

Polymorphisms/Haplotypes in DNA Repair Genes and Smoking: A Bladder Cancer Case-Control Study

Giuseppe Matullo,^{1,2} Simonetta Guarrera,¹ Carlotta Sacerdote,³ Silvia Polidoro,¹ Laura Davico,³ Sara Gamberini,² Margaret Karagas,⁶ Giovanni Casetta,⁴ Luigi Rolle,⁵ Alberto Piazza,² and Paolo Vineis^{1,3,7}

¹Institute for Scientific Interchange Foundation; ²Dip. Genetica, Biologia e Biochimica, University of Torino; ³Unità di Epidemiologia dei Tumori, Dip. Scienze Biomediche e Oncologia Umana; ⁴Divisione di Urologia 2 and ⁵Divisione di Urologia 1, Ospedale S. Giovanni Battista, Turin, Italy; ⁶University of Dartmouth, Hanover, New Hampshire; and ⁷Imperial College, London, United Kingdom

Abstract

Bladder cancer is associated with tobacco smoking and occupational exposure. The repair of DNA damage has a key role in protecting the genome from the insults of cancer-causing agents. We analyzed 13 polymorphisms in seven DNA repair genes belonging to different repair pathways [*X-ray repair cross-complementing group 1* (*XRCC1*): 26304C>T, 26651A>G, 28152A>G; *xeroderma pigmentosum-D* (*XPD*): 23591A>G, 35931A>C; *excision repair complementing defective in Chinese hamster, group 1* (*ERCC1*): 19007C>T; *XRCC3*: 4541T>C, 17893A>G, 18067C>T; *proliferating cell nuclear antigen* (*PCNA*): 6084G>C; *ERCC4*: 30028C>T, 30147A>G; and *XRCC2-31479A>G*] in 317 incident bladder cancer patients and 317 controls. After adjustment for age and smoking, the *PCNA-6084C* variant was significantly associated with an increased risk of bladder cancer [CC + CG versus GG, odds ratio (OR), 1.61; 95% confidence interval (95% CI), 1.00-2.61], as well as the *XRCC1-26651G* variant (GG+AG versus AA: OR, 1.73; 95% CI, 1.17-2.56). After stratifying by smoking habits, an elevated risk for carriers of the *XRCC3-18067T* allele was detected both in current (TT versus CC: OR,

2.65; 95% CI, 1.21-5.80; CT versus CC: OR, 1.96; 95% CI, 1.09-3.52) and never smokers (TT versus CC: OR, 4.34; 95% CI, 1.14-16.46; CT versus CC: OR, 2.02; 95% CI, 0.72-5.66), whereas an opposite and slightly weaker effect was associated to the *XRCC3-17893G* allele in current smokers (GG versus AA: OR, 0.30; 95% CI, 0.11-0.82; AG versus AA: OR, 0.73; 95% CI, 0.42-1.27). *XRCC3*, *XRCC1*, *ERCC4*, and *XPD-ERCC1* haplotype frequencies were estimated by the maximum likelihood method. The *XRCC3-TAT* haplotype was associated with an enhanced risk in the current smokers group (OR, 1.62; 95% CI, 1.15-2.29), whereas a reduction of the risk in the overall sample was observed in the presence of the *XRCC3-TAC* (OR, 0.69; 95% CI, 0.50-0.97). A significant protective effect of the *XPD-ERCC1-ACC* haplotype was observed among never smokers (OR, 0.16; 95% CI, 0.03-0.81). Our results suggest that polymorphisms and/or haplotypes in *XRCC3*, *XRCC1*, and *PCNA* genes and spanning *XPD-ERCC1* region may modulate bladder cancer risk and that some of these effects may preferentially affect current smokers. (Cancer Epidemiol Biomarkers Prev 2005;14(11):2569-78)

Introduction

In industrialized countries, bladder cancer is the fifth most common cancer in men. Although development of bladder cancer is associated with exposure to tobacco (which explains >50% of bladder cancers) and occupational exposure (~10-20%), only a small proportion of exposed individuals will develop cancer, suggesting the involvement of predisposing genetic factors.

Environmental and occupational chemical carcinogens, such as polycyclic aromatic hydrocarbons, aromatic amines, or *N*-nitroso compounds, mainly form DNA adducts repaired mostly through the nucleotide excision repair pathway [e.g., *xeroderma pigmentosum-D* (*XPD*) gene and *excision repair complementing defective in Chinese hamster, group 1* (*ERCC1*)]. These agents can produce interstrand cross-links repaired by genes involved in both nucleotide excision repair [e.g., *excision repair complementing defective in Chinese hamster, group 1* (*ERCC1*) and *ERCC4*] and homologous recombinational repair

[e.g., *X-ray repair cross-complementing group 2-3* (*XRCC2-3*)] pathways. Reactive oxygen species can also induce base damage, abasic sites, single-strand breaks, and double-strand breaks: Single-strand breaks are repaired through the base excision repair pathway [e.g., *XRCC1* and *proliferating cell nuclear antigen* (*PCNA*)], whereas double-strand breaks are corrected by either homologous recombination (e.g., *XRCC2-3*) or nonhomologous end-joining pathways.

Hundreds of polymorphisms in DNA repair genes have been identified; however, the effect on repair phenotype and cancer susceptibility remains uncertain for many of these (see also dbDNP database: <http://www.ncbi.nlm.nih.gov/SNP/>; refs. 1-3).

Functions of *XRCC1*, *XRCC2*, *XRCC3*, *XPD*, *ERCC1*, and *ERCC4* proteins have been described extensively (4-11).

Studies to date indicate that variation in DNA repair genes in each of the pathways may influence bladder cancer susceptibility, including polymorphisms in *XRCC1-28152G>A* (Arg³⁹⁹Gln), *XRCC1-26304C>T* (Arg¹⁹⁴Trp), *XRCC3-18067C>T* (Thr²⁴¹Met), and possibly *XPD/ERCC2-35931A>C* (Lys⁷⁵¹Gln) (12-18); however, results are not fully consistent and other potentially important polymorphisms in these and other genes have not been explored yet. Some other polymorphisms, such as *XPD-23591G>A* (Asp³¹²Asn) (19-22), *XRCC3-4541T>C* (5'-UTR) (19, 23), *XRCC3-17893A>G* (IVS6-14) (23), *ERCC1-19007C>T* (Asn¹¹⁸Asn) (20, 24, 25), *XRCC2-31479G>A* (Arg¹⁸⁸His) (23), *ERCC4-30028C>T* (Ser⁸³⁵Ser) (20), *XRCC1-26651G>A* (Pro²⁰⁶Pro) (26),

Received 3/16/05; revised 8/26/05; accepted 8/5/05.

Grant support: Compagnia di San Paolo (Turin, Italy; P. Vineis) and of the Associazione Italiana per le Ricerche sul Cancro (G. Matullo).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Paolo Vineis, Department of Epidemiology and Public Health, Imperial College of Science, Technology and Medicine, Norfolk Place, London W2 1PG, United Kingdom. E-mail: p.vineis@imperial.ac.uk

Copyright © 2005 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-05-0189

have been studied for different malignancies, but not as yet for bladder cancer. Still others, such as *PCNA*-6084G>C(5'UTR) and *ERCC4*-30147G>A(Glu⁸⁷⁵Gly), have not been investigated with respect to cancer susceptibility.

The greatly increased density of markers in specific chromosomal regions now allows us to investigate the possible involvement of a number of genes in the development of diseases using linkage disequilibrium to associate markers and disease at the population level. Still, few molecular epidemiologic studies have been published on cancer susceptibility conferred by specific DNA repair gene haplotypes (12, 25-31) whose allelic combination might be more relevant in determining the possible functional change at the protein level than single low-penetrant single nucleotide polymorphisms (SNP). To date, none of the studies on DNA haplotypes examined bladder cancer risk.

In the present study, we expanded our DNA repair gene polymorphisms analysis (13) to 13 biallelic polymorphisms in seven DNA repair genes (Table 1) involved in different repair pathways: base excision repair, nucleotide excision repair, and double-strand break repair. Moreover, we examined the bladder cancer risk associated with estimated haplotypes for SNPs lying in the same gene/region.

Materials and Methods

Subjects. We did a hospital-based case-control investigation at two urology departments of S. Giovanni Battista hospital in Turin, where about half of the newly diagnosed bladder cancers in the Turin metropolitan area are treated. The case group comprised men ages 34 to 76 years, resident in the Turin metropolitan area with newly diagnosed, histologically confirmed bladder cancer treated from 1994 to 2003. Cases were identified by daily contact between a trained interviewer and the urology departments, followed by histologic confirmation from the pathology departments. Controls were recruited daily in random fashion (a) from patients treated at the same urology department for benign diseases, mainly prostatic hyperplasia and cystitis (all newly diagnosed), and (b) from patients treated at the medical and surgical departments for hernias, vasculopathies, diabetes, heart failure, asthma, or other benign diseases (none being represented in >10% of controls). Patients with cancer, liver, or renal diseases and smoking-related conditions were excluded. Like the cases, controls were men ages 34 to 76 years and living in the Turin metropolitan area. Before therapy began, and after signature of an informed consent form, a trained interviewer administered a detailed questionnaire to cases and controls to retrieve data on individual tobacco smoking history (including brands and tobacco type), occupational history, and a 24-hour recall interview to collect information about dietary habits and drug use. Smoking habits were defined as current (plus ex-smokers

since <1 year), former (who ceased smoking since at least 1 year), and never smokers. Blood samples were collected before therapy.

Overall, 386 cases and 324 controls were eligible for interview, of whom 14 cases and 4 controls refused.

DNA Repair Gene Polymorphisms. WBC DNA was isolated and purified from stored buffy coats by enzymatic digestion of RNA and proteins, followed by phenol-chloroform extraction for the first half of the collected samples (32) and by a standard salting out procedure for the second half of the study (33). A list of the studied polymorphisms is reported in Table 1. Out of 372 cases and 320 controls who accepted to be interviewed, the DNA was exhausted for 55 cases and 3 controls recruited at the beginning of the study, leaving 317 cases and 317 controls for genetic analyses. Some additional DNA samples have been exhausted during the course of the present study; thus, for some polymorphisms, the sums do not add up to 317 cases and 317 controls.

We used a variety of genotyping techniques, choosing the most efficient approaches (i.e., reliable and cost-effective) for any given SNP and applying newer technologies during the course of the study.

PCR-RFLP Analysis. PCR followed by enzymatic digestion was used for the genotyping of the *XRCC1*-28152, *XPD*-35931, and *XRCC3*-18067 polymorphisms on the first 288 subjects. For details on primers, PCR conditions, and other technical aspects, see ref. (13).

Primer Extension/Denaturing High-Performance Liquid Chromatography. Five polymorphisms (*PCNA*-6084, *XRCC1*-26304, *XRCC1*-26651, *XRCC2*-31479, and *XRCC3*-17893) have been genotyped by the primer extension technique on a denaturing high-performance liquid chromatography instrument (34) by Varian, Inc. (Walnut Creek, CA) in the first 288 subjects; the same assay has been used for *XRCC3*-4541 genotyping in the overall sample. The primer extension technique, or single base extension, consists in a specific elongation of a primer by the dideoxynucleotide complementary to the base allelic substitution; the rate of appropriate dideoxynucleotide incorporation completely overwhelms misincorporations and heterozygotes are easily detectable after separation of the extended primers through a denaturing high-performance liquid chromatography column.

5' Nuclease Assay (TaqMan). 5' Nuclease assay with fluorogenic minor groove binder probes was used to genotype seven polymorphisms (*XPD*-35931, *PCNA*-6084, *XRCC1*-26304, *XRCC1*-26651, *XRCC1*-28152, *XRCC3*-17893, and *XRCC3*-18067) in the 346 remaining subjects, and other two polymorphisms (*XPD*-23591 and *ERCC1*-19007) in the overall sample.

Table 1. List of genes/polymorphisms analyzed

Gene name	Polymorphism	Nucleotide substitution	Nucleotide position	Reference sequence	Chromosomal location
<i>XPD</i>	Asp ³¹² Asn	G>A	23591	rs1799793	19q13.2-13.3
	Lys ⁷⁵¹ Gln	A>C	35931	rs1052559	19q13.2-13.3
<i>XRCC1</i>	Arg ¹⁹⁴ Trp	C>T	26304	rs1799782	19q13.2
	Pro ²⁰⁶ Pro	G>A	26651	rs915927	19q13.2
	Arg ³⁹⁹ Gln	G>A	28152	rs25487	19q13.2
<i>PCNA</i>	3'-UTR	G>C	6084	rs3626	20pter-p12
<i>XRCC3</i>	5'-UTR	T>C	4541	rs1799794	14q32.3
	IVS6-14	A>G	17893	rs1799796	14q32.3
	Thr ²⁴¹ Met	C>T	18067	rs861539	14q32.3
<i>ERCC1</i>	Asn ¹¹⁸ Asn	C>T	19007	rs3177700	19q13.2-13.3
<i>ERCC4</i>	Ser ⁸³⁵ Ser	C>T	30028	rs1799801	16p13.3-13.11
	Glu ⁸⁷⁵ Gly	A>G	30147	rs1800124	16p13.3-13.11
<i>XRCC2</i>	Arg ¹⁸⁸ His	G>A	31479	rs3218536	7q36.1

Primer Extension/Sequencing. A multiplex primer extension reaction followed by fragment analysis (SNaPshot technique, Applied Biosystems, Foster City, CA) on an automated sequencer ABI PRISM 310 (Applied Biosystems) was used to genotype two polymorphisms within the same PCR fragment in the *ERCC4* gene (*ERCC4*-30028 and *ERCC4*-30147); the following extension primers were used: *ERCC4*-30028 5'-GGCCATTACAGCAGATTC-3' and *ERCC4*-30147 5'-ATTAGCAGCCCTGCACA AGACGA-3'. Technical details on primers and experimental conditions can be given on request.

DNA Typing Quality Control. Methodologic validation included a comparison between PCR-RFLP, denaturing high-performance liquid chromatography, and TaqMan assay. Moreover, at least 10% of the genotyping have been randomly repeated for each polymorphism. Concordance was in the range between 99% and 100% for all the comparisons; discordant genotypes were excluded from the analysis.

Statistical Analysis. We calculated allele and genotype frequencies by gene counting method and tested for Hardy-Weinberg equilibrium. Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) were computed by logistic regression methods, adjusting for age and smoking status: (a) current smoker, (b) former smoker (quit smoking since at least 1 year), and (c) never smoker. In addition, each analysis was repeated stratifying by smoking status. We tested for interaction under a multiplicative model between smoking status and each genotype (assuming the number of variant alleles as a continuous variable) by including an interaction term in the logistic regression models. Similarly, we tested for gene-gene interactions for genotypes found to have main effects in the primary analysis. *D'* linkage disequilibrium values were calculated in the control population using the Maximum Likelihood method (Arlequin version 2.000) and haplotype frequencies were estimated in cases and controls. ORs and 95% CIs were computed to identify possible unfavorable (risk)/favorable (protective) haplotypes; each haplotype was tested against all the others. Additionally, we computed a "risk score" (risk allele presence/absence) for those polymorphisms for which consistent *a priori* information was available in bladder cancer studies (*XPD*-35931A>C, *XRCC1*-26304C>T, *XRCC1*-28152A>G, and *XRCC3*-18067C>T; refs. 12-18). These risk alleles are not necessarily the least frequent. Indeed, the minor allele is considered a risk allele only for *XRCC3*-18067C>T, whereas the minor alleles of the other three SNPs were considered protective.

Taking into account that all the variant allele frequencies were in the range 0.08 to 0.42 (except for *ERCC4*-30147 *G* = 0.03), the size of the study was sufficient to detect an OR of between 2.03 and 1.56, with $\alpha = 0.05$ and $\beta_{(\text{type II error})} = 20\%$. All analyses were done by the SPSS 10.0.1 package for personal computers (SPSS, Inc., Chicago, IL) and values of $P < 0.05$ were considered statistically significant.

A major challenge of studies on gene-environment interactions is the distinction between true-positive results and the false-positive results generated by the multiple comparisons allowed by high-throughput technologies. Wacholder et al. (35) proposed a simple Bayesian approach based on the estimation of a prior probability and the calculation of posterior probability. The prior probability can be represented by previous studies, biochemical or molecular information (e.g., gene expression) that support the function of a SNP, or other types of evidence such as alignment conservation information. Then, the new observations are weighed (a) with prior evidence and (b) power of the study to obtain a rate of false-positive results. We have weighed our observations on the basis of a meta-analysis we have conducted, based on a website on DNA repair that we maintain (<http://perseus.isi.it/huge>). Similarly to a recent paper on DNA repair (36), which

used a range of probabilities between 0.5 and 0.001, we gave a high prior probability (0.25) when (a) the biological plausibility was high and (b) the existing epidemiologic evidence of association with bladder cancer was fair; a prior probability of 0.1 when the prior epidemiologic evidence was poor but biological plausibility was high; and a prior probability of 0.01 when both were poor. The available epidemiologic evidence did not allow higher prior probabilities in any case.

