys genotype was observed in 40% and 33.3 % , Lys/Gln in 50% and 53.3% , Gln/Gln in 10% and 13.3% of patients with Sr. PSA levels of more than 50 ng/ml and less than 50 ng/ml respectively. No significant association was observed between the genotypes stratified by Serum PSA levels and Carcinoma Prostate.

DISCUSSION:

The Arg/ Gln genotype in XRCC1 and Lys/Gln genotype in XPD were found in 60 % and 52 % of Ca.Prostate patients and found to be statistically significant with the risk of Prostate cancer. All the subjects included in our study were unrelated. The other genotypes did not show a significant risk of developing Ca.Prostate.

The genotype association with Ca.Prostate based on the age, Gleason score and Sr.PSA was not found to be statistically significant. The statistical assessment is limited by the small number of patients .

Rybicki et al examined the XRCC1 codon 399 and XPD codons 312 and 751 polymorphisms in relation to prostate cancer risk in a large sample of primarily Caucasian sibships. Only the XPD codon 312 Asn allele showed a modest association with increased prostate cancer risk, 60%, when two copies of the allele were present. Perhaps more revealing, however, was the potential interaction between the XPD codon 312 Asn allele and the XRCC1 codon 399 Gln allele. When both alleles were present in their homozygous states, the risk for prostate cancer increased 4.8-fold.

Only a few studies of more common DNA repair genetic variants and prostate cancer risk exist in the literature. Xu et al. studied 18 different genetic variants of the DNA repair enzyme gene hOGG1, involved in base excision repair, and found the genotype frequency of two sequence variants (11657A/G and Ser326Cys) was significantly different between prostate cancer cases and controls. They also confirmed the association with the 11657A/G variant in a family-based association study. Van Gils et al 50 studied three genetic variants in another base excision repair enzyme gene, XRCC1, and found no association between XRCC1 polymorphisms and prostate cancer when only comparing genotype frequencies in cases and controls. However, when they stratified the study population by intake of several different dietary antioxidants, the more common XRCC1 codon 399 Arg/Arg genotype was associated with prostate cancer in those with low vitamin E or lycopene intake.

Rybicki et al , on the other hand, found the less common XRCC1 codon 399 Gln/Gln genotype to be a potential modifying factor for prostate cancer risk associated with the XPD codon 312 Asn/ Asn genotype. It is not inconceivable that interactions at the XRCC1 codon 399 locus are dependent on genotype, with some genetic or environmental risk factors preferentially interacting with the Arg/Arg genotype and others more likely to interact with the Gln/Gln genotype. The unadjusted ORs for the XRCC1 codon 399 Gln/Gln genotype in the study of van Gils et al. was 0.77, compared with Rybicki et al OR estimate of 0.88. Previous studies of the XRCC1 codon 399 polymorphism are equivocal with some finding increased risk for the Gln allele but others finding an increased risk for the Arg allele . Rybicki et al family-based study had several strengths, which include the size of the study population, the elimination of potential bias due to population genetic substructure, and full utilization of sibship data (without parental genotypes) that were composed of numerous configurations including sibships with only affected brothers.

CONCLUSION:

The XRCC 1 Arg/Gln genotype and XPD Lys/Gln genotype were significantly associated with an increased risk of developing Prostate cancer. The XRCC 1 and XPD genotypes stratified by age, grade and Sr.PSA levels did not show any significant risk of developing Prostate cancer. The present study of DNA Repair gene polymorphisms predicts risk of developing Prostate cancer and would enable identification of genetically predisposed individuals

REFERENCES

1. Carter, B. S., Beaty, T. H., Steinberg, G. D., Childs, B., and Walsh, P. C. Mendelian inheritance of familial prostate cancer. Proc. Natl. Acad. Sci. USA,1992; *89:* 3367–3371
2. Coughlin, S. S., and Hall, I. J. A review of genetic polymorphisms and prostate cancer risk. Ann. Epidemiol. 2002; *12:* 182–196.
3. Nwosu, V., Carpten, J., Trent, J. M., and Sheridan, R. Heterogeneity of genetic alterations in prostate cancer: evidence of the complex nature of the disease. Hum. Mol. Genet.2001; *10:* 2313–2318.
4. Bova, G. S., and Isaacs, W. B. Review of allelic loss and gain in prostate cancer. World J. Urol.1996; *14:* 338–346.
5. Dong, J. T., Suzuki, H., Pin, S. S., Bova, G. S., Schalken, J. A., Isaacs, W. B., Barrett, J. C., and Isaacs, J. T. Down-regulation of the KAI1 metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. Cancer Res.1996; *56:* 4387–4390.
6. Wang, C. Y., Debiec-Rychter, M., Schut, H. A., Morse, P., Jones, R. F., Archer, C., King, C. M., and Haas, G. P. N-Acetyltransferase expression and DNA binding of N-hydroxyheterocyclic amines in human prostate epithelium. Carcinogenesis (Lond.).1999; *20:* 1591–1595.
7. Nelson, C. P., Kidd, L. C., Sauvageot, J., Isaacs, W. B., De Marzo, A. M., Groopman, J. D., Nelson, W. G., and Kensler, T. W. Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione *S*-transferase P1. Cancer Res.2001;*61:* 103–109.
8. Fleshner, N. E., and Kucuk, O. Antioxidant dietary supplements: Rationale and current status as chemopreventive agents for prostate cancer. Urology 2001; *57:* 90–94.
9. Caldecott, K. W., Tucker, J. D., Stanker, L. H., and Thompson, L. H. Characterization of the XRCC1-DNA ligase III complex *in vitro* and its absence from mutant hamster cells. Nucleic Acids Res.1995; *23:* 4836–4843.
10. 10. Dianov, G. L., Prasad, R., Wilson, S. H., and Bohr, V. A. Role of DNA polymerase _ in the excision step of long patch mammalian base excision repair. J. Biol. Chem.1999; *274:* 13741–13743.
11. Thompson, L. H., and West, M. G. XRCC1 keeps DNA from getting stranded. Mutat. Res.2000; *459:* 1–18.

12. Masson, M., Niedergang, C., Schreiber, V., Muller, S., Menissier-de Murcia, J., and 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:* 3563–3571.
13. Zhang, Y., Weksler, B., Wang, L., Schwartz, J., and Santella, R. Immunohistochemical detection of polycyclic aromatic hydrocarbon-DNA damage in human blood vessels of smokers and non-smokers. Atherosclerosis.1998;*140:* 325–331.
14. Lunn, R. M., Langlois, R. G., Hsieh, L. L., Thompson, C. L., and Bell, D. A. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res.1999; *59:* 2557–2561.
15. Duell, E. J., Wiencke, J. K., Cheng, T., Varkonyi, A., Zuo, Z., Ashock, T. D., Mark, E. J., Wain, J. C., Christiani, D. C., and Kelsey, K. T. Polymorphisms in the DNA repair genes *XRCC1* and *ERCC2* and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis (Lond.).2000; *21:* 965–971.
16. Abdel-Rahman, S. Z., and El-Zein, R. A. 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:* 63–71.
17. Evans, E., Moggs, J.G.,Hwang, J.R., Egly, J.M.,and Wood, R. D. Mechanism of open complex and dual incision formation by human nucleotide excision repair factors. EMBO J.1997;*16:* 6559–6573.
18. Moncollin, V., Vichi, P., and Egly, J. M. TFIIH: a transcription factor involved in DNA repair and cell-cycle regulation. *In:* J. A. Nickoloffn and M. F. Hoekstra (eds.), DNA Damage and Repair, pp. 143–159. Totowa, New Jersey: