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]

[1]Institute for Scientific Interchange Foundation; [2]Dip. Genetica, Biologia e Biochimica, University of Torino; [3]Unità di Epidemiologia dei Tumori, Dip. Scienze Biomediche e Oncologia Umana; [4]Divisione di Urologia 2 and [5]Divisione di Urologia 1, Ospedale S. Giovanni Battista, Turin, Italy; [6]University of Dartmouth, Hanover, New Hampshire; and [7]Imperial 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($Arg^{399}Gln$), *XRCC1*-26304C>T($Arg^{194}Trp$), *XRCC3*-18067C>T($Thr^{241}Met$), and possibly *XPD/ERCC2*-35931A>C($Lys^{751}Gln$) (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($Asp^{312}Asn$) (19-22), *XRCC3*-4541T>C(5′-UTR) (19, 23), *XRCC3*-17893A>G(IVS6-14) (23), *ERCC1*-19007C>T($Asn^{118}Asn$) (20, 24, 25), *XRCC2*-31479G>A($Arg^{188}His$) (23), *ERCC4*-30028C>T($Ser^{835}Ser$) (20), *XRCC1*-26651G>A($Pro^{206}Pro$) (26),

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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

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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(Glu^{875}Gly), 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
XPD	Asp^{312}Asn	G>A	23591	rs1799793	19q13.2-13.3
	Lys^{751}Gln	A>C	35931	rs1052559	19q13.2-13.3
XRCC1	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
PCNA	3′-UTR	G>C	6084	rs3626	20pter-p12
XRCC3	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
ERCC1	Asn^{118}Asn	C>T	19007	rs3177700	19q13.2-13.3
ERCC4	Ser^{835}Ser	C>T	30028	rs1799801	16p13.3-13.11
	Glu^{875}Gly	A>G	30147	rs1800124	16p13.3-13.11
XRCC2	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′-ATT-AGCAGCCCTGCACA 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)
XPD-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)
XPD-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)
PCNA-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)
XRCC1-26304						
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)
XRCC1-26651						
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)
	$P = 0.05$					
XRCC1-28152						
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)
XRCC3-4541						
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)
XRCC3-17893						
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)
XRCC3-18067						
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)
				$P = 0.05$		
ERCC1-19007						
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)
	$P = 0.033$			$P = 0.026$		
ERCC4-30028						
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)
ERCC4-30147						
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)
XRCC2-31479						
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 *XPD/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 ofthe *XRCC1*-26651A allele. A significantly reduced risk associated to the *XPD/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 *XPD*-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; $P_{trend} = 0.045$).

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 nonexisting 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	
P = 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	
P = 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	
P = 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 polymorphisms 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]

[9] G. 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 Asn[118]Asn 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 *XPD/ERCC1* genes. Although *XRCC1* and *XPD/ER*CC1 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 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 ([241]Met) 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 (Pro[206]Pro) compared with the

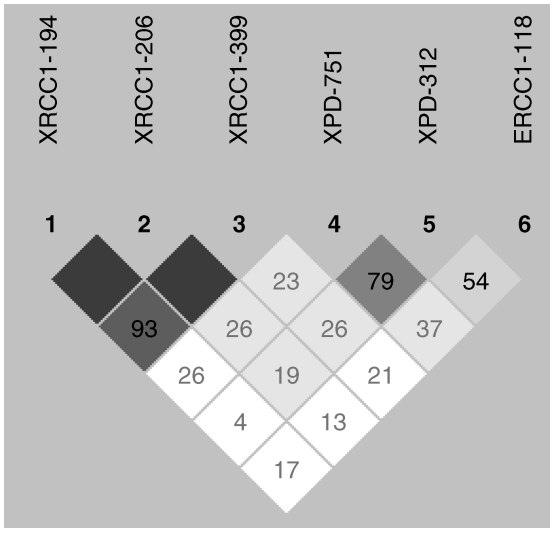

Figure 1. *D′* linkage disequilibrium values (×100) between polymorphisms in the region 19q32. *Black,* complete linkage disequilibrium.

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)
XRCC3 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		
XRCC1 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		
XPD/ERCC1 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		
ERCC4 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.

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
XPD-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
PCNA-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
XRCC1-28152											
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
XRCC3-18067											
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 ($Arg^{194}Trp$), 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'' ($^{194}Trp/^{280}Arg/^{399}Arg$) 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 (^{194}Trp), 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 *XPD-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 *ASEI*). 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 *XPD* 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 (*XPD*-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 *XPD-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.

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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	30.76	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

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

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

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