Results

We analyzed 13 DNA repair SNPs in 317 male bladder cancer patients (mean age 63.6 ± 7.2 years) and in 317 male controls (mean age 57.1 ± 9.7 years). All polymorphisms were in Hardy-Weinberg equilibrium both in control and in case groups. Although it seems unlikely that the analyzed SNPs could strongly influence aging or survival, when we tested the genotype distribution by age tertiles, the *XPD*-35931 C allele (Gln) seemed to be underrepresented in the third tertile (>66 years) in cases (tertiles: I, 28.0%; II, 16.8%; III, 7.9%) but not in controls (tertiles: I, 8.9%; II, 23.9%; III, 14.7%), with a significant linear trend only for cases ($P = 0.003$).

The variant allele frequencies in controls/cases were, respectively, as follows: *XRCC1*-26304T = 0.09/0.06, *XRCC1*-26651G = 0.38/0.45, *XRCC1*-28152A = 0.38/0.35, *XPD*-23591A = 0.41/0.42, *XPD*-35931C = 0.42/0.43, *ERCC1*-19007C = 0.39/0.36, *XRCC3*-4541C = 0.20/0.19, *XRCC3*-17893G = 0.26/0.26, *XRCC3*-18067T = 0.40/0.44, *PCNA*-6084C = 0.08/0.11, *ERCC4*-30028C = 0.32/0.34, *ERCC4*-30147G = 0.03/0.03, *XRCC2*-31479A = 0.08/0.08. Genotype frequencies and ORs (95% CI) for cases and controls in the overall group are shown in Table 2.

After adjustment for age and smoking habits, there was a significantly increased risk associated with the C variant allele of *PCNA*-6084G>C polymorphism in the overall group (CC+CG versus GG: OR, 1.61; 95% CI, 1.00-2.61). We also found an elevated risk associated with the G variant allele of the *XRCC1*-26651A>G polymorphism both in the overall group (GG+AG versus AA: OR, 1.73; 95% CI, 1.17-2.56) and in the current smokers.

Gene-Smoking and Gene-Gene Interactions. We observed a decreased risk for the *ERCC1*-19007C variant allele (CC+CT versus TT: OR, 0.62; 95% CI, 0.41-0.95), which was consistent across smoking groups. For the *XRCC3*-17893A>G, no overall association was observed, although the variant allele was related to a reduced risk among current smokers. For the *XRCC3*-18067C>T polymorphism, an elevated risk for the T variant allele was observed specifically among current smokers and never smokers. A significant decreased risk was found for the *XRCC3*-4541T>C polymorphism among never smokers (CC+CT versus TT: OR, 0.27; 95% CI, 0.10-0.77) and a borderline increase in risk was observed for the *XPD*-35931C variant homozygotes among current smokers. The other individual polymorphisms we examined did not seem to be clearly related to either reduced or enhanced bladder risk.

A test for interaction under a multiplicative model between smoking history and each genotype was done using logistic regression for *XRCC1*-26651, *XRCC3*-18067, *XRCC3*-17893, *PCNA*-6084, and *ERCC1*-19007 polymorphisms. A significant interaction was observed for *XRCC3*-18067 and *XRCC3*-17893 ($P = 0.0027$ and 0.0198 , respectively). A borderline significant gene-gene interaction was found only for *XRCC1*-26651**XRCC3*-18067 and *ERCC1*-19007**XRCC3*-17893 ($P = 0.07$ and 0.05 , respectively; Table 3).

Haplotype Analysis. We reconstructed maximum likelihood haplotypes for *XRCC3*, *XRCC1*, *XPD/ERCC1*, and *ERCC4* genes (Table 3). Linkage disequilibrium *D'* measures for the control population are reported in Fig. 1; two different blocks

Table 2. Case-control distribution of genotypes for the different DNA repair gene polymorphisms

	Overall			Current smokers		
	Controls, n (%)	Cases, n (%)	OR (95% CI)	Controls, n (%)	Cases, n (%)	OR (95%CI)
<i>XPB</i> -23591						
G/G	103 (33.8)	92 (31.5)	1.00	34 (32.1)	52 (31.0)	1.00
A/G	155 (50.8)	153 (52.4)	1.05 (0.70-1.57)	58 (54.7)	92 (54.8)	1.06 (0.59-1.91)
A/A	47 (15.4)	47 (16.1)	1.14 (0.65-1.98)	14 (13.2)	24 (14.3)	1.36 (0.56-3.31)
<i>XPB</i> -35931						
A/A	100 (31.8)	97 (30.7)	1.00	40 (36.0)	57 (31.3)	1.00
A/C	166 (52.9)	167 (52.8)	1.10 (0.72-1.59)	57 (51.4)	92 (50.5)	1.13 (0.64-1.99)
C/C	48 (15.3)	52 (16.5)	1.28 (0.74-2.22)	14 (12.6)	33 (18.1)	2.23 (0.96-5.18)
<i>PCNA</i> -6084						
G/G	248 (84.6)	225 (79.2)	1.00	92 (86.0)	132 (79.5)	1.00
C/G	42 (14.3)	53 (18.7)	1.57 (0.95-2.60)	14 (13.1)	29 (17.5)	1.46 (0.70-3.06)
C/C	3 (1.0)	6 (2.1)	1.99 (0.43-9.21)	1 (0.9)	5 (3.0)	2.97 (0.33-26.49)
<i>XRCC1</i> -26304						
C/C	260 (83.1)	275 (87.3)	1.00	90 (81.8)	157 (86.3)	1.00
C/T	51 (16.3)	40 (12.7)	0.69 (0.47-1.14)	19 (17.3)	25 (13.7)	0.69 (0.35-1.39)
T/T	2 (0.6)	0 (0.0)	0.03 (0-8.86e5)	1 (0.9)	0 (0.0)	0.01 (0-4.04e9)
<i>XRCC1</i> -26651						
A/A	116 (40.0)	87 (30.4)	1.00	47 (43.5)	53 (31.5)	1.00
A/G	125 (43.1)	139 (48.6)	1.77 (1.17-2.70)	41 (38.0)	81 (48.2)	1.96 (1.08-3.56)
G/G	49 (16.9)	60 (21.0)	1.63 (0.96-2.75)	20 (18.5)	34 (20.2)	1.61 (0.77-3.37)
	<i>P</i> = 0.05					
<i>XRCC1</i> -28152						
G/G	120 (38.5)	136 (43.7)	1.00	46 (41.8)	81 (45.3)	1.00
A/G	145 (46.5)	135 (43.4)	0.78 (0.54-1.15)	48 (43.6)	79 (44.1)	0.79 (0.45-1.38)
A/A	47 (15.1)	40 (12.9)	0.71 (0.40-1.25)	16 (14.5)	19 (10.6)	0.49 (0.20-1.17)
<i>XRCC3</i> -4541						
T/T	201 (63.8)	207 (65.5)	1.00	70 (63.1)	119 (65.0)	1.00
C/T	102 (32.4)	98 (31.0)	0.92 (0.63-1.35)	37 (33.3)	56 (30.6)	0.97 (0.55-1.68)
C/C	12 (3.8)	11 (3.5)	0.71 (0.27-1.85)	4 (3.6)	8 (4.4)	0.93 (0.24-3.55)
<i>XRCC3</i> -17893						
A/A	166 (53.4)	171 (55.3)	1.00	57 (51.4)	106 (59.6)	1.00
A/G	126 (40.5)	117 (37.9)	0.96 (0.66-1.39)	43 (38.7)	63 (35.4)	0.73 (0.42-1.27)
G/G	19 (6.1)	21 (6.8)	0.90 (0.44-1.84)	11 (9.9)	9 (5.1)	0.30 (0.11-0.82)
<i>XRCC3</i> -18067						
C/C	117 (36.9)	99 (31.2)	1.00	42 (37.8)	48 (26.2)	1.00
C/T	148 (46.7)	155 (48.9)	1.38 (0.93-2.05)	54 (48.6)	94 (51.4)	1.96 (1.09-3.52)
T/T	52 (16.4)	63 (19.9)	1.45 (0.87-2.42)	15 (13.5)	41 (22.4)	2.65 (1.21-5.80)
				<i>P</i> = 0.05		
<i>ERCC1</i> -19007						
T/T	86 (34.5)	99 (43.2)	1.00	29 (30.5)	56 (42.4)	1.00
C/T	132 (53.0)	94 (41.0)	0.53 (0.34-0.84)	56 (58.9)	54 (40.9)	0.55 (0.29-1.02)
C/C	31 (12.4)	36 (15.7)	1.06 (0.55-2.02)	10 (10.5)	22 (16.7)	1.33 (0.51-3.43)
	<i>P</i> = 0.033			<i>P</i> = 0.026		
<i>ERCC4</i> -30028						
T/T	106 (49.8)	92 (45.1)	1.00	41 (52.6)	53 (43.1)	1.00
C/T	79 (37.1)	87 (42.6)	1.45 (0.90-2.35)	25 (32.1)	50 (40.7)	1.56 (0.79-3.07)
C/C	28 (13.1)	25 (12.3)	1.06 (0.52-2.16)	12 (15.4)	20 (16.3)	1.57 (0.63-3.92)
<i>ERCC4</i> -30147						
A/A	272 (93.5)	274 (94.8)	1.00	103 (96.3)	158 (92.9)	1.00
A/G	19 (6.5)	15 (5.2)	0.75 (0.33-1.71)	4 (3.7)	12 (7.1)	1.61 (0.46-5.57)
<i>XRCC2</i> -31479						
G/G	94 (86.2)	133 (85.3)	1.00	33 (86.8)	83 (91.2)	1.00
A/G	13 (11.9)	22 (14.1)	1.78 (0.77-4.12)	4 (10.5)	8 (8.8)	1.27 (0.31-5.16)
A/A	2 (1.8)	1 (0.6)	0.54 (0.04-6.50)	1 (2.6)	0 (0.0)	0.002 (0-1.97e16)

NOTE: OR and 95% CI values are reported; ORs were adjusted for age and smoking in the overall population and for age in the stratified analysis. χ^2 *P* values from 3 × 2 contingency tables are shown when significant.

are evident for the *XRCC1* and *XPB/ERCC1* polymorphisms, although they are in the same chromosomal location; thus, we reconstructed haplotypes separately. We found a slightly elevated odds ratio associated with the *XRCC3*-TAT haplotype that was more pronounced among current smokers (OR, 1.62; 95% CI, 1.15-2.29) and of borderline significance among never smokers (OR, 1.69; 95% CI, 0.94-3.04). A reduced risk was observed for the *XRCC3*-TAC haplotype in the overall sample and for the *XRCC3*-TGC haplotype (OR, 0.70; 95% CI, 0.48-1.02) in current smokers.

Finally, we found a marginally statistically significant risk increase associated to *XRCC1*-CGA and *XRCC1*-CGG haplotypes and a slightly reduced risk related to the *XRCC1*-CAA and *XRCC1*-TAG haplotypes, possibly due to the

presence of the *XRCC1*-26651A allele. A significantly reduced risk associated to the *XPB/ERCC1*-ACC haplotype was observed among never smokers (OR, 0.16; 95% CI, 0.03-0.81). None of the *ERCC4* haplotypes was statistically significant (Table 4).

Risk Score. As described, we computed a risk score based on *a priori* data relative to *XPB*-35931A>C, *XRCC1*-26304C>T, *XRCC1*-28152A>G, and *XRCC3*-18067C>T polymorphisms (risk alleles were considered Lys = A, Arg = C, Arg = G, and Met = T, respectively). We then divided subjects in three categories (0-4, 5, and 6-8 risk alleles), roughly corresponding to the tertiles of the number of individuals [cases (311)/controls (310): I, 76 (24.4%)/101 (32.6%); II, 108 (34.7%)/106 (34.2%); III, 127 (40.8%)/103 (33.2%)]. Using this approach, we found an

Table 2. Case-control distribution of genotypes for the different DNA repair gene polymorphisms (Cont'd)

Ex-smokers			Never smokers		
Controls, n (%)	Cases, n (%)	OR (95% CI)	Controls, n (%)	Cases, n (%)	OR (95% CI)
33 (31.7)	30 (30.9)	1.00	36 (37.9)	10 (37.0)	1.00
50 (48.1)	47 (48.5)	1.02 (0.51-2.02)	47 (49.5)	14 (51.9)	1.08 (0.41-2.85)
21 (20.2)	20 (20.6)	1.02 (0.44-2.34)	12 (12.6)	3 (11.1)	0.96 (0.21-4.33)
31 (29.5)	28 (26.9)	1.00	29 (29.6)	12 (40.0)	1.00
55 (52.4)	61 (58.7)	1.28 (0.65-2.52)	54 (55.1)	14 (46.7)	0.61 (0.24-1.57)
19 (18.1)	15 (14.4)	0.88 (0.36-2.17)	15 (15.3)	4 (13.3)	0.79 (0.20-3.08)
88 (88.0)	74 (78.7)	1.00	68 (79.1)	19 (79.2)	1.00
12 (12.0)	19 (20.2)	1.87 (0.81-4.34)	16 (18.6)	5 (20.8)	1.40 (0.42-4.67)
0 (0.0)	1 (1.1)	156.14 (0-4.84e13)	2 (2.3)	0 (0.0)	0.003 (0-3.41e19)
88 (84.6)	90 (87.4)	1.00	82 (82.8)	28 (93.3)	1.00
16 (15.4)	13 (12.6)	0.83 (0.35-1.93)	16 (16.2)	2 (6.7)	0.39 (0.08-1.91)
0 (0.0)	0 (0.0)		1 (1.0)	0 (0.0)	0.04 (0-3.67e17)
41 (39.8)	27 (27.8)	1.00	28 (35.4)	7 (33.3)	1.00
48 (46.6)	48 (49.5)	1.84 (0.94-3.62)	36 (45.6)	10 (47.6)	1.00 (0.31-3.22)
14 (13.6)	22 (22.7)	1.97 (0.82-4.73)	15 (19.0)	4 (19.0)	0.97 (0.22-4.25)
35 (33.7)	42 (41.2)	1.00	39 (39.8)	13 (43.3)	1.00
54 (51.9)	44 (43.1)	0.75 (0.40-1.44)	43 (43.9)	12 (40.0)	0.82 (0.32-2.13)
15 (14.4)	16 (15.7)	0.79 (0.32-1.91)	16 (16.3)	5 (16.7)	1.29 (0.36-4.59)
72 (69.2)	64 (62.1)	1.00	59 (59.0)	24 (80.0)	1.00
28 (26.9)	37 (35.9)	1.48 (0.78-2.80)	37 (37.0)	5 (16.7)	0.25 (0.08-0.76)
4 (3.8)	2 (1.9)	0.54 (0.09-3.26)	4 (4.0)	1 (3.3)	0.46 (0.04-4.89)
58 (55.2)	51 (50.5)	1.00	51 (53.7)	14 (46.7)	1.00
43 (41.0)	41 (40.6)	1.20 (0.65-2.21)	40 (42.1)	13 (43.3)	1.23 (0.50-3.06)
4 (3.8)	9 (8.9)	3.04 (0.82-11.25)	4 (4.2)	3 (10.0)	2.12 (0.40-11.13)
36 (34.3)	44 (42.3)	1.00	39 (38.6)	7 (23.3)	1.00
44 (41.9)	45 (43.3)	0.82 (0.43-1.56)	50 (49.5)	16 (53.3)	2.02 (0.72-5.66)
25 (23.8)	15 (14.4)	0.48 (0.21-1.11)	12 (11.9)	7 (23.3)	4.34 (1.14-16.46)
28 (35.4)	32 (40.5)	1.00	29 (38.7)	11 (61.1)	1.00
39 (49.4)	35 (44.3)	0.59 (0.28-1.27)	37 (49.3)	5 (27.8)	0.33 (0.09-1.17)
12 (15.2)	12 (15.2)	0.94 (0.34-2.66)	9 (12.0)	2 (11.1)	0.75 (0.11-5.13)
32 (47.1)	35 (50.0)	1.00	33 (49.3)	4 (36.4)	1.00
27 (39.7)	31 (44.3)	1.16 (0.54-2.49)	27 (40.3)	6 (54.5)	2.56 (0.57-11.47)
9 (13.2)	4 (5.7)	0.41 (0.11-1.63)	7 (10.4)	1 (9.1)	1.69 (0.14-19.9)
90 (91.8)	93 (97.9)	1.00	79 (91.9)	23 (95.8)	1.00
8 (8.2)	2 (2.1)	0.24 (0.05-1.24)	7 (8.1)	1 (4.2)	0.74 (0.07-7.37)
39 (86.7)	38 (74.5)	1.00	22 (84.6)	12 (85.7)	1.00
5 (11.1)	12 (23.5)	2.81 (0.82-9.65)	4 (15.4)	2 (14.3)	1.97 (0.20-18.9)
1 (2.2)	1 (2.0)	1.16 (0.07-20.02)	0 (0.0)	0 (0.0)	

increased risk for patients carrying more than five risk alleles, after adjustment for age and smoking (6-8 versus 0-4: OR, 1.76; 95% CI, 1.12-2.76; 5 versus 0-4: OR, 1.38; 95% CI, 0.88-2.18), and a dose-effect of borderline statistical significance assuming the number of alleles as continuous variable (OR, 1.16; 95% CI, 0.99-1.35; $P_{\text{trend}} = 0.045$).

False-Positive Report Probability and Meta-Analysis. We used, as described above, prior probabilities ranging from 0.5 to 0.001 to weigh our observed odds ratios. In fact, proper meta-analyses, based on a DNA repair website,⁸ were possible only for the *XPD*-35931A>C, *XRCC1*-28152G>A, and *XRCC3*-18067C>T SNP. For other SNP/haplotypes, the evidence is

almost nonexistent in the case of bladder cancer. For *XPD*-35931A>C, we found meta-odds ratios (fixed-effect model) varying between 0.87 (95% CI, 0.61-1.25) for CA+CC versus AA and 1.05 (0.75-1.47) for CC versus AA (i.e., no evidence of association). For *XRCC1*-28152G>A, the only statistically significant meta-OR was for the comparison GA versus GG (OR, 1.18; 95% CI, 1.00-1.40), but a protective effect was suggested when comparing AA versus GG (OR, 0.86; 95% CI, 0.65-1.15) in agreement with our current results. For *XRCC3*-18067C>T, the OR was 1.26 (0.93-1.7) considering the TT versus CC comparison (four studies included in meta-analysis). Therefore, there is some prior evidence from meta-analyses only for *XRCC1* and *XRCC3* genes. Concerning the biological plausibility, although the three genes are probably relevant to carcinogenesis, we know little about the functional significance of the SNPs we have investigated. On this basis, we decided to

⁸ In preparation.

Table 3. ORs and 95% CIs for the joint effects of XRCC1-26651A>G versus XRCC3-18067C>T, XRCC1-26651A>G versus XRCC3-17893A>G, and ERCC1-19007T>C versus XRCC3-18067C>T polymorphisms

XRCC1-26651		XRCC3-18067							
C/C		C/T		T/T					
Controls, n (%)	Cases, n (%)	OR (95% CI)	Controls, n (%)	Cases, n (%)	OR (95% CI)	Controls, n (%)	Cases, n (%)	OR (95% CI)	
A/A	38 (36.5)	26 (29.9)	1.00	55 (40.7)	46 (33.1)	1.00	23 (45.1)	15 (25.0)	1.00
A/G	47 (45.2)	49 (56.3)	1.57 (0.78-3.16)	55 (40.7)	61 (43.9)	1.91 (1.01-3.63)	23 (45.1)	29 (48.3)	2.66 (0.93-7.70)
G/G	19 (18.3)	12 (13.8)	1.17 (0.45-3.06)	25 (18.5)	32 (23.0)	1.18 (0.56-2.52)	5 (9.8)	16 (26.7)	9.77 (2.18-43.71)
	104	87		135	139		51	60	
<i>P</i> = 0.07, test for interaction.									
XRCC3-17893									
XRCC1-26651		A/A		A/G		G/G			
Controls, n (%)	Cases, n (%)	OR (95% CI)	Controls, n (%)	Cases, n (%)	OR (95% CI)	Controls, n (%)	Cases, n (%)	OR (95% CI)	
A/A	68 (44.4)	50 (31.3)	1.00	40 (33.3)	32 (29.6)	1.00	8 (47.1)	5 (27.8)	1.00
A/G	65 (42.5)	72 (45.0)	1.85 (1.05-3.27)	55 (45.8)	56 (51.9)	1.40 (0.71-2.80)	5 (29.4)	11 (61.1)	3.52 (0.75-16.38)
G/G	20 (13.1)	38 (23.8)	2.92 (1.36-6.24)	25 (20.8)	20 (18.5)	0.98 (0.42-2.27)	4 (23.5)	2 (11.1)	0.80 (0.10-6.10)
	153	160		120	108		17	18	
<i>P</i> = 0.11, test for interaction.									
XRCC3-17893									
ERCC1-19007		A/A		A/G		G/G			
Controls, n (%)	Cases, n (%)	OR (95% CI)	Controls, n (%)	Cases, n (%)	OR (95% CI)	Controls, n (%)	Cases, n (%)	OR (95% CI)	
T/T	43 (33.6)	54 (41.9)	1.00	39 (37.5)	32 (40.5)	1.00	3 (20.0)	12 (70.6)	1.00
C/T	67 (52.3)	49 (38.0)	0.48 (0.25-0.89)	56 (53.8)	39 (49.4)	0.82 (0.40-1.71)	9 (60.0)	4 (23.5)	0.11 (0.14-0.73)
C/C	18 (14.1)	26 (20.2)	1.38 (0.59-3.25)	9 (8.7)	8 (10.1)	1.28 (0.37-4.45)	3 (20.0)	1 (5.9)	0.08 (0.01-0.88)
	128	129		104	79		15	17	
<i>P</i> = 0.05, test for interaction.									

NOTE: ORs were adjusted for age and smoking.

consider a prior probability of 0.25 for XRCC1 and for XRCC3, 0.1 for XPD, and 0.01 for PCNA. Table 5 shows the false-positive report probability for the statistically significant associations we observed. Only the XRCC3-18067C>T polymorphism seems to be considered as a true association in the current smoker group (Table 5) at a prior probability level of 0.25 and using a stringent false-positive report probability cut point of 0.2.

Discussion

Our study has investigated the relationships between 13 polymorphisms in seven DNA repair genes belonging to different repair pathways, their haplotypes, and smoking history in 317 bladder cancer patients and 317 controls. Each of the genotype frequencies lies in the range of published gene frequencies for Caucasoid populations (dbDNP database: <http://www.ncbi.nlm.nih.gov/SNP/>; refs. 2, 3). Many of the analyzed polymorphisms did not show any significant difference in the genotype distribution between cases and controls, which can be due to lack of statistical power, particularly for gene-smoking or gene-gene interactions. However, several associations emerged from our data.

The XRCC1-26651A>G polymorphism was associated with a significantly increased risk for carriers of the G variant allele. The XRCC1-26651A>G nucleotide substitution does not lead to an amino acid substitution as the proline in position 206 is maintained. For this reason, this polymorphism is unlikely to cause a functional change directly, and this may explain why it has not been studied extensively (37). However, the contrasting results reported on the well-studied XRCC1-26304C>T and XRCC1-28152A>G polymor-

phisms both in bladder cancer (12, 13, 15-18) or in other cancer studies (2, 3) suggest that other polymorphisms and, in particular, a combination of SNPs in the XRCC1 gene, could be more important than single SNPs.

We found an association between the XRCC3-18067T variant allele and an enhanced risk of bladder cancer. Similar results have been reported by Stern et al. (15) in current smokers although their results were close to statistical significance only when combined together with the XRCC1-26304C allele.

A previous study (16) described a putative protective effect of the XRCC3-18067T variant allele, whereas Sanyal et al. (17) found no significant association. In our present study, the T allele was associated with an increased risk among both current and never smokers, whereas we previously described an association in the never smokers group only (13). Other case-control studies recently investigated the role of the XRCC3-18067C>T polymorphism on cancer risk. A significantly increased risk of melanoma (19) and a nonstatistically significant increased risk of head and neck cancer have been reported (38). No association has been reported for cancers of the lung (39-41), skin (42-44), acute myeloblastic leukemia (45), breast cancer (23, 44), and gastric carcinoma (46).

Our findings about the XRCC3-17893 variant were opposite to those for the XRCC3-18067 variant (these SNPs are in strong linkage disequilibrium), in agreement with the opposite trend we observed measuring DNA adduct levels (47).⁹

⁹G. Matullo et al., unpublished data.

We also found that the *PCNA*-6084C variant allele was significantly associated with an increased risk of bladder cancer, but due to the very low frequency of the variant allele (0.08) results must be interpreted with particular caution and verified in the context of larger studies. No previous data described a possible involvement of this polymorphism in cancer association studies but the essential role played by this protein in base excision repair and other pathways (7) justifies the attempt to investigate *PCNA* gene polymorphisms in the future.

The *ERCC1*-19007C>T polymorphism leads to the silent Asn¹¹⁸Asn change, thus its possible role could be explained as for *XRCC1*-26651A>G. We observed a decreased risk for the *ERCC1*-19007C variant allele, in particular among heterozygotes, but also when pooling them with the variant homozygotes. Yin et al. (48) showed an increased risk of basal cell carcinoma for the *ERCC1*-19007 TT genotype versus CC genotype. Other studies reported some evidence of a borderline significant association alone or in combination with other polymorphisms (49, 50), with some *ERCC1* SNPs correlating with specific alterations also in *in vitro* studies on tumor cell lines (51). Positive findings have been described in cancer survival studies with better survival for the *ERCC1*-19007C variant homozygotes in non-small cell lung cancer patients (52) and, in combination with other metabolic and DNA repair polymorphisms, in colorectal cancer patients (53).

We believe that in most multifactorial diseases (e.g., most cancers), single polymorphisms in single genes unlikely alter the expression or function of specific proteins to the extent of producing a pathologic phenotype. Rather, it is possible that the combined effect of different SNPs in a gene/locus produces a change in expression/function.

In our study, we further estimated haplotype frequencies for *XRCC3*, *ERCC4*, *XRCC1*, and *XPB/ERCC1* genes. Although *XRCC1* and *XPB/ERCC1* are in the same chromosomal location (19q32.2), we did not find any significant linkage disequilibrium between these two loci as previously described (25, 28, 30).

A borderline significance has been observed for the possible unfavorable TAT haplotype of *XRCC3* gene in the overall population, with a statistically significant effect in the current smokers group. On the other hand, a significant protective effect has been found for the TAC haplotype in

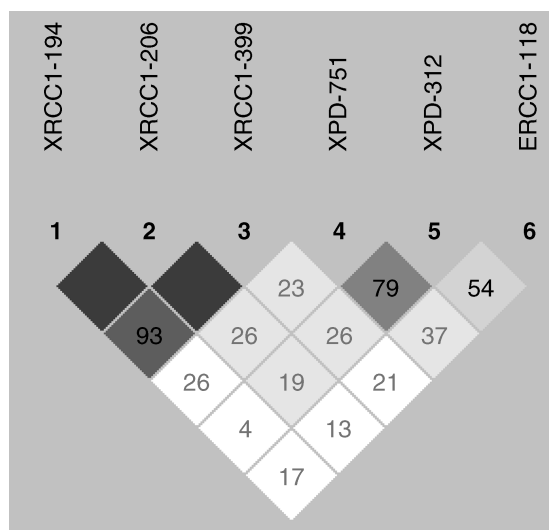


Figure 1. *D'* linkage disequilibrium values ($\times 100$) between polymorphisms in the region 19q32. *Black*, complete linkage disequilibrium.

Table 4. Overall case-control distribution of estimated haplotypes for the different DNA repair gene polymorphisms

Haplotype	Cases, n (%)	Controls, n (%)	P	OR (95% CI)
<i>XRCC3</i> 4541-17893-18067				
CAC	116 (18.7)	117 (19)	0.904	0.98 (0.74-1.31)
CAT	0 (0)	2 (0.3)	0.213	—
CGC	2 (0.4)	4 (0.7)	0.474	0.55 (0.11-2.85)
TAC	66 (10.7)	91 (14.7)	0.034	0.69 (0.50-0.97)
TAT	277 (44.8)	246 (39.8)	0.074	1.23 (0.98-1.54)
TGC	157 (25.4)	156 (25.3)	0.985	1.00 (0.78-1.30)
TGT	0 (0)	1 (0.2)	0.222	—
	618	618		
<i>XRCC1</i> 26304-26651-28152				
CAA	180 (31.6)	215 (37.1)	0.049	0.78 (0.61-1.00)
CAG	95 (16.8)	93 (16)	0.706	1.06 (0.78-1.45)
CGA	13 (2.2)	4 (0.8)	0.043	2.91 (0.98-8.59)
CGG	243 (42.8)	218 (37.7)	0.074	1.24 (0.98-1.57)
TAA	2 (0.3)	0 (0)	0.241	9.44 (0.10-934.71)
TAG	35 (6.2)	49 (8.4)	0.147	0.72 (0.46-1.13)
	568	580		
<i>XPB/ERCC1</i> 23591-35931-19007				
AAC	27 (5.9)	31 (6.3)	0.806	0.93 (0.55-1.60)
AAT	4 (1)	2 (0.4)	0.301	2.35 (0.44-12.44)
ACC	81 (18.1)	100 (20.4)	0.37	0.86 (0.62-1.19)
ACT	69 (15.3)	70 (14.3)	0.687	1.08 (0.75-1.54)
GAC	41 (9)	53 (10.8)	0.376	0.82 (0.54-1.27)
GAT	189 (42.1)	207 (42.3)	0.941	0.99 (0.76-1.28)
GCC	14 (3.2)	8 (1.5)	0.092	2.11 (0.87-5.15)
GCT	25 (5.4)	19 (3.9)	0.274	1.40 (0.76-2.59)
	450	490		
<i>ERCC4</i> 30028-30147				
CA	126 (30.9)	123 (28.9)	0.526	1.10 (0.82-1.48)
CG	11 (2.7)	12 (2.8)	0.915	0.95 (0.42-2.19)
TA	271 (66.4)	291 (68.3)	0.561	0.92 (0.69-1.23)
	408	426		

NOTE: ORs and 95% CIs have been reported for all the groups.

the overall population and for the TGC haplotype in current smokers. Only few studies investigated the *XRCC3* haplotypes and the risk of cancer. One of these (9) described an association with the GAT haplotype (corresponding to our CAT haplotype), suggesting that the last two SNPs of the haplotype are possibly more important for the definition of "at risk" individuals. Another study (23) investigated *XRCC3* involvement in skin cancer, and it found that the *XRCC3*-18067T (²⁴¹Met) allele and its associated haplotypes were significantly inversely associated with the risk of squamous and basal cell carcinoma; however, their haplotypes are not comparable with ours because they analyzed different SNPs, except for the *XRCC3*-18067 polymorphism. Finally, Jacobsen et al. (54) showed that the haplotype *XRCC3*-AAC (our *XRCC3*-TAC), and in particular another polymorphism *XRCC3*-IVS6-1571C>T (in linkage disequilibrium with the AAC haplotype), was associated with the risk of lung cancer; on the other hand, we observed a significant protection for *XRCC3*-AAC carriers. However, the same investigators reported an increased risk for the *XRCC3*-17893A and the *XRCC3*-18067T alleles, in agreement with our data. These discrepancies could be due both to cancer and/or population-specific differences, but also to the low frequencies involved in the subgroup analyses; additional work to select tagging SNPs in DNA repair genes in different populations is needed to better define possible high-risk haplotypes in relation to different ethnic/population-specific distributions.

A borderline increase in risk has been observed for *XRCC1*-CGA and for the *XRCC1*-CGG haplotypes (indicating a possible more important role of the CGN haplotype), with some evidence of a protective role of the *XRCC1*-TAG haplotype. Although the *XRCC1*-26651A/G polymorphism consists of a silent substitution (Pro²⁰⁶Pro) compared with the

Table 5. False-positive report probability

Polymorphism	Overall			Prior probability					Current smokers		
	OR (95% CI)	P	Power	0.5	0.25	0.1	0.01	0.001	OR (95% CI)	P	Power
<i>XPB</i> -35931											
A/C vs A/A	1.10 (0.72-1.60)	0.612	0.95	0.39	0.66	0.85	0.98	1.00	1.13 (0.64-2.00)	0.672	0.84
C/C vs A/A	1.28 (0.74-2.20)	0.380	0.71	0.35	0.61	0.83	0.98	1.00	2.23 (0.96-5.20)	0.062	0.18
(C/C+A/C) vs A/A	1.11 (0.76-1.60)	0.594	0.94	0.39	0.66	0.85	0.98	1.00	1.31 (0.76-2.20)	0.324	0.69
<i>PCNA</i> -6084											
C/G vs G/G	1.57 (0.95-2.60)	0.080	0.43	0.16	0.36	0.63	0.95	0.99	1.46 (0.70-3.10)	0.316	0.53
C/C vs G/G	1.99 (0.43-9.20)	0.379	0.36	0.51	0.76	0.90	0.99	1.00	2.97 (0.33-26.50)	0.330	0.27
(C/C+C/G) vs G/G	1.61 (1.00-2.60)	0.053	0.39	0.12	0.29	0.55	0.93	0.99	1.58 (0.78-3.20)	0.202	0.44
<i>XRCC1</i> -28152											
A/G vs G/G	0.78 (0.54-1.20)	0.210	0.79	0.21	0.44	0.71	0.96	1.00	0.79 (0.45-1.40)	0.408	0.72
A/A vs G/G	0.71 (0.40-1.30)	0.235	0.59	0.29	0.55	0.78	0.98	1.00	0.49 (0.20-1.20)	0.108	0.24
(A/A+A/G) vs G/G	0.77 (0.53-1.10)	0.151	0.79	0.16	0.37	0.63	0.95	0.99	0.72 (0.43-1.20)	0.222	0.61
<i>XRCC3</i> -18067											
C/T vs C/C	1.38 (0.93-2.10)	0.111	0.66	0.14	0.33	0.60	0.94	0.99	1.96 (1.09-3.50)	0.024	0.19
T/T vs C/C	1.45 (0.87-2.40)	0.155	0.55	0.22	0.46	0.72	0.97	1.00	2.65 (1.21-5.80)	0.015	0.08
(T/T+C/T) vs C/C	1.40 (0.97-2.03)	0.076	0.64	0.11	0.26	0.52	0.92	0.99	2.12 (1.21-3.70)	0.008	0.11

NOTE: Prior probabilities ranging from 0.5 to 0.001, with the estimated statistical power to detect an OR of 1.5 or 0.67 (if observed ORs were >1 or <1, respectively) with α level equal to the observed *P* value. Bold type indicates the false-positive report probability for the most likely prior probabilities.

nonconservative substitution of *XRCC1*-26304C>T (Arg¹⁹⁴Trp), it is possible that one of the real nonfunctional variants of the *XRCC1* protein is derived from a haplotype combination in which the *XRCC1*-26651G is in stronger linkage disequilibrium with other disadvantageous variants.

Few studies have been published on *XRCC1* haplotypes and cancer (26, 29, 31, 48) but the results are not directly comparable due to the genotyping of different numbers of SNPs and of different polymorphisms. In particular, we genotyped the *XRCC1*-26651A>G polymorphism, previously investigated only by Han et al. (26). Lee et al. (29) showed a protective effect on gastric cancer of the *XRCC1* haplotype "A" (¹⁹⁴Trp/²⁸⁰Arg/³⁹⁹Arg) corresponding to our T(A)G haplotype, for which we observed a non significant reduction of bladder cancer risk; they also found an increased risk for the haplotype "D" corresponding to our C(G)G/C(A)G haplotypes.

Along the same lines, Han et al. (23) described the protective effect on breast cancer of the *XRCC1* haplotype defined by the *XRCC1*-26304T variant (¹⁹⁴Trp), but the results were significant only when considering the single polymorphism and not its haplotypes. No significant association has been shown by Hao et al. (31) between esophageal squamous cell carcinoma and *XRCC1* SNPs/haplotypes, except for the *XRCC1*-77T>C polymorphism. Likewise, no association was found by Yin et al. (46) with basal cell carcinoma.

We observed a significant protective effect of the *XPB-ERCC1*-ACC haplotype among never smokers. These genes are included in the 19q13.2-13.3 chromosomal location and comprise two other genes (*RAI* and *ASE1*). This region seems to be candidate for susceptibility to basal cell carcinoma (25, 28) and postmenopausal breast cancer before the age of 55 years (30); in particular, the most significant results refer to the association with the polymorphisms in the *RAI* gene. Previous studies have found markers in *XPB* located near *RAI* to be associated with the development of melanoma, glioma, and lung cancer (24, 27, 55, 56), thus, it is possible that this limited region is important for other cancers as well. On the other hand, further studies are needed to clarify whether the same haplotype could have different roles in different cancers as it seems to be suggested by the rapidly accumulating data on single SNPs.

We investigated the combined effect of SNPs in different genes by defining an "at risk repair score" for bladder cancer on the basis of four polymorphisms (*XPB*-35931A>C, *XRCC1*-26304C>T, *XRCC1*-28152A>G, and *XRCC3*-18067C>T) with *a priori* information. A significant increase

in risk has been observed for patients carrying more than five risk alleles, suggesting a dose effect. As data accumulate on the effect of individual DNA repair gene polymorphisms and specific cancer risks, application of such scores may prove useful.

Due to the very low contribution that every single low-penetrant DNA repair polymorphism is expected to confer to the overall risk of bladder cancer, future studies should be designed to consider more tagging SNPs of a specific gene to confirm its involvement in the absence (total or partial) of functional evidence. Our results suggest that polymorphisms and/or haplotypes in *XRCC3*, *XRCC1*, and *PCNA* genes and spanning *XPB-ERCC1* region may modulate bladder cancer risk and that some of these effects may preferentially affect current smokers, as shown by our gene-smoking interaction analyses. However, when we applied a method for the estimation of the number of false-positive results, only the *XRCC3*-18067C>T polymorphism seems to be considered a true association in the current smoker group at a prior probability level of 0.25 and using a stringent false-positive report probability cut point of 0.2. Further investigations of the combined effects of polymorphisms within the same and different DNA repair genes, smoking, and other risk factors may help to estimate the effect of genetic variation on the bladder carcinogenic process.

References

- Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J Natl Cancer Inst* 2000;92:874-97.
- Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002; 11:1513-30.
- Berwick M, Matullo G, Vineis P. Studies of DNA repair and human cancer: an update. In: Wilson SH, Suk WA, editors. Biomarkers of environmentally associated disease: technologies, concepts and perspectives. New York: Lewis Publishers, CRC Press LLC; 2002. p. 83-107.
- Thompson LH, Brookman KW, Jone NJ, et al. Molecular cloning of the human *XRCC1* gene, which corrects defective DNA strand break repair and sister chromatid exchange. *Mol Cell Biol* 1990;10:6160-71.
- Liu N, Lamerdin JE, Tebbs RS, et al. *XRCC2* and *XRCC3*, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol Cell* 1998;1:783-93.
- Lunn RM, Helzlsouer KJ, Parshad R, et al. *XPB* polymorphisms: effects on DNA repair proficiency. *Carcinogenesis* 2000;4:551-5.
- Ma X, Jin Q, Forst A, et al. Single nucleotide polymorphism analyses of the human proliferating cell nuclear antigen (*PCNA*) and flap endonuclease (*FEN1*) genes. *Int J Cancer* 2000;88:938-42.

Table 5. False-positive report probability (Cont'd)

Prior probability					Never smokers			Prior probability				
0.5	0.25	0.1	0.01	0.001	OR (95% CI)	P	Power	0.5	0.25	0.1	0.01	0.001
0.45	0.71	0.88	0.99	1.00	0.61 (0.24-1.57)	0.305	0.43	0.42	0.68	0.87	0.99	1.00
0.26	0.51	0.76	0.97	1.00	0.79 (0.20-3.08)	0.734	0.60	0.55	0.79	0.92	0.99	1.00
0.32	0.58	0.81	0.98	1.00	0.64 (0.26-1.58)	0.333	0.46	0.42	0.68	0.87	0.99	1.00
0.37	0.64	0.84	0.98	1.00	1.40 (0.42-4.67)	0.584	0.54	0.52	0.76	0.91	0.99	1.00
0.55	0.79	0.92	0.99	1.00	0.003 (0.00-~)	0.823	0.42	0.66	0.86	0.95	0.99	1.00
0.31	0.58	0.80	0.98	1.00	1.21 (0.37-3.98)	0.754	0.64	0.54	0.78	0.91	0.99	1.00
0.36	0.63	0.84	0.98	1.00	0.82 (0.32-2.13)	0.684	0.66	0.51	0.76	0.90	0.99	1.00
0.31	0.57	0.80	0.98	1.00	1.29 (0.36-4.59)	0.694	0.59	0.54	0.78	0.91	0.99	1.00
0.27	0.52	0.77	0.97	1.00	0.93 (0.39-2.23)	0.871	0.77	0.53	0.77	0.91	0.99	1.00
0.12	0.28	0.54	0.93	0.99	2.02 (0.72-5.66)	0.181	0.29	0.39	0.66	0.85	0.98	1.00
0.16	0.36	0.63	0.95	0.99	4.34 (1.14-16.46)	0.031	0.06	0.34	0.61	0.82	0.98	1.00
0.07	0.18	0.40	0.88	0.99	2.42 (0.91-6.46)	0.078	0.17	0.31	0.58	0.80	0.98	1.00

8. Rafii S, O'Regan P, Xinarianos G, et al. A potential role for the XRCC2 R188H polymorphic site in DNA-damage repair and breast cancer. *Hum Mol Genet* 2002;11:1433-8.
9. Kuschel B, Auranen A, McBride S, et al. Variants in DNA double-strand break repair genes and breast cancer susceptibility. *Hum Mol Genet* 2002;11:1399-407.
10. Brookman KW, Lamerdin JE, Thelen H, et al. ERCC4 (XPF) encodes a human nucleotide excision repair protein with eukaryotic recombination homologs. *Mol Cell Biol* 1996;16:6553-62.
11. De Silva IU, McHugh PJ, Clingen PH, et al. Defects in interstrand cross-link uncoupling do not account for the extreme sensitivity of ERCC1 and XPF cells to cisplatin. *Nucleic Acids Res* 2002;30:3848-56.
12. Stern MC, Umbach DM, van Gils CH, et al. DNA repair gene XRCC1 polymorphisms, smoking, and bladder cancer risk. *Cancer Epidemiol Biomarkers Prev* 2001;10:125-31.
13. Matullo G, Guarrera S, Carturan S, et al. DNA repair gene polymorphisms, bulky DNA adducts in white blood cells and bladder cancer in a case-control study. *Int J Cancer* 2001;92:562-7.
14. Stern MC, Johnson LR, Bell DA, et al. XPD codon 751 polymorphism, metabolism genes, smoking, and bladder cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002;11:1004-11.
15. Stern MC, Umbach DM, Lunn RM, et al. DNA repair gene XRCC3 codon 241 polymorphism, its interaction with smoking and XRCC1 polymorphisms, and bladder cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002;11:939-43.
16. Shen M, Hung RJ, Brennan P, et al. Polymorphisms of the DNA repair genes XRCC1, XRCC3, XPD, interaction with environmental exposures, and bladder cancer risk in a case-control study in northern Italy. *Cancer Epidemiol Biomarkers Prev* 2003;12:1234-40.
17. Sanyal S, Festa F, Sakano S, et al. Polymorphisms in DNA repair and metabolic genes in bladder cancer. *Carcinogenesis* 2004;25:729-34.
18. Kelsey KT, Park S, Nelson HH, et al. A population-based case-control study of the XRCC1 Arg³⁹⁹Gln polymorphism and susceptibility to bladder cancer. *Cancer Epidemiol Biomarkers Prev* 2004;13:1337-41.
19. Winsey SL, Haldar NA, Marsh HP, et al. A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. *Cancer Res* 2000;60:5612-6.
20. Spitz MR, Wu X, Wang YW, et al. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. *Cancer Res* 2001;61:1354-7.
21. Xing D, Qi J, Miao X, et al. Polymorphisms of DNA repair genes XRCC1 and XPD and their associations with risk of esophageal squamous cell carcinoma in a Chinese population. *Int J Cancer* 2002;100:600-5.
22. Rybicki BA, Conti DV, Moreira A, et al. DNA repair gene XRCC1 and XPD polymorphisms and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2004;13:23-9.
23. Han J, Hankinson SE, Ranu H, et al. Polymorphisms in DNA double-strand break repair genes and breast cancer risk in the Nurses' Health Study. *Carcinogenesis* 2004;25:189-95.
24. Tomescu D, Kavanagh G, Ha T, et al. Nucleotide excision repair gene XPD polymorphisms and genetic predisposition to melanoma. *Carcinogenesis* 2001;22:403-8.
25. Yin J, Rockenbauer E, Hedayati M, et al. Multiple single nucleotide polymorphisms on human chromosome 19q13.2-3 associate with risk of Basal cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 2002;11:1449-53.
26. Han J, Hankinson SE, De Vivo I, et al. Prospective study of XRCC1 haplotypes and their interaction with plasma carotenoids on breast cancer risk. *Cancer Res* 2003;63:8536-41.
27. Butkiewicz D, Rusin M, Enewold L, et al. Genetic polymorphisms in DNA repair genes and risk of lung cancer. *Carcinogenesis* 2001;22:593-7.
28. Rockenbauer E, Bendixen MH, Bukowy Z, et al. Association of chromosome 19q13.2-3 haplotypes with basal cell carcinoma: tentative delineation of an involved region using data for single nucleotide polymorphisms in two cohorts. *Carcinogenesis* 2002;23:1149-53.
29. Lee SG, Kim B, Choi J, et al. Genetic polymorphisms of XRCC1 and risk of gastric cancer. *Cancer Lett* 2002;187:53-60.
30. Nexo BA, Vogel U, Olsen A, et al. A specific haplotype of single nucleotide polymorphisms on chromosome 19q13.2-3 encompassing the gene RAI is indicative of post-menopausal breast cancer before age 55. *Carcinogenesis* 2003;24:899-904.
31. Hao B, Wang H, Zhou K, et al. Identification of genetic variants in base excision repair pathway and their associations with risk of esophageal squamous cell carcinoma. *Cancer Res* 2004;64:4378-84.
32. Peluso M, Airoldi L, Magagnotti C, et al. White blood cell DNA adducts and fruit and vegetable consumption in bladder cancer. *Carcinogenesis* 2000;21:183-7.
33. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
34. Hoogendoorn B, Owen MJ, Oefner PJ, et al. Genotyping single nucleotide polymorphisms by primer extension and high performance liquid chromatography. *Hum Genet* 1999;104:89-93.
35. Wacholder S, Chanock S, Garcia-Closas M, et al. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst* 2004;96:434-42.
36. Hung RJ, Brennan P, Canzian F, 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.
37. 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;58:604-8.
38. Shen H, Sturgis EM, Dahlstrom KR, et al. A variant of the DNA repair gene XRCC3 and risk of squamous cell carcinoma of the head and neck: a case-control analysis. *Int J Cancer* 2002;99:869-72.
39. David-Beabes GL, Lunn RM, London SJ. No association between the XPD (Lys⁷⁵¹Gln) polymorphism or the XRCC3 (Thr²⁴¹Met) polymorphism and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 2001;10:911-2.
40. Misra RR, Ratnasinghe D, Tangrea JA, et al. Polymorphisms in the DNA repair genes XPD, XRCC1, XRCC3, and APE/ref-1, and the risk of lung cancer among male smokers in Finland. *Cancer Lett* 2003;191:171-8.
41. Medina PP, Ahrendt SA, Pollan M, et al. Screening of homologous recombination gene polymorphisms in lung cancer patients reveals an association of the NBS1-185Gln variant and p53 gene mutations. *Cancer Epidemiol Biomarkers Prev* 2003;12:699-704.
42. Duan Z, Shen H, Lee JE, et al. DNA repair gene XRCC3²⁴¹Met variant is not associated with risk of cutaneous malignant melanoma. *Cancer Epidemiol Biomarkers Prev* 2002;11:1142-3.
43. Bertram CG, Gaut RM, Barrett JH, et al. An assessment of a variant of the DNA repair gene XRCC3 as a possible nevus or melanoma susceptibility genotype. *J Invest Dermatol* 2004;122:429-32.
44. Jacobsen NR, Nexo BA, Olsen A, et al. No association between the DNA repair gene XRCC3 T241M polymorphism and risk of skin cancer and breast cancer. *Cancer Epidemiol Biomarkers Prev* 2003;12:584-5.
45. Seedhouse C, Bainton R, Lewis M, et al. The genotype distribution of the XRCC1 gene indicates a role for base excision repair in the development of therapy-related acute myeloblastic leukemia. *Blood* 2002;100:3761-6.

46. Shen H, Wang X, Hu Z, et al. Polymorphisms of DNA repair gene XRCC3 Thr²⁴¹Met and risk of gastric cancer in a Chinese population. *Cancer Lett* 2004;206:51–8.
47. Matullo G, Vineis P, Polidoro S, et al. DNA polymorphisms in XRCC1, XPD, XRCC2, ERCC4, PCNA genes and DNA-adduct levels in healthy subjects. *J UOEH* 2003;25 Suppl:107–10.
48. Yin J, Vogel U, Gerdes LU, et al. Twelve single nucleotide polymorphisms on chromosome 19q13.2-13.3: linkage disequilibria and associations with basal cell carcinoma in Danish psoriatic patients. *Biochem Genet* 2003;41:27–37.
49. Sturgis EM, Dahlstrom KR, Spitz MR, et al. DNA repair gene ERCC1 and ERCC2/XPD polymorphisms and risk of squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg* 2002;128:1084–8.
50. Chen P, Wiencke J, Aldape K, et al. Association of an ERCC1 polymorphism with adult-onset glioma. *Cancer Epidemiol Biomarkers Prev* 2000;9:843–7.
51. Yu JJ, Lee KB, Mu C, et al. Comparison of two human ovarian carcinoma cell lines (A2780/CP70 and MCAS) that are equally resistant to platinum, but differ at codon 118 of the ERCC1 gene. *Int J Oncol* 2000;16:555–60.
52. Ryu JS, Hong YC, Han HS, et al. Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. *Lung Cancer* 2004;44:311–6.
53. Stoecklacher J, Park DJ, Zhang W, et al. A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. *Br J Cancer* 2004;91:344–54.
54. Jacobsen NR, Raaschou-Nielsen O, Nexø BA, et al. XRCC3 polymorphisms and risk of lung cancer. *Cancer Lett* 2004;213:67–72.
55. Zhou W, Liu G, Miller DP, et al. Polymorphisms in the DNA repair genes XRCC1 and ERCC2, smoking, and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 2003;12:359–65.
56. Vogel U, Laros I, Jacobsen NR, et al. Two regions in chromosome 19q13.2-3 are associated with risk of lung cancer. *Mutat Res* 2004;546:65–74.

Polymorphisms/Haplotypes in DNA Repair Genes and Smoking: A Bladder Cancer Case-Control Study

Giuseppe Matullo, Simonetta Guarrera, Carlotta Sacerdote, et al.

Cancer Epidemiol Biomarkers Prev 2005;14:2569-2578.

Updated version Access the most recent version of this article at:
<http://cebp.aacrjournals.org/content/14/11/2569>

Cited articles This article cites 53 articles, 22 of which you can access for free at:
<http://cebp.aacrjournals.org/content/14/11/2569.full#ref-list-1>

Citing articles This article has been cited by 6 HighWire-hosted articles. Access the articles at:
<http://cebp.aacrjournals.org/content/14/11/2569.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cebp.aacrjournals.org/content/14/11/2569>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.